



Toxicological profile for Aluminum silicate Kaolin

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Oxo-oxoalumanyloxy-[oxo(oxoalumanyloxy)silyl]oxysilane, dihydrate (PubChem)

1.2. Synonyms

aa kaolin; acidic white clays; alfaplate; alphacoat; alphagloss; altowhite; altowhites; aluminium silicate dihydrate; aluminum silicate (hydrated); aluminum silicate hydroxide; apsilex; arcilla blanca; argiflex; argilla; argilla alba; asp (mineral); asp ultrafine; astra-glaze; bentone; beta coat; bolus alba; burgess 10; china clay; clay (kaolin); clay nanopowder; clays, white, acidic; comalco; comalco kaolin; epa pesticide chemical code 100104; expanded perlite powder; expanded perlite powder / nanopowder; halloysite clay nanotubes; halloysite nanotubes properties; hydrated aluminum silicate; hydrite; kaolin (clay); kaolinum; (PubChem) 310-194-1 [EINECS]; Aluminum, dioxo- μ -[silicic acid (H_2SiO_3)ato(2-)- κO , (hydroxy- κO)oxosilyl ester]-, hydrate (1:2); GF1670500; MFCD11045913 (ChemSpider)

1.3. Molecular formula

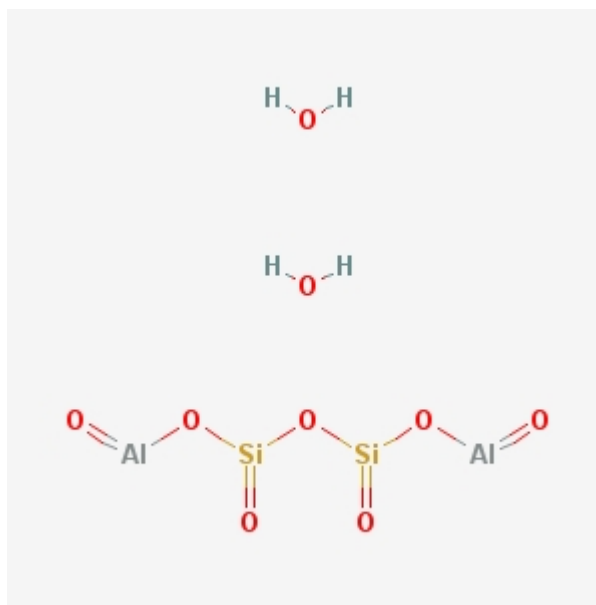
$\text{H}_2\text{Al}_2\text{Si}_2\text{O}_8 \cdot \text{H}_2\text{O}$

$\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$

$\text{Al}_2\text{H}_4\text{O}_9\text{Si}_2$

(PubChem)

1.4. Structural Formula



(PubChem)

1.5. Molecular weight (g/mol)

258.16 (PubChem)

1.6. CAS registration number

1332-58-7

1.7. Properties

1.7.1. Melting point

1760°C (VSDB, 2022)

1.7.2. Boiling point

No data available to us at this time.

1.7.3. Solubility

Insoluble in water, ether, dilute acids and alkali hydroxides (HSDB, 2013); 5.0 mg/L at 20°C (VSDB, 2022)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

No data available to us at this time.

1.7.6. Flammability limits (vol/vol%)

Not combustible (HSDB, 2013)

1.7.7. (Auto)ignition temperature

Not combustible (HSDB, 2013); "Not expected to self-ignite; Not highly flammable" (PPDB, 2021)

1.7.8. Decomposition temperature

"Does not sublime or decompose" (PPDB, 2021).

1.7.9. Stability

No rapid reaction with air; no rapid reaction with water; kaolin is stable and chemically unreactive under ordinary conditions (PubChem)

1.7.10. Vapor pressure

0 mm Hg (approx) (PubChem); "Non-volatile" (PPDB, 2021)

1.7.11. log Kow

Not applicable (VSDB, 2022)

2. General information

2.1. Exposure

Probable Routes of Human Exposure:

According to the 2006 TSCA Inventory Update Reporting data, the number of persons reasonably likely to be exposed in the industrial manufacturing, processing, and use of kaolin is 1000 or greater; the data may be greatly underestimated(1). [(1) US EPA; Inventory Update Reporting (IUR). Non-confidential 2006 IUR Records by Chemical, including Manufacturing, Processing and Use Information. Washington, DC: U.S. Environmental Protection Agency. Available from, as of Mar 25, 2013: <http://cfpub.epa.gov/iursearch/index.cfm> **PEER REVIEWED**

Occupational exposure to kaolin may occur through inhalation of dust and dermal contact with this compound at workplaces where kaolin is produced or used. Use data indicate that the general population may be exposed to kaolin via inhalation of, ingestion of, and dermal contact with consumer products containing kaolin(SRC).

Kaolin occupational exposure(1).

Total Dust Exposure

Year	Exposure group	No. area samples	Avg/range (mg/cu m)
1981	Car loader	3	10/7-15
1981	Bin operator	7	20/10-30
1981	Mill operator	8	20/10-80
1981	Baghouse operator	3	5/1-9

[(1) IPCS; Environmental Health Criteria 231. Bentonite, Kaolin, and Selected Clay Minerals. Geneva, Switzerland: World Health Org, Internl Prog Chem Safety (2005)] **PEER REVIEWED**

Body Burden:

Kaolin occupational exposure - personal samples(1).

Respirable Dust

Year	Exposure group	No. area samples	Avg/range (mg/cu m)
1981	Miner	10	0.2/0.1-0.35
1981	Car loader	3	1/0.7-1.2
1981	Bin operator	3	2/1.3-2.5
1981	Mill operator	3	1/0.8-1.2
1981	Baghouse operator	5	2/0.3-5
1951	Kaolin mining & processing		max value 377
1960	Kaolin mining & processing		361
1979	Kaolin mining and processing		<5
1977	Kaolin processing	14	3.9 mean

1978	Kaolin processing	9	3.8 mean
1980	Kaolin processing	5	5.3 mean
1981	Kaolin processing	14	1.8 mean
1981	Kaolin processing	68	1.74 mean
1980	Maintenance area	15	0.9 mean
1981	Maintenance area	11	0.1 mean
1981	Kaolin mine	12	0.8 mean
1981	Kaolin mine	5	0.1 mean
1980	Kaolin mine	4	0.14
1990	"UK" China Clay industry	8000	1.6
1978	Attritor mill	500	4.7 (9.32 mean)
1990	Attritor mill		2.1 (3.36 mean)
1978	Dryers		3.5 (5.41 mean)
1990	Dryers		1.7 (2.78 mean)
1978	Calciners		3.5 (3.93 mean)
1990	Calciners		2.32 (3.25 mean)
1978	Slurry paints	2	1.6 (mean)
1990	Slurry paints		1.2 (2.26 mean)
1984-1986	Attritor mill	114	2.7
1984-1986	Dryers	681	1.9
1984-1986	Calciners	63	2.5
1984-1986	Slurry paints	69	1.1
1984-1986	Tube presses	5	0.5

[(1) IPCS; Environmental Health Criteria 231. Bentonite, Kaolin, and Selected Clay Minerals. Geneva, Switzerland: World Health Org, Internl Prog Chem Safety (2005)] **PEER REVIEWED**

HUMAN EXPOSURE: Kaolin is a natural component in soil and found in ambient air. Kaolin mining and refining involve considerable exposure and significant exposure is expected in paper, rubber and plastics production.

Kaolin's production and use in paper, domestic products, petroleum industry, construction materials, medications and cosmetics(1) may result in its release to the environment through

various waste streams(SRC). Its use in insecticide formulations(1) will result in its direct release to the environment(SRC). [(1) Murray HH; Kirk-Othmer Encyclopedia of Chemical Technology. (1999-2013). New York, NY: John Wiley & Sons; Clays, Uses. Online Posting Date: 20 Dec 2002] **PEER REVIEWED**

Kaolin-coated paper is used in the manufacture of cigarettes(1). [(1) IPCS; Environmental Health Criteria 231. Bentonite, Kaolin, and Selected Clay Minerals. Geneva, Switzerland: World Health Org, Internl Prog Chem Safety (2005)] **PEER REVIEWED**

As taken from HSDB, 2013.

Kaolin is used as an abrasive, absorbent, anticaking, bulking, colorant and opacifying agent, and halloysite is used as an absorbent, anti-sebum, colorant and viscosity controlling agent in cosmetics in the EU (both CAS RN 1332-58-7). CI 77004 (CAS RNs 1302-78-9/1327-36-2/1332-58-7) is used as a cosmetic colourant in the EU. As taken from CosIng (undated).

Kaolin clay (CAS RN 1332-58-7) is listed as an ingredient (at given concentrations, where specified) in home maintenance (0-70%, includes 'old' products), hobby/craft (1-<50%), commercial/institutional (1-5%), inside the home (0-30%, includes 'old' products), landscaping/yard (1-30%, includes 'old' products), personal care (0-10%), pet care (0-15%), pesticides (1-<95%, includes 'old' products) and auto products (1-70%) by the CPID.

"Regarding to humans, pulmonary exposure is the most frequent, and although clays are usually mixed with other minerals, they have been reported to induce pneumoconiosis per se. Oral exposure is also common both intentionally and unintentionally. Although they do not show a high toxicity through this pathway, toxic effects could be induced due to the increased or reduced exposure to mineral elements." As taken from Maisanaba S et al. 2015. Environ. Res. 138, 233-54. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25732897>

The general population exposure to low concentrations of Mt, kaolinite, and other clay minerals in their natural form is ubiquitous (WHO, 2005). The incorporation of the clay minerals into polymers can result in non-intentional exposure to the consumer, and it is thus necessary to evaluate not only the microstructured clay but also the imbued nanomaterial in the polymer due to the possible migration to the food product (EFSA. European Food Safety Authority, 2011a, Guillard et al., 2010 and Song et al., 2011). Moreover, humans can absorb clays through occupational and environmental exposures and also due to their presence in consumer products (pharmaceutical formulations, spas, and beauty therapy) (Carretero et al., 2006). In contrast, the presence of clays in the environment and their effects on wildlife can also be derived from intentional and non-intentional sources. Maisanaba S et al. (2015). Toxicological evaluation of clay minerals and derived nanocomposites: a review. Environ. Res. 138, 233-54. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25732897>

Permissible exposure limits (PEL) for kaolin (respirable dust containing no asbestos and <1% crystalline silica, CAS RN 1332-58-7): 2 mg/m³ (Cal/OSHA).

Kaolin (CAS RN 1332-58-7) is used as an abrasive, absorbent, anticaking, bulking, clarifying, diluent and surfactant - suspending agent in non-medicinal natural health products (Health Canada, 2021).

Clay ingredients may be used in products that can be incidentally ingested; for example, Kaolin is used in lipstick (at up to 14.5%).¹⁷ Additionally, clay ingredients have been reported to be used in products that may come into contact with the eyes and mucous membranes; for example, Kaolin is used in several types of eye makeup preparations (at up to 8.5% in eyeshadows) and in personal cleanliness preparations (at up to 5% in bath soaps and detergents). (...) Kaolin is reported to be used in the paper industry to fill and coat the surface of paper, as a filler in rubber and plastics, paint extender, ceramics manufacture, ink, adhesives, insecticides, medicines, food additives, bleaching, adsorbents, cement, fertilizers, crayons, pencils, detergents, porcelain enamels, paste,

foundries, linoleum, floor tiles, and textiles. It has been classified by the National Formulary as a tablet and/or capsule diluent.

CIR (2022)

2.2. Combustion products

“The most commonly observed forms of aluminum, silicon, and titanium in tobacco products are aluminum silicates (e.g., kaolin), silica, and titanium(IV) oxide. These compounds are neither water soluble nor volatile at cigarette combustion temperatures. Rather, they are transported in mainstream tobacco smoke as particles after being freed by combustion from the tobacco filler and can induce pulmonary inflammation when inhaled. Aluminum silicate particles are the most frequently observed particles in the pulmonary macrophages of smokers and have become known as "smokers' inclusions." A relatively new technique, single particle triple quadrupole inductively coupled plasma-mass spectrometry (sp-QQQ-ICP-MS) was used to analyze aluminum, silicon, and titanium-containing particle deliveries in cigarette and little cigar mainstream tobacco smoke, and to collect information on solid inorganic particles. The mass concentration of aluminum containing particles transmitted in mainstream smoke was low (.89-.56 ng/cigarette), which was not surprising because aluminum silicates are not volatile. Although the collective masses (ng/cigarette) of aluminum, silicon, and titanium containing particles under 100 nm diameter transported in mainstream smoke were low, an abundance of "ultrafine" particles (particles <100nm or nanoparticles) was observed. Limitations of the particle background equivalent diameter (BED-the smallest detectable particle size (MassHunter 4.5 Software) (1, 2) due to the environmentally ubiquitous silicon background restricted the determination of silica nanoparticles, but silica particles slightly below 200 nm diameter were consistently detected. Aluminum and titanium containing nanoparticles were observed in all cigarette and little cigar samples, with titanium(IV) oxide particle deliveries consistently fewer in number and smaller in diameter than the other two types of particles. The highest concentrations of aluminum-containing particles (as kaolin) were in the nanoparticle range with much lower concentrations extending to the larger particle sizes (>100nm). The number and range of particle sizes determined in mainstream smoke is consistent with pulmonary deposition of aluminum silicates described by other researchers as contributing to the "smokers' inclusions" observed in pulmonary macrophages.” As taken from Fresquez MR et al. 2020. J. Anal. Toxicol. Epub ahead of print. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33044491/>

2.3. Ingredient(s) from which it originates

Naturally occurring substance, kaolin (CI 77004)

CI 77004 is classed chemically as an inorganic colour. It consists of a natural hydrated aluminum silicate.

As taken from CosIng (undated).

Natural Pollution Sources:

Kaolinite is found in most weathering zones and soil profiles.

Kaolin related clays occur in several different types of deposits. Many kaolin deposits throughout the world are in the form of tabular lenses and discontinuous beds in sedimentary rock. Extensive sedimentary deposits of this type occur in the Georgia-South Carolina kaolin belt, Arkansas bauxite region and Lone district California.

Kaolins occur on every continent.

Atmospheric Concentrations:

Kaolin and the clay mineral kaolinite are natural soil components and occur in the ambient air as floating dust.

As taken from HSDB, 2013

Kaolin's structure is composed of a single silica tetrahedral sheet and a single alumina octahedral sheet combined in a unit so that the tips of the silica tetrahedrons and one of the layers of the octahedral sheet form a common layer. All the tips of the silica tetrahedrons point in the same direction and toward the center of the unit made by the silica and octahedral sheets. Composite octahedral-tetrahedral layers are formed due to the similarity between the sheets a and b dimensions. The common layer between the octahedral and tetrahedral groups consists of two-thirds of shared atoms between silicon and aluminum that become O instead of OH. Analyses of Kaolin have shown there is little substitution within the lattice. In a small percentage of cases, iron and/or titanium has replaced aluminum. This has only been seen in the relatively poor crystalline varieties of Kaolin. (...)

A typical mineral composition of Kaolin (reported as kaolinite) is approximately 45% silicon dioxide, 39%, aluminum oxide, 0.8% iron (III) oxide, 0.08% magnesium oxide, 0.08% calcium oxide, 0.1% potassium oxide, 0.7% sodium oxide, 0.2% titanium (IV) oxide, and 14% water. According to the Food Chemicals Codex, Kaolin is a purified clay consisting mainly of alumina, silica, and water.

CIR (2022)

3. Status in legislation and other official guidance

OSHA Standards:

"Permissible Exposure Limit: Table Z-1 8-hr Time Weighted Avg: 15 mg/cu m, total dust; 5 mg/cu m, respirable fraction". [29 CFR 1910.1000 (USDOL); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of April 15, 2008: <http://www.gpoaccess.gov/ecfr>]**PEER REVIEWED**

Threshold Limit Values:

"8 hr Time Weighted Avg (TWA): 2 mg/cu m; particulate matter containing no asbestos and <1% crystalline silica; respirable fraction". [American Conference of Governmental Industrial Hygienists TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati, OH, 2008, p. 36]**QC REVIEWED**

"Excursion Limit Recommendation: Excursions in worker exposure levels may exceed 3 times the TLV-TWA for no more than a total of 30 minutes during a work day, and under no circumstances should they exceed 5 times the TLV-TWA, provided that the TLV-TWA is not exceeded". /Particulate matter containing no asbestos and <1% crystalline silica; respirable fraction/ [American Conference of Governmental Industrial Hygienists TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati, OH, 2008, p. 5]**QC REVIEWED**

A4; Not classifiable as a human carcinogen. [American Conference of Governmental Industrial Hygienists TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati, OH, 2008, p. 36]**QC REVIEWED

NIOSH Recommendations:

Recommended Exposure Limit: 10 Hr Time-Weighted Avg: 10 mg/cu m (total). [NIOSH. NIOSH Pocket Guide to Chemical Hazards & Other Databases CD-ROM. Department of Health & Human Services, Centers for Disease Prevention & Control. National Institute for Occupational Safety & Health. DHHS (NIOSH) Publication No. 2005-151 (2005), p.]**PEER REVIEWED**

Recommended Exposure Limit: 10 Hr Time-Weighted Avg: 5 mg/cu m (respirable fraction). [NIOSH. NIOSH Pocket Guide to Chemical Hazards & Other Databases CD-ROM. Department of Health & Human Services, Centers for Disease Prevention & Control. National Institute for Occupational Safety & Health. DHHS (NIOSH) Publication No. 2005-151 (2005), p.]**PEER REVIEWED**

FDA Requirements:

Clay (kaolin) ... consists of hydrated aluminum silicate. The commercial products of clay (kaolin) contain varying quantities of alkalies and alkaline earths. Clay (kaolin) is a white to yellowish or grayish fine powder. There are at least three different minerals, kaolinite, dickite, and nacrite, classified as kaolin. Kaolinite or china clay is whiter, less contaminated with extraneous minerals, and less plastic in water. (b) In accordance with 186.1(b)(1), the ingredient is used as an indirect human food ingredient with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as an indirect human food ingredient is based upon the following current good manufacturing practice conditions of use: (1) The ingredient is used in the manufacture of paper and paperboard that contact food. (2) The ingredient is used at levels not to exceed current good manufacturing practice. (c) Prior sanctions for this ingredient different from the uses established in this regulation do not exist or have been waived. [21 CFR 186.1256; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of March 28, 2013: <http://www.ecfr.gov/cgi-bin/ECFR?page=browse>] **PEER REVIEWED**

As taken from HSDB, 2013.

8 hr TWA : 2 mg/m³ (HSE, 2020).

KAOLIN- Occupational Exposure Values				
	TWA		STEL/CEIL (C)	
	ppm	mg/m ³	ppm	mg/m ³
ACGIH TLVs		2R E		
OSHA PELs		15 (total dust) 5 (respirable fraction)		
NIOSH RELs		10 (total dust) 5 (respirable fraction)		
Carcinogenicity category	MAK-3B* (Substances which are suspected of being germ cell mutagens because of their genotoxic effects in mammalian somatic cells in vivo; in exceptional cases, substances for which there are no in vivo data but which are clearly mutagenic in vitro and structurally related to known in vivo mutagens); TLV-A4 (Not Classifiable as a Human Carcinogen)			

R = Measured as respirable fraction of the aerosol E = The value is for particulate matter containing no asbestos and < 1% crystalline silica * quartz content must be considered separately.

As taken from ACGIH, 2021a.

TLV basis: Pneumoconiosis.

As taken from ACGIH, 2021b

Kaolin clay is listed in the US EPA InertFinder Database (2023) as approved for food and non-food use pesticide products. For food use it is covered under 40 CFR section 180.1180 Kaolin; exemption from the requirement of a tolerance. (US EPA, 2023b).

Kaolin (CAS RN 1332-58-7) is not registered under REACH (ECHA).

Kaolin (CAS RN 1332-58-7) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023).

Clay (Kaolin) is included on the FDA's List of Indirect Additives Used in Food Contact Substances, is affirmed as generally recognised as safe (GRAS) for indirect food use under 21 CFR 186.1256 (Clay (kaolin)) and is also covered under 21 CFR sections 176.170 (Components of paper and paperboard in contact with aqueous and fatty foods) and 178.3297 (Colorants for polymers) (FDA, 2023a,b).

Kaolin, in the form of particles with a thickness less than 100 nm, does not raise a safety concern for the consumer when incorporated at up to 12 % in ethylene-vinyl alcohol copolymer (EVOH). EVOH "is used as an inner layer of a plastic multi-layer structure, in contact with foods and beverages stored at room temperature up to 6 months. The EVOH inner layer containing the particles should be used behind a functional barrier that prevents migration of kaolin particles into the food" (EFSA, 2014).

Kaolin (CAS RN 1332-58-7) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule). The Chemical Data Reporting (CDR) Rule requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR.

US EPA Substance Registry Services (SRS)

Kaolin (CAS RN 1332-58-7) is included on the US EPA's list of Safer Chemical Ingredients (US EPA, 2023).

Kaolin (CAS RN 1332-58-7) has been identified as "low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach" and "poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework"

Kaolin clay (CAS RN 1332-58-7) is listed by the US EPA Office of Pesticide Programs (undated) and was first registered as a biochemical and "conventional chemical" pesticide on 12 March 1985. It is noted as being undergoing registration review. Halloysite clay is also listed by the US EPA Office of Pesticide Programs, with no further details.

Aluminium silicate (kaolin; E 559) is included on the European Commission's database of food additives and is authorised for use as a food additive in the EU, as a member of Group E 551-559 (Silicon dioxide – silicates), under EU Regulation No. 2018/1497 (European Commission, database for food additives).

Kaolin is included on the US FDA's list of inactive ingredients for approved drug products. It is permitted for use as an ingredient in various products, at the following maximum potencies per unit dose and maximum daily exposure:

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)
KAOLIN	ORAL	CAPSULE	1332587	24H4NWX5CO	NA	
KAOLIN	ORAL	CAPSULE, COATED PELLETS	1332587	24H4NWX5CO	14.61 mg	

KAOLIN	ORAL	CAPSULE, EXTENDED RELEASE	1332587	24H4NWX5CO	21 mg	
KAOLIN	ORAL	POWDER, FOR SUSPENSION	1332587	24H4NWX5CO	NA	
KAOLIN	ORAL	SYRUP	1332587	24H4NWX5CO	10 mg/1ml	
KAOLIN	ORAL	TABLET	1332587	24H4NWX5CO	150 mg	
KAOLIN	ORAL	TABLET, COATED	1332587	24H4NWX5CO	52.44 mg	
KAOLIN	ORAL	TABLET, DELAYED RELEASE	1332587	24H4NWX5CO	18.5 mg	
KAOLIN	ORAL	TABLET, EXTENDED RELEASE	1332587	24H4NWX5CO	66 mg	
KAOLIN	ORAL	TABLET, SUGAR COATED	1332587	24H4NWX5CO	NA	
KAOLIN	TOPICAL	POWDER	1332587	24H4NWX5CO		10056 mg
KAOLIN	TOPICAL	SYSTEM	1332587	24H4NWX5CO		420 mg

As taken from FDA, 2023b

Substance	Natural hydrated aluminium silicate, $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$, containing calcium, magnesium or iron carbonates, ferric hydroxide, quartz-sand, mica, etc. as impurities
CAS #	1302-78-9
EC #	215-108-5
Colour index Number / Name of Common Ingredients Glossary	CI 77004
INN/ISO/AN	
Regulation	(EC) No 2009/1223
Regulated By	88/667/EEC
Other Directives/Regulations	
Annex/Ref #	IV/119
Colour	White

Product Type, body parts	
Maximum concentration in ready for use preparation	
Other	
Wording of conditions of use and warnings	
SCCS opinions	
Chemical/IUPAC Name	Natural hydrated aluminium silicate, $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$, containing calcium, magnesium or iron carbonates, ferric hydroxide, quartz-sand, mica, etc. as impurities
Identified INGREDIENTS or substances e.g.	• ALUMINUM SILICATE • BENTONITE • CI 77004 • KAOLIN
Note	
Current Version	v.1

As taken from CosIng (undated).

Kaolin (CAS RN 1332-58-7) is classified as a natural health product (NHP), for medicinal use, under Schedule 1, item 7 (a mineral) of the Natural Health Products Regulations (Health Canada, 2021).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

Energy metabolism in kaolin-induced hydrocephalic rat brain. Assessed by phosphorus (31P) magnetic resonance spectroscopy and the diversity of lactate-dehydrogenase and its isoenzyme patterns (Abstract). Energy metabolism in kaolin-induced hydrocephalic rat brain was assessed by means of 31-phosphorus magnetic resonance spectroscopy and measurement of the activity of lactate dehydrogenase (LDH) as well as its isoenzyme patterns. Decreases in beta-adenosine triphosphate and phosphocreatine were observed in hydrocephalic rat brains. While the intracellular pH was decreased at 2 to 4 weeks, it showed some recovery 6 weeks after injection of kaolin. The activity of LDH increased in the hydrocephalic state, and its isoenzyme-pattern changes were as follows: the LDH5 fraction was predominant in 1-week to 4-week rats while the LDH4 fraction was predominant in 4-week rat brains, and at 6-weeks, the LDH4 and LDH5 fractions were decreased. These data from rat brains with kaolin-induced hydrocephalus indicate that anaerobic glycolysis is the primary pathway of energy metabolism in the acute hydrocephalic state, while in the chronic state the emphasis shifts to aerobic glycolysis". M. Matsumae et al. Child's Nervous System Volume 6, Number 7 / November, 1990. As taken from PubMed, 2009 available at: <http://www.ncbi.nlm.nih.gov/pubmed/1669248>

4.2. Absorption, distribution and excretion

"Kaolin and pectin are not absorbed following oral administration. [McEvoy, G.K. (ed.). American Hospital Formulary Service--Drug Information 94. Bethesda, MD: American Society of Hospital Pharmacists, Inc. 1994 (Plus Supplements)., p. 1879]**PEER REVIEWED**

As taken from HSDB, 2013

"Poorly absorbed from the gastrointestinal tract, has a short half-life and is nearly all excreted unchanged."

As taken from VSDB, 2022.

4.3. Interactions

... "Kaolin will /impair absorption/ when administered with erythromycin or digoxin". [Booth, N.H., L.E. McDonald (eds.). Veterinary Pharmacology and Therapeutics. 5th ed. Ames, Iowa: Iowa State University Press, 1982., p. 21]**PEER REVIEWED**

"Kaolin and pectin preparations may impair the absorption of oral lincomycin by up to 90% when the drugs are administered concomitantly". [McEvoy, G.K. (ed.). American Hospital Formulary Service--Drug Information 94. Bethesda, MD: American Society of Hospital Pharmacists, Inc. 1994 (Plus Supplements)., p. 1879]**PEER REVIEWED**

"The authors/ treated cultured tracheas from hamsters with Georgia kaolin (composition not indicated; diameter 3-5 um) and kaolin coated with 3-methylcholanthrene, implanted the tracheas after 4 weeks into syngeneic hamsters, and followed the animals until moribund at 105-110 weeks. Animals treated with kaolin did not develop tumors, but a high incidence of pulmonary tumors, often fatal, was observed in animals treated with kaolin coated with 3-methylcholanthrene. Animals treated with 3-methylcholanthrene coated hematite or carbon particles also developed a similar spectrum of tumors (carcinomas, sarcomas, undifferentiated tumors)". [IPCS INCHEM; Environmental Health Criteria (EHC) Monographs. Bentonite, kaolin, and selected clay minerals (EHC 231). Available from, as of June 25, 2007: <http://www.inchem.org/pages/ehc.html>]**PEER REVIEWED**

As taken from HSDB, 2013

"PURPOSE: We investigated the possible neuroprotective effects of the free radical scavenger edaravone in experimental hydrocephalus. METHODS: Seven-day-old Wistar rats were divided into three groups: control group (C), untreated hydrocephalic (H), and hydrocephalic treated with edaravone (EH). The H and EH groups were subjected to hydrocephalus induction by 20% kaolin intracisternal injection. The edaravone (20 mg/kg) was administered daily for 14 days from the induction of hydrocephalus. All animals were daily weighed and submitted to behavioral test and assessment by magnetic resonance imaging. After 14 days, the animals were sacrificed and the brain was removed for histological, immunohistochemical, and biochemical studies. RESULTS: The gain weight was similar between groups from the ninth post-induction day. The open field test performance of EH group was better ($p < 0.05$) as compared to untreated hydrocephalic animals. Hydrocephalic animals (H and EH) showed ventricular ratio values were higher ($p < 0.05$), whereas magnetization transfer values were lower ($p < 0.05$), as compared to control animals. Astrocyte activity (glial fibrillary acidic protein) and apoptotic cells (caspase-3) of EH group were decreased on the corpus callosum ($p > 0.01$), germinal matrix ($p > 0.05$), and cerebral cortex ($p > 0.05$), as compared to H group. CONCLUSIONS: We have demonstrated that administration of edaravone for 14 consecutive days after induction of hydrocephalus reduced astrocyte activity and that it has some beneficial effects over apoptotic cell death." As taken from Garcia CA et al. 2017. Childs Nerv. Syst. 33(3), 419-428. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/27988876>

"PURPOSE: To elucidate the potential role of erythropoietin (EPO) as a neuroprotective agent against reactive astrogliosis and reducing the thinning rate of subventricular zone (SVZ) in kaolin-

induced hydrocephalic rats. **METHOD:** Thirty-six ten-week-old Sprague-Dawley rats were used in this study. Hydrocephalus was induced with 20% kaolin suspension injected into the cistern of thirty rats and leaving the six rats as normal group. The hydrocephalic rats were randomly divided into hydrocephalic and treatment group. The treatment group received daily dose of recombinant human erythropoietin (rhEPO) from day 7 to day 21 after induction. The animals were sacrificed at 7 (only for hydrocephalic group) and 14 or 21 (for both groups) days after induction. Brain was removed and was prepared for histological analysis by hematoxylin and eosin staining as well as immunohistochemistry for 4-HNE, GFAP, Iba-1, and Ki-67. **RESULTS:** Histopathological analysis showed that animals treated with rhEPO had a reduced astrocyte reactivity displayed by lower GFAP expression. Hydrocephalic rats received rhEPO also displayed reduced microglial activation shown by lower Iba-1 protein expression. Exogenous rhEPO exerted its protective action in reducing astrogliosis by inhibiting lipid peroxidation that was documented in this study as lower expression of 4-HNE than non-treated group. The SVZ thickness was progressively declining in hydrocephalus group, while the progression rate could be reduced by rhEPO. **CONCLUSION:** Erythropoietin has a potential use for inhibiting lipid peroxidation, and reactive astrogliosis in hydrocephalic animal model. The reduced thinning rate of SVZ demonstrated that EPO also had effect in reducing the hydrocephalus progressivity. Further research is warranted to explore its efficacy and safety to use in clinical setting." As taken from Suryaningtyas W et al. 2019. Childs Nerv. Syst. 35(3), 469-476. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30661113>

5. Toxicity

5.1. Single dose toxicity

Reported Fatal Dose: Practically non-toxic: probable oral lethal dose (human) above 15 g/kg; more than 1 quart (2.2 lb) for 70 kg person (150 lb). [Gosselin, R.E., R.P. Smith, H.C. Hodge. Clinical Toxicology of Commercial Products. 5th ed. Baltimore: Williams and Wilkins, 1984., p. II-95] ****PEER REVIEWED****

/OTHER TOXICITY INFORMATION/ The daily variation in an intensity of kaolin-induced writhing reaction was examined in mice kept under conditions of light; 07:00 - 19:00 and dark; 19:00 - 07:00. The number of writhes was counted for 30 minutes after a single intraperitoneal injection of kaolin at 00:00, 02:00, 04:00, 06:00, 08:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00 and 22:00. The number of writhes showed a daily variation with a peak at 18:00 and a trough at 06:00. The intensity of writhing reaction was significantly reduced by pretreatment with the bradykinin B1 (Des-Arg9-[Leu8]-BK) and B2 (icatibant) receptor antagonists. Significant daily variation in this parameter was still observed in the group with the B1 antagonist, but disappeared in the B2 antagonist-treated group. ... [Sakamoto K et al; Life Sci 61 (13): 1253-9 (1997)] ****PEER REVIEWED**** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ /In/ an acute oral study in which 120 rats were fed doses of Kaolin ranging from 100 to 210 g/kg. Fourteen rats were controls. Kaolin was inert and nonstatic except for the danger of bowel obstruction resulting in perforation. The clinical signs were listlessness, anorexia, oliguria, hypothermia, and dyspnea. These were a pathological reaction from over distension of the alimentary canal by an inert solid. The number of fatalities and the incidence and advance of bowel obstruction along the small intestine were dose related. The dose that killed 50% of the rats by bowel obstruction was 149 g/kg. [Cosmetic Ingredient Review; Final Report of the Cosmetic Ingredient Review Expert Panel; Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite; International Journal of Toxicology 22 (Suppl 1): 37-102 (2003)] ****PEER REVIEWED****

/LABORATORY ANIMALS: Acute Exposure/ /Investigators/ fed a group of 10 male Sprague-Dawley rats a control diet plus 0.5 mL Kaolin 20%-pectin 1%. The control diet was then fed for 48 hr and 72 hr later stool samples were collected. The samples were analyzed for volume, sodium, potassium, and fat content. The results were 103% increase in sodium; 184% increase in potassium; fat excretion remained at baseline. [Cosmetic Ingredient Review; Final Report of the Cosmetic Ingredient Review Expert Panel; Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite; International Journal of Toxicology 22 (Suppl 1): 37-102 (2003)] **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ ... The effects of kaolin by inhalation in guinea-pigs until 3 months after the exposure, /were only/ mild alveolar proliferation... Thereafter, patchy bronchopneumonia occurred, with massive eosinophil infiltration. By 6 months, plaque formation and capillary bronchitis were observed. [IPCS INCHEM; Environmental Health Criteria (EHC) Monographs. Bentonite, kaolin, and selected clay minerals (EHC 231). Available from, as of June 25, 2007: <http://www.inchem.org/pages/ehc.html> **PEER REVIEWED**/LABORATORY

ANIMALS: Acute Exposure/ ... caused no dermal irritation in rabbits treated with 0.5 g kaolin for 4 hours. No toxicity or clinical abnormalities were observed throughout the study. [USEPA/Office of Pesticide Programs; Reregistration Eligibility Decision Document - Kaolin (1332-58-7; PC 100104) (April 2000). Available from, as of April 1, 2013: <http://iaspub.epa.gov/apex/pesticides/f?p=chemicalsearch:1> **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ After the instillation of a single dose of commercial acid-washed kaolin containing 8% hydrated free silica and 12% mica, grade 2-3 fibrosis was observed in rats after 8 months (grade 1 = minimal reticulin fibrosis, grade 4 = maximal fibrosis, as induced by quartz). [IPCS INCHEM; Environmental Health Criteria (EHC) Monographs. Bentonite, kaolin, and selected clay minerals (EHC 231). Available from, as of June 25, 2007: <http://www.inchem.org/pages/ehc.html> **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ /Investigators/ gave subplantar injections of 0.05 mL of a 5% solution of Bentonite to male Wistar rats. The rats either received both hind paw injections at an interval of 24 hr or their left paw was injected with Bentonite and their right paw injected with 0.05 mL of a 10% solution of Kaolin. The injection was of Kaolin. Subcutaneous Bentonite granulomas were produced on the left side, both dorsally and ventrally. Simultaneously Kaolin granulomas were produced on the right side analogous to the Bentonite injection. Sodium salicylate and prednisone suppressed the Bentonite edema during the first 24 hr. The presence of mononuclear cells was confirmed. [Cosmetic Ingredient Review; Final Report of the Cosmetic Ingredient Review Expert Panel; Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite; International Journal of Toxicology 22 (Suppl 1): 37-102 (2003)] **PEER REVIEWED**

As taken from HSDB, 2013

Species	Route	Dose data	Toxic effects	Reference
Guinea pig	Intrapleural	TDLo: 150 mg/kg	Lungs, Thorax, or Respiration - fibrosis, focal (pneumoconiosis)	INHEAO Industrial Health. (National Institute of Industrial Health, 6-21-1 Nagao, Tama-ku, Kawasaki, 213 Japan) V.1- 1963- Volume(issue)/page/year:

				19,85,1981
Guinea pig	Intratracheal	TDLo: 150 mg/kg/2M	Lungs, Thorax, or Respiration - fibrosis (interstitial)	INDHE* Industrial Health (journal) V.19- 1981- Volume(issue)/page/year: 19,85,1981
Rat	Intracerebral	TDLo: 125 mg/kg/2M	Brain and Coverings - changes in cerebral spinal fluid Brain and Coverings - other degenerative changes Immunological Including Allergic - increase in cellular immune response	IJNUB7 International Journal of Neuroscience. (Gordon & Breach Science Pub. S.A., 50 W. 23rd St., New York, NY 10010) V.1- 1970- Volume(issue)/page/year: 92,9,1997
Rat	Subcutaneous	TDLo - 94.3 mg/kg	Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 111,44,2018

As taken from RTECS, 2018

“Single-dose (acute) toxicity-oral...kaolin, 149 g/kg in rats (death due to bowel obstruction)”

As taken from CIR, 2016.

Acute oral rat LD₅₀ >5000 mg/kg bw

Acute dermal rat LD₅₀ 5000 mg/kg bw

As taken from VSDB, 2022.

5.2. Repeated dose toxicity

“Carleton (1924) studied the effects of kaolin (see Table 14) by inhalation in guinea-pigs. Until 3 months after the exposure, mild alveolar proliferation only was observed. Thereafter, patchy bronchopneumonia occurred, with massive eosinophil infiltration. By 6 months, plaque formation and capillary bronchitis were observed”.

“Mossman & Craighead (1982) treated cultured tracheas from hamsters with Georgia kaolin (composition not indicated; diameter 3–5 µm) and kaolin coated with 3-methylcholanthrene, implanted the tracheas after 4 weeks into syngeneic hamsters, and followed the animals until moribund at 105–110 weeks. Animals treated with kaolin did not develop tumours, but a high incidence of pulmonary tumours, often fatal, was observed in animals treated with kaolin coated with 3-methylcholanthrene. Animals treated with 3-methylcholanthrene-coated haematite or carbon particles also developed a similar spectrum of tumours (carcinomas, sarcomas, undifferentiated tumours)”.

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

Effects of repeated exposure to clays in experimental animals

Species/ gender/ number	Dose / study details / period of experiment	Findings	Reference
Guinea-pig / number and	Inhalation exposure to china clay "as supplied to the potteries" at	Until 3 months after the exposure, pulmonary reaction was slight, limited	Carleton

gender not given	unspecified concentration 2 h/day every day, every second day, twice weekly, or weekly; follow-up until 6 months	to mild alveolar proliferation. Thereafter, thickening of the alveolar walls, emphysema, and patchy bronchopneumonia with capillary congestion and massive eosinophil infiltration occurred. By 6 months, plaque formation and capillary bronchitis were observed.	(1924)
Rat, SD(SD)BR/ male /6 per group	Exposed by inhalation to dust from the town Mexicali, containing illite, calcium montmorillonite, kaolinite, alpha-quartz (23%), and small amounts of feldspars, calcite, volcanic glass; diameter approximately 10 µm, exposure 12 mg/m ³ for 3 h, tissue analysis after 0 h, 24 h, 8 days, 1 month	Accumulation of mononuclear cells and macrophages by 24 h in the airways and alveolar spaces; disappearance of particles and cellular changes by 8 days.	Osornio-Vargas et al. (1991)

"Guinea pigs and rats /were exposed/ to airborne kaolin dust for 6 hours/day, 5 days/week for 1 year. Guinea pigs exposed at 23.4 mg/cu m showed slight pleural mottling. The lymph nodes contained large masses of dust cells. Rats exposed at 27.1 mg/cu m developed scattered dust foci; collagenous fibers were observed between the cells and the lymph nodes contained large masses of dust. Intratracheal injection of rats with kaolin dust was associated with similar reactions, but the coarser particles elicited a large number of foreign body giant cells. The silica content of the kaolin was not specified."

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ ... Guinea pigs and rats /were exposed/ to airborne kaolin dust for 6 hours/day, 5 days/week for 1 year. Guinea pigs exposed at 23.4 mg/cu m showed slight pleural mottling. The lymph nodes contained large masses of dust cells. Rats exposed at 27.1 mg/cu m developed scattered dust foci; collagenous fibers were observed between the cells and the lymph nodes contained large masses of dust. Intratracheal injection of rats with kaolin dust was associated with similar reactions, but the coarser particles elicited a large number of foreign body giant cells. The silica content of the kaolin was not specified. [American Conference of Governmental Industrial Hygienists. Documentation of the TLV's and BEI's with Other World Wide Occupational Exposure Values. CD-ROM Cincinnati, OH 45240-1634 2006.] **PEER REVIEWED**"

As taken from HSDB, 2013.

Species	Route	Dose data	Toxic effects	Reference
Rat	Inhalation	TCLo: 300 mg/m ³ /12W (intermittent)	Lungs, Thorax, or Respiration - other changes	17IPC* Proceedings of the VIIth International Pneumoconiosis Conference, Pittsburgh, Pennsylvania, August 23-26, 1988. NIOSH, U.S. Department of Health and Human Services, DHHS (NIOSH). Volume(issue)/page/year: -,897,1990
Rat	Inhalation	TCLo: 30 mg/m ³ /96W (intermittent)	Lungs, Thorax, or Respiration - fibrosis (interstitial) Lungs, Thorax, or Respiration - other changes Lungs, Thorax, or Respiration -	8COLD* Proceedings, Eighth International Conference on Occupational Lung Diseases, Prague, Czechoslovakia, September 14-17, 1993 Volume(issue)/page/year: 1,480,1993

			tumors	
Rat	Inhalation	TCLo: 9 mg/m3/96W (intermittent)	Lungs, Thorax, or Respiration - fibrosis (interstitial)	8COLD* Proceedings, Eighth International Conference on Occupational Lung Diseases, Prague, Czechoslovakia, September 14-17, 1993 Volume(issue)/page/year: 1,480,1993
Hamster	Inhalation	TCLo: 30 mg/m3/72W (intermittent)	Lungs, Thorax, or Respiration - fibrosis (interstitial) Lungs, Thorax, or Respiration - other changes Gastrointestinal - tumors	8COLD* Proceedings, Eighth International Conference on Occupational Lung Diseases, Prague, Czechoslovakia, September 14-17, 1993 Volume(issue)/page/year: 1,480,1993
Rat	Oral	TDLo: 370 gm/kg/37D (intermittent)	Blood - normocytic anemia Blood - other changes Blood - changes in erythrocyte (RBC) count	JONUAL Journal of Nutrition. (Subscription Dept., 9650 Rockville Pike, Bethesda, MD 20014) V.1- 1928- Volume(issue)/page/year: 107,2020,1977
Rat	Inhalation	TCLo: 30 mg/m3/48W (intermittent)	Lungs, Thorax, or Respiration - fibrosis (interstitial)	EPASR* United States Environmental Protection Agency, Office of Pesticides and Toxic Substances. (U.S. Environmental Protection Agency, 401 M St., SW, Washington, DC 20460) History unknown. Volume(issue)/page/year: 89- 900000360
Hamster	Inhalation	TCLo: 30 mg/m3/72W (intermittent)	Lungs, Thorax, or Respiration - fibrosis (interstitial) Lungs, Thorax, or Respiration - tumors	EPASR* United States Environmental Protection Agency, Office of Pesticides and Toxic Substances. (U.S. Environmental Protection Agency, 401 M St., SW, Washington, DC 20460) History unknown. Volume(issue)/page/year: 89- 900000360
Rat	Inhalation	TCLo: 30 mg/m3/96W (intermittent)	Lungs, Thorax, or Respiration - other changes Lungs, Thorax, or Respiration - tumors	EVHPAZ EHP, Environmental Health Perspectives. (U.S. Government Printing Office, Supt of Documents, Washington, DC 20402) No.1- 1972- Volume(issue)/page/year: 102,207,1994
Hamster	Inhalation	TCLo: 30 mg/m3/24W (intermittent)	Lungs, Thorax, or Respiration - other changes Lungs, Thorax, or Respiration - fibrosis (interstitial)	EVHPAZ EHP, Environmental Health Perspectives. (U.S. Government Printing Office, Supt of Documents, Washington, DC 20402) No.1- 1972- Volume(issue)/page/year: 102,207,1994
Hamster	Inhalation	TCLo: 30 mg/m3/48W (intermittent)	Lungs, Thorax, or Respiration - fibrosis (interstitial) Lungs, Thorax, or Respiration - tumors	INHTE5 Inhalation Toxicology. (Published by Taylor & Francis Health, 11 New Fetter Lane, London EC4P 4EE) V.1- 1989- Volume(issue)/page/year: 7,503,1995

As taken from RTECS, 2018

Severe hypokalaemia from kaolin and morphine abuse. (Abstract). A 34 year old man, registered disabled as a result of muscle weakness, was admitted to hospital because of increasing

weakness. He had profound hypokalaemia and admitted to taking up to 1400 ml of kaolin and morphine mixture daily. After considerable potassium replacement muscle power recovered completely. The hypokalaemia is likely to have been caused by the combination of liquorice extract and sodium bicarbonate in kaolin and morphine. Kirkham B, Cowell R, Rees J.

Postgrad Med J. 1987 Jul; 63(741):589-90. As taken from PubMed, 2009 available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=3658872&query_hl=1&itool=pubmed_docsum

"There are five cross-sectional studies on occupational exposure to kaolin dust that include information on exposure levels. Three are from kaolin workers in Georgia, USA, and two are from kaolin workers in the UK.....The critical effects of kaolin exposure involve the lungs, with pneumoconiosis as the most important clinical observation" (As taken from DECOS, 2007).

5.3. Reproduction toxicity

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ /Investigators/gave 12 fetal lambs and six fetal monkeys subarachnoid injections of Kaolin. A sterile suspension of 2% Kaolin in saline was injected into the cisterna magna. Fetal lambs received 1 to 3 mL of Kaolin and fetal rhesus monkeys received 0.5 to 1.0 mL. After injection the fetuses were replaced into the uterus. Prenatal ultrasound monitoring was used to document the progression of fetal ventriculomegaly. Cesarean sections were scheduled for 140 to 145 days for the sheep and 160 to 165 days for monkeys. Newborn animals with gross head enlargement were killed 2 hr after birth and necropsy was performed. Brains were sectioned for gross and microscopic examination. Five lambs and one monkey underwent ventriculoamniotic shunting at 120 days after gestation. Ventricular dilatation was apparent at 1 week following Kaolin injections. The cerebral mantle was markedly thinned, with relative preservation of the cortex and severe attenuation of the white matter. The average cortical thickness of the cingulate gyrus in the Kaolin-injected sheep was 716 u compared to 1225 u in control animals. The corpus callosum was an average of 125 u in thickness in the sheep compared to 475 u in control animals. Microscopic examination of the cortical neurons were well preserved and contained the complexity and density of neural processes. A mild-to-moderate fibrotic reaction and inflammatory cell response along the basal meninges was apparent. A large number of macrophages containing Kaolin infiltrated the subarachnoid space. In five fetuses, Kaolin was injected mistakenly into either the epidural tissues superficial to the cisterna magna or into the cervical musculature. None of these fetuses had hydrocephalus at birth. [Cosmetic Ingredient Review; Final Report of the Cosmetic Ingredient Review Expert Panel; Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite; International Journal of Toxicology 22 (Suppl 1): 37-102 (2003)] **PEER REVIEWED**"

As taken from HSDB, 2013

"Thirty-six Sprague-Dawley rats were fed a control diet, 20% kaolin (air-floated Georgia kaolin) diet, and iron-supplemented 20% kaolin diet 37–117 days before mating and during pregnancy (Patterson & Staszak, 1977). Dams receiving kaolin diet developed anaemia, whereas anaemia was not observed in dams receiving iron supplementation. The birth weight of the pups of the dams receiving kaolin was 9% smaller than that of the control dams ($P < 0.01$); again, iron supplementation prevented this decrease. There was no effect on the litter size or macroscopic malformations."

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

Species	Route	Dose data	Reproductive effects	Reference

Rat	Oral	TDLo: 590 g/kg (female 37 day(s) pre-mating 1-22 day(s) after conception)	Reproductive - Effects on Newborn - growth statistics (e.g.%, reduced weight gain)	JONUAL Journal of Nutrition. (Subscription Dept., 9650 Rockville Pike, Bethesda, MD 20014) V.1- 1928- Volume(issue)/page/year: 107,2020,1977
Rat	Oral	TDLo: 370 g/kg (female 37 day(s) pre-mating 1-22 day(s) after conception)	Reproductive - Maternal Effects - other effects Reproductive - Effects on Newborn - other neonatal measures or effects	JONUAL Journal of Nutrition. (Subscription Dept., 9650 Rockville Pike, Bethesda, MD 20014) V.1- 1928- Volume(issue)/page/year: 107,2020,1977

As taken from RTECS, 2018

"The effectiveness of two inert dusts namely "Kaolin" and "Katelsous" with serial doses of 5000, 10000, 20000, 40000, 60000, 80000 and 100000 ppm were tried along with permethrin dust (2.5, 5, 7.5 and 10.0 ppm), and fenitrothion dust (at the rate of 0.25, 0.50, 1.00, 1.25 and 2.5 ppm) against newly emerged adults of *Trogoderma granarium* Everts. All the tested dusts were mixed with conditioned rice grains. Based on adult mortality percentages (1, 2, 4, 7 and 9 days after treatment), treated adults demonstrated 100% mortality 4 days after treatment with higher concentrations of the tested materials. With lower concentrations that level was achieved after 7-9 days. Untreated beetles dead, naturally, after 17 days. When LC50 values were worked out, Fenitrothion proved to be the most toxic dust (0.5367 ppm), followed by permethrin (2.571 ppm), "Kaolin" (37620 ppm, equivalent to 3.67% W/W) and "Katelsous" (39680 ppm, equivalent to 3.97% W/W). The slopes of the four tested dusts were 1.1922 for fenitrothion, 1.7438 for kaolin, 1.8139 for permethrin and 2.8485 for katelsous. In all cases, males were more sensitive to dusts than females. All dusts affected the insect reproductive capacity to extent that no offspring was produced by adults exposed to either fenitrothion, permethrin or to the high concentrations of the two inert dusts. Adults exposed to low concentrations (0.5 - 4%) of "Kaolin" produced (8-1) dead larvae. Also low concentrations (2-8%) of "Katelsous" produced (29-1) dead larvae, in comparison with 416 alive larvae produced by untreated "control" females. After two months of exposure to all four tested dusts, parents did not produce any offspring as compared with 2800 insects produced by the untreated beetles". Mostafa TS and Al-Moagel NH. 1989. Ann. Agric. Sci. (Cairo) 34(1), 381-400. As taken from DART, 2014 available at: <http://toxnet.nlm.nih.gov/newtoxnet/dart.htm>

"Female rats receiving a 20% kaolin diet exhibited maternal anemia but no reduction in birth weight of the pups was recorded".

As taken from CIR, 2016.

"BACKGROUND: Calabash chalk, a kaolin-base substance is a common geophagic material mostly consumed by pregnant women. This study investigated its effect on the histomorphology of the foetal cerebral cortex. METHODS: Twelve gestating Wistar rats were divided equally into groups 1 and 2. On pregnancy day seven (PD7), group 2 animals were administered 200 mg/kg body weight of calabash chalk suspension, while group 1 animals served as the control and received 1 ml of distilled water, by oral gavages and for 14 days (PD7-PD20). On PD21, the dams were sacrificed, and the foetuses removed, examined for gross malformations, weighed and culled to two foetuses per mother. Their whole brains were excised, weighed and preserved using 10% buffered formalin, and routinely processed by haematoxylin and eosin, and Luxol fast blue methods. RESULTS: The foetuses showed no morphological change, but their mean body weights was higher (p=0.0001). Histomorphological sections of the cerebral cortex showed hypertrophy and hyperplasia of cells in all the cortical layers, with less demonstrated Nissl and higher (p=0.001) cellular population compared with the control group. CONCLUSION: Calabash chalk cause body weight increase and histomorphological changes in the cerebral cortex of foetuses." As taken from Ekanem TB et al. 2015. Malays. J. Med. Sci. 22(4), 17-22. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26715904>

5.4. Mutagenicity

“No information was available on the genotoxicity of clays or clay minerals. Information on quartz has been summarized by IARC (1997) and IPCS (2001). Quartz did not test positively in standard bacterial mutagenesis assays. Results of genotoxicity studies of quartz conflict, and a direct genotoxic effect for quartz has not been confirmed or ruled out (IPCS, 2001)”.

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

The genotoxic effects of kaolin nano/microparticles have been examined in an in vitro micronucleus (MN) test conducted with a human lung cancer cell line (A549 cells). In addition, DNA damage and mutations were analyzed by in vivo assay systems using male C57BL/6J or gpt delta transgenic mice which were intratracheally instilled with single or multiple doses of 0.2 mg per animal of particles. In the in vitro genotoxicity assay, dose-related increased MN frequencies were observed. DNA damage was also seen in the lungs of C57BL/6J mice measured by the comet assay. Moreover, single or multiple instillations increased both gpt and Spi⁺ mutant frequencies in the lungs of gpt delta transgenic mice. Mutation spectra analysis showed transversions were predominant, and more than 60% of the base substitutions occurred at G:C base pairs in the gpt genes. The G:C to C:G transversion was commonly increased (Totsuka et al. 2009).

“Introduction: Kaolin is a clay mineral with the chemical composition $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$. It is an important industrial material, and is also used as a white cosmetic pigment. We previously reported that fine particles of kaolin have genotoxic potency to Chinese hamster ovary CHO AA8 cells, and to the lungs of C57BL/6 J and ICR mice. In the present study, we evaluated the genotoxicity of different particle sizes of kaolin using primary normal human diploid epidermal keratinocytes and primary normal human diploid dermal fibroblasts, in addition to a CHO AA8 cell line. Findings: After 6-h treatment with kaolin micro- and nano-particles of particle sizes 4.8 μm and 0.2 μm (200 nm), respectively, the frequencies of micronucleated cells increased in a dose-dependent manner. The frequency increased 3- to 4-fold by exposure to the particles at 200 $\mu\text{g/mL}$ (i.e., 31.4 $\mu\text{g/cm}^2$) in all cells tested. Two-way ANOVA revealed a significant main effect of particle size, and the nano-particles tended to have a higher potency of micronucleus (MN) induction. However, the cell type did not significantly affect the MN frequencies. In addition, one-hour treatment with the kaolin particles increased DNA damage in a dose-dependent manner in a comet assay. The %tail DNA was increased 8- to 20-fold by exposure to the particles at 200 $\mu\text{g/mL}$, for all cells tested. The kaolin nano-particles had higher DNA-damaging potency than the micro-particles. Furthermore, treatment with kaolin particles dose-dependently increased the production of reactive oxygen species (ROS) in all cells. Again, we observed that kaolin nano-particles induced more ROS than the micro-particles in all cells. Conclusion: Kaolin particles demonstrated genotoxicity in primary normal human diploid epidermal keratinocytes and fibroblasts as well as in CHO AA8 cells. Although no significant difference was observed among these three types of cells, fine particles of kaolin tended to have higher genotoxic potency than coarse particles. Since studies on its genotoxicity to skin have been scarce, the findings of the present study could contribute to safety evaluations of kaolin particles when used as a white cosmetic pigment.” As taken from Kawanishi M et al. 2020. Genes Environ. 42, 16. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32322315/>

“Increased gpt and Spi⁺ mutant frequencies were observed in the lungs of the mice following intratracheal instillation with either single or multiple doses of 0.2 mg nano-sized Kaolin. A mutation spectra analysis showed > 60% of G:C to C:G transversion occurred in the gpt genes. In another Comet assay, rats were given 2 oral doses of up to 1000 mg/kg bw cation-exchanged montmorillonite clay. There was no statistically significant difference in % tail DNA between the negative controls and the different treatment groups for any of the cells (liver, kidneys, colon) tested.”

CIR (2022)

5.5. Cytotoxicity

“Gormley et al. (1979) studied the cytotoxicity of 30 respirable mine dust specimens with varying compositions from the United Kingdom using a macrophage-like P388 D1 cell line. Positive correlation was found between cytotoxicity and the kaolin and mica (combined) content of dusts containing >90% coal, but not in dusts containing less coal. In the study described in Table 16 on bentonite, Gormley & Addison (1983) studied the cytotoxicity of two standard kaolinites, KGa-1 and KGa-2, with particle sizes of 3.2 and 3.9 μm , which do not contain quartz or cristobalite. They showed very little effect on cell viability at 20 $\mu\text{g/ml}$; even at 80 $\mu\text{g/ml}$, the cell viability remained at 60% or more”.

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

“Styles & Wilson (1973) studied the effect of a large number of mineral dusts and polymers on the alveolar and peritoneal macrophages of rat. The kaolin sample did not damage the macrophages. Five out of six kaolin samples decreased TTC reduction by peritoneal macrophages, but only one of them also induced a release of LDH to the medium (Adamis & Timar, 1976, 1986; Timar et al., 1979, 1980; Adamis et al., 1986; Adamis & Krass, 1991) (Table 17). The surface cationic groups may play a role in the cytotoxic effect of aluminium silicates (kaolin, illite, bentonite). This is suggested by the observed decrease in the cytotoxic effect of aluminium silicate by adsorption of paraquat cations (Adamis & Timár, 1976). The cytotoxic effect increases with increasing adsorption capacity of the kaolin sample (Adamis & Timár, 1980; Adamis et al., 1985). This relationship has also been observed in connection with methylene blue, paraquat cation, serum protein, serum lipid, and serum phospholipid adsorption. After dry grinding the kaolin sample in an oscillating mill for 32 h, the adsorption and, at the same time, the macrophage cytotoxic effect decreased (Juhász et al., 1978). The above indicates the importance of the nature of the crystal surface and the magnitude of the adsorption capacity on the sample's cytotoxicity”.

“Quartz, asbestos, and kaolinite increased the elastase secretion of peritoneal macrophages. The increase, however, was not as great as that observed after phagocytosis of latex particles. With alveolar macrophages, a difference was also found between the inert latex and the dust samples. This difference probably reflects the cytotoxicity of the dust. Kaolinite has a cytotoxic effect on both peritoneal and alveolar macrophages (White & Kuhn, 1980)”.

“Davies (1983) and Davies et al. (1984) studied the cytotoxicity of Cornwall (United Kingdom) and Georgian (USA) kaolinite on mouse peritoneal macrophages. The Cornwall sample contained 98% kaolinite and 2% mica, and 98% of the particles were smaller than 5 μm in diameter; the Georgia kaolin contained 99% kaolinite and no quartz, mica, or feldspar. Both caused cytoplasmic LDH liberation from macrophages, and the cytotoxicity was apparently caused by kaolinite, not the other dust components. After polyvinylpyridine-N-oxide adsorption, the cytotoxic effect of kaolinite specimens from Cornwall decreased significantly (Davies & Preece, 1983; Davies et al., 1984). Davies et al. (1984) concluded that the amorphous silica-rich gel coating the kaolinite particles was probably responsible for the cytotoxic effect of kaolinite. The flake-like morphology of kaolinite is unlikely to play a role”.

“Low et al. (1980), studying rabbit alveolar macrophage cells, found that the presence of kaolinite inhibited the incorporation of amino acids into proteins through the non-competitive inhibition of amino acid transport”.

“Brown et al. (1980) compared different macrophage experimental systems. Kaolin proved to be cytotoxic; after thermal treatment (calcined kaolin), however, the damaging effect decreased significantly”.

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

System	Treatment	Findings	Reference

Other cell types and in vitro systems			
Tracheal epithelial (cloned cell line from Syrian hamster, strain 87.20) in log growth phase in monolayer	Kaolinite 90% <2 µm in diameter at four concentrations, 0.003-0.1 mg/ml, incubated in cell suspension for 24 h	Cells phagocytized clay particles; dose-dependent damage to plasma membrane as evidenced by loss of ⁵¹ Cr from cells; loss of ⁵¹ Cr after 24 h approximately 40% with 0.1 mg/ml, twice that of quartz.	Woodworth et al. (1982)
Human umbilical vein endothelial cells	South Carolina kaolinite (non-specified, mainly 1-2 µm diameter) incubated at 0.1 mg/ml in cell suspension for 24 h	Kaolinite induced a statistically significant 50% increase in LDH activity in the medium and killed 90% of the cells in 24 h.	Murphy et al (1993b)
Macrophage-like cell line P338D ₁	Three kaolinites, 1 with "high crystallinity," 1 with "medium crystallinity," and 1 with "low crystallinity," diameter <5 µm, incubated at 80 µg/ml for 48 h	Kaolinites caused a 78-91 % decrease in the viability of the cells and induced leakage of LDH and beta-N-acetylglucosaminidase. Adsorption of nitrous oxide on the minerals slightly decreased the effect on viability.	Robertson et al. (1982)
V79-4 Chinese hamster lung cell line	Non-specified kaolin incubated with the cells for 6-7 days	LD ₅₀ 20 mg/ml for kaolin, which was the most toxic of the 21 particulate and fibrous materials tested.	Brown et al. (1980)
Macrophage-like cell line P338D ₁	Thirty respirable dust specimens from coal mines in United Kingdom; cytotoxicity index developed from effects on trypan blue exclusion, release of LDH, glucosaminidase, and lactic acid production	A positive correlation between ash content and cytotoxicity of the dusts. In dusts with >90% coal, there was also a correlation between kaolin + mica content and cytotoxicity.	Gormley et al. (1979)
Macrophage-like cell line P338D ₁	Two kaolinites (KGa-1, KGa-2) from Source Clays repository, particle sizes 3.2 and 3.9 µm, with no cristobalite or quartz, incubated for 48 h	Cell viability not changed at 20 µg/ml, and 60-70% at 80 µg/ml.	Gormley & Addison (1983)
Isolated human leukocyte elastase	Cornwall kaolinite and four different illite clays (composition and particle size not specified), 5 µg/ml or 20 µg/ml	Kaolinite (5 µg/ml) caused 90% inhibition of the enzyme, illites (20 µg/ml), 10-53% inhibition.	Oberson et al. (1996)
Artificial organelles			
Liposomes (artificial phospholipid membrane vesicles ^b 0.1-2 µm diameter) entrapping	Kaolinite, 90% <2 µm in diameter, at five concentrations, 0.1-10 mg/ml, incubated in cell suspension for 1 h	Dose-dependent loss of chromate from vesicles; loss of chromate after 1 h (in excess of spontaneous rate) approximately 20% with 10 mg/ml; spontaneous rate was 4-6%.	Woodworth et al. (1982)

dissolved chromate (CrO ₄ ²⁻)			

“BACKGROUND: Aluminum silicates have been used to control bleeding after severe traumatic injury WoundStat (WS) is the most recent such product. We recently observed that WS caused vascular thrombosis when applied to stop bleeding. This study investigated the cellular toxicity of WS in different cell types that may be exposed to this mineral and compared the results with other minerals such as... kaolin... METHODS: Human umbilical vein endothelial cells (HUVEC), HeLa cells, and RAW267.4 mouse macrophage-like cells (RAW) were incubated directly with different concentrations of each mineral for 24 hours. Cell viability was determined metabolically using the AlamarBlue fluorescent technique. In another experiment, minerals were exposed to HUVEC via Transwell inserts with a polycarbonate filter (0.4-µm pore size) to prevent direct contact between cells and minerals for determining whether direct exposure or leaching compounds from minerals cause cytotoxicity. RESULTS: Incubation of HUVEC and RAW cells with 1 to 100 µg/mL of the minerals for 24 hours resulted in differential toxicities. The cytotoxicity of WS was higher than kaolin Neither cell type survived for 24 hours in the presence of 100 µg/mL WS ... In the second HUVEC experiment, a 10 times higher concentration of these compounds placed in Transwell inserts yielded no decrease in cell viability. This result indicates that leaching toxicants or binding of nutrients by the ion-exchange properties of minerals did not cause the toxicity. CONCLUSIONS: Although aluminum silicates seem relatively innocuous to epithelial cells, all produced some toxicity toward endothelial cells and macrophages. WS was significantly more toxic than kaolin The cytotoxic effect seemed to be caused by the direct contact of the minerals with the cells present in wounds” (Bowman et al. 2011).

/ALTERNATIVE and IN VITRO TESTS/ Apoptosis was measured in rat alveolar macrophage NR8383 cells challenged in vitro with respirable quartz or kaolin dust and with the dusts pretreated with dipalmitoyl phosphatidylcholine (DPPC) to model conditioning of respired dusts by interaction with a primary phospholipid component of pulmonary surfactant. Quartz dust is known to induce apoptosis in vitro and in vivo. For this study, quartz and kaolin were compared as dusts of similar cytotoxicity in some in vitro assays but of differing pathogenic potential: quartz can cause significant pulmonary fibrosis while kaolin generally does not. NR8383 cells exposed to native quartz at concentrations from 50 to 400 ug/mL for 6 hr showed a dose-dependent increase in apoptosis measured by the TdT-mediated dUTP-fluorescein nick end labeling (TUNEL), cell death ELISA, and DNA ladder formation assays, while native kaolin induced significant response only at the higher concentrations and only in the TUNEL and ELISA assays. For cell challenge from 6 hr to 5 days at 100 ug/mL of dust, quartz was active at all times while kaolin was active only at 5 days. DPPC pre-treatment suppressed quartz activity until 3 days and kaolin activity through 5 days. Cellular release of lactate dehydrogenase, measured in parallel experiments to compare dust apoptotic and necrotic activities, indicated that components of serum as well as surfactant may affect kaolin in vitro expression of those activities. [Gao N et al; Toxicol Appl Pharmacol 175 (3): 217-25 (2001)] **PEER REVIEWED** PubMed Abstract

/ALTERNATIVE and IN VITRO TESTS/ ... Washed sheep erythrocytes /were exposed/ to kaolin that was simply characterized as "native dust." The kaolin was found to be significantly hemolytic and had less than a 5 um particle size. [American Conference of Governmental Industrial Hygienists. Documentation of the TLV's and BEI's with Other World Wide Occupational Exposure Values. CD-ROM Cincinnati, OH 45240-1634 2006.] **PEER REVIEWED**

ALTERNATIVE and IN VITRO TESTS/ ... In vitro cytotoxicity tests /were conducted/ with sheep erythrocytes and rat alveolar macrophages. Kaolin was found to be more cytotoxic than quartz. ... Treatment with lecithin resulted in suppression of the cytotoxicity. The results imply that the kaolin can damage cells through a cell membrane and dust interaction. By coating kaolin particles with a component of surfactant, it is possible to reduce or eliminate the in vitro cytotoxicity for a period of time. [American Conference of Governmental Industrial Hygienists. Documentation of the TLV's and BEI's with Other World Wide Occupational Exposure Values. CD-ROM Cincinnati, OH 45240-1634 2006.] **PEER REVIEWED**

As taken from HSDB, 2013

The nature of the clay mineral plays an important role in the observed toxicity. In this regard, Murphy et al. (1993a) reported the following toxicity scale in HUVE cells after 24 h of incubation with 0.1 mg/mL clay minerals: Mt>bentonite=kaolinite<erionite. In addition to the above-mentioned work, most studies have evaluated the toxicity of Mt and bentonite. To the best of our knowledge, the toxicity of kaolinite has only been studied by Gao et al. (2000), who compared the results with another silicate, namely quartz. Both compounds induced cytotoxic effects on rat pulmonary alveolar macrophage cells starting on the first day of exposure, although pretreatment with surfactant delayed the toxicity... The morphological changes induced by different clay minerals have been studied in HUVE cells, which were markedly changed after 24 h of exposure to 0.1 mg/mL Mt, bentonite and kaolinite, including cell lysis (Murphy et al., 1993a). The lysis was greater in the case of Mt compared with bentonite and kaolinite, with only limited lysis observed after exposure to erionite. This finding agreed with the cytotoxicity assay results. Maisanaba S et al. (2015). Toxicological evaluation of clay minerals and derived nanocomposites: a review. Environ. Res. 138, 233-54. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25732897>

"The problems of antibiotic overuse compel us to seek alternative antibacterial agents. Some clays have been shown to kill antibiotic-resistant human pathogens and may provide an alternative to known antibiotics. Here we show that Al toxicity plays a central role in the antibacterial action of a kaolin-rich clay from the Colombian Amazon (AMZ). Antibacterial susceptibility testing shows minimum inhibitory concentrations of 80 mg/mL against a model Escherichia coli (ATCC 25922). The clay buffered the media pH to ~4.6 and Eh values to +360 mV. Chemical analysis of AMZ and bacteria showed that Al, P, and transition metals (Fe, Cu, Mn, and Zn) were exchanged during incubation at 37 °C. Only Al derived from the clay exceeded the minimum inhibitory concentrations for E. coli under acidic conditions. Ion imaging showed elevated Al levels in the bacterial membrane, and high intracellular Fe levels, relative to those of untreated controls. Phosphorus depletion in E. coli after reaction with AMZ, together with evidence of membrane permeabilization, suggests that Al reacts with membrane phospholipids, enhancing intracellular transport of metals. These results highlight the importance of dissolved Al for amplifying the toxicity of transition metals to human pathogens." As taken from Londono SC et al. 2017. Environ. Sci. Technol. 51(4), 2401-2408. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28121138>

"Objectives: The impact of kaolinite on human periodontal cells is yet unknown. The aim of the study was to assess the response of human periodontal cells to kaolinite. Methods: Human periodontal cells were treated with kaolinite at reducing concentrations from 30 to 0.0015 mg/mL and with conditioned medium, which was depleted of kaolinite. Cell viability was evaluated with a resazurin-based toxicity assay, Live-Dead staining, and MTT assay and staining. The pro-angiogenic factors vascular endothelial growth factor (VEGF) and interleukin (IL)-6 and IL-8 were quantified via ELISA in periodontal fibroblasts. L-929, a standard cell-line used for cytotoxicity studies, served as control cell line. Composition of kaolinite was verified using energy-dispersive X-ray spectroscopy. Results: Kaolinite in suspension but not in conditioned medium impaired cell viability dose-dependently. VEGF, IL-6, and IL-8 production was not substantially modulated by kaolinite or the conditioned medium in periodontal cells. Conclusion: Overall, kaolinite can decrease cell viability dose-dependently while conditioned medium showed no toxic effect. No pronounced impact of kaolinite on VEGF, IL-6, and IL-8 production was observed. This study

provided first insights into the impact of kaolinite on human periodontal cells thereby inferring to the basis for the evaluation of kaolinite as a carrier in regenerative dentistry. Clinical relevance: Kaolinite, a clay mineral, is successfully used in medicine due to its favorable properties. Also, applications in conservative dentistry are described. However, the response of oral cells to kaolinite is still unclear. Here, we assessed the impact of kaolinite on human periodontal cells.” As taken from Müller AS et al. 2020. Clin. Oral. Investig. 24(3), 1205-1215. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/31420747/>

“In this research, the organelle damage, apoptosis and necrosis induced by PM_{2.5}, BC and Kaolin were studied using human bronchial epithelial (16HBE) cells. PM_{2.5}, BC and Kaolin all induce cell death, LDH release and excess intracellular ROS generation. For the organelle injuries, Kaolin and high-dose PM_{2.5} (240 µg/mL) cause lysosomal acidification, but BC causes lysosomal alkalization (lysosomal membrane permeabilization, LMP). BC and Kaolin cause the loss of mitochondrial membrane potential (MMP), while PM_{2.5} does not. For the cell death mode, PM_{2.5} causes both apoptosis and necrosis. However only necrosis has been detected in the BC and Kaolin treated groups, indicating the more severe cellular insult. Excess ROS generation is involved in the organelle damage and cell death. ROS contributes to the BC-induced LMP and necrosis, but does not significantly affect the Kaolin-induced MMP loss and necrosis. Therefore, the BC component in PM_{2.5} may cause cytotoxicity via ROS-dependent pathways, the Kaolin component may damage cells via ROS-independent mechanisms such as strong interaction. The PM_{2.5}-induced apoptosis and necrosis can be partially mitigated after the removal of ROS, indicating the existence of both the ROS-dependent and ROS-independent mechanisms due to the complicated PM_{2.5} components. BC represents the anthropogenic source component in PM_{2.5}, while Kaolin represents the natural source component. Our results provide knowledge on the toxic mechanisms of typical PM_{2.5} components at the cellular and subcellular levels.” As taken from Shan X et al. 2021. Sci. Total Environ. 782, 146785. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33838376/>

5.6. Carcinogenicity

“A4; Not classifiable as a human carcinogen.”

“ALTERNATIVE and IN VITRO TESTS/ /The authors/ treated cultured tracheas from hamsters with Georgia kaolin (composition not indicated; diameter 3-5 µm) and kaolin coated with 3-methylcholanthrene, implanted the tracheas after 4 weeks into syngeneic hamsters, and followed the animals until moribund at 105-110 weeks. Animals treated with kaolin did not develop tumors, but a high incidence of pulmonary tumors, often fatal, was observed in animals treated with kaolin coated with 3-methylcholanthrene. Animals treated with 3-methylcholanthrene coated hematite or carbon particles also developed a similar spectrum of tumors (carcinomas, sarcomas, undifferentiated tumors). [IPCS INCHEM; Environmental Health Criteria (EHC) Monographs. Bentonite, kaolin, and selected clay minerals (EHC 231). Available from, as of June 25, 2007: <http://www.inchem.org/pages/ehc.html> **PEER REVIEWED**”

As taken from HSDB, 2013.

“Wagner and co-workers (1987) studied the carcinogenicity of palygorskite and attapulgite in an inhalation study and used “coating grade” kaolin as a negative control: 20 male and 20 female Fischer rats were exposed by inhalation (10 mg/m³, 91.4% of the particles <4.6 µm in diameter) for 6 h/day, 5 days/week, until they died. However, at 3, 6, and 12 months, four rats were killed from each group for ancillary studies (leaving only 28 animals for the carcinogenicity study). At the end of the study, full autopsy and microscopic analysis of the lungs, liver, kidney, spleen, and other organs were performed. In two kaolin-exposed rats, bronchoalveolar hyperplasia but no benign or malignant tumours of the lungs or pleura were observed. However, the number of tumours in the positive control group (crocidolite treatment at the same exposure level) was also low (one adenocarcinoma only). The mean fibrosis grading in the rats at interim sacrifices and at the end of the experiment was between 2.1 and 2.8 on a scale of 1–8 (1 being normal; 2, dust in

macrophages; 3, early interstitial reaction; 4, first signs of fibrosis; 5, 6, 7, increasing fibrosis; 8, severe fibrosis)".

"As another part of the study on the carcinogenicity of mineral fibres, Wagner et al. (1987) injected a single dose (amount not given) of kaolin intrapleurally into 20 male and 20 female Fischer rats and followed the animals until moribund or dead (survival of animals in different groups not given). None of the kaolin-treated rats developed mesothelioma, whereas 34 of 40 of those given crocidolite did".

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

"The role of silica in the causation of lung cancer is an ongoing debate. In order to explore whether observed association between silica exposure and lung cancer is confounded by exposure to other occupational carcinogens, we updated a previously nested case-control study among a cohort of male workers in 29 Chinese mines and factories on the basis of an extended follow-up. Five hundred and eleven lung cancer cases and 1,879 matched controls were selected. Exposure to respirable silica as well as relevant occupational confounders were quantitatively assessed based on historical industrial hygiene data. The relationship between exposure to silica and lung cancer was analyzed by conditional logistic regression analysis adjusted for exposure to arsenic, polycyclic aromatic hydrocarbons (PAHs), radon, and smoking. In a crude analysis adjusted for smoking only, a significant trend of increasing risk of lung cancer with exposure to silica was found for tin, iron/copper miners, and pottery workers. But after adjustment for relevant occupational confounders, no relationship between silica and lung cancer can be observed. Instead, there is a significant association between lung cancer mortality and cumulative exposure to inorganic arsenic (OR = 1.86, 95% CI: 1.14, 3.04 for each mg/m(3)-year increase) and carcinogenic PAHs (OR = 1.35, 95% CI: 1.08, 1.69 for each 100 microg/m(3)-year increase). This analysis does not provide any evidence to show that exposure to silica causes lung cancer in the absence of confounding factors" (Chen et al. 2007).

"There is a lack of epidemiological data showing increased lung tumour incidence in kaolin workers. Lung tumours have only been found incidentally in epidemiological or case studies. Therefore, the committee concludes that there is insufficient epidemiological evidence for carcinogenicity of kaolin dust under occupational exposure conditions" (As taken from DECOS, 2007).

In a case-controlled study of Canadian workers exposed to a wide range of occupational chemical agents, clay (kaolin?) was identified as potentially exhibiting bladder carcinogenic properties (Richardson et al. 2007).

"In an inhalation study and in a study using intrapleural injection, kaolin did not induce tumours in rats."

"Wagner and co-workers (1987) studied the carcinogenicity of palygorskite and attapulgite in an inhalation study and used "coating grade" kaolin as a negative control: 20 male and 20 female Fischer rats were exposed by inhalation (10 mg/m³, 91.4% of the particles <4.6 µm in diameter) for 6 h/day, 5 days/week, until they died. However, at 3, 6, and 12 months, four rats were killed from each group for ancillary studies (leaving only 28 animals for the carcinogenicity study). At the end of the study, full autopsy and microscopic analysis of the lungs, liver, kidney, spleen, and other organs were performed. In two kaolin-exposed rats, bronchoalveolar hyperplasia but no benign or malignant tumours of the lungs or pleura were observed. However, the number of tumours in the positive control group (crocidolite treatment at the same exposure level) was also low (one adenocarcinoma only). The mean fibrosis grading in the rats at interim sacrifices and at the end of the experiment was between 2.1 and 2.8 on a scale of 1–8 (1 being normal; 2, dust in macrophages; 3, early interstitial reaction; 4, first signs of fibrosis; 5, 6, 7, increasing fibrosis; 8, severe fibrosis)."

"As another part of the study on the carcinogenicity of mineral fibres, Wagner et al. (1987) injected a single dose (amount not given) of kaolin intrapleurally into 20 male and 20 female Fischer rats

and followed the animals until moribund or dead (survival of animals in different groups not given). None of the kaolin-treated rats developed mesothelioma, whereas 34 of 40 of those given crocidolite did.”

“Mossman & Craighead (1982) treated cultured tracheas from hamsters with Georgia kaolin (composition not indicated; diameter 3–5 µm) and kaolin coated with 3-methylcholanthrene, implanted the tracheas after 4 weeks into syngeneic hamsters, and followed the animals until moribund at 105–110 weeks. Animals treated with kaolin did not develop tumours, but a high incidence of pulmonary tumours, often fatal, was observed in animals treated with kaolin coated with 3-methylcholanthrene. Animals treated with 3-methylcholanthrene-coated haematite or carbon particles also developed a similar spectrum of tumours (carcinomas, sarcomas, undifferentiated tumours).”

Carcinogenicity Studies:

Species:	Rat
Strain/Sex:	Wistar/Female (48/group)
Route:	Intratracheal
Dose:	6 mg instillations of 0.01-0.03 µm particles given as 10 or 20 doses wk 26, study duration 115 or 121 wk or no treatment
Tumor Site/ Type of Lesion:	Lung: Bronchiolo-alveolar adenoma (12/48 and 11/47 in experimental animals vs 1/46 control animals), bronchiolo-alveolar carcinoma (10/48 and 22/47 vs 0/46), adenosquamous cell carcinoma (0/48 and 1/47 vs 0/46), squamous cell carcinoma (3/48 and 11/47 vs 0/46), cystic keratinizing epithelioma (6/48 and 5/47 vs 0/46), non-keratinizing epithelioma (1/48 and 0/47 vs 0/46), >1 tumor type (7/48 and 11/47 vs 0/46)
Results:	Positive
Reference:	[MOHR,U, ERNST,H, ROLLER,M, AND POTT,F; PULMONARY TUMOR TYPES INDUCED IN WISTAR RATS OF THE SO-CALLED "19-DUST STUDY"; EXPERIMENTAL AND TOXICOLOGIC PATHOLOGY 58:13-20, 2006]

As taken from CCRIS, 2008

Species	Route	Dose data	Toxic effects	Reference
Rat	Inhalation	TCLo: 30 mg/m ³ /96W (intermittent)	Lungs, Thorax, or Respiration - tumors	8COLD* Proceedings, Eighth International Conference on Occupational Lung Diseases, Prague, Czechoslovakia, September 14-17, 1993 Volume(issue)/page/year: 1,480,1993
Hamster	Inhalation	TCLo: 30 mg/m ³ /72W (intermittent)	Gastrointestinal - tumors	8COLD* Proceedings, Eighth International Conference on Occupational Lung Diseases, Prague, Czechoslovakia, September 14-17, 1993 Volume(issue)/page/year: 1,480,1993
Rat	Inhalation	TCLo: 30 mg/m ³ /72W (intermittent)	Lungs, Thorax, or Respiration - tumors	EPASR* United States Environmental Protection Agency, Office of Pesticides and Toxic Substances. (U.S. Environmental Protection Agency, 401 M St., SW, Washington, DC 20460) History unknown. Volume(issue)/page/year: 89-900000360

Hamster	Inhalation	TCLo: 30 mg/m ³ /72W (intermittent)	Lungs, Thorax, or Respiration - tumors	EPASR* United States Environmental Protection Agency, Office of Pesticides and Toxic Substances. (U.S. Environmental Protection Agency, 401 M St., SW, Washington, DC 20460) History unknown. Volume(issue)/page/year: 89-900000360
Rat	Inhalation	TCLo: 30 mg/m ³ /96W (intermittent)	Lungs, Thorax, or Respiration - tumors	EVHPAZ EHP, Environmental Health Perspectives. (U.S. Government Printing Office, Supt of Documents, Washington, DC 20402) No.1- 1972- Volume(issue)/page/year: 102,207,1994
Hamster	Inhalation	TCLo: 30 mg/m ³ /48W (intermittent)	Lungs, Thorax, or Respiration - tumors	INHTE5 Inhalation Toxicology. (Published by Taylor & Francis Health, 11 New Fetter Lane, London EC4P 4EE) V.1- 1989- Volume(issue)/page/year: 7,503,1995

As taken from RTECS, 2018

5.7. Irritation/immunotoxicity

“Although kaolin is used extensively in cosmetics, no reports have been found on the possible adverse effects (irritation/sensitisation) of kaolin upon direct skin contact” (As taken from DECOS, 2007).

Causes moderate eye irritation. Avoid contact with eyes or clothing. May cause irritation of the respiratory system. Avoid breathing dust (OPP, 2000).

“caused no dermal irritation in rabbits treated with 0.5 g kaolin for 4 hours. No toxicity or clinical abnormalities were observed throughout the study. [USEPA/Office of Pesticide Programs; Reregistration Eligibility Decision Document - Kaolin (1332-58-7; PC 100104) (April 2000). Available from, as of April 1, 2013: <http://iaspub.epa.gov/apex/pesticides/f?p=chemicalsearch:1> **PEER REVIEWED**”

As taken from HSDB, 2013.

System/ species/gender	Dose (mg/ml)/ treatment	Findings	References
Peritoneal macrophages			
Rat (Sprague-Dawley CFY)	Two types of kaolin (90% kaolinite, 4% quartz; or 93% kaolinite, 4% quartz; diameter not given) incubated with cells for 24 h either as such or after dry milling for 32 h	Dry milling decreased the methylene blue adsorption to a third and the inhibition of TTC reduction by one-half to two-thirds.	Juhasz et al. (1978)
Mouse (Swiss T.O.)	Kaolin (non-specified), 100 µg/ml, incubation for 18 h	Approximately 25% release of LDH and beta-glucuronidase release with native kaolin; one-half to two-thirds of the activity lost upon calcination.	Brown et al. (1980)
Rat (Sprague-Dawley CFY), male	Six different kaolins, kaolinite content 51-95%, quartz content 5-20%; 1.0 mg/ml (<5 µm	All samples considered cytotoxic based on TTC reduction, except one (30%), which had 71% kaolinite	Adamis & Timár (1976); Timár et al. (1979,

	diameter, median 1-2 μm) incubated in cell suspension for 1 h	and 22% quartz. All considered inert based on small LDH release, except the one with the highest quartz concentration (29%); the kaolinite concentration in this specimen was 67%.	1980); Adamis et al., (1986); Adamis & Krass (1991)
	One sample of an illite clay (28% illite, 28% quartz); 1.0 mg/ml (<5 μm diameter, median 1-2 μm) incubated in cell suspension for 1 h	Cytotoxic based on TTC reduction, but not cytotoxic based on small LDH release.	
Rat (Sprague-Dawley CFY), male	Four different kaolins (from Hungary, silica content 4, 5, 18, and 30%, <5 μm diameter, otherwise not specified) incubated in cell suspension for 24 h; similar experiment with a non-specified illite clay	Three out of four kaolins and illite considered cytotoxic based on TTC reduction; no relationship between quartz concentration and cytotoxicity. The clays studied did not induce release of LDH, but illite decreased intracellular LDH activity.	Adamis & Timár (1980); Adamis et al. (1985)
Mouse (T.O.), female	Culture of unstimulated macrophages with Cornwall kaolinite (not specified) for 18 h	Kaolinite induced LDH release from macrophages; this was prevented by polyvinylpyridine-N-oxide.	Davies & Preece (1983)
Mouse (T.O.), female	Cornwall kaolin (98% kaolinite, 2% mica), 98% <5 μm in diameter, incubated with cells at 40 $\mu\text{g}/\text{ml}$ for 18 h	Kaolinite induced a 70% LDH release to the medium; the release was partly prevented by treatment of kaolin with polyvinylpyridine-N-oxide and fully prevented by additional treatment with polyacrylic acid.	Davies (1983)
Rat (Wistar, SPF), both sexes	Kaolin (composition not specified, diameter 0.2-25 μm), 0.5 mg/ 10^6 cells incubated for 2h	Of all cells with particles, 0.6% and 1.6% dead cells with particles at 1 and 2 h (lowest toxicity group of three).	Styles & Wilson (1973)
Mouse	Fifteen respirable dust samples from kaolin drying and calcining plants in England (kaolinite content 84-96%, mica 3-6%, quartz 1 %, feldspar 0-7%), a sample of Cornish kaolin (K1, 98% kaolinite, no quartz or feldspar, 2% mica), and a sample of Georgia kaolin (K2, 99% kaolinite, no quartz, mica, or feldspar, and reference quartz DQ12, mica, gibbsite, and titanium dioxide as controls; incubation for 18 h with macrophages, LDH release measured	All dust samples were cytotoxic. The quartz content could not explain the cytotoxicity. The kaolinite samples showed a dose-dependent cytotoxicity, which could not be explained by their content of ancillary materials.	Davies et al. (1984)
		Polyacrylic acid treatment of kaolin has only a small effect on its cytotoxicity, indicating that the positive charge at the edge of the	

		mineral (blocked by acrylic acid) is not a major determinant of the toxicity.	
Alveolar macrophages			
Rat (Wistar, SPF), both sexes	Kaolin (composition not specified, diameter 0.2-25 μm), 0.5 mg/10 ⁶ cells incubated for 2h	Of all cells with particles, 3.7% and 4.2% dead cells with particles at 1 and 2 h (lowest toxicity group of three).	Styles & Wilson (1973)
Rabbit (New Zealand)	Kaolinite (>99% pure), >99% respirable size, incubated with cells at 0.25-2.5 mg/ml	Kaolinite caused an inhibition of amino acid incorporation into protein in a dose-dependent manner, 65% inhibition at 1 mg/ml. Inhibition reversed by addition of serum.	Low et al. (1980)
Guinea-pig	Kaolin (non-specified), 100 $\mu\text{g}/\text{ml}$, incubation for 18 h	Approximately 30% release of LDH and beta-glucuronidase release with native kaolin; >90% of the activity lost upon calcination.	Brown et al. (1980)
Rabbit (New Zealand)	Kaolin (unspecified) and illite clay (unspecified) (<5 μm diameter), 0.5 mg/ml incubated in cell suspension for 24 h	Kaolin induced a 15.3% release of LDH and a 7% release of alkaline phosphatase. Illite clay induced a 2% release of LDH and a 1.3% release of alkaline phosphatase. For quartz, the figures were 51 % and 16%.	Daniel & Le Bouffant (1980)
Rat (Sprague-Dawley), male	Georgia kaolin ($\geq 96\%$ kaolinite, no quartz, >95% >5 μm in diameter), incubated with cells for 1 h at 0.1-1 mg/litre	Kaolin induced a dose-dependent release of LDH, beta-glucuronidase, and beta-N-acetylglucosaminidase of 60-80%. The effect was largely (9-15% release) abolished by lecithin.	Wallace et al (1985)
Rat (strain not specified)	Kaolin, 1.0 mg/ml (<5 μm diameter, MMAD 2.1 μm) incubated in cell suspension for 2 h	Kaolin induced an 80% release of LDH and a 60% release of beta-glucuronidase and beta-N-acetylglucosaminidase, being most cytotoxic of all minerals studied, quartz included.	Vallyathan et al. (1988)
Rat (Wistar), male	Alveolar macrophages incubated with Mexicali dust (see Table 13); LDH release measured	Concentration- and time-dependent release of LDH, which reacted 50% at 0.5 mg/ml and was much more pronounced than with quartz.	Osornio-Vargas et al. (1991)
Leukocytes			
Human phagocytic cells from one donor	Well crystallized standard kaolinite KGa-1, no quartz, cristobalite, or mica, particle size 3.2 μm median volume diameter	Kaolinite at concentrations of approximately 1 mg/ml induced luminol-dependent chemiluminescence as an expression of generation of reactive oxygen species in both monocytes and	Gormley et al. (1985)

		neutrophils when opsonized and when not opsonized.	

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

“Kaolinite treated with hydrochloric acid did not increase the liberation of LDH from macrophages. The activity of quartz samples was considerably increased by treatment with hydrochloric acid (Kriegseis et al., 1987)”.

“Bruch & Rosmanith (1985) found that mixed dusts containing kaolinite, illite, and quartz inhibit TTC reduction and induce liberation of LDH from guinea-pig alveolar macrophages; no apparent relationship emerged between the toxicity and dust composition”.

“Sluiter et al. (1987) intraperitoneally injected random-bred female Swiss mice (number of animals unknown) once with kaolin (0.5% w/v in saline; no data available on composition). This evoked a substantial increase in the number of granulocytes in the peripheral blood (6-12 hours after injection), and the peritoneal cavity (6-24 hours after injection). Lewis et al. (1976) used kaolin to produce a local inflammatory response (oedema) in the rat hind paw in order to screen new anti-inflammatory drugs, and to investigate the inflammatory process. Male Wistar rats (10/group) were administered 50, 100 and 150 mg/ml kaolin (no data available on composition) in 0.9% w/v saline by subplantar injection. Kaolin produced dose-related increases in paw volume” (Taken from DECOS, 2007).

“OBJECTIVE: intra-articular co-injection of kaolin with carrageenan (CGN) in rodents is widely used as an experimental model of arthritis. However, the ability of kaolin to cause arthritis and related immune responses when administered alone is unclear. We evaluated the contribution of prostanoids and sensory C-fibres (and their neuropeptide substance P) to kaolin-induced inflammation in the rat knee. METHODS: Wistar rats, 8-10 weeks old, received an intra-articular injection of kaolin (1-10 µg/joint) or saline into the knee joint. Knee inflammation, proinflammatory cytokines, pain behaviour and secondary tactile allodynia were assessed over 5 h, when synovial leukocyte counts, histopathological changes and proinflammatory cytokine levels were evaluated. RESULTS: The intra-articular injection of kaolin caused a dose- and time-dependent knee swelling and impairment of motion that were associated with secondary tactile allodynia, elevated concentrations of IL-1β, IL-6 and TNFα, leukocyte infiltration, and histopathological changes in the ipsilateral hindpaw. The neurokinin-1 (NK1) receptor antagonist SR140333 or neonatal treatment with capsaicin markedly reduced the inflammatory parameters, cytokines and allodynia but failed to significantly inhibit the impaired motion. The cyclo-oxygenase inhibitor indomethacin partially inhibited knee oedema and allodynia but did not affect the leukocyte influx, myeloperoxidase activity or impaired motion in the kaolin-injected rat. CONCLUSIONS: We show the first evidence that intra-articular injection of kaolin without CGN produced severe acute monoarthritis. This was highly dependent on substance P (released from C-fibres) and NK1 receptor activation, which stimulated local production of proinflammatory cytokines. This model may be of critical importance for mechanistic studies and screening new anti-inflammatory/analgesic drugs.” As taken from Camargo LL et al. 2015. Neuroimmunomodulation 22(6), 373-84. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26088412>

“Possible respiratory sensitiser”.

Skin and Eye irritant

As taken from VSDB, 2022.

“The potential for ocular irritation from a clay mask with 14.5% Kaolin was investigated in a tissue equivalent assay with EpiOcular™ cultures. The EpiOcular™ human cell constructs were exposed to 100 µl test material under standard culture conditions for 4, 8, 16, and 24 h. Tissue viabilities were then examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2Htetrazolium bromide (MTT) assay. The duration of exposure resulting in a 50% decrease of tissue viability (t50) was calculated to be 5.2 h (at 4 h, tissue viability was 63.4%). The positive control yielded expected results. As

residual test article may bind to the tissue and result in a false MTT reduction signal, a freeze-killed tissue control was used, and calculations were performed to correct for the amount of MTT reduced directly by the test article residues in the tissues. The clay mask with 14.5% Kaolin was predicted to not be an ocular irritant in this assay. this class is prepared by the controlled heat this assay.”

CIR (2022)

5.8. All other relevant types of toxicity

Haemolysis

“Different kaolin samples were found to have a significant haemolysing effect on red cells (Table 17). Mányai et al. (1970) investigated a water-washed Hungarian kaolin sample. The non-heat-treated samples had a haemolytic effect. The sample pretreated by heating at 200 or 350 °C for 90 min had a somewhat stronger haemolytic effect on the red cells than the original sample”.

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

System/ species/gender	Dose (mg/ml)/treatment	Findings	References
Human washed erythrocytes	Erythrocytes incubated with Hungarian water-cleaned kaolin (composition not indicated; <5 µm in diameter), as such or after heat treatment at 290-900 °C for 90 min	Kaolin was strongly haemolytic; heating for 90 min to 200 or 350 °C increased, but heating to 500 or 650 °C practically abolished, the haemolytic potency. Kaolin heated at 800 or 950 °C was at least as potent a haemolyser as non-treated kaolin.	Mányai et al. (1970)
Sheep, plasma-free erythrocytes	Kaolin (source and composition unspecified), <5 µm in diameter, incubated with cells at 1 mg/ml for 2 h	Kaolin caused 40% haemolysis; acid and alkali treatments of the clay decreased its haemolytic potency.	Narang et al. (1977)
Rat (strain not specified)	Kaolin (unspecified) and illite clay (unspecified) (<5 µm in diameter) 1.0 mg/ml incubated in cell suspension for 1 h	Kaolin caused 98% haemolysis, illite 24% (quartz 48%).	Daniel & Le Bouffant (1980); Le Bouffant et al. (1980)
Rabbit	Kaolin (non-specified), incubation for 50 min	Twenty per cent haemolysis caused by 1.3 mg kaolin in a total volume of 4 ml; 11.6 mg of calcined kaolin was needed for the same effect.	Brown et al. (1980)
Sheep (citrated blood)	Kaolin (median volume diameter 4.7 µm) incubated in cell suspension	Haemolysis 60% in 30 min; haemolytic potency similar to that of quartz.	Ottery & Gormley (1978)
Sheep	Kaolin, 1.0 mg/ml (<5 µm diameter, MMAD 2.1 µm) at five concentrations, 0.1-1 mg/ ml, incubated in cell suspension for 50 min	Linear, dose-dependent haemolysis; about 20% haemolysis with 0.5 mg/ml; 35% with 1 mg/ml; approximately 2 times as potent as quartz.	Vallyathan et al. (1988)
Sheep (citrated blood, single	Kaolinite 90% <2 µm in diameter at six concentrations, 0.005-0.25	More haemolytic per mg than silica or talc; 20% haemolysis with	Woodworth et

donor)	mg/ml, incubated in cell suspension for 1 h	0.25 mg/ml; 95% with 25 mg/ml.	al. (1982)
Sheep	Georgia kaolin ($\geq 96\%$ kaolinite, no quartz, $>95\%$ $>5\ \mu\text{m}$ in diameter), incubated with cells for 1 h	Kaolin induced a dose-dependent haemolysis, which reached 42% at 1 mg/ml. The effect was completely abolished by lecithin.	Wallace et al. (1985)
Bovine erythrocytes washed with buffered saline	South Carolina kaolinite (composition not specified) incubated in cell suspension for 1 h	A concentration of 0.6 mg/ml induced 50% haemolysis of erythrocytes. Polyvinylpyridine-N-oxide, a hydrogen-bonding material, partly inhibited the haemolysis.	Oscarson et al. (1981)
Bovine erythrocytes washed with buffered saline	South Carolina kaolinite (composition not specified) at several concentrations of different particle sizes incubated in cell suspension for 1 h	A concentration of 0.6 mg/ml induced 50% haemolysis of erythrocytes. Of the minerals studied, kaolinite was least haemolytic, the potency being 1/20 that of silica. Particles of 0.2-2 μm diameter were most active; particles $<0.2\ \mu\text{m}$ or $>20\ \mu\text{m}$ diameter had no or little haemolytic activity; reduction of surface charge and cation exchange capacity by coating particles with an aluminium-hydroxy polymer largely eliminated haemolytic capacity.	Oscarson et al. (1986)
Human (citrate blood)	Six different kaolins, kaolinite content 51-95%, quartz content 5-20%; 1.0 mg/ml ($<5\ \mu\text{m}$ diameter, median 1-2 μm) incubated in cell suspension for 1 h	Haemolysis 60-90% for all samples except one (30%), which had 71% kaolinite and 22% quartz.	Adamis & Krass (1991)
	One sample of an illite clay (28% illite, 28% quartz); 1.0 mg/ml ($<5\ \mu\text{m}$ diameter, median 1-2 μm) incubated in cell suspension for 1 h	Haemolysis 95%.	
Human	One "bentonite," containing 50% illite, 25% montmorillonite, 25% quartz; two undefined illite clays, one kaolinite with dickite and nakrite as main components and quartz as a minor component, one kaolin with kaolinite as the main component, and two unspecified kaolins ground in a ball mill to diameter $<5\ \mu\text{m}$, incubated in cell suspension for 1 h	Fifty per cent haemolysis caused by 1.5-4 mg/ml kaolinites (0.06-0.115 m^2/ml); and 1.0-4.0 mg/ml (0.039-0.12 m^2/ml) illites. Haemolytic activity roughly proportionate to surface area of mineral powder; haemolytic activity largely lost after heating to over 500 °C.	Mányai et al. (1969)
Human red blood cells from normal donors	Dust from the town Mexicali (see Table 14)	Concentration of 2 mg/ml produced a $95 \pm 3\%$ haemolysis in 1 h; the haemolysis was stronger than with quartz.	Osornio-Vargas et al. (1991)

"Kennedy et al. (1989) studied lipid peroxidation of membranes in human erythrocytes suspensions. They found that kaolin (1 mg/ml) significantly increased malondialdehyde content ($p < 0.05$), and caused marked in vitro lysis of 50% of the human red blood cells within 1 hour ($p < 0.001$)" (As taken from DECOS, 2007).

"The problems of antibiotic overuse compel us to seek alternative antibacterial agents. Some clays have been shown to kill antibiotic-resistant human pathogens and may provide an alternative to known antibiotics. Here we show that Al toxicity plays a central role in the antibacterial action of a kaolin-rich clay from the Colombian Amazon (AMZ). Antibacterial susceptibility testing shows minimum inhibitory concentrations of 80 mg/mL against a model *Escherichia coli* (ATCC 25922). The clay buffered the media pH to ~4.6 and Eh values to +360 mV. Chemical analysis of AMZ and bacteria showed that Al, P, and transition metals (Fe, Cu, Mn, and Zn) were exchanged during incubation at 37°C. Only Al derived from the clay exceeded the minimum inhibitory concentrations for *E. coli* under acidic conditions. Ion imaging showed elevated Al levels in the bacterial membrane, and high intracellular Fe levels, relative to those of untreated controls. Phosphorus depletion in *E. coli* after reaction with AMZ, together with evidence of membrane permeabilization, suggests that Al reacts with membrane phospholipids, enhancing intracellular transport of metals. These results highlight the importance of dissolved Al for amplifying the toxicity of transition metals to human pathogens." As taken from Londono SC et al. 2017. *Environ. Sci. Technol.* 51(4), 2401-2408. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28121138>

6. Functional effects on

6.1. Broncho/pulmonary system

/CASE REPORTS/ /The author/ reported in detail on six workers who had been working in the drying and bagging of kaolin /from Cornwall mines, in England/. ...Medical and radiological examinations /were performed/ of those occupationally exposed to kaolin. All had radiological pneumoconiosis, and two were further studied in autopsy. In one case, characteristic silicotic-type nodulation together with progressive tuberculosis were found. Large quantities of kaolinite and amorphous quartz were found in the lung. In another case, large quantities of pure kaolinite (as much as 20-40 g) were found in the lung without tuberculosis but with severe fibrosis. The disease was like the pneumoconiosis of coal miners and differed from classic silicosis. In the upper part of the lung, greyish or blue-greyish massive confluent lesions were described, which were not as hard on palpation as the silicotic conglomerates. [IPCS INCHEM; Environmental Health Criteria (EHC) Monographs. Bentonite, kaolin, and selected clay minerals (EHC 231). Available from, as of June 25, 2007: <http://www.inchem.org/pages/ehc.html> **PEER REVIEWED**

/CASE REPORTS/ A 62-year-old white male employed for 43 years in the polishing room of a cotton textile mill was admitted to a tertiary care center with progressive dyspnea and productive cough that had not responded to therapy for tuberculosis. In spite of aggressive antibiotic therapy and respiratory support, the patient died as a consequence of respiratory failure. Small rounded and irregular opacities had been noted on the chest radiograph. Review of job-site spirometry demonstrated a worsening restrictive pattern over a 4-year period prior to his death. Additional occupational history revealed long-term exposure to kaolin in the polishing room, and pathologic examination of lung tissue confirmed extensive fibrosis and substantial quantities of kaolin. Kaolinosiis is a disease typically found among individuals involved in mining or processing this material rather than in user industries. This case illustrates the importance of obtaining a complete occupational history in reaching a diagnosis. [Levin JL et al; *Am J Ind Med*; 29 (2): 215-21 (1996)] **PEER REVIEWED** PubMed Abstract

/CASE REPORTS/ Evaluation of lungs of Cornish china clay workers found nodular changes in those exposed to china stone and irregular changes with interstitial fibrosis in those exposed to china clay (kaolinite). The complicated pneumoconiosis progresses more slowly than silicosis; progressive massive fibrosis and Caplan's syndrome /SRP: (pulmonary nodules in Rheumatoid

Arthritis patients who also have pneumoconiosis)/ may also occur. [Rom, W.N. (ed.). Environmental and Occupational Medicine. 2nd ed. Boston, MA: Little, Brown and Company, 1992., p. 484]
PEER REVIEWED

/CASE REPORTS/ ... Two cases of pneumoconiosis /were reported/ in workers who had primarily worked with kaolin during their working lifetime. Postmortem lung examination showed extensive nodular fibrosis in the upper regions. One individual suffered severe dyspnea. No information was provided concerning smoking habits. The only exposure information provided was that the workers had worked under dusty conditions. [American Conference of Governmental Industrial Hygienists. Documentation of the TLV's and BEI's with Other World Wide Occupational Exposure Values. CD-ROM Cincinnati, OH 45240-1634 2006.] **PEER REVIEWED**

/CASE REPORTS/ ... Seven cases of pneumoconiosis /were reported/, primarily involving baggers and millers of kaolin. ... All but one of the seven reported cases of pneumoconiosis had more than 20 years of exposure; the one individual who was free from respiratory symptoms had 14 years of exposure. All of the cases showed extensive fibrosis radiographically. Chemical analysis of autopsied lung samples from two of the cases did not indicate the presence of crystalline silica and tuberculosis was not considered a factor. No smoking history was provided. [American Conference of Governmental Industrial Hygienists. Documentation of the TLV's and BEI's with Other World Wide Occupational Exposure Values. CD-ROM Cincinnati, OH 45240-1634 2006.] **PEER REVIEWED**

/CASE REPORTS/ In the case of a 36-year-old man who worked in /a kaolin/ plant for 17 years, chest films were taken at the end of his career and detected lesions of extensive confluent consolidation and nodule formation of advanced pneumoconiosis with infection. Autopsy and microscopic findings included alveolar spaces uniformly expanded, three areas of whorled fibrous tissue, scattered areas of cystic spaces, hilar nodes heavily pigmented, deposits of brownish black particulate matter, a large vessel with recent thrombus, hemorrhage, and necrosis, marked fibrous thickening of the pleura, and dense fibrous scarring of the lymph nodes. The final diagnosis was pneumoconiosis (kaolinosis) with pulmonary thrombosis and infarction of the lungs. [Cosmetic Ingredient Review; Final Report of the Cosmetic Ingredient Review Expert Panel; Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite; International Journal of Toxicology 22 (Suppl 1): 37-102 (2003)] **PEER REVIEWED**

/CASE REPORTS/ /A/ second case study was a 35-year-old man who worked in a Kaolin-processing plant for 21 years. Within his last 3 years, he had dyspnea and a slight cough with small amounts of dark colored sputum. The sputum was negative for bacteria. Chest films revealed advanced pneumoconiosis with infection, confluent consolidation, nodular infiltration, cavitation, and emphysema. Autopsy and microscopic findings included nodules in the right and middle lobes, pleural spaces were thickened and shaggy, large bulbous emphysematous blebs, a pulmonary artery with organizing thrombus, heavily pigmented hilar lymph nodes, whorled fibrous collagenous tissue, and spaces and walls with macrophages. The final diagnosis was pneumoconiosis (kaolinosis). [Cosmetic Ingredient Review; Final Report of the Cosmetic Ingredient Review Expert Panel; Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite; International Journal of Toxicology 22 (Suppl 1): 37-102 (2003)] **PEER REVIEWED**

/EPIDEMIOLOGY STUDIES/ Several epidemiologic studies have evaluated kaolin workers in Georgia where 70% of commercial USA kaolin is strip mined. A cross-sectional survey of 459 workers in three kaolin plants representing 85% of the total eligible population had an overall prevalence of simple pneumoconiosis of 9.2%; pneumoconiosis correlated with duration of

exposure longer than 15 years in job categories associated with greater dust exposure. The pneumoconiosis was not associated with respiratory symptoms and had minimal effect on pulmonary function, even in complicated cases. [Rom, W.N. (ed.). Environmental and Occupational Medicine. 2nd ed. Boston, MA: Little, Brown and Company, 1992., p. 483] **PEER REVIEWED**

/SURVEILLANCE/ ... A 13% prevalence rate of pneumoconiosis /was found/ in current Georgia kaolin workers and 9% in former workers who had greater than 5 years of exposure. Seven of eight workers had only worked in the milling area, with only one from the mine. These workers' exposures ranged from 15 to 29 years. No pneumoconiosis was seen in workers with less than 5 years' experience. Lung function testing showed reduction in forced vital capacity, forced expiratory volume 1, and peak flow rate. Environmental exposure ranged from 1 to 2 mg/cu m in the milling and bagging operation and 0.2 mg/cu m in the mining operation. Adjacent to the pulverizers and bag house, the dust fraction smaller than 10 um constituted more than 50% of the mass. In other locations, this size fraction constituted 10% to 20% of the mass. No crystalline silica was found. Smoking did not appear to be a factor in the pneumoconiosis; however, the study population may have been too small to address the factors. [American Conference of Governmental Industrial Hygienists. Documentation of the TLV's and BEI's with Other World Wide Occupational Exposure Values. CD-ROM Cincinnati, OH 45240-1634 2006.] **PEER REVIEWED**

/SURVEILLANCE/ A study of 2000 Georgia kaolin workers found adjusted prevalence rates of 3.2% for simple pneumoconiosis and 0.63% for complicated disease. They concluded that complicated pneumoconiosis had a modest effect on ventilatory function and that reduced forced expiratory volume (FEV) was seen in calcined clay workers. [Rom, W.N. (ed.). Environmental and Occupational Medicine. 2nd ed. Boston, MA: Little, Brown and Company, 1992., p. 483] **PEER REVIEWED**

/SURVEILLANCE/ ... /The authors/ evaluated 350 workers at a kaolin plant in Georgia. Thirty-two workers with abnormal chest radiographs were clinically evaluated. Twelve workers with mild kaolinitis but without any evidence of pleural changes on chest roentgenograms had computed tomography (CT) of the chest to assess the presence of pleural thickening. Nineteen workers had radiographic changes consistent with kaolin pneumoconiosis (prevalence 5.4%), and four workers had evidence of pleural plaques (prevalence 1.1%). Five of the 12 workers had evidence of pleural thickening on CT. Chaudhary BA et al; South Med J 90 (11): 1106-9 (1997)] **PEER REVIEWED** PubMed Abstract

OTHER TOXICITY INFORMATION/ Long-term exposure to kaolin may lead to a relatively benign pneumoconiosis, known as kaolinitis. Deterioration of lung function has been observed only in cases with prominent radiological alterations. Based on data from china clay workers in the United Kingdom, it can be very roughly estimated that kaolin is at least an order of magnitude less potent than quartz. [IPCS INCHEM; Environmental Health Criteria (EHC) Monographs. Bentonite, kaolin, and selected clay minerals (EHC 231). Available from, as of June 25, 2007: <http://www.inchem.org/pages/ehc.html> **PEER REVIEWED**

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Five hundred female SPF Sprague-Dawley rats were divided into groups each with 10 animals. The rats were exposed over a period of 3 months to 50-mg/rat intratracheal instillations of Kaolin. The following assessments were made: weight of the fresh lungs; macroscopic and microscopic lesions in the lungs; amount of collagen and dust present in the lungs; and calculation of the toxicity index from the amount of collagen formed per mg of dust. The weight of fresh lungs subjected to Kaolin was 1.76 g. Collagen formed per lung was 23.9 mg. The dust per lung was 30.2 mg and the collagen/dust ratio was 0.79. Microscopic examinations of the lungs showed no alveolar proteinosis but Kaolin was detected in the bronchiolovascular lymphoid sheaths. No information regarding nonexposed lungs was presented. The opinion of the investigators was that exposure to Kaolin results in "pulmonary toxicity" and possesses "fibrogenic capacity" [Cosmetic Ingredient Review; Final Report of the Cosmetic Ingredient Review Expert Panel; Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium

Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite; International Journal of Toxicology 22 (Suppl 1): 37-102 (2003)] **PEER REVIEWED**

As taken from HSDB, 2013

"Clear-cut deterioration of respiratory function and related symptoms have been reported only in cases with prominent radiological findings. The composition of the clay — i.e., quantity and quality of minerals other than kaolinite — is an important determinant of the effects."

"In the landmark paper in which intratracheal instillation as a means of studying the effects of dust on lungs was described, no collagen production was detected in the lung of a guinea-pig 336 days after the administration (whereas fibrosis was observed after similar injection of quartz) (Kettle & Hilton, 1932). Similarly, intratracheal instillation of kaolin from South Wales or untreated or ignited Cornish kaolin did not induce fibrosis in rats (King et al., 1948)."

"Intratracheal instillation of kaolin "prepared to the specification of the Society of Leather Trades" with atypical mycobacteria (not Mycobacterium tuberculosis) induced fibrotic reaction in the lungs of guinea-pigs (Byers & King, 1959). No control experiment with kaolin alone was performed."

"Similarly, when kaolin (75 mg, not specified qualitatively) was injected into guinea-pigs intratracheally together with Candida albi-cans, thick reticulin fibres and collagenous fibrosis ensued; acute inflammatory reaction followed by formation of thick reticulin fibres were observed after kaolin alone (Zaidi et al., 1981)."

"Schmidt & L  chtrath (1958) studied the effects of intratracheal administration of a 2:3 mixture of kaolin and quartz. Kaolin alone did not cause significant reaction in the lung. The mixture of kaolin and quartz caused significant changes in the lung; after 9 months, the fibrosis was grade 5. (Using pure quartz, the development of silicosis was somewhat quicker, but after 9 months, the difference compared with the kaolin-quartz mixture was insignificant.)"

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

Effects of intratracheal instillation of kaolin and illite on the respiratory tract

Species/gender ^a / number	Treatment	Findings	Reference
Guinea-pig /2	Intratracheal instillation of an unstated amount of kaolin, follow-up for 14 and 336 days	No collagen production in 336 days; in animals treated similarly with quartz, fibrosis observed.	Kettle & Hilton (1932)
Rat/12	Intratracheal instillation of a single dose of commercial acid-washed kaolin containing 8% hydrated free silica and 12% mica; two animals killed between days 3 and 6; the rest kept for life (up until 8 months)	Grade 2-3 fibrosis observed in rats after 8 months (grade 1 = minimal reticulin fibrosis, 4 = maximal fibrosis, as induced by quartz).	Belt & King (1945)
Rat / 6 or 10 per group	A single intratracheal instillation (50-60 mg) of washed South Wales kaolin, untreated Cornish kaolin, ignited Cornish kaolin, quartz; follow-up up to 6 months	South Wales kaolin (10 rats): Up to 60 days, no fibrous reaction; thereafter, local reticulinoses, no fibrosis or emphysema.	King et al. (1948)
		Cornish kaolin (10): Eight animals died within 10 days; the remaining two showed no	

		fibrous reaction.	
		Ignited Cornish kaolin (6): Only four rats available for study; their survival was 14, 28, 73, and 140 days; in the last-mentioned, there was local reticulinosi.	
		Quartz (6): Severe nodular silicosis in all five animals available for study; survival 68, 121, 130, 207, and 240 days.	
Rat	Intratracheal administration of kaolin, kaolin baked for 1 h at 900 or 1200 °C	Active phagocytosis, local storage of the dust without reticular fibres, and nodules were observed. In the case of heat-treated kaolin samples, the reaction was somewhat stronger, although fibrosis reached grade 1 in only a few cases (Belt-King scale). Histological signs resembling silicosis did not develop.	Schmidt & L��chtrath (1958)
Rat / 25 per group	A single intratracheal administration of kaolin containing kaolinite and quartz in ratios 82/18, 70/30, or 35/65; follow-up to 1 year	Foreign body reaction with the two samples containing 82 or 70% kaolinite, productive fibrosis with the sample containing 65% quartz.	T��m��r et al. (1966)
Rat/10-15 per group	Single intratracheal instillation of kaolin (sericite and quartz as main impurities, 1 % quartz), particle size <5 ��m; histological analysis of lungs after 4 months	Cellular lesions, some loose reticulin, with either no collagen or a few collagen fibres.	Goldstein & Rendall (1969)
Rat/10 per group	Intratracheal instillation of 50 mg non-specified kaolin, 3-month follow-up	Amount of collagen / amount of dust in lung after kaolin exposure 3 times higher than in titanium dioxide-treated control and 2.4 times more than in coal-treated animals, but only 15% of that in the quartz-treated animals.	Martin et al. (1977)
Rat, strain not specified / number and gender not specified	40 mg of illite clay F, nominal composition 100% illite (diameter <2 or 2-5 ��m), kaolin S (82% kaolinite, 18% quartz; diameter <3 ��m), or kaolin Sz (95% kaolinite, 5% quartz; diameter <2 or 2-5 ��m), instilled to rats; animals killed after 5, 15, 40, and 365 days and histological analysis performed	Illite F and kaolin Sz <2 ��m caused "storage foci," kaolin S, "foreign body" reaction, and kaolin Sz 2-5 ��m, mainly storage foci, rarely foreign body reaction.	T��m��r et al. (1979)
Rat, strain not	60 mg of illite (not described) instilled intratracheally and followed for 6	"Storage foci" observed in	Tim��r et al.

specified /10	months; lung weight, lipid, phospholipid, and hydroxyproline were analysed, and histological and histochemical studies on collagen performed	lungs of illite-treated rats.	(1980)
Rat, Sprague-Dawley / female /10 per group	30 or 50 ^b mg non-specified kaolin or illite clay injected intratracheally, followed for 3 and 12 months	Kaolin increased the lung weight 9 and 4 mg and collagen formed <26 and <7 mg/mg of injected dust at 3 and 12 months (all normalized to quartz = 100).	Le Bouffant et al. (1980)
		Illite clay induced alveolar proteinosis and thus increased the lung weight 17 and 6 mg, and collagen formed <26 and 11 mg/mg of injected dust at 3 and 12 months (all normalized to quartz = 100).	
Rat, Sprague-Dawley / female /10 per group	30 or 50 ^b mg non-specified kaolin or illite clay injected intratracheally, followed for 3 and 12 months	Correlation to haemolysis (rat erythrocytes) and release of LDH ^c and alkaline phosphatase from rabbit alveolar macrophages (see Table 17) weak.	Le Bouffant et al. (1980)
Rat, Fischer F344 / 10 per group	5 mg kaolin (non-specified), MMAD ^d 2.1 µm, instilled intratracheally, killed 1 day, 3 days, 7 days, 3 months, and 6 months later	Acute inflammatory reaction on day 1; thereafter, a slight interstitial cell thickening. At 3 and 6 months, lungs were normal.	Vallyathan et al. (1988)
Rat / male / 5 per group	Intratracheal injection of 10 mg of a kaolin containing 67% kaolinite and 23% quartz; bronchopulmonary lavage after 15 days, LDH activity and protein content and, after 15, 30, and 60 days, phospholipid content of supernatant measured	Kaolin did not induce significant LDH, protein, or phospholipid leakage to the supernate fraction. No change in the LDH or protein leakage was observed after similar exposure to eight other minerals, including two quartz samples. The quartz samples, however, increased the phospholipid content at 30 and 60 days.	Adamis & Krass (1991)
Rat, SD/male	Intratracheal injection of 10 mg of a kaolin containing 86% kaolinite, 4% quartz, 5% illite, and 5% amorphous silica; bronchopulmonary lavage after 14, 30, and 90 days, number of cells, activities, LDH activity, protein and phospholipid content of supernatant measured	Kaolinite caused a statistically significant increase in the protein content at 14 days, which returned to control level thereafter. No changes were observed in the other parameters studied.	Adamis et al. (1998)
Mouse / 70 per group	Intratracheal instillation of 5 mg of kaolin, 91% >3.62 µm in diameter; follow-up 210 days	Fibroblast reaction from 60 days post-exposure, prominent from day 120. Grade 2 fibrosis	Sahu et al. (1978)

		by day 210 (Belt-King scale).	
Rat, Wistar/female/ 20 per group	Kaolinite 1 (K1) contained 2% muscovite; K2, 1% quartz and 9% muscovite; K3, <1% quartz and anatase and 1 % muscovite; and K4, 1 % quartz and anatase and 2% muscovite; mean value for volume distribution was 3.6 and 2.6 μm for K3 and K4, not analysed for K1 or K2; instilled once intratracheally at 50 mg/kg of body weight; autopsy after 7 months; lung weight, histology, amount of dust, hydroxyproline, and total lipid content analysed; two samples of quartz also investigated, dose given 5 mg/kg of body weight	All kaolinite samples fibrogenic. Absolute amount of hydroxyproline roughly similar after exposure to kaolinites and quartz, hydroxyproline / retained dust in kaolinite-treated animals 1/10 that in quartz-treated animals. Absolute increase of lung weight one-sixth and of total lipids roughly 10% of that in quartz-treated animals.	Rosmanith et al. (1989)
Rats, SD(SD)BR/ male / 6 per group	Exposed by intratracheal instillation to 50 mg dust from the town Mexicali (see above), mean diameter 3.2 μm ; lung analysis after 30 days	Multifocal interstitial lung disease. Mononuclear cell accumulation and presence of collagen fibres.	Osornio-Vargas et al. (1991)

^a Where available. ^b Composition or particle size not given. ^c LDH = lactate dehydrogenase. ^d MMAD = mass median aerodynamic diameter.

“After the instillation of a single dose of commercial acid-washed kaolin containing 8% hydrated free silica and 12% mica, grade 2–3 fibrosis was observed in rats after 8 months (grade 1 = minimal reticulin fibrosis, grade 4 = maximal fibrosis, as induced by quartz) (Belt & King, 1945).”

“Following intratracheal dust treatment in rats, the histological reaction was found to depend on the composition of the dust (Tímár et al., 1966). Foreign body reaction as an effect of kaolinite was observed in all cases where the crystalline quartz content of the dust was less than or equal to 30%. The sample containing 65% quartz and 35% kaolinite caused progressive fibrosis. In addition to the composition, particle size also played a role in the development of the tissue reaction. Kaolin samples containing particles less than 2 μm caused storage foci (Tímár et al., 1979), while the kaolin samples containing bigger particles (particle size between 2 and 5 μm) caused mainly storage foci but also, to a smaller extent, foreign body reaction.”

“Goldstein & Rendall (1969) observed cellular reaction with minimal fibrosis: some loose reticulin with either no collagen in some animals or a few collagen fibres in other rats 4 months after an intratracheal instillation of kaolin.”

“Martin et al. (1977) observed an increase in the collagen content in the lungs of rats 3 months after an intratracheal instillation of non-specified kaolin; the reaction was considerably weaker than after a similar treatment with quartz.”

“Sahu et al. (1978) described the development of grade 2 fibrosis in mice after 7 months of exposure to non-specified kaolin.”

“Rosmanith et al. (1989) compared the fibrogenicity of four kaolinite samples with that of quartz in rats using intratracheal instillation. The fibrogenicity of kaolinites, as measured by the increase in hydroxyproline content in relation to the amount of dust retained, was approximately 1/10 that of quartz, but the inflammatory reaction was considerably less.”

“No significant LDH, protein, or phospholipid leakage to the supernate fraction was observed in bronchoalveolar fluid 15–60 days after an intratracheal instillation of kaolin to rats. In this system, effects of quartz became apparent towards the end of the observation period (Adamis & Krass, 1991).”

Based on a large-scale cross-sectional study of English china clay workers, it was predicted that 40 years of exposure to 2 mg/m³ respirable kaolin dust would lead to a mean decrement in forced expiration volume (FEV₁) at age 60 of approximately 220 ml (HSE 1994; cited in HSE 2007).

“There are five cross-sectional studies on occupational exposure to kaolin dust that include information on exposure levels. Three are from kaolin workers in Georgia, USA, and two are from kaolin workers in the UK.....The critical effects of kaolin exposure involve the lungs, with pneumoconiosis as the most important clinical observation” (As taken from DECOS, 2007).

“In occupational exposure studies of mineral dusts, fibrosis and pneumoconiosis has been documented in workers involved in the mining and processing of aluminum silicate, calcium silicate, zirconium silicate, fuller's earth, kaolin, montmorillonite, pyrophyllite, and zeolite.”

As taken from CIR, 2016.

“Inhalation may cause pneumoconiosis (Kaolinosis).”

As taken from VSDB, 2022.

“Nano-sized Kaolin (primary particle size 4.8 µm) was instilled intratracheally in groups of 4 male gpt delta mice as either a single dose of 0.2 mg/animal or multiple doses of 0.2 mg/animal/wk for 4 consecutive instillations.²⁹ Control mice received solvent alone intratracheally. The mice were killed at 12 wk after instillation (for single dose) or 8 wk after the last instillation (for multiple doses). Tissues, particularly the lungs and kidneys, underwent histopathological examination. Kaolin-phagocytized alveolar macrophages were found, diffusely distributed in the lungs. Focal granulomatous formation, with or without phagocytized alveolar macrophages, were also frequently observed in the lungs of mice that received multiple instillations. Similar observations were made in the mice that received a single instillation, but with a slight degree of particle accumulation and granuloma formation in the lungs. No abnormalities were observed in the kidneys. (...)

Toxicity of some of the minerals present in coal-mine dust was examined in groups of 10 SPF Sprague-Dawley rats. The rats were exposed over a period of 3 mo to 50 mg/animal intratracheal instillations of Kaolin. The following assessments were made: weight of the fresh lungs; macroscopic and microscopic lesions in the lungs; amount of collagen and dust present in the lungs; and calculation of the toxicity index from the amount of collagen formed per mg of dust. The weight of fresh lungs subjected to Kaolin was 1.76 g. Collagen formed per lung was 23.9 mg. The dust per lung was 30.2 mg and the collagen/dust ratio was 0.79. Microscopic examinations of the lungs showed no alveolar proteinosis but Kaolin was detected in the bronchovascular lymphoid sheaths. No information regarding nonexposed lungs was presented. The opinion of the investigators was that exposure to Kaolin results in “pulmonary toxicity” and possesses “fibrogenic capacity. (...)

The ability of Kaolin to increase susceptibility to bacterial pneumonia was studied in mice. The animals were injected intratracheally with 100 µg Kaolin. In vivo bacterial infectivity screening assays were conducted by exposing the animals to aerosolized Group C Streptococcus species. The severity of infection was calculated by recording the deaths of the mice over a 15-d period. Control animals were exposed to titanium dioxide. A 100-µg dose of Kaolin caused statistically significant but modest (< 50%) increased death due to infection by a large dose. Mortality was calculated at 38.9%. Control dusts at 100 µg produced only a 5% increase in mortality. (...)

Case Report

A patient was reported with multiple pulmonary Kaolin granulomas.¹ The man had a history of bilateral recurrent pneumothorax. Both pleural spaces were destroyed with a suspension of liquid Kaolin. Recurrent right-sided pneumothorax devolved and reobliteration was again performed. In a follow-up chest radiograph, multiple well-defined peripheral nodules were in both lungs and pathological analysis revealed a bland acellular material surrounded by chronic inflammatory cells. By light microscopy, the particles were consistent with Kaolin. It was presumed that Kaolin entered the lungs through pleuroalveolar or pleurobronchial openings.

The lungs of 62 recently deceased men between the years of 1968 to 1981 were taken for an assessment of the severity of lung disease. Fifty-four of the 62 men worked with Kaolin or related kaolinized mineral stone. All the test subjects were employed in the mining industry. Chest radiographs were available for 39 of the 62 cases. Sections of lung tissue were examined microscopically for nodular and interstitial fibrosis and an overall grade ranging from 0 (none) to 3 (severe). Samples from 42 cases were analyzed for mineral content by x-ray diffraction and lung-dust concentrations. Radiographic lesions included 13 cases of progressive massive fibrosis and 22 cases of simple pneumoconiosis. Only four cases had no evidence of any disease. Nodular opacities tended to reflect a high quartz content, whereas high-Kaolin lung content had interstitial changes and irregular radiological changes. An increasing quartz concentration appears to be related to nodular fibrosis. The degree of interstitial fibrosis appeared to be more related to dust lung concentrations, although these results failed to reach statistical significance.”

CIR (2022)

6.2. Cardiovascular system

“Washed sheep erythrocytes /were exposed/ to kaolin that was simply characterized as "native dust." The kaolin was found to be significantly hemolytic and had less than a 5 um particle size.”

As taken from HSDB, 2013.

“Different kaolin samples were found to have a significant haemolysing effect on red cells. Mányai et al. (1970) investigated a water-washed Hungarian kaolin sample. The non-heat-treated samples had a haemolytic effect. The sample pretreated by heating at 200 or 350 °C for 90 min had a somewhat stronger haemolytic effect on the red cells than the original sample.”

“Mányai et al. (1970) established that the haemolytic activity of the kaolin samples is determined by the crystal structure and hydration of the surface.”

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

“Background: The effective hemostasis after minor debridement in an outpatient clinic is important and essential. This study is aimed at evaluating the hemostatic efficacy and safety of the kaolin-impregnated dressing for diabetic foot ulcer patient who take an anticoagulant. Methods: A prospective, randomized, clinical trial study was performed in twenty-six patients with diabetic foot ulcers who take an anticoagulant requiring minor debridement in an outpatient clinic. Minor debridement and removal of break down skin were performed by one orthopedic surgeon. Hemostasis on wound bed was achieved using kaolin-impregnated gauze (study group) and standard sterilized dry gauze (control group). Two randomized groups were compared for hemostatic efficacy and side effect. Results: For the purpose of this study, the presence or absence of hemostasis was assessed at 5 and 10 minutes after the application of dressing material. Treatment was evaluated as successful if bleeding was ceased adequately, and no extra hemostatic measures were required within 10 minutes. At 5 minutes, 80% of patients using the kaolin-impregnated gauze successfully achieved complete hemostasis versus 40% in the control group that controlled bleeding partially. With kaolin-impregnated gauze, 100% of patients show complete hemostasis at 10 minutes versus 58.3% in the standard gauze (P < .001). An adverse effect was not noted in all patients. Conclusions: The use of kaolin-impregnated gauze appears to be a safe and feasible option in managing bleeding after debridement of diabetic foot ulcers, and merits to patient who had a bleeding tendency is high. This trial is registered with CRIS registration number KCT0003894.” As taken from Hwang YG et al. 2019. J. Diabetes Res. 2019, 9316380. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31828170/>

6.3. Nervous system

System	Treatment	Findings	Reference
Neural cells			
Neuroblastoma (N1E-115) cells with differentiation induced by dimethylsulfoxide	Standard kaolinite KGa-1 0.1-1.0 mg/ml incubated in cell suspension	Within minutes, resting potential depolarized and ability to maintain action potentials in response to stimulation was lost; within 30 min, severe morphological deterioration of cells.	Banin & Meiri (1990)
Neuroblastoma (N1E-115) cells and oligodendroglial (ROC-1) cells	South Carolina kaolinite (non-specified, mainly 1-2 µm diameter) incubated at 0.1 mg/ml in cell suspension for 24 h	No alteration of LDH activity in medium for either cell type; no decrease of the viability (assessed by trypan blue exclusion) of N1E-115 cells after 24 h.	Murphy et al. (1993b)

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

Immunological aspects of kaolin-induced hydrocephalus (Abstract). Adult female Sprague Dawley rats were administrated 0.1 ml Kaolin (250 mg/ml) into cisterna magna. One, 4 and 8 weeks later, brains were analyzed using antibodies against MHC class I (OX18), MHC class II (OX6), CD4 (OX38), CD8 (OX8), OX42, ED1, NF, GFAP, AChE and TH. Remarkably high numbers of T lymphocytes, and OX42- and ED1-positive macrophages were found aggregated in subarachnoid spaces, and in the third and fourth ventricles. Marked aggregations of ED1-positive reactive microglial cells were also found in paraventricular structures, medial septum, retrosplenic cortex and commissural structures. However, no such cells were found in hippocampus. ED1-positive areas were also positive for round cells with a rim of MHC I fluorescent cytoplasm as well as for OX42-positive cells and MHC II positive microglial cells. At week 1, in ventro-frontal areas of cortex, CD8-positive cells and MHC I positive astroglial fibers were detected. At week 1, MHC I positive ramified microglial cells were also recognized in almost the entire brain. The positive cells gradually decreased with time and finally remained rounded with a rim of fluorescent cytoplasm. In addition, ED1 positive partly ramified microglial cells could be recognized in corpus callosum, probably representing cells in transition between ramified and reactive microglia. CD8+ cells entered ventral brain structures, and were found in the horizontal diagonal band at week 4, and had disappeared at week 8. Finally in cortex, ED1 positive microglial cells could be identified only in the retrosplenic cortex, and there were also "dark shrunken neurons" in light microscopic stainings. However, there was only a moderate GFAP positive gliosis. In conclusion, kaolin-induced hydrocephalus leads to immune reactions in several defined areas such as cholinergic systems, corpus callosum, circumventricular organs, pontine cerebellar peduncles and the vestibular nucleus". Shinoda M Int J Neurosci. 1997 Nov; 92(1-2):9-28. As taken from PubMed, 2009 available at: <http://www.ncbi.nlm.nih.gov/pubmed?term=9522252>

"Kaolin induction of hydrocephalus in animals (rat, sheep, rhesus monkey) by injecting kaolin into the cisterna magna, is one of the most studied models of congenital hydrocephalus in the literature" (Taken from DECOS, 2007).

"Hydrocephalus is associated with reduced blood flow in periventricular white matter. To investigate hypoxic and oxidative damage in the brains of rats with hydrocephalus, kaolin was injected into the cisterna magna of newborn 7- and 21-day-old Sprague-Dawley rats, and ventricle size was assessed by magnetic resonance imaging at 7, 21, and 42 days of age. We conclude that hypoxia, via peroxidation and nitrosylation, contributes to brain changes in young rodents with hydrocephalus and that compensatory mechanisms are negligible" (Del Bigio et al 2012).

"Hydrocephalus is a common neurological disease in humans, but a uniform and particularly effective hydrocephalic animal model amenable to proper appraisal and deep study has not yet been established. In this study, we attempted to construct a high-efficiency model of hydrocephalus

via intraventricular kaolin injection. Adult male Sprague-Dawley rats were randomly divided into 2 groups: the control group (n = 15) and the experimental group (n = 30). Kaolin was injected into the lateral ventricle of experimental animals. Control rats underwent the same procedure but received sterile saline injection instead of kaolin. All animals with kaolin injection into the lateral ventricle developed hydrocephalus according to magnetic resonance imaging (MRI) results (success rate up to 100%). Also, the Morris water maze (MWM) test demonstrated disturbed spatial learning and memory. Furthermore, there were significant differences between groups with respect to the histological changes in the periventricular tissue. Our results indicate that experimental hydrocephalus induced by lateral ventricle injection of kaolin in adult rats is feasible and may be widely used." As taken from Shaolin Z et al. 2015. *Folia Neuropathol.* 53(1), 60-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25909876>

"Hydrocephalus is a common neurological disorder in children characterized by abnormal dilation of cerebral ventricles as a result of the impairment of cerebrospinal fluid flow or absorption. Clinical presentation of hydrocephalus varies with chronicity and often shows cognitive dysfunction. Here we used a kaolin-induction method in rats and studied the effects of hydrocephalus on cerebral cortex and hippocampus, the two regions highly related to cognition. Hydrocephalus impaired rats' performance in Morris water maze task. Serial three-dimensional reconstruction from sections of the whole brain freshly froze in situ with skull shows that the volumes of both structures were reduced. Morphologically, pyramidal neurons of the somatosensory cortex and hippocampus appear to be distorted. Intracellular dye injection and subsequent three-dimensional reconstruction and analyses revealed that the dendritic arbors of layer III and V cortical pyramid neurons were reduced. The total dendritic length of CA1, but not CA3, pyramidal neurons was also reduced. Dendritic spine densities on both cortical and hippocampal pyramidal neurons were decreased, consistent with our concomitant findings that the expressions of both synaptophysin and postsynaptic density protein 95 were reduced. These cortical and hippocampal changes suggest reductions of excitatory connectivity, which could underlie the learning and memory deficits in hydrocephalus." As taken from Chen LJ et al. 2017. *Brain Pathol.* 27(4), 419-436. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/27411167>

"BACKGROUND: In hydrocephalus, the impairment of cognitive and motor functions is thought to be partly due to injury to the myelin sheath of axons in the central nervous system. The exact nature of this injury is not completely understood. METHODS: We induced hydrocephalus in 3-week-old rats with an intracisternal injection of kaolin suspension (0.04 ml of 200 mg/ml) and examined paraffin and ultrathin sections of the subcortical white matter from coronal slices of the cerebrum obtained at the level of the optic chiasm after sacrifice at weekly intervals for 4 weeks. RESULTS: Over time, there was a progression of injury to the myelin sheath consisting of attenuation, lamella separation and accumulation of myelin debris, focal degeneration, and the appearance of casts and loops. CONCLUSION: The results suggest that myelin injury in kaolin-induced hydrocephalus progresses with the duration and severity of ventriculomegaly." As taken from Ayannuga OA et al. 2016. *Pediatr. Neurosurg.* 51(2), 61-8. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26761462>

"PURPOSE: We investigated the possible neuroprotective effects of the free radical scavenger edaravone in experimental hydrocephalus. METHODS: Seven-day-old Wistar rats were divided into three groups: control group (C), untreated hydrocephalic (H), and hydrocephalic treated with edaravone (EH). The H and EH groups were subjected to hydrocephalus induction by 20% kaolin intracisternal injection. The edaravone (20 mg/kg) was administered daily for 14 days from the induction of hydrocephalus. All animals were daily weighed and submitted to behavioral test and assessment by magnetic resonance imaging. After 14 days, the animals were sacrificed and the brain was removed for histological, immunohistochemical, and biochemical studies. RESULTS: The gain weight was similar between groups from the ninth post-induction day. The open field test performance of EH group was better ($p < 0.05$) as compared to untreated hydrocephalic animals. Hydrocephalic animals (H and EH) showed ventricular ratio values were higher ($p < 0.05$), whereas magnetization transfer values were lower ($p < 0.05$), as compared to control animals. Astrocyte

activity (glial fibrillary acidic protein) and apoptotic cells (caspase-3) of EH group were decreased on the corpus callosum ($p > 0.01$), germinal matrix ($p > 0.05$), and cerebral cortex ($p > 0.05$), as compared to H group. CONCLUSIONS: We have demonstrated that administration of edaravone for 14 consecutive days after induction of hydrocephalus reduced astrocyte activity and that it has some beneficial effects over apoptotic cell death." As taken from Garcia CA et al. 2017. Childs Nerv. Syst. 33(3), 419-428. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/27988876>

"BACKGROUND: The pathophysiology of hydrocephalus induced brain damage remains unclear. Long non-coding RNAs (lncRNAs) have been demonstrated to be implicated in many central nervous system diseases. However, the roles of lncRNAs in hydrocephalus injury are poorly understood. METHODS: The present study depicted the expression profiles of lncRNAs and messenger RNAs (mRNAs) in C57BL/6 mice with kaolin-induced hydrocephalus and saline controls using high-throughput RNA sequencing. Afterward, Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed to identify potential targets that correlated with hydrocephalus. In addition, co-expression networks and cis- and trans-regulation were predicted using bioinformatics methods. Finally, representative lncRNAs and mRNAs were further validation using quantitative real-time polymerase chain reaction. RESULTS: A total of 1575 lncRNAs and 1168 mRNAs were differentially expressed (DE) in hydrocephalus. GO and KEGG analyses indicated several immune and inflammatory response-associated pathways may be important in the hydrocephalus. Besides, functional enrichment analysis based on co-expression network showed several similar pathways, such as chemokine signaling pathway, phagosome, MAPK signaling pathway and complement and coagulation cascade. Cis-regulation prediction revealed 5 novel lncRNAs might regulate their nearby coding genes, and trans-regulation revealed several lncRNAs participate in pathways regulated by transcription factors, including BPTF, FOXM1, NR5A2, P2RX5, and NR6A1. CONCLUSIONS: In conclusion, our results provide candidate genes involved in hydrocephalus and suggest a new perspective on the modulation of lncRNAs in hydrocephalus." As taken from Shi YH et al. 2019. Gene 697, 184-193. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30797995>

"Aim: To demonstrate progression of acute and chronic endocrinopathies in a kaolin-induced hydrocephalus model using light microscopy. Material and methods: Adult male Sprague-Dawley rats ($n = 48$) were divided into six groups. Hydrocephalus was induced by intracisternal injection of kaolin solution in the acute and chronic kaolin groups, whereas an identical volume of sterile saline was injected into the sham groups. Results: Somatotrophic cell concentrations were lower in the kaolin groups compared with their controls, but there was no difference in somatotrophic cell concentration between the acute and chronic kaolin groups. Corticotrophic cell concentrations were higher in the acute kaolin and sham groups compared with acute controls. Thyrotrophic cell numbers were higher in the acute sham and kaolin groups compared with their controls, and although thyrotrophic cell concentrations were higher in the acute kaolin group than the acute sham group. No differences were observed between the acute and chronic controls and sham and kaolin groups regarding mammotrophic and gonadotrophic cell concentrations. Conclusion: Somatotrophic cells are most affected by hydrocephalus that causes pituitary dysfunction, and this effect was more prominent under acute and chronic phases." As taken from Nurhat RH et al. 2019. Turk. Neurosurg. 29(3), 420-429. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/30649829/>

"INTRODUCTION: The pathophysiology of normal-pressure hydrocephalus and the correlation with its symptomatology is not well understood. OBJECTIVE: To monitor and evaluate the enlargement patterns of the ventricular system for each ventricle and its correlation with the presenting symptoms. METHODS: Bilateral kaolin injection into the subarachnoid space overlying the cranial convexities was done in 18 adult rats. Magnetic resonance imaging was performed on an 11.7-T scanner 15, 60, 90, and 120 days after injection. Volumes of the ventricular system were measured for each ventricle and correlated with biweekly behavioral findings. RESULTS: There was a progressive increase in the ventricular volume for the lateral ventricles since day 15 in the kaolin-injected animals. There was a nonsignificant trend in volume growth for the third ventricle, but its

enlargement was synchronous with the lateral ventricles. No significant change for the fourth ventricle. No symptoms were detected in the first 60 days. Association was found between the ventricular volume and locomotor changes. In addition, the odds of locomotor symptoms increased by 3% for every additional cubic millimeter of volume in the left ($P < 0.001$) and right ($P = 0.023$) ventricles, and for the total magnetic resonance imaging volume by 1% ($P = 0.013$). CONCLUSIONS: Expansion of the lateral ventricles maintained similar proportions over time, accompanied by a synchronous third ventricular expansion with less proportion and a nonsignificant fourth enlargement. Lateral ventricles enlarged most in those animals that were to develop late locomotor deterioration. Further research using this animal model combined with different radiologic imaging techniques, such as diffusion tensor imaging and perfusion studies, is recommended.” As taken from Vivas-Buitrago T et al. 2018. World Neurosurg. 120, e1120-e1127. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30217783>

“Background: Syringomyelia is a pathological cavitation of the spinal cord. In this study, we examined whether a syrinx cavity would limit itself with axonal regeneration and stem cell activity in the cavity, and we evaluated subjects on a functional basis. Methods: Groups were designated as kaolin, trauma, kaolin-trauma, and saline groups. Also divided out of the syringomyelia treated groups were those given human mesenchymal stem cells (hMSCs). All groups were evaluated with immunohistochemistry, electron microscopy, confocal microscopy and functionally. Results: The kaolin-trauma group had a significant correction of BBB score with hMSCs therapy. The syrinx cavity measurements showed significant improvement in groups treated with hMSCs. The tissue surrounding the syrinx cavity, however, appeared to be better organized in groups treated with hMSCs. The process of repair and regeneration of damaged axons in the lesion were more improved in groups treated with hMSCs. Using confocal microscopy, fluorescence of hMSCs was observed in the central canal, in the ependymal tissue, and around the lesion. Conclusions: It was concluded that axonal repair accelerated in groups receiving stem cells, and thus, stem cells may be effective in recovery of neural tissue and myelin damage in syringomyelia.” As taken from Bal E et al. 2020. J. Neurosurg. Sci. Epub ahead of print. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33056946/>

6.4. Other organ systems, dependent on the properties of the substance

“Kaolin and pectin preparations have essentially no adverse effects. Constipation may occur but is usually mild and transient; however, constipation may rarely lead to fecal impaction, especially in infants or debilitated geriatric patients.”

/OTHER TOXICITY INFORMATION/ Although ... generally considered ... innocuous, granuloma of the stomach has been reported from its use ... [Goodman, L.S., and A. G. Gilman. (eds.). The Pharmacological Basis of Therapeutics. 4th ed. New York: Macmillan Co., 1970., p. 990] **PEER REVIEWED**

As taken from HSDB, 2013.

“In a study of a large number of organic and inorganic particulates, kaolin (composition not specified, particle size 0.25–25 μm) injected intraperitoneally into rats produced a granulomatous reaction at 1 and 3 months, but no fibrosis. In vitro, it had low toxicity: it killed less than 2% of peritoneal macrophages and approximately 4% of alveolar macrophages (Styles & Wilson, 1973). For the group of some 20 particulate materials, the authors considered that there was a good correlation between toxicity to macrophages and fibrogenicity after intraperitoneal injection.”

As taken from EHC 231, 2005, available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm>

“May produce gastrointestinal disturbances as high doses.”

As taken from VSDB, 2022.

“It has a role as an excipient and an antidiarrhoeal drug.”

As taken from PubChem

"Iron deficiency anemia is a common diagnosis encountered in the nutrition, primary care, and gastroenterology fields. Iron deficiency anemia most often leads to evaluation for various malabsorption disorders and colonoscopy to exclude colon cancer as an etiology. We present a case of iron deficiency anemia that was caused by geophagia. After the culprit dietary habit was stopped, the patient's iron deficiency anemia subsequently resolved." As taken from Attarha BO et al. 2021. Cureus 13(3), e13796. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33842169/>

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

No data available to us at this time.

9. Heated/vapor emissions toxicity

Aerosol from heated tobacco stick(s) containing Aluminum silicate was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	4.31	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	4.31	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	4.31	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

10. Ecotoxicity

10.1. Environmental fate

"Kaolin related clays occur in several different types of deposits. Many kaolin deposits throughout the world are in the form of tabular lenses and discontinuous beds in sedimentary rock. Extensive sedimentary deposits of this type occur in the Georgia-South Carolina kaolin belt, Arkansas bauxite region and lone district California". [Lefond SJ, ed; Industrial Minerals & Rocks, 5th ed, V1 p.618 (1983)]**PEER REVIEWED**

"Kaolins occur on every continent, but only a few (Georgia, England, Brazil, Czechoslovakia, Eastern Germany, Spain, the former Soviet Union, and Australia) can be mined and beneficiated to meet the rigid industrial specifications required for use as a filler, extender pigment, or ceramic raw material. Only those in Georgia, England, and Brazil can meet specifications for coating clays for paper". [Murray HH; Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (2005). NY, NY: John Wiley & Sons; Clays. Online Posting Date: Dec 15, 2000.]**PEER REVIEWED**

"The English kaolins that occur in southwest England in Cornwall. A large sedimentary deposit of kaolin of Pliocene age is mined along the Jari River, a tributary of the Amazon, on the border of the states of Para and Amapa. Another large sedimentary kaolin area in Brazil is being mined and

processed. This kaolin is coarse, somewhat similar to the Cretaceous kaolins in Georgia. Former Czechoslovakia is the principal producer of kaolin in eastern Europe. In Germany, both primary and secondary deposits are being mined. In the eastern part of Germany, near Dresden, are two large primary deposits at Caminau and Kemmlitz. Kaolin is mined in Spain in two locations. A primary kaolin are mined in the northwestern corner of Spain in Galicia. These deposit are residues resulting from the weathering of a large granitic body. A secondary or sedimentary kaolin of Cretaceous age is mined in the state of Guadalajara, east of Madrid. In the Ukraine, a large primary deposit is being mined and processed near Proyanovski. In China, several kaolin deposits are being mined. The largest is located near Suzhou, southwest of Shanghai. The Suzhou deposit is primary, resulting from both weathering and hydrothermal alteration of granites and other acid igneous rocks". [Murray HH; Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (2005). NY, NY: John Wiley & Sons; Clays. Online Posting Date: Dec 15, 2000.]**PEER REVIEWED**

As taken from HSDB, 2013.

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that kaolin is persistent in the environment

Data accessed February 2017 on the OECD website:
<http://webnet.oecd.org/CCRWeb/Search.aspx>

Soil degradation (aerobic) DT50 1000 days (very persistent).

"Naturally occurring mineral. Does not degrade".

As taken from VSDB, 2015

10.2. Aquatic toxicity

LC50 Water flea (*Daphnia pulex*) (24 & 48 hr) >1.1 g/liter". /Conditions of bioassay not specified in source examined/ [IPCS INCHEM; Environmental Health Criteria (EHC) Monographs. Bentonite, kaolin, and selected clay minerals (EHC 231). Available from, as of June 25, 2007: <http://www.inchem.org/pages/ehc.html>]**PEER REVIEWED**

/AQUATIC SPECIES/ No effect on the hatching success or egg development rate of four marine fish species - red seabream (*Pagrus major*), black porgy (*Acanthopagrus schlegeli*), striped knifefish (*Oplegnathus fasciatus*), and threeline grunt (*Parapristipoma trilineatum*) - was observed at kaolinite concentrations up to 10 g/L for 24 hr. Larvae were more sensitive to kaolinite: the 12-hr LC50 values were 170 and 710 mg/L for *P. trilineatum* and *O. fasciatus*, respectively; mortality was also observed for *P. major* at concentrations of 1000 mg/L and above. [IPCS INCHEM; Environmental Health Criteria (EHC) Monographs. Bentonite, kaolin, and selected clay minerals (EHC 231). Available from, as of June 25, 2007: <http://www.inchem.org/pages/ehc.html> **PEER REVIEWED**

/AQUATIC SPECIES/ Georgia kaolin caused <10% mortality of sea urchin (*Strongylocentrosus purpuratus*), Japanese clam (*Tapes japonica*), hermit crab (*Pagurus hirsutiusculus*), isopod (*Sphaeroma pentodon*), mud snail (*Nassarius obsoletus*), blue mussel (*Mytilus edulis*), and tunicates (*Molgula manhattensis* and *Styela montereyensis*) within 5-12 days. [IPCS INCHEM; Environmental Health Criteria (EHC) Monographs. Bentonite, kaolin, and selected clay minerals (EHC 231). Available from, as of June 25, 2007: <http://www.inchem.org/pages/ehc.html> **PEER REVIEWED**

/AQUATIC SPECIES/ To evaluate the effects of suspended inert particles on the early life stages of the eggs and larvae of four marine fish species, *Pagrus major* (Red seabream), *Acanthopagrus*

schlegeli (Japanese black porgy), *Oplegnathus fasciatus* (Striped beakfish) and *Parapristipoma trilineatum* were exposed to kaolinite suspensions up to 10,000 mg L⁻¹. Hatching success and developmental rates of eggs all species were not significantly affected by 10,000 mg L⁻¹ suspension over a 24-hr exposure period. Exposure of larvae of each species to suspensions ranging from 32 to 10,000 mg L⁻¹ for 1, 3 and 12 hr, however, resulted in over 50% mortality at 1000 mg L⁻¹ for 12 hr. Larvae were much less tolerant to kaolinite exposure than eggs probably due to their relatively larger and more delicate body surface compared to the tough outer membrane of eggs. Kaolinite particles adhered more readily to eggs and larvae of *P. major* than to those of the other species. [Isono RS et al; Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. 120 (3): 449-55 (1998)] **PEER REVIEWED** PubMed Abstract “

As taken from HSDB, 2013.

“Georgia kaolin caused <10% mortality of sea urchin (*Strongylocentrosus purpuratus*), Japanese clam (*Tapes japonica*), hermit crab (*Pagurus hirsutiusculus*), isopod (*Sphaeroma pentodon*), mud snail (*Nassarius obsoletus*), blue mussel (*Mytilus edulis*), and tunicates (*Molgula manhattensis* and *Styela montereyensis*) within 5–12 days. The 200-h LC₁₀ values for coast mussel (*Mytilus californianus*), black-spotted bay shrimp (*Crangon nigromaculata*), migrant prawn (*Palaemon macrodactylus*), dungeness crab (*Cancer magister*), and the polychaete *Neanthes succinea* were 26, 16, 24, 10, and 9 g/litre, respectively. The 100-h LC₁₀ values for the tunicate *Ascidia ceratodes*, amphipod *Anisogammarus confervicolus*, and shiner perch (*Cymatogaster aggregata*) were 7, 38, and 1 g/litre, respectively (McFarland & Peddicord, 1980)”.

“No effect on the hatching success or egg development rate of four marine fish species — red seabream (*Pagrus major*), black porgy (*Acanthopagrus schlegeli*), striped knifefish (*Oplegnathus fasciatus*), and threeline grunt (*Parapristipoma trilineatum*) — was observed at kaolinite concentrations up to 10 g/litre for 24 h. Larvae were more sensitive to kaolinite: the 12-h LC₅₀ values were 170 and 710 mg/litre for *P. trilineatum* and *O. fasciatus*, respectively; mortality was also observed for *P. major* at concentrations of 1000 mg/litre and above (Isono et al., 1998)”.

As taken from INCHEM available at: <http://www.inchem.org/documents/ehc/ehc/ehc231.htm#6.1.2>

The Ecological Categorization Results from the Canadian Domestic Substances List state that kaolin not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	0.089
Experimental result iT (mg/l)	0.089
Test species iT (Latin)	<i>Hyallela azteca</i>
Final EndPoint iT	LC50
Exposure duration iT (hours)	168
Comment iT	tested in 10% Lake Ontario water based on measured concentrations of dissolved metal originating from AA standards and complex metal anions.
Reference iT	Unpublished report. NWRI Laboratory. Dr Uwe Borgmann

Data accessed February 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>

Fish, acute 96-hour LC₅₀ >2500 mg/L

Aquatic invertebrate acute 48-hour EC₅₀ >2500 mg/L

As taken from VSDB, 2015

10.3. Sediment toxicity

Impact of clay minerals on sulfate-reducing activity in aquifers (Abstract). Previous studies have shown that sulfate-reduction activity occurs in a heterogeneous manner throughout the terrestrial subsurface. Low-activity regions are often observed in the presence of clay minerals. Here we report that clays inhibit sulfate reduction activity in sediments and in a pure culture of *Desulfovibrio vulgaris*. Clay minerals including bentonite and kaolinite inhibited sulfate reduction by 70-90% in sediments. Intact clays and clay colloids or soluble components, capable of passing through a .2-microm filter, were also inhibitory to sulfate-reducing bacteria. Other adsorbent materials, including anion or cation exchangers and a zeolite, did not inhibit sulfate reduction in sediments, suggesting that the effect of clays was not due to their cation-exchange capacity. We observed a strong correlation between the Al₂O₃ content of clays and their relative ability to inhibit sulfate reduction in sediments ($r^2 = .82$). This suggested that inhibition might be a direct effect of Al³⁺(aq) on the bacteria. We then tested pure aluminum oxide (Al₂O₃) and showed it to act in a similar manner to clay. As dissolved aluminum is known to be toxic to a variety of organisms at low concentrations, our results suggest that the effects of clay on sulfate-reducing bacteria may be directly due to aluminum. Thus, our experiments provide an explanation for the lack of sulfate-reduction activity in clay-rich regions and presents a mechanism for the effect. [D Wong et al. Microb Ecol. 2004. Jan ;47 \(1\):80-6.](#)

As taken from PubMed, 2009 at <http://www.ncbi.nlm.nih.gov/pubmed/15259272>

10.4. Terrestrial toxicity

Role of clay content in partitioning, uptake and toxicity of zinc in the earthworm *Eisenia fetida* (Abstract). We studied the effect of clay content on the bioavailability of zinc to pre-clitellate earthworm, *Eisenia fetida* in the laboratory using OECD artificial soil adjusted to 5%, 20%, and 40% clay. Batches of worms were exposed to a wide range of zinc concentrations. Mortality, growth, maturation (% clitellate), cocoon production, and body zinc concentrations were assessed over and after a period of 4 weeks. Total, DTPA, and CaCl₂ extractable zinc in the substrates were also determined. The results of the biological responses showed that interaction of clay and zinc had a significant influence on mortality but not on the other biological parameters. None of the three extraction methods showed consistent and significant effect of clay content on zinc partitioning. Although total, DTPA, and CaCl₂ extracts of zinc correlated strongly with one another and were in similar relation with the observed biological responses, only the CaCl₂ extract revealed a time dependent availability of this metal. It is concluded that clay content had no significant influence on sub-lethal toxicity of zinc to this earthworm over the range of exposure concentrations. Owojori OJ et al. *Ecotoxicol Environ Saf.* 2009 Jan; 72(1):99-107. As taken from PubMed, 2009 available at: [http://www.ncbi.nlm.nih.gov/pubmed?term=Ecotoxicol%20Environ%20Saf.%202009%20Jan%3B72\(1\)%3A99-107](http://www.ncbi.nlm.nih.gov/pubmed?term=Ecotoxicol%20Environ%20Saf.%202009%20Jan%3B72(1)%3A99-107).

“Although recent research has demonstrated that clays provide satisfactory control of some agricultural insect pests, the effect of clays on gall wasps that damage forest trees has not been previously reported. The aim of the current study is to evaluate the effectiveness of the clay kaolin in the laboratory and in the field in reducing the damage caused by the eulophid *Ophelimus maskelli* (Ashmead) on seedlings of eucalyptus (*Eucalyptus* L'Hér.) species. In the laboratory, kaolin + wetting agent significantly reduced the percentage of infested leaves and the number of galls per leaf. In the nursery, gall number per leaf was not correlated with leaf area with kaolin + wetting agent but was related to leaf area for all other treatments (wetting agent alone, imidacloprid, and untreated control). In the nursery, gall number per leaf was lower with kaolin + wetting agent and with imidacloprid than with the other two treatments. Overall, kaolin effectively

reduced eulophid infestations, and its effect was more persistent than that of imidacloprid. Although application of kaolin might not be feasible on large forested areas, kaolin could represent a valuable control method in nurseries, where the repeated application with more toxic chemicals can result in high concentrations of residual pesticides in the soil". As taken from Lo Verde G et al. 2011. J. Econ. Entomol. 104, 180-187. PubMed, 2013 available at: <http://www.ncbi.nlm.nih.gov/pubmed/21404856?dopt=AbstractPlus>.

"Lethal and sublethal effects of kaolin clays and two copper-based products on four natural enemies found in olive orchards *Anthocoris nemoralis* (F.) (Hem. Anthocoridae), *Chelonus inanitus* (L.) (Hym. Braconidae), *Chilocorus nigritus* (F.) (Col. Coccinellidae) and *Scutellista cyanea* Motschulsky (Hym. Pteromalidae) are described. Both kaolin and copper can be applied for controlling the olive fruit fly and the olive moth, two important pests of this crop. The products did not increase the mortality of any of the insects studied, with the exception of *A. nemoralis*. The sublethal effects, however, differed depending on the parameter evaluated and the insect studied. Both kaolin and coppers slightly, but significantly, reduced the life span of *C. inanitus* and *S. cyanea*. Number of eggs laid by *A. nemoralis* females were reduced, but not significantly compared to the controls. In the behavioural experiments, clear preference for remaining on kaolin-untreated surfaces when insects were able to choose was observed. Despite having some negative effects, the negative impact on natural enemies was lower than the impact caused by products commonly applied in this crop against the pests stated above. Therefore, both kaolin and copper can be considered as alternative products to be applied in olive orchards if an effective resistance management programme is to be developed. Furthermore, both of them are allowed in organic farming, in which the number of products that can be applied is more restricted". As taken from Bengochea P et al. 2013. Chemosphere 91(8), 1189-95. PubMed, 2014 available at: <http://www.ncbi.nlm.nih.gov/pubmed/23415488>.

LD50; Species: /*Apis mellifera*/ (Honeybee) dietary >100 ug/bee for 48 hr [USEPA/Office of Pesticide Programs; Reregistration Eligibility Decision Document - Kaolin (1332-58-7; PC 100104) (April 2000). Available from, as of April 1, 2013: <http://iaspub.epa.gov/apex/pesticides/f?p=chemicalsearch:1> **PEER REVIEWED**"

As taken from HSDB, 2013

Record for kaolin (CAS RN 1332-58-7):

Spec. Sci. Name Spec. Common Name	Resp. Type Exp. Dur. (Days)	Media Type Test Loc.	Exp. Site Chem. Anal.	Dose# Res. Sample Unit	Endpoint BAF/BCF	Effect Effect Meas.	Signif. Sig. Level	Dose Dose Stat. Meth.
Flowers, Trees, Shrubs, Ferns								
Malus pumila Paradise Apple	3 d	UKN LAB	SP U	2	LOEL	REP GERM	ASIG 0.05	F 59.2/ (NR/-NR/) g/L
Malus pumila Paradise Apple	3 d	CUL LAB	SP U	2	LOEL	REP GERM	ASIG 0.05	F 59.2/ (NR/-NR/) g/L

Eucalyptus camaldulensis Red River Gum	WO ~ 182.64 d	NONE FIELDA	FS U	3	NOEL	GRO DMTR	ANOSIG	F 50/ (NR/- NR/) g/L
Eucalyptus camaldulensis Red River Gum	WO ~ 182.64 d	NONE FIELDA	FS U	3	NOEL	GRO HGHT	ANOSIG	F 50/ (NR/- NR/) g/L
Eucalyptus camaldulensis Red River Gum	WO ~ 182.64 d	NONE FIELDA	FS U	3	NOEL	GRO NLEF	ANOSIG	F 50/ (NR/- NR/) g/L
Pyrus communis	FR	NONE	SP	2	NOEL	INJ	ANOSIG	F 28.0 kg/ha
Pear	2 gs	FIELDN	U			DAMG	>0.05	
Pyrus communis	FR	NONE	SP	2	NOEL	INJ	ANOSIG	F 28.0 kg/ha
Pear	2 gs	FIELDN	U			DAMG	>0.05	
Pyrus communis	FR	NONE	SP	2	NOEL	INJ	ANOSIG	F 28.0 kg/ha
Pear	2 gs	FIELDN	U			DAMG	>0.05	
Pyrus communis	FR	NONE	SP	2	NOEL	INJ	ANOSIG	F 28.0 kg/ha
Pear	2 gs	FIELDN	U			DAMG	>0.05	
Pyrus communis	FR	NONE	SP	2	NOEL	INJ	ANOSIG	F 28.0 kg/ha
Pear	4 gs	FIELDN	U			DAMG	>0.05	
Eucalyptus camaldulensis Red River Gum	LE ~ 182.64 d	NONE FIELDA	FS U	3	NOEL	MPH AREA	ANOSIG	F 50/ (NR/- NR/) g/L
Malus pumila Paradise Apple	PU 3 d	CUL LAB	SP U	2	NOEL	MPH LGTH	ANOSIG 0.05	F 59.2/ (NR/-NR/) g/L
Malus pumila		UKS	HS	2	NOEL	REP	ANOSIG	F 3370/ (NR/-NR/)

Paradise Apple	42 d	FIELDA	U			FRUT	0.05	L/ha
Insects/Spiders								
Toxoptera aurantii Black Citrus Aphid	21 d	NONE FIELDN	SP U	2	LOEC	POP ABND/	ASIG <0.05	F 1.4/ (NR/- NR/) %
Synanthedon scitula		NONE	SP	2	LOEL	POP	ASIG	A 21.54 ai kg/379 l
Dogwood Borer	< 7	FIELDN	U			ABND	0.05	
Lacanobia subjuncta		NONE	SP	2	LOEL	POP	SIG/	A 56069.0 Al g/ha
Speckled Cutworm Moth		FIELDN	U	SO		CNTL	0.05/	
Lacanobia subjuncta		NONE	SP	2	LOEL	POP	SIG	A 56069.0 Al g/ha
Speckled Cutworm Moth		FIELDN	U	SO		CNTL	0.05	
Lacanobia subjuncta		NONE	SP	2	LOEL	POP	SIG/	A 56069.0 Al g/ha
Speckled Cutworm Moth		FIELDN	U	SO		CNTL	0.05/	
Ophelimus maskelli Eucalyptus Gall Wasp	~ 182.64 d	NONE FIELDA	SP U	3	LOEL	POP ABND/	ASIG 0.05	F 50/ (NR/- NR/) g/L
Ophelimus maskelli Eucalyptus Gall Wasp	~ 182.64 d	NONE FIELDA	SP U	3	LOEL	POP ABND/	ASIG 0.05	F 50/ (NR/- NR/) g/L
Toxoptera aurantii Black Citrus Aphid	21 d	NONE FIELDN	SP U	2	LOEL	POP ABND/	ASIG <0.05	F 1.4/ (NR/- NR/) %
Aphis spiraeicola Spirea Aphid	21 d	NONE FIELDN	SP U	2	LOEL	POP ABND/	ASIG <0.05	F 1.4/ (NR/- NR/) %

Hymenoptera Bee Order	21 d	NONE FIELDN	SP U	2	LOEL	POP ABND	ASIG <0.05	F 1.4/ (NR/- NR/) %
Chrysoperla carnea Common Green Lacewing	21 d	NONE FIELDN	SP U	2	LOEL	POP ABND	ASIG <0.05	F 1.4/ (NR/- NR/) %
Coccinella septempunctata Sevenspotted Lady Beetle	21 d	NONE FIELDN	SP U	2	LOEL	POP ABND	ASIG <0.05	F 1.4/ (NR/- NR/) %
Adalia decempunctata Ten-spot Ladybird Beetle	21 d	NONE FIELDN	SP U	2	LOEL	POP ABND	ASIG <0.05	F 1.4/ (NR/- NR/) %
Coccinellidae Ladybird Beetle Family	21 d	NONE FIELDN	SP U	2	LOEL	POP ABND	ASIG <0.05	F 1.4/ (NR/- NR/) %
Arachnida Spider/Mite Class	21 d	NONE FIELDN	SP U	2	LOEL	POP ABND	ASIG <0.05	F 1.4/ (NR/- NR/) %
Aphis spiraeicola Spirea Aphid	21 d	NONE FIELDN	SP U	2	LOEL	POP ABND/	ASIG <0.05	F 1.4/ (NR/- NR/) %
Coccinellidae Ladybird Beetle Family	21 d	NONE FIELDN	SP U	2	NOEC	POP ABND	ANOSIG <0.05	F 1.4/ (NR/- NR/) %
Psytalia concolor Braconid Parasitoid		NONE LAB	SA U	2	NOEL	BEH PNPY/	ANOSIG <=0.05	A 5/ (NR/- NR/) kg/hL
Psytalia concolor		NONE	EN	2	NOEL	BEH	ANOSIG	A

Braconid Parasitoid	4 d	LAB	U			PNPY/	<=0.05	5/ (NR/-NR/) kg/hL
Psytalia concolor Braconid Parasitoid	4 d	NONE LAB	EN U	2	NOEL	BEH PNPY/	ANOSIG <=0.05	A 5/ (NR/-NR/) kg/hL
Psytalia concolor Braconid Parasitoid	7 d	NONE LAB	EN U	2	NOEL	BEH PNPY/	ANOSIG <=0.05	A 5/ (NR/-NR/) kg/hL
Psytalia concolor Braconid Parasitoid		NONE LAB	SA U	2	NOEL	DVP EMRG/	ANOSIG <=0.05	A 5/ (NR/-NR/) kg/hL
Popillia japonica		NONE	CH	2	NOEL	FDB	ANOSIG	F 60 g/L
Japanese Beetle	0.25	LAB	U			FCNS	<0.05	
Popillia japonica		NONE	FD	2	NOEL	FDB	ANOSIG	F 60 g/L
Japanese Beetle	0.75	LAB	U			FCNS	<0.05	
Popillia japonica		NONE	FD	2	NOEL	FDB	ANOSIG	F 60 g/L
Japanese Beetle	0.25	LAB	U			FCNS	<0.05	
Popillia japonica		NONE	FD	2	NOEL	MOR	ANOSIG	F 60 g/L
Japanese Beetle	0.75	LAB	U			MORT	<0.05	
Popillia japonica		NONE	FD	2	NOEL	MOR	ANOSIG	F 60 g/L
Japanese Beetle	0.25	LAB	U			TKNO	<0.05	
Psytalia concolor Braconid Parasitoid	7 d	NONE LAB	EN U	2	NOEL	MOR MORT/	ANOSIG <=0.05	A 5/ (NR/-NR/) kg/hL
Ophelimus maskelli Eucalyptus Gall Wasp	~ 182.64 d	NONE FIELDA	SP U	3	NOEL	POP ABND/	ANOSIG 0.05	F 50/ (NR/-NR/) g/L

Episyrphus balteatus Hover Fly	21 d	NONE FIELDN	SP U	2	NOEL	POP ABND	ANOSIG ----- <005	F 1.4/ (NR/- NR/) %
Hymenoptera Bee Order	21 d	NONE FIELDN	SP U	2	NOEL	POP ABND	ANOSIG -<0.05	F 1.4/ (NR/- NR/) %
Chrysoperla carnea Common Green Lacewing	21 d	NONE FIELDN	SP U	2	NOEL	POP ABND	ANOSIG -<0.05	F 1.4/ (NR/- NR/) %
Coccinella septempunctata Sevenspotted Lady Beetle	21 d	NONE FIELDN	SP U	2	NOEL	POP ABND	ANOSIG -<0.05	F 1.4/ (NR/- NR/) %
Adalia decempunctata Ten-spot Ladybird Beetle	21 d	NONE FIELDN	SP U	2	NOEL	POP ABND	ANOSIG -<0.05	F 1.4/ (NR/- NR/) %
Stethorus punctillum Lady Beetle	21 d	NONE FIELDN	SP U	2	NOEL	POP ABND	ANOSIG -<0.05	F 1.4/ (NR/- NR/) %
Rodolia cardinalis Vedalia Beetle	21 d	NONE FIELDN	SP U	2	NOEL	POP ABND	ANOSIG -<0.05	F 1.4/ (NR/- NR/) %
Scymnus sp. Lady Beetle	21 d	NONE FIELDN	SP U	2	NOEL	POP ABND	ANOSIG -<0.05	F 1.4/ (NR/- NR/) %
Arachnida Spider/Mite Class	21 d	NONE FIELDN	SP U	2	NOEL	POP ABND	ANOSIG -<0.05	F 1.4/ (NR/- NR/) %
Psytalia concolor Braconid Parasitoid		NONE LAB	SA U	2	NOEL	REP PROG/	ANOSIG -<=0.05	A 5/ (NR/- NR/) kg/hL

Psytalia concolor Braconid Parasitoid	4 d	NONE LAB	EN U	2	NOEL	REP PROG	ANOSIG ≤0.05	A 5/ (NR/- NR/) kg/hL
Psytalia concolor Braconid Parasitoid	4 d	NONE LAB	EN U	2	NOEL	REP PROG	ANOSIG ≤0.05	A 5/ (NR/- NR/) kg/hL
Psytalia concolor Braconid Parasitoid	7 d	NONE LAB	EN U	2	NOEL	REP PROG/	ANOSIG ≤0.05	A 5/ (NR/- NR/) kg/hL
Tamarixia radiata Eulophid Parasitoid	3 d	LIT LAB	SP U	2 OR		MOR MORT		A 30.0/ (NR/-NR/) g/L
Tamarixia radiata Eulophid Parasitoid	3 d	LIT LAB	EN U	2 OR		MOR MORT		A 30.0/ (NR/-NR/) g/L
Tamarixia radiata Eulophid Parasitoid	3 d	LIT LAB	EN U	2		MOR MORT		A 30.0/ (NR/-NR/) g/L
Tamarixia radiata Eulophid Parasitoid	3 d	LIT LAB	EN U	2		MOR MORT		A 30.0/ (NR/-NR/) g/L

As taken from the EPA ECOTOX database, accessed February 2017, available at http://cfpub.epa.gov/ecotox/advanced_query.htm

Bird acute LD₅₀ >5000 mg/kg bw.

Honeybee acute oral 48-hour LD₅₀ >1000 µg/bee

Earthworm (*Eisenia foetida*) acute 14-day LC₅₀ >2000 mg/kg

As taken from VSDB, 2015

“*Araniella cucurbitina* (Araneae: Araneidae) is a widespread orb-weaver spider commonly found in agroecosystems. Mineral particle films such as kaolin, due to their protective or anti-feeding action, can represent an alternative to pesticides, especially in organic farming systems, but little is known about its effects on *A. cucurbitina*. Therefore, we tested the effect of kaolin sprays on the life span of *A. cucurbitina* under laboratory conditions. Four treatments were tested encompassing different exposure routes. Thus, kaolin sprays were applied on (i) the surface, (ii) the prey (fly), (iii) the spider and (iv) both spider & prey. A control group was tested with water in each treatment. Results showed that sprays of kaolin significantly affected the survival of *A. cucurbitina* when applications were done on the surface and on both spider & prey registering a reduction of 48% and 56%, respectively. Spiders in control obtained higher probability of reaching alive at the end of the assay than those treated with kaolin. Differences observed can be explained by the feeding behavior of the species and may depend on the consumption of the web by the spider and the ratio spider/fly for body size.” As taken from Benhadi-Marín J et al. 2016. Chemosphere 144, 918-24. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26432533>

10.5. All other relevant types of ecotoxicity

“In a series of 100 necropsies among 11 mammalian and eight avian species in the San Diego Zoo, California, USA, interstitial fibrosis was observed in 20% of the mammals studied. In birds, granulomas around tertiary bronchi, but no fibrosis, were observed. Electron and X-ray diffraction showed that the minerals present in the lungs were muscovite mica and illite (Brambilla et al., 1979). As the authors noted, the finding does not infer causality between clays present in the lungs and pulmonary effects”.

As taken from INCHEM at <http://www.inchem.org/documents/ehc/ehc/ehc231.htm#6.1.2>

According to Canadian Categorization Criteria, the bioaccumulative potential of kaolin in the environment has not been determined.

Data accessed February 2017 on the OECD website:
<http://webnet.oecd.org/CCRWeb/Search.aspx>

11. References

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13. Last audited

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SCIENTIFIC OPINION

Scientific Opinion on the safety assessment of the substances, kaolin and polyacrylic acid, sodium salt, for use in food contact materials¹

EFSA Panel on Food Contact Materials, Enzymes,
Flavourings and Processing Aids (CEF)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion of the EFSA Panel on Food Contact Materials, Enzymes, Flavorings and Processing Aids (CEF) deals with the safety assessment of kaolin which is used in a combination with polyacrylic acid, sodium salt and is incorporated in a gas barrier layer of ethylene-vinyl alcohol (EVOH) copolymer. The EVOH copolymer is used as an inner layer in multi-layer food contact materials. Morphological studies show stacks of kaolin clay platelets of different sizes dispersed in the EVOH and some of these platelets are below 100 nm in the thickness dimension. Nevertheless, exfoliation into individual platelets does not occur for kaolin as confirmed by WAXS. No migration of the kaolin particles from the EVOH layer was shown. There was no detectable migration of aluminium or magnesium (inorganic constituents of kaolin) from a typical multi-layer plastic material, with detection limits of 0.07 mg/kg and up to 2.2 mg/kg, respectively. These values indicate that migration of these inorganic components is not expected to be significant. Migration of polyacrylic acid sodium salt is not expected to exceed the SML of 6 mg/kg foodstuffs at the conditions of use foreseen. Therefore, the CEF panel concluded that the substance kaolin in the form of particles with a thickness less than 100 nm does not raise a safety concern for the consumer when incorporated up to 12 % in EVOH copolymer. This material is used as an inner layer of a plastic multi-layer structure, in contact with foods and beverages stored at room temperature up to 6 months. The EVOH inner layer containing the particles should be used behind a functional barrier that prevents migration of kaolin particles into the food.

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KEY WORDS

kaolin, polyacrylic acid sodium salt, food contact materials, safety evaluation

¹ On request from Agencia Española de la Seguridad Alimentaria y Nutrición, Spain, Question No EFSA-Q-2013-00023, adopted on 27 March 2014.

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SUMMARY

Within the general task of evaluating substances intended for use in materials in contact with food according to the Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with foodstuffs, the CEF Panel received a request from a competent Member State Authority for safety evaluation of a substance following a corresponding application from the industry.

In the context of this evaluation procedure, following a request from the Agencia Española de la Seguridad Alimentaria y Nutrición, Spain, the Panel on Food Contact Materials, Enzymes and Processing aids (CEF) was asked to deliver an opinion on kaolin which is used in a combination with and polyacrylic acid, sodium salt, for incorporation in an a gas barrier layer of a multi-layer food contact material, not in direct contact with food.

Kaolin (99.5 %) in a combination with polyacrylic acid, sodium salt (0.5 %) intended to be incorporated in EVOH copolymer in order to enhance its gas barrier properties. The mixture is incorporated typically at 4 % in the EVOH copolymer, but depending on the application the concentration can range from 1 % to 12 %. The EVOH copolymer is intended to be used in food contact materials for beverages (alcoholic and non-alcoholic), sauces and dressings, sausages and wet and dry pet food, stored at room temperature up to 6 months.

Both substances have been evaluated and authorised for use as additives in plastic materials and articles in contact with foods.

Polyacrylic acid, sodium salt is used as an agent to disperse the kaolin in the EVOH. Particle size distribution of kaolin in an aqueous dispersion showed values larger than 0.92 μm (90 %) and larger 2.17 μm (50 %). The specific surface area was 3.2 m^2/g . Morphological studies show stacks of kaolin clay platelets of different sizes dispersed in the EVOH. These particles have shown strong interfacial adhesion to the polymer. Some of these particles when dispersed in the polymer are below 100 nm in the thickness dimension. Nevertheless, exfoliation into individual platelets does not occur for kaolin as confirmed by WAXS.

From a toxicological point of view, migration of aluminium and magnesium ions from kaolin (constituents of the clay) is of interest. Specific migration from a typical multi-layer was not detectable; less than 0.07 mg/kg for aluminium and less than 2.2 mg/kg for magnesium. These values indicate that migration of these inorganic components is not expected to be significant and would result in exposure estimates much lower than the TWI established in 2008 for aluminium and lower than the Tolerable Upper Intake level of 250 mg/day for magnesium.

Migration of polyacrylic acid sodium salt, estimated assuming total migration from EVOH layers up to 15 μm , containing 4 % of substance was well below of the SML of 6 mg/kg foodstuffs. This indicates that migration of polyacrylic acid sodium salt at the intended conditions of use does not exceed the SML.

Therefore, the CEF panel concluded that the substance kaolin in the form of particles with a thickness less than 100 nm does not raise a safety concern for the consumer when incorporated up to 12 % in EVOH copolymer. This material is used as an inner layer of a plastic multi-layer structure, in contact with foods and beverages stored at room temperature up to 6 months. The EVOH inner layer containing the particles should be used behind a functional barrier that prevents migration of kaolin particles into the food.

TABLE OF CONTENTS

Abstract	1
Summary	2
Table of contents	3
Background as provided by the legislation	4
Terms of reference as provided by the legislation.....	4
Assessment	5
1. Introduction	5
2. General information.....	5
3. Data available in the dossier used for this evaluation.....	5
4. Evaluation.....	6
4.1. Non-toxicological data.....	6
4.2. Toxicological data.....	6
Conclusions	7
Documentation provided to EFSA	7
References	7
Abbreviations	8

BACKGROUND AS PROVIDED BY THE LEGISLATION

Before a substance is authorised to be used in food contact materials and is included in a positive list EFSA's opinion on its safety is required. This procedure has been established in Articles 8 and 9 of the Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food⁴.

According to this procedure the industry submits applications to the Member States competent Authorities which in their turn transmit the applications to the EFSA for their evaluation. The application is supported by a technical dossier submitted by the industry following the SCF guidelines for the "presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation" (EC, 2001).

In this case, EFSA received an application from the Agencia Española de la Seguridad Alimentaria y Nutrición, Spain, requesting the evaluation of kaolin, with the CAS number 1332-58-7 and FCM Substance No 410 which is used in a combination with polyacrylic acid, sodium salt, with the CAS number 9003-04-7 and FCM Substance No 70.

TERMS OF REFERENCE AS PROVIDED BY THE LEGISLATION

According to Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food EFSA is asked to carry out an assessment of the risks related to the intended use of the substances and to deliver a scientific opinion.

⁴ Commission Regulation (EC) No 1935/2004 of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. OJ C 117, 30.4.2004, p. 1.

ASSESSMENT

1. Introduction

The European Food Safety Authority was asked by the Agencia Española de la Seguridad Alimentaria y Nutrición, Spain, to evaluate the safety kaolin, with the CAS number 1332-58-7 and FCM Substance No 410, used in a combination with polyacrylic acid, sodium salt, with the CAS number 9003-04-7 and FCM Substance No 70. The request has been registered in the EFSA's register of questions under the number EFSA-Q-2013-00023. The dossier was submitted by the applicant, NanoBioMatters Industries, S.L., Spain.

2. General information

According to the applicant, kaolin (99.5 %) in a combination with polyacrylic acid, sodium salt (0.5 %) intended to be incorporated in EVOH copolymer in order to enhance its gas barrier properties. The EVOH is used as inner layer in a multi-layer, not in direct contact with the food, and contains typically 4 % of the mixture, but depending on the application the concentration can range from 1 % to 12 %.

The multi-layers are intended to be used in contact with beverages (alcoholic and non-alcoholic), sauces and dressings, sausages and wet and dry pet food, at room temperature up to 6 months.

The substances are listed in Regulation (EU) No 10/2011⁵:

- Kaolin is authorised as additive for plastic materials and articles in contact with foods (FCM Substance No 410), with no specific restriction;
- Polyacrylic acid, sodium salt, is authorised as additive for plastic materials and articles in contact with foods (FCM Substance No 70), with a specific restriction of 6 mg/kg, expressed as acrylic acid.

3. Data available in the dossier used for this evaluation

The studies submitted for evaluation followed the SCF guidelines for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation (EC, 2001).

Non-toxicity data:

- Data on identity
- Data on physical and chemical properties
- Data on intended use and authorisation
- Data on migration of the substance
- Data on residual content of the substance
- Data on worst-case migration

Toxicity data:

- None

⁵ Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food OJ L 12. 15.1.2011, p. 1-89.

4. Evaluation

4.1. Non-toxicological data

Kaolin (99.5 %) in combination with polyacrylic acid, sodium salt (0.5 %) is intended to be incorporated in EVOH copolymer in order to enhance its gas barrier properties by creating a tortuous path to gases diffusing through the polymer. According to the applicant the incorporation is performed by conventional polymer processing techniques, in a concentration up to 12 %.

Polyacrylic acid, sodium salt is used as an agent to disperse the kaolin in the EVOH. Particle size distribution of kaolin in an aqueous dispersion showed values larger than 0.92 μm (90 %) and larger 2.17 μm (50 %). The specific surface area was 3.2 m^2/g . Morphological studies show stacks of clay platelets of different sizes dispersed in the EVOH. These particles have shown strong interfacial adhesion to the polymer. Some of these particles when dispersed in the polymer are below 100 nm in the thickness dimension. Nevertheless, exfoliation into individual platelets does not occur for kaolin as confirmed by WAXS (Wide-angle X-ray scattering).

The EVOH containing the additive is used as intermediate layer in plastic multi-layers and does not make direct contact with the food. Given the nature of the substances, decomposition or degradation is not expected at the production and use conditions.

Migration tests for aluminium and magnesium from the kaolin were performed in food simulants water, 10 % ethanol and 3 % acetic acid at 40 °C for 10 days and in isooctane at 20 °C for 2 days. A sample with a typical multi-layer structure was used: PE/EVOH + 4 % substance/PE with thickness 40/6/40 μm , with a surface area to volume ratio of 2 dm^2/kg . There was no detectable migration, as values were similar to the blanks. The migration of magnesium was less than 2.2 mg/kg and migration of aluminium was less than 0.07 mg/kg, for the conventional surface area to mass ratio of 6 dm^2/kg food or food simulant.

Results from TEM (Transmission Electron Microscopy) analysis of a sample of material with structure representative of the intended use, composed of PE/EVOH + 4 % substance/PE with thickness 40/6/40 μm indicate no migration of the kaolin from the EVOH layer into the food contact layer and therefore, no transfer to the food is to be expected.

Migration of polyacrylic acid sodium salt, estimated assuming total mass transfer from a 15 μm EVOH sample, containing 4 % of substance, was 0.216 mg/kg foodstuffs, which is well below the SML of 6 mg/kg. This indicates that migration of polyacrylic acid sodium salt at the intended conditions of use does not exceed the SML.

4.2. Toxicological data

The substances in the mixture have already been evaluated from the toxicological point of view and they are authorised in the EU legislation for plastic in contact with food. For the purpose of this evaluation, no further toxicological data are necessary.

The Panel took note of the opinion of the AFC Panel (EFSA, 2008) setting a TWI of 1 mg/kg bw /week for aluminium. Based on a conservative scenario of consumption of 1 kg of food per day containing aluminium migrated at the level of detection of the method used of 0.07 mg/kg food, an adult of 60 kg body weight in one week time would be exposed to 0.008 mg Al/kg bw/ per week, corresponding less than 1 % of the TWI set in 2008.

Concerning magnesium, the Panel noted that magnesium occurs naturally in foods and is an authorised food supplement (Annex I of Regulation (EC) No 1925/2006⁶ and Annex I of Directive 2002/46/EC⁷).

⁶ Regulation (EC) No 1925/2006 of the European Parliament and the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ L404, 30.12.2006, p.26-38.

⁷ Directive 2002/46/EC of the European Parliament and the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements, OJ L 183, 12.7.2002, p.51-63.

In 2001, the SCF established a Tolerable Upper Intake level of 250 mg/day for readily dissociable magnesium salts (SCF, 2001). Therefore, no safety concern is raised at the detected migration level.

CONCLUSIONS

Having considered the above mentioned data, the CEF Panel concluded that the substance kaolin in the form of particles with a thickness less than 100 nm does not raise a safety concern for the consumer when incorporated up to 12 % in EVOH copolymer. This material is used as an inner layer of a plastic multi-layer structure, in contact with foods and beverages stored at room temperature up to 6 months. The EVOH inner layer containing the particles should be used behind a functional barrier that prevents migration of kaolin particles into the food.

DOCUMENTATION PROVIDED TO EFSA

1. O2Block®4x4 C1.33. November 2012. Submitted by the applicant NanoBioMatters Industries, S.L., Spain

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SCF (Scientific Committee for Food), 2001. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Magnesium, 16pp.
http://ec.europa.eu/food/fs/sc/scf/out105_en.pdf

ABBREVIATIONS

AFC	Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials
bw	body weight
CAS	Chemical Abstracts Service
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EVOH	Ethylene-vinyl alcohol
FCM	Food Contact Materials
PE	Polyethylene
SCF	Scientific committee on food
SML	Specific Migration Limit
TEM	Transmission Electron Microscopy
TWI	Tolerable Weekly Intake
WAXS	Wide-angle X-ray scattering

SCIENTIFIC OPINION

Scientific Opinion on safety assessment of the active substance, iron (0) modified kaolinite as oxygen absorber, for use in active food contact materials¹

EFSA Panel on Food Contact Materials, Enzymes,
Flavourings and Processing aids (CEF)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion of EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids deals with the safety assessment of the iron (0) modified kaolinite, intended to be incorporated in monolayer or multilayer packages or in sachets for absorbing oxygen from the food environment. All starting substances have been evaluated and approved for use as additives in plastic or as food additives. Based on migration results, specific migration limit of iron is not expected to be exceeded under the intended conditions of use. Migration of boron was higher than the total specific migration limit of 6 mg/kg but in the range of the tolerable upper intake level (UL) of 10 mg boron/person per day set by the EFSA NDA Panel in 2004. Migration of aluminium is expected to occur at levels corresponding to less than 1 % of the TWI set in 2008. Therefore the CEF Panel concluded that the use of iron (0) modified kaolinite does not raise a safety concern when used as oxygen absorber incorporated without compatibilisers in polyolefin articles used for long term of storage at room temperature or refrigeration i) at level up to 3 % w/w incorporated in polyolefin layer in direct contact with all types of food including beverages, or ii) at higher levels, when the food is separated from the active material by a layer of polyolefin that does not contain the oxygen absorber formulation and offering a barrier to diffusion against inorganic species at least equivalent to 10 µm polypropylene. The substance equally does not raise a safety concern when it is used in sachets, placed in the headspace of the packaging, that prevent the physical release of their contents into the food and are not in direct contact with liquid foods, exudates, or foods with external aqueous liquid phase.

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¹ On request from the Agencia Española de Seguridad Alimentaria y Nutrición, Spain, Question No EFSA-Q-2011-00192, adopted on 27 September 2013.

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KEY WORDS

Iron (0) modified kaolinite; Boron; Aluminium; Food Contact Materials; Active and intelligent materials; Safety assessment; Evaluation

SUMMARY

According to the Commission Regulation (EC) No 450/2009⁴ of the Commission of European Communities of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food, substances responsible for the active or intelligent function need first to be evaluated by the EFSA before their inclusion into a positive Community list. The procedure of the evaluation and the tasks of EFSA are described in the Regulation (EC) No. 1935/2004⁵ of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.

In the context of this evaluation procedure, following a request from the Agencia Española de Seguridad Alimentaria y Nutrición, Spain, the EFSA Panel on Food Contact Materials, Enzymes and Processing aids (CEF) was asked to deliver an opinion on the safety of iron (0) modified kaolinite (FCM Substance No 01037), made from the starting substances iron (III) chloride anhydrous (CAS No 7705-08-0), kaolinite (CAS No 1332-58-7, FCM Substance No 410) and sodium borohydride (CAS No 16940-66-2, FCM substance No 981), for use as oxygen absorber. The substance is intended to be incorporated in monolayer or multilayer packages or in sachets for absorbing oxygen from the food environment. The dossier was submitted by the applicant, NanoBioMatters Industries, S.L., Spain.

The main active ingredient of the oxygen absorber system is iron which reacts with oxygen in the presence of water. The oxygen absorber is intended to be incorporated at level up to 3 % w/w in a polyolefin in contact with any type of foods for long term of storage at room temperature or below. It may also be used in sachets made of HDPE, which are intended to be placed in the headspace of the primary packaging or in contact with solid foodstuffs, but should not be in contact with liquid foods, or foods with external aqueous liquid phase.

All starting substances have been evaluated and approved for use as additives in plastic food contact materials or as food additives. The iron (0) modified bentonite is intended and expected to be present in the final article as non-nanoform.

From a toxicological point of view, migration of iron (incorporated), aluminium from kaolinite (naturally present) and boron produced in the modification process of the natural clay, is of interest.

Based on migrations tests carried out with HDPE layer in direct contact with food simulants, it is expected that migration of iron into foods is lower than the SML value of 48 mg/kg, set in Regulation EU No 10/2011, if the oxygen absorber is used under the intended conditions of use. The Panel noted that the SML of iron is based on the PMTDI of 0.8 mg/kg bw established by the Joint FAO/WHO Expert Committee on Food Additives in 1983 and agreed by the SCF in 1990. However in 2004, the EFSA NDA Panel concluded that the data available are insufficient to establish a tolerable upper intake level for iron.

Migration of boron from an HDPE layer using the maximum amount of active substance foreseen (3 % w/w) was up to 10 mg/kg food when using the 10 % ethanol. This value is higher than the SMLT of 6 mg/kg (expressed as boron) set in the Regulation (EU) No 10/2011⁶. The Panel noted that any use of the active substance would have to comply with the SMLT. It also noted that this migration value is in the range of the tolerable upper intake level (UL) of 10 mg boron/person per day set by the EFSA NDA Panel in 2004.

⁴ Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. OJ L 135, 30.5.2009, p. 3–11

⁵ Regulation (EC) No 1935/2004 of the European parliament and of the council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. OJ L 338, 13.11.2004, p. 4–17

⁶ Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food OJ L 12. 15.1.2011, p. 1-89

Under the intended conditions of use, migration of aluminium was estimated to be up to 0.045 mg/kg in 3 % acetic acid solution, corresponding to less than 1 % of the Tolerable Weekly Intake set by the AFC Panel in 2008.

The substance may also be used in sachet, placed in the headspace of the packaging. Such sachets must prevent the physical release of their contents into the food and should not be in direct contact with liquid foods, exudates, or foods with external aqueous liquid phase. Under these conditions of use, migration of iron, boron and aluminium is not expected.

Therefore, the CEF Panel concluded that the use of the substance iron (0) modified kaolinite does not raise a safety concern when used as oxygen absorber incorporated without compatibilisers in polyolefin articles used for long term of storage at room temperature or refrigeration:

- i) At level up to 3 % w/w incorporated in a polyolefin layer in direct contact with all types of food including beverages,
- ii) At higher levels, when the food is separated from the active material by a layer of polyolefin that does not contain the oxygen absorber formulation and offering a barrier to diffusion against inorganic species at least equivalent to 10 µm polypropylene.

The substance equally does not raise a safety concern when it is used in sachets, placed in the headspace of the packaging, that prevent the physical release of their contents into the food and are not in direct contact with liquid foods, exudates, or foods with external aqueous liquid phase.

The Panel noted that the estimated migration of boron from an HDPE layer using 3% active mixture, in direct contact with 10% ethanol exceeded the SML value of 6 mg/kg food set in Regulation (EU) No 10/2011. The Panel stressed that the final article should comply with specific migration limits applicable for plastic materials.

TABLE OF CONTENTS

Abstract	1
Key words	2
Summary	3
Table of contents	5
Background as provided by the Legislation	6
Terms of reference as provided by the Applicant	6
Assessment	7
1. Introduction	7
2. General information.....	7
3. Data available in the dossier used for this evaluation.....	8
4. Evaluation.....	8
4.1. Non-toxicological data.....	8
4.2. Toxicological data.....	9
Documentation provided to EFSA	10
References	10
Glossary and abbreviations	12

BACKGROUND AS PROVIDED BY THE LEGISLATION

Regulation (EC) No 450/2009 of the Commission of European Communities is a specific measure that lays down specific rules for active and intelligent materials and articles intended for contact with foodstuffs in addition to the general requirements established in Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food. Active materials and articles are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food.

The substance(s) responsible for the active and/or intelligent function of the material should be included in a positive list by the Commission following a safety evaluation by EFSA according to the procedure described in the abovementioned regulations.

According to this procedure the industry submits applications to the Member States competent Authorities which in their turn transmit the applications to EFSA for evaluation. The application is supported by a technical dossier submitted by the industry following the EFSA guidelines on “submission of a dossier for safety evaluation by EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food” (EFSA, 2009).

In this context, EFSA received an application from the Agencia Española de Seguridad Alimentaria y Nutrición, Spain, requesting the evaluation of the substance iron (0) modified kaolinite for use as oxygen scavenger.

TERMS OF REFERENCE AS PROVIDED BY THE APPLICANT

EFSA is required to carry out a risk assessment on the risks originating from the migration into food of the substance iron (II) modified kaolinite, made from the starting substances iron (III) chloride anhydrous, kaolinite and sodium borohydride, used in oxygen absorbing components in food contact materials, and deliver a scientific opinion, according to the Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food.

The opinion of EFSA will be considered by the Commission for adoption of a Community list of authorised substances where according to the Regulation (EC) No 450/2009 there will be specified:

- (a) the identity of the substance(s);
- (b) the function of the substance(s);
- (c) the reference number;
- (d) if necessary, the conditions of use of the substance(s) or component;
- (e) if necessary, restrictions and/or specifications of use of the substance(s);
- (f) if necessary, conditions of use of the material or article to which the substance or component is added or into which it is incorporated.

ASSESSMENT

1. Introduction

The European Food Safety Authority was asked by the Agencia Española de Seguridad Alimentaria y Nutrición to evaluate the safety of the oxygen scavenger iron (0) modified kaolinite (FCM Substance No 01037), made from the starting substances iron (III) chloride anhydrous (CAS No 7705-08-0), kaolinite (CAS No 1332-58-7, FCM Substance No 410) and sodium borohydride (CAS No 16940-66-2, FCM Substance No 981). The request has been registered in the EFSA's register of received questions under the number EFSA-Q-2011-000192. The dossier was submitted by the applicant, NanoBioMatters Industries S.p.a.

2. General information

According to the applicant, the active oxygen absorber is iron (0) modified kaolinite. The natural kaolinite clay is surface modified by cation exchange between sodium and added iron which has oxygen scavenging properties. The active substance is intended to be incorporated up to 3 % w/w in a polyolefin layer in direct or indirect contact with any type of foods including beverages under conditions of long term of storage at room temperature or refrigeration. It may also be used in sachets made of HDPE, placed in the headspace of the primary packaging or in contact with solid foodstuffs, but should not be in contact with liquid foods (i.e. dressings, soups, beverages) or foods with external aqueous liquid phase (i.e. sliced fruits, fresh meat and poultry).

The iron (0) modified kaolinite has not been evaluated by EFSA in the past. However, non-modified kaolinite and iron powder are listed in Regulation (EU) 10/2011. The by-products formed during the process are boric acid and sodium chloride.

- Kaolinite is authorised as additive for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with no specific restriction (FCM Substance No 410).
- Iron powder is authorised as additive for plastic materials and articles in contact with foods with a specific restriction of 48 mg iron/kg food based on a Provisional Maximum TDI (PMTDI) of 0.8 /kg bw set by JECFA/WHO (1983) and agreed by the SCF (1990) (FCM Substance No 983).

Iron (II) has also been evaluated for use in oxygen scavenger mixtures incorporated in polyolefin layers (EFSA CEF Panel, 2012a; EFSA CEF Panel, 2013). The CEF Panel concluded that the active substances do not raise a safety concern a) when incorporated without compatibilisers in polyolefin layers of food packages up to 15 % w/w (EFSA CEF Panel, 2012 a and b) when incorporated in polyethylene and in polypropylene articles used for long time storage and/or hot fill up to 95 °C for several minutes in i) direct contact with dry and fatty foods and ii) indirect contact with aqueous or acidic foods, separated from the active material by a layer of at least 10 µm polyethylene or polypropylene that does not contain the oxygen absorber formulation (EFSA CEF Panel, 2013).

- Boric acid is authorised as a monomer and additive for plastic materials and articles in contact with foods (Regulation (EU) No 10/2011) with a combined specific migration limit (SML) with other substances containing boron of 6mg/kg (expressed as boron) (FCM Substance No 584). Sodium borohydride was also evaluated as active FCM substance (EFSA CEF Panel, 2012b). In that case the CEF Panel concluded that there is no safety concern for the consumer when the sodium borohydride is only used behind a plastic layer to prevent direct contact with the packaged food.
- Sodium chloride is covered by the sodium salt of hydrochloric acid authorised as an additive for plastic materials and articles in contact with foods, with no specific restriction (FCM Substance No 507). In addition, sodium chloride (common salt) may be used as an ingredient in the preparation of food (Regulation (EC) No 1925/2006). Sodium chloride has also been

evaluated for use in oxygen scavenger mixtures incorporated in polyolefin layers (EFSA CEF Panel, 2012a; EFSA CEF Panel, 2013) with the same conclusion as quoted above for iron.

3. Data available in the dossier used for this evaluation

The studies submitted for evaluation followed the EFSA guidelines on submission of a dossier for safety evaluation by EFSA of active or intelligent substances present in active and intelligent materials and articles intended to come into contact with food (EFSA, 2009).

Non-toxicity data:

- Identity of the active system
- Physical/chemical characteristics of the active system, including particle size distribution and morphology studies
- Manufacturing process of the active system
- Intended use of the active system
- Existing authorisations
- Migration data (specific migration of iron, aluminium and boron, and overall migration).

Toxicity data:

- None

4. Evaluation

4.1. Non-toxicological data

The active substance is iron (0) modified kaolinite, produced from the starting substances iron (III) chloride anhydrous (46 %), kaolinite (29 %), sodium borohydride (26 %) and water as solvent. Aluminium is naturally present in kaolinite clay. The natural clay is surface modified with the attachment of iron which has oxygen scavenging properties and the process for kaolinite modification is a physical blending with iron (III), following reduction to iron (0) at a maximum temperature of 80 °C. In the process, boric acid and sodium chloride are formed. The soluble inorganic products are mainly washed off with the process water. However analysis by ICP-MS showed that final clay contained approx. 5 % total boron.

Iron (0) modified kaolinite is incorporated in a polyolefin layer intended for direct or indirect (multilayer) contact with any type of foods. The incorporation of modified iron (0) kaolinite into the passive polyolefin matrix involves physical blending and moulding by conventional plastics processing operations, at maximum temperature of 250 °C. Decomposition or degradation of the active substance is not expected at the production and use conditions. It may also be used in sachets made of HDPE. The iron (0) modified kaolinite is insoluble in water and organic solvents.

Particle size distribution of iron (0) modified kaolinite showed values in the micron range: higher than 3 µm for more than 90 % of the particles and with an average particle size (50th percentile) between 10 and 20 µm. The specific surface area is lower than 1 m²/g. Morphology studies of the clay incorporated in passive material do not indicate exfoliation. Given the non-polar nature of the polyolefin and the processing conditions without compatibilizer addition, the iron (0) modified kaolinite is intended and expected to be present in the final article as non-nanoform. Analyses of simulants from the migration experiments, targeting the evaluation of nanoparticles, gave negative results. However, the formation of nanoparticles due to exfoliation cannot be excluded if the substance were to be incorporated in non-polar polymers with compatibilisers, or in polar polymers without such additives.

Specific migration of iron, boron and aluminium were measured in a HDPE layer containing 10 % iron (0) modified kaolinite in direct contact with the food simulants. Long term contact at room temperature was simulated by testing with water, acetic acid 3 % and ethanol 10 % for 10 days at 40 °C. Migration test was also conducted with isooctane for 2 days at 20 °C. Migration of iron, boron and aluminium was found to be up to 17.9 mg/kg, 9.3 mg/kg and 0.15 mg/kg in 3 % acetic acid, respectively. Migration of boron into 10% ethanol was 34 mg/kg.

Using the maximum amount of active substance foreseen (3 % w/w), maximum migration of iron, boron and aluminium from an HDPE layer in direct contact with acidic foods can be estimated to be lower than the measured one by a factor of 3.33, i.e. 5.4 mg/kg, 2.8 mg/kg and 0.045 mg/kg foodstuffs, respectively. Migration of boron into ethanol 10 % can be estimated to be 10.2 mg/kg.

When the layer containing the active formulation is used in multilayer food contact materials, i.e. indirect contact with foods, no significant migration of any of the inorganic active components is likely to occur through a polyolefin barrier layer and the specific migration limits for iron and boron are not expected to be exceeded.

The overall migration limit tested in the same conditions as used for specific migrations testing was up to 15 mg/dm² food into 3 % acetic acid. When used at levels up to 3 % w/w into polyolefin layer in direct contact with acidic foods, the overall migration of 10 mg/dm² food set for plastics is not expected to be exceeded.

The substance may also be used in sachet, placed in the headspace of the packaging. Such sachets must prevent the physical release of their contents into the food and should not be in direct contact with liquid foods, exudates, or foods with external aqueous liquid phase. Under these conditions of use, migration of iron, boron and aluminium is not expected.

4.2. Toxicological data

The substances used for the manufacture of the iron (0) modified kaolinite, iron (III) chloride, sodium borohydride and kaolinite, have been evaluated and approved for use as additives in plastic food contact materials or as food additives. Boric acid and sodium chloride are formed in the process as by-products. Iron and sodium chloride have also been evaluated for use in oxygen scavenger mixtures incorporated in polyolefin layers.

From a toxicological point of view, migration of iron (incorporated), aluminium (naturally present) from kaolinite and boron (used in the process) is of interest.

From the migration results, it is expected that migration of iron into foods is lower than the SML value of 48 mg/kg, set in Regulation EU No 10/2011, if the oxygen absorber is used under the intended conditions of use. The Panel noted that the SML of iron is based on the PMTDI of 0.8 mg/kg bw established by the Joint FAO/WHO Expert Committee on Food Additives (WHO, 1983) and the SCF (1990). However in 2004, the EFSA NDA Panel concluded that the data available are insufficient to establish a tolerable upper intake level for iron (EFSA, 2004).

The migration of boron from an HDPE layer using the maximum amount of active substance foreseen (3 % w/w) was up to 10 mg/kg food when using the 10 % ethanol. This value is higher than the SML established for substances containing boron (boric acid, barium tetraborate, sodium tetraborate and boron nitride) which are authorised additives and listed in the Regulation (EU) No 10/2011, with a combined specific migration limit (SML) of 6 mg/kg (expressed as boron). The Panel noted that any use of the active substance would have to comply with this SML value. Nevertheless, based on results from repeated dose studies on reproduction and development and on boron toxicokinetic, the CEF Panel agreed in 2012 with the tolerable upper intake level (UL) of 10 mg boron/person per day which was set by the EFSA NDA Panel (EFSA, 2004) (EFSA CEF Panel, 2012b). Considering the various sources of exposure to boron, other than food contact materials, such as foodstuffs, mineral water, food supplements and a wide range of consumer products, e.g. medicinal products, cosmetics, toys,

detergents and adhesives (EFSA, 2004 and 2005; ECHA, 2008), the Commission may wish to take note of these other sources of exposure if setting a restriction for boron.

Migration of aluminium can be estimated to be up to 0.045 mg/kg in 3 % acetic acid solution. The Panel took note of the opinion of the AFC Panel (EFSA, 2008) setting a TWI of 1 mg/kg bw/week for aluminium. Based on a conservative scenario of consumption of 1 kg of acidic food per day containing aluminium migrated at the level of 0.045 mg/kg, an adult of 60 kg bodyweight in one week time would be exposed to 0.00525 mg Al/kg bw/week corresponding to less than 1 % of the TWI set in 2008.

Therefore, the Panel considered that under the intended conditions of use, the oxygen absorber is toxicologically acceptable.

CONCLUSIONS

The CEF Panel concluded that the use of the substance iron (0) modified kaolinite does not raise a safety concern when used as oxygen absorber incorporated without compatibilisers in polyolefin articles used for long term of storage at room temperature or refrigeration:

- i) At level up to 3 % w/w incorporated in polyolefin layer in direct contact with all types of food including beverages,
- ii) At higher levels, when the food is separated from the active material by a layer of polyolefin that does not contain the oxygen absorber formulation and offering a barrier to diffusion against inorganic species at least equivalent to 10 µm polypropylene.

The substance equally does not raise a safety concern when it is used in sachets, placed in the headspace of the packaging, that prevent the physical release of their contents into the food and are not in direct contact with liquid foods, exudates, or foods with external aqueous liquid phase.

The Panel noted that the estimated migration of boron from an HDPE layer using 3% active mixture, in direct contact with 10 % ethanol exceeded the SML value of 6 mg/kg food set in Regulation EU No 10/2011. The Panel stressed that the final article should comply with specific migration limits applicable for plastic materials.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier referenced: iron based oxygen scavenger, dated: 8 February 2011. Submitted by NanoBioMatters Industries, S.L.

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- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013. Scientific Opinion on the safety evaluation of the active substances, iron, polyethyleneglycol, disodium pyrophosphate, monosodium phosphate and sodium chloride for use in food contact materials. EFSA Journal 2013;11(6):3245
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1983. Evaluation of certain additives and contaminants. 27th report. WHO Techn. Report Series, No 696.52pp
- SCF (Scientific Committee of Food), 1990, First series of food additives of various technological functions, Report 25th Series http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_25.pdf.

GLOSSARY AND ABBREVIATIONS

Overall migration: The sum of the amounts of volatile and non volatile substances, except water, released from a food contact material or article into food or food simulant

Specific migration: The amount of a specific substance released from a food contact material or article into food or food stimulant

AFC	Food additives, Flavourings, processing aids and materials in Contact with food
bw	body weight
CAS No	Chemical Abstract Service Number
CEF	Food Contact Materials Enzymes, Flavourings and Processing Aids
EC	European Commission
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
FCM	Food Contact Materials
HDPE	High Density Polyethylene
JECFA	The Joint FAO/WHO Committee on Food Additives
ICP-MS	Inductively coupled plasma-mass spectroscopy
NDA	Dietetic Products, Nutrition and Allergies
PMTDI	Provisional Maximum Tolerable Daily Intake
SCF	Scientific committee on food
SML(T)	Specific Migration Limit (temporary)
TDI	Tolarable daily Intake
TWI	Tolerable Weekly Intake
WHO	World Health Organization
w/w	Weight by weight

Safety Assessment of Silicates as Used in Cosmetics

Status: Re-Review for Panel Review
Release Date: May 23, 2018
Panel Meeting Date: June 4-5, 2018

The 2018 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett, Senior Scientific Analyst/Writer
Date: May 23, 2018
Subject: Silicate-Related Ingredients Re-Review

Enclosed is the Re-Review of the Safety Assessment of Silicate-Related Ingredients as Used in Cosmetics. (It is identified as *silica06208rep* in the pdf document).

The CIR Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite was published in 2003. Based on the available data at the time, the Panel concluded that the 17 silicates named in that report are safe for use in cosmetic products. Because it has been 15+ years since this document was published, it is time for a re-review.

In addition to the ingredients that were included in the 2003 review, there are 16 possible "add-ons" that have not yet been looked at by CIR. Further, the related safety assessment on Potassium Silicate, Sodium Metasilicate, and Sodium Silicate was published in 2005. These would be additional ingredients for consideration for inclusion: a re-review of this safety assessment would be due soon. The Panel may also consider adding the ingredients from the 2009 safety assessment on Silica and Related Cosmetic Ingredient. This safety assessment was never published in the *International Journal of Toxicology* and will be due for a re-review in approximately 6 years.

In a cursory review of the literature available on PubMed and SciFinder, minimal new pertinent data was found on the original ingredients and those reviewed in 2005 and 2009. There were no pertinent data on the proposed add-on ingredients that have not been previously reviewed. Because of time constraints, a partial review of certain databases, such as ECHA, was performed, but a full review would be conducted if the Panel decides to re-open the original silicates report to include the proposed add-on ingredients. Most of the data that was included in the re-review did not seem to indicate any new toxicological concerns.

According to 2018 VCRP data, Silica has the most reported uses in cosmetic products, with a total of 8024; the majority of the uses are in leave-on eye makeup preparations and makeup preparations. Kaolin has the second most reported uses in cosmetic products, with a total of 1794; the majority of the uses are also in leave-on eye makeup preparations and makeup preparations. The reported numbers of uses for the remaining ingredients in this report are much lower. The uses for both of these ingredients have greatly increased since the original safety assessments were finalized: in 2009, Silica was reported to have 3276 uses and in 1998, Kaolin was reported to have 509 uses. The results of the concentration of use survey conducted in 2018 by the Council indicate Kaolin has the highest reported maximum concentration of use; it is used at up to 53% in "other" manicuring products and up to 35% in rinse-off "other" skin care preparations (*silica062018data1* and *silica062018data2*). Zeolite is used at up to 37.8% in in paste masks and mud packs and up to 35.7% in hair tonics, dressings and other hair grooming aids. According to the original safety assessments, the maximum use concentration for Kaolin was 100% in leave-on "other" skin care preparations and the maximum use concentration for Hectorite was 100% in rinse-off skin cleansing preparations (the maximum leave-on concentration was 15% in makeup foundations). Silica was reported to be used at up to 44% in eye shadows. Several proposed add-on ingredients have not been surveyed by the Council yet.

The previous reports are attached for your use:

- Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite (2003) [*silica062018origrep*]
- Final Report on the Safety Assessment of Potassium Silicate, Sodium Metasilicate, and Sodium Silicate (2005) [*silica062018_silicates2005*]

- Final Report on the Safety Assessment of Silica and Related Cosmetic Ingredients (2009) [*silica062018_silica2009*]

Minutes from the original deliberations of the original review, and from the other reports named above, have been included:

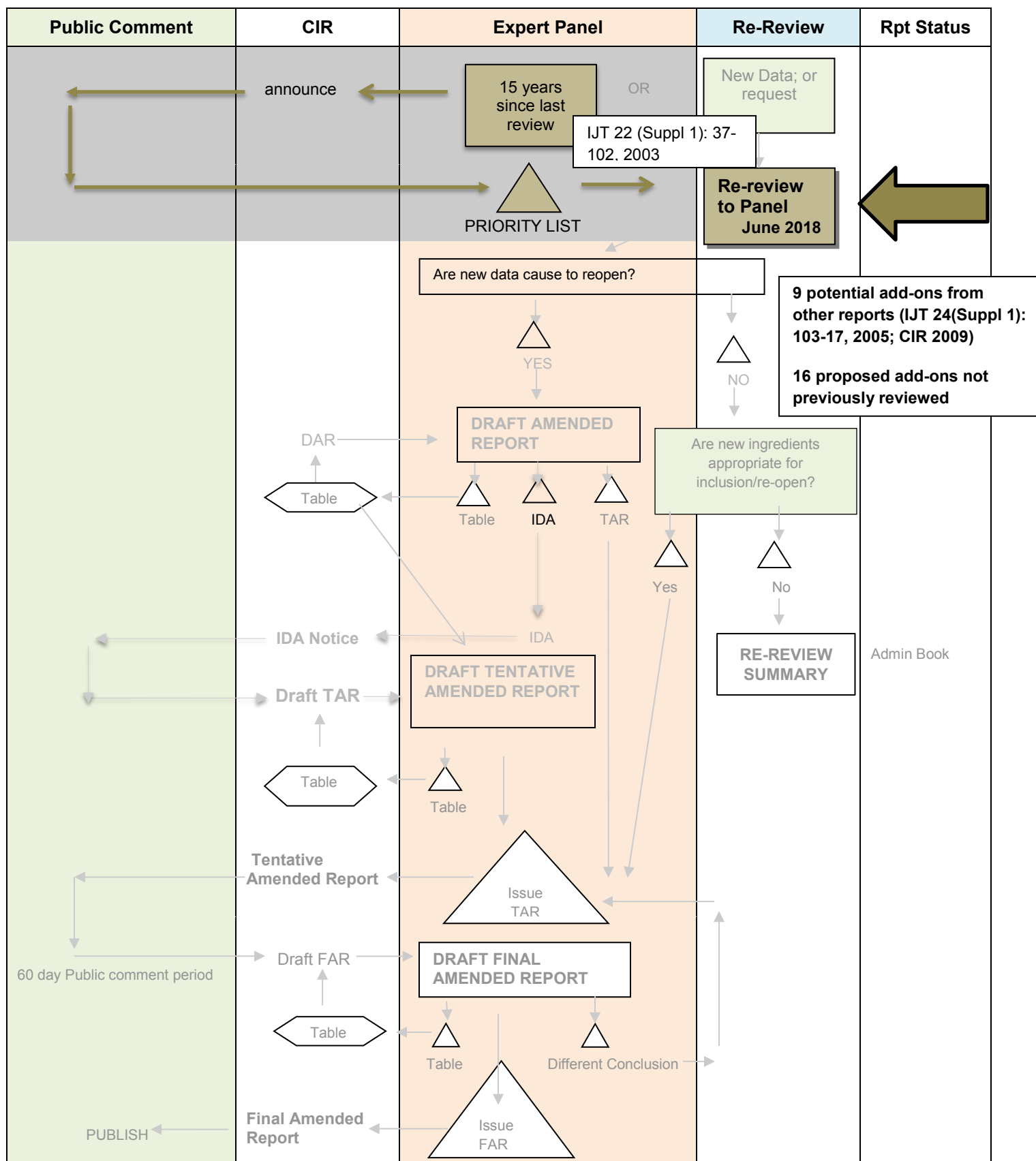
- September 1999 and February 2000 - Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite [*silica062018min1_origsilicates*]
- December 1999, May 2000, December 2000 and June 2001 - Potassium Silicate, Sodium Metasilicate, and Sodium Silicate [*silica062018min2_saltsilicates*]
- June 2009 and September 2009 - Silica and Related Cosmetic Ingredients [*silica062018min3_silica*]

Based on the similarity of the proposed add-on ingredients to those in the original report and on the topics of discussion from the previous deliberations of all of these ingredients, the Panel may want to re-open the original report to include all of the proposed add-on ingredients in one report on Silica and Silicate ingredients.

RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Silicates

MEETING June 2018



*If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

Silicates History

2003– The CIR’s Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller’s Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite in the *IJT* after the report was finalized by the Panel in 2000. Based on the available animal and clinical data available at that time, the Panel concluded that these ingredients are safe as cosmetic ingredients in the practices of use and concentrations as described in the safety assessment.

2005 – The CIR’s Final Report on the Safety Assessment of Potassium Silicate, Sodium Metasilicate, and Sodium Silicate in the *IJT* after the report was finalized by the Panel in 2001. Based on the available animal and clinical data available at that time, the Panel concluded that these ingredients are safe for use in cosmetic products in the practices of use and concentration described in the safety assessment when formulated to avoid irritation.

2009 – The CIR issued a Final Report on the Safety Assessment of Silica and Related Cosmetic Ingredients, which has not been published in the *IJT*. Based on the available animal and clinical data available at that time, the Panel concluded that Silica, Alumina Magnesium Metasilicate (now called Magnesium Aluminometasilicate), Aluminum Calcium Sodium Silicate, Aluminum Iron Silicates, Hydrated Silica, and Sodium Potassium Aluminum Silicate are safe as cosmetic ingredients in the practices of use and concentrations as described in the safety assessment.

April/May 2018 – Review of the available published literature since 2000 was conducted in accordance to CIR Procedure regarding re-review of ingredients after ~15 years.

[illegible]

Silica and Silicates Data Profile –June 2018 – Writer, Christina Burnett

[illegible]

Silica and Silicates Data Profile –June 2018 – Writer, Christina Burnett

	In-Use	Physical/Chemical Properties	Method of Manufacturing	Composition/Impurities	Acute Toxicity	Repeated Dose Toxicity	Genotoxicity	Reproductive and Developmental Toxicity	Carcinogenicity	Other Relevant Toxicity Studies	Irritation/Sensitization - Nonhuman	Irritation/Sensitization - Human	Ocular/Mucosal	Phototoxicity	Clinical Studies/Case Reports	Toxicokinetics
Sodium Potassium Aluminum Silicate	X															
Re-Review – New Add-on Ingredients																
Activated Clay																
Aluminum Calcium Magnesium Potassium Sodium Zinc Silicates																
Aluminum Iron Calcium Magnesium Germanium Silicates																
Aluminum Iron Calcium Magnesium Zirconium Silicates																
Ammonium Silver Zeolite																
Ammonium Silver Zinc Aluminum Silicate	X															
Calcium Magnesium Silicate																
Gold Zeolite																
Silver Copper Zeolite																
Silver Zinc Zeolite																
Sodium Magnesium Aluminum Silicate		X			X											
Sodium Silver Aluminum Silicate																
Titanium Zeolite																
Tromethamine Magnesium Aluminum Silicate																
Zinc Silicate		X					X				X		X			
Zinc Zeolite	X															

“X” indicates that data were available in the category for that ingredient.

Silicates

Ingredient	CAS #	InfoB	SciFin	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Re-Review Ingredients																				
Aluminum Silicate	1327-36-2	√	√	√		CFRs	√	√		√			√		√					
Aluminum Calcium Sodium Silicate	1344-01-1	√	√	√			√	√					√							
Aluminum Iron Silicates		√	√	√			√	√					√							
Attapulgite	12174-11-7; 1337-76-4	√	√	√	IARC 1987	CFRs	√	√		√			√		√					
Bentonite	1302-78-9	√	√	√		CFRs	√	√		√			√		√					
Calcium Silicate	1344-95-2	√	√	√		CFRs	√	TBC		YES			√		√					
Fuller's Earth	8031-18-3	√	√	√			√	√		√			√		√					
Hectorite	12173-47-6; 68084-71-9	√	√	√		CFRs	√	√		√			√		√					
Hydrated Silica	10279-57-9; 112926-00-8; 1343-98-2; 63231-67-4; 7631-86-9	√	√	√		CFRs	√						√							
Kaolin	1332-58-7	√	√	√		CFRs OTC	√	√		√			√		√					
Lithium Magnesium Silicate	37220-90-9	√	√	√			√	√		√			√		√					
Lithium Magnesium Sodium Silicate	53320-86-8	√	√	√			√	TBC		√			√		√					
Magnesium Aluminometasilicate	12408-47-8	√	√	√			√						√							
Magnesium Aluminum Silicate	12199-37-0; 12511-31-8	√	√	√		CFRs OTC	√	TBC		√			√		√					
Magnesium Silicate	1343-88-0	√	√	√		CFRs	√	TBC		√			√		√					
Magnesium Trisilicate	14987-04-3	√	√	√		CFRs OTC	√	√		√			√		√					
Montmorillonite	1318-93-0	√	√	√			√	√		√			√		√					

Ingredient	CAS #	InfoB	SciFin	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Pyrophyllite	11349-10-3; 113349-11-4; 113349-12-5; 12269-78-2; 141040-73-5; 141040-74-6	√	√	√		CFRs	√	√		√			√		√					
Silica	112945-52-5; 60676-86-0; 7631-86-9	√	√	√	IARC?	CFRs	√	TBC					√							
Sodium Magnesium Silicate		√	√	√			√	√		√			√		√					
Sodium Potassium Aluminum Silicate	12736-96-8; 66402-68-4	√	√	√			√	TBC					√I							
Zeolite	1318-02-1	√	√	√	IARC 1997		√	TBC		YES HERA			√		√					
Zirconium Silicate	10101-52-7; 1344-21-4	√	√	√		CFRs	√	√		√			√		√					
Sodium Silicate	1344-09-8	√	√	√		CFRs	√	√		YES			√		√					
Sodium Metasilicate	6834-92-0	√	√	√		CFRs	√	√		YES			√		YES					
Potassium Silicate	1312-76-1	√	√	√		CFRs	√	√		YES			√		√					
Potential Add-ons																				
Sodium Silver Aluminum Silicate		√	√	√			√	√		√			√		√					
Tromethamine Magnesium Aluminum Silicate		√	√	√			√	√		√			√		√					
Activated Clay		√	√	√			√	√		√			√		√					
Ammonium Silver Zinc Aluminum Silicate		√	√	√			√	√		√			√		√					
Ammonium Silver Zeolite		√	√	√			√	√					√							
Aluminum Calcium Magnesium Potassium Sodium Zinc Silicates		√	√	√			√	√		√			√		√					

Ingredient	CAS #	InfoB	SciFin	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Aluminum Iron Calcium Magnesium Germanium Silicates		√	√	√			√	√		√			√		√					
Aluminum Iron Calcium Magnesium Zirconium Silicates		√	√	√			√	√		√			√		√					
Calcium Magnesium Silicate	12765-06-9	√	√	√			√	√					√							
Silver Copper Zeolite	130328-19-7; 168042-42-0	√	√	√	IARC?		√	√					√							
Silver Zinc Zeolite	under development	under development	√	√	IARC?		√	√					√							
Sodium Magnesium Aluminum Silicate	12040-43-6	√	√	√			√	√		√			√		√					
Zinc Silicate	13597-65-4	√	√	√			√	√		√			√		√					
Gold Zeolite		√	√	√			√	√		√			√		√					
Zinc Zeolite		√	√	√			√	√		√			√		√					
Titanium Zeolite		√	√	√	IARC?		√	√					√							

TBC = To be completed upon re-opening

Typical Search Terms

- INCI names
- CAS numbers
- chemical/technical names
- additional terms will be used as appropriate

Total references ordered/downloaded from initial searches = 45 (some relevant hits were duplicates)

Search Strategy: Re-review ingredients limited time frame from 2000-2018, except where noted

PubMed

Re-review ingredients

Aluminum Silicate – 11825 hits, limited with toxicity = 770 hits, limited with irritation = 14 hits (4 relevant), limited with sensitization = 9 (0 relevant), limited with dermal = 20 hits (5 relevant)

Aluminum Calcium Sodium Silicate – 6 hits (0 relevant)

Aluminum Iron Silicates - 281 hits, limited with toxicity = 27 hits, limited with irritation = 0 hits, limited with sensitization = 0 hits, limited with dermal = 0 hits

Attapulgit – 231 hits, limited with toxicity = 13 hits (3 relevant), limited with irritation = 0 hits, limited with sensitization = 0 hits, limited with dermal = 0 hits

Bentonite – 2155 hits, limited with toxicity = 161 hits, limited with irritation = 3 hits (1 relevant), limited with sensitization = 0 hits, limited with dermal = 7 hits (1 relevant)

Calcium Silicate – 1181 hits, limited with toxicity = 79 hits, limited with irritation = 0 hits, limited with sensitization = 0 hits, limited with dermal = 1 hit (0 relevant)

Fuller's Earth – 50 hits (12 relevant)

Hectorite – 89 hits (0 relevant)

Hydrated Silica (limited to 2009-2018) – 12440 hits, limited with toxicity = 1764 hits, limited with irritation = 14 hits (4 relevant), limited with sensitization = 9 hits (0 relevant), limited with dermal = 28 hits (6 relevant)

Kaolin – 1964 hits, limited with toxicity = 160 hits (4 relevant), limited with irritation = 2 hits (0 relevant), limited with sensitization = 17 hits (0 relevant), limited with dermal = 1 hit (0 relevant)

Lithium Magnesium Silicate – 3 hits (0 relevant)

Lithium Magnesium Sodium Silicate – 2 hits (1 relevant)

Magnesium Aluminometasilicate – 24 hits (0 relevant)

Magnesium Aluminum Silicate – 80 hits (1 relevant)

Magnesium Silicate – 776 hits, limited with toxicity = 31 hits (3 relevant), limited with irritation (0 relevant), limited with sensitization = 1 hit (0 relevant), limited with dermal = 1 hit (0 relevant)

Magnesium Trisilicate – 198 hits (2 relevant)

Montmorillonite – 3385 hits, limited with toxicity = 214 hits (8 relevant), limited with irritation = 6 hits (1 relevant), limited with sensitization = 0 hits, limited with dermal = 8 hits (1 relevant)

Pyrophyllite – 946 hits, limited with toxicity = 60 hits (0 relevant), limited with irritation = 3 hits (0 relevant), limited with sensitization = 0 hits, limited with dermal = 0 hits

Silica (limited to 2009-2018) – 47,440 hits, limited with toxicity = 3342 hits, limited with irritation = 30 hits (2 relevant), limited with sensitization = 41 hits (0 relevant), limited with dermal = 84 hits (3 relevant)

Sodium Magnesium Silicate – 66 hits (1 relevant)

Sodium Potassium Aluminum Silicate – 8 hits (0 relevant)

Zeolite – 6699 hits, limited with toxicity = 193 hits (1 relevant), limited with irritation = 4 hits (1 relevant), limited with sensitization = 6 hits (0 relevant), limited with dermal = 5 hits (1 relevant)

Zirconium Silicate – 350 hits (0 relevant)

Sodium Silicate – 338 hits (3 relevant)

Sodium Metasilicate – 84 hits (5 relevant)

Potassium Silicate – 912 hits, limited with toxicity = 25 hits (1 relevant), limited with irritation = 4 hits (0 relevant), limited with sensitization = 0 hits, limited with dermal = 0 hits

Add-on ingredients

Sodium Silver Aluminum Silicate - 18 hits (0 relevant)

Tromethamine Magnesium Aluminum Silicate – 0 hits

Activated Clay – 383 hits (0 relevant)

Ammonium Silver Zinc Aluminum Silicate – 1 hit (0 relevant)

Aluminum Calcium Magnesium Potassium Sodium Zinc Silicates - 2 hits (0 relevant)

Aluminum Iron Calcium Magnesium Germanium Silicates – 0 hits

Aluminum Iron Calcium Magnesium Zirconium Silicates – 0 hits

Sodium Magnesium Aluminum Silicate – 19 hits (0 relevant)

Zinc Silicate - 70 hits (0 relevant)

Ammonium Silver Silicate – 4 hits (0 relevant)

Gold Zeolite - 62 hits (0 relevant)

Zinc Zeolite – 222 hits (0 relevant)

SciFinder: Re-review ingredients limited time from from 2000-2018, except where noted, and to Adverse Effects and English

Re-review ingredients

Aluminum Silicate – 10 hits, 0 relevant (CAS#1335-30-4), 25 hits, 0 relevant (CAS#1327-36-2)

Aluminum Calcium Sodium Silicate (from 2005-2018) – 15 hits, 2 relevant

Aluminum Iron Silicates (from 2005-2018)

Attapulgite – 11 hits, 5 relevant

Bentonite – 119 hits, 7 relevant

Calcium Silicate - 5 hits, 1 relevant (CAS# 10034-77-2), 60 hits, 4 relevant (CAS#1344-95-2)

Fuller's Earth – 13 hits, 0 relevant

Hectorite – 4 hits (CAS# 12173-47-6)

Hydrated Silica (from 2005-2018) - 0 hits (CAS#870616-37-8), 0 hits (CAS#68918-35-4), 1 hit, 1 relevant (CAS#112926-00-8), 18 hits, 0 relevant (CAS#63231-67-4), 2 hits, 0 relevant (CAS#10279-57-9), 54 hits, 1 relevant (CAS#1343-98-2)

Kaolin – 132 hits, 1 relevant

Lithium Magnesium Silicate – 0 hits

Lithium Magnesium Sodium Silicate – 1 hit, 1 relevant

Magnesium Aluminometasilicate – 0 hits

Magnesium Aluminum Silicate – 4 hits, 1 relevant (CAS#1327-43-1), 0 hits (CAS#12511-31-8), 20 hits, 0 relevant (CAS#12199-37-0)

Magnesium Silicate – 11 hits, 1 relevant

Magnesium Trisilicate – 7 hits, 1 relevant

Montmorillonite – 47 hits, 7 relevant

Pyrophyllite – 0 hits (CAS#141040-74-6), 0 hits (CAS#141040-73-5), 0 hits (CAS#13349-12-5), 0 hits (CAS#113349-11-4), 0 hits (CAS#113349-10-3), 0 hits (CAS #12269-78-2)

Silica (from 2005-2018) – 3606 hits, further limited by dermal OR irritation OR sensitization OR cosmetic = 6 hits, 0 relevant;

Sodium Magnesium Silicate – 0 hits

Sodium Potassium Aluminum Silicate (from 2005-2018) – 0 hits

Zeolite – 140 hits, 1 relevant

Zirconium Silicate – 5 hits, 0 relevant (CAS#10101-52-7), 5 hits, 0 relevant (CAS#1344-21-4)

Sodium Silicate – 16 hits, 1 relevant

Sodium Metasilicate – 13 hits, 5 relevant

Potassium Silicate – 0 hits (CAS#10006-28-7), 5 hits, 0 relevant (CAS#1312-76-1)

Add-on ingredients

Sodium Silver Aluminum Silicate – 0 hits

Tromethamine Magnesium Aluminum Silicate – 0 hits

Activated Clay- 0 hits

Ammonium Silver Zinc Aluminum Silicate – 0 hits

Aluminum Calcium Magnesium Potassium Sodium Zinc Silicates – 0 hits

Aluminum Iron Calcium Magnesium Germanium Silicates – 0 hits

Aluminum Iron Calcium Magnesium Zirconium Silicates - 0 hits

Ammonium Silver Zeolite – 0 hits

Sodium Magnesium Aluminum Silicate – 0 hits

Zinc Silicate – 0 hits (CAS #127734-84-3), 0 hits (CAS#126755-25-7), 0 hits (CAS#13814-85-2), 1 hit, 0 relevant (CAS#13597-65-4) 0 hits (CAS#11126-29-7)

Ammonium Silver Silcate – 0 hits

Gold Zeolite – 0 hits

Calcium Magnesium Silicate – 1 hit, 0 relevant

Silver Copper Zeolite – 0 hits

Silver Zinc Zeolite – 0 hits

Zinc Zeolite – 0 hits

Titanium Zeolite – 0 hits

LINKS

Search Engines

- Pubmed (- <http://www.ncbi.nlm.nih.gov/pubmed>)
- Toxnet (<https://toxnet.nlm.nih.gov/>); (includes Toxline; HSDB; ChemIDPlus; DART; IRIS; CCRIS; CPDB; GENE-TOX)
- Scifinder (<https://scifinder.cas.org/scifinder>)

appropriate qualifiers are used as necessary

search results are reviewed to identify relevant documents

Pertinent Websites

- wINCI - <http://webdictionary.personalcarecouncil.org>
- FDA databases <http://www.ecfr.gov/cgi-bin/ECFR?page=browse>
- FDA search databases: <http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm>;
- EAFUS: <http://www.accessdata.fda.gov/scripts/fcn/fcnavigation.cfm?rpt=eafuslisting&displayall=true>
- GRAS listing: <http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm>
- SCOGS database: <http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm>
- Indirect Food Additives: <http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives>
- Drug Approvals and Database: <http://www.fda.gov/Drugs/InformationOnDrugs/default.htm>
- <http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf>
- FDA Orange Book: <https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm>
- OTC ingredient list: <https://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135688.pdf>
- (inactive ingredients approved for drugs: <http://www.accessdata.fda.gov/scripts/cder/iig/>)
- HPVIS (EPA High-Production Volume Info Systems) - <https://ofmext.epa.gov/hpvis/HPVISlogin>
- NIOSH (National Institute for Occupational Safety and Health) - <http://www.cdc.gov/niosh/>
- NTIS (National Technical Information Service) - <http://www.ntis.gov/>
- NTP (National Toxicology Program) - <http://ntp.niehs.nih.gov/>
- Office of Dietary Supplements <https://ods.od.nih.gov/>
- FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
- EU CosIng database: <http://ec.europa.eu/growth/tools-databases/cosing/>
- ECHA (European Chemicals Agency – REACH dossiers) – <http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1>
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - <http://www.ecetoc.org>
- European Medicines Agency (EMA) - <http://www.ema.europa.eu/ema/>
- IUCLID (International Uniform Chemical Information Database) - <https://iuclid6.echa.europa.eu/search>
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- <http://webnet.oecd.org/hpv/ui/Search.aspx>
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- <https://www.nicnas.gov.au/>
- International Programme on Chemical Safety <http://www.inchem.org/>
- FAO (Food and Agriculture Organization of the United Nations) - <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/>
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Potassium Silicate, Sodium Magnesium Silicate, Sodium Metasilicate, Sodium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite

September 9-10, 1999

Dr. Belsito noted that this group of ingredients consists mostly of clay-like materials, but that salts (i.e., Potassium Silicate, Sodium Metasilicate, Sodium Silicate, and, possibly, Zirconium Silicate) are also included. He also recalled studies indicating that the salts, but not the clays, were irritants, and that his Team recommended that these four salts should be included in a separate report. The Belsito Team also concluded that the remaining ingredients are safe as used in cosmetic products. Dr. Belsito said that his Team will make a decision on specific data requests after the current report has been divided into two separate reports.

Dr. Schroeter said that his Team agreed that the ingredients in this review could be separated into two groups, soluble salts, which may be active (Sodium Metasilicate, Potassium Silicate, and Sodium Silicate) and minerals of solids (or clays) within the same report. He noted that the clays have no absorption and are basically safe, except for the possibility of irritation. Dr. Schroeter also noted that cosmetic use includes sprays and that the issue of inhalation exposure could be addressed in the report discussion as a cautionary item. Furthermore, he said that the irritation potential of clays could be addressed in the report discussion by stating that concentrations in formulation that induce irritation should be avoided.

Dr. Andersen said that according to yesterday's Team discussions, the principal issue concerning the soluble salts relates to irritation. Therefore, he said that if the conclusion on this group of ingredients could reflect the need to formulate so that products are not irritating, then that concern could be eliminated.

Dr. Andersen also said that it may be possible for the Panel to issue a tentative conclusion on this group of ingredients. He recalled that, except for the issue of inhalation exposure to clays, there are no other safety issues and, thus, the clays could be considered safe as used.

Dr. Belsito agreed that a safe as used conclusion could be issued on the clays. He also said that it could be stated in the report discussion that data on the use of clays in aerosolized products are insufficient.

Dr. Shank expressed concern over the possibility of silicosis following inhalation exposure to dust particles.

Dr. McEwen said that silicosis is not a concern because these ingredients are not composed of crystalline silicone. However, he noted that pneumoconiosis may be a concern.

Dr. Andersen noted that crystalline forms do exist.

Dr. Belsito proposed dividing the current document into two reports. One of the reports will contain a safe as used conclusion on the clays and the other report on the salts will be re-reviewed as a separate document. Dr. Belsito speculated that the issue of irritation will be the only safety issue relating to the salts.

The Panel agreed with Dr. Belsito's proposal.

Dr. Schroeter confirmed that the issue of inhalation relating to the clays will be addressed in the report discussion.

The Panel voted unanimously in favor of issuing a Tentative Report with a safe as used conclusion (and appropriate report discussion) on the clays.

The Panel also voted unanimously in favor of incorporating the data on the soluble salts from the current report into a separate document that will be reviewed by the Panel.

Dr. Bergfeld stated that the report on the soluble salts will be reviewed at the next Team meeting.

February 14-15, 2000

Dr. Schroeter stated that a Tentative Report with a safe as used conclusion was issued at the September 9-10, 1999 Panel meeting. He then noted that one of the ingredients included in this review, Magnesium Silicate, had been considered talc, and that FDA informed the Panel that there is a considerable amount of data indicating that talc may have carcinogenic potential and that this issue is being addressed. Dr. Schroeter pointed out that the structure and CAS number of Magnesium Silicate are different from those associated with talc, and that this should be clarified in the CIR report.

Dr. Belsito said that the fact that talc is not one of the ingredients in this review should be stated in the report introduction and discussion, and also noted that talc will be the subject of another review by the CIR Expert Panel. The Panel voted unanimously in favor of issuing a Final Report with a safe as used conclusion on the Aluminum Silicate ingredient family.

Because of the number of ingredients to date for which the issue of particle size (relating to inhalation or aerosol exposure) has been raised, Dr. Bergfeld asked Dr. Belsito to review the caveat relating to particle size that has been included in CIR reports. Dr. Bergfeld informed the Panel that this caveat will be discussed at the upcoming Panel meeting.

Dr. Bergfeld also noted that because it is likely that the Panel will review talc at some point, the Panel's prioritization of this ingredient for review should be considered.

Dr. Belsito added that it is his understanding that FDA has reviewed talc and has not found that the data warrant any immediate action. He said that talc should be added to the CIR Priority List, but should not necessarily be added at the top of the list.

Dr. Bailey said that there are some aspects of talc that would be of interest, more so from the perspective of setting standards or specifications for talc in terms of particle size. He noted that the results of an NTP inhalation study (animals) on talc indicated exposure-related carcinogenic effects that were attributed to particle size. In this study, the particle size of the talc was smaller than that used in cosmetics. Dr. Bailey added that he has not reviewed any comprehensive data that address the particle size of talc that is used in cosmetics (i.e., the particle size distribution). In light of the NTP finding, he also said that in order for one to have a higher level of confidence relative to inhalation exposure, data on particle size distribution (in cosmetics) would be very useful.

Dr. McEwen said that the NTP study results were not linked directly to the talc, but to the overload and a secondary mechanism. He also said that the effects of talc in miners and millers of this chemical have been studied over a period of 50 to 60 years. The magnitude of the lung effects seen in a specific talcosis is basically pneumoconiosis, which can be identified by the crystalline structure in X-rays. Dr. McEwen added that lung cancer has never resulted from exposure to talc itself. However, talc that is mined from asbestiform-containing mineral deposits has been implicated in cancer, specifically, the asbestiform particulate. According to Dr. McEwen, the specification for cosmetic grade talc indicates that it contains no asbestiform particulate.

Dr. Bailey wanted to know the extent of industry compliance with the CTFA specification for cosmetic grade talc. He said that it would be nice to have some assurance that the standard is being implemented.

Dr. McEwen said that relevant sampling would have to be done in order to insure this.

Dr. Bailey said that the Expert Panel could request these data, and that the Panel's efforts may be more successful than those of FDA.

Dr. Bailey also said that another issue relates to perineal use of talc and ovarian cancer, and that, based on the available data, FDA has not arrived at any conclusion relative to this issue.

Dr. Bergfeld said that information relating to particle size will be retrieved from CIR reports for review. She noted that the Panel has been faced with issues relating to aerosol exposure to cosmetic ingredients, and that previous statements regarding particle size need to be captured for future use in safety assessments.

Potassium Silicate, Sodium Metasilicate, and Sodium Silicate

December 20-21, 1999

Dr. Schroeter recalled that at the September 9-10, 1999 Panel meeting, these three silicate salts were removed from the CIR report on these ingredients and Silicate Minerals and Clays.

The Panel issued the following informal data request:

- (1) Physical and chemical properties, including octanol/water partition coefficient and impurities data
- (2) UV absorption (while these ingredients are not expected to have significant UV absorption, the Panel believes the report would be improved if these data were available rather than assumed)
- (3) Gross pathology and histopathology in skin and other major organ systems associated with repeated dermal exposures; and, if these data are suggestive, reproductive and developmental toxicity data may be needed
- (4) Dermal irritation and sensitization (what is the highest non-irritating dose?)
- (5) Mammalian genotoxicity data
- (6) Ocular irritation, if available; with the view of establishing the highest non-irritating dose

May 18-19, 2000

Dr. Schroeter noted that no response to the following informal data request issued at the December 20-21, 1999 Panel meeting was received:

- (1) Physical and chemical properties, including octanol/water partition coefficient and impurities data
- (2) UV absorption (while these ingredients are not expected to have significant UV absorption, the Panel believes the report would be improved if these data were available rather than assumed)
- (3) Gross pathology and histopathology in skin and other major organ systems associated with repeated dermal exposures; and, if these data are suggestive, reproductive and developmental toxicity data may be needed
- (4) Dermal irritation and sensitization (what is the highest non-irritating dose?)
- (5) Mammalian genotoxicity data
- (6) Ocular irritation, if available; with the view of establishing the highest non-irritating dose

He also stated that his Team determined that item 6 above is unnecessary and should be deleted from the list of data requests.

Dr. Schroeter also noted that the hypersensitivity test on Sodium Metasilicate that is being conducted by the National Toxicology Program study is nearing completion and that the preliminary data appear to be negative.

Concerning item 5 above, Dr. Belsito noted that the Panel has negative Ames test data on the silicate, but no test data on the metasilicate. Thus, the Belsito Team determined that Ames test data on the metasilicate and mammalian genotoxicity data on the silicate and metasilicate are needed.

Dr. Slaga recalled that Ames mutagenicity test data on Sodium Silicate are included in the CIR report.

Dr. McEwen did not see the need for another non-mammalian mutagenicity assay, considering that assays of this type are included in the report.

Dr. Klaassen noted that bacterial mutagenicity data on Sodium Metasilicate are not included in the CIR report and need to be requested.

Dr. McEwen said that based on the negative Ames test data on Sodium Silicate, it is expected that the other two ingredients also are not mutagenic. He did not see the need for additional mutagenicity tests on either of the three ingredients.

Dr. Belsito noted that his Team did not mention specific ingredients in any of the other data requests and asked whether this should be done because of differences in chemical structure.

Dr. Slaga noted that the three chemicals in this review are very similar and it is possible that data on one chemical may be used to evaluate the safety of another.

The Panel voted unanimously in favor of issuing an insufficient data announcement with the following data requests:

- (1) Physical and chemical properties, including the octanol/water partition coefficient and impurities data
- (2) Gross pathology and histopathology in skin and other major organ systems associated with repeated dermal exposures; and if these data are suggestive, reproductive and developmental toxicity data may be needed
- (3) Human dermal irritation and sensitization (specifically, the Panel wants to know the highest non-irritating dose)
- (4) Two genotoxicity studies for Sodium Metasilicate, one of which should be in a mammalian system; and one mammalian genotoxicity study for either Potassium or Sodium Silicate
- (5) Ocular irritation data, if available (again with the view of establishing a non-irritating dose)

December 4-5, 2000

At the May 18-19, 2000 Panel meeting, the Panel voted unanimously in favor of issuing an insufficient data announcement with the following data requests:

- (1) Physical and chemical properties, including the octanol/water partition coefficient and impurities data
- (2) Gross pathology and histopathology in skin and other major organ systems associated with repeated dermal exposures; and if these data are suggestive, reproductive and developmental toxicity data may be needed
- (3) Human dermal irritation and sensitization (specifically, the Panel wants to know the highest non-irritating dose)
- (4) Two genotoxicity studies for Sodium Metasilicate, one of which should be in a mammalian system, and one mammalian genotoxicity study for either Potassium or Sodium Silicate
- (5) Ocular irritation data, if available (again, with the view of establishing a non-irritating dose)

Dr. Schroeter stated that unpublished data from industry were received in response to the preceding announcement and that the Panel also received additional published studies. He then noted that his Team concluded that the available data on Potassium Silicate, Sodium Metasilicate, and Sodium Silicate are no longer insufficient for the following reasons, addressing each item on the list of data requests:

- (1) Data on chemical and physical properties (Item 1) are available and further information is not needed. The octanol/water partition coefficient (Item 1) is not needed because these ingredients are probably poorly absorbed through the skin.
- (2) Item 2 above is not needed because there was no evidence of developmental toxicity and these ingredients are probably poorly absorbed through the skin.
- (3) Item 3 is not needed. Irritancy may be a problem, but appropriate formulations should decrease the likelihood of skin irritation.
- (4) Item 4 is not needed. On the basis of limited skin absorption, mutagenicity and genotoxicity data are not necessary.
- (5) Item 5 is not needed. Ocular irritation may be avoided by formulation in rinse-off products to create a non-irritating product. Leave-on product cautionary statements may also be developed.

Dr. Schroeter said that, based on the preceding comments, the reasons why the data originally requested are no longer needed should be stated in the report discussion.

Dr. Belsito noted that Sodium Silicate is used in skin cleansing products, which include cleansing lotions, liquids, and pads (which may be considered rinse-off products) and cold creams (which may be considered leave-on products). He also noted that Sodium Silicate is used in skin cleansing products at concentrations up to 10.0%, and that any leave-on product containing 10.0% Sodium Silicate may be irritating to the skin. Dr. Belsito added that a safe as used conclusion for ingredient use at this concentration in a cold cream would probably be inappropriate, given the uncertainty as to whether or not the skin cleansing cold creams are classified as leave-on products.

The possibility of concentration limits for Sodium Silicate (up to 4.0%, based on available data) as well as Sodium Metasilicate in leave-on products was also mentioned, taking into consideration that Sodium Metasilicate has a different type of irritation potential when compared to Sodium Silicate. Dr. Belsito said that a concentration limit for Sodium Metasilicate needs to be determined.

Dr. Shank noted that Sodium Metasilicate is used only in rinse-off products.

Dr. Schroeter said that the irritation potential of Sodium Silicate should be addressed by indicating in the report discussion that products containing this ingredient should be formulated to avoid skin irritation. He did not feel that a concentration limit should be established for this ingredient.

Dr. Belsito agreed that Potassium Silicate, Sodium Metasilicate, and Sodium Silicate are safe as used in cosmetic products when formulated to avoid skin irritation, and proposed this statement for the report conclusion. The Panel voted unanimously in favor of issuing a Tentative Report with the following conclusion: Based on the animal and clinical data included in this report, the CIR Expert Panel concludes that Potassium Silicate, Sodium Metasilicate, and Sodium Silicate are safe as used in cosmetic products when formulated to avoid skin irritation.

June 4-5, 2001

Dr. Schroeter recalled that a Tentative Report with the following conclusion was issued at the December 4-5, 2000 Panel meeting: The CIR Expert Panel concludes that Potassium Silicate, Sodium Metasilicate, and Sodium Silicate are safe as used in cosmetic products when formulated to avoid skin irritation.

Dr. Schroeter also noted that unpublished data (clinical skin irritation studies on Sodium Silicate and Sodium Metasilicate) considered by the Panel at its December 2000 meeting have been incorporated into the report text, and that these data do not warrant any change in the Panel's tentative conclusion.

The Panel voted unanimously in favor of issuing a Final Report with the following conclusion: Based on the available data contained within this report, the CIR Expert Panel concluded that Potassium Silicate, Sodium Metasilicate, and Sodium Silicate are safe when formulated to avoid irritation in cosmetic formulations.

Silica and Silicates

June 29-30, 2009

Presentation:

DR. ANDERSEN: The next item on the agenda is to hear from the folks from SASSI which is the Synthetic Amorphous Silica and Silicate Industry Association. Dave Pavlich is the association manager and has a PowerPoint presentation for us. There are limited numbers of copies, but certainly enough for the panel to look at. The rest of you can take notes on what Dave is saying. We're going to try and get this up onto the screen. Dave, take a deep breath and let's see what we can do.

MR. PAVLICH: As for the acronym, when I reserved the domain name I thought for sure I'd get some interesting calls from people to buy it, but that didn't happen. I'm also sure that there are people who go to that website and they're disappointed by what they found, and amorphous silica is probably not what they had intended to see.

The Synthetic Amorphous Silica and Silicate Industry Association is an association that has been around for a number of years but was actually incorporated and formed in July 2007. The eight founding companies that are listed here, J.M. Huber, Evonik, Wacker Chemical, Cabot Corporation, Rhodia, PPG Industries, PQ Corp. and W.R. Grace, you may or may not recognize them as being the major global producers of synthetic amorphous silica, but they are. We are also associated with a group that's a subgroup called the Amorphous Silica and Silicate Producers, so we've done some work with them in doing research, that's a group that we're associated with and we meet with them every year. I'm also here with two other representatives from SASSI companies, Dr. Jim Hathaway from Rhodia, and Dr. Gregg Daum from W.R. Grace. Dr. Hathaway is going to come up here in a little bit and go through some of the details of our comments, but at this point I'll give an introduction of why we're here.

The basic reason is that the circumstance of CIR's review of silica fits our mission particularly well, and our association's mission is to further the understanding of synthetic amorphous silica and silicate health and safety within the industry, to monitor the regulation of synthetic amorphous silica and silicates by government, to educate the public and government on the views of the industry, and to consult and cooperate with state officials and state agencies on matters having industry-wide significance, and I would add other groups like CIR. That's our purpose here.

We'd like to thank Dr. Andersen for working with us. He did attend our spring meeting in March and gave us an overview of the CIR process for reviewing silica, and then gave us the opportunity to review the March 25 scientific literature review. We did send comments in on May 12 on that review and a number of those were incorporated into the latest version of the scientific literature review, but there were a number of things that we felt were not addressed, and those are the comments that we're going to make today. I'll highlight here seven issues that we'd like to address, and then I'll introduce Dr. Hathaway to go through those in detail.

First of all, obviously a reason for our existence is to differentiate synthetic amorphous silica from other forms of silica. In the SLR we definitely feel that there is a need to have fair and accurate differentiation of SAS from other forms of silica. Also the SLR we feel needed to focus more on just synthetic amorphous silica since that's the form used in cosmetics and limit the discussion in reference to other forms of silica. This is a document that's published and is going to be available, and obviously SASSI members are concerned about misinterpretation of information.

Similarly, there are a number of manufacturing processes that are mentioned in the summary that are not contemporary and do not reflect the processes that are used for commercial manufacturing of synthetic amorphous silica, and we feel that there is too much emphasis on those noncommercial processes and the composition of the materials from those processes. Along the same lines, those references also give some information about the impurity levels which we feel incorrectly represent synthetic amorphous silica. In the toxicological studies that are referenced, there are a number of factors that Dr. Hathaway will emphasize that are important in interpreting the judging the applicability of the studies on synthetic amorphous silica. The bibliography of the SLR is very lengthy. We feel that it's relatively comprehensive but that because of the number of studies that are referenced that there is little effort to identify the more current information that's available and to emphasize the importance of that data. Finally, we were relatively surprised that in the summary, the information quoted was there were 3,276 products that contain synthetic amorphous silica and the only specific reference was to hair spray with little identification of other

cosmetic products of routes of exposure that are suspected for those products. I'll now introduce Dr. Hathaway to go through those comments in detail. Thank you.

DR. HATHAWAY: I appreciate the opportunity to provide additional comments to the CIR expert panel. I think the thing that we're most concerned about is having a very clear and accurate differentiation from synthetic amorphous silica and other forms of silica particularly crystalline silica or products that contain crystalline silica.

Unfortunately, there's a tremendous amount of confusion between these that is occurring all the time. Just a couple of years ago the insurance carriers for all of the member companies wanted to have an exclusion against any product liability for silica. Part of the problem is there's one cast number for all forms of silica and a lot of people don't understand the difference. Companies had to have extensive discussions with their insurance carriers. Once they understood the difference they limited it to crystalline silica, but to the extent that the document which will be available publicly has some confusion in it, we'd very much appreciate it if those things could be corrected so that we don't get something else out there that misinforms or confuses the public.

Instead of using the term silica, we would prefer that every time that you're referring to synthetic amorphous silica, that either that full term be used or that it be abbreviated SAS and be very clear that the abbreviation stands for synthetic amorphous silica.

Also the document contains a lot of references to the other forms of silica which I don't think adds anything of benefit to the review, and we would prefer that you have something that I'll show you in a couple more slides, a very limited discussion of the other forms of silica, and then following that strictly limiting the rest of it to synthetic amorphous silica.

Hopefully you can read it a little bit better in the document that you have. There are some things here that are not quite as clear as I had hoped they would be. If you look right here, that's the form of amorphous silica, it's called fused silica. It's essentially made by melting crystalline silica to a molten form. You form a kind of glass. In the document this was listed as if it were the major form of production of synthetic amorphous silica. I guess it's a synthetic amorphous silica, but it's not what goes into cosmetics. It would be a hunk of glass and it's not ground up and put into synthetic amorphous silica at all.

Over here in this group here, that's natural diatomaceous earth from diatoms. In nature it contains about 2 to 3 percent crystalline silica and the rest is amorphous silica. The ones that are further down that list are calcined, and when you calcine diatomaceous earth, a lot of this is used as a filter aid for filtering various products in their manufacturing processes, you form up to 70 percent crystalline silica. There are a lot of problems with the epidemiology studies that are talking about amorphous silica because in some cases they found cases of silicosis, but these are ones where there was exposure to the calcine diatomaceous earth which is up to 70 crystalline silica, and so it's very important to make a very clear distinction between what we're calling synthetic amorphous silica and these other forms that can actually contain crystalline silica themselves.

Right here, this particular area is what we think should be the focus of the document. These are the synthetic amorphous silicas that would be used in cosmetics. There are two essential processes here. One is the way process that produces precipitated silica and also silica gel, and the other one is a thermal process that produces pyrogenic silica. Unfortunately, the historic name for pyrogenic silica was fumed silica and this has the potential for tremendous confusion. Let me just show you. Over there there's a thing called silica fume. It sounds a lot like fumed silica. Unfortunately, silica fume contains crystalline silica. I think the person who drafted the document had some confusion between these two and we would of course like that cleared up as well and we would strongly prefer that the thermal process, synthetic amorphous silica, always be called pyrogenic to help avoid this confusion in terms of terminology.

As I mentioned before on that fused silica where they essentially melt crystalline silica, this is really not a commercial process that's used in anything that goes into there. It would probably be best that it be taken out of the document. You can show the kind of table that we presented in the previous slide and then after that limit the discussion to the true synthetic amorphous silicas that are used in cosmetics.

I think I've pretty much discussed both of these things already, the confusion with heating the crystalline silica to form a type of glass, and the confusion between silica fume and pyrogenic silica.

In some of the discussion of pyrogenic silica which in the document is referred to as fumed silica, they have a reference saying that it may contain up to 6 to 8 percent crystalline silica. We're pretty sure that was confusion with silica fume. Then it follows immediately after that reference with a reference from Cabot saying that their stuff is 99.8 percent pure as if there's maybe some discrepancy in which one do you want to believe. The pyrogenic silica from Cabot Corporation is indeed 99.8 percent, and any of the producers of the pyrogenic form have a very high level of purity. The precipitated silica is less pure mostly because it contains a certain amount of water and the pyrogenic is very dry. It appears to call into question the claims about Cabot Corporation about the purity and we think the way these things are juxtaposed they are potentially very misleading to reader.

In terms of the toxicology studies, I think the ones that discuss oral toxicity and dermal toxicity are pretty much fine. This is a compound that is considered safe to use in food products. In terms of skin exposure there is very little in the way of issues. Synthetic amorphous silica can absorb water, so if you put the powder directly on your skin it may cause some drying of the skin and some irritation. I don't imagine that this would be an issue the way it's used as ingredients within cosmetics, however. It could be an issue in the workplace. But there is a very key thing when we're considering inhalation or intratracheal injection studies. One of the things that creates an anomaly here is that these products as they're produced are about 100 microns in diameter and for some applications they are milled down into the maybe the 10 or 20 micron range, and that's actually a relatively smaller percentage of the total. Most of the material is actually in 100 micron range or at least about 30 or 40 microns in diameter as it would be used in most cosmetic ingredients. But if you're going to do an inhalation study and you have material that's big, anything above 10 microns is not going to get down into the lungs. So the various groups like OECD that do the toxicology protocols require that these things be broken up into something that averages 4 microns in diameter, and indeed all of the toxicology studies have had to do this in order to comply with these protocols. So you get an artificial situation where this material can now be inhaled or it can be injected down into the trachea. What happens when this is done is you have the smaller particles that have a higher surface area and although synthetic amorphous silica if you look up some of the references on solubility, they will say it's insoluble; everything is relative. Crystalline silica for example is pretty much insoluble. Synthetic amorphous silica is relatively insoluble. As you get to a larger surface area for the mass of material, you do get some of this material dissolved and it dissolves to form silicic acid. If you do break up these particles either by dispersion or by milling or by whatever means and you have an inhalation toxicology study, you're going to get some silicic formed on the alveoli of the experimental animals and you're going to get some corrosive effects from the acidic silicic acid. This is not something that you would see from even inhalation of cosmetic products or from the manufacture of these things in the protocols that workers might be exposed to during the manufacturing process because they're just not respirable in the form that they're being used. In a sense it's almost an artificial situation, and they do cause inhalation toxicity if they are broken up to those smaller sizes. One of the interesting things is though that because they are somewhat soluble under these circumstances, there are a number of clearance studies that show that this material is completely cleared from the lungs and that the reason that the studies don't find fibrosis that you would find with crystalline silica. It's something that we would recommend that before they go into the animal inhalation studies that they talk about this particle size and the fact that it's an artificial situation with all of the inhalation and intratracheal toxicology studies that were done and that you would not see this with the larger particles that are used commercially.

As Dave mentioned, the review covers an enormous number of studies. Unfortunately, there's not a lot of I guess what you'd call interpretation of weighing of which of the studies are most significant. We would like to see a little bit more of this done and perhaps more emphasis be given to some of the newer or more credible studies rather than just simply listing them all and leaving it up to the reader to try to judge which ones are most important. Most of the older inhalation toxicity studies did not discuss this particle size difference in terms of the materials being dispersed or milled down to a small particle size, that's unfortunate, and so the abstracts don't discuss that at all, but the reader is going to wonder which ones of these are really the correct situation. If you want, we would be willing to go and try to give some assistance here in terms of pointing out what we think are the more reliable and credible studies.

As Dave mentioned, we were surprised that there wasn't more discussion of the actual applications in cosmetics. That really is not a big issue with us, but it's something that you might want to consider in terms of improving the document. Here we have the spelling of our names and our website and so forth.

I'd be happy to address any questions that any of the panel members have.

DR. SLAGA: Just to have it straight, the 100 percent that is supplied to the cosmetic industry is between 10 and 100 microns?

DR. HATHAWAY: Correct.

DR. SLAGA: And only in some of the studies did an inhalation was it at 4 micron?

DR. HATHAWAY: Correct. They either break it up and disperse it some form or mill it down to that smaller diameter so it can get in there. In fact, in order to comply with the testing protocols they have to do this even though it's not representative of the material that would be involved in worker exposure or consumer exposure.

DR. LIEBLER: I appreciate the silica family tree that you provided us. I think it's helpful in organizing our thinking about this. I have two questions that relate to this. One is are there any sort of milestone dates in terms of synthetic amorphous silica manufacturing processes that would be useful in helping us interpret some of the older literature? In other words, in the 1970s or 1980s or sometime were there any changes in manufacturing processes that yielded the materials that are used in cosmetics now?

DR. HATHAWAY: I'm going to go out on a limb and make some guesses. I'm thinking that these processes probably were introduced in the 1950s or earlier. There is certainly much more production now than there was in that timeframe. But many of these articles that are from the 1950s to the 1970s still are talking about older processes and maybe there was some use of this glass that was formed from melting crystalline silica and I'm not sure what it would be today. I think a relatively small amount of these materials go into the cosmetic field. I know of the stuff that our company produces probably 80 percent goes into tires to reduce rolling friction and most of the other 20 percent goes into toothpaste, so relatively small amounts go into the cosmetic industry, but it's probably widely used in a lot of products.

DR. LIEBLER: It doesn't sound like there's a clear dividing line of any sort in the manufacturing process that would be helpful to us.

DR. HATHAWAY: Unfortunately not, but I would say the studies that would have dates after 1990 certainly would be probably more credible than ones that had dates before that.

DR. LIEBLER: I have one other question. What is the analytical method that's used to determine the content of crystalline silica in a background of synthetic amorphous silica?

DR. HATHAWAY: Usually this would be a microscopic thing. I was recently at our plant that manufactures this and even though we expected to find no crystalline silica, we went ahead and had some industrial hygiene sampling done at the site to reassure our own employees, and they do a microscopic analysis for the three forms of crystalline silica and they found nondetectable levels at extremely low levels, whereas when they measured total particulates which would include the larger particles, indeed there was a certain amount of dust exposure during the manufacturing process.

DR. LIEBLER: So the analytical methodology then probably would have been the same for a long time if you say it's microscopic evaluation?

DR. HATHAWAY: I believe so, yes.

DR. LIEBLER: So you're counting particles in microscopic fields. Right?

DR. HATHAWAY: I believe so, yes.

DR. BELSITO: As you may or may not be aware, and I guess that's my question, we're already issued two prior reports on silicates and this is the third to capture all of the ingredients that we failed to capture before. Did you have the opportunity to review those two prior published reports?

DR. HATHAWAY: This would have been before the one that came out in March?

DR. BELSITO: Yes. This is before this SLR.

DR. HATHAWAY: I don't think we were aware of that. Dave, were you aware of anything?

MR. PAVLICH: No, we haven't seen that.

DR. MARKS: In the manufacturing of the cosmetics, are there any physical changes that would occur as with a natural amorphous silica where there would be more crystalline silica produced in the end product or the use?

DR. HATHAWAY: I'm not familiar with how they're done in cosmetics. I would assume these are simply blendings, and unless you have a process that introduces very high heat, I don't believe you'd form any crystalline silica.

DR. HILL: I want to get clarification in that regard. So if they were truly amorphous rather than crystalline and they were inhaled because it was in some powdered product or some spray, what we would expect is dissolution to silicic acid and the lung is able to clear that and you'd probably talking about small amounts where there wouldn't be a toxicity issue. Is that what I heard you to say?

DR. HATHAWAY: I have a hard time imagining very much is going to be inhaled from any cosmetic use.

DR. HILL: Clearly not toothpaste.

DR. HATHAWAY: But even hair spray I have a hard time imagining.

DR. HILL: Because of the particles produced by the spray.

DR. HATHAWAY: There was one reference in the review that talked about hair spray and I believe that they said that the particles were in the 30 to 50 micron range for that particular product which is way above the respirable size and I would imagine that the proportion that might be below 10 would be very, very small. Most of the things that we've looked at have been at least 99 percent above 10 in terms of total mass of the dust. So these the amount of fines that might be below 10 is going to be below 1 percent and I would suspect considerably below 1 percent.

DR. HILL: And what is produced under those circumstances you're telling us that the lungs should be able to clear?

DR. HATHAWAY: Even when they used very large amounts of the dispersed material, this clears in the lungs. I think the half-life is less than 30 days even with a significant amount. Between SASSI and ASAP, the European equivalent of ours, we've done these dissolution studies to demonstrate this. There is also a study of three German manufacturing for synthetic amorphous silica in terms of epidemiology studies where they're looking both at pulmonary function and chest X-rays and I believe this is in the process of being written up for publication and they've found no evidence of any fibrosis.

DR. MARKS: There was one reference in which Epstein in the early 1960s injected colloidal silica subcutaneously and developed granuloma formation. Is this an example where we don't really know what was in that colloidal silica that was not synthetic amorphous silica?

DR. HATHAWAY: I'm really not sure. Colloidal sounds like it would probably be amorphous, but I have a hard time knowing. I'm not familiar with that particular article.

DR. MARKS: Can you comment about granuloma formation?

DR. HATHAWAY: There are an awful lot of things that can cause granulomas. If you implant a diamond in a rat you're going to get granulomas forming. I'm not sure it's directly related to any kind of an inhalation type of toxicity or exposure to the exterior of the skin. There are lots of things just because of their geometric shape and so forth

will cause granulomas in experimental animals especially rats. When I was in the Army and we were looking at issues with the safety of Kevlar for bulletproof vests, they implanted some Kevlar in the rats and you form granulomas around them.

DR. MARKS: I was thinking more in terms of application to damaged skin.

DR. SNYDER: Related to slide 7, the reliability of the studies, do you have additional data that you could provide us that are not in the reports?

DR. HATHAWAY: We provided two documents that are relatively recent and I think pretty thorough reviews of the issue. One of these is called the Jack Report that was produced in Europe. It's a very, very large document. Then we recently produced another document that I think is around 50 pages long that's maybe a condensed summary of a lot of that information and we did this as a voluntary effort with the Environmental Protection Agency because they're concerned about nanoparticle issues. In the manufacturing process, you start off with nanosized amorphous silica and it forms aggregates and then agglomerates to get up to the 100 micron size so the primary particles are nanosized and this was the reason that we provided that summary. It's a shorter summary but I think it covers the manufacturing processes very well. There are pretty good summaries on the epidemiology and the toxicology studies. It also talks about the bonding of the agglomerates. These things do not break up under normal circumstances. You have to go to pretty significant mechanical forces to get these things to break up into smaller particles. Thank you very much.

Belsito's Team Meeting:

DR. BELSITO: Okay, Silica and Silicates. Now, folks, we've really got to concentrate on this because the Marks Group report's in public session, and we got to get ready to attack them.

Okay, so this is -- we got some pages here, information that was not in the book, unpublished data, and basically it was two human studies, 27 individuals each, exposed to 17 percent concentration of hydrated silica with negative sensitization.

And then we got a guinea pig sensitization study on hydrated silica -- the shortest study I've ever seen, or at least the shortest summary -- and this was 10 percent in distilled water with a challenge that induction from 1 to 20 percent, and that was on 10 animals, and that was negative.

And then we got a summary from the FDA on line just lots of data that I didn't think had added anything to the report.

And then we had a rather SASSI talk this morning, so hopefully you've heard more than you need to hear already today on silicates.

And so the question here is, what are we -- where are we going with this? And I thought probably, you know, safe as used, I sort of did agree with the comments this morning that we need to be very careful about the amorphous silica because I got like really confused reading the document as to where we're going.

And I guess the other questions I have are, how do we handle these prior reports from 2003 and 2005? Do we want to lump them now? Do we want to wait till 2018 when the first 2003 report comes up for re-review, and then lump them all together? Or where do we go with those? And table 10 was missing from my document.

SPEAKER: (off mike)

DR. BELSITO: Table 12 was where -- table 12 continued was where Table 10 was supposed to be, so --

MS. BECKER: Right. So table 12 continues -- started a page early.

B - SPEAKER: Microphone.

DR. BELSITO: Oh, so table 12 on page 78 is really table 10.

MS. BECKER: Correct.

DR. BELSITO: And table 12 continued belongs after belongs after table 12? Okay. Or is table 12 continued -- really Table 10? Which one is table --

SPEAKER: The first table 12 continued on page 78, the table --(off mike).

DR. BELSITO: Oh. So it's really not table 12 continued. Oh, okay, good. Well, that helped me out there. (Inaudible chatter) I thought I was Hebrew reading from right to left.

Okay, those are my comments. Curt and Paul?

DR. SNYDER: So all of that data, that other data has been added in?

MS. BECKER: Everything's in.

DR. SNYDER: That's everything?

MS. BECKER: That I have except for what just this little bit you got handed.

DR. LIEBLER: I would quite agree with the revision suggested in the staff, the presentation this morning, on clarifying the definition of the form to use and minimizing the emphasis on crystalline silica.

DR. BERGFELD: Minimizing or deleting?

DR. LIEBLER: Excuse me?

DR. BERGFELD: Minimizing or deleting.

DR. LIEBLER: Minimizing.

DR. BERGFELD: Do you think leaving it in leads to confusion?

DR. LIEBLER: No, I -- well, I got the impression that it's good to know that what is being discussed is not crystalline silica.

DR. BERGFELD: That will be really a very great delete.

DR. LIEBLER: Very brief, right, minimizing.

DR. BELSITO: And probably just summarizing that a) we're not talking about crystalline silica, and so that means things in the nature of silicosis, pneumoconiosis, those types of issues that people associate with silica are going away.

DR. LIEBLER: Exactly. But if you don't mention that, if you don't mention it's not crystalline silica, then people are going to get confused and asked why you're ignoring all that stuff.

DR. BERGFELD: But there are a lot of citations here that --(off mike).

DR. LIEBLER: Yeah. And I guess I had a question, in the older literature where it may not have been clear what form of silica was actually used in some of these older studies, and what effects are valued? Should those studies be included?

That's why I asked the question this morning, was there any milestone or change in manufacturing process that would have sort of invalidated earlier studies, because the material that was tested was no longer applicable to current cosmetic use. And it sounds like it's not that straightforward.

But where it's not clear that the material study was the material that is used in current cosmetic use, I think that stuff should be deleted.

MS. BECKER: Whenever I was unclear on whether it's crystalline or amorphous, I left it out. I only did think I could definitely say were amorphous from the test to this paper.

DR. BERGFELD: You have the fume silica. Fume silica, that would be one of --

MS. BECKER: If they called it fumed silica. If it says silica fume, I never saw that.

DR. BERGFELD: Okay, but --

MS. BECKER: But I saw fumed silica.

DR. SNYDER: That's all the tox data, is fumed silicic.

DR. BERGFELD: But that's different from the crystalline form, then.

MS. BECKER: I guess.

DR. BAILEY: I would recommend taking from the presentation this morning that in some detail the characterization and definition and so forth, and put that into the appropriate part of this report, because there is a huge amount of confusion. And your assessment will be for the synthetic amorphous silica. And I think you need to, in the discussion, strongly make that distinction and separate from the crystalline silica, because this is an area where there's so much confusion, and the terms used and, you know, questions about silicosis and, you know, being related to silicos used in cosmetics. That really needs to be clarified.

And your expectations need to be clarified that we're talking about, you know, that we're talking about the, you know, synthetic amorphous silica in the process.

DR. BELSITO: Right. But as Paul pointed out, you know, a lot of the studies, and I guess what really threw, you know, a monkey wrench into -- and again I think it's safe as used -- but I mean I think you want to present the correct data and not incorrect data that you have to argue against: Was the fumed silica versus silica fumed, and one is actually crystalline and not amorphous, and the other is amorphous.

DR. BAILEY: Right. Right, exactly.

DR. BELSITO: And so when we're referring a lot of the studies in here is a fumed silica which -- is that silica fumed or fumed silica? Is it amorphous, or is it crystalline?

And, you know, I don't -- you know, based upon that, I don't know how to proceed with this. Should we --

DR. HATHAWAY: Can I make a --

DR. BELSITO: Yeah. (Chatter -- inaudible)

DR. HATHAWAY: Unfortunately, there's different categories of amorphous silica. What's used in the cosmetics are synthetic amorphous silica, and there is the two processes, you know, the wet and the thermal that was described. There is also a whole bunch of other things that are called amorphous silica that typically contain a certain amount of crystalline silica. And so that adds to additional confusion. You have the diatomaceousers which contain a little bit to start with.

When they're calcine they contain a lot. And then you have on the other side of the other amorphous ones is silica fume, which is usually called an amorphous silica, but it contains a certain amount of crystalline silica.

So it presents a potential point of confusion, and we offered to work with -- I forget your name --

MS. BECKER: Lillian.

DR. HATHAWAY: Lillian -- you know, to try to come up with some, you know, perhaps a better way of presenting the characterization of the material, you know, early on in the report.

DR. SNYDER: I think that's a good idea.

DR. BELSITO: So should we table this --

DR. BERGFELD: Yeah.

DR. BELSITO: -- and ask Lillian to work with the SASSI -- but we don't want to get too sassy in the process, Lillian -- and go through and look at each of the references that are here and make sure that a) that when they're talking about fume silica, it's the amorphous and not the crystalline.

And then spoof up the document and have it come back to us with the notion that at least I'm comfortable, Paul and Curt, with the idea that this is going to be safe as used, but we want the information in the document to be what is actually used in cosmetics and not what is not used in cosmetics or --

Lillian, do you feel that you've already done that --

MS. BECKER: I --

DR. BELSITO: -- or were you confused by their presentation?

MS. BECKER: I was. That's one of the things that took me so long in getting started on this particular -- was going through and combing through all of that and figuring out what was amorphous and what was not.

DR. BELSITO: Okay.

MS. BECKER: And all I have to go on is what, you know, the writers wrote. And what the writers wrote I did quote in there. If they say colloidal, I put it in there; if they say silica so I'll put it in there so that I did not interpretation other than its amorphous or not.

So unless they know something I don't know about the papers, I don't think I can -- you know.

DR. BELSITO: Okay.

DR. BAILEY: (off mike) -- these are really editorial changes. I mean they may be more editorial than we're maybe used to, but I think if you feel comfortable with the, you know, conclusion, and working with SASSI folks to make these corrections and editorial changes, that, you know, we would -- I would recommend going ahead and giving your conclusion and moving the documents forward.

DR. BELSITO: Okay.

DR. BAILEY: With the idea that you'll have a chance to look at it, you know, with those editorials when corrected.

MS. BECKER: And then when we get through, it might be more clear --

DR. BELSITO: Okay.

MS. BECKER: -- without the --

DR. BELSITO: So, then, why don't -- well, then, the suggestion is we move forward, tentative final, safe as used.

Lillian will get together with the SASSI people for editorial corrections, and I guess I would like to see done what was done for the cyclomethicone report where there are comments that we should delete something that is currently in here. If they could keep it, then just underline that whole paragraph with a comment that, yeah, in review we're recommending this be deleted because in reality it was not amorphous silica, and then we can say, oh, okay. You know, rather than just having a whole bunch of material disappear from this document and us now knowing why it disappeared.

DR. BERGFELD: Well, can I offer another suggestion that maybe, when they're working on their draft, that they do that? But then they give us the second draft of deleting all of that, because I think it's going to be confusing with all that fume stuff in there. It's everywhere.

DR. SNYDER: But isn't the fume silica the pyrogenic silica which is one of the forms of a --

DR. BERGFELD: Well, maybe. You did say, Dr. Hathaway, that some of that had a high crystalline level.

DR. SNYDER: That's silica fume.

DR. BERGFELD: I know. I know. I know, but --

DR. HATHAWAY: Unfortunately, the two terms are very similar. That's why, you know, and the editorial changes we would strongly recommend that you use the term "pyrogenic" instead of fume silica. We're trying to do that in the industry to, you know, avoid that confusion.

DR. ANDERSEN: Don, I think with due respect to the industry input that we've received today, I'm not prepared to turn this report over to industry for writing it.

DR. BELSITO: Well, I don't think for writing, but for comment that we can review.

DR. ANDERSEN: Comment can be made by any interested party when the tentative document is issued for public review. If we get some further input, I'd love to receive that.

DR. BELSITO: Okay.

DR. ANDERSEN: But there is nothing that I've heard in terms of fundamental flaws that says this should stop. Should we include up front maybe a further glossary that explains to the reader that some of the terminology you're going to be seeing may look strange, and, in fact, the current preferred term for fume silica is pyrogenic silica. We can put that up front so that the explanation is provided.

But if the author of the published study called it fume silica, we can't recreate what was said in that published study. The fact that we think that's pyrogenic silica, you can in fact state positively that we think it is.

DR. BELSITO: Okay.

DR. ANDERSEN: That's a fine way to deal with it. But I don't -- I'm not hearing anything that says that there is a fundamental flaw in this document. The data that are there don't raise particularly any safety issues. It is axiomatic that we must be clear that this is not a safety assessment of crystalline silica.

DR. BELSITO: Mm-hmm.

DR. ANDERSEN: So however we do that, you know, it's going to be like teaching high school history: Tell 'em what you're going to tell 'em; tell 'em and tell 'em what you told 'em. If we don't say it that many times, we will not have done our job. So we can look at it from that point of emphasis, but unless there is a study that's included in here that is known to be crystalline silica and shouldn't be in there, there's nothing to deal with here.

DR. BELSITO: Okay. So then we're not going to make any changes, except to perhaps a little stronger emphasis in the --

DR. SNYDER: Prologue.

DR. ANDERSEN: Except to be responsive to Dan Liebler's --

DR. BELSITO: Right.

DR. ANDERSEN: -- point about minimizing the crystalline silica part, that I don't have a problem with that.

DR. BELSITO: Mm-hmm.

DR. ANDERSEN: But that is indeed, as John pointed out, it's editorial. And that's just a level of finessing this that is important. I mean I think we heard clearly this morning that to any reader they better see clearly the focus of this is away from crystalline silica.

DR. BELSITO: Okay.

DR. ANDERSEN: I think we're very close to that anyway, but a little more emphasis can't hurt.

DR. BAILEY: I mean I -- from the industry's perspective, I would like to see this document, you know, set sort of a framework for future use of terms, and understanding of what is what. A glossary would certainly do that.

DR. BELSITO: Okay, sure.

DR. ANDERSEN: It could help.

DR. LIEBLER: You had a figure 1 that's sort of like what I called the silica family tree --

DR. ANDERSEN: Right.

DR. LIEBLER: -- earlier in this morning's presentation. And I think, you know, maybe sitting here with a couple cups of coffee and listening to it here to get off to the second time made it more clear to me that there were some nice distinctions that emerged from this morning's presentation that I just didn't get first time reading it. And that might have been me, not you, but I think it's worth making the point about pyrogenic silica being -- also being called in the literature "fumed silica," and how that can lead to confusion of that material with "silica fume."

And if that can be briefly explained in the introductory material so that it allows, then, the reader to go to the original language in the literature report and not be baffled.

DR. BELSITO: Right. Okay.

DR. ANDERSEN: That works.

SPEAKER: That we know how to do.

DR. BELSITO: Okay. And then just to get back to my prior point about we have two prior reports out, the 2003, 2005, do we want to collapse all of those ingredients into this report? And then a final point is in one of those two our conclusion was when formulated not to be irritating. In this series of reports we really don't have any data to suggest that these materials are in fact irritating when used.

But now this will be the third silica document. One I think was safe as used, and one had a conclusion that -- that potassium, sodium, and silicate is the one that says "when formulated to avoid irritation." And then the other one was just "are safe as used in cosmetic products."

DR. BERGFELD: You could -- you could handle that with "should not be irritating in the product." I mean like we did before.

DR. BELSITO: I understand that, but my point, Wilma, is that in this current document --

DR. BERGFELD: Yeah.

DR. BELSITO: -- with these current ingredients, we have no data to suggest that irritation is, in fact, the problem. And, in fact, the irritation that we had --

MS. BECKER: Was only with the potassium study only.

DR. SNYDER: Sodium potassium.

DR. BELSITO: -- was only with potassium, sodium --

DR. BERGFELD: Silica.

DR. BELSITO: -- metasilicate and sodium silicate.

DR. BERGFELD: Which was the --

DR. BELSITO: And that was, you know, I think because we had data as in all cases where, you know, 100 percent there was some irritation or something, and this was back when we -- I don't know what we were doing -- but so if we don't combine these documents, then we're going to have two reports on silicates that say safe as used, and one that says safe as used when formulated not to be irritating.

And it's just, to me, if I were not on this panel, and I'm looking, okay, so what's the difference between calcium and sodium silicate, and why can one be safe as used and one only be safe as used when it's formulated not to be irritating?

DR. ANDERSEN: Well, I think that the answer is not in this report, but the answer is in fixing the other report. The only data that suggested a concern was actually sodium metasilicate.

DR. BELSITO: Right.

DR. ANDERSEN: And the conclusion could have focused on that ingredient in the earlier report; we just didn't do that. But all of the other simple salts were not irritating, continuing the pattern. So I don't think that you lose anything by not perpetuating the problem here but rather when we come back to the previous report fixing it. Or if industry is particularly concerned, they can suggest an amendment as needed to the earlier safety assessment.

DR. BELSITO: Okay.

DR. HATHAWAY: I might be able to add a little bit of clarification. The sodium metasilicate is a very alkaline material, and that may be the reason why in some formulations, if it's not very careful to adjust the overall pH of the product, you could end up with an irritating situation. We really didn't comment on that; we focused really only on the synthetic amorphous silica things, but that's probably why it was there in the older ones.

DR. BERGFELD: And our summary from the older document mentions that.

DR. BELSITO: Okay, so we're going ahead with the safe as used. We're going to do -- clean just as little bit, strengthen the introduction to clarify exactly what we're looking at, the fume versus fumed silicate -- silicate fumes, and, I gather from what Alan said, we're not going to add in the ingredients from the reports we previously did, we're worry for the 2018 people.

So you'll remember this discussion, Wilma, in 2018.

DR. BERGFELD: Me, too.

Marks' Team Meeting:

DR. MARKS: Okay. Let's move on. We've got another non-contentious ingredient named silica.

SPEAKER: Lillian will come up.

DR. SLAGA: I recommend we table this.

DR. MARKS: Okay.

DR. HILL: And I second that.

DR. MARKS: Let me see -- and I'm the one that --

DR. SLAGA: It's too complicated. Unless all these things are changed and number two, I would like to see us relook at -- even if informal -- the other two that we -- has been approved in the past.

DR. MARKS: Well, one of --

MS. BECKER: (off mike)

DR. MARKS: Go ahead, Lillian.

DR. SLAGA: Why?

MS. BECKER: I'm just trying to catch up, so I think I'm just going to sit in while I listen here.

DR. SLAGA: Because I think somewhere (off mike).

DR. SHANK: But they're already finished.

DR. MARKS: No. Not -- actually nothing was said. Basically the suggestion was made that this be tabled so we can go ahead and integrate the presentation we heard this morning. Thank you. And then also look at the two previous safety assessments that were done and, in fact, one of the -- the potential suggestion --

DR. SLAGA: Well, even Belsito wanted them to look at those two. I mean --

DR. MARKS: Yeah.

DR. SLAGA: -- because that'll be brought out tomorrow, I'm sure.

DR. MARKS: Well, one of the potential tacts I thought was we just reopen the old safety assessments and group all of this together.

DR. SLAGA: That could be wise.

DR. MARKS: And that could be -- so it could be tabled with that idea also as to consider do we group all -- all of the safety assessments.

DR. SLAGA: Who's presenting this one?

DR. MARKS: I'm tomorrow. So it will be easy if it's tabled. But I should think we need to know what we want other than obviously integrating the data we've heard today.

MS. BECKER: Well, of the data you heard today, the papers that they talked about are already integrated into the report. I got the information in time to integrate it for you guys. So you've already read everything they've given us.

DR. SLAGA: Oh, okay. It's been changed over what they were --

MS. BECKER: Right, right.

DR. SLAGA: Oh, okay.

MS. BECKER: Yes. They gave me the stuff -- I already -- I stayed up late at night putting all this stuff in so you guys could have it. You have it.

DR. SHANK: You made it clear about the crystalline versus amorphous. Is that what you're talking about?

MS. BECKER: Um.

DR. SLAGA: I didn't think that was --

DR. MARKS: Yeah, it's in the introduction -- the second paragraph. It's very clear. There are two categories -- crystalline and amorphous -- and only the amorphous ones are used in cosmetics. That's page one.

DR. HATHAWAY: Part of the problem is that a lot of amorphous forms are not used. It's really only the synthetic amorphous forms. And -- you know -- we would appreciate it if even though the data is in there, particularly the section on the silicas -- you know -- I think it could be clarified so that it would be a lot less confusing to other people. It may not affect your safety assessment, but since it would be a public document -- you know -- we would like it to be -- you know -- as straight forward and easy for -- you know -- someone else to read and understand it.

MS. BECKER: When I was going through all the papers in many papers it is very difficult to figure out which type of silica it was and I gave their -- the author's description of the silica as given and that is clear as I can make it. If you know something I don't as in -- you know -- we know this guy only worked on this type of silica, which is never used, we could -- you know --

DR. SLAGA: We probably don't know that.

MS. BECKER: We don't as far as I know.

DR. SLAGA: I would based on the (off mike).

DR. MARKS: (off mike)

MS. BECKER: But, I used the description of the authors provided.

DR. HATHAWAY: No, I understand that. The confusion comes as I mentioned this morning. This one amorphous silica that's formed by melting crystalline silica -- you know -- ends up forming a solid object, you know. Maybe it doesn't become a glass like this, but it's a type of glass. So it's not really relevant to this and then the confusion between silica fume, which is considered another amorphous form but has a certain amount of crystalline silica in it and the pyrogenic silica -- that a synonym is fumed silica -- you know there's a problem there. You know we would just like -- we would very much appreciate it if -- you know -- all of these terminologies were clarified so there would be not confusion on the part of an outsider reader and -- you know -- we're willing to work with you to try to get that squared away.

SPEAKER: We can do that. We can help.

MS. BECKER: Yeah, because as far as -- my information -- with the information I have, it's as clear as I can make it. So if you've got better information --

DR. SLAGA: Some of the publications won't be clear. That's -- I think that's the point you make.

MS. BECKER: Yes.

DR. ANSELL: Well, a lot of them do have scriptors -- precipitated study, aerosols, silica, undescribed, (off mike) silica. Perhaps we could clarify --

DR. HATHAWAY: No. There's no question the revision is a lot better than the -- than the initial draft, but there's still, we feel, could be improved to avoid -- you know -- confusion by people reading it.

SPEAKER: Well, I --

DR. ANSELL: Could you identify which of these are the cosmetic silicas as opposed to the (off mike) silicas?

DR. HATHAWAY: Correct. Yeah.

DR. SHANK: Inclusion of that flow sheet that you gave us this morning would be helpful.

DR. MARKS: It is actually in there.

MS. BECKER: It is in there.

DR. MARKS: Page 62. Yeah. It's page 62.

DR. SHANK: I forgot that.

DR. MARKS: Yeah. It's in there which -- so I think we're -- if we decide to issue a tentative report, that gives the opportunity for you to comment and do some of this suggestions you have.

DR. HATHAWAY: I mean -- yeah. I mean if you'd be willing to -- you know -- have us work with you --

MS. BECKER: Um-hmm. Sure.

DR. HATHAWAY: I think the section on describing the forms of silica is an area that we would like to see changed.

DR. MARKS: Sure.

DR. HATHAWAY: And maybe some introduction on the inhalation intratracheal thing on particle size -- you know -- just to clarify. I mean you have it in there, but it was right at the very beginning -- you know -- that kind of prefacing all of these studies, even though many of the studies may not have specifically referenced particle size -- particularly some of the older ones.

DR. MARKS: And sometimes that appears in the discussion and the discussion at this point hasn't actually been written, so these nuances are often included in the discussion to put it in perspective. So that can -- all that can be done.

MS. BECKER: Yeah. The inhalation boilerplate is in there.

DR. MARKS: Yeah.

DR. HATHAWAY: On the form (off mike) size?

DR. MARKS: Yes.

DR. HATHAWAY: Okay.

SPEAKER: Let's help through the discussion focus on the most relevant studies versus (off mike).

SPEAKER: Dr. Marks?

DR. MARKS: Yes.

SPEAKER: May I make an administrative request here --

DR. MARKS: Sure.

SPEAKER: -- which is that we do have an administrative process which includes a time period during which comments are solicited and welcome and we really would like people to submit valuable comments -- to submit them during that time frame, not afterwards. Because that really messes up our process and it doesn't allow us to incorporate the changes in a timely way so that you can see them before the panel meeting and the panel can see them.

DR. MARKS: Right. And that's a 60 day time period, so we'll have plenty of --

SPEAKER: Well, okay -- wait -- yeah -- we --

DR. MARKS: -- plenty of time to add these wording. We haven't even seen the discussion on this. We are basically today to decide --

SPEAKER: Okay.

DR. MARKS: -- one, do we want to issue a tentative safety assessment.

DR. PAVLICH: As we understood the process when Dr. Andersen visited, we were given the opportunity to look at the first draft of the scientific review and we sent in our comments and then he told me that we probably wouldn't have a chance to get those comments incorporated into the review before this meeting and therefore just to come and give our presentation. So that was our -- that's how we understood the process. So -- I mean -- we had these prepared last week, but we -- our understanding was that it wouldn't make it any difference if we sent them in early or not.

SPEAKER: Not the case (off mike).

DR. PAVLICH: Not the case.

DR. MARKS: Okay.

DR. PAVLICH: We stand corrected.

DR. MARKS: So, Ron, Ron and Tom -- Ron, Tom and Ron -- whichever way I want to go is -- do you want to move this forward to make a conclusion on these ingredients as a cosmetic ingredient and keep it as such? Do you want to group this with the other reviews (off mike)?

DR. SLAGA: Well, based on a lot of changes have already made, there's -- we can do it with the others later. We don't have to deal with them (off mike).

SPEAKER: She can't hear you.

DR. SLAGA: I think we have to deal with this --

DR. MARKS: Okay.

DR. SLAGA: -- and right now, I don't think we have to based on what we have already heard that we have to deal with the other two that have been already out in literature.

DR. MARKS: Is there -- so can the conclusion be safe?

DR. SHANK: Yes.

DR. MARKS: Okay.

DR. SHANK: With one question. The iron -- the which is it called -- aluminum iron silicate. We have almost no data on any of the metal silicates. But calcium silicate, sodium silicate -- that doesn't bother me. But the adding aluminum iron -- especially if it's inhaled with a high oxygen content of the lung -- the iron atom could produce oxidative damage which would not be expected by any of the other silicates. So I would not include aluminum iron silicate without data. The others I can add. That's the only change.

DR. MARKS: So that would be insufficient?

DR. SHANK: Insufficient for -- well, these are add-ons, aren't they or whatever?

DR. MARKS: No.

SPEAKER: No. It's the original assessment.

DR. SHANK: So it would be insufficient for the aluminum iron silicate and you'd need inhalation data unless -- you could say since it's not respirable.

DR. HATHAWAY: Well, I don't think any of our member companies produce that compound, so I don't have any information.

DR. SHANK: Okay. We had no data on it. But if it's -- if it's not respirable, then it's not a problem.

SPEAKER: I have no idea what the (off mike) for that is.

DR. SHANK: But, since we have no data on it, we would need some data.

SPEAKER: That's a good compromise.

DR. MARKS: So we'll move that this be a tentative safety assessment and it's these ingredients are safe with the exception of aluminum iron silicates, which would be insufficient data --

DR. SHANK: Inhalation needed.

DR. MARKS: Yeah. We need the inhalation.

DR. SLAGA: Unless that can be shown --

DR. SHANK: Well --

DR. MARKS: Yeah.

DR. SHANK: -- if it can show it's not inhaled then --

SPEAKER: Unless someone shows (off mike).

DR. MARKS: So we'll issue a tentative safety --

DR. HILL: And I'm answering a question while you're writing sort of from this morning is -- and I'm thinking in particular of hairspray formulations where there -- the amounts are small anyway. I understood you to say that as manufactured -- according to your knowledge -- there are large enough aggregates that even assuming that whatever

liquid accompanied the droplets evaporated before somebody inhales this, that the particle sizes are still too large to go any farther than the trachea. So I --

DR. HATHAWAY: Correct. When they're -- when they're in a solution -- whether it's aqueous or a combination of other solvents or whatever -- it's not going to disaggregate.

DR. HILL: So then my question became at least based on your knowledge of the companies that are manufacturing this stuff, that are in products available to Americans at least, that there are no nanosize particles -- anything smaller than four microns that are fines -- what we always called fines working with silica in the lab -- in products as they are manufactured, but the finished products -- the hairsprays and such.

DR. HATHAWAY: We ran it by -- you know -- the companies on both sides of the Atlantic. Initially we had down there 16 to 100 microns because that's pretty much what all the people on this side had and they recommended we drop it to 10, because I guess they must have some products that are down closer to 10.

DR. HILL: Okay. Okay.

DR. HATHAWAY: But -- you know -- we checked with -- you know -- the eight companies are the same companies on both sides of the Atlantic. They may have different plants and have -- you know -- slightly different product mix, so we certainly checked with all of the European and the North American manufacturers.

MS. BECKER: Could we get a letter or memo saying that so I can put it in the document?

DR. PAVLICH: It's in the -- it's in our summary. Ten to 100 was quoted in that summary.

MS. BECKER: Okay. Thank you.

DR. MARKS: Okay. Any other comments? We'll issue -- we will move -- I will move since I'm the one that will be presenting this -- issue a tentative safety assessment with a finding that aluminum magnesium (off mike) aluminum calcium, sodium silicate, hydrated silica and a sodium potassium aluminum silicate are safe for use in cosmetic ingredients. And that the aluminum iron silicates -- there's insufficient data and we need the inhalation data to decide whether that's safe. And, Ron, if there's any discussion --

DR. SHANK: Unless it's in the 10 to the 100 --

DR. MARKS: Well, that would be essentially the --

DR. ANSELL: Yeah, that would be formulated to be nonrespirable.

SPEAKER: Do we have anything you want in the discussion other than inhalation?

DR. SHANK: Did Lillian catch what we just said?

DR. ANSELL: And formulated to be nonrespirable.

MS. BECKER: Yes. Writing it down.

DR. MARKS: And we can use the same words as they used in the 2004 report.

SPEAKER: Just finishing silica.

MS. BECKER: For which?

DR. SHANK: In the discussion for this document, you can just use the discussion on inhalation with cosmetic sprays --

MS. BECKER: Okay.

DR. SHANK: -- that we used in 2004 --

MS. BECKER: Okay.

DR. SHANK: -- which was the potassium and (off mike).

MS. BECKER: Alright. That works for me.

DR. MARKS: Okay. Any other?

SPEAKER: Anything else?

DR. HATHAWAY: Just to say that although I mentioned hairspray, face powders is in here. So this is a totally theoretical question.

SPEAKER: Alright.

DR. MARKS: Not really. Thank you very much for your patience and comments.

SPEAKER: Thank you.

SPEAKER: Thank you.

MS. BECKER: Thank you.

SPEAKER: Very helpful.

DR. MARKS: This morning and also right now. Thank you.

DR. PAVLICH: Good. Well thank you for having us.

Full Panel Meeting:

DR. BERGFELD: We're going to move forward then. This (off mike) this table to answer the questions that have been so stated, and we'll be moving on to the next group, Dr. Marks presenting on silica and silicates.

DR. MARKS: In the March meeting of the CIR Panel, a scientific literature review was announced, and we're now seeing the draft report on silica, alumina magnesium metasilicate, aluminum calcium sodium silicate, aluminosilicates, hydrated silica, sodium potassium aluminosilicate. And we had the presentation yesterday by the SASSI group clarifying the difference between synthetic amorphous silica, which is used in cosmetics and other forms of silica, and based on the information that we reviewed, we move to issue a tentative safety assessment that has the ingredients safe with the exception of aluminum iron silicates. We move that that be insufficient data, because of concern about inhalation toxicity.

DR. SLAGA: Unless it's (off mike).

DR. MARKS: Ron can -- well, that would be the inhalation data. If it's not respirable, then it's not an issue.

DR. BERGFELD: Any other -- that's a motion?

DR. MARKS: Yes.

DR. BERGFELD: And there's no other comment on the motion? Second? Second, Ron? Discussion?

DR. BELSITO: Respirable? We have that boilerplate. How would it be respirable?

DR. MARKS: We don't know. We can consider that it's not.

DR. SHANK: I worry about the -- or I have concern about the iron atom going into the lung, high-oxygen environment. There could be oxidative damage, which would not expect that the other silicates --

DR. BELSITO: But I --

DR. SHANK: If not, we can say this is formulated (off mike). That takes care of the issue. But we don't have that information.

DR. BELSITO: I thought the information we have was that cosmetic formulations -- the particle size was such that it's in a pump or a spray it's not respirable.

DR. SHANK: Okay, but this one doesn't have a stated use, does it?

DR. BELSITO: But how would it be used as an aerosol other than as a hairspray?

DR. SHANK: Don't know. Lack of information is not proof of safety.

DR. BELSITO: But -- then I just --

DR. BERGFELD: Alan?

DR. ANDERSEN: We did have the information yesterday from the synthetic amorphous silica group that said the particle size of the amorphous silica material is between 10 and 100 microns in diameter, so independent of what happens to it after that, the particle size as produced by the suppliers is already of a size to be not respirable.

DR. BELSITO: Correct.

DR. ANDERSEN: From that point, it doesn't matter what formulation it goes into. Not much can happen beyond that, and in order to conduct the inhalation toxicity studies that were described, further unnatural reduction particle size had to be done, but that doesn't represent what's actually on the market from the suppliers. So, we could rely on that information that was presented to make the assertion that in fact the panel does not expect that these particles are respirable and put that burden on the industry for all of the amorphous silica, including the iron one. So, it would be a way to assert the panel's expectation of non-respirable.

DR. BERGFELD: So, you're going to amend?

DR. MARKS: I'll retract the previous motion with that clarification, and that being captured in the discussion so that all these silica cosmetic ingredients would be safe and that we issue a tentative safety assessment of that conclusion.

DR. BELSITO: Second.

DR. BERGFELD: Second. And with the assumption that the discussion will take the place or support your worry.

DR. MARKS: Yeah.

DR. BERGFELD: Okay. Any other discussion? John?

DR. BAILEY: Yeah, at yesterday's team meeting I emphasized the importance of stating clearly in this document the synthetic amorphous silica as the material that's used in cosmetics, because we have a lot of confusion inquiries coming in that rather it's crystal and we're amorphous and we'd like to be able to use this document to clear that up both for people who have concerns in the public but also for the users of the ingredients so that that's clearly communicated to them what they're supposed to do.

DR. BERGFELD: I think that I sat on Don Belsito's team and he discussed that and which to do with that, did you not?

DR. BELSITO: Yeah, a very strong statement up front in the introduction going over basically that slide of silica production and where we are and that this is not crystal and then the amorphous, so I think Dan had -- Did you actually beef up your introduction, or --

DR. LIEBLER: I provided comments.

DR. BELSITO: Right.

DR. LIEBLER: But I think it's most important to -- because some of the original literature refers to silica forms -- for example, as fume silica -- and that there's a preferred term now, pyrogenic silica, for that, but there needs to be a very clear sort of glossary in the introduction section to provide the reader with some guidance as they go forward in the report, because we did have some discussion about whether to change in the body of the report reference to fume silica -- change that to pyrogenic -- but that would be essentially, as Alan pointed out, replacing -- revising the literature inappropriately, and I agree with that, so a glossary up front that clarifies the terminology for names and points out where you're going to have confusion between silica fume and fume silica, for example.

DR. BERGFELD: Paul, any comment? Greg? Coming over here. Don? Ron? Rob? Jim? Is there any other comments? Okay. Motion has been placed that this ingredient is safe and discussant points have been added, so all those in favor please raise your hand for a safe review. Thank you very much. It's a unanimous vote.

September 24-25, 2009

Belsito's Team Meeting:

DR. BELSITO: So now we get into the silica and the silicates (off mike). Okay, so where are we here? Silicates. Okay. So, we got -- is this a handout from today from Sassi?

MS. BECKER: You got that in the e-mail.

DR. BELSITO: In the e-mail. Oh, I printed out an e-mail. Okay.

MS. BECKER: Yes.

DR. BELSITO: I must have addressed it. Okay. I thought, Lillian, you did a great job and I really thought that Figure 1 and 2 were really great in this report. And I think it really addressed concerns of our team, at least particularly Dan's concern of getting everything up front and making it clear. I like the way you've boxed that out in Figure 1.

DR. LIEBLER: Yeah.

DR. BELSITO: It was really a superb way of handling that to show exactly where we're focusing.

DR. LIEBLER: One little note on that on Figure 1 -- so I completely echo Don's praise for your work on this -- but one little thing I would change is under the box where you've got -- where it says the types of silica in this safety assessment, and it's got a little box around that -- that that actually kind of hides that because everything else in the figure has a box around it. And that's actually a message that you want to stand out and putting the box around it makes it blend in. So what I would say is take the box out of it and then use a bigger font and italicize it just so it --

DR. BELSITO: And put it into the box maybe even.

DR. LIEBLER: Or right under the box or, yeah, lower the box a little bit and stick that in the box. Yeah, that's a good idea.

MS. BECKER: Yeah. That's what I'm drawing right now actually.

DR. LIEBLER: Okay. But that's just a tweak. It's very nice. Huge improvement.

DR. BELSITO: On page 17, under parenteral silica one, two, three, four, five, six lines down, it says lymphocytes were less numerous and new.

MS. BECKER: Okay, few. Probably -- yeah, few. Probably something (off mike) said.

DR. BELSITO: So if it's less numerous and few, then you don't even need few.

MS. BECKER: Yeah.

DR. BELSITO: Just less numerous.

MS. BECKER: So we will check that.

DR. KLAASSEN: Probably don't need numerous either. There were lots of lymphocytes.

MS. BECKER: There's some pretty weird wording on some of these.

DR. BELSITO: Page 29. Things were moved around so perhaps I missed it, but in the prior document there was an ECETOC 2006 report of two subchronic oral and toxicity studies that I couldn't find again.

MS. BECKER: If I remember correctly, a couple of short-term -- I'm sorry, long-term and chronic got moved around just because of dates, but I don't -- did you check to see if it's just in a different time section?

DR. BELSITO: I tried to do that and I couldn't find it, but, I mean, it's entirely possible. The reference though is gone, at least as an ECETOC 2006 reference, so I'm wondering if someone recommended it be deleted or was it, in fact, maybe published under a different title?

DR. BRESLAWEK: Here's ECETOC 2006 on 31.

MS. BECKER: There was also a couple that were thought to be duplicates of the other large document I had and we picked one or the other.

DR. BELSITO: Okay.

MS. BECKER: So that might be --

DR. BELSITO: So it may be --

MS. BECKER: It might be under UNEP instead.

DR. BELSITO: Okay. But then that ECETOC 2006 reference doesn't occur in your references.

MS. BECKER: I see what you're saying.

DR. BRESLAWEK: If you look at the reference there's nothing that says ECETOC.

DR. BELSITO: And there are some ECETOC on page 31 that refers to some 2006 unpublished studies, which are different from the studies that I was talking about.

MS. BECKER: European Centre for Ecotoxicology and Toxicology of Chemicals.

DR. BELSITO: Okay.

MS. BECKER: On page 57. It's in the references.

DR. BELSITO: There it is. ECETOC 2006. Sorry, I stand corrected.

Okay. On page 35, the fifth line up the bottom, starting from the line above that it says although there was a trend of more frequent incidence in those exposed to pyrogenic silica, it was obscured in some control animals. Interstitial fibrosis was associated with yadda, yadda, yadda. And some of the rats of the control treatment groups, although there was a trend to more frequent incidence in those exposed, but was obscured in some control animals. I'm assuming it wasn't significant because it was seen in control animals or --

MS. BECKER: If I remember correctly, yes.

DR. BELSITO: I just think that needs to be stated a little bit more clearly.

MS. BECKER: You got it.

DR. BELSITO: Page 54, the third paragraph, silica subcutaneously instilled in humans. Next sentence, the cells --

MS. BECKER: I'm sorry?

DR. BELSITO: -- invaded blood vessels. Invaded blood vessels?

MS. BECKER: Page 54?

DR. BELSITO: Page 54.

MS. BECKER: Third paragraph?

DR. BELSITO: From the bottom.

MS. BECKER: Oh, sorry.

DR. BELSITO: Silica subcutaneously instilled in humans caused granulomatous inflammation with seven days and persisted for months. The cells invaded blood vessels?

MS. BECKER: That was --

DR. BELSITO: Invaded blood vessels?

MS. BECKER: Something like that would have been stolen wording. Yes, that was wording from Epstein 63. That's 47 and that would have been his wording. That's not something I would have picked up, but I try not to interpret too much. Is that 47? Epstein 63.

DR. LIEBLER: Just strike the whole sentence. It doesn't add anything.

MS. BECKER: Okay.

DR. BELSITO: Okay. In our conclusion, do we really need to isolate aluminum iron silicates?

MS. BECKER: That was Dr. Shank and his concern about the iron.

DR. BELSITO: I understand and I remember the discussion, but we decided that it wasn't going to be (off mike) even in the current form that it was used. So do we need to put that in the conclusion or just the discussion? I mean, I think, you know, its ingredients and practice of use in concentration as described in the safety assessment is sufficient. If there's any concern that could go in the discussion that the size of these particles, irrespective of how they would be used that was captured in the minutes, would not be respirable.

Beyond that, we know that the way pumps and sprays are formulated it wouldn't be respirable either. But as Alan pointed out at the last meeting, you literally would have to break down the silicates in order to make them of a size where they would be respirable. So, taking that out and putting it into the conclusion I think is a bit much.

MS. BECKER: Okay.

DR. BELSITO: I would just move that to the discussion if there's any concern at all.

DR. LIEBLER: So simply in the conclusion --

MS. BECKER: Just the first sentence.

DR. LIEBLER: -- just use the first sentence and then add aluminum iron silicates to the first sentence.

DR. BELSITO: Exactly.

MS. BECKER: Got it.

DR. BELSITO: The last thing that I couldn't find and maybe you can tell me where it was is -- and maybe it was decided to get rid of it -- but in the old document there was a statement about natural silica levels in rabbits. And I couldn't find where that was moved to, but the reference was retained.

MS. BECKER: Do you have the reference offhand?

DR. BELSITO: Yes, Ammon and Moan, 1959.

MS. BECKER: Ammon with an A?

DR. BELSITO: A-M-M-O-N.

MS. BECKER: I think that was marked as not necessary.

DR. BELSITO: I would agree that it's not necessary. Then we just need to delete the reference if it's not in the document. I mean, just check because -- I mean, you could just do a quick word search and see if it pops up someplace in the document other than the references.

MS. BECKER: Okay. That's going to be my major task this weekend actually.

DR. BELSITO: I hope not this weekend.

MS. BECKER: Oh, yeah.

DR. BELSITO: I think that's all that I had. So before we address the Sassi comments, any other comments?

DR. LIEBLER: Page 2 and 3, I recommend a couple of additional tweaks. Just moving sections to make the presentation more logical in terms of the flow.

So, on top of page 2, you have Chemistry, major heading, then subhead Definition and Structure, and then Amorphous versus Crystalline Silica. You have two paragraphs. Then you've got Silica, which is really the very most introductory information about silica. And I suggested moving that stuff there that's subtitled Silica, beginning with the CAS Number 7631, all the way through the top of the next page where it says, "The current terminology for silicon dioxide fumed is pyrogenic silica." That whole chunk, move it up between Definition and Structure and Amorphous versus Crystalline Silica. And I pointed -- I drew it on my copy for you.

MS. BECKER: Okay. All right. We're changing a lot of things as CIR, but normally we keep all of the definitions of all the ingredients together. Would that -- I'm just asking if that -- separating silica out separately from the aluminum magnesium, metasilicate, et cetera, would that be confusing?

DR. LIEBLER: I don't think so because the way you have it now you begin by talking about amorphous versus crystalline silica. And then at the bottom of page 2, you start out by explaining what silica is. It seems like you should start out by explaining what silica is and then get into the distinction between amorphous versus crystalline. It just seems more logical to me.

MS. BECKER: Okay.

DR. LIEBLER: And so you can move that section up to the top. And then you also have, at the bottom of page 3, you have the section on hydrated silica. That can go right after amorphous versus crystalline silica. And then you get into the aluminum magnesium salts and the silicates.

MS. BECKER: Okay.

DR. LIEBLER: And so that way you're doing pure silica first, then the salts, to just introduce the chemistry.

MS. BECKER: Okay.

DR. BELSITO: Except I guess the only issue that I'd have with that, Dan, is that we're doing -- you know, we're looking only at the amorphous. So then you would have amorphous and then you'd mix in amorphous with crystalline and then go back to the amorphous forms. That could be confusing. So that I guess if you wanted to move things around would be to move the amorphous and crystalline to the end of the whole thing and list the things that we're discussing first and then making the point of the difference between amorphous and crystalline at the end.

MS. BECKER: That would be more my inclination, but.

DR. LIEBLER: So, instead of moving the things I moved, just take amorphous versus crystalline and move it to the end?

DR. BELSITO: Yes.

DR. LIEBLER: I'm fine with that. That accomplishes the same thing. I just thought that you have amorphous versus crystalline at the top of the description of all the silica and silicates and it was premature to address that at that point. So, Don's suggestion takes care of that as well and I agree with it.

MS. BECKER: Okay.

DR. SNYDER: I had some issues with the nomenclature again. It's just really confusing because on page 5 we introduce silica gel and precipitated silica for the first time. And then on page 6 we introduce colloidal silica. And on page 8 we bring in the sodium metasilicate, hydrated silica, and silica solution. So I was a little confused as to where those all --

DR. LIEBLER: So under hydrated silica, Lillian has these bullets of synonyms, and silica gel and precipitated silica are listed there. So the reader will have encountered those definitions before they got to where you're concerned about.

MS. BECKER: Right.

DR. LIEBLER: And colloidal silica, is that one here?

DR. SNYDER: Yeah, page 6.

DR. LIEBLER: Page 6.

DR. SNYDER: On the third paragraph on down, silica sols, colloidal silica.

DR. LIEBLER: Does colloidal fall under one of these? Lillian, do you know?

MS. BECKER: I thought it was under hydrate.

DR. LIEBLER: I want to double-check that. If it can be defined there, that's a good place to put it if that's correct.

MS. BECKER: The issue was that through the literature the naming conventions are not consistent. And unless they gave me something that said I can identify it as exactly what we have as our definition, I used the terminology of the author.

DR. LIEBLER: So in Table 1 with the box around the forms that are defined in the safety assessment on page 61, you have silica gel or colloidal silica.

MS. BECKER: Right.

DR. LIEBLER: So the reader will have seen this figure at that point. It's just that colloidal silica isn't listed under the bullets that you have on pages 2 and 3.

MS. BECKER: Right. Yeah, and what I just explained is also in the introduction that I did not guess what the authors were trying to say.

DR. LIEBLER: Okay.

MS. BECKER: Unless they gave me real evidence.

DR. LIEBLER: Right.

DR. BELSITO: So colloidal silica is silica gel?

MS. BECKER: Yes.

DR. BELSITO: And silica gel is hydrated silica?

MS. BECKER: Pretty much.

DR. LIEBLER: Right.

DR. BRESLAWEK: So on page 3, you said include that in the bullets there?

DR. BELSITO: Under hydrated silica.

DR. LIEBLER: And colloidal silica.

MS. BECKER: So that -- well, okay, but that's another reference, so that would be slightly different.

DR. BELSITO: But you could add it just so it's clear and just put that reference so we know where each of them falls.

DR. LIEBLER: I mean, there must have been a basis for in Figure 1 including colloidal silica with silica gel.

MS. BECKER: Right. Right.

DR. LIEBLER: So that would presumably be the same reference.

MS. BECKER: Yes. That's what ARTS did. Yes.

DR. LIEBLER: Okay.

DR. SNYDER: And then there's in the nomenclature you get all the way to page 19 and we start talking about ultra fine and then fine silica. And we haven't defined that (off mike).

MS. BECKER: And neither did the authors.

DR. LIEBLER: I'm sure it's just particle sizing.

DR. SNYDER: So then for this use, does this have an aerosol use?

DR. LIEBLER: You know what? I'm sorry, just to -- fine versus ultra fine, on page 5, under Particle Size and Form, we've got amorphous silicas are composed of very fine particles, average 20 microns. Very fine, ultra fine, fine.

SPEAKER: Super fine.

DR. LIEBLER: Super fine.

SPEAKER: The point comes into question that we do have data here that says that the sum of the particles are respirable size, certainly the .01 to .1 micron diameter particles.

DR. LIEBLER: I don't know if there's a standard nomenclature of, you know, fine, ultra fine, very fine, that actually corresponds to giant particle diameter ranges. It might be something to look for and see because you list very fine in a way that just might mean it's sort of a kind of ordinary colloquia descriptor as opposed to whether or not very fine

means a particular size range. And if there is any definition in the literature that assigns the term "fine," "very fine," "ultra fine," the size range, this would be a good place to put it. This would be the ideal place to put it. So if there is any additional information you could find that would put it there, that would be useful there.

MS. BECKER: Okay.

DR. LIEBLER: I mean, I realize this whole area is a mess, but, you know.

MS. BECKER: Right. And it's a 1961 reference, so.

DR. LIEBLER: Yeah. I'm not sure where you could ask, but someone might be able to point you in the right direction. I forget who was here last time that made -- the Sassi people, I guess, you know, provided some input on -- some clarification on the nomenclature and forms. They may know something or be able to point you in the direction on sizing nomenclature. If there is any and if it's referred to in the types of particle study, it probably should be up front in this report.

MS. BECKER: Okay.

DR. KLAASSEN: On page 5, about the fifth or sixth line, it says there that very fine particles had an average of 20 micrometers.

DR. LIEBLER: Yeah, that's what I was pointing to. Yeah. Yeah. So I'm assuming fine is more than 20 micrometers and ultra fine is less.

MS. BECKER: Well, I think the phrase that might solve all of it, is right after the 20, is "which tends to aggregate loosely in the air." So something that size doesn't exist very long. It adheres onto others and makes larger particles.

DR. BELSITO: And I think that's what we were told before.

MS. BECKER: Yes.

DR. BELSITO: And then going on it says aggregates assemble in chains, fumed or clusters precipitated in gel. Agglomerates are assembled -- assemblies of aggregates held together by strong physical adhesion forces and not in a dispersible nano-size less than 100 nanometers.

MS. BECKER: I think that kind of solves it.

DR. BELSITO: So the concept of very fine is a laboratory concept, not a real concept in nature?

MS. BECKER: At least not in the air.

DR. BELSITO: At least not as it would be formulated into a cosmetic product. I seem to remember them telling us that, too. They rapidly sort of adhere together.

DR. LIEBLER: I was just looking for a way to address Paul's question about whether --

DR. SNYDER: I mean, it was deep in the document and all of a sudden this popped up. And I thought if we could pop in appropriate information to define that, that would be useful. If any of the subsequent literature refers to particle size and distinguishes effects on the basis of anything having to do with particle size, then I think we need to deal with it.

DR. BELSITO: But then we could put -- we could move that issue of the aggregation of these very fine particles into the discussion as well since there is a hairspray use. And I see that the panel noted data on the use of very fine, fine molecular structures, average of 20 microns.

SPEAKER: (off mike) they are in respirable range of diameter.

DR. BELSITO: Right. But it is our understanding that these aggregate into chains, fumed or clusters, precipitated in gel to particle sizes that would not be respirable in cosmetic formulations.

DR. KLAASSEN: In general, to get things down into the alveoli you want to have it between 1 and 10. Larger than 10 they don't get to the alveoli very well and if they're smaller than 1 they don't settle in the alveoli. They just blow them back out again. So, even at this 20 microns here they're relatively safe as far as getting them into the alveoli. You still have them in the bronchi, et cetera. So, what Don said I agree with. This just gives us even further protection.

DR. SNYDER: We have a statement on page 53 in the fourth paragraph, the last sentence, related to -- in relation to monkey status, it says the frequency and the size of the cell aggregates vary with the type of silica precipitated in greater (off mike) and greater than gel. So that's what we should capture there.

DR. HOWARD: It is in the discussion -- I mean, the summary. So you want that clearly in the discussion?

DR. SNYDER: Well, I mean, I think that's just some more data --

DR. BELSITO: Well, I think we did, particularly now that we've moved the aluminum iron silicates out of the conclusion. We're going to make mention about it in the discussion anyway. So then we could just expand upon it a little bit if the panel noted data on use of very fine silicas, average molecular size 20 microns. However, we noted that these tend to aggregate into -- help me.

DR. SNYDER: Tend to form aggregates.

DR. BELSITO: Tend to form aggregates of a size that would not be respirable.

DR. LIEBLER: But is the passage you're referring to, Paul, is that referring to the silica particles or cells aggregating? Because it says the frequency and size of the cells aggregates.

DR. BELSITO: Oh, oh.

DR. LIEBLER: That's why I'm reading.

DR. SNYDER: Oh, I see. Yeah.

DR. LIEBLER: I'm trying to see if there's anything that's being said about clumps or aggregates of, like, lymphocytes.

DR. SNYDER: No, I mean, to me (off mike) the other way. I read it that it was the aggregates as in aggregates of silica. I mean, I guess there's nothing in that paragraph to suggest otherwise, is there?

DR. LIEBLER: So I'm just wondering what that actually refers to. Because the preceding paragraph refers to considerable cellular infiltration of the alveoli and the alveolar septa.

DR. SNYDER: There's nothing about aggregation there.

DR. LIEBLER: And with the extension and accumulation of acetate and macrophages. See, that could easily be referring to clumps of macrophages, perhaps. That's how I would read that. So maybe check that language there.

DR. KLAASSEN: And if true, then this sentence should go up in the other paragraph.

DR. LIEBLER: Yeah.

DR. BELSITO: So where are you moving this, Curt?

DR. KLAASSEN: Well, into the previous paragraph. It really has to do with macrophage. If it really has to do with cells and the aggregation of cells, then it probably is more appropriate in the previous paragraph. But we, first of all, need to make sure what's going on here.

MS. BECKER: It's the study on page -- it starts at the very bottom of 35.

DR. SNYDER: Yeah. It's macrophage and (off mike) aggregate. So just change that to "Frequency and size of the inflammatory cell aggregates varied with the type of silica," and move it up to the previous paragraph, to the paragraph that begins, "Rabbits and (off mike)."

DR. BELSITO: But these weren't rabbits; these were monkeys.

DR. SNYDER: Oh, so the monkeys then.

DR. BELSITO: So that's the paragraph above it?

DR. SNYDER: Yes.

MS. BECKER: These are two different experiments. I'm sorry. Say what you want to change again.

DR. BELSITO: The paragraph above is a different study.

MS. BECKER: Right.

DR. BELSITO: These are two different studies in monkeys. So that paragraph has to -- I mean, you can't move it anywhere.

DR. SNYDER: Okay. All right.

DR. BELSITO: The monkeys were exposed to different types of silica. The precipitated silica had lower lung volumes. No change in parameters, ventilatory performance, mechanical parameters, dynamic lung compliance and FEP. When exposed to silica gel, the frequency and size of cellular aggregates varied with the type of silica.

DR. SNYDER: I would just change that sentence. So the frequency and size of inflammatory cell aggregates varied with the type of silica.

DR. BELSITO: Okay.

MS. BECKER: Okay.

DR. BELSITO: So the frequency and size of inflammatory cell aggregates varied with the type of silica. Okay.

DR. SNYDER: That'll do it.

DR. BELSITO: Okay. Industry comments from Sassi, page 53. It says: In our opinion the following statement on page 53 does not accurately describe the commercial pyrogenic process used to manufacture synthetic amorphous silica. Amorphous silica is the product of a high heat process applied to crystalline silica. The contemporary pyrogenic process is accurately described on page 6 of the report and should be substituted for the description on page 53.

I'm just reading what was sent out to us.

DR. SNYDER: I can't make it out.

MS. BECKER: Maybe 6 and 3?

DR. SNYDER: It's on page 50, actually.

MS. BECKER: Page 50.

DR. BELSITO: They may be referring to the old report. I don't know.

DR. SNYDER: Here it is on page 50, the second paragraph. (off mike) pyrogenic silica is a product of high heat process applied to crystalline and silica.

MS. BECKER: You're on 50?

DR. SNYDER: Page 50.

DR. BELSITO: Fifty, the first and second paragraph.

DR. SNYDER: Second paragraph, first line.

DR. BELSITO: So --

MS. BECKER: You want to get rid of that sentence?

DR. BELSITO: Well, no. They're saying that it's not the way it's produced. The way it's produced is --

DR. SNYDER: On page 6.

DR. BELSITO: What we said on page 6: Precipitated silica and silica gels are produced from an alkaline metal silicate dissolved in water and then acid, usually sulfuric.

DR. LIEBLER: No, they are actually probably referring to the bottom of page 5 on the current report, amorphous pyrogenic silica.

DR. BELSITO: Yes. Okay.

DR. LIEBLER: So here it says, on page 5, the bottom of our current report, it says, "Amorphous pyrogenic silica is manufactured by the hydrolysis of volatile silanes, usually silica and tetrachloride, in the flame of an oxygen hydrogen burner."

And then page 50, second paragraph of our second report, it says, "Amorphous pyrogenic silica is the product of a high heat process applied to crystalline silica."

MS. BECKER: Right. Which is of that.

DR. BELSITO: Right. So what they're saying is that's not -- the contemporary pyrogenic process is the process currently described on page 5.

MS. BECKER: Right. The rest of the paragraph, I think --

DR. LIEBLER: We agreed.

MS. BECKER: So we just want to get rid of the --

DR. BELSITO: You want the amorphous pyrogenic silica, you just want to --

DR. SNYDER: Capture from page 6.

DR. BELSITO: -- capture from page -- no, 5.

DR. LIEBLER: Yeah, right. So the sentence on page 50, the first sentence of the second paragraph, amorphous pyrogenic silica through -- applied to crystalline silica, delete that sentence and in its place copy the sentence from the bottom of page 5, "Amorphous pyrogenic silica through oxygen hydrogen burner." It's the same definition word for word from the bottom of page 5 instead.

MS. BECKER: Thank you.

DR. BELSITO: Okay. The next comment from Sassi is we noted in our earlier comments the lack of differentiation between silica fumes and the commercial product called fumed silica, i.e., pyrogenic silica, leads to a clear misunderstanding of the significance of the statement on page 8 -- may be different -- regarding the high level of crystalline silica impurity 6 to 8 percent noted in the Swensson 1971 study.

DR. SNYDER: On page 7, first sentence.

DR. BELSITO: Since silica fume is a commercial product not classified as synthetic amorphous silica, we recommend deleting this reference on the basis of irrelevance. So, that's now on page 7.

DR. SNYDER: First sentence, (off mike) composition of the fumes.

DR. KLAASSEN: So you want to eliminate the first paragraph? Is that what we're talking about?

DR. LIEBLER: Maybe the first sentence.

DR. BELSITO: The first sentence.

DR. LIEBLER: Swensson, et al., through courts.

DR. BELSITO: You have the Cabot Corporation. They're not saying anything about --

DR. LIEBLER: So that's two (off mike).

DR. BELSITO: So it would just be, "Cabot Corporation 2004 states that its silicate products are greater than 99.8 percent pure." The moisture content, yeah, treated silicas are susceptible to.

Okay. Next comment from Sassi. On page 3, two references to Spiron as a technical name for silica are noted. Our members are not aware of this technical name and suspect it is a trade name.

MS. BECKER: For silica?

DR. BELSITO: That may have already been removed because I'm not seeing Spiron here anywhere.

SPEAKER: I don't see it.

MS. BECKER: You have that little table somewhere (off mike)?

DR. BELSITO: No, it would have just -- it would have been on the page. I mean, maybe it was in the old report and it's already been deleted. Again, this letter seems to be addressing the old report and not --

MS. BECKER: Yeah, because they would have got the version that I produced right after the last panel meeting.

DR. BELSITO: Okay.

MS. BECKER: It's been edited since then one more time before you got it.

DR. BELSITO: Okay. What you may want to do, Lillian, again, just do a word search for "Spiron." Make sure that it's been deleted.

Okay. Page 24, a reference to a UNEP 2004 study mentioned the LC50 of 691 milligrams per liter. We believe the greater than symbol was omitted in error on the LC50, so.

SPEAKER: On page 21? Under inhalation, third paragraph?

DR. BELSITO: Okay. So you need to check and be certain that they're correct, that the LC50 was greater than 691.

MS. BECKER: Sure.

DR. BELSITO: Okay. In the discussion we noted that the last sentence to the paragraph was incomplete. It appears a word may have been omitted. I didn't notice a word omitted, so this may again --

DR. LIEBLER: Last sentence of which paragraph?

DR. BELSITO: It just says, "in the discussion session section on page 50A." Well, it's not relevant anymore. We noted that the last sentence of the paragraph was incomplete.

MS. BECKER: Okay. Okay, it wasn't quite the last sentence. My guess is that the whole section was completely removed.

DR. BELSITO: Right. Okay. That was it from the silica council.

DR. SNYDER: They changed that sentence anyway. So instead of saying no pursuant silica is used -- to the panel determined that silicosis is not an issue since crystalline silica is not an ingredient used in cosmetics.

MS. BECKER: Okay.

DR. BELSITO: Okay.

DR. LIEBLER: I had a -- again, in the front on pages 4 and 5 of our current document, the use of subheads under, for example, Physical and Chemical Properties and then Properties.

MS. BECKER: I'm sorry. Where?

DR. LIEBLER: On page 4, Physical and Chemical Properties. And then you have Properties and then you have the subhead Silica. And then there's no other compound like silicates referred to.

I think when you don't have any other compound referred to, you can just delete the Silica subheading. I made a few notes like that, but then I stopped. I think it's a question if there's going to be silica and then you're going to do aluminum magnesium silicate, then you have the subheads for each. Otherwise, you just delete silica subheads.

MS. BECKER: Okay.

DR. BELSITO: Except that I find it helpful because then you know exactly what information you have under that particular heading. You can quickly, very visually see it rather than -- I know what you're saying.

DR. LIEBLER: Yeah.

DR. BELSITO: It's like you look at properties and the only properties we're going to get are on silica. It's not going to be on something else.

DR. LIEBLER: Okay. I'm an editorial slasher by nature, so.

DR. KLAASSEN: You can have silica property.

DR. BRESLAWEC: May I suggest that we do whatever the JAMA format requires us to do in terms of the IJT publication on that?

DR. BELSITO: Okay.

DR. BRESLAWEC: We'll follow the guidance there.

DR. BELSITO: Sure. Okay, good. That sounds reasonable. Anything else on this silica?

DR. LIEBLER: When judgment fails, fall back on policy.

SPEAKER: What?

DR. LIEBLER: When judgment fails, fall back on policy.

DR. BELSITO: Okay. So no other comments. We'll move to sodium and potassium bromate.

MS. BECKER: So we're going final?

DR. BELSITO: We're going final. And I think the change moving the aluminum iron out of the conclusion is really editorial. I mean, it's not substantive so I don't think we need to send it out again.

Marks' Team Meeting:

DR. MARKS: You're welcome. Next is silica and silicates. We have in front of us a "Tentative Report of Silica and Related Ingredients." There were comments from industry. There's a September 3 letter from SASSI, the Synthetic Amorphous Silica and Silicate Industry Association, who characterized their comments as being relatively minor. The conclusion is that these ingredients are safe as cosmetic ingredients, that aluminum iron silicate is safe as a cosmetic ingredient in the practices and uses described in the safety assessment when formulated to be nonrespirable, and I think there are some potential comments about that. Ron Shank?

DR. SHANK: I think the conclusion is okay. The SASSI suggestions I agree with to put in as they have requested. There is a UNEP report of 2004. On page 21 SASSI refers to the LC50, we say 0.691 and SASSI says it should be less than 0.691. That can be checked by going to the UNEP report. On page 45 under "Clinical Assessment of Safety," it says that the oral lethal dose is 15 grams per kilogram. That would be over 1 kilo per person, so that there is something wrong there, and it really doesn't add anything. I would just throw it out. It's an FDA comment or something. With due respect to FDA, I don't think that could possibly be correct that the oral lethal dose is 15 grams per kilo. It would be pretty hard to take a kilogram for an adult. Those are my only comments.

DR. MARKS: Lillian will capture those then. I'm not sure we need to mention that tomorrow unless you feel we need to.

DR. SLAGA: Right.

DR. MARKS: Are there any other comments? Ron Hill?

DR. BERGFELD: I'd like to make a comment that I thought it was nicely reorganized and redefined so that we were not as confused in reading it. Thank you.

DR. MARKS: I suspect tomorrow that our team will be seconding a motion that a final report be issued with the conclusions as stated on page 55, that these are safe and with the proviso that aluminum iron silicates are not respirable. Let's take a break for 5 minutes. You have more comments? After your comments, Jay.

DR. ANSELL: Just on the wording, this is the first report we hit where we put in this "were ingredients in this group not currently to be used in the future. The expectation is that they be used in product categories and concentrations comparable to others in the group," is a little tortured.

DR. MARKS: We've discussed that at some time in the past, Jay. Do you have a proposal to make it clear and less tortured?

DR. ANSELL: No, not right now.

DR. MARKS: When you come up with the proposed change, let us look at it. I know we all worked on it, and Alan particularly.

Full Panel Meeting:

DR. BERGFELD: We're moving on to "Silica," Dr. Belsito.

DR. BELSITO: Yes, at the last meeting we issued a tentative safety assessment that these ingredients -- silica, aluminum magnesium metasilicate, aluminum calcium sodium silicate, hydrated silica, sodium potassium aluminum silicate -- are safe as cosmetic ingredients in the practice of use in concentrations as described in the safety assessment, and put a caveat in that conclusion that aluminum ion silicate is safe as a cosmetic ingredient, the practice of use in concentration as described in the safety assessment when formulated to be non-respirable.

We thought that we would like to make a minor editorial change in that conclusion and just put aluminum ion silicate into the list of other silica products that are safe as used and move the discussion of the ability of these particles to be inhaled, which essentially they cannot be because of their size -- they tend to aggregate into large molecular sizes -- into the discussion rather than putting it into the conclusion.

DR. BERGFELD: And that's a motion.

DR. BELSITO: That's a motion.

DR. BERGFELD: Is there a second? Second, Paul? Discussion? Ron?

DR. SHANK: That's okay.

DR. BERGFELD: It's okay? Any other discussion?

DR. BELSITO: Yeah, in the discussion itself just putting -- stressing that these do tend to aggregate into larger molecular weight particles in formulation, stressing that a little bit more.

DR. BERGFELD: Any other discussion? Seeing none, I call the question. All those in favor of this conclusion please indicate by raising your hands. Unanimous. No abstainers. Okay.

Safety Assessment of Silicates as Used in Cosmetics

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The 2018 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.

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INTRODUCTION

The CIR Expert Panel (Panel) previously reviewed the safety of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite in a report that was published in 2003.¹ The Panel concluded that these ingredients were safe as used in cosmetic products. In accordance with its procedures, the Panel evaluates the conclusions of previously-issued reports every 15 years, and it has been at least 15 years since this assessment has been issued.

The Panel also previously reviewed Potassium Silicate, Sodium Metasilicate, and Sodium Silicate in a report that was published in 2005 and concluded that these ingredients were safe for use in cosmetic products in the practices of use and concentration described in the safety assessment when formulated to avoid irritation.² In 2009, the Panel issued a final report on the safety of Silica, Alumina Magnesium Metasilicate (now called Magnesium Aluminometasilicate), Aluminum Calcium Sodium Silicate, Aluminum Iron Silicates, Hydrated Silica, and Sodium Potassium Aluminum Silicate with the conclusion that these ingredients are safe as cosmetic ingredients in the practices of use and concentrations as described in the safety assessment. Because of the similarity of these 9 ingredients to those of the original 2003 assessment, the Panel may be consider adding on these ingredients and 16 other related silicate ingredients, that were not previously reviewed, to this assessment. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*; see Table 1), the majority of these ingredients function as abrasives, absorbents, bulking agents, and/or deodorant agents in cosmetic products.³

Related ingredients that were previously reviewed by the Panel but are not up for consideration as add-ons include ammonium hectorites (Disteardiomonium Hectorite, Dihydrogenated Tallow Benzylmonium Hectorite, Stearlkonium Hectorite, and Quaternium -18 Hectorite) and silylates and surface-modified siloxysilicates (Silica Silylate, Silica Dimethyl Silylate, Trimethylsiloxysilicate, and Trifluoropropyldimethyl/Trimethylsiloxysilicate).^{4,5} The conclusion of the former report was safe as used in cosmetic ingredients and the conclusion of the latter report was safe as used in cosmetics when formulated and delivered in the final product not to be irritating or sensitizing to the respiratory tract.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (<http://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites>; <http://www.cir-safety.org/supplementaldoc/cir-report-format-outline>). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Excerpts from the summary of the 2003 report, as well as the 2005 and 2009 reports of the potential add-ons, are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text*. (This information, except for chemical and physical properties, is not included in the tables or the summary section.) The original reports that were published in 2003, 2005, and 2009 are available on the CIR website (<http://www.cir-safety.org/ingredients>).

CHEMISTRY

Definition

The definitions and functions of the silicate ingredients included in this re-review are provided in Table 1.

Physical and Chemical Properties

Physical and chemical properties of the silicate ingredients are provided in Table 2. These ingredients generally are not soluble in water.

Method of Manufacturing

No published methods of manufacturing were discovered, and no unpublished data were submitted for the proposed, not previously reviewed add-on ingredients.

Composition/Impurities

No composition/impurities data were discovered, and no unpublished data were submitted for the proposed, not previously reviewed add-on ingredients.

USE **Cosmetic**

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2018 VCRP data, Silica has the most reported uses in cosmetic products, with a total of 8024; the majority of the uses are in leave-on eye makeup preparations and makeup preparations (Table 3 and Table 4).⁶ Kaolin has the second most reported uses in cosmetic products, with a total of 1794; the majority of the uses are also in leave-on eye makeup preparations and makeup preparations. The reported numbers of uses for the remaining ingredients in this report are much lower. The uses for both of these ingredients have greatly increased since the original safety assessments were finalized: in 2009, Silica was reported to have 3276 uses and in 1998, Kaolin was reported to have 509 uses.^{1,2,7} The results of the concentration of use survey conducted in 2018 by the Council indicate Kaolin has the highest reported maximum concentration of use; it is used at up to 53% in “other” manicuring products and up to 35% in rinse-off “other” skin care preparations.⁸ Zeolite is used at up to 37.8% in paste masks and mud packs and up to 35.7% in hair tonics, dressings and other hair grooming aids. According to the original safety assessments, the maximum use concentration for Kaolin was 100% in leave-on “other” skin care preparations and the maximum use concentration for Hectorite was 100% in rinse-off skin cleansing preparations (the maximum leave-on concentration was 15% in makeup foundations).¹ Silica was reported to be used at up to 44% in eye shadows.⁷ Cosmetic ingredients with no reported uses in the VCRP database are listed in Table 5. The Council is currently performing a concentration of use survey on Aluminum Calcium Sodium Silicate, Aluminum Iron Silicate, Aluminum Silicate, Calcium Magnesium Silicate, Hydrated Silica, Magnesium Aluminometasilicate (previously Alumina Magnesium Metasilicate), Silica, Silver Copper Zeolite, Silver Zinc Zeolite, Sodium Potassium Aluminum Silicate, and Titanium Zeolite.

Many of the silicate ingredients described in this safety assessment may be used in products that can be incidentally ingested or come into contact with mucous membranes; for example, use is reported in lipsticks and oral hygiene products.⁶ In the 2009 report, Hydrated Silica was reported to be in dentifrices at up to 34%.⁷ Additionally, these ingredients have been reported to be used in products that may come into contact with the eyes, such as eye shadows, eye liners, and mascaras. In the 2003 report, Kaolin was reported to be used at up to 48% in eyeliner.¹ Moreover, these ingredients are reported to be used in spray products and powders that could possibly be inhaled, like hair sprays and face powders. In the original reports, Silica was reported to be used at up to 6% in a fragrance product and Kaolin was reported to be used at up to 30% in face powders.^{1,7} In practice, 95% to 99% of the droplets/ particles released from cosmetic sprays have aerodynamic equivalent diameters $> 10 \mu\text{m}$, with propellant sprays yielding a greater fraction of droplets/particles below $10 \mu\text{m}$ compared with pump spray.⁹⁻¹² Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.^{9,11} Silicates are also used in powders, and these products could possibly be inhaled. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.¹³⁻¹⁵

In regulations on cosmetic products in the European Union, zirconium and its compounds (including Zirconium Silicate) are listed under Annex II-substances prohibited in cosmetic products, with the exception of the substances listed under reference number 50 in Annex III, and the zirconium lakes, pigments or salts of the coloring agents when listed in Annex IV.¹⁶ Aluminum Silicate, Bentonite, and Kaolin are listed under Annex IV – colorants allowed in cosmetic products. The remaining silicate-related ingredients listed in this report are not restricted from use in any way under the rules governing cosmetic products in the European Union.

According to Australia’s National Industrial Chemicals Notification and Assessment Scheme (NICNAS), the following ingredients are Tier I chemicals (not considered to pose an unreasonable risk to the health of workers and public health on the basis of the Tier I IMAP assessment): Aluminum Silicate, Bentonite, Calcium Silicate, Fuller’s Earth, Kaolin, Magnesium Silicate, Magnesium Trisilicate, Montmorillonite, Sodium Potassium Aluminum Silicate, Zeolite, and Silica (as amorphous, fumed, crystalline-free; gel; gel-precipitated, crystalline-free; and vitreous).¹⁷ Attapulgite, Potassium Silicate, Sodium Silicate, Sodium Metasilicate, and Silica (crystalline) are Tier II chemicals (require risk management measures to be instituted for safe use for human health or the environment). The remaining silicates have no NICNAS determination.

Non-Cosmetic

Aluminum Silicate is approved as an indirect food additive in the Code of Federal Regulations (CFR).¹ Bentonite is considered GRAS as a direct food additive and Kaolin is considered GRAS as an indirect food additive. Pyrophyllite is listed as a naturally occurring color additive in the CFR. Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Trisilicate, Attapulgit, Hectorite, and Kaolin are all used in over-the-counter drug products.

Potassium Silicate and Sodium Silicate were reported as being used in industrial cleaners and detergents.² Sodium Metasilicate is a GRAS food ingredient.

Hydrated Aluminum Calcium Sodium Silicate countered the effects of aflatoxin in animal feed.⁷ Silica is used in food preparation as an anticaking agent, dispersion agent, suspending agent, and thickening agent. It is a defoaming and conditioning agent in malt beverages. Silica is a thickener in pastes and ointments.

Calcium Silicate

Calcium Silicate is used in endodontics in root canal sealer preparations and dental cements.¹⁸⁻²⁰

Fuller's Earth

Fuller's Earth is reported to be used as military decontaminant for removal of hazardous materials from the skin.²¹

Sodium Magnesium Aluminum Silicate

Sodium Magnesium Aluminum Silicate is reported to be used in print enhancement (imparting high brightness and opacity), paper filler, and carbonless copy intensifier.²²

Zeolite

The use of Zeolite has been investigated for use in oral drug delivery of acidic medications, such as aspirin.²³

Zinc Silicate

Zinc Silicate is reported to be used as phosphors (in television screens), in spray ingredients, and to remove traces of copper from gasoline.^{22,24}

TOXICOKINETICS

Absorption, Distribution, Metabolism, Distribution

All silicates have the great ability to absorb, especially the clays.¹ Reports describe drugs, bacteria, viruses, and toxins absorbed to clays due to the physical structure of clays and their cationic nature.

No statistically significant absorption of aluminum and elevated levels of silicon were recorded in assayed plasma samples given Magnesium Trisilicate and Zeolite orally.¹ The urinary excretion of silica was 5.2% in males given 20 g of Magnesium Trisilicate. Ten percent Bentonite in the diets of rats overcame T-2 toxicosis completely. Various Zeolites were added to the diets of pigs. No adverse effects were noted by the supplementation.

A sample of Aluminum Silicate was toxic to pulmonary alveolar macrophages and LDH activity and β -GAL release were increased.¹ Aluminum Silicate had relatively no effect on the hemolysis of rat RBCs. Synthetic Calcium Silicate samples and higher concentrations of Calcium Silicate caused increased hemolysis of human RBCs; a greater fibrous character of Calcium Silicate samples caused increased LDH and β -Gal release. Many clays (Attapulgit, Bentonite, Hectorite, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite) demonstrated cytotoxicity to several macrophage type cell lines and have hemolytic activity towards several species' RBCs. Particle size, fibrogenicity, concentration, and mineral composition had the greatest effect on toxicity. Larger particle size and longer and wider fibers cause more adverse effects. In most of the studies, a dose-dependent effect on cytotoxicity or lysis was observed. Most mineral samples were not 100% pure and many samples already contained toxic dusts or minerals like quartz or cristobalite.

Sodium Silicate administered orally acts as a mild alkali and was readily absorbed from the alimentary canal and excreted in the urine.² Urinary excretion of Sodium Silicate given orally to rats at 40 and 1000 mg/kg was 18.9% and 2.8%, respectively.

Orally administered Silica was mostly excreted through the urine in guinea pigs.⁷ Some studies showed accumulation of Silica from oral exposure whereas others did not. Silica intraperitoneally injected

into guinea pigs was mostly excreted through the urine. When Silica is inhaled by rats, it accumulates in the lungs and lymph nodes initially. The accumulated amount remains at a steady state with continued treatment. When treatment ceases, the Silica decreases. When subcutaneously injected into rats, Silica is absorbed over a few months. When Silica is inhaled or intratracheally instilled into rats, mice, and rabbits, there is a transient inflammatory response that resolves in days. Incubated macrophages ingested fewer Silica particles than *C. albicans* or *S. cerevisiae*. Ultra-fine Silica (14 nm) did more damage to the lungs during the inflammation than did fine Silica (213 nm).

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The following are a list of acute oral LD₅₀ determinations: Calcium Silicate, 3400 mg/kg in rats; Magnesium Aluminum Silicate, 50,000 mg/kg in mice; Zirconium Silicate, > 200 g/kg in mice; Hectorite, > 5 g/kg in rats; Kaolin, 149 g/kg in rats (death due to bowel obstruction); 15 natural Zeolites, 10 g/kg in rats.¹ The acute dermal LD₅₀ was > 3.5 g/kg of rabbits exposed to Magnesium Aluminum Silicate.

The toxicity of Potassium Silicate, Sodium Metasilicate, and Sodium Silicate has been related to the molar ratio of SiO₂/Na₂O and the concentration.² The acute oral LD₅₀ of Sodium Metasilicate ranged from 847 mg/kg in male rats to 1349.3 mg/kg in female rats, and from 770 mg/kg in female mice to 820 mg/kg in male mice. Gross lesions of variable severity were found in the oral cavity, pharynx, esophagus, stomach, larynx, lungs, and kidneys of dogs receiving 0.25 g/kg or more of a commercial detergent containing Sodium Metasilicate. Similar lesions were seen in pigs given the same detergent and dose as in the previous study. Male Sprague-Dawley rats orally administered 464 mg/kg of a 20% solution containing either 2.0 or 2.4 ratio to 1.0 ratio of sodium oxide showed no signs of toxicity, whereas doses of 1000 and 2150 mg/kg produced gasping, dyspnea, and acute depression. Acute intraperitoneal injections of a neutralized 2% solution of Sodium Metasilicate in white rats resulted in a decrease in spleen weight and relative enlargement of the kidneys.

Silica was reported to have an oral LD₅₀ of > 40,000 mg/kg in rats and > 8000 mg/kg in mice.⁷ The oral lethal dose of pyrogenic silica in humans is 15 g/kg. Orally administered Aluminum Calcium Sodium Silicate had no adverse effects up to 800 mg/kg in mice. The acute dermal NOEL for silica is > 2000 mg/kg for rabbits. When applied as an aqueous paste, there were no adverse effects. The LD₅₀ was > 5000 mg/kg for precipitated (hydrated) Silica and > 2000 mg/kg for silica gel on intact and abraded rabbit skin. Mild erythema was observed. The acute inhalation of silica at 477 mg/m³ by rats resulted in restlessness, droopy eyelids, lethargy, and dyspnea during treatment. Clinical signs resolved quickly after treatment and necropsies were unremarkable.

Acute dermal, oral, and inhalation data from recent literature searches are summarized in Table 6. Potassium Silicate (30%) had a dermal LD₅₀ greater than 5 g/kg in rats.²⁵ In oral rat studies, the LD₅₀ for Aluminum Silicate (concentration not reported), Calcium Silicate (20%), Potassium Silicate (concentration not reported), Sodium Magnesium Aluminum Silicate (concentration not reported), and Sodium Silicate were > 2 g/kg, > 10g/kg, > 5 g/kg, > 2 g/kg, and up to 8.65 g/kg, respectively.²⁵⁻³⁰ An oral LD₅₀ for Sodium Silicate in mice was 6.60 g/kg.²⁷ In an inhalation study, the LC₅₀ for 30% Potassium Silicate in rats was > 2.06 mg/l.²⁵

Short-Term and Subchronic Toxicity Studies

In short-term oral toxicity studies, no adverse effects were seen in mice or rabbits dosed up to 5 g/kg Magnesium Aluminum Silicate; beagle dogs and rats fed Aluminum Silicate had no renal lesions.¹ Dogs and rats fed Magnesium Trisilicate for 4 weeks had polydipsia and polyuria, and all dogs had renal cortical lesions. Guinea pigs had renal lesions after 4 months of drinking Magnesium Trisilicate in their tap water. Rats fed 10% Magnesium Aluminum Silicate had slightly elevated silicon levels of the spleen and dogs and rats fed 10% Magnesium Aluminum Silicate had no negative responses in 90-day feeding studies.

Beagle dogs fed 2.4 g/kg/day of Sodium Silicate for 4 weeks had gross renal lesions but no impairment of renal function. In an oral subchronic study (drinking water containing 600 and 1200 ppm of added silica), there were body weight gains in male rats, but decreases in female rats. No apparent effect of the treatment in the drinking water was found on the longevity in rats having started treatment after weaning.

Short-term oral doses of silica at 8000 mg/kg/d produced no clinical effects in dogs. Short-term oral doses of silica produced no clinical effects for rats; [highest no effect levels] (HNELS) were up to 1000 mg/kg/d. At 16,000 mg/kg, clinical signs observed were shyness, dirty fur, reduced activity, cachexia,

hemorrhages of mucous membranes of the eyes and nose. Short-term dermal application of silica to intact and abraded skin resulted in no dermal toxicity in rabbits.

Short-term inhalation of silica up to 668 mg/m³ resulted in respiratory distress during treatment and a short-term inflammation response in the lungs, which resolved quickly when treatment ceased in rats. The lung clearance half-life was ~50 day for 50.5 and 154 mg/m³. The NOAEL was 10.1 mg/m³ in one study. In other studies, the NOEL was 1 and 46 mg/m³.

The oral subchronic HNEL was 5000 mg/kg/d, the NOEL was 500 mg/kg/d and the lowest effect level was 500 mg/kg/d for rats. There were no clinical signs up to 7950 and 8980 mg/kg/d for male and female rats, respectively. There were no gross findings of toxicity up to 50,000 ppm in feed.

Subchronic inhalation of silica at 53 mg/m³ caused 44% mortality in rats from pulmonary vascular obstruction and emphysema. There was increased respiration rates and decreased weight gain during treatment. Necropsy findings included congestion of the lungs, lymph node enlargement, emphysema, vacuolated cells within alveolar spaces, and increased lung weights and collagen content. There were no mortalities at 31 mg/m³. The LOAEL was 53 mg/m³. The NOEL was 1.3 mg/m³.

Montmorillonite

In a 90-day subchronic study of an organo-modified clay mineral derived from Montmorillonite, Wistar rats were exposed orally to 40 mg/kg/day to the clay.³¹ No adverse effects were observed in the main organ tissues and no significant effects were observed in blood clinical biochemistry parameters, reduced/oxidized glutathione ratio, or interleukin-6 leakage in serum. The authors concluded that the modified Montmorillonite clay did not cause toxic effects in this oral study in rats.

In a 28-week study, male and female Sprague–Dawley rats were fed 0%, 0.25%, 0.5%, 1.0% or 2.0% (0, 2500, 5000, 10,000 or 20,000 ppm, respectively) Montmorillonite clay (calcium montmorillonite; 9.34% total Al).³² This corresponded to a daily clay consumption of 75, 150, 300 and 600 mg/kg/day, respectively. There were no significant differences in either gender regarding feed consumption, body weight gain, feed efficiency or relative organ weights. There were no differences in gross lesions or histopathological changes in liver, kidney, lung, heart, brain, spleen, tibia, uteri or ovaries in any of the treated groups when compared to the control. There were no differences in hematologic parameters or in clinical chemistry parameters. Serum and hepatic vitamins A and E remained unaffected by clay ingestion. The authors concluded that feeding up to 2.0% (20,000 ppm or 600 mg/kg/day) Montmorillonite [equivalent to 1860 ppm total Al as the hydrated aluminum silicate] to rats did not elicit signs of systemic toxicity.

Chronic Toxicity Studies

No lesions were found in rats dosed up to 1000 mg/kg Zeolite for 104 weeks.

Silica incorporated into the feed of rats at up to 10% for 6 months or more produced unremarkable necropsies.⁷ Females had increased leukocytes and males had increased eosinophils at 10% in feed. Females had reduced liver weights at 12 and 24 months at 5%. Mice treated with Silica in their feed had similar results for up to 103 weeks.

In inhalation studies, mice bred to be susceptible to tumors exposed to aerosolized Silica at 0.5 g/d for a year had increased incidence of lung tumors with no obvious fibrosis of the lung tissue but fibrotic nodules in the tracheo-bronchial lymph nodes.⁷ At 53 mg/m³ for a year, treatment related deaths were 75% in rats from pulmonary vascular obstruction and emphysema starting in the 4th month. Guinea pigs exposed to Silica at 1.5 mg/ft³ for up to 24 months had no deaths. A chronic reaction of the lung tissue was established at 4 months and emphysema after 4 to 8 months. Histologically, there [were] periductal and peribronchiolar intra-alveolar accumulations of the giant cells. In the lymphoid tissue, medullary hyperplasia with the formation of slight amounts of reticulum was prominent during the second year of exposure. There were no macroscopically visible anomalies after 1 year of recovery.

Rabbits chronically exposed to 0.2 and 5.0 mm aerosolized Silica had formation of nodular fibrotic or diffuse fibrotic changes in the lungs.⁷ Rats exposed to aerosolized Silica at 15 mg/m³ for 12 months had a few macrophages aggregated in the lungs. The LOAEL was 6 to 9 mg/m³. At 126 mg/m³ of Silica for up to 24 months, guinea pigs and rabbits had increased lung weights and particle phagocytosing macrophages accumulated in alveoli, bronchioles, and lymphoid tissue. There was complete reversibility of Silica retention and inflammatory responses in guinea pigs within 6 months of recovery. Silicotic processes were completely absent. Rabbits exposed to aerosolized silica at 1.5 mg/ft³ for 12 months had progressive functional incapacitation and elevation of hematocrit levels observed in the majority of the rabbits, possibly due to the combined effect of pulmonary vascular obstruction and emphysema. During recovery, the cellular reactions

and emphysema regressed but minor focal alveolar mural collagen persisted. In rabbits exposed to 360 mg/m³ for a year, emphysema, pulmonary emphysema, vascular stenosis, alveolar cell infiltration, sclerosis, and epithelization granulomatosis, macrophage catarrh were observed. Lesions were observed in the liver, spleen and kidney. The LOAEL was 28 mg/m³.

Monkeys exposed to aerosolized silica at 15 mg/m³ for 12 months had initial decreased activity and body weight gain.⁷ There was emphysema at 3 months and considerable cellular infiltration of the alveoli and alveolar septa associated with distention of alveoli or accumulation of exudate and macrophages. After 12 months, the lesions were marked pulmonary emphysema, alveolar wall sclerosis, vascular occlusions, and cor pulmonale. The silica content remained low and decreased over time. At 50 and 100 mg/m³, there was interstitial fibrosis which did not resolve after 24 months. When monkeys were exposed to different types of Silica, the precipitated (hydrated) silica group had lower lung volumes. There were no changes in lung volume parameters, but in ventilatory performance and mechanical parameters, dynamic lung compliance, and forced expiratory flow when exposed to silica gel. The frequency and size of inflammatory cell aggregates varied with the type of Silica.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

Calcium Silicate (250 to 1600 mg/kg) had no discernible effect on nidation or on maternal or fetal survival in rabbits.¹ Magnesium Aluminum Silicate (6000 mg/kg) had neither a teratogenic nor adverse effects on the mouse fetus. Female rats receiving 20% Kaolin diet exhibited maternal anemia but no significant reduction in birth weight of the pups was recorded. Type A Zeolite produced no adverse effects on the dam, embryo, or pups in either rats or rabbits at any dose level (74 or 1600 mg/kg). Clinoptilolite had no effect on female rat reproductive performance.

Rats given Sodium Silicate (600 and 1200 ppm of added silica) in the drinking water in reproductive studies produced a reduced number of offspring; to 67% of controls at 600 ppm and to 80% of controls at 1200 ppm.² Three adult rats injected intratesticularly and subcutaneously with 0.8 mM/kg of Sodium Silicate showed no morphological changes in the testes and no effect on the residual spermatozoa in the ductus deferens.

No reproductive or teratological effects were observed following the oral administration of Silica in rabbits at 1600 mg/kg/d, hamsters at 1600 mg/kg/d, mice 1340 mg/kg/d, and rats up to 1350 mg/kg/d.⁷ An oral NOEL of 500 mg/kg/d was reported for rats; an oral NOAEL of 1350 mg/kg/d was also reported.

GENOTOXICITY STUDIES

No increase in mutation frequencies were seen in the *Salmonella* TA-1530 or G-46 assay and no significant increase in recombinant activity in the *Saccharomyces* D3 assay treated with Calcium Silicate.¹ A subacute dose of 150 mg/kg of Calcium Silicate produced 3% breaks in bone marrow cells arrested in c-metaphase. In a metaphase spread of bone marrow cells, Calcium Silicate produced no significant increase in the number of aberrations compared to controls and in a dominant lethal assay did not induce any dominant lethal mutations. In the *S. typhimurium* LT2 spot test (TA98, TA100, TA1535, TA1537, and TA1538) with or without metabolic activation, Magnesium Aluminum Silicate and Hectorite were found to be nonmutagenic. In primary hepatocyte cultures, the addition of Attapulgate at 10 µg/cm² caused significant increases in UDS in rat pleural mesothelial cells. Zeolite particles (< 10 µm) produced statistically significant increase in the percentage of aberrant metaphases, mostly chromatid breaks.

Sodium Metasilicate was nonmutagenic in a DNA damage and repair assay without metabolic activation using *B. subtilis*.² Sodium Silicate was nonmutagenic in studies using *E. coli* strains B/Sd-4/1,3,4,5 and B/Sd-4/3,4.

Silica was not mutagenic using the Rec or Ames test up to 10 M.⁷ Silica was not mutagenic in Ames test up to 1580 µg/plate. In a single-cell gel/Comet assay using Chinese hamster fibroblasts, there was an increase in DNA migration in a dose-dependent manner. A chromosomal aberration test was negative up to 300 µl/ml without and 1000 µl/ml with metabolic activation. A HGPRT was negative up to 250 µm/ml without and 500 µm/ml with metabolic activation. An unscheduled DNA synthesis test was negative up to 1000 µm/ml. Silica was not mutagenic to CHO cells, hamster fibroblasts, rat hepatocytes, and human embryonic lung cells. Silica was not mutagenic to mice or rats. A positive sister chromatid exchange test of AFB₁ showed inhibition by 10⁻⁵ M Aluminum Calcium Sodium Silicate. Hydrated Aluminum Calcium Sodium Silicate at 0.5% in the feed of rats inhibited the effects of AF.

In Vitro

Genotoxicity data from recent literature searches are summarized in Table 7. Aluminum Silicate, Sodium Metasilicate, Sodium Silicate, and Zinc Silicate were not genotoxic in Ames tests, HPRT gene mutation assays, or chromosome aberration tests.^{27,28,33,34} Potential genotoxicity was observed in Montmorillonite in an Ames test and a cytokinesis block micronucleus cytome assay.^{35,36}

CARCINOGENICITY STUDIES

The International Agency for Research on Cancer (IARC) has ruled Attapulgite fibers > 5µm as group 2B, possibly carcinogenic to humans, and fibers < 5µm as group 3, not classified as to their carcinogenicity to humans.¹ The natural Zeolites (clinoptilolite, phillipsite, mordenite, and nonfibrous Japanese zeolite) and synthetic Zeolites cannot be classified as to their carcinogenicity to humans (group 3) according to IARC.

Oral administration of silica to rats for 24 months was not carcinogenic up to 100 mg/kg/d.⁷ In an inhalation study, Silica at 0.5 g/d administered to mice for a year increased the incidence of overgrowth or hyperplasia of the tracheobronchial lymph nodes from 14.3% to 29.5%.

OTHER RELEVANT STUDIES

Cytotoxicity

Silica is not cytotoxic to Chinese hamster V79 cells at up to 160 µg/l⁷. Pyrogenic silica and silica gel were cytotoxic to macrophages at 13.5 µg/cm²; precipitated silica was less cytotoxic. Micronuclei were induced in Chinese hamster cells when incubated with 80 and 160 mg/ml. Micronuclei were induced with the presence of silica. There was ~85% lysis of sheep blood erythrocytes incubated with silica. Mesothelial cells incubated in silica were observed to accumulate silica in the cytoplasm, around the nucleus, and vacuoles. When incubated in pyrogenic silica, both macrophages and neutrophils were inhibited in their ability to phagocytose sheep red blood cells. Silica caused intracellular alteration in calcium homeostasis in renal cells. Alveolar macrophages exposed to silica had increased protein kinases, NO_x production, and cell death. Human primary fibroblasts exposed to silica produced COX-2 and PGE₂ in a dose dependent manner. COX-1 was not affected. Silica was not cytotoxic to human mesothelioma and rodent fibroblast cells. Sodium Potassium Aluminum Silicate, in the form of Mexicali dust, induced anaphasic alterations in Balb3T3 cells.

Montmorillonite

The cytotoxicity of 3 different organo-modified Montmorillonite clays was assessed using the human cell lines Caco-2 and HepG2.³⁷ The cells were exposed to the clay materials for 0, 24, or 48 h at concentrations dependent on the type of modifications to the clay material (maximum concentrations were 8µg/ml, 125 µg/ml, or 62.5 µg/ml, respectively). Only one clay material induced toxic effects in both cell lines. In calculating the mean effective concentration, it was determined that Caco-2 was more sensitive than HepG2. Mechanistic biomarkers were utilized to determine the toxicity mechanism of the clay that affected both cell lines; it was found that glutathione content decreased in HepG2, but interleukin leakages and generation of intracellular reactive oxygen species were not observed. DNA damage, as determined with a comet assay, was induced in both cell lines at the highest concentration tested. The authors concluded that toxicity of the modified clay materials was dependent on the type of clay modifications, the concentration, and the type of cell line tested.

In another cytotoxicity study by the same group of researchers, unmodified and organo-modified Montmorillonite clays were studied using human umbilical vein endothelial cells.³⁶ The unmodified clay was tested at up to 62.5 µg/ml and the modified clays were tested at up to 250 µg/ml. Two of the modified clays induced cytotoxic effects, while the third modified clay and the unmodified clay did not induce cytotoxicity.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal Irritation

Magnesium Aluminum Silicate (4%) was a weak primary skin irritant in rabbits and had no cumulative skin irritation in guinea pigs. No gross effects were reported in any of these studies. Sodium Magnesium Silicate (4%) had no primary skin irritation in rabbits and had no cumulative skin irritation in guinea pigs. Hectorite was nonirritating to the skin of rabbits in a Draize primary skin irritation study. Applications of 2 g of Magnesium Aluminum Silicate made to the skin of two humans daily for 1 week caused no effects.

Dermal irritation of Potassium Silicate, Sodium Metasilicate, and Sodium Silicate ranged from negligible to severe, depending on the species tested and the molar ratio and concentration tested.² Sodium Metasilicate/carbonate detergent (37% Sodium Metasilicate) mixed 50/50 with water was considered a severe skin irritant when tested on intact and abraded human skin. Detergents containing 7%, 13%, and 6% Sodium Silicate mixed 50/50 with water, however, were negligible skin irritants to intact and abraded human skin. Sodium Silicate (10% of a 40% aqueous solution) was negative in a repeat-insult predictive patch test in humans. The same aqueous solution of Sodium Silicate was considered mild under normal use conditions in a study of cumulative irritant properties. Sodium Metasilicate and Sodium Silicate were studied in modified soap chamber tests. No burning or itching was observed and low erythema + edema scores were noted. Sodium Metasilicate and Sodium Silicate, tested in elbow crease studies and semiocluded patch tests, produced low grade and transient irritation.

Silica at 100% was nonirritating to intact and abraded rabbit skin.⁷

Dermal Sensitization

Sodium Metasilicate was negative in the local lymph node assay, but a delayed-type hypersensitivity response was observed in mice.²

A guinea pig sensitization study of 20% Hydrated Silica resulted in no reactions.⁷ Hydrated Silica and Silica was non-sensitizing in humans at up to 21.7% and 45%, respectively.

In vitro and animal dermal irritation and animal sensitization data from recent literature searches are summarized in Table 8. Aluminum Silicate and Zinc Silicate were predicted to be not irritating in EpiDerm™ skin assays.^{28,33} In rabbit studies, the irritation potential of Potassium Silicate (up to 36%) and Sodium Metasilicate (up to 97%) were dependent on concentration.^{25,30,34} Aluminum Silicate (up to 25%) and Zinc Silicate (up to 50%) were not sensitizing in LLNA studies.^{28,33} Potassium Silicate (30%) was not sensitizing in a Buehler sensitization test in guinea pigs.²⁵

OCULAR IRRITATION STUDIES

A 4% solution of Magnesium Aluminum Silicate and a 4% solution of Sodium Magnesium Silicate caused minimal eye irritation in a Draize eye irritation test.¹ Bentonite caused severe iritis after injection into the anterior chamber of the eyes of rabbits. When injected intralamellarly, widespread corneal infiltrates and retrocorneal membranes were recorded. In a primary eye irritation study in rabbits, Hectorite was moderately irritating without washing and practically nonirritating to the eye with a washout. Rats tolerated a single dose of Zeolite A without any adverse reaction to the eye.

Potassium Silicate was nonirritating in two acute eye irritation studies in rabbits.² Sodium Metasilicate (42.4% H₂O) was corrosive to the rabbit eye. Sodium Silicate was a severe eye irritant in acute eye irritation studies. A skin freshener (10% of a 40% aqueous solution) containing Sodium Silicate was nonirritating. Sodium Silicate in another three Draize eye irritation studies was highly irritating, irritating, and nonirritating, respectively.

Silica was a non- to mild ocular irritant in rabbits up to 100 mg.⁷

In vitro and animal ocular irritation data from recent literature searches are summarized in Table 9. Aluminum Silicate (tested pure) was predicted to be not irritating using the hen's egg test chorioallantoic membrane (HET-CAM) method.²⁸ Sodium Metasilicate (concentration not reported) was predicted to be corrosive in an in vitro method using rabbit eyes, and Zinc Silicate (20%) was predicted to be irritating in a bovine corneal opacity and permeability (BCOP) test.^{33,34} Potassium Silicate was not irritating to slightly irritating when tested at up to 35%.^{25,30}

CLINICAL STUDIES

Clinical Assessment of Safety

Oral ingestion of Silica up to 1250 mg in humans resulted in a rapid increase of Silica blood levels and a rapid elimination in the urine over 8 to 24 h with no adverse effect reported.⁷

Montmorillonite

The safety of the oral use of Montmorillonite to protect against the adverse effects of the ingestion of aflatoxins was studied in 50 human subjects for 2 weeks.³⁸ The clinical study was randomized and double-blinded.

The 23 males and 27 females ranged in age from 20–45 years. The low-dose group received nine capsules containing 1.5 g/day processed Montmorillonite, and the high-dose group received nine capsules containing 3.0 g/day processed Montmorillonite. Blood and urine samples were collected before and after the study for analysis. All participants completed the trial and compliance was 99.1%. Mild gastrointestinal effects were reported, including abdominal pain (6%, 3/50), bloating (4%, 2/50), constipation (2%, 1/50), diarrhea (2%, 1/50), and flatulence (8%, 4/50). No statistical significance was found between the two groups for these adverse effects ($p > 0.25$). No significant differences were shown in hematology, liver and kidney function, electrolytes, vitamins A and E, and minerals in either group.

Case Reports

Colloidal Sodium Metasilicate was fatal to one man and neutralized Sodium Silicate produced vomiting, diarrhea, and gastrointestinal bleeding in another man in separate case reports.²

Sodium Metasilicate

Acute kidney injury was reported in a 52-year-old man who had ingested approximately 150 ml of a plate developer solution containing Sodium Metasilicate.³⁹ The patient also developed severe upper airway obstruction due to laryngeal edema, severe inflammation of the upper gastrointestinal tract with narrowing of the esophagus and pyloric region. The patient succumbed to his injuries a few months after the ingestion incident.

Reactive airway dysfunction syndrome was reported in 43-year-old man who had inhaled dishwasher detergent powder containing Sodium Metasilicate.⁴⁰ The patient was employed as an apprentice cook and accidentally inhaled the detergent while preparing to use an institutional dishwasher.

Occupational Exposure

Occupational exposure to mineral dusts has been studied extensively. Fibrosis and pneumoconiosis has been documented in workers involved in the mining and processing of Aluminum Silicate, Calcium Silicate, Zirconium Silicate, Fuller's Earth, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite.

Workers in environments with aerosolized silica had few signs of silicosis or pulmonary disease up to 100 mg/m³.⁷ Smoking and exposure to silica synergize to induce small airway disease. Exposure to hydrated silica also had no evidence of silicosis or pulmonary disease. There were signs of dermal irritation due to the desiccative and defatting properties of silica.

Bentonite

In a toxicological and epidemiological review of Bentonite, the authors concluded Bentonite is probably not more toxic than any other particulate. However, because some forms may contain variable amounts of respirable crystalline silica, prudent management and adherence to occupational exposure limits is appropriate.⁴¹

Zeolite

In a safety assessment of synthetic zeolites (zeolites A, P, X, and Y) used in detergents, the author concluded that these ingredients are safe for consumers under the conditions of recommended use.⁴² The author further stated that due to irritant effects of undiluted zeolite material on mucous membranes and the respiratory tract, the exposure of workers should be controlled.

SUMMARY

The Panel previously reviewed the safety of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite in a report that was published in 2003 and concluded that these ingredients were safe as used in cosmetic products. In accordance with its procedures, the Panel evaluates the conclusions of previously-issued reports every 15 years, and it has been at least 15 years since this assessment has been issued. Proposed ingredients to "add-on" to this report are the previously reviewed ingredients Potassium Silicate, Sodium Metasilicate, and Sodium Silicate that the Panel concluded were safe when formulated to avoid irritation, and the previously review ingredients Silica, Alumina Magnesium Metasilicate, Aluminum Calcium Sodium Silicate, Aluminum Iron Silicates, Hydrated Silica, and Sodium Potassium Aluminum Silicate that the Panel concluded were safe for use in cosmetic. Because of the similarity of these 9 ingredients to those of the original

2003 assessment, the Panel may be consider adding on these ingredients, plus 16 other related silicate ingredients that were not previously reviewed to this assessment.

According to 2018 VCRP data, Silica has the most reported uses in cosmetic products, with a total of 8024; the majority of the uses are in leave-on eye makeup preparations and makeup preparations. Kaolin has the second most reported uses in cosmetic products, with a total of 1794; the majority of the uses are also in leave-on eye makeup preparations and makeup preparations. The reported numbers of uses for the remaining ingredients in this report are much lower. The uses for both of these ingredients have greatly increased since the original safety assessments were finalized: in 2009, Silica was reported to have 3276 uses and in 1998, Kaolin was reported to have 509 uses. The results of the concentration of use survey conducted in 2018 by the Council indicate Kaolin has the highest reported maximum concentration of use; it is used at up to 53% in “other” manicuring products and up to 35% in rinse-off “other” skin care preparations. Zeolite is used at up to 37.8% in in paste masks and mud packs and up to 35.7% in hair tonics, dressings and other hair grooming aids. According to the original safety assessments, the maximum use concentration for Kaolin was 100% in leave-on “other” skin care preparations and the maximum use concentration for Hectorite was 100% in rinse-off skin cleansing preparations (the maximum leave-on concentration was 15% in makeup foundations). Silica was reported to be used at up to 44% in eye shadows.

Potassium Silicate (30%) had a dermal LD₅₀ greater than 5 g/kg in rats. In oral rat studies, the LD₅₀ for Aluminum Silicate (concentration not reported), Calcium Silicate (20%), Potassium Silicate (concentration not reported), Sodium Magnesium Aluminum Silicate (concentration not reported), and Sodium Silicate were > 2 g/kg, > 10g/kg, > 5 g/kg, > 2 g/kg, and up to 8.65 g/kg, respectively. An oral LD₅₀ for Sodium Silicate in mice was 6.60 g/kg. In an inhalation study, the LC₅₀ for 30% Potassium Silicate in rats was > 2.06 mg/l.

In oral subchronic rat studies up to 28 weeks, an organo-modified Montmorillonite clay (40 mg/kg /day) and an unmodified clay (up to 600 mg/kg/day) did not cause systemic toxicity.

Aluminum Silicate, Sodium Metasilicate, Sodium Silicate, and Zinc Silicate were not genotoxic in Ames tests, HPRT gene mutation assays, or chromosome aberration tests. Potential genotoxicity was observed in Montmorillonite in an Ames test and a cytokinesis block micronucleus cytome assay.

Aluminum Silicate and Zinc Silicate were predicted to be not irritating in EpiDerm™ skin assays. In rabbit studies, the irritation potential of Potassium Silicate (up to 36%) and Sodium Metasilicate (up to 97%) were dependent on concentration. Aluminum Silicate (up to 25%) and Zinc Silicate (up to 50%) were not sensitizing in LLNA studies. Potassium Silicate (30%) was not sensitizing in a Buehler sensitization test in guinea pigs.

Aluminum Silicate (tested pure) was predicted to be not irritating using the HET-CAM method. Sodium Metasilicate (concentration not reported) was predicted to be corrosive in an in vitro method using rabbit eyes, and Zinc Silicate (20%) was predicted to be irritating in a BCOP test. Potassium Silicate was not irritating to slightly irritating when tested at up to 35%.

A 2-week clinical oral study of Montmorillonite resulted in only mild gastrointestinal complaints in 50 subjects. Case reports of severe injury were reported from ingestion and inhalation of Sodium Metasilicate. Occupational exposures to Bentonite and Zeolite should be limited.

ORIGINAL REPORT DISCUSSIONS

2003 Silicates Report

The CIR Expert Panel determined that the data provided in this report are sufficient to assess the safety of the tested ingredients: Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite. The Panel did note a concern about inhalation of these ingredients due to reported cases of pneumoconiosis and fibrosis in humans and pulmonary lesions in animals. However, extensive pulmonary damage in humans was the result of direct occupational inhalation of the dusts and lesions seen in animals were affected by particle size, fiber length, and concentration. The Panel recognizes that most of the formulations are not respirable and of the preparations that are respirable, the concentration of the ingredient is very low. Even so, the Panel considered that any spray containing these solids should be formulated to minimize their inhalation.

2005 Potassium Silicate, Sodium Metasilicate, and Sodium Silicate Report

The CIR Expert panel determined that the data provided in this report are sufficient to address the safety of the tested ingredients Potassium Silicate, Sodium Metasilicate, and Sodium Silicate. The Panel recognized the irritation potential of these ingredients, especially in leave-on products. However, because

these ingredients have limited dermal absorption and Sodium Metasilicate is a GRAS direct food substance, the Panel deemed the ingredients safe as currently used, when formulated to avoid irritation.

2009 Silica and Related Ingredients Report

The CIR Expert Panel emphasized that the Silica considered in this safety assessment is synthetic amorphous silica (gel, hydrated, and fumed/pyrogenic) and does not include any form of crystalline silica.

The Panel recognizes that there are data gaps regarding use and concentration of these ingredients. However, the overall information available on the types of products in which these ingredients are used and at what concentrations indicate a pattern of use, which was considered by the Expert Panel in assessing safety.

The Panel was concerned about the possibility of iron atoms reaching the lungs if Aluminum Iron Silicates were to be used in a spray. In the absence of inhalation toxicity data, the Panel determined that Aluminum Iron Silicates can be used safely in hair sprays, because the ingredient particle size is not respirable. The Panel reasoned that the particle size of aerosol hair sprays (38 μm) and pump hair sprays (>80 μm) is large compared to respirable particulate sizes (10 μm). The Panel recognizes that most of the formulations are not respirable and of the preparations that are so, the Panel considered that any spray containing these solids should be formulated to minimize their inhalation potential. Aluminum Iron Silicates is safe as a cosmetic ingredient because the particles for aggregates and agglomerates that are too large to be respirable.

The Panel determined that silicosis was not an issue since crystalline silica is not used in cosmetics.

TABLES

Table 1. Definitions, idealized structures, and functions of the ingredients in this safety assessment.³

Ingredient & CAS No.	Definition & Structure	Function(s)
Activated Clay	Activated Clay is the inorganic compound obtained by heating natural aluminum silicate with sulfuric acid.	Absorbents; Bulking Agents
Aluminum Calcium Magnesium Potassium Sodium Zinc Silicates	Aluminum Calcium Magnesium Potassium Sodium Zinc Silicates is a ceramic powder consisting mainly of silicon dioxide, aluminum oxide, calcium oxide, magnesium oxide, potassium oxide, sodium oxide and zinc oxide.	Antimicrobial Agents
Aluminum Calcium Sodium Silicate 1344-01-0	Aluminum Calcium Sodium Silicate is a complex silicate refined from naturally occurring minerals.	Bulking Agents
Aluminum Iron Calcium Magnesium Germanium Silicates	Aluminum Iron Calcium Magnesium Germanium Silicates is a ceramic powder consisting mainly of silicon dioxide, aluminum oxide, ferric oxide, calcium oxide, magnesium oxide and germanium oxide.	Anticaries Agents; Antifungal Agents; Antimicrobial Agents; Antioxidants
Aluminum Iron Calcium Magnesium Zirconium Silicates	Aluminum Iron Calcium Magnesium Zirconium Silicates is a ceramic powder consisting mainly of silicon dioxide, aluminum oxide, ferric oxide, calcium oxide, magnesium oxide and zirconium oxide.	Bulking Agents
Aluminum Iron Silicates	Aluminum Iron Silicates is a ceramic powder consisting mainly of silicon dioxide, aluminum oxide, and ferric oxide.	Abrasives; Bulking Agents
Aluminum Silicate 1327-36-2	Aluminum Silicate is a complex inorganic salt that has a composition consisting generally of 1 mole of alumina and 1 to 3 moles of silica.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents; Slip Modifiers
Ammonium Silver Zeolite	Ammonium Silver Zeolite is the ammonium salt of the product obtained by the reaction of silver nitrate and Zeolite.	Absorbents; Deodorant Agents; Preservatives
Ammonium Silver Zinc Aluminum Silicate	Ammonium Silver Zinc Aluminum Silicate is a complex silicate formed from the reaction of zinc nitrate, Ammonium Nitrate, and Silver Nitrate with Zeolite.	Absorbents; Deodorant Agents; Preservatives
Attapulgit 12174-11-7 1337-76-4	Attapulgit is a variety of Fuller's Earth found typically near Attapulgas, Georgia. It is characterized by having a chain structure rather than the usual sheet structure of other clay minerals.	Abrasives; Absorbents; Bulking Agents; Opacifying Agents; Viscosity Increasing Agents - Aqueous
Bentonite 1302-78-9	Bentonite is a native hydrated colloidal aluminum silicate clay.	Absorbents; Bulking Agents; Dispersing Agents - Nonsurfactant; Emulsion Stabilizers; Opacifying Agents; Viscosity Increasing Agents - Aqueous
Calcium Magnesium Silicate 12765-06-9	Calcium Magnesium Silicate is a synthetic silicate clay consisting chiefly of calcium and magnesium silicates	Absorbents; Deodorant Agents
Calcium Silicate 1344-95-2	Calcium Silicate is a hydrous or anhydrous silicate with varying proportions of calcium oxide and silica.	Absorbents; Bulking Agents; Opacifying Agents
Fuller's Earth 8031-18-3	Fuller's Earth is a non-plastic variety of kaolin containing an aluminum magnesium silicate.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents
Gold Zeolite	Gold Zeolite is the product obtained by the reaction of gold chloride with Zeolite.	Absorbents; Cosmetic Astringents; Skin Protectants; Skin-Conditioning Agents - Miscellaneous
Hectorite 12173-47-6 68084-71-9	Hectorite is one of the montmorillonite minerals that are the principal constituents of bentonite clay.	Absorbents; Bulking Agents; Dispersing Agents - Nonsurfactant; Opacifying Agents; Viscosity Increasing Agents - Aqueous

Table 1. Definitions, idealized structures, and functions of the ingredients in this safety assessment.³

Ingredient & CAS No.	Definition & Structure	Function(s)
Hydrated Silica 10279-57-9 112926-00-8 1343-98-2 (silicic acid) 63231-67-4 7631-86-9	Hydrated Silica is the inorganic oxide that conforms generally to the formula $\text{SiO}_2 \cdot x\text{H}_2\text{O}$.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents; Oral Care Agents; Skin-Conditioning Agents – Misc.; Viscosity Increasing Agents - Aqueous
Kaolin 1332-58-7	Kaolin is a native hydrated aluminum silicate with an approximate composition of $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents; Skin Protectants; Slip Modifiers
Lithium Magnesium Silicate 37220-90-9	Lithium Magnesium Silicate is a synthetic silicate clay consisting mainly of lithium and magnesium silicates.	Binders; Bulking Agents; Viscosity Increasing Agents - Aqueous
Lithium Magnesium Sodium Silicate 53320-86-8	Lithium Magnesium Sodium Silicate is a synthetic silicate clay consisting mainly of lithium, magnesium and sodium silicates.	Bulking Agents; Viscosity Increasing Agents - Aqueous
Magnesium Aluminometasilicate 12408-47-8	Magnesium Aluminometasilicate is the inorganic compound consisting of varying amounts of magnesium oxide, aluminum oxide and silica.	Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents; Slip Modifiers; Viscosity Increasing Agents – Aqueous; Viscosity Increasing Agents – Nonaqueous
Magnesium Aluminum Silicate 12199-37-0 12511-31-8	Magnesium Aluminum Silicate is a complex silicate refined from naturally occurring minerals.	Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents; Slip Modifiers; Viscosity Increasing Agents - Aqueous
Magnesium Silicate 1343-88-0	Magnesium Silicate is an inorganic salt of variable composition which consists mainly of $\text{MgO} \cdot \text{SiO}_2 \cdot x\text{H}_2\text{O}$.	Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents; Slip Modifiers; Viscosity Increasing Agents - Aqueous
Magnesium Trisilicate 14987-04-3	Magnesium Trisilicate is the inorganic compound that conforms generally to the formula $2\text{MgO} \cdot 3\text{SiO}_2 \cdot x\text{H}_2\text{O}$.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents; Slip Modifiers; Viscosity Increasing Agents - Aqueous
Montmorillonite 1318-93-0	Montmorillonite is a complex aluminum/magnesium silicate clay.	Abrasives; Absorbents; Bulking Agents; Emulsion Stabilizers; Opacifying Agents; Viscosity Increasing Agents - Aqueous
Potassium Silicate 1312-76-1	Potassium Silicate is a potassium salt of silicic acid.	Corrosion Inhibitors

Table 1. Definitions, idealized structures, and functions of the ingredients in this safety assessment.³

Ingredient & CAS No.	Definition & Structure	Function(s)
Pyrophyllite 113349-10-3; 113349-11-4; 113349-12-5; 12269-78-2; 141040-73-5; 141040-74-6	Pyrophyllite is a naturally occurring mineral substance consisting predominantly of a hydrous aluminum silicate represented as $\text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 \cdot \text{H}_2\text{O}$.	Absorbents; Colorants; Opacifying Agents
Silica 112945-52-5 60676-86-0 7631-86-9	Silica is the inorganic oxide that conforms to the formula SiO_2 .	Abrasives; Absorbents; Anticaking Agents; Bulking Agents; Dispersing Agents – Nonsurfactant; Opacifying Agents
Silver Copper Zeolite 130328-19-7 168042-42-0	Silver Copper Zeolite is the product obtained by the reaction of Zeolite with silver nitrate and cupric nitrate.	Absorbents; Deodorant Agents
Silver Zinc Zeolite	Definition under development.	Not reported
Sodium Magnesium Silicate	Sodium Magnesium Silicate is a synthetic silicate clay with a composition mainly of magnesium and sodium silicate.	Binders; Bulking Agents
Sodium Magnesium Aluminum Silicate 12040-43-6	Sodium Magnesium Aluminum Silicate is the complex silicate obtained by the reaction of Sodium Silicate and Sodium Aluminate in an aqueous solution of Magnesium Nitrate.	Absorbents
Sodium Metasilicate 6834-92-0	Sodium Metasilicate is the inorganic salt that conforms to the formula Na_2SiO_3 .	Chelating Agents; Corrosion Inhibitors
Sodium Potassium Aluminum Silicate 12736-96-8 66402-68-4	Sodium Potassium Aluminum Silicate is a complex silicate refined from naturally occurring minerals, or derived synthetically.	Bulking Agents
Sodium Silicate 1344-09-8	Sodium Silicate is a sodium salt of silicic acid.	Buffering Agents; Corrosion Inhibitors; pH Adjusters
Sodium Silver Aluminum Silicate	Sodium Silver Aluminum Silicate is the complex silicate obtained by the reaction of sodium silicate with sodium aluminate in an aqueous solution of sodium nitrate, sodium hydroxide and silver nitrate.	Absorbents; Deodorant Agents
Titanium Zeolite	Titanium Zeolite is the product obtained by the reaction of Zeolite with titanium tetrachloride.	Absorbents; Light Stabilizers; Skin- Conditioning Agents – Misc.
Tromethamine Magnesium Aluminum Silicate	Tromethamine Magnesium Aluminum Silicate is a reaction product of Tromethamine (q.v.) and Magnesium Aluminum Silicate.	Viscosity Increasing Agents - Aqueous
Zeolite 1318-02-1	Zeolite is a hydrated alkali aluminum silicate.	Absorbents; Deodorant Agents
Zinc Silicate 13597-65-4	Zinc Silicate is an inorganic salt consisting of variable amounts of zinc oxide and silica.	Deodorant Agents
Zinc Zeolite	Zinc Zeolite is the product obtained by the reaction of Zeolite with zinc chloride.	Absorbents; Cosmetic Astringents; Skin Protectants; Skin- Conditioning Agents - Miscellaneous
Zirconium Silicate 10101-52-7 1344-21-4	Zirconium Silicate is the inorganic compound that conforms to the formula ZrSiO_4 .	Abrasives; Opacifying Agents

Table 2. Physical and chemical properties

Property	Value	Reference
Aluminum Silicate		
Physical Form	Light brown to brown, odorless beads	28
Molecular Weight (Da)	162.05 - 426.05	1
Density (g/cm ³ @ 20°C)	3.156; 3.247	1
Attapulgite		
Physical Form	White, gray, or transparent, dull, elongated, lath-shaped crystals in bundles that comprise thin sheets of minute interlaced fibers; surface is protonated and hydrated	1
Density (g/cm ³)	2.2	1
Bentonite		
Physical Form	Crystalline, claylike material, available as an odorless, pale buff or cream to grayish-colored fine powder, which is free from grit; dioctahedral	1
Molecular Weight (Da)	359.16	1
pH	9.5-10.5 (2% aqueous solution)	1
Calcium Silicate		
Physical Form	White or slightly cream-colored free-flowing powder	1
Molecular Weight (Da)	116.16	1
Density (g/cm ³ @ 25°C)	0.227	29
Melting Point (°C)	1710	29
Water Solubility (mg/L @ 20°C)	260	29
Fuller's Earth		
Physical Form	Nonplastic variety of Kaolin; sheet structure	1
Hectorite		
Physical Form	Translucent colorless mineral when mined and turns white when dried; tridecahedral	1
Specific Gravity (g/cm ³)	2.65	1
pH	8.5 (5% slurry)	1
Kaolin		
Physical Form	White or yellowish white, earthy mass or white powder; unctuous when moist	1
Molecular Weight (Da)	258.2	1
Magnesium Aluminum Silicate		
Physical Form	Off-white to creamy white small flakes or micronized powder	1
Molecular Weight (Da)	262.4	1
pH	9.0-10.0 (5% aqueous solution)	1
Magnesium Silicate		
Physical Form	Fine, white, odorless, tasteless powder, free from grittiness	1
Magnesium Trisilicate		
Physical Form	Fine, white, odorless, tasteless powder, free from grittiness	1
Potassium Silicate		
Physical Form	Yellowish to colorless, translucent to transparent, hygroscopic	2
Density (g/cm ³ @ 20°C)	1.26-1.60	25
Vapor Pressure (mmHg @ 1175°C)	0.00772	25
Melting Point (°C)	905	25
Silica		
Physical Form	White fluffy powder (fumed hydrophobic or precipitated hydrophobic); white or white, milky (gel or colloidal)	7
Molecular Weight (Da)	60.1 (fumed hydrophobic)	7
Density (g/cm ³ @ 20°C)	2.2 (fumed hydrophobic)	7
Specific Gravity (g/cm ³)	2.2-2.65 (fumed hydrophobic); 1.9-2.2 (precipitated hydrophobic); 1.8-2.2 (gel); 1.0-1.4 (colloidal.)	7
Vapor Pressure (mmHg)	0 (fumed hydrophobic)	7
Melting Point (°C)	2230 (fumed hydrophobic)	7
Boiling Point (°C)	1700-1710 (fumed hydrophobic)	7
Water Solubility (mg/L @ 20°C)	15-68 (pH 5.5-6.6; fumed hydrophobic)	7
pH	3.7-4.5 (fumed hydrophobic); 5-9 (precipitated hydrophobic); 3-8 (gel); 3-5 or 8-11 (colloidal)	7
Sodium Magnesium Silicate		
pH	8.5-10.5 (2% aqueous dispersion)	1
Sodium Magnesium Aluminum Silicate		
Physical Form	White powder	26
Density (g/cm ³ @ 20°C)	2.11	26
Melting Point (°C)	> 400	26
Water Solubility (mg/L @ 20°C)	2.24	26

Table 2. Physical and chemical properties

Property	Value	Reference
<i>Sodium Metasilicate</i>		
Physical Form	Nonahydrate, efflorescent platelets	2
Molecular Weight (Da)	122.08	2
Density (g/cm ³)	2.614	2
Vapor Pressure (mmHg @ 1175°C)	0.00772	34
Melting Point (°C)	1089	2
Water Solubility (g/L @ 20 °C)	210	34
pH	12 (0.1% solution)	2
<i>Sodium Silicate</i>		
Physical Form	Colorless to white or grayish-white, crystal-like clumps or aqueous solutions	2
Density (g/cm ³)	1.26 - 1.71	27
Vapor Pressure (mmHg)	0.00120	27
Melting Point (°C)	730 - 870	27
Water Solubility (mg/L @ 20 °C)	115	27
Acidity/Alkalinity	Strongly alkaline	2
<i>Zeolite</i>		
Physical Form	Crystalline, hydrated alkali-aluminum silicates	1
<i>Zinc Silicate</i>		
Physical Form	White crystals or white powder	22,24
Molecular Weight (Da)	222.90	24
Density (g/cm ³)	4.103	22
Melting Point (°C)	1509	22
Water Solubility (µg/L @ 20 °C)	162.01	33
<i>Zirconium Silicate</i>		
Physical Form	Bipyramidal crystals, colorless unless has impurities and radioactive bombardment; red or various colored crystals	1
Molecular Weight (Da)	183.31	1
Density (g/cm ³)	4.56	1
pH	6-7.5 (10% aqueous slurry)	1

Table 3. Current (2018) and historical frequency and concentration according to duration and type of exposure for previously reviewed silicates^{1,2,6,7}

	Aluminum Calcium Sodium Silicate				Aluminum Silicate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018	2009	2018	2008	2018	1998	2018	1999
Totals*	250	7	NS	0.4-6	68	10	NS	0.5-37
Leave-On	250	7	NS	0.4-6	46	6	NS	0.5-3
Rinse-Off	NR	NR	NS	NR	22	4	NS	2-37
Diluted for (Bath) Use	NR	NR	NS	NR	NR	NR	NS	NR
Eye Area	65	NR	NS	0.5	1	2	NS	0.5
Incidental Ingestion	125	NR	NS	6	NR	NR	NS	37
Incidental Inhalation-Spray	13 ^a ; 3 ^b	1 ^a	NS	NR	6; 12 ^a ; 23 ^b	1 ^a	NS	NR
Incidental Inhalation-Powder	3; 3 ^b	1	NS	NR	23 ^b	NR	NS	NR
Dermal Contact	92	3	NS	0.4-6	58	8	NS	2-3
Deodorant (underarm)	NR	NR	NS	NR	NR	NR	NS	NR
Hair - Non-Coloring	1	NR	NS	NR	4	NR	NS	NR
Hair-Coloring	NR	NR	NS	NR	6	NR	NS	NR
Nail	32	4	NS	0.5	NR	NR	NS	NR
Mucous Membrane	125	NR	NS	6	4	NR	NS	37
Baby Products	NR	NR	NS	NR	NR	NR	NS	NR
	Attapulgite				Bentonite			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018	1998	2018	1999	2018	1998	2018	1999
Totals*	4	10	NR	8	339	94	0.00002-29.7	0.5-80
Leave-On	1	5	NR	8	157	37	0.00008-15	0.8-8
Rinse-Off	3	5	NR	8	180	57	0.00002-29.7	0.5-80
Diluted for (Bath) Use	NR	NR	NR	NR	2	NR	0.14	5
Eye Area	1	NR	NR	NR	38	8	0.00025-2	0.8-5
Incidental Ingestion	NR	NR	NR	NR	7	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	8 ^b	1; 36 ^a ; 27 ^b	5 ^a ; 7 ^b	0.1	1-3 ^a ; 2-5 ^b
Incidental Inhalation-Powder	NR	5	NR	8 ^b	3; 27 ^b ; 1 ^c	7 ^b	2; 0.35-14.3 ^c	2-5 ^b
Dermal Contact	4	10	NR	8	276	88	0.00002-29.7	0.5-80
Deodorant (underarm)	NR	NR	NR	NR	5 ^a	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	16	4	0.1	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	17.3	NR
Nail	NR	NR	NR	NR	21	1	0.00008-0.88	1
Mucous Membrane	NR	NR	NR	NR	17	3	0.14-0.35	0.5-5
Baby Products	NR	NR	NR	NR	1	NR	NR	NR
	Calcium Silicate				Fuller's Earth			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018	1998	2018	1999	2018	1998	2018	1999
Totals*	101	132	0.00013-5	0.3-10	15	3	NR	NR
Leave-On	91	115	0.00013-5	0.3-10	3	1	NR	NR
Rinse-Off	1	1	1.5	8	12	2	NR	NR
Diluted for (Bath) Use	9	16	0.86-1.3	NR	NR	NR	NR	NR
Eye Area	7	11	1	1-8	1	NR	NR	NR
Incidental Ingestion	NR	3	0.00013	0.5	1	NR	NR	NR
Incidental Inhalation-Spray	1 ^b	NR	0.005	NR	1 ^b	NR	NR	NR
Incidental Inhalation-Powder	61; 1 ^b	75	0.25-5; 4.7-5 ^c	0.3-10	1 ^b	NR	NR	NR
Dermal Contact	100	128	0.25-5	0.3-10	14	3	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	1.5	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	0.005	NR	NR	NR	NR	NR
Nail	1	1	NR	NR	NR	NR	NR	NR
Mucous Membrane	9	19	0.00013-1.3	0.5	2	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

Table 3. Current (2018) and historical frequency and concentration according to duration and type of exposure for previously reviewed silicates^{1,2,6,7}

	Hectorite				Hydrated Silica*			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018	1998	2018	1999	2018	2009	2018	2008
Totals*	83	18	0.1-3.2	0.4-100	449	176	NS	0.001-34
<i>Leave-On</i>	59	10	0.1-3.2	0.7-15	197	90	NS	0.001-4
<i>Rinse-Off</i>	24	8	0.5	0.4-100	244	78	NS	0.01-34
<i>Diluted for (Bath) Use</i>	NR	NR	NR	NR	8	8	NS	0.4-4
Eye Area	34	4	0.1-0.8	0.7	12	8	NS	0.06-2
Incidental Ingestion	NR	NR	NR	NR	83	25	NS	0.003-34
Incidental Inhalation-Spray	2 ^a ; 4 ^b	1 ^a	NR	8 ^b	13 ^a ; 10 ^b	10 ^a ; 12 ^b	NS	0.04-2 ^a ; 0.06-2 ^b
Incidental Inhalation-Powder	3; 4 ^b	NR	0.8-1.1 ^c	8 ^b	31; 10 ^b	33; 12 ^b	NS	2-4; 0.06-2 ^b
Dermal Contact	62	10	0.1-3.2	0.4-100	333	117	NS	0.001-17
Deodorant (underarm)	NR	1 ^a	NR	0.7 ^a	1 ^a	NR	NS	2 ^a
Hair - Non-Coloring	8	NR	NR	1	4	NR	NS	0.04-2
Hair-Coloring	1	5	NR	NR	9	20	NS	2
Nail	1	2	0.7	NR	16	13	NS	1-2
Mucous Membrane	NR	1	NR	NR	249	50	NS	0.003-34
Baby Products	NR	NR	NR	NR	NR	NR	NS	NR
	Kaolin				Lithium Magnesium Silicate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018**	1998	2018	1999	2018	1998	2018	1999
Totals*	1794	509	0.0001-53	0.01-100	2	NR	0.3-5	NR
<i>Leave-On</i>	1171	501	0.0001-53	2-100	2	NR	0.3-5	NR
<i>Rinse-Off</i>	609	7	0.0005-35	0.01-84	NR	NR	NR	NR
<i>Diluted for (Bath) Use</i>	14	1	NR	NR	NR	NR	NR	NR
Eye Area	515	231	0.75-27.2	3-48	NR	NR	NR	NR
Incidental Ingestion	90	6	0.97-11.9	12-30	2	NR	NR	NR
Incidental Inhalation-Spray	69 ^a ; 84 ^b	NR	0.0096; 0.15-25 ^a ; 4 ^b	2-25 ^a ; 3 ^b	NR	NR	0.4; 0.3 ^a	NR
Incidental Inhalation-Powder	125; 84 ^b ; 3 ^c	98	2-23.1; 4 ^b ; 1-25 ^c	5-30; 3 ^b	NR	NR	5 ^c	NR
Dermal Contact	1566	466	0.0005-35	0.01-100	NR	NR	0.4-5	NR
Deodorant (underarm)	5 ^a	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	41	5	0.0096-25	4-15	NR	NR	0.3	NR
Hair-Coloring	25	1	0.4-31	NR	NR	NR	NR	NR
Nail	10	NR	0.0001-53	53-54	NR	NR	NR	NR
Mucous Membrane	147	8	0.05-11.9	3-30	2	NR	NR	NR
Baby Products	3	NR	NR	NR	NR	NR	NR	NR
	Lithium Magnesium Sodium Silicate				Magnesium Aluminometasilicate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018	1998	2018	1999	2018	2009	2018	2008
Totals*	56	NR	0.0005-6	NR	3	NR	NS	0.002-0.01
<i>Leave-On</i>	35	NR	0.0005-6	NR	2	NR	NS	0.002-0.01
<i>Rinse-Off</i>	21	NR	0.4	NR	1	NR	NS	NR
<i>Diluted for (Bath) Use</i>	NR	NR	NR	NR	NR	NR	NS	NR
Eye Area	5	NR	0.0005-4	NR	NR	NR	NS	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NS	NR
Incidental Inhalation-Spray	4 ^a ; 2 ^b	NR	6	NR	1 ^b	NR	NS	0.002-0.01 ^b
Incidental Inhalation-Powder	2 ^b	NR	3 ^c	NR	1 ^b	NR	NS	0.002-0.01 ^b
Dermal Contact	31	NR	0.0005-4	NR	3	NR	NS	0.002-0.01
Deodorant (underarm)	NR	NR	0.5	NR	NR	NR	NS	NR
Hair - Non-Coloring	12	NR	6	NR	NR	NR	NS	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NS	NR
Nail	10	NR	NR	NR	NR	NR	NS	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NS	NR
Baby Products	NR	NR	NR	NR	NR	NR	NS	NR

Table 3. Current (2018) and historical frequency and concentration according to duration and type of exposure for previously reviewed silicates^{1,2,6,7}

	Magnesium Aluminum Silicate				Magnesium Silicate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018	1998	2018	1999	2018	1998	2018	1999
Totals*	917	632	0.0004-11	0.1-5	70	NR	0.001-21.6	NR
<i>Leave-On</i>	734	509	0.0008-11	0.1-5	68	NR	0.001-21.6	NR
<i>Rinse-Off</i>	183	122	0.0004-10	0.1-5	2	NR	NR	NR
<i>Diluted for (Bath) Use</i>	NR	1	NR	NR	NR	NR	NR	NR
Eye Area	207	76	0.0025-4.5	0.2-5	24	NR	3-21.6	NR
Incidental Ingestion	5	3	0.93	0.7	14	NR	10	NR
Incidental Inhalation-Spray	1; 144 ^a , 148 ^b	2; 106 ^a , 75 ^b	0.5-0.8 ^a	0.3-5 ^{a,b}	2 ^a ; 3 ^b	NR	NR	NR
Incidental Inhalation-Powder	30; 148 ^b , 8 ^c	75 ^b	1; 0.3-11 ^c	0.3-5 ^b	5; 3 ^b	NR	1 ^c	NR
Dermal Contact	862	583	0.0004-11	0.1-5	55	NR	0.001-21.6	NR
Deodorant (underarm)	8 ^a	5 ^a	0.5-0.7	0.5-1 ^a	NR	NR	NR	NR
Hair - Non-Coloring	15	10	0.19-0.8	1-2	NR	NR	NR	NR
Hair-Coloring	6	1	NR	2	NR	NR	NR	NR
Nail	1	2	NR	NR	1	NR	NR	NR
Mucous Membrane	34	19	0.93	0.5-2	14	NR	10	NR
Baby Products	8	NR	NR	NR	NR	NR	NR	NR
	Magnesium Trisilicate				Montmorillonite			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018	1998	2018	1999	2018	1998	2018	1999
Totals*	17	NR	NR	NR	177	NR	0.05-8.2	NR
<i>Leave-On</i>	NR	NR	NR	NR	101	NR	0.05-6	NR
<i>Rinse-Off</i>	17	NR	NR	NR	75	NR	0.5-8.2	NR
<i>Diluted for (Bath) Use</i>	NR	NR	NR	NR	1	NR	NR	NR
Eye Area	NR	NR	NR	NR	39	NR	0.3-2.8	NR
Incidental Ingestion	NR	NR	NR	NR	13	NR	1.7	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	12 ^a , 7 ^b	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	5; 7 ^b , 1 ^c	NR	2.5-6 ^c	NR
Dermal Contact	16	NR	NR	NR	145	NR	0.05-6	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	9	NR	8.2	NR
Hair-Coloring	1	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	23	NR	0.5-1.7	NR
Baby Products	NR	NR	NR	NR	1	NR	NR	NR
	Potassium Silicate				Silica***			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018	2001	2018	1999/2000	2018	2009	2018	2008
Totals*	1	2	NR	NR	8024	3276	NS	0.0000003-44
<i>Leave-On</i>	NR	1	NR	NR	7416	2937	NS	0.00004-44
<i>Rinse-Off</i>	1	1	NR	NR	558	316	NS	0.0000003-16
<i>Diluted for (Bath) Use</i>	NR	NR	NR	NR	50	23	NS	0.02-2
Eye Area	NR	NR	NR	NR	2403	867	NS	0.0004-44
Incidental Ingestion	NR	NR	NR	NR	1583	551	NS	0.01-21
Incidental Inhalation-Spray	NR	NR	NR	NR	131; 473 ^a , 382 ^b	19; 247 ^a , 183 ^b	NS	0.0005-6; 0.00004-8 ^a ; 0.02-10 ^b
Incidental Inhalation-Powder	NR	NR	NR	NR	507; 382 ^b , 3 ^c	248; 183 ^b , 1 ^c	NS	1-26; 0.02-10 ^b
Dermal Contact	1	1	NR	NR	5297	2298	NS	0.0000003-44
Deodorant (underarm)	NR	NR	NR	NR	31 ^a	38 ^a	NS	0.02-9 ^a
Hair - Non-Coloring	NR	1	NR	NR	111	51	NS	0.0005-3
Hair-Coloring	NR	NR	NR	NR	187	149	NS	0.002-6
Nail	NR	NR	NR	NR	549	92	NS	0.3-9
Mucous Membrane	NR	NR	NR	NR	1792	624	NS	0.0000003-21
Baby Products	NR	NR	NR	NR	7	2	NS	0.003-10

Table 3. Current (2018) and historical frequency and concentration according to duration and type of exposure for previously reviewed silicates^{1,2,6,7}

	Sodium Magnesium Silicate				Sodium Metasilicate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018	1998	2018	1999	2018	2001	2018	1999/2000
Totals*	139	34	0.13-0.2	0.08-5	137	191	0.001-15	13-18
Leave-On	84	33	0.13	0.08-5	1	NR	0.001	NR
Rinse-Off	54	1	0.2	0.3-5	136	191	1.2-15	13-18
Diluted for (Bath) Use	1	NR	NR	NR	NR	NR	0.1	NR
Eye Area	13	13	NR	0.08-0.4	NR	NR	NR	NR
Incidental Ingestion	9	1	NR	0.3-3	NR	NR	NR	NR
Incidental Inhalation-Spray	37 ^a ; 5 ^b	2 ^a ; 5 ^b	NR	1-5 ^a ; 0.1-5 ^b	1 ^a	NR	NR	NR
Incidental Inhalation-Powder	7; 5 ^b	4; 5 ^b	NR	0.4; 0.1-5 ^b	NR	NR	NR	NR
Dermal Contact	127	31	0.13-0.2	0.08-5	NR	2	0.001-1.2	NR
Deodorant (underarm)	NR	NR	NR	0.5 ^a	NR	NR	NR	NR
Hair - Non-Coloring	2	1	NR	NR	1	1	NR	NR
Hair-Coloring	NR	NR	NR	NR	136	188	5-15	13-18 ^d
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	13	1	NR	0.3	NR	NR	0.1	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Sodium Potassium Aluminum Silicate				Sodium Silicate			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2018	2009	2018	2008	2018	2001	2018	1999/2000
Totals*	12	1	NS	0.001-4	91	22	0.017-35	0.06-55
Leave-On	10	NR	NS	0.001-4	15	2	NR	0.6-1
Rinse-Off	2	1	NS	NR	76	20	0.017-35	0.06-55
Diluted for (Bath) Use	NR	NR	NS	NR	NR	NR	NR	NR
Eye Area	NR	NR	NS	NR	4	1	NR	NR
Incidental Ingestion	NR	NR	NS	NR	1	NR	0.44	0.6
Incidental Inhalation-Spray	5 ^a	NR	NS	NR	1 ^a ; 6 ^b	1 ^b	NR	NR
Incidental Inhalation-Powder	NR	NR	NS	NR	6 ^b	1 ^b	NR	NR
Dermal Contact	12	1	NS	NR	35	14	0.017-1.5	0.06-10
Deodorant (underarm)	NR	NR	NS	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NS	NR	1	NR	NR	NR
Hair-Coloring	NR	NR	NS	NR	54	8	15-35	1-55 ^e
Nail	NR	NR	NS	0.001-4	NR	NR	NR	NR
Mucous Membrane	1	NR	NS	NR	8	2	0.44-1.4	0.06-7
Baby Products	NR	NR	NS	NR	NR	NR	NR	0.6
	Zeolite				NR = Not reported. S = Survey in progress. † Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses. ^a It is possible these products may be sprays, but it is not specified whether the reported uses are sprays. ^b Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation. ^c It is possible these products may be powders, but it is not specified whether the reported uses are powders. ^d Hair bleaches were diluted from 13% to 18% to 7% to 14% before use. ^e Hair bleaches were diluted from 16%-55% to 1%-20% before use. *Includes entries for Hydrated Silica and Silicic Acid from the VCRP database. ** Includes entries for Kaolinite from the VCRP database. *** Includes entries for Silica; Silica, Amorphous; Silica, Fumed; and Silicon Dioxide, Colloidal from the VCRP database.			
	# of Uses		Max Conc of Use (%)					
	2018	1998	2018	1999				
Totals*	157	NR	0.0043-37.8	NR				
Leave-On	140	NR	0.03-35.7	NR				
Rinse-Off	17	NR	0.0043-37.8	NR				
Diluted for (Bath) Use	NR	NR	NR	NR				
Eye Area	25	NR	0.032-3.6	NR				
Incidental Ingestion	3	NR	3	NR				
Incidental Inhalation-Spray	3; 15 ^a ; 11 ^b	NR	1; 35.7 ^a	NR				
Incidental Inhalation-Powder	45; 11 ^b	NR	0.6-1.1; 0.5-1 ^c	NR				
Dermal Contact	146	NR	0.0043-37.8	NR				
Deodorant (underarm)	NR	NR	NR	NR				
Hair - Non-Coloring	8	NR	1-35.7	NR				
Hair-Coloring	NR	NR	5	NR				
Nail	NR	NR	NR	NR				
Mucous Membrane	5	NR	3	NR				
Baby Products	NR	NR	NR	NR				

Table 4. 2018 frequency and concentration of use according to duration and type of exposure for potential Silicate add-on ingredients⁶

	<i># of Uses</i>	<i>Max Conc of Use (%)</i>	<i># of Uses</i>	<i>Max Conc of Use (%)</i>
	Ammonium Silver Zinc	Aluminum Silicate	Zinc Zeolite	
Totals[†]	17	0.001	1	NR
<i>Duration of Use</i>				
Leave-On	16	NR	NR	NR
Rinse Off	1	0.001	1	NR
Diluted for (Bath) Use	NR	NR	NR	NR
<i>Exposure Type</i>				
Eye Area	13	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR
Incidental Inhalation-Powder	1	NR	NR	NR
Dermal Contact	17	0.001	NR	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	1	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR

NR = Not reported. S = Survey in progress.

[†] Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.**Table 5.** Ingredients not reported to be in use.⁶

Activated Clay
 Aluminum Calcium Magnesium Potassium Sodium Zinc Silicates
 Aluminum Iron Calcium Magnesium Germanium Silicates
 Aluminum Iron Calcium Magnesium Zirconium Silicates
 Aluminum Iron Silicates*[‡]
 Ammonium Silver Zeolite
 Calcium Magnesium Silicate[‡]
 Gold Zeolite
 Pyrophyllite*
 Silver Copper Zeolite[‡]
 Silver Zinc Zeolite[‡]
 Sodium Magnesium Aluminum Silicate
 Sodium Silver Aluminum Silicate
 Titanium Zeolite[‡]
 Tromethamine Magnesium Aluminum Silicate
 Zinc Silicate
 Zirconium Silicate*

*Additionally, no uses were reported in original safety assessment.

[‡] Not surveyed.

Table 6. Acute toxicity studies

Ingredient/Concentration/Vehicle	Dose/Study Protocol	Results	LD₅₀ or LC₅₀	Reference
<i>Dermal</i>				
Bentonite (2/3 weight) and Zeolite (1/3 weight) in a hemostatic agent	~8 g applied on wounded skin to 12 male Sprague-Dawley rats, another 12 rats served as controls; wounds were circular, full-thickness and 2 cm in diameter; skin sampled excised and evaluated stereologically after scarification; 6 rats from test group and 6 rats from control group were killed on days 12 and 21	At day 12 termination, reduction in the length density of the blood vessels (31%) and diameter of the large and small vessels (38% and 16%, respectively) was observed in the rats that received the test material; at day 21 termination, volume density of both the dermis and collagen bundles was reduced by 25% in the treated rats when compared to the controls; authors concluded hemostatic agent containing Bentonite and Zeolite may cause vasoconstriction and inhibition of neoangiogenesis	Not measured	⁴³
30% Potassium Silicate solution in water; molar ratio = 2.47	5000 mg/kg bw applied for 24 h to 5 male and 5 female Sprague-Dawley rats; test sites were occluded	Erythema and alopecia noted at application site of 4 females and 1 male between days 1 and 8; no other adverse effects during observation period or necropsy	> 5 g/kg	²⁵
<i>Oral</i>				
Aluminum Silicate in water; concentration not reported	2000 mg/kg bw; 3 female Sprague-Dawley rats via gavage	No mortality occurred from dosing; no clinical signs of toxicity; no treatment-related effects at necropsy	> 2 g/kg bw	²⁸
20% Calcium Silicate in feed	10 g/kg bw; 10 male and 10 female Wistar rats via diet	No mortality occurred from dosing; no significant clinical findings; no treatment-related effects at necropsy	> 10 g/kg bw	²⁹
Potassium Silicate; concentration and vehicle not reported	3.30, 3.96, 4.75, 5.70, or 6.86 g/kg bw; 5 male and 5 female Cpb; Wu Wistar rats per dose; method of delivery not reported	Deaths per dose = 1/10 at 2.50 ml/kg, 2/10 at 3.00 ml/kg, 3/10 at 4.32 ml/kg, and 10/10 at 5.20 ml/kg; sedation, signs of abdominal discomfort, sluggishness and unconsciousness were all reversible; no treatment-related effects at necropsy	5.70 g/kg bw	³⁰
Potassium Silicate; undiluted; no further details reported	5.0 g/kg bw in 3 female Sprague-Dawley rats via gavage	No deaths occurred following treatment; no clinical or gross macroscopic signs of toxicity observed	> 5.0 g/kg bw	²⁵
Sodium Magnesium Aluminum Silicate in water; no further details reported	2.0 g/kg bw in 6 female Sprague-Dawley rats via gavage	No deaths occurred following treatment; no clinical or gross macroscopic signs of toxicity observed	> 2.0 g/kg bw	²⁶

Table 6. Acute toxicity studies

Ingredient/Concentration/Vehicle	Dose/Study Protocol	Results	LD₅₀ or LC₅₀	Reference
Sodium Silicate; molar ratio 3.27; concentration and vehicle not reported	3.43, 4.11, 4.93, 5.89, 7.12, or 8.49 g/kg bw; 5 male and female Cpb:Wu Wistar rats per dose via gavage	Deaths per dose = 0/10 at 3.43 g/kg, 2/10 at 4.11 g/kg, 9/10 at 4.93, 5.89, and 7.12 g/kg, and 10/10 at 8.49 g/kg; sedation, signs of abdominal discomfort, sluggishness and unconsciousness; no treatment-related effects at necropsy	5.15 g/kg bw	²⁷
Sodium Silicate in water; molar ratio = 3.38	Male Wistar rats; no additional details provided	Breathing difficulties, staggering gait, reduced motility; additional effects not reported	8.65 g/kg bw	²⁷
Sodium Silicate; molar ratio = 3.3; no additional details provided	Rats; no additional details provided	No details provided	> 2.00 g/kg bw	²⁷
Sodium Silicate; molar ratio = 3.35; no additional details provided	Male mice; no additional details provided	No details provided	6.60 g/kg bw	²⁷
Inhalation				
30% Potassium Silicate solution in water; molar ratio 2.47	2.06 mg/l; whole body exposure for 4.4 h to 5 male and 5 female Sprague-Dawley rats; particle size distribution = 4% at 9 µm, 8.3% at 5.8 µm, 11.1% at 4.7 µm, 12% at 3.3 µm, 32% at 2.1 µm, 2.6% at 1.1 µm, 7.4% at 0.7 µm, and 2.6% at 0.4 µm	Animals had hunched posture and hypoactivity during exposure that reversed; no deaths or adverse effects during observation period or necropsy	> 2.06 mg/l	²⁵

Table 7. Genotoxicity studies

Ingredient/Concentration/Dose	Species/Strain/Cell	In Vitro		Method	Results	Reference
Aluminum Silicate in water, up to 5017 µg/plate, with or without metabolic activation	<i>Salmonella typhimurium</i> strains TA97a, TA98, TA100, TA102, and TA1535	Ames test			Not genotoxic	28
Aluminum Silicate in DMSO; up to 250 µg/ml without metabolic activation; up to 500 µg/ml with metabolic activation	Chinese hamster ovary	HPRT gene mutation assay			Not genotoxic	28
Montmorillonite in unmodified (1 type) and organo-modified clay forms (3 types); unmodified tested at up to 125 µg/ml and the modified clays were tested at up to 250 µg/ml, with and without metabolic activation	<i>S. typhimurium</i> strains TA97a, TA98, TA100, TA102, TA104	Ames test			Two modified clays had significant increases in revertant colonies in strain TA98 with metabolic activation; no significant changes were observed in TA98 without metabolic activation or in the other strains; no mutagenic activity observed in the third modified clay or in the unmodified clay	36
Montmorillonite; 15.65, 31.25 or 62.5 µg/ml	Human hepatoma cell line (HepG2)	Cytokinesis block micronucleus cyto assay			Potentially genotoxic; an increased number of micro nuclei was observed but there were no effect observed in nucleoplasmic bridges or nuclear buds	35
Sodium Metasilicate; up to 5000 µg/plate, with or without metabolic activation	<i>S. typhimurium</i> strains TA98, TA100, TA 1535, TA 1537 and <i>Escherichia coli</i> WP2	Ames test			Not genotoxic	34
Sodium Metasilicate; up to 675 µg/ml without metabolic activation and up to 1800 µg/ml with metabolic activation	Chinese hamster V79 cells	HPRT gene mutation assay			Not genotoxic	34
36% Sodium Silicate; molar ratio = 3.3; up to 156.3 µg/ml with and without metabolic activation	Chinese hamster V79 cells	Chromosome aberration test			Not genotoxic	27
36% Sodium Silicate; molar ratio = 3.35; up to 675 µg/ml without metabolic activation and up to 1800 µg/ml with metabolic activation	Chinese hamster V79 cells	HPRT gene mutation assay			Not genotoxic	27
Zinc Silicate; 100, 316, 1000, 3160 or 5000 µg/plate with or without metabolic activation	<i>S. typhimurium</i> strains TA98, TA100, TA102, TA1535, and TA1537	Ames test			Not genotoxic	33

Table 8. Dermal irritation and sensitization

Ingredient/Concentration/ Dose/Vehicle	Test System	Method	Results	Reference
<i>Irritation – In Vitro</i>				
Aluminum Silicate; 25 mg in Dulbecco's phosphate buffered saline	EpiDerm tissue	EpiDerm human skin model; material applied for 30 min	Not irritating	²⁸
Zinc Silicate; undiluted; 25 mg	EpiDerm tissue	EpiDerm reconstructed human epidermis model in accordance with OECD Test Guideline 439; test material applied to 0.63 cm ² test tissue for 60 min	Not irritating	³³
<i>Irritation - Animal</i>				
36% (weight) Potassium Silicate; molar ratio = 2.0; 0.5ml in water	1 female New Zealand White rabbit	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and semi-occluded for 4 h before being rinsed off with water; test site examined for up to 5 days	Slightly irritating; transient erythema observed cleared by day 5; primary dermal irritation index (PDII) = 1	^{25,30}
33% (weight) Potassium Silicate; molar ratio = 3.0; 0.5ml in water	1 male New Zealand White rabbit	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and semi-occluded for 4 h before being rinsed off with water; test site examined for up to 5 days	Moderately irritating; well-defined erythema and very slight edema persisted for at least 5 days; PDII = 3	^{25,30}
35% (weight) Potassium Silicate; molar ratio = 3.4; 0.5ml in deionized water	3 New Zealand White rabbits; sex not reported	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and occluded for 4 h before being rinsed; test site examined for up to 7 days	Not irritating; slight erythema after 1 h that cleared after 48 h; PDII = 0.17	^{25,30}
25% dilution of 35% (weight) Potassium Silicate; molar ratio = 3.4; 0.5 ml in deionized water	3 New Zealand White rabbits; sex not reported	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and occluded for 4 h before being rinsed; test site examined for up to 7 days	Not irritating; very slight erythema after 24 and 48 h after treatment; PDII = 0	^{25,30}
25% dilution of 29% (weight) Potassium Silicate; molar ratio = 3.9; 0.5 ml in deionized water	5 New Zealand White rabbits; sex not reported	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and occluded for 4 h before being rinsed; test site examined for up to 7 days	Not irritating; PDII = 0	^{25,30}
29% (weight) Potassium Silicate; molar ratio = 3.9; 0.5 ml in deionized water	5 New Zealand White rabbits; sex not reported	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and occluded for 4 h before being rinsed; test site examined for up to 7 days	Not irritating; slight erythema cleared by 24 h ;PDII = 0.25	^{25,30}
Sodium Metasilicate (anhydrous); 0.5 g in water	1 male New Zealand White rabbits	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and semi-occluded for 4 h before being rinsed; test site examined for up to 5 days	Corrosive; necrosis observed; PDII = 8; no erythema or edema observed when applied as dry powder	³⁴
Sodium Metasilicate (pentahydrate); 0.5 g in water	1 female New Zealand White rabbits	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and semi-occluded for 4 h before being rinsed; test site examined for up to 5 days	Corrosive; necrosis observed; PDII = 8; no erythema or edema observed when applied as dry powder	³⁴
10% aq. Sodium Metasilicate; 0.5 ml in water	3 rabbits; strain and sex not reported	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and semi-occluded for 4 h before being rinsed; test site examined for up to 72 h	Slightly irritating; severity of erythema reduced but persisted through day2; edema in 1 animal reversed by day 2; PDII = 1.22	³⁴
50% aq. Sodium Metasilicate; 0.5 ml in water	3 rabbits; strain and sex not reported	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and semi-occluded for 4 h before being rinsed; test site examined for up to 72 h	Irritating; PDII = 3.67	³⁴

Table 8. Dermal irritation and sensitization

Ingredient/Concentration/ Dose/Vehicle	Test System	Method	Results	Reference
57.5% (weight) Sodium Metasilicate (pentahydrate); 0.5 g	3 white landrace rabbits; sex not reported	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and semi-occluded for 4 h before being rinsed; test site examined for up to 14 days	Corrosive; 2/3 animals had acute skin necrosis and the 3 rd had pigmented necrosis; wounds persisted for more than 14 days; PDII = 7.8	³⁴
97% (weight) Sodium Metasilicate (anhydrous); 0.5 g	3 white landrace rabbits; sex not reported	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and semi-occluded for 4 h before being rinsed; test site examined for up to 14 days	Corrosive; 2/3 animals had acute skin necrosis with well-defined edema; wounds persisted for more than 14 days; third animal had wounds that were observed at up to 72 h but had healed by day 14; PDII = 5.1	³⁴
Sodium Metasilicate; concentration not reported; fine powder with pH of 12.4 tested undiluted; 0.5 g	3 New Zealand White rabbits; sex not reported	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and semi-occluded for 4 h before being rinsed; test site examined for up to 14 days	Not irritating; 1/3 animals had erythema and edema 1 h post-treatment that cleared by 72 h; PDII = 0.17	³⁴
83% (w/w) Sodium Metasilicate as aqueous paste; pH 12.4; 0.5 g/0.10 purified water; 0.3 ml applied	3 male New Zealand hybrid rabbits	Dermal irritation study in accordance with OECD Test Guideline 404; test material applied to shaved test site and semi-occluded for 4 h before being rinsed; test site examined for up to 14 days	Corrosive; erythema persisted for at least 14 days; edema observed 1 h post-treatment but cleared by 72 h; necrosis persisted 7-14+ days; PDII = 4.67	³⁴
<i>Sensitization - Animal</i>				
0%, 5%, 10%, or 25% (w/v) Aluminum Silicate in dimethyl sulfoxide; application volume = 25 µl	4 female CBA/CaOlaHsd mice/dose group	Local lymph node assay (LLNA)	Not sensitizing; stimulation indices (SI) below 3	²⁸
30% Potassium Silicate solution; molar ratio = 2.47	20 male Hartley guinea pigs received test material; 10 animals served as control	Buehler sensitization test; animals were induced with undiluted test material and challenged at 75%	Not sensitizing	²⁵
0%, 10%, 25%, or 50% Zinc Silicate in acetone/olive oil (4:1; v/v)	6 female NMRI mice/dose group	LLNA	Not sensitizing; SI below 1.4; irritant response noted	³³

Table 9. Ocular irritation

Ingredient/Concentration/ Dose/Vehicle	Test System	Method	Results	Reference
<i>In Vitro</i>				
Aluminum Silicate tested pure; no vehicle; 164.3 mg	Lohmann Leghorn chicken eggs	HET-CAM method; treatment duration = 5 min	Not irritating	²⁸
Sodium Metasilicate; concentration not reported; undiluted; 50 mg	New Zealand White rabbit eyes	In vitro rabbit eye study; treatment duration = 0.17 min; eyes studied for opacity for up to 4 h post-treatment	Corrosive	³⁴
Zinc Silicate; 20% suspension in 750 µl of physiological saline solution (0.9% NaCl)	Bovine corneas	Bovine corneal opacity and permeability test (BCOP); exposure was 4 h	Irritating; mean opacity score of 3 corneas was 6.31; mean fluorescein retention/leakage score was < 0.01	³³
<i>Animal</i>				
29% (weight) Potassium Silicate; molar ratio = 3.9; 0.1 ml in water	6 New Zealand White rabbits; sex not reported	Ocular irritation study in accordance with OECD Test Guideline 405; eyes not rinsed; observed for up to 7 days post-treatment	Not irritating	^{25,30}
25% dilution of 29% (weight) Potassium Silicate; molar ratio = 3.9; 0.1 ml in deionized water	6 New Zealand White rabbits; sex not reported	Ocular irritation study in accordance with OECD Test Guideline 405; eyes not rinsed; observed for up to 7 days post-treatment	Not irritating	^{25,30}
35% (weight) Potassium Silicate; molar ratio = 3.4; 0.1 ml in water	3 New Zealand White rabbits; sex not reported	Ocular irritation study in accordance with OECD Test Guideline 405; eyes not rinsed; observed for up to 7 days post-treatment	Slightly irritating; redness and chemosis of the conjunctivae (scores 1.0-1.3 and 1.3-1.5, respectively) observed up to 7 days post-treatment	^{25,30}
25% dilution of 35% (weight) Potassium Silicate; molar ratio = 3.4; 0.1 ml in water	3 New Zealand White rabbits; sex not reported	Ocular irritation study in accordance with OECD Test Guideline 405; eyes not rinsed; observed for up to 7 days post-treatment	Not irritating	^{25,30}
~30% Potassium Silicate in water; molar ratio = 2.47; 0.1 ml	3 New Zealand White rabbits; sex not reported	Ocular irritation study; eyes not rinsed; observed for up to 7 days post- treatment	Slightly irritating	²⁵

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Final Report on the Safety Assessment of Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite¹

This report reviews the safety of Aluminum, Calcium, Lithium Magnesium, Lithium Magnesium Sodium, Magnesium Aluminum, Magnesium, Sodium Magnesium, and Zirconium Silicates, Magnesium Trisilicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite as used in cosmetic formulations. The common aspect of all these claylike ingredients is that they contain silicon, oxygen, and one or more metals. Many silicates occur naturally and are mined; yet others are produced synthetically. Typical cosmetic uses of silicates include abrasive, opacifying agent, viscosity-increasing agent, anticaking agent, emulsion stabilizer, binder, and suspending agent. Clay silicates (silicates containing water in their structure) primarily function as adsorbents, opacifiers, and viscosity-increasing agents. Pyrophyllite is also used as a colorant. The International Agency for Research on Cancer has ruled Attapulgite fibers $>5\ \mu\text{m}$ as possibly carcinogenic to humans, but fibers $<5\ \mu\text{m}$ were not classified as to their carcinogenicity to humans. Likewise, Clinoptilolite, Phillipsite, Mordenite, Nonfibrous Japanese Zeolite, and synthetic Zeolites were not classified as to their carcinogenicity to humans. These ingredients are not significantly toxic in oral acute or short-term oral or parenteral toxicity studies in animals. Inhalation toxicity, however, is readily demonstrated in animals. Particle size, fibrogenicity, concentration, and mineral composition had the greatest effect on toxicity. Larger particle size and longer and wider fibers cause more adverse effects. Magnesium Aluminum Silicate was a weak primary skin irritant in rabbits and had no cumulative skin irritation in guinea pigs. No gross effects were reported in any of these studies. Sodium Magnesium Silicate had no primary skin irritation in rabbits and had no cumulative skin irritation in guinea pigs. Hectorite was nonirritating to the skin of rabbits in a Draize primary skin irritation study. Magnesium Aluminum Silicate and Sodium Magnesium Silicate

caused minimal eye irritation in a Draize eye irritation test. Bentonite caused severe iritis after injection into the anterior chamber of the eyes of rabbits and when injected intralaminally, widespread corneal infiltrates and retrocorneal membranes were recorded. In a primary eye irritation study in rabbits, Hectorite was moderately irritating without washing and practically nonirritating to the eye with a washout. Rats tolerated a single dose of Zeolite A without any adverse reaction in the eye. Calcium Silicate had no discernible effect on nidation or on maternal or fetal survival in rabbits. Magnesium Aluminum Silicate had neither a teratogenic nor adverse effects on the mouse fetus. Female rats receiving a 20% Kaolin diet exhibited maternal anemia but no significant reduction in birth weight of the pups was recorded. Type A Zeolite produced no adverse effects on the dam, embryo, or fetus in either rats or rabbits at any dose level. Clinoptilolite had no effect on female rat reproductive performance. These ingredients were not genotoxic in the Ames bacterial test system. In primary hepatocyte cultures, the addition of Attapulgite had no significant unscheduled DNA synthesis. Attapulgite did cause significant increases in unscheduled DNA synthesis in rat pleural mesothelial cells, but no significant increase in sister chromosome exchanges were seen. Zeolite particles ($<10\ \mu\text{m}$) produced statistically significant increase in the percentage of aberrant metaphases in human peripheral blood lymphocytes and cells collected by peritoneal lavage from exposed mice. Topical application of Magnesium Aluminum Silicate to human skin daily for 1 week produced no adverse effects. Occupational exposure to mineral dusts has been studied extensively. Fibrosis and pneumoconiosis have been documented in workers involved in the mining and processing of Aluminum Silicate, Calcium Silicate, Zirconium Silicate, Fuller's Earth, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite. The Cosmetic Ingredient Review (CIR) Expert Panel concluded that the extensive pulmonary damage in humans was the result of direct occupational inhalation of the dusts and noted that lesions seen in animals were affected by particle size, fiber length, and concentration. The Panel considers that most of the formulations are not respirable and of the preparations that are respirable, the concentration of the ingredient is very low. Even so, the Panel considered that any spray containing these solids should be formulated to minimize their inhalation. With this admonition to the cosmetics industry, the CIR Expert Panel concluded that these ingredients are safe as currently used in cosmetic formulations.

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¹Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Amy R. Elmore, former Scientific Analyst and Writer. Address correspondence to F. Alan Andersen, Cosmetic Ingredient Review Director, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

The Panel did note that the cosmetic ingredient, Talc, is a hydrated magnesium silicate. Because it has a unique crystalline structure that differs from ingredients addressed in this safety assessment, Talc is not included in this report.

INTRODUCTION

Various silicates and silicate clays are used in cosmetics, largely for their adsorbent, anticaking, bulking, and other similar properties. They are created synthetically in some cases, e.g., Lithium Magnesium Silicate, or are refined from naturally occurring minerals, e.g., Magnesium Aluminum Silicate. In either case, variations in composition occur. Thus the Zeolite group of hydrated aluminosilicates has forms that are crystalline or fibrous, and contain interchangeable cations.

This report reviews the safety of these ingredients. Because the issues of safety are likely to be similar, many ingredients have been grouped. Although there are not data on each and every ingredient, it is expected that the data will be broadly applicable among the following ingredients: Aluminum Silicate (CAS no. 1327-36-2); Calcium Silicate (CAS no. 1344-95-2); Magnesium Aluminum Silicate (CAS no. 12199-37-0, 1327-43-1, 12511-31-8); Magnesium Silicate (CAS no. 1343-88-0); Magnesium Trisilicate (CAS no. 14987-04-3); Sodium Magnesium Silicate; Zirconium Silicate (CAS no. 14940-68-2); and the silicate clays/clay minerals: Attapulgite (CAS no. 1337-76-4, 12174-11-7); Bentonite (CAS no. 1302-78-9); Fuller's Earth (CAS No. 8031-18-3); Hectorite (CAS no. 12173-47-6); Kaolin (CAS no. 1332-58-7); Lithium Magnesium Silicate; Lithium Magnesium Sodium Silicate (CAS no. 53320-86-8); Montmorillonite (CAS no. 1318-93-0); Pyrophyllite (CAS no. 12269-78-2); and Zeolite (CAS no. 1318-02-1) used in cosmetics.

It is important to note that the cosmetic ingredient, Talc, is not included in this safety assessment. Talc is a hydrated magnesium silicate with the CAS no. 14807-96-6, but it should not be confused with any of the silicates in this report. Talc is differentiated by its definition, a hydrated magnesium silicate, and its unique crystalline form.

The safety of Quaternium-18 Hectorite and Quaternium-18 Bentonite have been previously reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel; the final conclusion indicated that "Quaternium-18 Hectorite and Quaternium-18 Bentonite are safe as cosmetic ingredients in the present practices of use and concentration" (CIR 1980).

CHEMISTRY

Given the large number of ingredients, a tabular presentation of basic information concerning the chemical description has been provided (Table 1).

Zeolites

The Zeolite group is very diverse. Over 100 structural types of Zeolites, both natural and synthetic, have been reported, 40

of which are natural Zeolites (IARC 1997). Even though these Zeolites are considered to be a group, the formulas of the most common are listed in tabular form in Table 2 so the reader can understand the diversity in this category.

Physical and Chemical Properties

In alphabetical order according to the cosmetic ingredient name as specified in the *International Cosmetic Ingredient Dictionary and Handbook* (Wenninger et al. 2000), Table 3 provides information on the various synonyms used to describe each cosmetic ingredient, lists the available information on physical properties, and, if available, provides the specifications for the cosmetic grade of the ingredient.

Clay Structure

According to Grim (1967), clays in general have atomic lattices consisting of two structural units. One unit consists of two sheets of closely packed oxygens or hydroxyls as shown in Figure 1. Aluminum, iron, or magnesium atoms are embedded within these sheets in octahedral coordination, so that they are equidistant from the oxygen or hydroxyl groups.

The second unit is composed of silica tetrahedrons as shown in Figure 2. Assuming there are no distortions in each tetrahedron, a silicon atom is equidistant from four oxygens or hydroxyls, if needed to balance the structure, arranged in the form of a tetrahedron with a silicon atom in the center. The silica tetrahedral groups are arranged in a hexagonal network, which is repeated infinitely to form a sheet of composition $\text{Si}_4\text{O}_6(\text{OH})_4$. The tips of the tetrahedrons all point in the same direction and the bases are all in the same plane. Substantial distortion of these units occurs in order to fit into determined unit-cell dimensions of minerals (Grim 1967).

Attapulgite

The general attributes of structure and composition of the minerals are not very well known. The structurally important element is the amphibole double silica chain oriented with its long direction parallel to the *c* axis as shown in Figure 3. Attapulgite

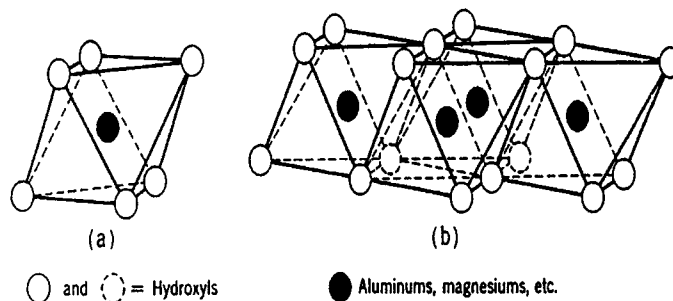


FIGURE 1

(a) Single octahedral unit; (b) Sheet of units (taken from Grim 1967 with permission).

TABLE 1
Chemical formulas and compositions of Silicates and Silicate Clays used in cosmetics

Ingredient	Description	Reference
Aluminum Silicate	$\text{Al}_2\text{O}_3 \cdot \text{SiO}_2$ Complex inorganic salt that has a composition of consisting generally of 1 mole of alumina and 1 to 3 moles of silica	Wenninger et al. 2000 Wenninger et al. 2000
Calcium Silicate	Varying CaO and SiO_2 Hydrous or anhydrous silicate with varying proportions of calcium oxide and silica	Wenninger et al. 2000 Wenninger et al. 2000
Magnesium Aluminum Silicate	$\text{Al}_2\text{MgO}_8\text{Si}_2$ Complex silicate refined from naturally occurring minerals	Budavari 1989 Wenninger et al. 2000
Magnesium Silicate	$\text{MgO} \cdot \text{SiO}_2 \cdot x\text{H}_2\text{O}$ Inorganic salt of variable composition	Wenninger et al. 2000 Wenninger et al. 2000
Magnesium Trisilicate	$2\text{MgO}_3 \cdot \text{SiO}_2 \cdot x\text{H}_2\text{O}$ Inorganic compound	Wenninger et al. 2000 Wenninger et al. 2000
Zirconium Silicate	ZrSiO_4 Inorganic compound Zircon sand or flour; specially sized grades of the mineral zircon—a naturally occurring zirconium silicate	Wenninger et al. 2000 Wenninger et al. 2000 American Minerals, Inc. 1998
Attapulgit	$[\text{Mg}(\text{Al}_{0.5-1}\text{Fe}_{0-0.5})\text{Si}_4\text{O}_{10}(\text{OH}) \cdot 4\text{H}_2\text{O}]$ Variety of Fuller's Earth (q.v.) found typically near Attapulgas, Georgia. It is characterized as having a chain structure rather than the usual sheet structure of other clays Hydrated magnesium aluminum silicate with magnesium partially replaced by aluminum, or to a lesser extent, iron Purified native magnesium aluminum silicate	IARC 1997 Wenninger et al. 2000 IARC 1997 Barr and Arnista 1957
Bentonite	$\text{Al}_2\text{O}_3 \cdot 4\text{SiO} \cdot 2\text{H}_2\text{O}^a$ (empirical formula) $\text{Na}_{0.33}[\text{Al}_{1.67}\text{Mg}_{0.33}]\text{Si}_4[\text{OH}]_2$ Native hydrated colloidal aluminum silicate clay Commercial term for clays containing montmorillonite type minerals formed by the alteration of volcanic ash	Informatics, Inc. 1974 Rheox Inc. 1999 Wenninger et al. 2000 Gamble 1986
Fuller's Earth	No specific formula Nonplastic variety of kaolin containing an aluminum magnesium silicate Porous colloidal aluminum silicate, a catch-all phrase for clay or other fine-grained earthy material suitable for use as an absorbent and bleach	Wenninger et al. 2000 Wenninger et al. 2000 Gamble 1986
Hectorite	$\text{Na}_{0.67}(\text{Mg},\text{Li})_6\text{Si}_8\text{O}_{20}(\text{OH},\text{F})_4^a$ $\text{Na}_{0.33}[\text{Mg}_{2.67}\text{Li}_{0.33}]\text{Si}_4\text{O}_{10}[\text{OH}]_2$ Montmorillonite mineral that is the principle constituent of bentonite clays Fluorine-bearing magnesium rich montmorillonite Almost a complete substitution of aluminum in the lattice structure of bentonite by magnesium in hectorite and the presence of lithium and fluorine	Budavari 1989 Rheox Inc. 1999 Wenninger et al. 2000 Grim 1972 United States Pharmacopeial Convention, Inc. 1994
Kaolin/Kaolinite	$\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$ Native hydrated aluminum silicate Kaolinite is the mineral that characterizes most Kaolins	Wenninger et al. 2000 Wenninger et al. 2000 Ross and Kerr 1931
Lithium Magnesium Silicate	No specific formula Synthetic clay consisting of mainly lithium and magnesium silicates	Wenninger et al. 2000 Wenninger et al. 2000

(Continued on next page)

TABLE 1
Chemical formulas and compositions of Silicates and Silicate Clays used in cosmetics (*Continued*)

Ingredient	Description	Reference
Lithium Magnesium Sodium Silicate	No specific formula Synthetic clay consisting mainly of lithium, magnesium, and sodium silicates	Wenninger et al. 2000 Wenninger et al. 2000
Montmorillonite	$R_{0.33}^+(Al,Mg)_2Si_4O_{10}(OH)_2$, where $R^+ = Na^+, K^+, Mg^{2+}$, or Ca^{2+} Complex aluminum/magnesium silicate clay Term used to describe a group of minerals with an expanding lattice, except vermiculite and also a specific mineral with a high-alumina end member of the montmorillonite group with some slight replacement of Al^{3+} by Mg^{++} and substantially no replacement of Si^{4+} by Al^{3+}	Budavari 1989 Wenninger et al. 2000 Grim 1972
Pyrophyllite	$Al_2O_3 \cdot 4SiO_2 \cdot 2H_2O$ Naturally occurring mineral substance consisting predominantly of a hydrous aluminum silicate	Wenninger et al. 2000 Wenninger et al. 2000
Sodium Magnesium Silicate	No specific formula Synthetic silicate clay with a composition mainly of magnesium and sodium silicate	Wenninger et al. 2000 Wenninger et al. 2000
Zeolite	$M_{2/n}O \cdot Al_2O_3 \cdot ySiO_2 \cdot xH_2O$ (M = a group IA or IIA element; n = cation valence; y = 2 or greater; x = the number of water molecules within the molecule) Hydrated alkali aluminum silicate Group of hydrated, crystalline aluminosilicates containing exchangeable cations of group IA and IIA elements such as sodium, potassium, magnesium, and calcium	IARC 1997 Wenninger et al. 2000 IARC 1997

TABLE 2
Zeolites (IARC 1997)

Zeolite	CAS no.	Chemical formula
Clinoptilolite (general)	12173-10-3	Not given
	12271-42-0	$Na(AlSi_5O_{12} \cdot xH_2O)$
	67240-23-7	$AlNaH_{16}(SiO_4 \cdot 4H_2O)$
Mordenite (general)	12173-98-7	Not given
	12445-20-4	$AlNaH_6(SiO_3)_5$
	66732-10-3	$Al_2CaH_{12}(SiO_3)_{10} \cdot H_2O$
	68652-75-5	$Na(AlSi_5O_{12})$
Phillipsite (general)	12174-18-4	Not given
	61027-84-7	$CaK[Al_3O(SiO_3)_5] \cdot 6H_2O$
	66733-09-3	$AlNa(SiO_4) \cdot 6H_2O$
Zeolite A	68989-22-0	$Na_{12}[(AlO_2)_{12}(SiO_2)_{12}] \cdot 27H_2O$
Zeolite X	68989-23-1	$Na_{86}[(AlO_2)_{86}(SiO_2)_{106}] \cdot 264H_2O$
Zeolite Y	Not specified	$Na_{56}[(AlO_2)_{56}(SiO_2)_{136}] \cdot 250H_2O$
Zeolite L	Not specified	$K_9[(AlO_2)_9(SiO_2)_{27}] \cdot 22H_2O$
ZSM-5	79982-98-2	$(NaTPA)_3[(AlO_2)_3(SiO_2)_{93}] \cdot 16H_2O^*$

*TPA = tetrapropylammonium.

consists of double silica chains situated parallel to the c axis with the chains linked together through oxygens at their longitudinal edges. Tetrahedral apexes in successive chains point in the opposite direction. The linked chains form a kind of double-ribbed sheet with two rows of tetrahedral apexes at alternate intervals in the top and bottom of the sheets. The ribbed sheets are arranged so that the apex oxygens of successive sheets point together and are held together by aluminum and/or magnesium in octahedral coordination between the apex oxygens of successive sheets. Chains of water molecules run parallel to the c axis and fill the interstices between the amphibole chains. Aluminum substitutions for silicon is considered probable (Grim 1967).

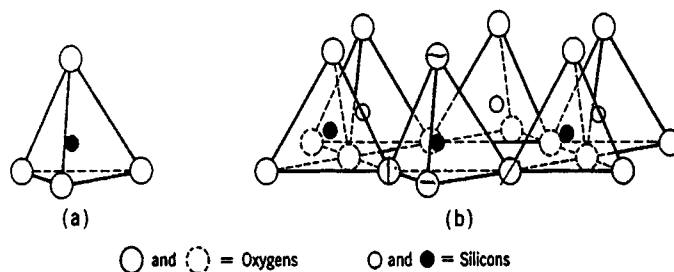


FIGURE 2

(a) Single tetrahedral unit; (b) Sheet of units (taken from Grim 1967 with permission).

TABLE 3
 Synonyms for, physical properties of, and specifications for Silicates and Silicate Clays used in cosmetics

Item	Description	Reference
Aluminum Silicate		
Synonyms	Anhydrous aluminum silicate, china clay, natural aluminum silicate, pyrophyllite, synthetic aluminum silicate, willinite	Wenninger et al. 2000
Form/description	Kaolin	Budavari 1989
	Aluminosilicate	Syracuse Research Corp. 1974
	Generally consisting of 1 mole of alumina and 1 to 3 moles of silica	Wenninger et al. 2000
Molecular weight	Four naturally occurring minerals (andalusite, cyanite, sillimainte, mullite); other associated minerals: anauxite, dickite, kaolinite, kochite, newtonite, pyrophyllite, takizolite, termierite, and ton	Budavari 1989
	Variable: ranging from 162.05 to 426.05 Da	Lide 1993
	Variable: 3.156, 3.247	Lide 1993
Solubility	Insoluble in water	Syracuse Research Corp. 1974
Calcium Silicate		
Synonyms	Silicic acid, calcium salt	Wenninger et al. 2000
Form/description	Hydrous or anhydrous silicate with varying proportions of calcium oxide and silica	Wenninger et al. 2000
Molecular weight	White or slightly cream colored free-flowing powder	Budavari 1989
	116.16 Da	Lide 1993
	Insoluble in water	Budavari 1989
pH	8.0–10.0 (aqueous slurry)	Budavari 1989
Magnesium Aluminum Silicate		
Synonyms	Aluminum magnesium silicate, magnesium aluminosilicate, complex colloidal, <i>Carrisorb</i> , <i>Gelsorb</i> , <i>VEEGUM</i>	Palmieri 1994
Form/description	Aluminosilicic acid, magnesium salt, aluminum magnesium silicate	Wenninger et al. 2000
	Complex silicate refined from naturally occurring minerals	Wenninger et al. 2000
	Off-white to creamy white small flakes or micronized powder	Palmieri 1994
Molecular weight	262.4 Da	Budavari 1989
Solubility	Insoluble in water, alcohol, and organic solvents	Palmieri 1994
pH	9.0–10.0 (5% aqueous solution)	Nikitakis and McEwen 1990b
Viscosity	225–2200 mPa	Palmieri 1994
CTFA specifications	Arsenic (as As), 3 ppm maximum	Nikitakis and McEwen 1990a
	Lead (as Pb), 10 ppm maximum	Nikitakis and McEwen 1990a
Magnesium Silicate		
Synonyms	Silicic acid, magnesium salt (1:1)	Wenninger et al. 2000
Form/description	Fine, white, odorless, tasteless, powder, free from grittiness	United States Pharmacopeial Convention, Inc. 1994
Solubility	Insoluble in water and alcohol	United States Pharmacopeial Convention, Inc. 1994
CTFA specifications	Arsenic (as As), 3 ppm maximum	Nikitakis and McEwen 1990a
	Lead (as Pb), 20 ppm maximum	Nikitakis and McEwen 1990a
Magnesium Trisilicate		
Synonyms	Silicic acid, magnesium salt (1:2)	Wenninger et al. 2000
Form/description	Fine, white, odorless, tasteless powder, free form grittiness	United States Pharmacopeial Convention, Inc. 1994
Solubility	Insoluble in water and alcohol	United States Pharmacopeial Convention, Inc. 1994
Sodium Magnesium Silicate		
Synonyms	Synthetic sodium magnesium silicate	Wenninger et al. 2000
Form/description	Synthetic silicate clay with a composition mainly of magnesium and sodium silicate	Wenninger et al. 2000

(Continued on next page)

TABLE 3

Synonyms for, physical properties of, and specifications for Silicates and Silicate Clays used in cosmetics (*Continued*)

Item	Description	Reference
Zirconium Silicate		
Synonyms	Silicic acid, zirconium salt (1:1) Zircon, zirconium orthosilicate Zirconium (IV) silicate (1:1)	Wenninger et al. 2000 Budavari 1989 Lewis 1993
Form/description	Bipyramidal crystals, colorless unless has impurities and radioactive bombardment Red or various colored crystals	Budavari 1989 Lewis 1993
Molecular weight	183.31 Da	Budavari 1989
Solubility	Insoluble in alcohol, aqueous solution, and alkali	Lide 1993
Density	4.56	Lide 1993
pH	6–7.5 (10% aqueous slurry)	American Minerals 1998
CTFA specifications	Arsenic (as As), 3 ppm maximum Lead (as Pb), 20 ppm maximum	Nikitakis and McEwen 1990a Nikitakis and McEwen 1990a
Attapulgit		
Synonyms	Activated attapulgit, Attaclay, Attagel, Attasorb, Min-u-gel, palygorskit, Permangel, Zeogel	Registry of Toxic Effects of Chemical Substances (RTECS) 1999
Form/description	Palygorskite Variety of Fuller's Earth; characterized by a chain structure rather than the sheet structure of other clay minerals White, gray, or transparent, dull, elongated, lath-shaped crystals in bundles that comprise thin sheets of minute interlaced fibers; surface is protonated and hydrated	IARC 1997 Wenninger et al. 2000 IARC 1997
Density	2.2	IARC 1997
Solubility	Insoluble in water	United States Pharmacopeial Convention, Inc. 1994
Bentonite		
Synonyms	CI 77004, soap clay Albangel Premium USP 4444, Bentonite magma, Hi-gel, Imvite I.G.B.A., Magbond, montmorillonite, Tixoton, Volclay, Wilkinitite, BentoPharm, E558, mineral soap, soap clay, taylorite, Veegum HS, wilkinitite	Wenninger et al. 2000 RTECS 1999 Belmonte 1994
Form/description	Native hydrated colloidal aluminum silicate clay Crystalline, claylike material, available as an odorless, palebuff or cream to grayish-colored fine powder, which is free from grit Diocahedral	Wenninger et al. 2000 Belmonte 1994 Rheox Inc. 1999
Molecular weight	359.16 Da	Belmonte 1994
Solubility	Practically insoluble in ethanol, fixed oils, glycerin, propan-2-ol and water	Belmonte 1994
pH	9.5–10.5 for a 2% aqueous solution	Belmonte 1994
Particle size	Mainly 50–150 μm along with 1–2 μm particles 0.8 $\times$ 0.8 $\times$ 0.01 μ	Belmonte 1994 Rheox Inc. 1999
Color	Grey to green	Rheox Inc. 1999
Swelling ability	15 $\times$	Rheox Inc. 1999
Iron	2.3%	Rheox Inc. 1999
Fuller's Earth		
Synonyms	English Fuller's earth	Wenninger et al. 2000
Form/description	Nonplastic variety of kaolin Sheet structure	Wenninger et al. 2000 Gamble 1986

(Continued on next page)

TABLE 3

Synonyms for, physical properties of, and specifications for Silicates and Silicate Clays used in cosmetics (*Continued*)

Item	Description	Reference
Hectorite		
Synonyms	Macaloid, Ben-A-Gel Bentone and Bentone Gel	Barr 1963 Rheox Inc. 1999
Form/description	Translucent colorless mineral when mined and turns white when dried	Barr 1963
Particle size	Tridecahedral $0.8 \times 0.08 \times 0.01 \mu$	Rheox Inc. 1999 Rheox Inc. 1999
pH	8.5 (5% slurry)	Rheox Inc. 1999
Iron	0.2% (typical)	Rheox Inc. 1999
Color	Light pink to tan; off-white	Rheox Inc. 1999
Swelling ability	35×	Rheox Inc. 1999
Odor	None	Rheox Inc. 1999
Specific gravity	2.65	Rheox Inc. 1999
Kaolin		
Synonyms	Bolbus Alba, China Clay, CI 77004, Kolite, Pigment White 19 Altowhites, Argilla, Bentone, China Clay, Emathlite, Fitrol, Glomax, Hydrite, Kaopaous, Langford, Mcnamee, Parclay, Porcelin Clay, Snow tex	Wenninger et al. 2000 RTECS 1999
	Bolbus alba, China clay, white bole, argilla, terra alba, porcelin clay White or yellowish white, earthy mass or white powder; unctous when moist	Informatics, Inc. 1974 Budavari 1989
Form/description	Native hydrated aluminum silicate	Wenninger et al. 2000
Molecular weight	258.2 Da	Budavari 1989
Solubility	Insoluble in water, cold acids, or in alkali hydroxides	Budavari 1989
Cation exchange capacity	3–15 mEq/100 g	Carrol 1959
CTFA specifications	Arsenic (as As), 3 ppm maximum Lead (as Pb), 20 ppm maximum	Nikitakis and McEwen 1990a Nikitakis and McEwen 1990a
Lithium Magnesium Silicate		
Synonyms	Silicic acid, lithium magesium salt	Wenninger et al. 2000
Form/description	Synthetic silicate clay consisting mainly of lithium and magnesium silicates	Wenninger et al. 2000
Lithium Magnesium Sodium Silicate		
Synonyms	Magnesium lithium sodium silicate; silicic acid, lithium, magnesium, and sodium salt	Wenninger et al. 2000
Form/description	Synthetic silicate clay consisting mainly of lithium, magnesium and sodium silicates	Wenninger et al. 2000
Montmorillonite		
Synonyms	Smectite	Grim 1972
Form/description	Complex aluminum/magnesium silicate clay	Wenninger et al. 2000
Cation exchange capacity	80–150 mEq/100 g	Carrol 1959
Pyrophyllite		
Synonyms	Pyrophyllite clay	Wenninger et al. 2000
Form/description	Naturally occurring mineral—predominantly hydrous aluminum silicate	Wenninger et al. 2000
Sodium Magnesium Silicate		
Synonyms	Synthetic sodium magnesium silicate	Wenninger et al. 2000
Form/description	Synthetic silicate clay with a composition mainly of sodium and magnesium silicate	Wenninger et al. 2000
pH	8.5–10.5 of 2% aqueous dispersion	Nikitakis and McEwen 1990b
Solubility	Insoluble in organic solvents and disperses in water	Nikitakis and McEwen 1990b

(Continued on next page)

TABLE 3

Synonyms for, physical properties of, and specifications for Silicates and Silicate Clays used in cosmetics (*Continued*)

Item	Description	Reference
	Zeolite	
Synonyms	Aluminosilicates, Bacterkiller, CS100, Sitton, Zeokar, Zeolith, Zeolum, Zeostar	Wenninger et al. 2000
	Clinoptilolite, Mordenite, Phillipsite, Zeolite A, Zeolite X, ZSM-5, Non-fibrous Japanese Zeolite	IARC 1997
Form/description	Crystalline, hydrated alkali-aluminum silicates	Budavari 1989; Wenninger et al. 2000

Kaolin

Kaolin's structure is composed of a single silica tetrahedral sheet and a single alumina octahedral sheet combined in a unit so that the tips of the silica tetrahedrons and one of the layers of the octahedral sheet form a common layer as shown in Figure 4. All the tips of the silica tetrahedrons point in the same direction and toward the center of the unit made by the silica and octahedral sheets. Composite octahedral-tetrahedral layers are formed due to the similarity between the sheets *a* and *b* dimensions. The common layer between the octahedral and tetrahedral groups consists of two thirds of shared atoms between silicon and aluminum that become O instead of OH. Analyses of Kaolin have

shown there is little substitution within the lattice. In a small percentage of cases, iron and/or titanium has replaced aluminum. This has only been seen in the relatively poor crystalline varieties of Kaolin (Grim 1967).

Smectites (Montmorillonites, Hectorite, and Bentonite)

Smectite units comprise of two silica tetrahedral sheets with a central alumina octahedral sheet as shown in Figure 5. All tetrahedral tips point in the same direction and toward the center of the unit. The tips of the tetrahedrons of each silica sheet and one of the hydroxyl layers of the octahedral sheet form a common layer. As in Kaolin, the atoms common to both the tetrahedral and octahedral layer become O instead of OH. These layers are continuous in the *a* and *b* directions and are stacked one above the other in the *c* direction. As a consequence, O layers in the units become adjacent and a very weak bond is created with the possibility of cleavage. The preeminent feature of smectites is the ability of water and other organic molecules to enter between unit layers and expand in the *c* direction. Expansion properties are reversible; however, the structure is completely collapsed by removal of interlayer polar molecules. Most smectites have substitutions within their lattices: aluminum or phosphorous for

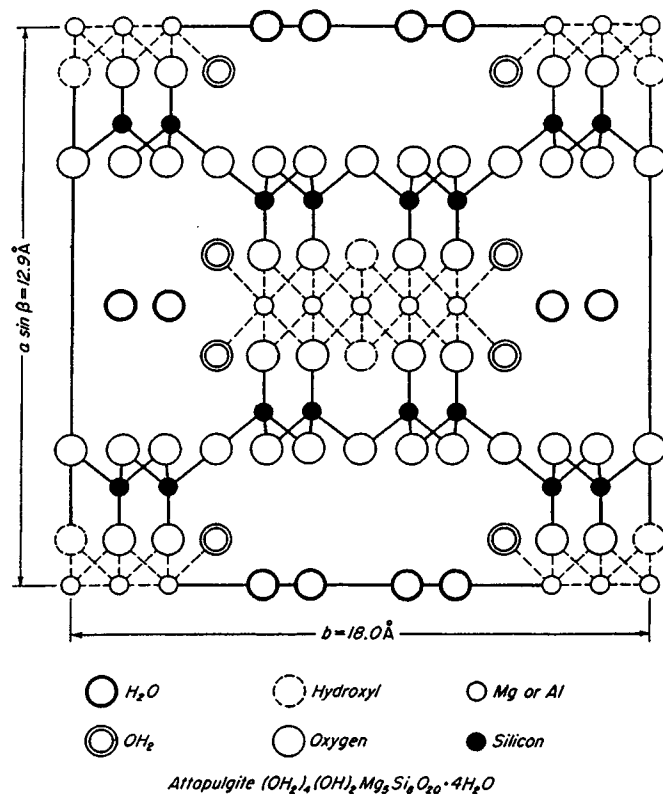


FIGURE 3

Attopulgite structure (taken from Grim 1967 with permission).

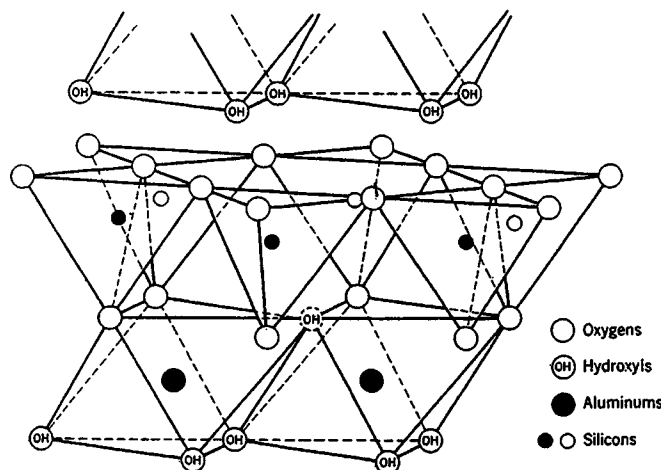
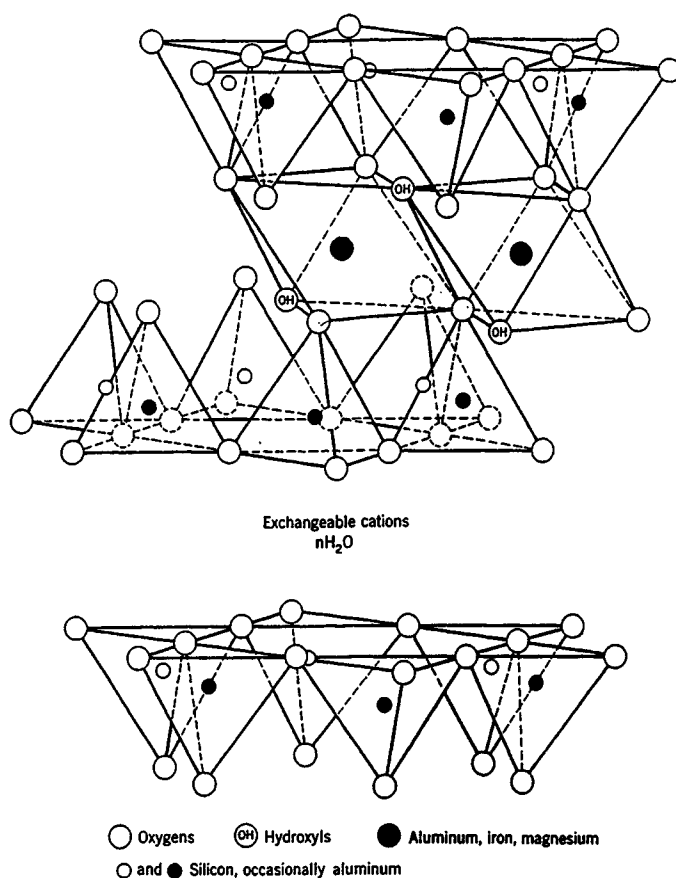


FIGURE 4

Kaolin layer (taken from Grim 1967 with permission).

**FIGURE 5**

Smectite structure (taken from Grim 1967 with permission).

silicon in the tetrahedral coordination and/or magnesium, iron, zinc, nickel, lithium, etc. for aluminum in the octahedral sheet (Grim 1967).

Natural Occurrence of Clays

Aluminum Silicate

Natural Aluminum Silicates are reportedly being mined in India, California, North Carolina, and Georgia (Gamble 1986).

Attapulgite

Attapulgite is mined in 10 countries: Australia, China, France, India, Russia, Senegal, South Africa, Spain, Turkey, and the United States (Informatics, Inc. 1974).

Bentonite

Large deposits of Bentonite have been discovered in Canada, China, France, Germany, Great Britain, Greece, Hungary, Italy, Japan, Mexico, New Zealand, North Africa, Poland, South Africa, the former Soviet Union, and the United States (Informatics, Inc. 1974).

Kaolin

Deposits of Kaolin have been found in England, the United States, France, Czechoslovakia, Germany, and Japan (Informatics, Inc. 1974).

Pyrophyllite

Gamble (1986) reported Pyrophyllite being mined primarily in North Carolina.

Zeolite

Natural Zeolites are mined in Japan, the United States, Hungary, Bulgaria, Cuba, Italy, and South Africa (Roskill Informations Services Ltd. 1988).

Method of Manufacture

Aluminum Silicate

Aluminum Silicate is a naturally occurring mineral as well as artificially produced. The naturally occurring Aluminum Silicate minerals are known as andalusite, sillimanite, and cyanite. Natural Aluminum Silicate is mined from an ore and synthetic Aluminum Silicate is formed by heating compositions of controlled proportions of silica, alumina, and alkalis under conditions to promote the specific structure (Syracuse Research Corp. 1981).

Attapulgite

Hevlin and Murray (1994) describe the mining process of Attapulgite as an opencast technique, stripping layers with heavy machines such as bulldozers, backhoes, and excavators. The clay is then transported to a processing plant where crushing, drying, classification, and pulverizing takes place. High-heat drying to remove water may occur to enhance absorbent qualities.

Bentonite

The mined ore of Bentonite is processed to remove grit and nonswelling materials (Belmonte 1994).

Kaolin

In a process described by Wells, Bhatt, and Flanagan (1985), Kaolin is extracted from kaolinized granite by washing it out with powerful and remote water hoses. The clay stream is then pumped to the separation plant where sand and mica are removed. The purified clay is filtered when wet and then dried. The very fine powder is formed by milling.

Magnesium Aluminum Silicate

Magnesium Aluminum Silicate is obtained from silicate ores of the montmorillonite group. The ores are blended with water to produce a slurry, which is then processed to remove impurities and separate out the colloidal fractions. Refined colloidal fractions are dried to form a small flake and then is microatomized to form various powder grades (Palmieri 1994).

Zeolite

Roskill Informations Services Ltd. (1988) reported natural Zeolites being recovered from deposits by selective opencast or strip mining processes. The raw material is then processed by crushing, drying, powdering, and screening. Synthetic Zeolite synthesis requires the following conditions: reactive starting materials; a high pH; a low-temperature hydrothermal state with concurrent low autogenous pressure at saturated water pressure; and a high degree of supersaturation of a large number of crystals.

Analytical Methods

Montmorillonite has been detected using far infrared spectra (Angino 1964). Bentonite and Kaolin are described by Angino (1964) using far infrared spectra and by Sadik (1971) using x-ray diffraction. Attapulgite has been detected with the use of transmission or scanning electron microscope (Zumwalde 1976), and by means of x-ray powder diffraction analysis (Keller 1979). The characterization of Hectorite was achieved through x-ray diffraction, infrared spectroscopy, and chemical analysis (Browne et al. 1980). Zeolites have been examined using scanning electron microscopy (Wright and Moatamed 1983; van Hoof and Roelofsen 1991) and x-ray diffraction (van Hoof et al. 1991). Magnetic angle spinning nuclear magnetic resonance (NMR) has confirmed the structural breakdown of Fuller's Earth (Drachman, Roch, and Smith, 1997).

IMPURITIES/COMPOSITION*Aluminum Silicate*

Other minerals associated with natural Aluminum Silicates are anauxite, dickite, kaolinite, kochite, mullite, newtonite, pyrophyllite, takizolite, terierite, and ton (Budavari 1989).

Attapulgite

Attapulgite commonly is found with smectites, amorphous silica, chert, and other minerals (Bish and Guthrie 1993).

A typical composition is shown in Table 4 (Keller 1979).

Bentonite

The principle constituent is Montmorillonite. However, other minerals such as illite, kaolinite, and nonargillaceous detrital minerals can be present. Most Bentonites appear relatively pure and other mineral contributions rarely exceed 10%. Cristobalite is often present. Montmorillonite compositions frequently vary either in its lattice structure or in the exchangeable ions present (Informatics, Inc. 1974).

A typical composition is shown in Table 4 (Belmonte 1994).

Fuller's Earth

Principle deposits of Fuller's Earth include Montmorillonite, Bentonite, Attapulgite, and sepiolite (Gamble 1986).

TABLE 4

Mineral composition of individual samples of Magnesium Aluminum Silicate, Attapulgite, Bentonite, Hectorite, Kaolinite, and Montmorrillonite (Barr 1963)

Mineral	Silicate clays analyzed					
	Magnesium Aluminum Silicate (%)	Attapulgite (%)	Bentonite (%)	Hectorite (%)	Kaolinite (%)	Montmorillonite (%)
SiO ₂	61.1	55.03	59.92	55.86	45.44	51.14
Al ₂ O ₃	9.3	10.24	19.78	0.13	38.52	19.76
Fe ₂ O ₃	—	3.53	—	0.03	0.80	0.83
FeO	0.9	—	2.96	—	—	—
MgO	13.7	10.49	1.53	25.03	0.08	3.22
CaO	2.7	—	0.64	Trace	0.08	1.62
K ₂ O	0.3	0.47	0.57	0.10	0.14	0.11
Na ₂ O	2.9	—	20.6	2.68	0.66	0.04
TiO ₂	0.1	—	—	—	0.16	—
CO ₂	1.8	—	—	—	—	—
LiO ₂	—	—	—	1.05	—	—
F	—	—	—	5.96	—	—
MnO	—	—	—	—	—	Trace
ZnO	—	—	—	—	—	0.10
H ₂ O	7.2	19.86	Not reported	12.14	14.20	22.80
Reference	Palmieri 1994	Keller 1979	Belmonte 1994	Keller 1979	Keller 1979	Keller 1979

Hectorite

Principle impurities include calcite, dolomite, silica crystals, and grit (Barr 1963). A typical composition is shown in Table 4 (Keller 1979).

Kaolin

Quartz, mica, and feldspar are often found associated with the crude mineral and is often removed through screening and elutriation (Informatics, Inc. 1974).

Ferreira and Freitas (1976) surveyed Kaolin for any potentially pathogenic organisms and a mean viable count. *Pseudomonas aeruginosa*, *Salmonella typhosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Clostridium tetani* were absent. The mean viable count was $74 \times 10^3/6$ M. The bacteria present were mostly gram-positive aerobic spore-formers.

A typical composition is shown in Table 4 (Keller 1979).

Magnesium Aluminum Silicate

One trade-name group of products contain 1% to 6% by volume weight crystalline silica in the form of cristaballite; they also comment that a few grades may contain quartz as well (Kelse 1997).

A typical composition is shown in Table 4 (Palmeiri 1994).

Montmorillonite

A typical composition of Montmorillonite is shown in Table 4 (Keller 1979).

Zeolite

Valatina, Pylev, and Lemjasev (1994) analyzed the chemical compositions of five samples of Zeolite dusts taken from mines in Russia (Table 5). The benzo[a]pyrene content in the dusts of natural Zeolite tuffs (rock deposits) ranged from 0.0 to 3.6 $\mu\text{g/kg}$.

TABLE 5
Zeolite mine dust chemical analysis (Valatina, Pylev, and Lemjasev 1994)

Dust sample	1	2	3	4	5
Molar ratio of $\text{SiO}_2/\text{Al}_2\text{O}_3$	9.0	8.3	9.8	7.4	9.4
Zeolite (%)	83	50.6	73	63	56
Silicon dioxide (%)	66.84	0	70.92	62.64	68.6
Aluminum oxide (%)	12.36	12.62	12.11	14.17	12.16
Iron (III) oxide (%)	0.92	4	1.03	2.65	0.2
Magnesium oxide (%)	1.53	1.34	0.53	1.19	0.93
Calcium oxide (%)	2.36	4.15	2.56	2.01	1.93
Sodium oxide (%)	2.65	0.15	0.62	1.75	2
Benzo[a]pyrene	2.5	3.6	0.1	1.3	0

USE

Cosmetic

According to the European Cosmetic Directive (EU reference no. 391 Annex II), Zirconium and its compounds are listed under substances that must not form part of the composition of cosmetic products, with the exception of complexes in Annex III, Part I. These complexes are aluminum zirconium chloride hydroxide complexes and the aluminum zirconium chloride hydroxide glycine products used in antiperspirants; and the zirconium lakes, salts, and pigments of coloring agents listed in reference 3 in Annex IV, Part I (Cosmetics Directive of the European Union 1995).

Aluminum Silicate, anhydrous, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Bentonite, Hectorite, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite are listed in the *Japanese Comprehensive Licensing Standards by Category* (CLS) (Rempe and Santucci 1998). Aluminum Silicate, anhydrous has no concentrations limits and is listed in all categories except eyeliner preparations and lip preparations. Calcium Silicate, is listed in all categories. Magnesium Aluminum Silicate, which is listed under Aluminum Magnesium Silicate, is listed in all categories. Magnesium Silicate is listed in all categories. Hectorite is listed in all categories except eyeliner preparations, lip preparations, and oral preparations. Montmorillonite is excluded from only eyeliner preparations. Pyrophyllite is listed in all groups except eyeliner, lip, oral, and bath preparations. Bentonite, Kaolin, and Zeolite are listed in all categories.

Information on use of ingredients in cosmetic formulations is available from the Food and Drug Administration (FDA) as part of a voluntary industry reporting program (FDA 1998). These data are presented in the first two columns of Table 6.

In addition, the Cosmetic, Toiletry, and Fragrance Association (CTFA) provides information from the industry directly to CIR on the current concentration of use (CTFA 1999a). In some cases a current concentration of use is provided even when there is no current use reported to FDA. It is presumed that an industry report of a current concentration of use means the ingredient is in use. These data are included in the third column of Table 6.

In those cases where there is a use reported to FDA, but there is no current concentration of use data available, the last column in Table 6 includes historical data from 1984 when FDA collected information on concentration as part of the voluntary reporting program described earlier (FDA 1984). If no historical data are available, no concentration is listed.

Aluminum Silicate

Aluminum Silicate functions as an abrasive, anticaking agent, bulking agent, and opacifying agent in cosmetics (Wenninger et al. 2000). In 1998 it was reported as an ingredient in 10 formulations in seven different categories (FDA 1998).

TABLE 6
Frequency of use and concentration of use as a function of product category

Product category (Number of formulations reported to FDA 1998)	Number of formulations containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999a) (%)	Historical concentration of use (FDA 1984) (%)
Aluminum Silicate			
Mascara (167)	2	0.5	
Blushers (all types) (238)	1	—	—
Dentifrices (38)	—	37	
Shaving cream (139)	1	—	—
Cleansing (653)	2	2	
Paste masks (mud packs) (255)	1	—	1-5
Skin fresheners (184)	1	—	0.1-1
Other skin preparations (692)	2	3	
1998 total uses of Aluminum Silicate	10		
Calcium Silicate			
Bath oils, tablets, and salts (124)	12	—	0.1-5
Bubble baths (200)	2	—	0.1-25
Other bath preparations (159)	2	—	0.1-25
Eye shadow (506)	11	1-8	
Powders (247)	35	2	
Blushers (all types) (238)	17	5-8	
Face powders (250)	40	0.3-10	
Foundations (287)	5	2-8	
Lipstick (790)	3	0.5	
Makeup bases (132)	1	0.5	
Rouges (12)	1	—	1-5
Other makeup preparations (135)	1	—	1-5
Other manicuring preparations (61)	1	—	1-5
Skin cleansing preparations (653)	1	8	
Men/s talcum (8)	—	8	
1998 total for Calcium Silicate	132		
Magnesium Aluminum Silicate			
Other bath preparations (159)	1	—	—
Eye makeup remover (84)	20	—	0.1-25
Eye shadow (506)	4	1	
Eye lotion (18)	1	1	
Eye makeup remover (84)	2	—	0.1-25
Mascara (167)	33	0.4-5	
Eyeliners (514)	—	0.2-0.5	
Eyebrow pencil (91)	—	0.5	
Other eye makeup preparations (120)	16	1-5	
Cologne and toilet waters (656)	1	—	—
Other fragrance preparations (148)	1	—	>0-1
Hair conditioners (636)	1	—	0.1-1
Hair straighteners (63)	3	—	0.1-1
Hair dyes and colors (1572)	—	2	
Shampoos (noncoloring) (860)	3	1-2	
Other hair preparations (276)	3	—	—
Hair rinses (coloring) (33)	1	—	—
Foundations (287)	130	0.4-5	
Lipstick (790)	3	—	0.1-1
Makeup bases (132)	60	1-2	

(Continued on next page)

TABLE 6
Frequency of use and concentration of use as a function of product category (*Continued*)

Product category (Number of formulations reported to FDA 1998)	Number of formulations containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999a) (%)	Historical concentration of use (FDA 1984) (%)
Makeup fixatives (11)	3	2	
Other makeup preparations (135)	24	0.8	
Cuticle softeners (19)	1	—	—
Nail creams and lotions (17)	1	—	0.1–5
Dentifrices	—	0.7	
Bath soaps and detergents (385)	1	0.5–1	
Deodorants (underarm) (250)	5	0.5–1	
Other personal cleanliness products (291)	14	2	
Aftershave lotion (216)	9	—	1–>50
Other shaving preparations (60)	2	—	0.1–5
Skin cleansing preparations (653)	41	0.1–5	
Face and neck skin care preparations (263)	16	0.6–3	
Body and hand skin care preparations (796)	56	0.3–5	
Foot powders and sprays (35)	3	—	—
Moisturizers (769)	70	0.3–4	
Night creams, lotions, powders, and sprays (188)	11	0.3–2	
Paste masks (mud packs) (255)	34	3–5	
Other skin care preparations (692)	33	0.1	
Suntan gels, creams, and liquids (136)	6	2–5	
Indoor tanning preparations (62)	19	0.5–2	
1998 total for Magnesium Aluminum Silicate	632		
	Attapulgate		
Powders (fragrance) (247)	5	—	—
Body and hand skin care preparations (796)	—	8	
Paste masks (mud packs) (255)	5	8	
1998 total for Attapulgate	10		
	Bentonite		
Bath, oils, tablets, and salts (124)	—	5	
Eyeline (514)	6	5	
Mascara (167)	1	0.8	
Other eye makeup preparations (120)	1	—	—
Hair conditioners (636)	1	—	—
Hair straighteners (63)	3	—	0.1–1
Foundations (287)	5	2–8	
Makeup bases (132)	3	1	
Cuticle softeners (19)	1	1	
Bath soaps and detergents (385)	1	0.5	
Other personal cleanliness products (291)	2	—	0.1–10
Skin cleansing preparations (653)	6	—	>0–10
Face and neck skin care preparations (excluding shaving) (263)	1	2–5	
Body and hand skin care preparations (excluding shaving) (796)	6	2–5	
Moisturizers (769)	2	3	
Night creams, lotions, powders, and sprays (188)	1	—	—
Paste masks (mud packs) (255)	44	12–80	
Skin fresheners (184)	1	—	—

(Continued on next page)

TABLE 6
Frequency of use and concentration of use as a function of product category (*Continued*)

Product category (Number of formulations reported to FDA 1998)	Number of formulations containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999a) (%)	Historical concentration of use (FDA 1984) (%)
Other skin preparations (692)	8	—	—
Suntan gels, creams, and liquids (136)	1	—	—
Other suntan preparations (38)	—	1	—
1998 total for Bentonite	73		
	Fuller's Earth		
Paste masks (mud packs) (255)	2	—	—
Other skin preparations (692)	1	—	25–50
1998 total for Fuller's Earth	3		
	Hectorite		
Eyeliners (514)	3	—	—
Mascara (167)	1	0.7	—
Shampoos (noncoloring) (860)	—	1	—
Hair bleaches (113)	5	—	—
Foundations	—	15	—
Other makeup preparations (135)	1	—	1–5
Basecoats and undercoats (manicuring) (48)	1	—	—
Nail polish and enamel (80)	1	—	—
Deodorants (underarm) (250)	1	0.7	—
Other personal cleanliness products (291)	1	—	—
Paste masks (mud packs) (255)	2	0.4	—
Skin cleansing preparations (653)	—	100	—
Body and hand creams, lotions, powders, and sprays (796)	—	8	—
Other skin preparations (692)	1	—	—
Paste masks (mud packs) (255)	—	8	—
Other suntan preparations (38)	1	—	—
1998 total for Hectorite	18		
	Sodium Magnesium Silicate		
Eyeliners	—	0.08	—
Eye shadow (506)	11	0.08	—
Mascara (167)	1	0.4	—
Other eye makeup preparations (120)	1	—	—
Powders (fragrance) (247)	1	—	—
Tonics, dressings, and other hair-grooming aids (549)	1	—	—
Blushers (all types) (238)	2	—	—
Face powders (250)	3	0.4	—
Foundations (287)	4	0.4	—
Lipstick (790)	1	3	—
Makeup bases (132)	—	0.1	—
Other makeup preparations (135)	1	—	—
Dentifrices (38)	—	0.3	—
Deodorants (underarm) (250)	—	0.5	—
Skin cleansing preparations (653)	—	0.5	—
Face and neck skin care preparations (excluding shaving) (263)	3	0.8–5	—
Body and hand skin care preparations (excluding shaving) (796)	2	0.1	—
Moisturizers (769)	1	1	—

(Continued on next page)

TABLE 6
Frequency of use and concentration of use as a function of product category (*Continued*)

Product category (Number of formulations reported to FDA 1998)	Number of formulations containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999a) (%)	Historical concentration of use (FDA 1984) (%)
Paste masks (mud packs) (255)	1	5	
Skin fresheners (184)	—	5	
Other skin preparations (692)	1	—	1–5
1998 total for Sodium Magnesium Silicate	34		
Kaolin			
Other bath preparations (159)	1		1–10
Eyebrow pencil (91)	5	15–17	
Eyeliners (514)	9	25–48	
Eye shadow (506)	171	3–29	
Mascara (167)	31	8–18	
Other eye makeup preparations (120)	15	20	
Powders (247)	40	5	
Hair conditioners (636)	5	4	
Tonics, dressings, and other hair-grooming aids (549)	—	15	
Other hair-coloring preparations (59)	1	5	
Blushers (all types) (238)	72	14–20	
Face powders (250)	58	30	
Foundations (287)	45	6–36	
Lipstick (790)	6	12–30	
Makeup bases (132)	24	7–25	
Rouges (12)	2	—	>0–50
Makeup fixatives (11)	3	—	1–5
Paste masks (mud packs) (255)	—	12–84	
Other makeup preparations (135)	20	10–24	
Bath soaps and detergents (385)	1	3	
Other manicuring preparations (61)	—	53–54	
Skin cleansing preparations (653)	—	0.01	
Face and neck skin care preparations (263)	—	3	
Moisturizers (769)	—	25	
Skin fresheners (184)	—	2	
Other skin care preparations (692)	—	3–100	
Suntan gels, creams, liquids (136)	—	25	
1998 total for Kaolin	509		

Attapulgate

Attapulgate functions as an abrasive, bulking agent, opacifying agent, and viscosity-increasing agent (Wenninger et al. 2000). The FDA reported in 1998 Attapulgate being used in 10 formulations (FDA 1998).

Bentonite

Bentonite functions as an absorbent, bulking agent, emulsion stabilizer, opacifying agent, suspending agent—nonsurfactant, and viscosity-increasing agent—aqueous in cosmetic formulations (Wenninger et al. 2000). In 1998, 94 formulations were reported (FDA 1998). Of the 94 formulations, 47% were reported within paste masks (mud packs) (FDA 1998).

Calcium Silicate

Calcium Silicate functions as an absorbent, bulking agent, and an opacifying agent in cosmetic formulations (Wenninger et al. 2000). The FDA reported 132 formulations containing Calcium Silicate in 1998, of which 30% of the formulations were face powders (FDA 1998).

Fuller's Earth

Fuller's Earth functions as an absorbent, anticaking agent, bulking agent, and opacifying agent (Wenninger et al. 2000). Fuller's Earth was reported in three formulations in 1998 (FDA 1998).

Hectorite

Hectorite functions as an absorbent, bulking agent, opacifying agent, suspending agent—nonsurfactant, and viscosity-increasing agent—aqueous (Wenninger et al. 2000). In 1998, Hectorite was reported in 18 formulations (FDA 1998). Rheox Inc. (1999a) reported Hectorite as being used in antiperspirants, suntan products, eye products, hair products, creams and lotions, lip products, facial masks, and nail products.

Kaolin

Kaolin functions as an abrasive, absorbent, anticaking agent, bulking agent, and opacifying agent in cosmetic formulations (Wenninger et al. 2000). Of the 509 formulations reported by FDA in 1998, 34% were eye shadows (FDA 1998).

Lithium Magnesium Silicate

Lithium Magnesium Silicate functions as a binder, bulking agent, and viscosity-increasing agent—aqueous in cosmetic formulations (Wenninger et al. 2000). There were no current uses reported to FDA.

Lithium Magnesium Sodium Silicate

Lithium Magnesium Sodium Silicate functions as a bulking agent and viscosity-increasing agent—aqueous (Wenninger et al. 2000). There were no current uses reported to FDA.

Magnesium Aluminum Silicate

Magnesium Aluminum Silicate functions as an absorbent, anticaking agent, opacifying agent, and viscosity-increasing

agent—aqueous in cosmetics (Wenninger et al. 2000). It was reported that Magnesium Aluminum Silicate was used in 629 formulations in 1998 (FDA 1998). Of those 629 formulations, 21% were used in foundations.

Magnesium Aluminum Silicate (VEEGUM) was reported by Carlson (1977) to typically be used at a concentration of 1% to 2%, consistent with the data in Table 6. Another source reported Magnesium Aluminum Silicate used at concentrations of 10% to 50% for adsorbents, 0.5% to 2.5% for stabilizing agents, 1% to 10% for suspending agents, 2% to 10% for tablet and capsule disintegrants, 2% to 10% tablet binders, and 2% to 10% viscosity-increasing agents, again consistent with data in Table 6 (Palmieri 1994).

Additional historical data on concentration of use of this ingredient are available from a Toilet Good Association survey. Table 7 is a summary of that information (Toilet Goods Association 1969).

Magnesium Silicate

Magnesium Silicate functions as an absorbent, anticaking agent, bulking agent, opacifying agent, and viscosity-increasing agent—aqueous in cosmetic formulations (Wenninger et al. 2000). There were no current uses reported to FDA.

Magnesium Trisilicate

Magnesium Trisilicate functions as an abrasive, absorbent, anticaking agent, bulking agent, opacifying agent, and viscosity-increasing agent—aqueous in cosmetics (Wenninger et al. 2000).

TABLE 7
Magnesium Aluminum Silicate in cosmetic preparations (Toilet Goods Association 1969).

Product category	Use in product	Concentration (%)
Face cream/lotion (cleansing, hormone, night, acne, astringent)	Thickener, binder, emulsion stabilizer	2.1
Hand cream/lotion	Thickener, binder, emulsion stabilizer	1.3
Body cream/lotion (moisturizer, suntan preparations)	Thickener, binder, emulsion stabilizer, slip agent	1.6
Makeup (lotion, cream, medicated, matte, highlight)	Thickener, binder, emulsion stabilizer, pigment suspender	1.8
Rouge (cream, liquid, blusher, toner)	Thickener, binder, pigment suspender	1.8
Face mask	Thickener, binder	8.9
Powder aerosol	Anticaking	8.0
Powder compact/pressed	Oil absorption	1.0
Leg makeup	Thickener	3.9
Deodorant/antiperspirant	Thickener, emulsion stabilizer	1.8
Eye makeup (eyeshadow, mascara, eyeliner)	Thickener, emulsion stabilizer, pigment suspender	2.0
Depilatory	Thickener	2.0
Shave preparations	Thickener	0.5
Shampoo	Thickener	3.5
Cream sachet	Thickener, emulsion stabilizer	0.8

Montmorillonite

Montmorillonite functions as an abrasive, absorbent, emulsion stabilizer, opacifying agent, and viscosity-increasing agent—aqueous in cosmetics (Wenninger et al. 2000). There were no current uses reported to FDA.

Pyrophyllite

Pyrophyllite functions as an absorbent, colorant, and opacifying agent (Wenninger et al. 2000). There were no current uses reported to FDA.

Sodium Magnesium Silicate

Sodium Magnesium Silicate functions as binder and bulking agent (Wenninger et al. 2000). In 1998, Sodium Magnesium Silicate was reported in 34 formulations (FDA 1998).

Zeolite

Zeolite functions as an absorbent and deodorant agent in cosmetic formulations (Wenninger et al. 2000). There were no current uses reported to FDA.

Zirconium Silicate

Zirconium Silicate functions as an abrasive and opacifying agent in cosmetic formulations (Wenninger et al. 2000). There were no current uses reported to FDA.

Noncosmetic*Aluminum Silicate*

Aluminum Silicate is approved, under the heading of indirect food additives, as a substance used as basic components of single or repeated use of the food contact surfaces cellophane (21 Code of Federal Regulations [CFR] 177.1200) and rubber (21 CFR 177.2600).

Attapulgate

Attapulgate is listed in the OTC Active Ingredient Status Report as proposed category I, as an antidiarrheal ingredient (FDA 1994). Attapulgate is listed by Gamble (1986) as being primarily used in absorbents, pesticides, oil and petroleum treatment, and as a filler in many products.

Bentonite

Bentonite is considered by FDA to be generally recognized as safe (GRAS) as a direct food additive (21 CFR 184.1155).

Bentonite is listed by Gamble (1986) as being used in foundry sand bonding, bleaching clay in oil refining and decolorizers, filtering agents, water impedance, animal feed, pharmaceuticals, paint, plasticity increasers, and iron-ore pelletizing. Another source reported Bentonite as being used as an adsorbent, emulsion stabilizer, and suspending agent (Belmonte 1994). Bentonite is categorized by the *National Formulary* as a suspending and/or viscosity-increasing agent (United States Pharmacopeial Convention, Inc. 1994).

Calcium Silicate

Calcium Silicate is listed in the OTC Active Ingredient Status Report as an external analgesic and skin protectant (FDA 1994). The *National Formulary* category is as a glident and/or anticaking agent (United States Pharmacopeial Convention, Inc. 1994).

The American Conference of Governmental Industrial Hygienists (ACGIH) TLV-TWA (threshold limit value–time weighted average) is 10 mg/m³ for inhalable dust (ACGIH 1997).

Hectorite

Hectorite has two listings of category IISE in the OTC Active Ingredient Status Report (FDA 1994). It is listed as being used as an external analgesic and skin protectant. Barr (1957) stated that the Federal Drug Administration (sic) has given approval for the use of Hectorite in internally and externally applied products, as well as dentifrices, cosmetics, and externally approved pharmaceuticals.

Kaolin

According to FDA, Kaolin is considered GRAS as an indirect food additive (21 CFR 186.1256). Kaolin is listed as being used in antacids, anorectals (external and interrectal), antidiarrheals, skin protectants, and digestive aids (colloidal Kaolin) in the OTC Active Ingredient Status Report. The final rulings are as follows: antacids: category IIE; anorectals (both): category I; and digestive aid: category IISE. Proposed rulings are as follows: antidiarrheal: category IIIE; skin protectant diaper rash: category I; skin protectant poison ivy: category I; and skin protectant: category I. Category III is designated as the conditions for which the available data are insufficient to permit final classification at this time.

Gamble (1986) reports Kaolin's main use in the paper industry to fill and coat the surface of paper. Kaolin is also reported being used as a filler in rubber, paint extender, filler in plastics, ceramics manufacture, ink, adhesives, insecticides, medicines, food additives, bleaching, adsorbents, cement, fertilizers, crayons, pencils, detergents, porcelain enamels, paste, foundries, linoleum, floor tiles, and textiles.

The *National Formulary* classifies Kaolin as a tablet and/or capsule diluent (United States Pharmacopeial Convention, Inc. 1994).

The *Food Chemicals Codex* specifies limits of impurities for clay (Kaolin) as: acid-soluble substances <2%; Arsenic (as As) <3 ppm; Heavy Metals (as Pb) <40 ppm; Lead <10 ppm (National Academy of Science 1996).

Magnesium Aluminum Silicate

Magnesium Aluminum Silicate (MAS) is listed as being used in acne treatments and in antacids in the OTC Active Ingredient Status Report (FDA 1994). As an antacid, MAS is a category I listing, meaning it is generally recognized as safe and effective and is not misbranded. However, MAS is a category IISE listing as used for acne. MAS was listed as category IISE due to safety and/or effectiveness.

Other uses for Magnesium Aluminum Silicate have been reported as: adsorbent, suspending agents, tablet and capsule disintegrant, tablet binder, and viscosity-increasing agent (Palmieri 1994).

The *National Formulary* classifies Magnesium Aluminum Silicate as a suspending and/or viscosity-increasing agent (United States Pharmacopeial Convention, Inc. 1994).

VEEGUM, a tradename for Magnesium Aluminum Silicate, has been designated by the FDA as a raw material with the following number: FD-CRMCS no. R0010045 and has an individual Chemical Abstract Registry (CAS) number 12199-37-0.

Magnesium Silicate

Magnesium Silicate is classified as a glidant or anticaking agent by the *National Formulary* (United States Pharmacopeial Convention, Inc. 1994).

Magnesium Trisilicate

Magnesium Trisilicate is listed in the OTC Active Ingredient Status Report as being used as antacids, digestive aids, and overindulgence remedy (FDA 1994). In antacids, FDA has listed Magnesium Trisilicate as category I (generally recognized as safe and effective). FDA concluded that Magnesium Trisilicate use in digestive aids is category IISE (not generally recognized as safe and effective). FDA has proposed that Magnesium Trisilicate use in overindulgence remedies is category I.

Pyrophyllite

Pyrophyllite is listed under Code of Federal Regulations (21 CFR 73.1400) as a naturally occurring color additive and must conform to the following specifications: lead (as Pb) not more than 20 ppm; and arsenic (as As) not more than 3 ppm. Also Pyrophyllite may be used safely for coloring externally applied cosmetics, in amounts consistent with good manufacturing practice (21 CFR 73.2400).

Pyrophyllite is listed by Gamble (1986) as being used in refineries, rubber, ceramics, insecticides, plastics, paint, roofing, bleaching powder, textiles, cordage, and wall board.

Zeolite

Zeolites are reported by Gamble (1986) as being used in CO₂ recovery from natural gas, aromatic separates dimension stones, filler in paper, isolation of radioactive wastes, water aeration, dietary supplements for animals, neutralization of acidic soils, carriers for pesticides and fungicides, sorbents for oil spills, polishing agent in toothpastes, and petroleum solvents. International Agency for Research on Cancer (IARC) (1997) lists the three main uses of synthetic Zeolite as: detergents, catalysts, and adsorbents or desiccants.

Zirconium Silicate

Zirconium Silicate is reported by Kleber and Putt (1986) as being used in chewing gum and in a dental prophylaxis paste.

GENERAL BIOLOGY

Adsorption

The large volume of general data available on the adsorption of various chemicals, cells, etc., to these silicate clays is presented in Table 8. In addition, to this general information, specific reactions are described using specific silicate clays—these data are described below.

Hectorite

Bujdak and Rode (1996) reported that Hectorite-catalyzed glycine and diglycine oligomerizations were performed as drying/wetting cycles. Approximately 7% of glycine was converted to diglycine and diketopiperazine on Hectorite after 7 days. It may be noted that the Hectorite sample was altered by substituting Li(I) for Mg(II), which caused a greater effect on oligomerizations.

Porter et al. (1998) reported condensation reactions of the amino acid glycine on the surface of Cu(II)-exchanged Hectorite. Polymerization of glycine oligomers was seen primarily at the edges or topmost layer. These reactions were facilitated by the availability of intergallery metal cations at the step edges or pores in the surface region.

Kaolin

Adenosine monophosphate molecules were adsorbed onto Kaolinite, modified with Mg²⁺ and irradiated with ultraviolet (UV) light. These synthesis products were tested for their bond types by enzymatic hydrolysis and analyzed by ion-exchange chromatography. Considerable portions of the products were phosphodiesterase hydrolyzed, which implies a 3'-5', 2'-5', or both, nature of the bonds (Strigunkova, Lavrentiev, and Ostroshchenko 1986).

Montmorillonite

Dougherty et al. (1985) incubated Montmorillonite saturated with magnesium chloride (10 mg) with 5×10^6 human neutrophils. Effects were determined by phase contrast microscopic examination and by the measurement of lactate dehydrogenase. Both untreated and clay treated with human albumin were used to stimulate neutrophil chemiluminescence. Montmorillonite was also incubated with human erythrocytes and the free hemoglobin was measured at 430 nm and the effect of clay on zymosan-activated serum was also investigated. Rapid neutrophil lysis was observed in cells exposed to untreated clay. After lysis, lactate dehydrogenase rapidly adsorbed to the surface of the clay. Clay pretreatment with human albumin blocked the enzyme surface adsorption and cell lysis. Neutrophil chemiluminescence was stimulated by untreated clay but not by clay pretreated with 5% albumin. Clay lysis of erythrocytes was incomplete as compared to neutrophil lysis. Zymosan-activated serum samples exposed to clay; complement activity as measured by neutrophil chemotaxis was suppressed in a dose-dependent manner.

TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays

Compound adsorbed	Experimental design	Results	Reference
Magnesium Aluminum Silicate			
Dicumarol	The drug dicumarol was given to dogs with 50% colloidal Magnesium Aluminum Silicate (MAS); the plasma level of dicumarol in dogs was measured	Significantly lower plasma levels and delayed appearance of dicumarol resulted from administration with 50% MAS; drug concentration at peak level was 16.7% (25.8% in controls) and peak plasma levels were seen at 12–24 h (8–12 h in controls)	Akers, Lach, and Fischer 1973
Streptomycin sulphate and neomycin sulphate	Adsorption studies were carried out in vitro in McIlvaine's Buffer and water	MAS had the greatest affinity for streptomycin sulphate in water (adsorption coefficient of $111 \cdot 10^{-3}$ for water and $33 \cdot 10^{-3}$) whereas the adsorption coefficient for MAS in water to neomycin sulphate was $34 \cdot 10^{-3}$	Ghazy, Kassem, and Shalaby 1984
Bromohexine HCL	MAS was mixed with bromohexine HCL to make tablets and were stored in polyethylene film for various times; the amount of bromohexine remaining in the tablet was determined	Bromohexine remaining in the tablets increased with increasing concentrations of MAS, indicating that MAS prevented the adsorption of bromohexine to polyethylene film; no bromohexine degradation was reported	Kukita et al. 1992
Tetracycline	In vitro and in vivo adsorption of tetracycline by VEEGUM was studied	The maximum serum concentration of tetracycline was decreased by 21%; the maximum adsorption in vitro occurred at pH 1.2, where the % adsorbed ranged from 91.5% to 97.2%	Healy et al. 1997
Trimethoprim	The concentration of trimethoprim in the blood was determined at 0, 15, and 30 min and 1, 2, 4, and 6 h	The mean decrease in the maximum blood concentration of trimethoprim was 49.94%	Babhair and Tariq 1983
Aminosidine sulphate, chloramphenicol, erythromycin, neomycin B sulphate, novobiocin sulphate, penicillin V, streptomycin sulphate, and tetracycline hydrochloride	Each antibiotic was added to 250 mg of magnesium trisilicate; the antibiotic activity was determined by cup-plate method using <i>Staphylococcus aureus</i>	Magnesium Trisilicate reduced the activity of all antibiotics except chloramphenicol	El-Nakeeb and Youssef 1968
Ampicillin and amoxycillin	In vitro adsorption and desorption studies were carried out at different pHs	Hydrated silica gel formed from decomposition of the antacid at pH 2.1 and Magnesium Trisilicate had no adsorptive effect on either antibiotic	Khali, Mortada, and El-Khawas 1984a
Attapulgit			
Strychnine, quinine, and atropine	Adsorption isotherms for each of the drugs and the clay was determined using spectrophotometric or colorimetric methods	Attapulgit adsorbed strychnine better than atropine than quinine; an increase in the hydrogen ion concentration was found to have a slight decreasing effect on the adsorptive ability for strychnine	Evcim and Barr 1955

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TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays (*Continued*)

Compound adsorbed	Experimental design	Results	Reference
Strychnine and atropine	Activated attapulgite was added to both compounds and adsorption isotherms were calculated	Both compounds were adsorbed by Attapulgite; optimum adsorbent properties were calculated at pH 6.8 and 7.2	Barr and Arnista 1957
<i>Agrobacterium radiobacter</i>	The measurement of O ₂ uptake by calculating the respiration quotients (Q _{O₂}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Attapulgite contained excess basic cations, which accounted for the initial high pH and the reduction on respiration elicited by the addition of buffer	Stotzky 1966
<i>Vibrio cholerae</i> and <i>Escherichia coli</i> enterotoxins	The toxins and Attapulgite were injected into the intestinal loop of rabbits	Attapulgite prevented the toxic effects caused by enterotoxins in the intestinal loop by adsorption; Attapulgite was effective when injected simultaneously with the toxin and before the toxin is injected	Drucker et al. 1977
Ampicillin and amoxycillin	In vitro adsorption and desorption studies were carried out at different pHs	Both drugs were adsorbed at pH 2.1; desorption experiments at pH values of 2.0 and 6.5 showed only partial release of the adsorbed antibiotics	Khali, Mortada, and El-Khawass 1984a
Bentonite			
<i>Escherichia coli</i> , <i>Serratia marcescens</i> , and <i>Bacillus</i> species	Each organism was cultivated in broth portions with 3% and 10% Bentonite	All organisms were absorbed by Bentonite at each concentration; <i>Bacillus</i> species was almost completely absorbed at each concentration	Novakova 1977
<i>Escherichia coli</i> 0111 endotoxins (ETU 144, 150, and 153)	In vitro and in vivo endotoxin binding was studied	In vitro, Bentonite was an effective endotoxin binder and binding was pH dependent (lower pHs yielded better results); 75 mg completely eliminated endotoxemia. At pH 3.0, the ED ₅₀ was 20 mg	Ditter, Urbaschek, and Urbascek 1985
Zearalenone and nivalenol	20 or 50 g/kg of Bentonite was added to the feed of pigs contaminated with zearalenone and nivalenol and was ingested for 29 days	Bentonite was unsuccessful at overcoming the estrogenic or depressed performance effects caused by the mycotoxins	Williams, Blaney, and Peters 1994
Aflatoxins B ₁ , B ₂ , G ₁ , G ₂ , M ₁	Various methods	2% Bentonite adsorbed 400 µg of B ₁ ; 2% adsorbed 89% of M ₁ ; 2.5% adsorbed 5 ppm of B ₁ and G ₁ and 0.5 to 5 ppm of B ₂ and G ₂ ; 10% adsorbed 70% B ₁	Ramos, Fink-Gremmels, and Hernandez 1996
Kaolin			
Strychnine and atropine	Kaolin was added to both compounds and adsorption isotherms were calculated	Both compounds were adsorbed by Kaolin	Barr and Arnista 1957

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TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays (*Continued*)

Compound adsorbed	Experimental design	Results	Reference
Aminosidine sulphate, chloramphenicol, erythromycin, neomycin B sulphate, novobiocin sulphate, penicillin V, streptomycin sulphate, and tetracycline hydrochloride	Each antibiotic was added to 250 mg of Kaolin; the antibiotic activity was determined by cup-plate method using <i>Staphylococcus aureus</i>	Kaolin adsorbed significant amounts of aminosidine, neomycin, streptomycin, and tetracycline; Kaolin had no effect on antibiotic activity	El-Nakeeb and Youssef 1968
<i>Agrobacterium radiobacter</i>	The measurement of O ₂ uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Kaolin did not maintain the pH therefore the bacteria could not maintain respiration even with an optimal pH for growth	Stotzky 1966
<i>Bacillus subtilis</i> , <i>Bacillus megaterium</i> , <i>Aerobacter aerogenes</i> , <i>Escherichia intermedia</i> , <i>Pseudomonas aeruginosa</i> and <i>P. aeruginosa</i> C-II, <i>Flavobacterium</i> species, <i>Proteus vulgaris</i>	The measurement of O ₂ uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Kaolin in unadjusted pH systems reduced respiration of the bacteria below that of cultures without clay; but in adjusted systems some stimulation of respiration with the addition of Kaolin was apparent	Stotzky and Rem 1966
Mycelial homogenates of 27 species of fungi	Fungal mycelium and Kaolinite were cultured together and the O ₂ uptake and pH were recorded	Kaolinite concentrations <4% generally did not effect respiration; respiration was only markedly inhibited at concentrations >40%	Stotzky and Rem 1967
Crystal violet	2 g of Kaolin was added to 100 ml of a crystal violet solution	Adsorption was examined over a pH range of 2.5–9.5; adsorption increased with increasing pH	Armstrong and Clarke 1971
<i>Staphylococcus aureus</i>	Suspension of the organism, Kaolinite, and NaCl were studied	Increasing electrolyte concentration was accompanied by increased edge-to-face Kaolinite flocculation and organism-Kaolin aggregates	Steel and Anderson 1972
<i>Escherichia coli</i>	<i>E. coli</i> was cultivated in broth portions with 3% and 10% Kaolinite	<i>E. coli</i> was absorbed by Kaolin at both concentrations; the greatest adsorption occurred at 10% Kaolin at all phases of bacterial growth	Novakova 1977
¹²⁵ I-labeled <i>Pseudomonas aeruginosa</i> toxin	The in vitro adsorption of the toxin by Kaolin was investigated over a range of pHs	The maximum adsorption occurred at pHs below 4.1; minimal values occurred at pH 4.1, 7.4, and 8	Said, Shibal, and Abdullah 1980
Acetohexamide, tolazamide, and tolbutamide	In vitro (pH 7.4) and in vivo (rats) adsorption studies were carried out	All 3 drugs bound and acetohexamide had the greatest binding; the hypoglycemic activity of the 3 drugs were suppressed and blood glucose concentrations were increased; desorption of the drugs from Kaolin ranged from 1.8% to 24.5%	Said and Al-Shora 1980

(Continued on next page)

TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays (*Continued*)

Compound adsorbed	Experimental design	Results	Reference
Coliphages T1 and T7 of <i>Escherichia coli</i>	1 ml suspensions of the coliphages were added to various concentrations of Kaolin	Adsorption of both coliphages by Kaolin were approximately the same 99%	Schiffenbauer and Stotzky 1982
Trimethoprim	The concentration of trimethoprim in the blood was determined at 0, 15, and 30 min and 1, 2, 4, and 6 h in the presence of Kaolin-Pectin	The mean decrease in the maximum blood concentration of trimethoprim was 29.42%	Babhair and Tariq 1983
Cationic surfactants: distearyl dimethyl ammonium chloride (74%); lauryl dimethylbenzyl ammonium chloride (50%)	A Kaolinite solution with added copper ions was added to surfactants and the metal ion uptake was recorded	Cationic surfactant result: the equilibrium between the metal ions and the organic cations was not effected	Beveridge and Pickering 1983
Anionic surfactants: sodium alkylbenzene sulphonate (80%); Monoethanolamine lauryl sulphate (34%); lauryl alcohol polyethylene condensate (28%)		Anionic surfactants: increased metal uptake by the clay was observed	
Nonionic surfactants: alcohol ethoxylates; tridecamyl ethoxylate (90%); cetystearyl alcohol ethoxylates; stearic acid ethoxylate; cocnut monoethanolamide ethoxylate; octadecylamine ethoxylate; castor oil ethoxylate; nonyl phenol ethoxylates; dinonyl pheno ethoxylate; polypropylene glycol ethoxylates		Nonionic surfactants: many surfactants had no effect and some caused enhanced loss of the metal ions from solution	
<i>Escherichia coli</i> 0111 endotoxins (ETU 144, 150, and 153)	In vitro and in vivo endotoxin binding to Kaolin	In vitro Kaolin was an effective endotoxin binder and binding was pH dependent (lower pHs yielded better results); 300 mg of Kaolin eliminated endotoxemia, at pH 7.4, the ED ₅₀ was 900 mg	Ditter, Urbascek, and Urbascek 1983
Reovirus type 3	Chymotrypsin, ovalbumin, and lysozyme were added to Kaolinite and reovirus type 3	Chymotrypsin and ovalbumin reduced the adsorption of reovirus but lysozyme did not	Lipson and Stotzky 1984
Ampicillin and amoxycillin	4 g of Kaolin was ingested and 2 h later, 500 mg of the drugs were administered. This protocol was repeated 2 h later and urine (human) samples were collected	All volunteers showed reduced drug bioavailability following treatment; after 8 h, the reduced bioavailability for ampicillin ranged from 51.2 to 76.3 and 63.6 to 80.6 for amoxycillin	Khali, Mortada, and El-Khawass 1984b

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TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays (*Continued*)

Compound adsorbed	Experimental design	Results	Reference
Ampicillin and amoxycillin	In vitro adsorption and desorption studies to Kaolin (light, natural, and fine) were carried out at different pHs	The 3 types of Kaolin adsorbed only ampicillin and adsorption decreased as the pH increased; only partial release of the antibiotics was seen at pH 2.0 and 6.5	Khali, Mortada, and El-Khawas 1984a
Reovirus type 3 and coliphage T1	Competitive adsorption studies were carried out with Kaolin in estuarine water and distilled water	Reovirus type 3 and coliphage T1 did not share common adsorption sites on Kaolin and the coliphage did not interfere with the reovirus adsorption in estuarine water; the reovirus had no apparent effect on the adsorption of the phage in estuarine water	Lipson and Stotzky 1985
LT toxins of <i>Vibrio cholerae</i> and <i>Escherichia coli</i> , the ST toxin of ETEC, and the verotoxin of EHEC	Not specified	Kaolin inactivated the LT toxin and adsorption was a result of hydrogen bonding; it was ineffective against the verotoxin when the pH was alkaline; Kaolin was only slightly effective against the ST toxin	Brouillard and Rateau 1989
Montmorillonite			
<i>Agrobacterium radiobacter</i>	The measurement of O ₂ uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Montmorillonite spurred bacterial respiration by maintaining the initial pH; when the pH was adjusted to 7.0 respiration was its highest and similar to the buffered systems	Stotzky 1966
<i>Bacillus subtilis</i> , <i>Bacillus megaterium</i> , <i>Aerobacter aerogenes</i> , <i>Escherichia intermedia</i> , <i>Pseudomonas aeruginosa</i> and <i>P. aeruginosa</i> C-II, <i>Flavobacterium</i> species, <i>Proteus vulgaris</i>	The measurement of O ₂ uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Montmorillonite increased the respiration of all species regardless of pH and characteristics of the bacteria primarily by maintaining the pH of the systems favorable for growth	Stotzky and Rem 1966
Mycelial homogenates of 27 species of fungi	Fungal mycelium and Montmorillonite were cultured together and the O ₂ uptake and pH were recorded	Montmorillonite concentrations <4% generally did not effect respiration; respiration was markedly inhibited at concentrations of 4% and above	Stotzky and Rem 1967
Cationic drugs: chlorpheniramine maleate, amphetamine sulfate, and propoxyphene hydrochloride; Anionic drugs: not specified Nonionic drugs: xanthines, theophylline, and caffeine	Dissolution and dialysis were carried out in vitro	All the cationic drugs and certain nonionic drugs bound tenaciously; the anionic drugs and nonionic drugs that exist as nonionics bound very weakly and rapidly pass into solution	McGinity and Lach 1976

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TABLE 8

Adsorption of various chemicals, cells, etc., to Silicate clays (*Continued*)

Compound adsorbed	Experimental design	Results	Reference
Carbon tetrachloride, ethylene dibromide, trichloroethylene	10–1000 ppb/water of the three compounds were exposed to aluminum-saturated Montmorillonite and calcium-saturated Montmorillonite	Aluminum-saturated Montmorillonite absorbed 17% of trichloroethylene and 6% of the other cmpds; calcium-saturated Montmorillonite did not absorb carbon tetrachloride or trichloroethylene	Rogers and MacFarlane 1981
Coliphages T1 and T7 of <i>Escherichia coli</i>	1 ml suspensions of the coliphages were added to various concentrations of Montmorillonite	Adsorption of T1 coliphages by Montmorillonite was 84% and T7 was 96%	Schiffenbauer and Stotzky 1982
Cationic surfactants: distearyl dimethyl ammonium chloride (74%); lauryl dimethylbenzyl ammonium chloride (50%)	A Montmorillonite solution with added copper ions was added to surfactants and the metal ion uptake was recorded	Cationic surfactant result: metal ion uptake was reduced by competing surface sites	Beveridge and Pickering 1983
Anionic surfactants: sodium alkylbenzene aulphonate (80%); monoethanolamine lauryl sulphate (34%); lauryl alcohol polyethylene condensate (28%);		Anionic surfactants: increased metal uptake by the clay was observed	
Nonionic surfactants: alcohol ethoxylates; tridecaml ethoxylate (90%); cetystearyl alcohol ethoxylates; stearic acid ethoxylate; coconut monoethanolamide ethoxylate; octadecylamine ethoxylate; castor oil ethoxylate; nonyl phenol ethoxylates; dinonyl pheno ethoxylate; polypropylene glycol ethoxylates		Nonionic surfactants: surfactants reduced the amount of metal ion adsorbed by the clay	
Reovirus type 3	Chymotrypsin, ovalbumin, and lyso-zyme were added to Montmorillonite and reovirus type 3	Chymotrypsin, ovalbumin, and lysozyme reduced the adsorption of reovirus	Lipson and Stotzky 1984
Poliovirus-1 (Lsc 2ab strain)	500, 15, 3 mg/L of Sodium Montmorillonite and the virus were suspended in seawater and the adsorption, desorption, and virus survival were studied	99.9% of the virus was absorbed in less than 30 min; 500 mg/L of Na-Montmorillonite significantly increased the survival duration of the virus and desorption tests showed elution of 76%	Gantzer, Quignon, and Schwartzbrod 1994
Reovirus type 3 and coliphage T1	Competitive adsorption studies were carried out with Montmorillonite in estuarine water and distilled water	Reovirus type 3 and coliphage T1 did not share common adsorption sites on Kaolin and the coliphage did not interfere with the reovirus adsorption in estuarine water or distilled water; the reovirus suppressed the adsorption of the coliphage in estuarine water	Lipson and Stotzky 1985

(Continued on next page)

TABLE 8
Adsorption of various chemicals, cells, etc., to Silicate clays (*Continued*)

Compound adsorbed	Experimental design	Results	Reference
Pyrophyllite			
<i>Agrobacterium radiobacter</i>	The measurement of O ₂ uptake by calculating the respiration quotients (Q_{O_2}) was performed on all species of bacteria in the presence of 2% Kaolin with either adjusted (7.0) or unadjusted pHs	Pyrophyllite did not maintain a favorable pH for sustained respiration in either buffered or nonbuffered systems	Stotzky 1966
Zeolite			
Zearalenone	5% of a synthetic anion-exchange zeolite and a cation-exchange zeolite and 250 μ g/g of zearalenone were added to the feed of rats	The anion-exchange zeolite was completely effective and the cation-exchange zeolite was not	Smith 1980
Aflatoxin B ₁	Two samples of natural Zeolites in different liquids were incubated with B ₁	The average aflatoxin retention rate was 605; effectiveness was lower in media containing nitrogen compounds	Dvora'k 1989

Bujdak and Rode (1996) reported peptide formation on the surface of three Montmorillonite samples. The Montmorillonite-catalyzed reaction produced diglycine and diketopiperazine from glycine.

Ferris et al. (1996) studied the catalytic properties of Na⁺-Montmorillonite by adding daily ImpA to a decanucleotide ([³²P]-dA(pdA)₈pA, where Im = imidazole; pA = adenosine-5'-phosphate; pdA = 3'-deoxyadenosine-5'-phosphate; ³²P = radioactively labeled phosphate group). Polyadenylates were formed after two additions of ImpA, with the main products being monomers ranging from 11 to 14. Polynucleotides, with more than 50 monomers, were formed after 14 additions. The principle oligomeric products contained 20 to 40 monomers.

Ertem and Ferris (1998) reported Montmorillonite-catalyzed ImpA and ImpA-A5' reactions. Oligomer yields decreased significantly when the addition of alkylammonium or aluminum poly oxo cations blocked the interlayer surfaces of the Montmorillonite particles.

Absorption, Distribution, Metabolism, and Excretion

Magnesium Trisilicate

Page, Heffner, and Frey (1941) measured the urinary excretion of silica in five men given 5 g of synthetic Magnesium Trisilicate orally for 4 consecutive days. Urine samples were collected for 24 h on the second day after the end of administration and analyzed for silica content. The mean 24-h excretion of all subjects was 16.2 mg of SiO₂. On the second, third, and fourth days after administration, the mean excretion rose to 172, 178, and 162 mg SiO₂. A total of 20 mg of Magnesium Trisilicate was taken and contained 9.2 g of SiO₂. An approximation of 5.2% SiO₂ excretion was estimated.

Benke and Osborn (1979) conducted a study in which groups of four to six male Sprague-Dawley Cox rats were fasted for 17 to 18 h and then were administered Magnesium Trisilicate orally in doses of 40, 200, or 1000 mg/kg of their body weight. Control animals received 10 ml of quartz-distilled water. All suspensions contained <0.5 ppm of silicon and aluminum. Urine samples were collected over an 8-h period, and the remaining urine in the bladder was collected afterwards. The concentrations of silicon was measured by induction-coupled radiofrequency (RF) plasma optical emission spectrometry. Silicon excretion was most rapid in the first 24 h after dosing. The control values were subtracted from the final values and the following number resulted. The urinary silicon excretion at 40, 200, and 1000 mg/kg Magnesium Trisilicate was 16.8%, 5.1%, and 1.5%, respectively.

Dobbie and Smith (1982) reported a 24-h urinary excretion study in which Si was determined by atomic absorption spectroscopy in one male and one female participant. A normal diet was given to the participants and four urine collections were made. A single dose of Magnesium Trisilicate was ingested at the beginning of the second 24-h collection. Magnesium Trisilicate doses given were as follows: 2, 5, and 10 g to the male subject and 2.5, 5, and 7.5, and 10 g in the female subject. The amount of Si excreted at the 5-g dose was greater than any other dose in the male subject and was greater than the 2.5- and 7.5-g doses in the female subject. The value of Si excretion for the male and female subjects were 3.63 and 3.31 mmol/day, respectively. Maximum excretion occurred in the first 24 h after ingestion.

The oral bioavailability of silicon and aluminum in Magnesium Trisilicate was studied by Cefali et al. (1995). Twelve female beagle dogs were administered a single 20-mg/kg dose of Magnesium Trisilicate and their blood was sampled at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after dosing. The plasma

samples were assayed for silicon and aluminum by graphite furnace atomic adsorption. No dogs displayed emesis, but four had soft stool. The area under the curve (AUC, $\text{mg} \cdot \text{h/L}$), concentration maximum (C_{max} , mg/L), and time maximum (T_{max} , h) for silicon absorption was 8.8, 0.75, and 6.9, respectively. The AUC ($\text{mg} \cdot \text{h/L}$), C_{max} (mg/L), and T_{max} (h) for aluminum absorption was 315, 24, and 5.7, respectively. There was no statistically significant absorption of aluminum from the aluminum containing compounds.

Montmorillonite

Retention of monodisperse and polydisperse Montmorillonite particles inhaled by dogs, rats, and mice was studied by Snipes, Boecker, and McClellan (1983a). Cations normally present in Montmorillonite were exchanged with ^{134}Cs . Polydisperse and monodisperse ^{134}Cs -labeled Montmorillonite suspensions were administered to groups of 40 rats and mice and to 120 beagle dogs by a multiport nose-only inhalation exposure system. Aerosol concentrations ranged from 10^{-3} to 10^{-1} mg of fused Montmorillonite per liter of air. Equal numbers of male and female rats and mice and 74 male and 46 female dogs were utilized. Exposure times for rats and mice ranged from 25 to 45 min and for dogs 15 to 50 min. All animals were whole-body counted for the labeled particles. Rats and mice were counted on exposure days 2, 4, 8, 16, 32, 64, 128, 256, 365, 512, 730, and 850 and the dogs were also counted on the same schedule, but also at 4, 5, 7, and 9 years after inhalation exposure. Excreta collections were made for animals from each exposure group. Five rats and five mice from each group were killed 4 h after exposure. The remaining rats and mice were killed at various times after exposure. Two dogs were scheduled for termination at times ranging from 4 h to 9 years. All animals were necropsied and tissues from lungs, lung-associated lymph nodes (LALNs), gastrointestinal tract, spleen, kidneys, abdominal lymph nodes, blood, skeleton, muscle, and skin were prepared for analysis of ^{134}Cs exposure. Results of the counts were converted into disintegrations per minute.

The mass of material deposited into the lungs of rats and mice was ~ 0.01 to 0.1 mg and for dogs was ~ 1 to 10 mg. The mass of Montmorillonite for all three species was < 0.1 mg per gram of lung. Clearance of the initial ^{134}Cs occurred by dissolution and mechanical clearance. Mechanical clearance from the nasopharynx was rapid, and the clearance rate was decreased to a negligible value for all three species within a few days. Most initial deposit cleared via the gastrointestinal tract. Long-term mechanical clearance from the pulmonary region occurred at a constant rate for all species. Solubilization was the primary factor in long-term lung clearance for most particles inhaled by dogs and mechanical clearance was dominant in rats and mice. Most of the long-term clearance of deposited particles went to LALNs in dogs and occurred at a slower rate as compared to rats and mice. Rats and mice had a rapid clearance from the pulmonary region, where most of the mechanical clearance occurred via the gastrointestinal tract. Long-term clearance of the particles

in dogs occurred at 3500-day half-time in the lymph nodes and 6900-day half-time clearance in the gastrointestinal tract. The transport rate of the particles in the dog was 0.0002 day^{-1} of the lung burden. The long-term biological clearance half-term day was 690 days for rats and 490 days for mice. The lymph node accumulation process was modeled by a short-term process that became negligible after a few days (Snipes, Boecker, and McClellan 1983a).

Snipes, Muggenburg, and Bice (1983b) instilled radio-labeled (^{134}Cs) fused Montmorillonite particles into specific lung lobes or injected intraperitoneally into 32 beagle dogs. Necropsy was performed at 34, 182, and 365 days later. Specific sites of instillation included right apical lobe, right cardiac lobe, right diaphragmatic lobe, right intermediate lobe, left apical lobe, left diaphragmatic lobe, and intraperitoneal. Initial burdens in the peritoneal cavity or the lungs ranged from 0.50 to $14 \mu\text{Ci}$ of ^{134}Cs for 29 dogs and from 42 to $64 \mu\text{Ci}$ of ^{134}Cs for lung burdens for the other three dogs. Effective translocation half-time of lung instillations was 390 days. The accumulation rate of ^{134}Cs -labeled particles in the lymph nodes was 0.03% per day. Individual lung lobes cleared particles to one or two lymph nodes, and specific lymph nodes accumulated particles from one to three lung lobes. Lymph nodes that collected particles from the lung included the left mediastinal node, left tracheobronchial lymph node (TBLN), right TBLN, left middle TBLN, and right middle TBLN. The destination for translocated particles were primarily the nodes proximate to the tracheal bifurcation. Particles injected into the peritoneal cavity were translocated mainly to mesenteric lymph nodes and left sternal and right sternal lymph nodes. A small percentage of particles went to the left TBLN.

Zeolite

The oral bioavailability of silicon and aluminum in Zeolite A was studied by Cefali et al. (1995). Twelve female beagle dogs were administered a single 20-mg/kg dose of Zeolite A and blood was sampled at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after dosing. The plasma samples were assayed for silicon and aluminum by graphite furnace atomic adsorption. No dogs displayed emesis but four had soft stool. The AUC ($\text{mg} \cdot \text{h/L}$), C_{max} (mg/L), and T_{max} (h) for silicon absorption was 9.5, 1.07, 7.9, respectively. The AUC ($\text{mg} \cdot \text{h/L}$), C_{max} (mg/L), and T_{max} (h) for aluminum absorption was 342, 29, and 3.5, respectively. The AUC and C_{max} values were elevated after the addition of the silicon containing compounds compared to the baseline and the AUC was significantly elevated. There was no statistically significant absorption of aluminum from the other aluminum-containing compounds.

In a study by Cefali et al. (1996), the bioavailability of silicon and aluminum in Zeolite A administered in either a capsule, an oral suspension, or an oral solution relative to an intravenous bolus infusion administered over a 1- to 1.5-min period was investigated. Twelve beagle dogs were given single doses of Zeolite A and their plasma samples, drawn at 0 and 36 h, were analyzed for silicon and aluminum concentrations by graphite furnace

atomic absorption. The plasma aluminum AUC values from the oral capsule and suspension were not statistically different from those during the control period. However, the aluminum AUC of the oral solution was statistically greater than the AUC of the corresponding control period. The extent of absorption of aluminum from the oral dosage forms was less than 0.1% relative to the intravenous infusion.

In Vitro Assays

Aluminum Silicate

Nadeau et al. (1987) tested Fiberfrax, an aluminum silicate, in several in vitro assays for red blood cell (RBC) hemolysis, lactate dehydrogenase activity (LDH), β -galactosidase (β -GAL) activity, lactic acid production, cellular ATP activity, and the cellular DNA contents. The mean length and diameter of this sample were determined to be 8.3 μm and 0.2 μm , respectively. Approximately 60% of this Fiberfrax sample was nonfibrous.

For the hemolysis assay, RBCs from rats were isolated and exposed to 100, 250, 500, 750, or 1000 $\mu\text{g}/\text{ml}$ of fibers for 1 h. The percentage of release of hemoglobin was compared with that of a fully lysed sample. The target cells for the other experiments were obtained by bronchoalveolar lavage from black hooded rats. Each of the experiments tested both fresh cell monolayers and 1-day-old monolayers. Fiber samples were added to the cultures at two doses, 33.3 $\mu\text{g}/\text{ml}$ and 166.7 $\mu\text{g}/\text{ml}$. LDH activity was based on the formation rate of NADH at 340 nm. The β -GAL activity was based on the measurement of *p*-nitrophenyl release. The amount of metabolite released from PAMs (pulmonary alveolar macrophages) into the medium was the measurement of lactic acid production. PAMs were treated with 1 ml of dimethyl sulfoxide to release the nucleotides and the ATP was measured later by a bioluminescence assay.

Fiberfrax particles produced no hemolytic activity at any concentration except 1000 $\mu\text{g}/\text{ml}$. Even at 1000 $\mu\text{g}/\text{ml}$, the particles had very weak hemolytic properties with only 2.0% hemolysis. In fresh PAM monolayers, Fiberfrax was very cytotoxic at 166.7 $\mu\text{g}/\text{ml}$. The extracellular releases of LDH and β -GAL were approximately 60% to 70% and 40% to 50%, respectively. A low cell viability was confirmed by an 80% decrease in ATP cell contents. Even at the lower dose, 33.3 $\mu\text{g}/\text{ml}$, a significant cytotoxic effect resulted, as judged by enzyme releases and ATP cell contents. Again in the day-old cultures, Fiberfrax was highly cytotoxic to PAM. LDH and β -GAL activities were as great and ATP cell contents were significantly decreased. At the lower dose, a moderate cytotoxic effect was observed. Decreases in lactic acid production were more pronounced at 166.7 $\mu\text{g}/\text{ml}$. No significant effect on total DNA cell content was noted in either the fresh or day-old cultures (Nadeau et al. 1987).

Attapulgit

Colony formation of human embryo intestinal cells (I-470) was examined by Reiss, Millette, and Williams (1980). At a dose of 0.001 to 1 mg/ml of Attapulgit with fibers <2 μm , colony

formation was not modified. Colony formation was inhibited by 35% and 43% at doses of 2.5 and 5.0 mg/ml, respectively.

Oscarson, Van Scoyoc, and Ahlrichs (1981) added Attapulgit to a culture of bovine RBCs to study the extent of hemolysis. Saline was added to cultures as a control and in a separate experiment, the polymer poly-2-vinylpyridine-*N*-oxide was also added to study its inhibiting effects. No other details were given. The concentration of Attapulgit that caused 50% hemolysis in 1 ml of a 3% solution of RBCs was determined as 0.06 mg Attapulgit/ml of silicate-erythrocyte-buffer suspension. A concentration of 0.2 and 1.0 $\mu\text{m}/\text{ml}$ of polymer caused 20% and 3% hemolysis, respectively. This was somewhat less hemolysis than without the polymer.

Chamberlain et al. (1982) tested two samples, one with short fibers and one with long fibers, of Attapulgit for their cytotoxicity in three cell lines: mouse peritoneal macrophages, human type II alveolar tumor (A549) cells, and Chinese hamster V79-4 lung cells. Attapulgit samples of 50, 100, and 150 $\mu\text{g}/\text{ml}^{-1}$ were added to mouse peritoneal macrophages for 18 h. The medium and cell lysates were assayed for LDH activity. The control received no dust sample. In the second experiment Attapulgit, 100 $\mu\text{g}/\text{ml}^{-1}$ and 200 $\mu\text{g}/\text{ml}^{-1}$, were added to A549 cultures and incubated for 5 days. The diameters of the cells were assessed for giant cell formation. The control treatment received no dust. In the last experiment, the survival of V79-4 cells in the presence of a series of concentrations of each dust was determined. Specific concentrations were not given. The cells and dust samples were incubated for 6 days and counted after the incubation. The controls received no dust.

The mouse macrophages released 57.7% LDH from interaction with 150 $\mu\text{g}/\text{ml}^{-1}$ of short fiber Attapulgit and was considered cytotoxic. However, the short fiber sample was considered inert to the A549 cells and V79-4 cells. The long fiber Attapulgit was cytotoxic to all three cell types. It was noted by investigators that mouse peritoneal macrophages are sensitive to both fibrogenic and carcinogenic dusts; whereas nonmacrophage cell lines such as V79-4 and A549 cells are insensitive to fibrogenic dusts but sensitive to the fiber morphology of carcinogenic dusts (Chamberlain et al. 1982).

Gormley and Addison (1983) investigated the cytotoxic effect of Attapulgit with a particle size of 2.6 μm . Clay suspensions, 20 and 80 $\mu\text{g}/\text{ml}$, were added to P388D1, a macrophage-type cell line for 48 h. Three sets of controls were included: a positive control, 20 μg of quartz DQ₁₂/ml; and two negative controls, 80 μg of TiO₂/ml, and an undusted set of cultures. The following assessments were made: cell viability; the activity of LDH; the activity of *p*-nitrophenyl-*N*-acetyl- β -D-glucosamide; L-(+)-Lactic acid production; and total cellular protein concentrations. Cellular viability was expressed as a percentage of the titanium dioxide control (100.0%) $\pm$ the standard deviation. The 20- $\mu\text{g}/\text{ml}$ dose of Attapulgit produced a 65.8% $\pm$ 9.2% viability and the 80 $\mu\text{g}/\text{ml}$ dose produced a 30.9% $\pm$ 17.4% viability. Cellular LDH activities fell with decreasing cell viability, whereas the percentage of LDH in the medium increased.

Similar results were seen with glucosamidase. Also, the amount of lactate produced decreased as cell viability decreased. However, little change in the total cellular protein was recorded.

The induction of squamous metaplasia in tracheal organ cultures was investigated by Woodworth, Mossman, and Craighead (1983). Suspensions of Attapulgitte at concentrations of 1, 4, and 16 mg/ml were added to the mucosal surface of the tracheal explants for 1 h. After experimental treatments, extracts were transplanted to another surface more suitable for cell attachment. Mucocillary differentiation was maintained for 4 weeks and the explants were examined at 2, 4, and 6 weeks after exposure to Attapulgitte. The extent of squamous metaplasia was evaluated by SEM (scanning electron microscope). The explants were labeled with [^3H]-thymidine and the labeling index was scored. Four weeks after exposure to Attapulgitte, the explants underwent both proliferative and metaplastic alteration. Attapulgitte induced an increase in metaplasia at low doses (1.0 and 4.0 mg/ml), but the increase was not statistically significant. The labeling index was also increased slightly but statistically significant. SEM was used to determine the association of fibers with metaplastic lesions. Most fibers aggregated at the margins of the explant, although small numbers of individual fibers were distributed along the mucosal surface. These fibers either rested on nonciliated cells or protruded into the mucosal surface. They were often encompassed by accumulations of epithelial cells. Metaplastic foci tended to be small. Many foci associated with the lesions but some were located at sites where no lesions could be seen.

The binding capacity, in vitro cytotoxicity, and percentage of hemolysis were investigated in a study by Harvey, Page, and Dumas (1984). Binding assays were carried out using the known carcinogens benzo(α)pyrene (B(α)P), nitrosonormicotine (NNN), and *N*-acetyl-2-aminofluorene (NAAF) and 2 mg/ml of Attapulgitte. A 2% suspension of sheep erythrocytes were added to 30 mg of Attapulgitte and incubated for 50 min. Cytotoxicity was measured using 1000 μg of Attapulgitte and macrophage-like P399D1 cells and using the Trypan blue dye exclusion method. Hemolysis was calculated by measuring the optical density at 540 nm. All experiments included the positive control UICC chrysotile A and the negative control titanium dioxide. Chrysotile binds significantly more to all three carcinogens than the other fibers ($p < .005$) except Attapulgitte. Attapulgitte and chrysotile had very comparable binding capacities. Again Attapulgitte and chrysotile had the greatest hemolysis and cytotoxicity compared to the negative control. On a scale of 1 to 5, 5 being the greatest, Attapulgitte scored a 3.72 and 4.26 in hemolysis and cytotoxicity, respectively.

The cellular interactions between Attapulgitte and rat hepatocytes were examined in a study by Denizeau et al. (1985a). Primary cultures of rat hepatocytes were exposed to 10 $\mu\text{g}/\text{ml}$ of Attapulgitte fibers for 20 h. Ultrastructural analysis was performed by transmission electron microscopy. Fiber length was not indicated in this study. Fibers are phagocytized by the cells and numerous phagolysosomes are distributed throughout the

cytoplasm. The phagolysosomes also appear in the vicinity of charged vacuoles. Invaginations of the plasma membrane engulfing fibers and formation of vacuoles are identifiable. Deeper in the cytoplasm vacuoles with various shapes show the presence of fibers.

Beck and Bignon (1985) incubated leukemic mouse cells with two samples of 10, 50, or 100 $\mu\text{g}/\text{ml}$ of Attapulgitte. Viable cell counts were taken at 0, 24, 48, and 72 h. A positive control consisting of UICC amosite and untreated negative controls were also used in this experiment. The majority of fibers in the Attapulgitte samples were $< 1.0 \mu\text{m}$. No evidence of cytotoxicity was measured over the 72-h period. The results from the Attapulgitte samples were indistinguishable from the untreated controls.

The cytotoxic effects of Attapulgitte on rabbit alveolar macrophages and rat pleural mesothelial cells were investigated by Jaurand et al. (1987). Attapulgitte samples with a mean fiber length of 0.77 μm were added at concentrations 4 and 8 $\mu\text{g}/\text{cm}^2$ to rabbit alveolar macrophage cultures for 4 and 20 h; control cultures received medium with no fibers. Enzyme release, activity of cytoplasmic LDH and lysosomal β -GAL was tested. The presence of LDH activity in cultures was the gauge of cytotoxicity and the presence of β -GAL was the gauge of cell stimulation. Attapulgitte at both concentrations was cytotoxic at 20 h. β -GAL release percentages for Attapulgitte and quartz after 20 h were almost identical.

Again Attapulgitte was added at concentrations of 1, 2, 4, and 10 $\mu\text{g}/\text{cm}^2$ to rat pleural mesothelial cells. The cell number was determined daily with the use of a Natch NS 1002 image analyzer. Attapulgitte was not cytotoxic except at 10 $\mu\text{g}/\text{cm}^2$. At the lower doses, cell number increases were comparable to that of the controls (Jaurand et al. 1987).

Nadeau et al. (1987) tested Attapulgitte for its effects on cells in several in vitro assays for RBC hemolysis, LDH activity, β -GAL activity, lactic acid production, cellular ATP activity, and the cellular DNA contents. The mean length and diameter of this sample were determined to be 0.8 μm and 0.1 μm , respectively. The same study was conducted on Aluminum Silicate and all protocol and procedures are explained under that section. Attapulgitte particles produced no hemolysis except at 1000 $\mu\text{g}/\text{ml}$. Even at 1000 $\mu\text{g}/\text{ml}$, the particles showed very weak hemolytic properties with only 2.0% hemolysis. Analysis with the fresh PAM monolayers revealed Attapulgitte to be very cytotoxic at 166.7 $\mu\text{g}/\text{ml}$. The extracellular releases of LDH and β -GAL were approximately 60% to 70% and 40% to 50%, respectively. A low cell viability was confirmed by an 80% decrease in ATP cell contents. Even at the lower dose, 33.3 $\mu\text{g}/\text{ml}$, a significant cytotoxic effect resulted, as judged by enzyme releases and ATP cell contents. Again in the day old cultures, Attapulgitte was highly cytotoxic to PAM. LDH and β -GAL activities were very large and ATP cell contents were significantly decreased. At the lower dose, a moderate cytotoxic effect was observed. Decreases in lactic acid production were more pronounced at 166.7 $\mu\text{g}/\text{ml}$. No significant effect on total DNA cell content was noted in either the fresh or day-old cultures.

Garcia, Dodson, and Callahan (1989) investigated the effects of Attapulgit on cultures of human umbilical vein and bovine artery endothelial cell monolayers. Chrysotile asbestos was also studied as a positive control. Rapid phagocytosis of Attapulgit and chrysotile particulates was evident in endothelial cell monolayers. Attapulgit was markedly toxic according to a gradient of time-dependent and concentration-dependent endothelial cell injury measured by specific ^{51}Cr release. Chrysotile was much less toxic. Responses of bovine pulmonary artery and human vein endothelial cells to fiber phagocytosis and fiber-induced injury were similar. Fiber-mediated stimulation in human umbilical cell monolayers of the arachidonate metabolite prostacyclin paralleled endothelial injury. Attapulgit was stimulatory in this experiment, whereas chrysotile was only weakly cytotoxic. Superoxide dismutase and catalase produced significant protection against fiber-mediated endothelial cell injury. Chelation by deferoxamine of elemental Fe in the fiber preparations was also protective.

Perderiset et al. (1989) reported the hemolytic activity of Attapulgit on human red blood cells at five concentrations (0.05, 0.1, 0.2, 0.4, and 0.5 mg/ml). Additional studies tested the hemolytic activity of dipalmitoyl phosphatidylcholine (DPPC) and bovine serum albumin (BSA)-treated Attapulgit (2 mg/ml). The mean fiber length was $<2\ \mu\text{m}$. The percentage of hemolysis was determined by measuring the absorbance of the supernatant at 540 nm. At 0.5 mg/ml, Attapulgit caused 82% hemolysis. The maximum amount of BSA adsorbed was $70 \pm 10\ \mu\text{g}/\text{mg}$ of Attapulgit, and the maximum occurred at an initial concentration of $200\ \mu\text{g}/\text{ml}$. For DPPC, the maximum amount of BSA adsorbed was $210 \pm 14\ \mu\text{g}/\text{mg}$ of Attapulgit, and the maximum occurred at an initial concentration of 250 to $300\ \mu\text{g}/\text{ml}$. Both compounds reduced the hemo-

lytic effect of Attapulgit due to adsorption on the particle's surface.

Nolen, Langer, and Herson (1991) tested nine different samples of Attapulgit for their membrane-lysing activity using human RBCs. The HC_{50} (concentration of particulate in $\mu\text{g}/\text{ml}$ required to lyse 50% of the erythrocytes in a suspension containing 1.8×10^8 cells/ml) was determined quantitatively. Three samples of Chrysotile were used as positive controls. No other details of the experiment were given. The fiber characteristics were determined by light microscopy and x-ray diffraction and the HC_{50} values are presented in Table 9.

Attapulgit's cytotoxicity was investigated in rat pleural mesothelial cells (RPMCs) by Yegles et al. (1995). A suspension of 0.5 mg/ml of Attapulgit was added to RPMCs, and a 3,(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) viability test and anaphase/telophase abnormalities test were performed. The clay sample had no fibers measuring greater than $4\ \mu\text{m}$. Cytotoxicity was expressed as the concentration that provides 75% of cell viability compared to untreated controls (IC_{75}). Attapulgit was only poorly toxic with an IC_{75} of $>100\ \mu\text{g}/\text{cm}^3$. Untreated controls averaged about 3.4% of abnormal anaphases; no significant anaphase abnormalities were seen with Attapulgit as well.

Bentonite

The hemolysis of human erythrocytes and methylene blue adsorption by two Bentonite samples were investigated by M'anyai et al. (1969). A white Bentonite sample consisted of 50% illite, 25% quartz, and 25% Montmorillonite; the yellow Bentonite sample consisted of predominately Montmorillonite. The data in Table 10 show that the hemolytic effect varied as a function of both of the amount of clay (mg) and the surface area (m^2).

TABLE 9
Fiber characteristics of nine Attapulgit samples tested for their membranolytic activity using human red blood cells (Nolen, Langer, and Herson 1991)

Sample	Fiber character	Fiber length (μm)				HC_{50}^* ($\mu\text{g}/\text{ml}$)
		<1.0	1.1–5.0	5.1–10.0	>10.0	
1	Fibrous	71.5	26.3	1.7	0.5	400
2	Fibrous	92.7	7.1	—	—	Inactive
3	Nonfibrous	90.2	9.3	0.3	0.3	746
4	Fibrous	78.0	21.3	0.7	0.2	211
5	Fibrous	75.1	22.4	2.0	0.6	369
6	Nonfibrous	91.1	8.7	0.1	0.1	76
7	Nonfibrous	83.4	16.6	—	—	83
8	Nonfibrous	83.1	16.8	—	—	109
9	Fibrous	59.4	37.5	2.6	0.6	51
Chrysotile 1	Fibrous	77.2	20.5	1.8	0.5	41
Chrysotile 2	Fibrous	84.9	13.6	0.6	0.4	82
Chrysotile 3	Fibrous	88.8	10.6	0.4	0.2	59

*The HC_{50} is the concentration of silicate clay (in $\mu\text{g}/\text{ml}$) required to lyse 50% of the erythrocytes in a 1.8×10^8 cells/ml suspension.

TABLE 10
Hemolysis and methylene blue adsorption results (M'anyai et al. 1969)

Mineral	Sample description	50% hemolysis in 1 ml of a 2% erythrocyte suspension as function of:		Amount of methylene blue adsorbed by 1 m ² clay surface (mg)
		Amount of clay (mg)	Surface area of clay (m ²)	
Bentonite	White	1.66	0.039	3.59
Bentonite	Yellow	1.0	0.135	2.13
Montmorillonite	Ca-substituted	5.0	0.50	1.46
Montmorillonite	+Quartz	0.8	0.02	—
Kaolin		2.0	0.06	1.09
Kaolin	Fat	1.5	0.07	1.60
Kaolin	White	4.0	0.06	0.12
Kaolin	Pink	5.0	0.115	0.19

Beck and Bignon (1985) dosed peritoneal macrophages with two samples of Bentonite and the triphenyltetrazolium chloride (TTC) reduction, LDH activity, and methylene blue adsorption were used to assess cytotoxicity. One sample of Bentonite contained 3% SiO₂ and the other 34%. Bentonite inhibited TTC reduction similar to the fibrogenic dusts such as quartz. However, the extracellular LDH activity was not increased and methylene blue adsorption was very high.

Hatch et al. (1985) examined the cytotoxicity of Bentonite to rabbit alveolar macrophages. The alveolar macrophages were incubated with 1.0 mg/ml of Kaolin for 20 h at 37°C. Control cultures received 1.0 mg/ml of TiO₂. The viability percentage of the macrophages and the ATP content of the cells as index of cytotoxicity were determined. Bentonite caused a large reduction in both the viability and ATP levels. The viability index and ATP levels were presented as percentage reductions and were 64.7% and 92.0%, respectively. Controls figures were 18.3% and 0.7%, respectively.

TTC reduction, LDH activity, and methylene blue adsorption were measured as an index of cytotoxicity in a study by Adamis et al. (1986). Bentonite was added to peritoneal macrophages obtained from rats. No specific dose of Bentonite or other details were stated. TTC reduction was much greater and proved Bentonite to be cytotoxic. Extracellular LDH was almost half for Bentonite compared to control values. Methylene blue adsorption was significantly higher for Bentonite.

Murphy, Roberts, and Horrocks (1993a) investigated the cytotoxicity of Bentonite to human umbilical vein endothelial (HUVE) cells, undifferentiated N1E-115 neuroblastoma cells, and ROC-1 oligodendroglial cells. Indices of cytotoxicity used in this study were morphological examination, LDH activity, and fatty acid release. A suspension of Bentonite (1 to 2 μ m in fiber length) was added to the cultures at concentrations of 0.1, 0.03, and 0.01 mg/ml and incubated for 1, 6, and 24 h.

Following incubations, the cells were examined morphologically. The medium and cells were extracted for free fatty acid quantitation. LDH activities were assayed after 24 h of incubation at a Bentonite concentration of 0.10 mg/ml.

Bentonite did not lyse ROC-1 oligodendroglial and the neuroblastoma cells and did not cause a dose-dependent increase in fatty acids at 24 h. No significant increases in LDH activity were detected utilizing any of these cell lines. However, Bentonite caused a dose-dependent increase in fatty acid concentrations only after 24 h of incubation. A 4.5-fold increase in fatty acid concentrations over control values was calculated. Increases over control activities of LDH were 141% with Bentonite. Within 1 h, Bentonite associated with the plasma membrane of HUVE cells and the morphology was drastically changed after treatment (no details given). Cell lysis was also apparent with treatment. After trypan blue staining, 94% of HUVE cells were nonviable with Bentonite treatment (Murphy, Roberts, and Horrocks 1993a).

In a separate study by Murphy et al. (1993b), the cytotoxicity of Bentonite was examined in two cell lines: primary murine spinal cord neurons and differentiated N1E-115 neuroblastoma cells. A clay suspension with a concentration of 0.1 mg/ml was added to the cultures. The neuronal cells were incubated for 1 h with Bentonite. Photomicrographs were taken at 5, 15, and 60 min following treatment. For the N1E-115 cells, incubation lasted 18 h and photomicrographs were taken at 5 and 15 min and 3, 6, and 18 h after the treatment. Morphological changes were observed using a phase contrast microscope. Within 5 min, clay particles were observed on the neuronal cell bodies. Cell bodies appeared granular within 15 min. The cells were completely lysed after 60 min and there was no evidence of any remaining cell bodies or processes. Cell membrane contact was apparent after 5 min in N1E-115 cultures. No morphological changes were apparent at this point. At 18 h, the cells were covered with

clay but cellular processes remained intact. N1E-115 cell lysis did not occur and no cytotoxicity was recorded as a result of Bentonite treatment.

Calcium Silicate

Hunt, Pooley, and Richards (1981) tested three samples of Calcium Silicate (A, B, and C) for biological reactivity in three *in vitro* test systems. Table 11 presents the differences in SiO₂ and Al₂O₃ percentages between the three samples.

In the first test system, 50, 100, 150, and 200 mg of the three samples of Calcium Silicate, UICC chrysotile (positive control), and titanium dioxide (negative control) were added to rabbit erythrocytes. The cultures were incubated for 50 min. The percentage of hemolysis was calculated. Rabbit erythrocytes were also incubated with 10, 30, and 50 mg heated, crushed samples of Calcium Silicate to calculate the percentage of hemoglobin binding. In the second study, rabbit alveolar macrophages were incubated with 5 mg of the Calcium Silicate samples for time intervals up to 60 min. The results were expressed as total viable cells. In the third study, sonicated Calcium Silicate samples (100 to 2000 μ g) were added to rabbit lung fibroblasts. On days 7, 10, 17, and 24 after treatment, the cultures were analyzed for cellular DNA, protein, other cellular material, and hydroxyproline. Cytological studies on the same cells were carried out using dust concentrations of 50 to 400 μ g and staining the cultures to visualize reticulin fibers.

In order to obtain 20% hemolysis, 0.4 mg of chrysotile, 2.8 mg of A, 25.0 mg of B, and 15.0 mg of C are required. Titanium dioxide did not produce 20% hemolysis at any concentration. Sonication of all samples enhanced hemolysis and a "respirable" preparation of A had the same hemolytic activity as chrysotile. Sample B binds more hemoglobin than A or C but not more than chrysotile. Samples B and C had enhanced hemolytic activity when heated above 300°C. Heating had no effect on sample A. All samples produced similar macrophage mortality and at concentrations of 5 mg, only 60% of the cells were surviving at 60 min. Chrysotile at 5 mg resulted in a 20% viability. Samples A and B produced greater DNA and protein concentrations at day 7. However, sample A induced greater protein concentrations at day 24 with normal hydroxyproline levels. Sample B at day 24 had decreased concentrations of protein and hydroxyproline with an increase in mineral concentration. Sample A produced few changes in fibroblast morphology and reticulin deposits.

TABLE 11

Aluminum and Silicon content in Calcium Silicate samples used in biological reactivity study (Hunt, Pooley, and Richards 1981)

Calcium Silicate sample	SiO ₂ %	Al ₂ O ₃ %
A	57.3	2.6
B	52.3	4.4
C	53.7	1.0

TABLE 12
Sample characteristics of five Calcium Silicates tested for hemolytic activity *in vitro* (Skaug and Gyseth 1983)

Sample	Chemical formula	SiO ₂ %	Fibrous character
CaSi A, natural wollastonite	CaSiO ₃	—	+++
CaSi B, natural wollastonite	CaSiO ₃	2	+
CaSi C, synthetic wollastonite	CaSiO ₃	9	—
CaSi D, synthetic tobermorite	Ca ₅ Si ₆ O ₁₇ · 2.5 H ₂ O	10	—
CaSi E, synthetic tobermorite	Ca ₅ Si ₆ O ₁₇ · 2.5 H ₂ O Ca ₆ Si ₆ O ₁₇ (OH) ₂	2	+

Sample B produced sparse and irregular deposition of reticulin (Hunt, Pooley, and Richards 1981).

Skaug, Davies, and Glyseth (1984) tested five Calcium Silicate dust samples for hemolytic activity *in vitro*. Electron microscopy and x-ray diffractions techniques were used to characterize the Calcium Silicates and the results are presented in Table 12. The Calcium Silicate samples A to E, chrysotile B (positive control), and titanium dioxide were added to RBCs at concentrations of 0, 5, and 10 mg/ml. The effect of sonication of the dust samples and the addition of 30 mM CaCl₂, EDTA, and EGTA were also investigated. Sample E produced the greatest hemolysis at nearly 40%. The hemolytic activity of the synthetic Calcium Silicate samples were greater. In all experiments, greater dust concentrations increased hemolysis. Sonication increased the hemolytic activity of the synthetic samples but had no effect on the natural samples. The 30 mM CaCl₂ increased the hemolysis of samples D and E, but not C. EDTA did not decrease hemolysis for samples D and C, and EGTA did not inhibit hemolysis of samples B, C, D, and E.

Five samples of Calcium Silicate also were used to test cytotoxic effects on mouse peritoneal macrophages *in vitro*. Calcium Silicate concentrations of 0, 20, 40, and 60 μ g/cm³ were added to mouse peritoneal macrophages for 18 h. The medium and cell lysates were assayed for LDH and β -glucuronidase (β -GLUC). The positive-control dust utilized was DQ12 quartz standard and the negative-control dust was magnetite. Characterization of the five samples were carried out by means of x-ray diffraction and scanning electron microscopy. The results of the mineral characterization are presented in Table 13. The samples A, B, C, and D had little effect on LDH release but sample E, the fibrous tobermorite, was clearly cytotoxic. Samples A and B caused release of large levels of β -GLUC. Sample E also caused the release of significant amounts of β -GLUC due to its cytotoxicity. Samples C and D caused the release of amounts comparable to the negative controls (Skaug, Davies, and Glyseth 1984).

TABLE 13
Mineral characterization of five samples of Calcium Silicate used to test cytotoxic effects on mouse peritoneal macrophages in vitro (Skaug, Davies, and Glyseth 1984)

Sample	Description	Chemical formula	% SiO ₂ added	Presence of fibers
A	US wollastonite	CaSiO ₃	—	+
B	Natural wollastonite	CaSiO ₃	2	+
C	Synthetic wollastonite	CaSiO ₃	9	—
D	Synthetic tobermorite	Ca ₅ Si ₆ O ₁₇ · 2.5 H ₂ O	10	—
E	Synthetic tobermorite and xonotlite	Ca ₅ Si ₆ O ₁₇ · 2.5 H ₂ O Ca ₆ Si ₆ O ₁₇ (OH) ₂	2	+

Hectorite

In a study by Gormley and Addison (1983) mentioned earlier, the cytotoxic effects of Hectorite were investigated. The Hectorite sample had a particle size of 2.8 μ m. The procedures are detailed in the study under the Attapulgitte heading. Cellular viability was expressed as a percentage of the titanium dioxide control (100.0%) $\pm$ the standard deviation. The 20- μ g/ml dose of Hectorite produced an 83.4% $\pm$ 10.9% viability and the 80 μ g/ml dose produced a 56.4% $\pm$ 13.3% viability. Cellular LDH activities decreased with decreasing cell viability while the activity of LDH in the medium increased. Similar results were seen with glucosaminidase. Also, the amount of lactate produced decreased as cell viability decreased. However, little change in the total cellular protein was recorded.

Banin and Meiri (1990) reported that they added Hectorite to murine neuroblastoma cells at a concentration range of 70 to 1000 μ g/ml, although details were not provided. They concluded that clear morphological signs of cell deterioration were evident and, at the concentrations listed, an acute toxic effect was seen.

Kaolin

Results from a study by M'anyai et al. (1969) on the hemolysis and methylene blue adsorption by Kaolin are presented in Table 10.

Kaolin was heated to temperatures of 290°C, 350°C, 500°C, 650°C, 800°C, and 950°C and changes in the internal structure and surface properties were investigated and compared to alterations in hemolytic activity in vitro. The measurement of methylene blue adsorption and investigation of the crystal structure by x-ray diffraction were made. In addition, Kaolin was added to human erythrocytes and the amount of lysed hemoglobin release was determined following an 1-h incubation. Complete dehydration of Kaolin resulted in the formation of metakaolinite between the temperatures 500°C to 650°C. The formation of metakaolinite resulted in complete loss of hemolytic activity. Heating to higher temperatures, 800°C and 950°C, resulted in the formation of γ -Al₂O₃ (mullite) or SiO₂ (cristobalite), which led to greater intensification of hemolytic activity. The extent of hemolysis depended on the crystal structure and hydration of the surface (M'anyai et al. 1970).

Oscarson et al. (1981) added Kaolin to a culture of bovine RBCs to study the extent of hemolysis. Saline was added to cultures as a control and in a separate experiment, the polymer poly-2-vinylpyridine-*N*-oxide was also added to study its inhibiting effects. No other details were given. The concentration of Kaolin that caused 50% hemolysis in 1 ml of a 3% solution of RBCs was determined as 0.6 mg Kaolin/ml of silicate-erythrocyte-buffer suspension. A concentration of 0.2 and 1.0 μ M/ml of polymer caused 50% and 20% hemolysis, respectively. This was somewhat less hemolysis than without the polymer.

Mossman and Craighead (1982) adsorbed 3-Methylcholanthrene (3MC) onto heat-sterilized preparations of Kaolin (4, 8, and 16 mg dust/ml medium). The tracheas of female golden Syrian hamsters were excised, and prepared for organ cultures and exposed to 3MC/Kaolin preparations. After 4 weeks in vitro, the organ cultures were examined morphologically or implanted subcutaneously into syngeneic weanling female hamsters. The hamsters were palpated for tumors at 3-week intervals and any masses >5 mm in diameter were excised. Animals with no tumors were killed at 105 to 110 weeks of age and the tracheal implants were removed. The tracheal organ cultures and tumors were fixed for microscopic examination. Explants exposed to Kaolin had differentiated mucociliary epithelium for periods of several weeks. In vitro the columnar mucosal cells acquired a cuboidal configuration and the foci of the epithelial hyperplasia appeared at sites where microscopically evident accumulations of particles were deposited on the tracheal epithelium. No keratinizing squamous metaplasia was evident. Neoplasms developed in the tracheal implants exposed to 3MC-coated Kaolin. Tumor development was dosage dependent. No sarcomas developed only carcinomas. In the highest Kaolin/3MC-treated group, 28% of the animals developed tumors. Tumors failed to develop in tissues treated with Kaolin alone.

The comparative effects of Kaolinite (Kaolinite is the raw mineral that comprises Kaolin) on cellular and artificial membranes were examined using three test systems: tracheal epithelial cells, sheep erythrocytes (RBCs), and preparations of phospholipid-cholesterol vesicles in a study by Woodworth, Mossman, and Craighead (1982). Kaolinite doses of 0.003, 0.01, 0.03, and 0.1 mg/ml were added to tracheal epithelial cells for 24 h. Control cultures received no particulate. The ⁵¹Cr release

was determined by liquid scintillation. Spontaneous release was determined from the control cultures. The second experiment, a hemolytic assay, combined RBC and Kaolinite doses of 0.1, 0.5, 1.0, 5.0, and 20.0 mg/ml were added at 37°C for 1 h. The optical density was determined at 540 nm. One milliliter of the preparation of liposomes (11.5 μg lipids) was added to 1 ml of a Kaolinite suspension. After 1 h, the optical density of the mixture was measured at 380 nm. The percentage of CrO_4^{2-} release was calculated. Control cultures received no particulate.

Kaolinite induced release of ^{51}Cr by tracheal epithelium was almost 50% at the highest dose. The cells phagocytized the particles, as demonstrated by SEM and phase-contrast microscopy. This process was most evident after 24 h. Cells containing intracellular particles demonstrated retraction of lamellopodial extensions, surface blebbing, and a change in morphology from flattened to round.

A dose-dependent relationship between mineral concentration and hemolysis was demonstrated. Hemolysis was rapid. Approximately 50% of the RBCs were hemolyzed within 10 min. SEM revealed remnants of RBCs in cultures with complete hemolysis.

CrO_4^{2-} release at 10 mg/ml of Kaolinite was $\sim 35\%$ after 1 h. A dose-dependent relationship between particle concentration and CrO_4^{2-} release was again demonstrated (Woodworth, Mossman, and Craighead 1982).

In a study by Gormley and Addison (1983) described earlier, the cytotoxic effects of two Kaolins (K-1 and K-2) were investigated. K-1 had a particle size of 3.2 μm , and K-2 had a particle size of 3.9 μm . The procedures are detailed in the study Gormley and Addison (1983) under the Attapulgitte heading.

Cellular viability was expressed as a percentage of the titanium dioxide control (100.0%) $\pm$ the standard deviation. The 20- $\mu\text{g}/\text{ml}$ dose of Kaolin (K-1) resulted in a 101.4% $\pm$ 6.7% viability and the 80- $\mu\text{g}/\text{ml}$ dose produced a 69.5% $\pm$ 6.5% viability. With a 20- $\mu\text{g}/\text{ml}$ dose of Kaolin (K-2), viability was 93.6% $\pm$ 4.5%, with the 80 $\mu\text{g}/\text{ml}$ dose, it was 60.0% $\pm$ 4.1%. It may be noted that K-1 has a finer particle size but a smaller surface area as compared to K-2. Cellular LDH activities decreased with decreasing cell viability, whereas the percentage of LDH in the medium increased. Similar results were seen with glucosaminidase. Also the amount of lactate produced decreased as cell viability decreased. However, little change in the total cellular protein was recorded (Gormely and Addison 1983).

The cytotoxicity of Kaolinite toward mouse peritoneal macrophages was examined in a study by Davies et al. (1984). This three-part study investigated whether or not respirable china clay (Kaolinite) was cytotoxic toward macrophages *in vitro*, the components responsible for the toxicity, and the factors responsible for the components toxicity. The assessment of toxicity was indicated by the activity of LDH assayed from the medium and cell lysates.

China clay dusts (60 $\mu\text{g}/\text{culture}$) from 12 separate drying plants were added to mouse peritoneal macrophage cultures and incubated for 18 h. The medium and cell lysates were collected

and assayed for LDH activity. All 12 cultures had changes that indicated dust cytotoxicity. Between 19.5% and 60.0% LDH was released from the cultures. Four other dust samples, three of quartz (5,10,15, 20 $\mu\text{g}/\text{culture}$) and one of magnetite, were also assayed. The cytotoxicity of quartz indicated a dose-dependent relationship and was quite toxic. The magnetite dust had little effect on LDH release.

Mineral composition of the dusts was determined using x-ray diffraction analysis. A summary of the dust samples' composition was as follows: Kaolinite (84% to 96%), mica (3% to 6%), quartz (1%), and feldspar (0% to 7%). Due to the possibility of other dust cytotoxicity, the biological effects of the ancillary minerals and Kaolin was studied. Two high-purity Kaolins were tested in the same method as above and were clearly cytotoxic toward the macrophages. By x-ray diffraction, these two Kaolins were both 98% pure Kaolin. The feldspar sample had lower activity than titanium dioxide, a material considered nonfibrogenic and is used as a control dust in cell studies. The mica dust samples were cytotoxic but much lower than that of the Kaolin. By mineral analysis, it was found that mica dusts had 34% Kaolinite. Quartz was ruled out as the cytotoxic agent due to the very low concentrations (1%) in the initial experiment.

In a separate experiment, Kaolin pretreated with poly-2-vinyl pyridine-*N*-oxide (PVPNO) (0.45 $\mu\text{g}/\text{mg}$), was added to mouse peritoneal macrophages. (Note: PVPNO has been demonstrated to reduce the cytotoxicity of Kaolin [Davies and Preece 1983]). Electron micrographs were taken of the macrophages with and without the pretreated Kaolin for analysis of the factors causing the toxicity. The ultrastructural alterations and number of particles within the cells appeared to be similar in both the treated and nontreated cultures. It was concluded that PVPNO has no effect on the inhibition of the uptake of Kaolin. Dust particles were found adjacent to cell surfaces and in membrane-bound intracytoplasmic vesicles. However, no particles penetrated or were seen penetrating the nucleus and no lysed cells were seen.

In the last set of experiments, the physical structure of Kaolin and how it relates to dust toxicity was studied. Four components of Kaolin's structure were examined: gibbsite or mica-like surfaces, positively charged edges, negative charged particles, and an amorphous 'gel' coating on kaolinite. Transmission electron micrographs of gibbsite or mica-like surfaces indicated low toxicity and were ruled out as a possible marked toxic factor. A colloidal gold decoration technique was to study the positively charged edges of Kaolinite. Gold binds to the positively charged particles of Kaolinite and treatment of polyacrylic acid abolishes the gold decoration. In this study, mouse peritoneal macrophages were incubated with polyacrylic treated Kaolin (120 $\mu\text{g}/\text{culture}$). Only a small drop in the cytotoxicity of Kaolin was observed. The electrophoretic mobility of negatively charged Kaolin particles was also studied. Increased amounts of ammonium chloride produced a significant decrease in electrophoretic mobility. It is important to note that the greater concentrations did not produce negatively charged Kaolin particles. These same aluminum-treated Kaolins were added to mouse

peritoneal macrophages (120 $\mu\text{g}/\text{culture}$) and the cytotoxicity changed very little based on the amount of LDH activity released. The last experiment examined the effect of the amorphous 'gel' coating of Kaolin and its cytotoxicity. Plasma-ashing and the same LDH assay were performed on the samples. The first group, Kaolin (40 mg/cm^3), was plasma-ashed after 24 h and no effect was observed. Plasma-ashing after 72 h did reduce cytotoxicity. The second group of Kaolin dusts were mixed with formalin-fixed lung tissue and then immediately plasma-ashed. The cytotoxicity was not reduced. The last groups included Kaolin recovered from air-dried lungs of Fischer rats exposed to china clay dust (10 mg/m^3) for 40 h/week for 1 year, left for 1 year, then ashed to a constant weight. Inhalation of these dusts was significantly less toxic. Reductions in cytotoxicity was probably due to alterations in the surface coating of Kaolin (Davies et al. 1984).

Beck and Bignon (1985) dosed peritoneal macrophages with a sample of Kaolin and the TTC reduction, LDH activity, and methylene blue adsorption were used to assess cytotoxicity. The sample contained 30% SiO_2 . The results from this study classified Kaolin as an inert dust and nontoxic. Methylene blue adsorption was slight.

Gormley, Kowolik, and Cullen (1985) used luminol-dependent chemiluminescence (CL) to assess the *in vitro* production of reactive oxygen species by human neutrophils and monocytes after exposure to Kaolinite. Either opsonized or nonopsonized Kaolinite dust was added to either neutrophil or monocyte suspensions and luminol. The suspensions were assayed for CL and measured in millivolt. Concentrations of dust ranged from the maximum of 3 mg/ml downwards. A control suspension of zymosan (2 mg/ml) was also assayed for CL production. Neutrophils challenged with opsonized dust had relatively low dose-dependent CL production compared to controls. However, when neutrophils challenged with nonopsonized dust, CL production peaked at 67%. Again dose-dependent responses were obtained when monocytes were tested. However, monocytes had a greater CL response in the presence of opsonized dust. These results were the reverse of the earlier neutrophil responses as a very low monocyte CL production was obtained with nonopsonized dust.

In a study by Wallace et al. (1985), the cytotoxicity of native and surface-modified Kaolin and the effect of pulmonary surfactant were studied. Cell membrane damage and cytotoxicity were measured by the release of alveolar macrophage cytoplasmic enzyme LDH, the lysosomal enzymes β -n-acetylglucosaminidase (β -NAG) and β -GLUC, and sheep blood cell hemolysis. Dipalmitoyl lecithin (DPL) emulsions made from synthetic L- α -lecithin β,γ -dipalmitoyl were added to Kaolin to produce a concentration of 7.5 mg dust/ ml . Controls of saline and Kaolin without DPL were also utilized. For the hemolysis assays, the mixtures were resuspended in phosphate-buffered saline (PBS) at a concentration of 2.0 mg dust/ ml PBS.

Fresh sheep blood erythrocytes were mixed with dust suspensions in concentrations of 0.1 to 1.0 mg/ml . Untreated Kaolin

and DPL-treated Kaolin erythrocytes were incubated for 1 h at 37°C. Negative controls were made with erythrocytes in PBS and positive controls were made by lysing erythrocytes. All samples were read at 540 nm using a spectrophotometer and the percentage of lysis was calculated. The lecithin treated Kaolin suppressed erythrocyte activity to near "background levels." The hemolysis value for the maximum nontreated Kaolin concentration (1 mg/ml) was 42%, whereas the hemolysis value for the lecithin-treated Kaolin at the same concentration was 2%. Adsorption isotherm data estimated that 0.1 mg Lecithin/ mg Kaolin would provide full surface coverage and suppress the hemolytic capacity to 97% lower than the native Kaolin.

In the second experiment of the same study, alveolar macrophage enzyme release studies were carried out using macrophages from Sprague-Dawley rats. Untreated Kaolin and DPL-Kaolin samples at a concentration of 1 mg/ml were mixed with macrophages and incubated for 2 h at 37°C. The results were similar as in the above experiment. The nontreated Kaolin caused release of enzymes: 570% LDH, 600% β -GLUC, and 570% β -NAG of the control values. The treated Kaolin did not cause the release of these enzymes. These results imply that Kaolin damages erythrocytes and macrophages through cell membrane-dust surface interactions and that pulmonary surfactants can absorb the mineral surfaces for a short time (Wallace et al. 1985).

Mossman and Be'gin (1989) conducted a study in which Kaolin samples were coated with the enzymes L-alpha-dipalmitoyl glycerophosphorylcholine (DGPL) and phospholipase A₂ (PLA₂) and the hemolytic potential of both coated and noncoated samples were studied *in vitro*. The samples were incubated with sheep erythrocytes and the optical density of the supernatant at 540 nm was determined to measure hemoglobin release. With increasing amounts of DGPL, neutralization of the hemolytic potential occurred at 75 to 85 mg DGPL/ g of Kaolin. The residual adsorbed value was 83.0 mg DGPL/ g Kaolin. The digestive removal of DGPL by Kaolin was measured at the applied specific activity of 0.96 units PLA₂ per molecule DGPL on Kaolin. Most of the produced lysolecithin remains adsorbed at 2 h.

Banin and Meiri (1990) added Kaolinite to murine neuroblastoma cells at concentrations of 100 to 1000 $\mu\text{g}/\text{ml}$. Within minutes, the Kaolinite increased the increasing permeability of the membranes, depolarized resting potential, and the maintaining action potentials in response to stimulation were lost. Within 30 min, the cells had alterations of morphological deterioration. Microvilli retracted, the surface assumed an unruffled, smooth appearance, and large holes developed in the plasma membrane.

Murphy, Roberts, and Horrocks (1993a) investigated the cytotoxicity of Kaolinite using three cell lines: HUVE cells, undifferentiated N1E-115 neuroblastoma cells, and ROC-1 oligodendroglial cells. Indices of cytotoxicity used in this study were morphological examination, LDH activity, and fatty acid release. Exact experimental details are provided in the Bentonite section under the same heading.

Kaolinite did not lyse ROC-1 oligodendroglia and the neuroblastoma cells and did not cause a dose-dependent increase in fatty acids at 24 h. No significant increases in LDH activity were detected utilizing either of these cell lines. However, Kaolinite increased fatty acid concentrations after 24 h of incubation in a dose-dependent fashion. A 1.7-fold increase in fatty acid concentrations over control values was calculated. Increases over control activities of LDH were 146% with Kaolinite. Within 1 h, Kaolinite associated with the plasma membrane of HUVE cells and the morphology was drastically changed after treatment (no details given). Cell lysis was also apparent. After trypan blue staining, 90% of HUVE cells were nonviable with Kaolinite treatment (Murphy, Roberts, and Horrocks 1993a).

Kaolinite dust was tested for potential human leukocyte elastase (HLE)-inhibiting effects (Oberson et al. 1996). HLE inhibition was evaluated by incubating 15 nM HLE for 1 h in the presence of 5 μ g of Kaolinite. Suc(Ala)₃pNA was then added for 30 min. Activity was measured at 410 nM. The 5 μ g Kaolinite abolished (90% inhibition) the activity of 0.45 μ g HLE.

Montmorillonite

Results from a study by M'anyai et al. (1969) on the hemolysis and methylene blue adsorption by Montmorillonite are presented in Table 10.

Oscarson, Van Scoyoc, and Ahlrichs (1981) added Montmorillonite to a culture of bovine RBCs to study the extent of hemolysis. Saline was added to cultures as a control and in a separate experiment, the polymer, poly-2-vinylpyridine-*N*-oxide, was also added to study its inhibiting effects. No other details were given. The concentration of Montmorillonite that caused 50% hemolysis in 1 ml of a 3% solution of RBCs was determined as 0.006 mg Montmorillonite/ml of silicate-erythrocyte-buffer suspension. A concentration of 0.2 and 1.0 μ M/ml of polymer reduced hemolysis to 23% and 0%, respectively.

The comparative effects of Montmorillonite on cellular and artificial membranes were examined using three test systems—tracheal epithelial cells, sheep erythrocytes (RBCs), and preparations of phospholipid-cholesterol vesicles—in a study by Woodworth, Mossman, and Craighead (1982). Montmorillonite doses of 0.003, 0.01, 0.03, and 0.1 mg/ml were added to tracheal epithelial cells for 24 h. Control cultures received no particulate. The ⁵¹Cr release was determined by liquid scintillation. Spontaneous release was determined from the control cultures. A second experiment, a hemolytic assay, combined RBC and Montmorillonite doses of 0.1, 0.5, 1.0, 5.0, and 20.0 mg/ml at 37°C for 1 h. The optical density was determined at 540 nm. Control cultures received no particulate. One milliliter of the preparation of liposomes (11.5 μ g lipids) was added to 1 ml of a Montmorillonite suspension. After 1 h, the optical density of the mixture was measured at 380 nm. The percentage of CrO₄²⁻ release was calculated. Control cultures received no particulate.

Montmorillonite induced release of ⁵¹Cr by tracheal epithelium was almost 60% at the highest dose. The cells phagocytized the particles, as demonstrated by SEM and phase-contrast

microscopy. This process was most evident at after 24 h. Cells containing intracellular particles demonstrated retraction of lamellopoidal extensions, surface blebbing, and a changed morphology from flattened to round.

A dose-dependent relationship between mineral concentration and hemolysis was demonstrated. Hemolysis was rapid. Approximately 50% of the RBCs were hemolyzed within 10 min. SEM revealed remnants of RBCs in cultures exhibiting complete hemolysis.

CrO₄²⁻ release at 10 mg/ml of Montmorillonite was ~40% after 1 h. A dose-dependent relationship between particle concentration and CrO₄²⁻ release was again demonstrated (Woodworth, Mossman, and Craighead 1982).

In the Gormley and Addison study (1983) described earlier, the cytotoxic effects of three samples of Montmorillonite (CaM-1, CaM-2, and NaM) were investigated. CaM-1 and -2 have calcium substitutions in their lattices whereas NaM has sodium substitutions. Particle sizes ranged from 2.0 to 3.1 μ m. The procedures are detailed under the Attapulgit heading. Cellular viability was expressed as a percentage of the titanium dioxide control (100.0%) $\pm$ the standard deviation. The 20- μ g/ml dose of CaM-1 with particle size of 3.1 μ m produced a 79.1% $\pm$ 19.2% viability and the 80- μ g/ml dose produced a 51.9% $\pm$ 15.6% viability; CaM-2 with a particle size of 2.5 μ m produced viabilities of 21.2% $\pm$ 3.5% (20 μ g/ml) and 13.1% $\pm$ 2.2% (80 μ g/ml); and NaM with a particle size of 2.0 μ m produced viabilities of 47.3% $\pm$ 7.4% (20 μ g/ml) and 37.2% $\pm$ 4.6% (80 μ g/ml). The sample CaM-1 had the largest surface area, whereas sample NaM, had the smallest. Sample CaM-2 had the lowest viability percentage despite the median particle size and surface area. Investigators attributed the marked toxicity of sample CaM-2 due to the presence of ~1% of quartz and 10% cristobalite in the sample. Sample NaM, which also exhibited a greater toxicity, contained ~5% quartz and ~2% calcite. Cellular LDH levels fell with decreasing cell viability whereas the percentage of LDH in the medium increased. Similar results were seen with glucosaminidase. Also, the amount of lactate produced decreased as cell viability decreased. However, little change in the total cellular protein was recorded.

Gormley, Kowolik, and Cullen (1985) used luminol-dependent CL to assess the in vitro production of reactive oxygen species by human neutrophils and monocytes on exposure to Montmorillonite. Either opsonized or nonopsonized Montmorillonite (containing a calcium as its exchange ion) dust was added to either neutrophil or monocyte suspensions and luminol. The suspensions were assayed for CL and measured in millivolt. Concentrations of dust ranged from the maximum of 3 mg/ml downwards. A control suspension of zymosan (2 mg/ml) was also assayed for CL production. Neutrophils challenged with opsonized dust resulted in relatively low dose-dependent CL production compared to controls. However, when neutrophils were challenged with nonopsonized dust, a marked response of CL peak production at 114% was elicited. Again dose-dependent responses were obtained when monocytes were tested. However,

monocytes elicited a slightly higher response in the presence of opsonized dust. These results proved to be the reversal of the earlier neutrophil responses. A very low monocyte CL production was obtained with nonopsonized dust.

Banin and Meiri (1990) reported a study in which Montmorillonite was added to murine neuroblastoma cells at a concentration range of 100 to 1000 $\mu\text{g/ml}$, but no details were given. The authors concluded that clear morphological signs of cell deterioration were evident and, at the concentrations listed, an acute toxic effect was seen.

Murphy, Roberts, and Horrocks (1993a) investigated the cytotoxicity of Montmorillonite to three cell lines: HUVE cells, undifferentiated N1E-115 neuroblastoma cells, and ROC-1 oligodendroglial cells. Indices of cytotoxicity used in this study were morphological examination, LDH activity, and fatty acid release. Exact experimental details are provided in the Bentonite section under the same heading.

Montmorillonite did not lyse ROC-1 oligodendroglia and the neuroblastoma cells and did not cause a dose-dependent increase in fatty acids at 24 h. No significant increases in LDH activity were detected utilizing either of these cell lines. However, Montmorillonite caused a dose-dependent increase in fatty acid levels only after 24 h of incubation. A 10-fold increase in FA levels over control values was calculated. Increases over control activities of LDH were 154%. Within 1 h, Montmorillonite associated with the plasma membrane of HUVE cells and the morphology was drastically changed after treatment (no details given). Cell lysis was also apparent with treatment. After trypan blue staining, 99% of HUVE cells were nonviable with Montmorillonite treatment (Murphy, Roberts, and Horrocks 1993a).

In a study by Murphy et al. (1993b), the cytotoxicity of Montmorillonite was examined in two cell lines: primary murine spinal cord neurons and differentiated N1E-115 neuroblastoma cells. A clay suspension with a concentration of 0.1 mg/ml was added to the cultures. The neuronal cells were incubated for 1 h with Montmorillonite. Photomicrographs were taken at 5, 15, and 60 min following treatment. For the N1E-115 cells, incubation lasted 18 h and photomicrographs were taken at 5 and 15 min and 3, 6, and 18 h after the treatment. Morphological changes were observed using a phase-contrast microscope. Within 5 min, clay particles were observed on the neuronal cell bodies. Cell bodies appeared granular within 15 min. The cells were completely lysed after 60 min and there was no evidence of any remaining cell bodies or processes. Cell membrane contact was apparent after 5 min in N1E-115 cultures. No morphological changes were apparent at this point. At 18 h, the cells were covered with clay but cellular processes remained intact. N1E-115 cell lysis did not occur and no cytotoxicity was recorded.

Montmorillonite dust was tested for potential HLE-inhibiting effects (Oberson et al. 1996). HLE inhibition was evaluated by incubating 15 nM HLE for 1 h in the presence of 5 μg of Montmorillonite. Suc(Ala)₃pNA was then added for 30 min. Activity was measured at 410 nM. The 5 μg Montmorillonite (98% inhibition) abolished the activity of 0.45 μg HLE.

Pyrophyllite

The cytotoxicity of Pyrophyllite dust on rat alveolar macrophages was investigated in a study by Zhang, Zhang, and Song (1997). Cytotoxicity was measured by the potassium content of the macrophages and the levels of LDH. Alveolar macrophages were isolated from bronchi alveolar lavages of male Wistar rats. These animals were divided into six groups based on the dust concentrations. The groups were as follows: quartz (75.72 $\mu\text{g/ml}$) dust group; Pyrophyllite mine (PM) dust group A, 200 $\mu\text{g/ml}$ (75.72 $\mu\text{g/ml}$ SiO₂ and 30.42 $\mu\text{g/ml}$ Al₂O₃); PM dust group B, 200 $\mu\text{g/ml}$ (75.72 $\mu\text{g/ml}$ SiO₂ and 30.42 $\mu\text{g/ml}$ Al₂O₃); Pyrophyllite carving mills (PCM) dust group A, 200 $\mu\text{g/ml}$ (31.68 $\mu\text{g/ml}$ SiO₂ and 40.58 $\mu\text{g/ml}$ Al₂O₃); PCM dust group B, 200 $\mu\text{g/ml}$ (31.68 $\mu\text{g/ml}$ SiO₂ and 40.58 $\mu\text{g/ml}$ Al₂O₃); normal control of saline. Both PM group B and PCM group B were imitated groups of the natural dusts from the mines used to study the toxicity of SiO₂ and Al₂O₃. They did not include the metals Fe, Cu, Ni, and Zn as did both samples A. The cell cultures were incubated at 37°C for 16 and 22 h.

The LDH activity of quartz was greater than all other groups except PM group A incubated at 22 h. When compared to the saline controls, all exposed groups had significantly lower increases in LDH activity. Both the LDH activities of the PM dust groups were greater than those of the PCM dust groups ($p < .5$). However, no differences between the PM groups A and B or between the PCM groups A and B were detected. The K⁺ content of the saline controls was greater than all exposed groups. The quartz group had the lowest concentrations of K⁺ followed by the PM dust groups and then the PCM dust groups. Again, no differences between either A or B groups was observed. It was concluded that Pyrophyllite dust exposure is cytotoxic to alveolar macrophages and people working in a PM have greater risk of respiratory problems than people working on PCMs.

Mineralogical analysis of the dust samples taken from the mines was performed using an atomic absorption spectrophotometer. The SiO₂ content was 37.9% higher in the PM group than in the PCM group 15.8%. Al₂O₃ concentrations were lower in the PM dust groups (15.2%) than in the PCM dust groups (20.3%). Toxicity due to metals in the samples A was ruled out. The samples B did not include the metals and had similar LDH activity as the samples A (Zhang, Zhang, and Song 1997).

Zeolite (Zeolite A)

Zeolite A at concentrations of 0.1 to 100 $\mu\text{g/ml}$ was incubated for 48 h with normal human osteoblast-like cells. An induction of a dose-dependent increase in DNA synthesis and the proportion of cells in mitosis occurred. This mitogenic action was dependent on cell seeding density. Alkaline phosphatase activity and osteocalcin release were also increased but no significant effect on collagen production per cell occurred. Zeolite treatment increased the steady-state mRNA levels of transforming growth factor β (Keeting et al. 1992).

Zeolite (Clinoptilolite)

Total degradation of rat peritoneal macrophages incubated with Clinoptilolite dust particles occurred during 15- and 30-min time periods at concentrations of 1.0 and 0.5 mg/ml, respectively. Dust particles measured $<5\ \mu\text{m}$. Thirty-eight percent of macrophages and 57.5% of RBCs were killed within 30 min at a Zeolite concentration of 0.25 mg/ml. Dose-dependent CL was observed in the first 10 to 20 s when luminol was added to the cultures. Catalase (30% to 50%) decreased the cytotoxic effects of Zeolite, whereas ethanol, sodium azide, and mannitol had no effect (Korkina et al. 1984).

Zeolite (Mordenite)

Syrian hamster and rat alveolar macrophages were exposed to nontoxic concentrations of Mordenite and the reduction of cytochrome *c* in the presence and absence of superoxide dismutase, and the amount of O_2 released were indicators of cytotoxicity. Other fibrous particles were used as positive controls. Mordenite as compared to the positive controls was less active at comparable concentrations (Hansen and Mossman 1987).

Zeolite (Nonfibrous Japanese Zeolite)

Japanese Nonfibrous Zeolite was incubated with two cell lines, Chinese hamster V79-4 and A579 at concentrations ranging from 5 to 100 $\mu\text{g}/\text{ml}$. Two samples of erionite and a sample of UICC crocidolite, a positive control, were also tested. Concentrations that inhibited plating were estimated using the LD_{50} . Compared to the positive control and the erionite samples, the Zeolite had a much greater LD_{50} value and was nontoxic in the A549 assay (Brown et al. 1980).

ANIMAL TOXICOLOGY**Acute Oral***Calcium Silicate*

Calcium Silicate FDA compound 71-41 was suspended in 0.85% saline and administered to 10 male rats by intubation. Each animal that received a dose of 5000 mg/kg died within 24 h. Doses of 100, 500, 1000, 2000, 3000, and 4000 mg/kg were selected to determine the acute LD_{50} using the Litchfield-Wilcoxon method. Groups of 5 male rats were administered the doses and were killed for necropsy. The LD_{50} was determined as 3400 mg/kg; at the highest dose, necropsy findings included bloody gastric mucosa with distension, hydrothorax, and congested lungs. In a second LD_{50} assessment, Calcium Silicate was prepared as 24.1% (w/v) suspension and administered orally to a group of 10 male rats at a single dose of 5000 mg/kg. No signs of toxicity or abnormal behavior were observed within a 7-day period. No deaths occurred. All animals were killed and on necropsy no gross findings were observed. The acute oral LD_{50} was considered to be greater than 5000 mg/kg (Litton Bionetics, Inc. 1974).

Hectorite

Five male and five female Sprague-Dawley rats were administered a single dose of 5 g/kg of the test article by gavage. The animals were observed the day of dosing and 15 days after for gross and visible toxic or pharmacological effect. No such effects were seen and none of the animals died. All animals were killed for necropsy. No findings were reported. The acute oral LD_{50} was $>5.0\ \text{g}/\text{kg}$ of body weight (FDRL Inc. 1980b).

Kaolin

A report by the Federation of American Societies for Experimental Biology (1977) included an acute oral study in which 120 rats were fed doses of Kaolin ranging from 100 to 210 g/kg. Fourteen rats were controls. Kaolin was inert and nonstatic except for the danger of bowel obstruction resulting in perforation. The clinical signs were listlessness, anorexia, oliguria, hypothermia, and dyspnea. These were a pathological reaction from overdistension of the alimentary canal by an inert solid. The number of fatalities and the incidence and advance of bowel obstruction along the small intestine were dose related. The dose that killed 50% of the rats by bowel obstruction was 149 g/kg.

McClurg, Beck, and Powers (1980) fed a group of 10 male Sprague-Dawley rats a control diet plus 0.5 ml Kaolin 20%–pectin 1%. The control diet was then fed for 48 h and 72 h later stool samples were collected. The samples were analyzed for volume, sodium, potassium, and fat content. The results were 103% increase in sodium; 184% increase in potassium; fat excretion remained at baseline.

Magnesium Aluminum Silicate

Suspensions of 1 ml of Magnesium Aluminum Silicate at doses of 100–2000, 5000, 10000, 20000, and 50000 mg/kg were administered to a series of 37 mice. At the greatest dose, the mortality rate was 33%. The LD_{50} was considered to be $>50,000\ \text{mg}/\text{kg}$ (Munch 1944).

Zirconium Silicate

In a study conducted by Stookey et al. (1967), the LD_{50} of Zirconium Silicate was determined. Oral intubations of a 60% aqueous slurry of Zirconium Silicate containing 1% carboxymethylcellulose to prevent settling was given to 80 albino mice. Doses ranged from 70 to 200 gm/kg body weight. A dosage of 200 g of Zirconium Silicate per kilogram body weight was not sufficient to create a 50% mortality rate in mice. Dosages greater than 200 g were not tested due to the limitations of the mouse gastrointestinal tract. A 37.5% mortality rate was recorded for the dosage of 200 g/kg of body weight.

Short-Term Oral*Bentonite*

Carson and Smith (1982) fed Bentonite at concentrations 0%, 2.5%, 7.5%, or 10% to male weanling rats to determine the most effective level to overcome the effects of T-2 toxicosis.

Increasing the concentration of Bentonite resulted in significant increases in body weight and feed consumption. The most effective concentration tested was 10%. Bentonite had no effect on the activity of nonspecific hepatic esterase.

The role of Bentonite in the prevention of T-2 toxicosis in rats was further investigated by Carson and Smith (1983). Groups of 10 male Wistar rats were fed diets containing 5% Bentonite for 2 weeks and the feed consumption and growth were recorded. Each diet was administered with or without 3 μ g T-2 toxin/g of feed for 2 weeks. Bentonite reduced the decreases in final body weight and feed consumption as compared to controls. The livers from this test group were excised and assayed for nonspecific esterase (E.C.3.1.1.1). Five percent Bentonite had no significant effect on the activity of this enzyme. In a second experiment, Bentonite was supplemented in the control diet at 2.5%, 5.0%, 7.5%, and 10%. Bentonite at 2.5% greatly increased feed consumption and final body weights and feeding. Ten percent Bentonite overcame the toxicosis completely. In a third study, rats were fed 0%, 5%, 7.5%, or 10% Bentonite for 2 weeks and then dosed with [^{3} H] T-2 toxin. The urine and feces were collected at 21 h and tissues were excised for determination of residual ^{3} H. Feeding Bentonite had little effect on the fraction of the dose excreted in the urine. Feeding 5%, 7.5%, and 10% Bentonite resulted in significant increases in the fecal excretion of ^{3} H when compared to controls. Bentonite had no effect on residual ^{3} H in the liver or kidneys but all concentrations reduced residual ^{3} H in muscle. Rats fed 5% Bentonite had more ^{3} H in the digesta in the small intestine and in the wall of the intestinal tissue when compared to controls. Intestinal transit time was reduced as well.

Bartko et al. (1983) fed a group of five sheep a diet containing 0.15 g/kg body weight of Zeolite for 3 months. Other sheep received no additions to their normal diet. At the end of the study, no difference in health effects was found between the two groups. The health effects included general behavior, total and acute acidity, content of volatile fatty acids in rumen contents, hematological values, content of microelements, transaminase activity, and acid-base homeostasis in the blood.

Magnesium Aluminum Silicate

Munch (1945) gave groups of 10 mice daily doses of either 5 or 10 g/kg of body weight orally for 10 days. Two days separated the first five doses from the second five doses. No signs were observed in any mouse at any time when administered 5 g/kg. The animals were killed and no pathological changes were seen at necropsy. No tissue was taken for further examination. One mouse died after five doses of 10 g/kg and one mouse died after nine doses of 10 g/kg. Neither mouse had lesions at postmortem examination.

This same author administered VEEGUM orally to 10 rabbits for a total of 10 doses. The first four animals were given 5 g/kg of body weight; the fifth animal was a control. The second four animals were given 10 g/kg of body weight; the fifth was also a control. No changes in body weight, no signs at toxicity, and no

deaths were recorded. All animals were killed and at necropsy no lesions were seen in the stomach, liver, kidneys, or other viscera. No tissue was taken for microscopic examination (Munch 1945).

Zeolite (Clinoptilolite)

In a 148-day feed-lot experiment reported by McCollum and Galyean (1983), 48 cross-bred steers were fed a 70% sorghum diet with Clinoptilolite substituted at 0%, 1.25%, and 2.5% of the diet dry matter. No differences were found among treatments in average daily weight gain, feed intake or feed efficiency.

Pond, Yen, and Crouse (1989) fed 32 castrated male pigs various diets of calcium, iron, and Clinoptilolite to study tissue storage of major and trace elements with the addition of Clinoptilolite. At day 84, all pigs were killed and analyzed. Dietary concentrations of calcium, iron, and Clinoptilolite had no effect on daily weight gain, daily feed intake, or the ratio of weight gain:feed intake of growing pigs.

Zeolite (Clinoptilolite and Sodium Zeolite A)

Weanling Landrace $\times$ Yorkshire pigs were fed diets containing 3% Clinoptilolite with or without 150 ppm cadmium chloride or 3% Sodium Zeolite A with or without 150 ppm cadmium chloride for 31 days. Pigs fed cadmium and Zeolites did not have decreased hematocrit and hemoglobin values similar to those of pigs fed diets without the Zeolites. Hepatic cadmium concentration was significantly reduced in animals fed with Clinoptilolite. Hepatic iron was not affected significantly by either Zeolite; hepatic iron and zinc were decreased by dietary cadmium. Hepatic zinc was increased by Sodium Zeolite A (Pond and Yen 1983b).

Zeolite A

Various diets containing no Zeolite, 0.3% Zeolite A, or 0.5% Clinoptilolite were fed to cross-bred pigs for 6 weeks. The average daily weight gain, average daily feed intake, and feed:weight gain ratio were unaffected by supplementation of either Zeolite. Energy utilization was improved by feeding diets containing either Zeolite (Shurson et al. 1984).

Subchronic Oral

Magnesium Aluminum Silicate

The Food and Drug Research Laboratories (FDRL 1958a) carried out a 90-day feeding study using 220 weanling albino rats divided into five groups. The largest dose group consisted of 10 male and 10 female rats; control animals totaled 25 rats of each sex. A commercial ration was supplemented with 2%, 5%, 10%, and 20% VEEGUM. Control diets were unmodified. Body weight and feed intake were recorded daily and the efficiency of feed utilization (EFU; gram gained per 100 g) was calculated. Hematological examinations were made at 6 and 12 weeks on half of the test group. Blood sugar and nonprotein nitrogen determinations and urine analyses were also completed. Four rats in the 20% group, four rats in the 10% group, and control group

were placed on a modified program to estimate the balance between the intake of dietary ash and the ash excreted. Rats fed the 20% diet were examined at 8 weeks and rats fed the 10% diet at 12 weeks. All animals were killed at the end of the 90-day period. Liver, kidneys, spleen, heart, and adrenal glands weights were determined. Microscopic examination of the liver, kidneys, spleen, and portions of the gastrointestinal tract of four rats of each sex and control, 10%, and 20% groups were carried out.

The average body weights and net gains were not adversely affected by the ingestion of VEEGUM up to 10% in the diet. Growth was diminished slightly but with statistical significance ($p = .05$) when 20% VEEGUM was fed to both sexes. With EFU corrections, only the 20% dose significantly lowered the observed EFU value. One male rat of the 2% group died and one of each sex of the 10% group died. These rats had fibrinous exudates in the thorax, hemorrhagic lungs, and evidence of respiratory infection at necropsy. Gross findings for the rest of the animals revealed no significant abnormalities other than in the lungs. The incidences of pulmonary lesions did not differ among controls and test animals. Organ weights fell within normal limits. Hematological observations were within normal limits, including the rats of the 20% group. Blood sugar and nonprotein nitrogen values were also within normal limits. Females of the 20% group had slightly increased values compared to controls but still were in the normal range. Silicon content of the spleens of control animals were about the same as in the 2% group. However, in the 5% and 10% groups, the silicon content was slightly increased. Microscopic examination disclosed no abnormalities in the liver, kidneys, and gastrointestinal tract. Ash data indicated that 81% of VEEGUM of the 20% group was excreted and 73% of the 10% group was excreted (FDRL 1958a).

FDRL (1958b) fed two groups of four mongrel dogs, two female and two male for each group, a basal diet and a diet supplemented with 10% VEEGUM for 90 days. At 6 and 12 weeks, complete blood counts were made and blood sugar and nonprotein nitrogen were determined. Urine specimens were examined at 12 weeks for acidity, sugar, albumin, and microscopic elements in the sediment. At the end of 90 days, all dogs were killed for necropsy. Silicon content of the spleen was also determined. Body weight did not change despite a depression of appetite with the addition of VEEGUM. No abnormalities were seen upon hematological examination at the 6- or 12-week periods. Two of the test animals had slightly increased blood sugar at the end of the testing period. All other values for sugar and nonprotein nitrogen levels were normal. No difference in organ weight was seen. Silicon concentration of the spleens of the test animals were slightly elevated compared to controls (143 versus 103 mg/spleen). No microscopic lesions were compound induced.

CTFA (1999b) reported that in feeding tests with dogs and rats ingesting large amounts of VEEGUM (10% of ration) for 90 days, all responses were negative and VEEGUM was considered nontoxic.

Magnesium Trisilicate

Page, Heffner, and Frey (1941) gave six white rats daily doses of 0.6 g of Magnesium Trisilicate for 6 months. A litter was born and divided into two groups, a control and a treated group. The treated group received Magnesium Trisilicate doses from the time of weaning that corresponded to a daily dose of 3 or 4 pounds for a healthy human. This litter was also mated. Tissues from the animals of the first and second generation were examined microscopically. No evidence of tissue changes were recorded.

Dobbie and Smith (1982) gave six male guinea pigs a suspension in tap water of 250 mg/L Magnesium Trisilicate over a 4-month period for 5 days each week. Atomic absorption spectroscopy established that the soluble Si in the suspension was 267 $\mu\text{mol/L}$. Normal tap water was given to six control animals 7 days a week and 2 days a week to the test guinea pigs. At 4 months, all animals were killed for necropsy. The kidneys were processed for microscopic examination. All six animals had renal lesions that involved the distal nephron. Lesions of the distal tubule were dilation or cystic change. Some tubules were plugged with proteinaceous material. The interstitium of the kidneys was expanded by chronic inflammatory cells and excess collagen fibers. No lesions were seen in control animals.

Chronic Oral

Zeolite (Synthetic Zeolite A)

Groups of 50 male and female Wistar rats were fed 1, 10, 100, or 1000 mg/kg of Synthetic Zeolite A in their diets for up to 104 weeks. Clinical signs, mortality, and gross and microscopic lesions were recorded. No differences in body weight gain or clinical parameters were observed between control and treated animals. Based on feed intake, the Zeolite intake of the 10-, 100-, and 1000-mg/kg groups was 0.62, 6.1, and 58.5 mg/kg body weight/day for males and 0.65, 6.53, and 62.2 mg/kg body weight/day for females, respectively. No significant treatment-related lesions were observed in any of the organs examined and there was no effect on the types or incidence of any neoplastic changes seen (Gloxhuber et al. 1983).

Acute Parenteral

Aluminum Silicate

Musk et al. (1988) exposed Syrian golden hamsters to saline suspensions of Aluminum Silicate at 3.75 and 0.75 mg/100 g body weight by intratracheal instillation and sacrificed the animals at day 1. Their lungs were lavaged and the lavage fluid was characterized using cellular and biochemical indicators (lactic dehydrogenase, albumin, macrophages, polymorphs, and RBCs) of pulmonary damage. Either dose did not alter the biological parameters tested in comparison to those animals only exposed to saline.

Lemaire et al. (1989) gave Fiberfrax, an aluminum silicate, by intratracheal instillation at doses of 1, 5, and 10 mg to groups of

five rats. The details of this experiment are explained by Lemaire et al. (1989) under the Attapulgit heading in this section. The average length of Fiberfrax fibers were 8.3 μm and <50% were under 5 μm . The significant inflammatory response was mainly numerous lymphocytes and epithelioid giant cells. The lesions were located predominantly around the terminal bronchioles. Areas of early fibrosis were seen in the lesions. Every test animal developed type C lesions, described above. A dose-dependent reaction was suggested due to more extensive lesions seen in animals dosed with 10 mg. The bronchoalveolar lavage fluid had macrophages as the predominant cells followed by neutrophils and then by lymphocytes.

Pigott and Ishmael (1992) studied the effects of intrapleural injections of Aluminum Silicate in rats. A single intrapleural injection of 20 mg of four Aluminum Silicate samples (Saffil, aged Saffil, aluminosilicates A and B) and chrysotile A asbestos was administered to dose and control groups consisting of 24 rats of each sex. The control group received only a saline injection. The predominant length of the fibers in each sample were Saffil, 10 to 20 μm ; aged Saffil, 20 to 40 μm ; aluminosilicate A, 20 to 40 μm ; and aluminosilicate B, 0 to 10 μm . Each rat was allowed to live out its lifespan or until it appeared distressed until 85% mortality was reached. All animals, were then killed and organs were taken for microscopic examination. Reactions to both forms of Saffil were very similar. In almost all animals, a minimal focal chronic pleurisy/fibrosis was minimal with adhesion formation. Pericardial adhesions and mesothelial proliferation with some Saffil fibers were seen. The reactions to both aluminosilicate samples were very similar. Minimal to moderate focal chronic pleurisy/fibrosis was often associated with mesothelial proliferation. Aluminosilicate B caused three malignant mesotheliomas, one pleural and two peritoneal. A benign testicular mesothelioma was seen in one rat dosed with Saffil, two dosed with aged Saffil, and four dosed with aluminosilicate A. Incidences of tumors are presented in Table 14.

Attapulgit

Pott et al. (1987) injected three samples of 25 mg of Attapulgit dust intraperitoneally into 40 Wistar rats. Electron microscopy of the sample revealed 37.5% of fibers <2 μm long and 70.0% <5 μm . All animals were observed until they died either spontaneously or were killed. Saline was injected into 80 control animals. The time required to produce the first tumor in the rats was 257 days and the tumor incidence rate was 65%.

Stanton et al. (1981) reported that two groups of 30 to 50 female Osbourne-Mendel rats received a single direct application to the left pleural surface by open thoracotomy of 40 mg of one of two Attapulgit samples. The samples were 90% pure with quartz being the other component. One dose consisted of fibers >4 μm and the other contained no fibers >4 μm . The rats were killed at the end of 2 years. Pleural sarcomas were seen in 2/29 rats. The incidences of pleural sarcomas in the untreated groups were 3/491 and 17/615 of the rats receiving the pleural implants of Attapulgit. Of rats receiving UICC crocidolite, 14/29 developed pleural mesotheliomas.

Be'gin et al. (1987) delivered Attapulgit with a mean fiber length of 0.8 μm and diameter of 0.02 μm to the lungs of sheep by bronchoscopic cannulation. The tracheal lobe of 16 sheep was subjected to a single exposure of 100 mg of Attapulgit in 100 ml of saline. A bronchoalveolar lavage (BAL) was conducted at 2, 12, 24, 40, and 60 days, and necropsy was conducted on day 60. Total BAL cells, macrophages, and neutrophils, fibronectin content, and LDH and β -GLUC activity were examined. Nine samples of the tracheal lobe of the lung were obtained each time for microscopic examination. The controls were saline-exposed sheep and had no changes in BAL or pulmonary morphology. The total BAL cells/ml and subpopulations increased significantly above control numbers at days 12, 24, and 40 but returned to control levels by day 60. Albumin and procollagen III did not differ from controls, whereas fibronectin, LDH, and β -GLUC activities were significantly above the controls. Microscopic examination revealed infiltrates that were predominantly alveolar and peribronchial lesions. Macrophagic alveolitis with minimal airway distortion was seen. Three sheep had lesions of peribronchiolar alveolitis.

Jaurand et al. (1987) injected samples (20 mg/ml of 0.9% NaCl) of Attapulgit fibers with the median length of 0.77 μm into the pleural cavities of 36 2-month-old Sprague-Dawley rats. Two control groups, untreated and saline-injected, were utilized. Necropsy was performed after the rats died or killed when moribund. No mesothelial neoplasms were found in either controls or in rats treated with Attapulgit. Survival times between the Attapulgit-treated group and the controls were not statistically different.

Wagner, Griffiths, and Munday (1987) injected 20 male and 20 female, SPF Fischer rats intrapleurally with single injections of Attapulgit. Three samples of Attapulgit named after the location of their discovery (Lebrija, Torrejon, and Leichester) were utilized in this study. No concentrations were provided.

TABLE 14
Tumors in rats treated with intrapleural injections of four Aluminum Silicate samples (Pigott and Ishmael 1992)

Tumor	Control	Chry. Asbestos	Saffil	Saffil aged	Alumosil. A	Alumosil. B
Total no. of animals	62	81	71	68	57	67
No. of benign	44	55	57	56	46	49
No. of malignant	17	26	16	14	10	19
Malignant mesothelioma	0	7	0	0	0	3

TABLE 15
Toxic reactions to intrapleural injections of Attapulgit
(Wagner, Griffiths, and Munday 1987)

Dust	Mesothelioma	Nonmesothelioma
Lebija Attapulgit	2	38
Torrejon Attapulgit	14	26
Leicester Attapulgit	30	2
Crocidolite	34	6
Kaolin	0	40
Saline	1	39

However, fiber length information was provided. Lebija Attapulgit had fiber lengths of $\leq 2 \mu\text{m}$. Torrejon Attapulgit contained at the most 0.54% of fibers $\geq 6 \mu\text{m}$. Leicester Attapulgit contained about 19% of fibers $\geq 6 \mu\text{m}$. The animals were allowed to live their life span but were killed if they appeared distressed. Upon death, necropsy and microscopic examination of tissue were performed. Dust extraction was obtained from granulomas removed from the diaphragm or mediastinal tissue. Two controls were used in this experiment; Kaolin and saline. One positive-control crocidolite was also used. The results from this experiment are summarized in Table 15.

Lebija Attapulgit dust extracted from the lung had fibers $\leq 2 \mu\text{m}$. Material examined from Torrejon Attapulgit was fibrous and have fiber length up to $8 \mu\text{m}$. Leicester Attapulgit fibers from extracted lungs were up to $25 \mu\text{m}$. The investigators considered these fibers to be tumorigenic. Kaolin was a nonfibrous dust and crocidolite was fibrous. The authors concluded that exposure to Torrejon, and Leicester Attapulgit should be avoided (Wagner, Griffiths, and Munday 1987).

Lemaire et al. (1989) reported a study in which groups of five rats received single intratracheal instillations of Attapulgit at 1, 5, and 10 mg. One month after treatment, BAL and microscopic examination of the lungs were performed. The average length of the fibers were $0.8 \mu\text{m}$ and 100% of the fibers were less than $3 \mu\text{m}$. Every test animal had type A lesions. Type A lesions are characterized by an accumulation of inflammatory cells mostly macrophages, and epithelioid cells around fiber deposits. These inflammatory cells form a compact cellular infiltrate at the periphery of the deposits and some are focally dispersed throughout the alveolar region. The BAL had mostly macrophages and a small number of neutrophils at 5- and 10-mg doses. At the 5-mg dose, 3.6% of the cells were lymphocytes.

In a study by Renier et al. (1989), intrapleural injections of 20 mg of different Attapulgit fiber samples in 1 ml of saline were given to 2-month-old Sprague-Dawley rats. The control group received only a saline injection. All rats were allowed to live full life span. The mean length of Attapulgit fibers in this experiment was $0.77 \mu\text{m}$. The number of groups were not reported; however, 36 rats were reported to comprise each group. Pulmonary and thoracic neoplasms were fixed and processed for histopathological examination. The survival time of the treated

groups (788 ± 155 days) was very similar to that of the control groups (809 ± 110 days). The incidence of mesothelioma was 0% for control groups and treated groups. Attapulgit in the present experiment was not carcinogenic (Renier et al. 1989).

Lemaire (1991) reported a study in which groups of five animals received doses of 1, 5, or 10 mg of Attapulgit by transtracheal injection to examine alveolar macrophage (AM) production of interleukin-1 (IL-1) and macrophages-derived growth factor (MDGF) from fibroblasts. Saline and UICC chrysotile B asbestos were used as controls. At 1 month, Attapulgit produced granulomas and the UICC chrysotile B produced fibrosis. At 8 months, the granulomatous reactions had either resolved or were greatly diminished, whereas the fibrosis persisted. Cells obtained by BAL included multinucleated giant macrophages in animals treated with Attapulgit, but not in those treated with UICC chrysotile B. Enhanced production of IL-1 was seen in all treated groups. MDGF production was only seen in animals with lung fibrosis.

Coffin, Cook, and Creason (1992) injected a single dose of 0.5, 2, 4, 8, 16, or 32 mg of Attapulgit intrapleurally into six groups of 25 Fischer 344 rats. Nearly all the fibers were $< 1 \mu\text{m}$ in length. Mesotheliomas were present in 2/140 treated rats compared to 1/79 incidences in control groups. The median life span was 839 days for Attapulgit-treated animals and 729 days for nontreated animals.

Bentonite

Sykes et al. (1982) investigated the effects of Bentonite dust administered by intratracheal instillation in rats. A 0.5-mg dose of Bentonite with a mean size of $0.3 \mu\text{m}$ was instilled intratracheally. Control animals were injected with sterile saline and TiO_2 (a nontoxic dust). Animals were killed at 1, 2, 6, 24, and 48 h; and 4 and 7 days after instillation. Bronchopulmonary lavage (BPL) was carried out and AMs and polymorphonuclear (PMN) leukocytes were recovered. The activity of LDH and protein content of the lavage fluid were also determined. In a second experiment, after instillation of 5 mg of Bentonite, the animals were killed at 1, 7, 49, and 100 days. In addition to the above, peroxidase and lysozyme activity were measured.

In the first experiment, a rapid influx of PMN leukocytes was detected at 6 h. PMN leukocyte response peaked at $\sim 19 \times 10^6$ cells after instillation and started declining more slowly up to 4 days. At 7 days, the PMN leukocyte numbers were 2.5×10^6 . The greatest increase in the numbers of AMs recovered occurred at 4 and 7 days. The mean diameter of macrophages increased from 11.0 to $12.5 \mu\text{m}$ over the first 48 h after instillation. The mean diameter decreased at 4 and 7 days. LDH activity at 24 h was maintained at 40 mU cm^{-3} and then increased (73 mU cm^{-3}) with the influx of PMN leukocytes into the lungs after 48 h. Protein concentration was calculated at $500 \mu\text{g cm}^{-3}$ for the first 24 h and was maintained for 48 h.

In the second experiment, large number of PMN leukocytes were recovered at day 1. However the severity of the response did not differ significantly from the 0.5 mg dose. By 7 days,

the numbers had decreased and was similar to control values. A significant decrease in the number of AMs compared to controls was observed at 24 h after instillation. This decrease was followed by a sharp increase that exceeded control values by 7 days. Total number estimates were similar to those of the first experiment. LDH activity and protein concentration from Bentonite and TiO₂ were very similar. The initial rise at day 1 following administration was short-lived. Peroxidase activity was minimal. Lysozyme activity rose sharply between 1 and 7 days, but returned to control values at 49 and 100 days (Sykes et al. 1982).

Marek and Blaha (1985) gave subplantar injections of 0.05 ml of a 5% solution of Bentonite to male Wistar rats. The rats either received both hind paw injections at an interval of 24 h or their left paw was injected with Bentonite and their right paw injected with 0.05 ml of a 10% solution of Kaolin. The injection was of Kaolin. Subcutaneous Bentonite granulomas were produced on the left side, both dorsally and ventrally. Simultaneously Kaolin granulomas were produced on the right side analogous to the Bentonite injection. Sodium salicylate and prednisone suppressed the Bentonite edema during the first 24 h. The presence of mononuclear cells was confirmed.

Tatrai et al. (1983) administered a single dose of 40 mg of Bentonite suspended in 1 ml of physiological saline containing 40,000 IU of crystalline penicillin intratracheally to male CFY rats. The Bentonite's composition consisted of 73% Montmorillonite, 18% cristobalite, 3% quartz, 3% feldspar, and 3% other minerals. Particle sizes were <2 μ m. The control group received 1 ml of physiological saline containing 40,000 IU of crystalline penicillin. Animals were killed 12, 24, 48, or 72 h or 90 days after exposure. Body and lung weight of the rats were measured. The right lung was fixed and sectioned for microscopic examination. The lipids and phospholipids were analyzed in the left lung.

The body weights of the rats were moderately decreased and the lung weight increased 72 h after Bentonite exposure. After 90 days, the lung weight was only slightly greater than that of the control animals. Upon microscopic examination at 12 h, Bentonite exposure had resulted in a nonspecific inflammation of mostly neutrophils with perivascular edema, alveolitis, and incipient bronchopneumonia. A small number of macrophages and lymphocytes were detected. Dust particles were observed in the leukocytes and macrophages or extracellularly in the alveoli. After the 24th h, bronchopneumonia was present after coalescence of the inflammatory foci; the pneumonia then became necrotizing and desquamative. Necrotic neutrophilic leukocytes and eosinophil leukocytes were observed. The reticular network collapsed between the 48th and 72nd h. Exposure after 90 days, included dust storage foci filled with large foamy cells with pale cytoplasm. Closely packed cells with dark cytoplasm and nuclei were located at the periphery.

After 12 and 24 h, the amount of lipids and phospholipids in the lungs was not altered. However, between 48 and 72 h, the lipid and phospholipid content increase but distribution remained the same. After 90 days, the value was the same as seen at 72 h. (Tatrai et al. 1983).

Hatch et al. (1985) assessed the ability of Bentonite to increase susceptibility to bacterial pneumonia. Bentonite was injected intratracheally into mice at concentrations of 1, 10, and 100 μ g. In vivo bacterial-infectivity screening assays were conducted by exposing the animals to aerosolized Group C *Streptococcus* species. The severity of infection was calculated by recording the deaths of the mice over a 15-day period. Control animals were exposed to TiO₂, a nontoxic dust. At the 100- μ g dose, Bentonite increased the infectivity of the bacteria. Mortality was 85%. Even at 10 μ g, Bentonite caused increased animal mortality (43.3%). Control dusts at 100 μ g produced only a 5% mortality (Hatch et al. 1985).

In a study by Tatrai et al. (1985), male CFY rats were given a single dose of 60 mg of Bentonite, in 1 ml of physiological saline containing 40,000 IU crystalline penicillin, by the intratracheal route. Bentonite particle size was less than 5 μ m. Control groups received 1 ml physiological saline containing 40,000 IU penicillin. Animals were killed at the end of 72 h, the 2nd and 4th week, and the 3rd, 6th, and 12th month. The acid phosphatase activity and the progression of fibrosis was determined. The lungs were processed for microscopic examination and fibrosis determined by Belt and King's classification. The results from this experiment are presented in Table 16. Acid phosphatase activity was increased at 72 h and had returned to normal by the first month.

Bentonite dust was administered intratracheally as a single 60-mg dose to Sprague-Dawley rats in a study by Adamis et al. (1986). The animals were killed 3, 6, and 12 months after exposure. The right lung was studied microscopically and the lipids, phospholipids, and hydroxyproline were determined. Significantly greater phospholipid values compared to controls were observed. Among the phospholipid fractions, the greatest quantitative increase was seen in phosphatidylcholine (more than twice the control) and the smallest increase was seen in phosphatidylethanolamine (less than 1.6 times). After 6 and 12 months, the values were similar. Lung lipids had a greater range of values than did the phospholipids (no details given). The wet weight of the lung in grams increased in 5% to 10% Bentonite-treated rats compared to controls at month 3. No

TABLE 16
Toxic effect of intratracheal instillation of Bentonite
(Tatrai et al. 1985)

End point	Time after instillation		
	72 hours	1st month	12th month
Acid phosphatase activity	72	—	—
Fibrosis	N/A	Loose reticulin fibrils, no collagen	Loose reticulin fibrils, no collagen

difference was detected at 6 and 12 months. Hydroxyproline content of treated rats (mg/g lung wet weight) was very similar to controls at 3, 6, and 12 months (Adamis et al. 1986).

Calcium Silicate

Bolton et al. (1986) injected three Calcium Silicate samples into the peritoneal cavity of three groups of 36 rats. Each rat was given a single injection of 25 mg of dust and allowed to live out their life span. At necropsy, little dust or dust-related fibrosis was visible in the peritoneal cavity. No mesotheliomas developed in any of the animals.

Richards, Tetley, and Hunt (1981) compared the biological reactivity of three samples of Calcium Silicate (A, B, and C) in vivo to that of chrysotile and titanium dioxide. Titanium dioxide and saline were considered negative controls, while chrysotile was considered a positive control. Groups of 32 female, MRC hooded rats were instilled intratracheally with 0.25, 0.50, 1.0, or 5.0 mg of Calcium Silicate. At weeks 1 and 4 after instillation, the control and treated rats were killed. The lungs were lavaged and the reactivity of the minerals to free cell populations, lavaged lung tissue, and pulmonary surfactant was conducted. All mineral doses of 5 mg induced an increase in the number of free cells at week 1. Only sample B increased in cell numbers at lower doses. At the end of 1 week, sample B was considered more reactive than either sample A or C, but chrysotile was considered more reactive than sample B. At 4 weeks, the effects seen from samples A and B are almost completely reversed and were comparable to that of titanium dioxide. Sample B at 4 weeks produced a greater or a comparable activity to chrysotile. No mineralogical analysis of the Calcium Silicate samples was provided.

Kaolin

Zaidi et al. (1981) investigated the effect of *Candida albicans* in modifying the fibrogenesis caused by Kaolin. Five groups of guinea pigs were injected intratracheally with *C. albicans* (500 μ g); Talc dust (75 mg); Talc and *C. albicans*; Kaolin (75 mg); or Kaolin and *C. albicans*. Two animals from each group were killed at 1, 7, 15, 30, 60, 90, 120, and 180 days after injection. The lungs were collected for bacteriological and microscopic examination. The combined effect of Kaolin and the organism incited an acute inflammatory reaction similar to Kaolin dust alone at day 1. However, Kaolin and the organism produced thick reticulin and collagenous fibrosis, unlike Kaolin alone. Talc produced only a thin reticulin fibrosis not enhanced by the presence of the organism. The enhanced fibrogenicity was attributed to the adjuvant activity of Kaolin with the polysaccharide glucan component of *C. albicans*.

Edwards et al. (1984) gave 12 fetal lambs and six fetal monkeys subarachnoid injections of Kaolin. A sterile suspension of 2% Kaolin in saline was injected into the cisterna magna. Fetal lambs received 1 to 3 ml of Kaolin and fetal rhesus monkeys received 0.5 to 1.0 ml. After injection the fetuses were replaced into the uterus. Prenatal ultrasound monitoring was used to document the progression of fetal ventriculomegaly. Cesarean

sections were scheduled for 140 to 145 days for the sheep and 160 to 165 days for monkeys. Newborn animals with gross head enlargement were killed 2 h after birth and necropsy was performed. Brains were sectioned for gross and microscopic examination. Five lambs and one monkey underwent ventriculoamniotic shunting at 120 days after gestation.

Ventricular dilatation was apparent at 1 week following Kaolin injections. The cerebral mantle was markedly thinned, with relative preservation of the cortex and severe attenuation of the white matter. The average cortical thickness of the cingulate gyrus in the Kaolin-injected sheep was 716 μ compared to 1225 μ in control animals. The corpus callosum was an average of 125 μ in thickness in the sheep compared to 475 μ in control animals. Microscopic examination of the cortical neurons were well preserved and contained the complexity and density of neural processes. A mild-to-moderate fibrotic reaction and inflammatory cell response along the basal meninges was apparent. A large number of macrophages containing Kaolin infiltrated the subarachnoid space. In five fetuses, Kaolin was injected mistakenly into either the epidural tissues superficial to the cisterna magna or into the cervical musculature. None of these fetuses had hydrocephalus at birth (Edwards et al. 1984).

Hatch et al. (1985) assessed the ability of Kaolin to increase susceptibility to bacterial pneumonia. Kaolin was injected intratracheally into mice at a dose of 100 μ g. In vivo bacterial-infectivity screening assays were conducted by exposing the animals to aerosolized Group C *Streptococcus* species. The severity of infection was calculated by recording the deaths of the mice over a 15-day period. Control animals were exposed to TiO₂, a nontoxic dust. A 100- μ g dose of Kaolin caused statistically significant but modest (<50%) increased death due to infection by a large dose. Mortality was calculated at 38.9%. Control dusts at 100 μ g produced only a 5% increase in mortality.

Wagner, Griffiths, and Munday (1987) used Kaolin as a negative control in a previous intrapleural injection study. The protocol and results are cited under Attapulgit in this section.

Fugiyoshi, Hayashi, and Oh-ishi (1989) reported a study in which Kaolin, a known activator of factor XII, was injected intraperitoneally into mice at 2.5 mg/mouse to study the Kaolin-induced writhing response. The writhing responses were observed in the 10 min after treatment and the mean number of responses was 9.2. Sixty minutes after the Kaolin injection, captopril (20 μ g/mouse) was injected and the writhing response was observed again for 10 min after injection. Captopril is an anti-hypertensive and vasodilator. A second study was conducted by administering bromelain (10 mg/kg intravenously) followed by the injection of Kaolin 30 min later. Bromelain is a standardized complex of proteases from the pineapple plant purported to have primarily antiedema, antiinflammatory, and coagulation-inhibiting effects. The response was not reproduced.

Montmorillonite

Heat-treated Montmorillonite in doses of 5, 15, and 45 mg was given to groups of four Sprague-Dawley rats by intratracheal

instillation. Following a 3-month postexposure period, the animals were killed and tissues were subjected to microscopic examination. The Montmorillonite particles were mainly restricted to alveoli within and adjacent to alveolar ducts regardless of dose. Most particles were contained within small to moderate numbers of pulmonary AMs. However, some particles were free in alveoli. Adjacent alveoli septae were mildly thickened. Interstitial fibrosis was present in all groups. At the 5- and 15-mg doses, fibrosis was mild to moderate, multifocal, and loose, meaning less collagen. The 45-mg dose produced dense fibrosis. Macrophages contained clay particles and lymphocytes were present in the lesions. Occasionally giant multinucleate cells were seen (Schneider, Culbertson, and Raabe 1985).

Zeolite

A single intratracheal administration of 50 mg of Zeolite dust was given to male rats and observations were made at 1 and 3 days, and 1 and 3 months after injection. Time-dependent increases in phagocytosis were observed. Morphological changes in the lungs was described as exogenous fibrous alveolitis (Kruglikov, Velichkovsky, and Garmash 1990).

Zeolite (Clinoptilolite)

Kruglikov et al. (1992) reported a study in which a single intratracheal instillation of 50 mg of Clinoptilolite was made to male rats. On days 1, 3 to 5, and 18 after injection, lung tissues were examined histopathologically. On the first day, the smallest Zeolite particles were phagocytized by neutrophils, whereas larger particles were phagocytized by macrophages. About a fourth of macrophages had phagocytized more than six dust particles per cell and <2% of macrophages were degenerated. At 3 to 5 days, no more particles were seen in neutrophils and their numbers had decreased. However, the percentage of macrophages containing more than six dust particles in the cytoplasm increased to 90%. Only 7% of macrophages degenerated. On day 18, the pattern of phagocytosis was similar to that at days 3 to 5, but 4% of macrophages were degenerated.

Tatrai and Ungváry (1993) instilled single intratracheal doses of 30 and 60 mg of Clinoptilolite particles to groups of 50 male and female (equal numbers) Wistar rats. The particles were <5 μm and were suspended in 40,000 IU crystalline penicillin. Controls received only saline instillations. All survivors were killed at the end of the study. Examination for gross and microscopic lesions were conducted. None of the treated groups had a significant increase in the incidence of any specific neoplasms compared to the controls. No positive trend was noted in the occurrence of neoplasms. Neoplasms seen within both control and treated animals were similar in the anatomical sites in which they were found and their histological feature.

Zeolite (Mordenite)

Suzuki (1982) gave two groups, one of 18 and one of 5 male Swiss albino mice, a single injection of 10 or 30 mg Zeolite intraperitoneally. The control animals were untreated. Ten

months after exposure, no neoplastic changes were observed in the treated animals. Nearly all (98%) of the sample particles were <5 μm .

Suzuki and Kohyama (1984) administered a single injection of 10 mg of Mordenite to a group of 50 male BALB/c mice. The control animals received saline injections. The Mordenite sample was comprised of 94% of particles <3 μm . No peritoneal tumors were observed in any of the control animals. Mild peritoneal fibrosis was seen in treated mice, but no peritoneal or any other organ neoplasms were observed between 7 to 23 months.

Tatrai, Wojnárovits, and Ungváry (1991) made intratracheal instillations of 60 mg of Mordenite to groups of 10 rats. The animals were killed at 1 week, and 1, 3, 6, and 12 months after exposure. Lesions in the lungs were observed. Nonspecific confluent bronchopneumonia was observed at 1 week after exposure and sequestration of macrophages at 1 month after exposure. Mild fibrosis was observed at later times. After 12 months, the aluminum:silicon ratio in macrophages was similar to the ratio in natural Zeolites.

Tatrai et al. (1992) reported the changes in cervical and hilar lymph nodes in the test animals treated in the above study as seen by electron microscopy and light microscopy. By the end of the first year, dust storing macrophage foci developed in the lymph nodes with minimal fibrosis. Also 3/10 of the rats had atypical hyperplasia. Electron microscopy showed the dust stored in macrophages without structural changes. However, dispersive x-ray microanalysis of the intracellularly stored dust revealed the ratio of the two main elements, aluminum and silicon, changed with respect to aluminum as compared to the original Zeolite sample.

Zeolite (Nonfibrous Japanese Zeolite)

A single intrapleural injection of 20 mg of Nonfibrous Japanese Zeolite was administered to two groups of 20 male and 20 female Fischer 344 rats. Control rats received saline injections alone. Mean survival time for control animals was 720 days and 715 days for treated animals. One pleural mesothelioma was found in the control group and one pleural and one peritoneal mesothelioma was found in the treated group (Wagner et al. 1985).

Zeolite (Synthetic Zeolite 4A)

A single intraperitoneal injection of 10 mg of Synthetic Zeolite 4A was given to groups of 50 male BALB/c mice. The average particle length of the sample was 2.24 μm . Treated animals were observed for 7 to 23 months after exposure and no mesothelioma were observed (Suzuki and Kohyama 1984).

Zeolite (Synthetic Zeolite MS4A and MS5A)

Maltoni and Minardi (1988) reported a study in which groups of 20 male and 20 female Sprague-Dawley rats received a single intraperitoneal injection of 25 mg of Zeolite MS4A (sodium aluminum silicate) or MS5A (calcium aluminum silicate) or water

only (control). Observations were made for the animal's entire life span and microscopic examination was performed. One peritoneal mesothelioma in an Zeolite MS4A-exposed rat was found at 141 weeks after treatment.

These same authors administered single intrapleural injections and single subcutaneous injections of 25 mg of Zeolite MS4A and MS5A or water to separate groups of 20 male and 20 female Sprague-Dawley rats. No difference in incidences of tumors was found among control and treated animals (Maltoni and Minardi 1988).

Zirconium Silicate

In a study by Harding (1948), a 3-ml dose of a 10% suspension of Zircon in milk and saline was injected intraperitoneally into three cavies (guinea piglike rodent). The animals were killed nearly a year later. At microscopic examination, a dry opaque material was embedded in the peritoneum of the abdominal wall over the small intestine, and in the omentum. Growth was not affected.

The accumulation of Zirconium Silicate in tissue was reported by Stookey et al. (1967). In one study, six young adult male rats were anesthetized and were given subcutaneous injections into their back. Half of the rats were injected with saline to serve as controls and the other half were injected with 0.3 ml of an aqueous 50% slurry of Zirconium Silicate. Three weeks after the injections, the animals were killed. Tissue surrounding the injection site was excised and prepared for microscopic examination. Zirconium Silicate deposits were observed as discrete nodules with a narrow surrounding connective tissue wall in the deep connective tissues of the back. Saline controls had no lesions and in some cases, healing was complete.

In another study in this report, eight young adult female rats were divided into four equal groups according to body weight and their tissues were subjected to microscopic examination following saline and Zirconium Silicate or sodium zirconium

lactate injections. Group 1, the control group, was given a single injection of 0.05 ml of isotonic saline in four different areas: subcutaneous injections in the right buccal mandibular mucosa; periosteal injections in the left buccal mandibular periosteum; intramuscular injections on the ventral side of the left thigh; subcutaneous injections in a shaved area on the back located about 1 inch behind the shoulders of the midline. Group 2 was similarly injected with 0.05 ml of a 20% slurry of Zirconium Silicate. Groups 3 and 4 were injected with 0.05 ml of a 20% solution of sodium zirconium lactate and a 20% slurry of flour of pumice. All animals were killed 1 week after the injections and tissue samples for histological sections were taken at each injection site. An identical study with the same experimental procedures as the above study used adult male guinea pigs. In each species, saline injections produced no effect, Zirconium Silicate caused minimal toxicity, and sodium zirconium lactate plus pumice was toxic. The results from these two studies are listed in Table 17.

The results pertain to both the rat and guinea pig studies. Zirconium Silicate deposits were described as well circumscribed masses of particulate material surrounded by a narrow zone of new connective tissue. Nonspecific muscle damage, without necrosis due to the presence of the particulate matter and the volume of injected material, was localized to the immediate vicinity of the injection site. Macrophages along a border of a mass of Zirconium Silicate had reflective material within their cytoplasm. Dispersed particles were phagocytized by macrophages, with little or no associated inflammatory response. No evidence of bone resorption was found adjacent to periosteal deposits.

In another study by these authors, skin and muscle tissue samples were taken for microscopic examination. Eight adult rats were anesthetized and a deep incision was made on the ventral side of the left rear leg. The incision was made in the quadratus femoris muscle. The animals were exposed to 50 mg of pumice flour, silica dioxide, and Zirconium Silicate, respectively. Insertion of the appropriate substance was made into the muscle

TABLE 17
Toxic reactions to injected Zirconium Silicate (Stookey et al. 1967)

Animal species	Agent injected	Concentration (%)	Degree* of tissue reaction			
			Oral mucosa	Subcutaneous tissues	Periosteal tissue	Intramuscular tissue
Rat	Saline		0	0	0	0
Rat	Zirconium Silicate	20	+	+	0	+
Rat	Sodium zirconium lactate and pumice	45 and 20	+++	+++	+++	+++
Guinea pig	Saline		0	0	0	0
Guinea pig	Zirconium Silicate	20	+	+	+	+
Guinea pig	Sodium zirconium lactate and pumice	45 and 20	+++	+++	+++	+++

*0 = reaction absent.

+ = mild inflammatory reaction of little consequence.

++ = mild reaction with granulomatous response.

+++ = destructive granulomatous reaction.

TABLE 18
Toxic reactions to implantation of Zirconium Silicate
in muscle tissue (Stookey et al. 1967)

Agent embedded in muscle	Amount (mg)	Degree of tissue reaction*	
		Subcutaneous tissue	Intramuscular tissue
Pumice	50.0	+	+
Silica dioxide	50.0	++	+++
Zirconium Silicate	50.0	+	+
Control		0	0

*0 = reaction absent.

+ = mild inflammatory reaction of little consequence.

++ = mild reaction with granulomatous response.

+++ = destructive granulomatous reaction.

incision and into the skin 1 cm lateral to the muscle incision. Control animals had the same muscle incision, but no foreign material was inserted. One animal from each group was sacrificed 10 days following surgery. The remaining animals were sacrificed 30 days from the incision. All tissue was fixed and prepared for microscopic examination. Table 18 presents the data from this experiment.

Adjacent tissues were free of inflammation or evidence of injury at 10 and 30 days. Deposits of Zirconium Silicate were identified and were surrounded by a narrow zone of new connective tissue. No necrosis was identified (Stookey et al. 1967).

Short-Term Parenteral

Attapulgit

Pott et al. (1987) conducted a study in which three samples of Attapulgit labeled Georgia, Lebrija, and Morimoiron were injected intraperitoneally to study their carcinogenic effects in rats. Each sample was injected one time each week for 9 weeks at 60 mg per injection. The number of female Wistar rats for each of the samples (Georgia, Lebrija, and Morimoiron) was 112, 115, and 114, respectively. Fiber analysis was made

of each of the samples Morimoiron, Georgia, and Lebrija. The <50% fiber length was 0.7, 0.5, and 0.8 μm , respectively, and a <50% fiber diameter of 0.07, 0.07, and 0.04 μm , respectively. Some rats died spontaneously or others in poor health were killed. Surviving animals were killed 2.5 years after treatment for necropsy. At necropsy, neoplasms or organs with suspected neoplasm tissue were fixed for microscopic examination. These three samples were noncarcinogenic. The results are presented in Table 19.

In another experiment by the same investigators, a fourth sample of Attapulgit from Caceres was tested. Intraperitoneal injections of 2, 4, and 4 mg were administered consecutively for 3 weeks. The fiber length and diameter of this sample were <50% 1.3 and 0.07 μm , respectively. Animals in poor health were killed. Surviving animals were killed 2.5 years after treatment for necropsy. At postmortem examination, parts of neoplasms or organs with suspected neoplasm tissue were fixed for microscopic examination. The results were considered moderate in relation to the dose. The Caceres Attapulgit sample results are also presented in Table 19 (Pott et al. 1987).

Kaolin

Toxicity of some of the minerals present in coal-mine dust was examined by Martin, Daniel, and Le Bouffant (1975). Five hundred female SPF Sprague-Dawley rats were divided into groups each with 10 animals. The rats were exposed over a period of 3 months to 50-mg/rat intratracheal instillations of Kaolin. The following assessments were made: weight of the fresh lungs; macroscopic and microscopic lesions in the lungs; amount of collagen and dust present in the lungs; and calculation of the toxicity index from the amount of collagen formed per mg of dust. The weight of fresh lungs subjected to Kaolin was 1.76 g. Collagen formed per lung was 23.9 mg. The dust per lung was 30.2 mg and the collagen/dust ratio was 0.79. Microscopic examinations of the lungs showed no alveolar proteinosis but Kaolin was detected in the bronchiolovascular lymphoid sheaths. No information regarding nonexposed lungs was presented. The opinion of the investigators was that exposure to

TABLE 19
Carcinogenic effect of intraperitoneal injection of Attapulgit from four sources (Pott et al. 1987)

			Lifespan (weeks) after treatment of					
Attapulgit sample source	No. of rats	% of rats with tumors	All rats			Rat with tumors		
			Time to death for <20% of all rats	Time to death for <50% of all rats	Time to death for <80% of all rats	All rats dead by this time	Time to death of first rat with tumor	Average time to death of rats with tumors
Mormoiron	114	3.5	92	116	138	164	47	92
Lebrija	115	3.5	95	116	134	164	98	114
Georgia	112	3.6	89	108	129	163	75	100
Caceres	30	40.0	94	109	132	142	74	116

Kaolin results in "pulmonary toxicity" and possesses "fibrogenic capacity" (Martin, Daniel, and Le Bouffant 1975).

Magnesium Silicate

An emulsion of Magnesium Silicate, 500 mg in 1 ml of saline, was injected subcutaneously into groups of 10 female Wistar rats once daily at 2, 4, 6, 13, or 20 days. As controls, 12 nontreatment rats were killed on the first experimental day and 12 rats were injected with 1 ml of saline once daily for 20 days. The trabecular bone, sinusoids, and hematopoietic cells were processed for microscopic examination. No significant change in the volume percentage of hematopoietic cells, sinusoids, or trabecular bone was present in the day-2 treatment group. After 4 days of treatment, the volume percentage of hematopoietic cells increased rapidly, sinusoids decreased rapidly, and trabecular bone decreased gradually. The volume percentage of hematopoietic cells was about 2.6 times normal, and that of sinusoids and trabecular bone was about 30% and 60% of normal, respectively, after 20 days of treatment. The tibia metaphyses had the following changes after 4, 6, 13, and 20 days of treatment; sinusoids were compressed by the markedly proliferated myelocytic element and severely narrowed the distance between the sinusoidal wall and the surface of trabecular bone was markedly increased. Atrophy of the thin trabecular bone was seen but no significant changes in osteocytes, osteoblasts, or osteoclasts were seen (Shibayama, Nishioto, and Nakata 1993).

Zeolite (Clinoptilolite)

Three intrapleural injections of 20 mg of Clinoptilolite were given in monthly increments to a group of 44 male and 49 female rats. Control animals received only saline injections. The Zeolite sample was described as having the formula: $(\text{Na}, \text{K}) \text{Ca}[\text{Al}_6\text{Si}_3\text{O}_{72}] \cdot 20\text{H}_2\text{O}$, with Cu, Pb, Zn, Ni, Co, Mo, Mn, Ti, Sr, Ba, and Hg contamination. Particle size measurements were recorded as follows: $<3 \mu\text{m}$, 6.5%; $5 \mu\text{m}$, 5.9%; $10 \mu\text{m}$, 5.9%; $10\text{--}30 \mu\text{m}$, 20.6%; $30\text{--}100 \mu\text{m}$, 35.1%; $100\text{--}500 \mu\text{m}$, 26.1%. Pulmonary lymphosarcomas, pleural and abdominal lymphosarcomas, and lymphatic leukemias were observed in 47/93 treated animals and 5/45 saline-treated animals. No mesothelioma or pulmonary neoplasms were observed in the controls. Mesothelioma and bronchial carcinoma were detected in 2/93 and 1/93 treated animals, respectively (Pylev et al. 1986).

Zeolite (Phillipsite)

Three intrapleural injections of 20 mg of Phillipsite given in monthly increments were administered to a group of 44 male and 49 female rats. Control animals received only saline injections. The Zeolite sample was described as having the formula: $(\text{Na}_{1.38}\text{K}_{0.53}\text{Ca}_{0.87}\text{Mg}_{0.25})(\text{Si}_{11.93}\text{Al}_{4.03}\text{O}_{32}) \cdot 9\text{H}_2\text{O}$. Particle size measurements were recorded as follows: $<5 \mu\text{m}$, 14.5%; $10\text{--}30 \mu\text{m}$, 32.8%; $50\text{--}70 \mu\text{m}$, 16%; $\geq 100 \mu\text{m}$, 36.7%. Neoplasms were found in 41/101 Zeolite-treated rats (50 tumors).

Tumor types included 1 pleural mesothelioma, 2 pulmonary adenocarcinoma, 29 hemoblastosis, 7 mammary gland neoplasms, and 11 neoplasms found at other sites. In control animals, 16 neoplasms (pulmonary, pleural, and abdominal lymphosarcomas, lymphocytic leukemias, and mammary gland neoplasms) were identified in 14/52 rats (Pylev et al. 1986).

Zirconium Silicate

Harding (1948) reported results when an adult rabbit received intravenously four doses over 1 week of a 5-ml suspension of a 10% solution of Zircon. The animal was killed 33 weeks later. At microscopic examination revealed small clumps of crystals were close to the portal tracts of the liver. The clumps were in the Kupfer cells. Fibrosis was detected. Small clumps of crystals were also observed in the spleen and alveolar walls and spaces of the lungs.

In another study in this report, six young rats were injected intratracheally with 1 ml of a 10% solution of Zircon. Three rats were killed after 7 and 9 months. The lungs were radiographed and sectioned for microscopic examination. Much of the material was found free within the alveoli and lymph vessels of the lungs. A small amount was found within phagocytic cells. Swollen histiocytes were seen in a few alveoli. Fibrosis was not evident (Harding 1948).

Inhalation

Attapulgate

Wagner, Griffiths, and Munday (1987) exposed 40 (20 male and 20 female) SPF Fischer rats to Attapulgate dust in an inhalation chamber. The rats were exposed to two samples of Attapulgate (named by the region in which they were mined, Lebrija and Leichester) at a concentration of 10 mg/m^3 for 6 h/day for 5 day/week until they were killed. At 3, 6, and 12 months, four animals were killed. All remaining rats were allowed to live their life span. All animals were subject to necropsy; the lungs, liver, spleen, kidneys, and other relevant organs were examined microscopically. Mineralogical analysis, examination of ashed lung sections and examination of macerated lung tissue, were also performed. Kaolin, the negative-control dust, and Crocidolite UICC, the positive-control dust, were also administered at a dose of 10 mg/m^3 .

At microscopic examination, one peritoneal mesothelioma, one adenocarcinoma, and three bronchoalveolar hyperplasia were found in rats treated with Lebrija Attapulgate. Thirty-five rats had no proliferative changes. In rats treated with Leichester Attapulgate, proliferative lesions observed included two mesothelioma, one peritoneal mesothelioma, one malignant alveolar neoplasm, two benign alveolar neoplasms, and eight bronchoalveolar hyperplasias. Twenty-seven rats had no proliferative lesions. Rats exposed to the negative-control Kaolin had two bronchoalveolar tumors. Rats in the positive-control Crocidolite group had one adenocarcinoma and three bronchoalveolar tumors. The mean fibrosis grades of each treatment group are presented in Table 20.

TABLE 20
Toxicity of inhaled Attapulgit dust (Wagner, Griffiths, and Munday 1987)

Dust source	Total no. of rats	Mean fibrosis grade as function of time after exposure			
		3 months	6 months	12 months	24 months
Lebrija Attapulgit	40	3.1	2.6	3.2	3.2
Leichester Attapulgit	40	3.0	3.1	4.0	—
Kaolin	40	2.8	2.75	2.4	2.1
Crocidolite UICC	40	4.1	3.3	3.1	3.8

The classification of proliferative lesions and neoplasms corresponding to the mean fibrosis grades are as follows: (1) bronchoalveolar hyperplasia—no malignant proliferation of the epithelia; (2) benign alveolar neoplasm; (3) malignant alveolar neoplasm; (4) adenocarcinoma; (5) squamous carcinoma; (6) adenosquamous carcinoma; and (7) mesothelioma.

The Lebrija Attapulgit dust extracted from the animal lungs did not have short fibers and the presence of granular material and long fibers. The Leichester Attapulgit dust also had the presence of long fibers. Kaolin is a nonfibrous dust. UICC Crocidolite is a fibrous dust but lengths were not published in this study (Wagner, Griffiths, and Munday 1987).

Calcium Silicate

Bolton et al. (1986) exposed white male Wistar rats to clouds of Calcium Silicate dust at a concentration of 10 mg/m³ for 7 h/day, 5 days/week, for a total of 224 days over an elapsed period of 12 calendar months. A total of four inhalation chambers were used with 48 animals/chamber. One chamber was reserved for control animals receiving only filtered air. The remaining three chambers were used to test three samples (A, B, and C) of Calcium Silicate. Twelve rats were killed from each of the chambers at the end of the dusting period. The final surviving animals were killed at the end of 19 months after exposure. At necropsy, tissue samples and one lung were taken from all major organs for microscopic examination. The other lung was taken for lung-dust analysis. The lung was dried and prepared for infrared analysis. Blood samples were taken 5 days prior to the start of the exposure and 3 days after the exposure.

All Calcium Silicate-treated groups had dust-containing macrophages scattered throughout the alveolar regions of the lung at the end of the exposure period. Occasional fibers were seen in animals with exposure to the Calcium Silicate 3. The frequency of dust-containing macrophages declined at the end of the dust exposure. Fewer dust-containing cells were in animals exposed to samples C than A or B. The number of animals with interstitial fibrosis for samples A, B, C, and controls were three, five, five, and five, respectively. In all cases, the alveolar septa were thickened with abnormal deposits of reticulin and in old animals with collagen. Although most cells were relatively flat in some areas, some cells were cuboidal and had the appearance of adenomatosis. Peribronchiolar fibrotic areas were close to the

respiratory bronchioles and small granulomatous nodules with macrophages and fibroblasts were seen in rats exposed to sample A. Mediastinal lymph nodes from all treated animals showed no particulate material at the end of exposure. Small primary neoplastic lesions were found in two animals exposed to sample B. One lesion was described as a small squamous cell carcinoma and the other as an adenoma. No pathological changes were observed in all other organs. All examined blood parameters were within normal ranges for both animals studied before and after exposure (Bolton et al. 1986).

Kaolin

Kaolin was used as a negative control in a previous inhalation study. The protocol and results are cited under Attapulgit in this section (Wagner, Griffiths, and Munday 1987).

Zeolite (Synthetic Zeolite A)

A group of 15 male and 15 female Wistar rats were exposed to 20 mg/m³ of Synthetic Zeolite A for 5 h/day, three times a week for 22 months. The Zeolite was characterized by (Na₁₂(Al)₂)(SiO₂)₁₂·27H₂O and consisted of particles ranging from 0.5 to 10 μm. Thirty untreated males were the control group. Histopathological examinations of the trachea and the lung were completed. Moderate to extensive respiratory disease was seen in treated and control groups. No neoplasms were observed in any group (Gloxhuber et al. 1983).

In another study by Gloxhuber et al. (1983), a chronic inhalation study of Zeolite A batch F 325 dust was conducted. Groups of 15 male and 15 female hamsters and 15 male and 15 female rats were exposed for 5-h periods three times a week for 12 months for hamsters and 22 months for rats. Control animals were exposed to untreated air. The trachea and lungs of the animals were examined microscopically. Microscopic examination was limited to the trachea and lungs of 10 treated hamsters and 8 controls and to 10 treated rats and 5 controls due to deaths caused by a specific infection. Both species had moderate signs of respiratory disease in the treated and controls. In Zeolite-exposed hamsters, macrophages with accumulations of foreign material were found, mainly in alveoli. No other lesions of inflammation or connective tissue reactions were seen. Rat lungs had grey-white deposits in macrophages of the alveoli and the peribronchiolar lymph nodes near the hilus. Isolated

clay deposits were found in the mediastinal lymph nodes but no reactions were seen about the deposits.

Zeolite (Synthetic Nonfibrous Zeolite)

Groups of 20 male and 20 female Fischer 344 rats were exposed in inhalation chambers to a mean respirable dust concentration of 0 or 10 mg/m³ of a Synthetic Nonfibrous Zeolite. Exposures were for 7 h/day, five days/week for 12 months. All animals were observed for their life span. Three males and three females per group were killed at 3, 6, 12, and 24 months after exposure. Erionite and UICC crocidolite were used as positive controls. The mean survival time for animals exposed to the Zeolite was 797 days, 504 days for animals exposed to erionite, 718 days for animals exposed to UICC crocidolite, and 738 days for untreated animals. One pleural mesothelioma and one pulmonary adenocarcinoma were seen in Zeolite-exposed rats. No neoplasms were found in controls; 27 mesotheliomas were found in erionite-treated rats and 1 squamous-cell carcinoma of the lungs was found in UICC crocidolite-treated rats (Wagner et al. 1985).

Dermal Irritation

Hectorite

A primary irritation study patterned after the Draize method was conducted using six white rabbits. Either a 0.5-ml or a 0.5-g sample of Hectorite was applied to two sites, one on abraded skin, and the other on intact skin of the backs of the rabbits. The test sites were occluded for 24 h. At the end of the 24 h, the binders were removed and the sites were gently wiped clean. One-half hour later, the sites were examined and scored for erythema and edema. The sites were examined again at 72 h. The average score was 0.0 and the test subject was nonirritating to the skin of rabbits (FDRL Inc. 1980a).

Magnesium Aluminum Silicate

VEEGUM (2 g) was applied daily to the external ears of four rabbits for 10 days. These applications were made to both abraded and intact skin. The abraded skin healed completely within 4 to 6 days after application. No gross effects were noted in any of the animals. No tissue was taken for microscopic examination (Munch 1944).

VEEGUM was applied to the closely clipped intact and abraded abdominal skin of two groups of four rabbits each. A nonabsorbent paper binder was placed onto the treated area. The dose was 3.4 g/kg of body weight. After 24 h, the binder was removed and any residual test material was removed by washing. Dermal irritation was recorded at 24 h and once daily after application for 7 days. All the animals were killed and necropsy was performed. No deaths and no systemic toxicity occurred from percutaneous absorption. The acute dermal LD₅₀ was >3.5 g/kg of body weight. Dermal irritation generally consisted of moderate erythema and slight edema. The edema completely subsided within an additional 24 h, and erythema completely subsided in

all animals between days 2 and 4. No major necropsy findings were reported (Hazelton Laboratories, Inc. 1968).

Eight male white rabbits were used in a primary skin irritation test with a solution of 4% MAS; 0.3 ml of the test substance was applied to the intact and abraded skin of the backs of four rabbits. The test substance was applied under occlusive patches for 24 h. The plaster was removed 24 h after application and the skin reactions were evaluated at 24 and 72 h. The primary irritation index was 0.1, suggesting that Magnesium Aluminum Silicate is a weak primary skin irritant (CTFA 1970a).

Three male guinea pigs were used in a cumulative skin irritation test with a solution of 4% MAS (in deionized water). The test substance (0.05) was applied to the flank of the animals once daily for 3 consecutive days. Skin reactions were evaluated at 24 h after each application. The cumulative irritation index was 0.0 and MAS had no cumulative skin irritation under the test conditions (CTFA 1970a).

Sodium Magnesium Silicate

CTFA (1970b) reported a study in which eight male, white rabbits were used in a primary skin irritation test with a solution of 4% Sodium Magnesium Silicate (in deionized water). The test substance (0.3 ml) was applied to the intact and the abraded skin on the backs of four rabbits. The test substance was applied under occlusive patches for 24 h. The plaster was removed 24 h after application and the skin reactions were evaluated at 24 and 72 h. The primary irritation index was 0.0, suggesting that Sodium Magnesium Silicate has no primary skin irritation under these test conditions.

CTFA (1970b) reported that three male guinea pigs were used in a cumulative skin irritation test with a solution of 4% Sodium Magnesium Silicate (in deionized water). The test substance (0.05 ml) was applied the flank of the animals once daily for 3 consecutive days. Skin reactions were evaluated at 24 h after each application. The cumulative irritation index was 0.0 and Sodium Magnesium Silicate had no cumulative skin irritation under the test conditions.

Ocular and Mucosal Irritation

Bentonite

Preparations of Prophypaste, Bentonite, tragacanth, trypsin, and sterile water were injected either intralaminally or directly into the anterior chamber of six adult New Zealand rabbits at concentrations ranging from 1 to 5 mg/ml. No significant reactions were recorded with sterile water, Prophypaste, tragacanth, or combinations of tragacanth and Bentonite. Bentonite caused severe iritis after injection into the anterior chamber, but no corneal or retrocorneal reaction was noted grossly or microscopically. In five of the eyes where Bentonite was injected intralaminally, widespread corneal infiltrates and retrocorneal membranes were observed within 2 to 5 days. The sixth eye had no reaction, only 0.1 ml of 0.25 mg/ml was injected. Anterior chamber taps of the eyes showed viscous mucopurulent material. Microscopic sections showed pseudoeosinophils, retrocorneal membranes,

and fibrovascular membranes in the anterior segment. Polarized light revealed highly birefringent particles were found at the injections sites, but not in the retrocorneal masses (Austin and Doughman 1980).

Hectorite

A primary eye irritation study using nine New Zealand white rabbits was carried out according to the Wolcott Procedure. A 0.1-ml liquid or semisolid (100 mg of the solid) sample was instilled into the one eye of each rabbit. Six of the nine animals' eyes were not rinsed and the eyes of three of the animals were rinsed approximately 4 s. All untreated eyes served as controls. The eyes were then examined with sodium fluorescein and an ultraviolet lamp at 24, 48, and 72 h and at 7 days. The mean score at 24 h was 2.0. All subsequent scores were 0.0. The test sample was considered moderately irritating to rabbit eyes without rinsing and practically nonirritating to the eyes with rinsing 4 s after instillation (FDRL Inc. 1981).

Magnesium Aluminum Silicate

Hazleton Laboratories, Inc. (1968) made a single application of 100 mg of VEEGUM or 0.1 ml of a 50% weight/volume to rabbit eyes. An aqueous suspension was made into the conjunctival sac of the left eye of each of six (undiluted) and three (50% suspension) rabbits. Three eyes (undiluted) were washed for 4 s after application and the remaining six eyes were not irrigated but held closed for 1 s. Control rabbits were not treated. Observations were made at 1, 4, 24, 48, and 72 h and at 4 and 7 days following application. Irritation was graded according to the Draize system. On day 7, the eyes were treated with 2% sodium fluorescein stain to provide evidence of corneal damage. Irritation generally consisted of moderate conjunctival hyperemia in all eyes and slight iritis in five of the eyes (one in the nonirrigated, undiluted group and two in each of the other groups). In the nonirrigated eye treated with the dry material, the iritis persisted until 72 h, whereas it was only present at the 1- and 4-h observations in the other eyes. The irritation gradually subsided completely in all within 2 to 4 days. The sodium fluorescein test was negative for corneal damage.

CTFA (1970a) reported that three male, white rabbits were used in an eye irritation test using a 4% solution of MAS. The test substance (0.01 ml) was instilled into the conjunctival sac of one eye of the animals without irrigation. Acute reactions were evaluated at 1 and 4 h, and 1, 2, 3, 6, and 7 days after application according to the Draize scoring system. The average irritation score at the time of maximum score (1 h) for the cornea, iris, and conjunctivae was 0, 0, and 6.7, respectively. The average total score was 6.7 suggesting that MAS produced minimal eye irritation under these test conditions.

Sodium Magnesium Silicate

Three male, white rabbits were used in an eye irritation test using a 4% solution of Sodium Magnesium Silicate (in deionized water). The test substance, 0.1 ml, was instilled into one

eye of the animals without irrigation. Eye reactions were evaluated at 1 and 4 h, and 1, 2, 3, 6, and 7 days after application according to the Draize scoring system. The average irritation score at the time of maximum score (1 h) for the cornea, iris, and conjunctivae was 0, 0, and 6.0, respectively. The average total score was 6.0, suggesting that Sodium Magnesium Silicate had minimal eye irritation under these test conditions (CTFA 1970b).

Zeolite (Zeolite A)

In an acute ocular study, rats tolerated a single dose of 10 g of Zeolite A without any adverse reaction (Gloxhuber et al. 1983).

Zirconium Silicate

Gingival tissue was histologically examined in a study conducted by Stookey et al. (1967). Six weanling albino rats were given an oral prophylaxis using a paste containing 75% Zirconium Silicate and 25% distilled water. The animals were anesthetized and given a routine prophylaxis for 30 s per mandibular hemijaw. Three of the animals were killed 1 h following treatment. The other three animals were killed 24 h following treatment. Gingival tissue of the buccal surface of the mandibular molar areas were removed for microscopic examination.

No unusual tissue response was observed in either group. At 1 h, scattered particles of Zirconium Silicate were noted on the surface of the gingiva. Occasional particles could be identified in the superficial epithelium. Only an occasional mild local inflammatory response was noted in the subepithelial tissue. It was presumed to be secondary to the prophylaxis procedure (Stookey et al. 1967).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Calcium Silicate

FDRL Inc. (1973) conducted a study in which adult, Dutch-belted female rabbits were artificially inseminated and received oral intubations of Calcium Silicate at doses of 250, 500, 750, 1000, 1250, 1500, and 1600 mg/kg on days 6 through 18 after insemination. On day 29, cesarean section was performed and the numbers of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were recorded. Body weights of live pups were recorded. The urogenital tracts of the animals were examined in detail. All fetuses underwent detailed gross examination. Calcium Silicate administered at 1600 mg/kg to pregnant rabbits for 13 consecutive days had no clear discernible effect on nidation or on maternal or fetal survival. Skeletal or soft tissue abnormalities did not differ from the number occurring in control groups.

Kaolin

Groups of 12 Sprague-Dawley female rats were fed three diets: control diet, 20% Kaolin diet, or iron-supplemented 20% Kaolin diet. The diets were fed for 37 to 86 days, 69 to 85 days, and 96 to 117 days prior to fertilization. These same diets were fed for the duration of the gestation period. The animals fed

the 20% Kaolin diet had significant reductions in hemoglobin, hematocrit, and RBC numbers, indicating maternal anemia. Significant reduction in the birth weight of the pups was observed. Animals fed the iron-supplemented diet maintained their hematocrit, hemoglobin, and RBC levels (Patterson and Staszak 1977).

Magnesium Aluminum Silicate

According to Sakai and Moriguchi (1975), "MAS has neither teratogenic nor had adverse effects on the mouse fetus." MAS was administered at doses of 600, 3000, and 6000 mg/kg/day orally to pregnant mice (ICR-JCL) for 6 days on the 7th to 12th day of gestation. No significant differences between MAS-administered and control groups were observed in body weight gain, gross lesions, implantations, resorbed or dead fetuses, or growth inhibition of live fetuses. Incidences of skeletal anomalies were significantly greater in MAS-exposed fetuses, but none resulted in skeletal malformation. Development, external differentiation, body weight gain, and behavior were normal in all offspring.

Zeolite (Type A)

Type A Zeolite containing 15.8% sodium 19.0% silicon, and 20.1% aluminum was tested for its teratogenic potential by Nolen and Dickerman (1983). Sprague-Dawley rats and New Zealand rabbits were utilized under the standard FDA Segment II protocol. Zeolite A in distilled water was given to rats by gavage at concentrations of 74 or 1600 mg/kg of body weight on days 6 to 15. Rabbits were given doses of 74, 345, and 1600 mg/kg of Zeolite A by oral gavage on days 6 to 18. Vehicle controls were included but no details were provided. Type A Zeolite produced no adverse effects on the dam, embryo, or fetus in either the rats or rabbits at any dose.

Zeolite (Clinoptilolite)

Pond and Yen (1983a) investigated whether Clinoptilolite offers protection against the toxic effect of long-term cadmium ingestion by examining the effects of long-term ingestion of Clinoptilolite on reproduction and on the postnatal development of the progeny. Four groups of female Sprague-Dawley rats were fed the following diets: control; control and Clinoptilolite; control plus cadmium; and control plus cadmium and Clinoptilolite. At 13 weeks, male rats were placed with the females for mating. The female reproductive performance was unaffected by any of the various diets. The supplemental level of Clinoptilolite resulted in reduced body weight during gestation; body weight at parturition and postpartum was similar for rats of all diet groups.

GENOTOXICITY

Attapulgit

DNA damage caused by Attapulgit was evaluated through the measurement of unscheduled DNA synthesis (UDS) in a

study conducted by Denizeau et al. (1985b). Hepatocytes-taken from male Sprague-Dawley rats were prepared according to the collagenase perfusion technique. Attapulgit fibers were added at concentrations of 1 and 10 $\mu\text{g}/\text{ml}$ to the primary cultures 2 h after the cells were seeded. 2-Acetylaminofluorene (AAF), a known UDS-inducing agent of rat hepatocytes, was added to the cultures at 0.05 and 0.25 $\mu\text{g}/\text{ml}$ for each concentration of Attapulgit. Therefore, Attapulgit was used alone in this UDS assay system or in combination with AAF. The cultures were incubated for 20 h. Labeled thymidine was added to final concentration of 4 $\mu\text{Ci}/\text{ml}$. The amount of thymidine in the DNA was evaluated by liquid-scintillation counting. Cytotoxicity was also measured in this study by measuring LDH activity using a spectrophotometer.

A significant increase in [^3H]-thymidine incorporation took place with the addition of AAF (0.05 and 0.25 $\mu\text{g}/\text{ml}$). However, at both Attapulgit concentrations, no significant increase in DNA-specific activity was observed. No alteration occurred in the UDS (induced by AAF) by secondary agents when both the fibers and AAF were applied. No statistically significant fiber effect of AAF-fiber interaction was recorded. Extracellular LDH activity was observed after 20-h incubations of Attapulgit at 1 and 10 $\mu\text{g}/\text{ml}$ applied to the cells. No significant differences were found between the LDH activity in the treated samples versus the controls (Denizeau et al. 1985b).

Beck and Bignon (1985) tested Attapulgit and UICC chrysotile asbestos B for UDS in primary hepatocyte cultures. Attapulgit fibers (96%) averaged 0.8 μm in length. Cells were also exposed to AAF alone and mixed with fibers. Within 20 h, both types of fibers were found in various cell structures, i.e., plasma membrane invaginations, cytoplasmic vacuoles, and phagolysosome-like components. Chrysotile B and Attapulgit did not induce a significant UDS response or modulate the response to AAF.

The UDS and cellular growth was studied utilizing rat pleural mesothelial cells (RPMCs) in a study conducted by Renier et al. (1989). RPMCs were cultured to confluence on glass coverslips in multiwell plates. Concentrations 2, 4, and 10 $\mu\text{g}/\text{cm}^2$ of Attapulgit and [^3H]-thymidine were added to cultures for 20 h. UDS was not modified at concentrations of 2 and 4 $\mu\text{g}/\text{cm}^2$ of Attapulgit. However, in one experiment, 10 $\mu\text{g}/\text{cm}^2$ produced a significant increase in UDS. Cellular growth was measured by counting in situ with an inverted phase-contrast microscope after 24 h of treatment of 1, 2, 4, and 10 $\mu\text{g}/\text{cm}^2$ of Attapulgit. Results were similar to that of the UDS. Attapulgit was considered noncytotoxic at concentrations of 1, 2, and 4 $\mu\text{g}/\text{cm}^2$. However, at 10 $\mu\text{g}/\text{cm}^2$, cell growth was inhibited. No specific details were given.

Adachi et al. (1992) studied the effect of asbestos fibers on DNA by measuring the yield of 8-hydroxy-2'-deoxyguanosine (8-OH-dGuo). 8-OH-dGuo is an OH adduct at the 8-position of a guanine base thought to induce an AT-to-GC transversion in DNA which may lead to a point mutation. For comparison purposes, Attapulgit was also studied. Results for

Attapulgit were not different from controls (Adachi et al. 1992).

Calcium Silicate

Litton Bionetics, Inc. (1974) conducted a study in which FDA compound 71-41, hydrated Calcium Silicate, was suspended in 0.85% saline at concentrations of 1000, 500, 200, 100, and 10 $\mu\text{g/ml}$ and applied to WI-38 cells in a logarithmic phase of growth. The cells were observed for cytopathic effects (CPEs) and the presence of mitosis at 24 and 48 h. Inhibition of mitosis was observed at all concentrations except 100 and 10 $\mu\text{g/ml}$. A closer range of concentrations, 200, 150, 100, 75, and 50 $\mu\text{g/ml}$, were employed and tested for the same findings. Mitosis was stopped only in the cells dosed at 200 $\mu\text{g/ml}$.

FDA compound 71-41, hydrated Calcium Silicate, was also tested for mutagenic properties in a host-mediated assay using the microorganisms *Salmonella* TA-1530 and G-46 and *Saccharomyces* D3. These experiments were carried out in mice orally administered (acute and subacute) 15, 150, and 1500 mg/kg of Calcium Silicate. No increased mutation frequencies were seen in *Salmonella* TA-1530 or G-46. *Saccharomyces* D3 had no significant increase in recombinant activity. In fact, a reduction in recombinant activity was produced by the compound. In a second host-mediated assay, Calcium Silicate was administered at 5000 mg/kg to mice against *Salmonella* TA-1530 and G46 and *Saccharomyces* D3. All tests were negative.

Cytogenetic studies in vivo examined bone marrow cells arrested in C-metaphase from rats exposed to FDA compound 71-41, Calcium Silicate. Rats were administered 15, 150, and 1500 mg/kg doses. The positive-control was triethylene melamine (TEM) and the negative-control was saline. The chromosomal abnormalities observed in the positive-control animals were significantly greater than those of either the negative control or the compound. The maximum effect of the positive control was observed at 48 h after administration. Calcium Silicate produced breaks in the range of 1% to 3% in all three acute dosage levels. However, these were not significantly higher than the negative controls. The subacute dose of 150 mg/kg produced breaks at 3%. The negative-control breaks were consistent with those of other experiments.

These same cytogenetic tests were observed in vitro. Cells (not specified) were observed in anaphase for chromosomal aberrations such as bridges, pseudochiasmata, multipolar cells, acentric fragments, etc. Doses of Calcium Silicate were as follows: 1.0, 10.0, and 100.0 $\mu\text{g/ml}$. Controls, both positive and negative, were the same as reported above. The positive control produced significantly greater percentages of chromosomal aberrations than the negative control or test compound. There were no aberrations observed due to Calcium Silicate.

In a third cytogenetic test, Calcium Silicate was administered to male rats in one dose and in five doses of 5000 mg/kg. A positive-control, TEM, and a negative-control, saline, were also tested. Metaphase spreads were prepared from the bone marrow cells of these animals and scored for chromosomal aberrations.

Neither the variety nor the number of the aberrations differed significantly from the negative controls. Calcium Silicate was nonmutagenic.

Dominant lethal assays were carried out in male rats administered FDA compound 71-41, hydrated Calcium Silicate, at doses of 15, 150, and 1500 mg/kg, both as one dose and as five doses. Also tested were the negative saline control and a positive TEM control. This assay measures the amount and type of fetal wastage that may occur following administration of a potential mutagen. Each treated male rat was mated with two virgin female rats each week for eight (acute) or seven (subacute) doses. Two weeks after mating, the female rats were sacrificed and the fertility index, preimplantation loss, and lethal effects were determined and compared with the same parameters calculated from the negative and positive controls. No significant findings were observed in the fertility index or preimplantation loss. The test compound was also administered at a dose of 5000 mg/kg. The protocol was the same as listed above. All parameter values did not differ significantly from that of the negative control. Comparing the data of both experiments indicates that hydrated Calcium Silicate does not induce dominant lethal mutations (Litton Bionetics, Inc., 1974).

Hectorite

Hectorite suspended in dimethylsulfoxide (DMSO) at concentrations of 10 to 3000 $\mu\text{g/plate}$ was subjected to spot test using five mutant strains of *Salmonella typhimurium* LT2, hisTA98, hisTA100, hisTA1535, hisTA1537, and hisTA1538, with and without metabolic activation. Positive controls were carried out utilizing Aroclor 1254. Hectorite was nonmutagenic in all five test strains (Inveresk Research International 1995).

Magnesium Aluminum Silicate

MAS was subjected to spot test using five mutant strains of *S. typhimurium* LT2, hisTA98, hisTA100, hisTA1535, hisTA1537, and hisTA1538. Positive and negative controls were carried out utilizing S9 mitochondrial preparations from the livers of Sprague-Dawley rats and 2-aminoanthracene. MAS was found to be nonmutagenic in all five test strains (Blevins and Taylor 1982).

Zeolite

Durnev et al. (1993) tested the clastogenic potential of Zeolite particles <10 μm in length in peripheral human blood lymphocytes. Chrysotile fibers were used as a positive control. Both fibers produced statistically significant increases in the percentage of aberrant metaphases, mostly from chromatid breaks. Superoxide dismutase (50 $\mu\text{g/ml}$) protected against the induction of aberrant metaphases by chrysotile asbestos, but not by Zeolite. However, catalase (20 $\mu\text{g/ml}$) protected against induction of aberrant metaphases by Zeolite, but not by chrysotile asbestos.

Chromosomal aberrations in cells of C57BL/6 mice were also investigated. The cells were collected by peritoneal lavage and

from the bone marrow of mice and were sampled at 1, 2, 7, and 28 days after the intraperitoneal injection of 100 $\mu\text{g}/\text{mouse}$ natural Zeolite particles. Chrysotile asbestos was used as a positive control. The lavage sample contained 20% lymphocytes, 20% to 30% macrophages, and 50% to 60% PMN leukocytes. The injection of the Zeolite induced a statistically significant increase in aberrant metaphases after 7 and 28 days in the peritoneal lavage cells. Chrysotile induced the aberrant metaphases at all times in both the peritoneal lavage and bone marrow cells (Durnev et al. 1993).

Valatina, Pylev, and Lemjasev (1994), tested the clastogenic effect on bone marrow cells of five dust samples from Zeolite tuffs. Presterilized dusts were administered intraperitoneally to BALB/C mice. The known clastogen mitomycin C was used as a positive control and 0.5 ml of saline as a negative control. The animals were killed 24 h after administration and mice bone marrow samples were taken. Polychromatophilic erythrocytes (PCEs), which contain micronuclei that are formed during mitosis on acentric fragments of the chromosomes as a result of clastogenic actions, were counted. Many of the dust samples were as potent a clastogenic agent as mitomycin C. A summary of the results is listed in Table 21.

CARCINOGENICITY

The IARC (1997) has placed Attapulgitic fibers $>5 \mu\text{m}$ in Group 2B, *possibly carcinogenic to humans*. Fibers $<5 \mu\text{m}$ cannot be classified as to their carcinogenicity to humans and were classified in group 3. The Utrecht University's Institute for Earth Sciences and Vening Meinesz Institute for Geodynamic Research (Engelhard 1998) analyzed Engelhard's Attapulgitic clay by transmission electron microscopy to determine the fiber length. The transmission electron microscopic analytical results was $<5 \mu\text{m}$.

TABLE 21

Micronuclei induced by Zeolite tuffs (Valatina, Pylev, and Lemjasev 1994)

Administered substance	Dose (mg/g)	Amount of PCEs with micronuclei (per 1000 PCEs)
Dust 1	2.0	8.33 ± 0.5
	0.8	5.83 ± 0.5
Dust 2	1.4	2.83 ± 0.3
	2.1	3.83 ± 0.6
Dust 3	3.15	0.5 ± 0.8
	1.26	3.8 ± 0.5
Dust 4	2.15	6.7 ± 0.5
	.86	5.2 ± 0.5
Dust 5	3.25	4.83 ± 0
	1.3	3.66 ± 0.5
Mitomycin C	0.16 mg/kg	7.70 ± 0.3
Saline control	0.5 ml	2.70 ± 0.03

Clinoptilolite, Phillipsite, Mordenite, Nonfibrous Japanese Zeolite, and synthetic Zeolites *cannot be evaluated as to their carcinogenicity to humans* (group 3) according to the IARC (1997).

Table 22 is a summary of carcinogenicity data, which were detailed earlier in the section *Animal Toxicology*.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation

Magnesium Aluminum Silicate

Applications of 2 g of VEEGUM were made to the skin of two human subjects in an 1-inch area daily for 1 week. No effects were noted and no other details were given (Munch 1944).

Inhalation

Aluminum Silicate

Musk et al. (1980) surveyed 17 workers exposed to the Aluminum Silicate dust, alunite. Respiratory questionnaires and occupational history, pulmonary function testing, and posterior-anterior chest radiographs were obtained. The alunite chemical analysis was that 48.5% of it was Al_2O_3 and 35.0% was SiO_2 . The average age of the subjects was 29.1 years. The mean transfer factor for carbon monoxide (T_L) predicted for the whole group was 85.8% and the mean ratio of T_L to effective alveolar volume (V_A) was 83.8%. The actual group T_L and T_L/V_A was less than predicted. Overall, the group had comparable predicted levels of forced expiratory volume (FEV) in 1 second, vital capacity (VC), and total lung capacity (TLC). Two subjects had small irregular opacities on chest films. Neither of these subjects had previous exposure.

Attapulgitic

Churg (1983) surveyed the total pulmonary nonasbestos mineral content in 20 patients who had no occupational dust exposure. The lungs were autopsied and 3- to 5-g pieces were dissolved in bleach and the treated sediment was transferred to an electron microscope grid. Mineral fibers were identified using electron diffraction and energy dispersive x-ray spectroscopy. No correlations were between numbers or types of fibers and age, sex, or smoking. Attapulgitic was identified in 12/20 patients and approximately 8400/106000 fibers (7.9%) were Attapulgitic. Further mineralogical analysis revealed 100% of the Attapulgitic fibers were 1 to 4.9 μm in length.

Kaolin

Churg (1983) surveyed the total pulmonary nonasbestos mineral content in 20 patients who had no occupational dust exposure. The lungs were autopsied and 3- to 5-g pieces were dissolved in bleach and the treated sediment was transferred to an electron microscope grid. Mineral fibers were identified using electron diffraction and energy dispersive x-ray spectroscopy. No correlations were between numbers or types of fibers and

TABLE 22
Summary of carcinogenicity data

Procedure	Dose/concentration	Result	Reference
Aluminum Silicate			
Single intrapleural injections of four samples into rats (lived life span)	20 mg (0–40 μ m)	3 malignant mesotheliomas (1 pleural and 2 peritoneal)	Pigott and Ishmael 1992
Calcium Silicate			
Single intraperitoneal injections into rats (lived life span)	25 mg	Little dust or dust-related fibrosis was visible; no mesotheliomas	Bolton et al. 1986
Chronic inhalation exposure for 1 year in rats	10 mg/m ³	Interstitial fibrosis, 1 small squamous cell carcinoma, 1 adenoma in lungs	Bolton et al. 1986
Attapulgate			
Single intraperitoneal injections into rats	25 mg	Tumor incidence rate was 67%	Pott, Huth, and Friedrichs 1974
Single direct pleural application to left pleural surface of rats (killed 2 years later)	40 mg	17/615 of treated rats developed pleural sarcomas	Stanton et al. 1981
Single intrapleural injections into rats (lived life span)	20 mg/ml of 0.9% NaCl (0.77 μ m)	No mesothelial neoplasms in either control or treated rats	Jaurand et al. 1987
Single intraperitoneal injections into rats (lived life span)	No concentrations given (fiber lengths ranged from 0 to 25 μ m)	46 mesotheliomas	Wagner, Griffiths, and Munday 1987
Single intrapleural injections into rats (lived life span)	20 mg (0.77 μ m)	No mesotheliomas	Renier et al. 1989
Single intrapleural injections into rats (lived life span)	0.5, 2, 4, 8, 16, or 32 mg (<1 μ m)	2/140 had mesotheliomas	Coffin, Cook, and Creason 1992
3 samples were injected one time each week for 9 weeks into rats (surviving animals were killed at 2.5 years)	60 mg (0.04 to 0.8 μ m)	Noncarcinogenic results for all three samples	Pott et al. 1987
Single intraperitoneal injections were administered for 3 weeks in rats (killed at 2.5 years)	2, 4, and 4 mg (1.3 and 0.07 μ m)	40% of 30 rats had neoplasms	Pott et al. 1987
Inhalation chamber exposure to rats for 6 h/day for 5 day/week (killed at 3, 6, and 12 months)	10 mg/m ³	2 mesotheliomas, 2 peritoneal mesotheliomas, 1 malignant alveolar neoplasm, 2 benign alveolar neoplasms, 11 bronchoalveolar hyperplasias	Wagner, Griffiths, and Munday 1987
Zeolite			
Oral administration for 104 weeks in rats	1, 10, 100, or 1000 mg/kg	No incidence of neoplastic changes	Glohuber et al. 1983
Single intratracheal instillations into rats (killed at end of study)	30 and 60 mg (< 5 μ m)	No significant increase in the incidence of any specific neoplasm	Tatrai and Ungvárý 1983
Single intraperitoneally injections into mice (10 month study)	10 or 30 mg (< 5 μ m)	No neoplastic changes were observed	Suzuki 1982
Single intraperitoneal injection into mice	10 mg (<3 μ m)	Mild peritoneal fibrosis but no neoplasms	Suzuki and Kohyama 1984

(Continued on next page)

TABLE 22
Summary of carcinogenicity data (*Continued*)

Procedure	Dose/concentration	Result	Reference
Single intraperitoneal injections into mice (7–23-month exposure)	10 mg (2.24 μm)	No mesotheliomas observed	Suzuki and Kohyama 1984
Single intrapleural injection into rats (chronic study)	20 mg	1 pleural and 1 peritoneal mesothelioma	Wagner et al. 1985
Single intraperitoneal injections into rats (141 weeks)	25 mg	1 peritoneal mesothelioma	Maltoni and Minardi 1988
Single intrapleural injections in rats	25 μm	No difference in tumor incidence between control and treated groups	Maltoni and Minardi 1988
Single subcutaneous injections	25 μm	No difference in tumor incidence between control and treated groups	Maltoni and Minardi 1988
3 intrapleural injections were given in monthly increments to rats	20 mg (3 to 500 μm)	2 mesotheliomas and 1 bronchial carcinoma/93 treated animals	Pyev et al. 1986
3 intrapleural injections were given in monthly increments to rats	20 mg (5 to 100 μm)	Neoplasms were found in 41/101 animals	Pyev et al. 1986
Inhalation exposure to rats for 7 h/day, 5 days/week for 1 year (lived life span)	10 mg/m ³	1 mesothelioma and 1 pulmonary adenocarcinoma	Wagner et al. 1985

age, sex, or smoking. Kaolin was identified in 12/20 patients and approximately 3500/106000 (3.3%) fibers were Kaolin. Further mineralogical analysis revealed 94% of the Kaolin fibers were 1 to 4.9 μm in length.

Morgan et al. (1988) surveyed and studied the prevalence of ventilatory impairment, chest symptoms, and radiographic abnormalities in over 2000 Kaolin workers representing over 95% of the current employees in the industry. Of the participants, 19% admitted having a cough. Of those participants with a cough, 17% had an abnormal FEV and 14% had an abnormal VC. Of those without a cough, 5.5% had an abnormal FEV and 7% had an abnormal VC. Also, 18% of the participants admitted to chronic sputum production. Of those with sputum production, 16% had abnormal FEV, and 12.5% had abnormal VC. Of those without the production, 6% had an abnormal FEV, and 7.5% had an abnormal VC. About 30% of the participants complained of shortness of breath, 3.1% was classified as severe. Wheezing was reported by 29% of the subjects. Satisfactory chest films for 2069 of the subjects were available for examination. Radiographic findings of 90 subjects revealed simple pneumoconiosis. Of these cases, 3.16% had category 2 pneumoconiosis, 1.0% had category 5, and 0.25% had category 3. Eighteen subjects (0.89%) had complicated pneumoconiosis. Of these cases, five had stage A, eight had stage B, and five had stage C. Of men with either case of pneumoconiosis, 51.1% were dry processors, compared to 6.3% of the men who worked in wet processing. Of the non-smoking participants (549), 542 and 537 men had a satisfactory FEV and forced vital capacity (FVC), respectively, in addition to an acceptable chest radiograph. Of these nonsmoking workers,

516 were studied for dust exposure and pulmonary function. Among the nonsmokers with no pneumoconiosis, those persons working in calcined clay had a greater prevalence of lung function abnormalities. This group had a significant increase in the risk of having an abnormal FEV but tended to have less incidences of pneumoconiosis. In short, ventilatory impairment was related to the presence of complicated pneumoconiosis, employment in clay calcining, and cigarette smoking. Also work in dry processing was associated with a greater risk of developing pneumoconiosis (Morgan et al. 1988).

Waxweiler et al. (1988) evaluated the possible health effects of occupational exposure to Attapulgit. A cohort study of 2302 men employed for at least 1 month at an Attapulgit mining and milling facility was followed through 1975. A significant deficit of mortality from nonmalignant respiratory disease (NMRD) was observed based on age, calendar year, and rates was observed. A marked deficit of NMRD was seen regardless of presumed dust exposure level, induction-latency period, or duration of employment. A statistically significant excess of mortality from lung cancer was observed among whites, but a deficit occurred among nonwhites. Lung-cancer risk in either race was not altered substantially with presumed dust exposure level, induction-latency period, or duration employed, with one exception—those employed for at least 5 years in high-exposure-level jobs. An increased mortality was observed for gastric cancer (six observed) and a deficit due to nonmalignant respiratory disease was observed (nine observed).

The lungs of 62 recently deceased men between the years of 1968 to 1981 were taken for an assessment of the severity

of lung disease (Wagner et al. 1996). Fifty-four of the 62 men worked with china clay or china stone. All the test subjects were employed in the mining industry. Test subjects were divided into groups according to their contact with the minerals: dusty china clay; wet, nondusty china clay; china stone; other dusty environments. The authors of this publication define china clay as "consisting mainly of the mineral kaolinite and in most other countries it is referred to as Kaolin." China stone "consists essentially of a mixture of quartz, feldspars, micas, and amorphous silicon dioxide." Chest radiographs were available for 39 of the 62 cases. Sections of lung tissue were examined microscopically for nodular and interstitial fibrosis and an overall grade ranging from 0 (none) to 3 (severe). Samples from 42 cases were analyzed for mineral content by x-ray diffraction and lung-dust concentrations.

Radiographic lesions included 13 cases of progressive massive fibrosis and 22 cases of simple pneumoconiosis. Only four cases had no evidence of any disease. Nodular opacities tended to reflect a high quartz content, whereas high-Kaolin lung content had interstitial changes and irregular radiological changes.

Mineralogical analysis of the 42 cases revealed two separate groups of mineral composition and one miscellaneous group. The china clay group was composed of $\geq 90\%$ Kaolinite in its samples consisted of 16 cases. The other distinct group, the clay and stone group, was composed of $< 90\%$ Kaolinite and greater contents of subsidiary components including quartz comprised 16 cases. The other group had a large variation of mineral composition. Lung-dust concentrations were greatest in the china clay group as shown in Table 23.

The grades of nodular fibrosis ranged in the china clay group from 0 (none) to 2 (moderate—up to 7 nodules/section or nodules of 3 to 6 mm in diameter). In china stone/clay group half, 8 of 16, were grade 3 (severe—more than 7 nodules/section or 6 to 10 mm in diameter). An increasing quartz concentration appears to be related to nodular fibrosis. Interstitial fibrosis in group ranged from 1 (slight—fibrosis located around respiratory bronchioles, which may extend into alveolar ducts and adjacent alveoli, but with areas remaining free of fibrosis between adjacent respiratory bronchioles) to 3 (severe—widespread diffuse fibrosis with few recognizable alveoli; honeycomb may or may not be present). No correlation was found between Kaolinite concentration and interstitial fibrosis grades; however, the china

clay group had little exposure to anything but china clay. The degree of interstitial fibrosis appears to be more related to dust lung concentrations, although these results failed to reach statistical significance (Wagner et al. 1996).

The ACGIH does not classify Kaolin as a human carcinogen and gives a TLV-TWA of 2 mg/m^3 for respirable dust and total dust (ACGIH 1997).

Zhang, Zhang, and Song (1997) reported the results of environmental monitoring and health surveillance performed on 781 Pyrophyllite miners and Pyrophyllite dust carvers from the years of 1954 to 1986. Routine radiographs of the workers lungs were studied for lesions of pneumoconiosis. The PM workers were divided into three groups, manual drillers (A), mechanical dry drillers (B), and mechanical wet drillers (C). The PCM workers were divided in two groups, carvers in factories (A) and carvers working at home (B).

PM workers, group B, had a greater incidence (43.5%) of pneumoconiosis than all other groups. In order to exclude the effect of the duration of exposure (DE), the DE-adjusted prevalence rate was calculated. The DE-adjusted rates are as follows, PM groups, 36.6% and PCM groups, 14.4% of pneumoconiosis (Zhang, Zhang, and Song 1997).

Case Reports

Aluminum Silicate

Sherwin (1979) found abnormal numbers of birefringent particles in the lungs of seven patients: five vineyard workers, one farmer, and one rural resident. A spectrum of early-to-late interstitial inflammation and fibrosis were seen. Nodular granulomas seen in silicosis were absent. Mineralogical analysis revealed mostly silicates, i.e., aluminum and potassium silicate.

Musk, Greville, and Tribe (1980) reported a case of a 42-year-old woman who had no history of previous exposure to Aluminum Silicate dust until she started working at an alunite-residue bagging mill. Chemical analysis of the alunite-residue showed 48.5% of constituents to be Al_2O_3 and 35.0% to be SiO_2 . Eight months after working, she noticed the onset of dry cough and shortness of breath. Within 3 months these signs lasted throughout the day. She remained working for 18 months and after leaving work, the cough completely subsided within 3 months. She also complained of pain and morning stiffness in joints, wrists, elbows, and right knee. Corticosteroid treatment was started after a lung biopsy. A chest film taken 3 months after the onset of symptoms had lesions of diffuse small irregular opacities throughout both lungs. Subsequently, pulmonary function tests revealed a decrease in transfer factor for carbon monoxide (TL) and effective alveolar volume (TL/VA) and abnormal transpulmonary pressure–lung volume relationships. Pulmonary lesions included examination interstitial infiltration with small round cells, variable fibrosis, and scattered granulomas. Alveoli were distorted and the granulomas were moderately well formed with multinucleate giant cells and epithelioid histiocytes. After corticosteroid treatment, no increase in severity of the lung lesions was seen.

TABLE 23

Dust concentrations in lung tissue of deceased men who worked in the mining industry (Wagner et al. 1996)

Mineral group	Lung dust concentrations (mg/g)		
	Minimum	Maximum	Median
China Clay (a)	7.6	289.3	40.0
China Stone/Clay (b)	4.1	44.8	15.0
Miscellaneous (c)	1.6	28.7	6.5

Calcium Silicate

A 23-year-old man was involved in the bagging process of a food additive. The food additive produced a white thin layer of powder that continuously covered the work floor. An antibiotic, carboxymethylcellulose, and Calcium Silicate comprised the food additive. On the third day of working, the patient experienced an itchy eruption on his face, neck, and forearms. The rash was erythematopapular with no vesicles. The redness was not diffuse and patches of erythema and papules were confluent on the neck and forearms. All signs faded the following morning. The rash occurred again when the patient returned to work. Patch tests were performed using the food additive, an antibiotic, carboxymethylcellulose, and Calcium Silicate. All tests were negative and there were no clinical signs of irritation at the test sites. No late reaction was recorded either. A sample of the food additive was examined under the microscope. Analysis revealed sharp-edged particles corresponding to Calcium Silicate. It was determined that the Calcium Silicate dust caused an "airborne irritant contact reaction." The problem was eliminated by increasing the humidity in the workplace and aspirating the air (Lachapelle 1984).

Bentonite

Phibbs, Sundin, and Mitchell (1971) reported many case studies involving Bentonite workers. Some milling plants had dangerous concentrations of silica that ranged from 2 to 10 times the safe maximal concentration according to the U.S. Bureau of Mines. Silicotuberculosis developed in four patients studied.

Austin and Doughman (1980) reported a 20-year-old dental assistant who noted a foreign body in her right eye after using a drill to polish a patient's teeth with Prophypaste. Immediately she noticed decreased vision and photophobia. Several opaque deposits superficially embedded in her right cornea were removed within 2 h. There was no evidence of corneal perforation or iritis. A residual superficial corneal infiltrate was noted paracentrally. An anterior uveitis developed and was treated. One month after the injury, the cornea was edematous with a superficial, peripheral ringlike stromal infiltrate and a deep inferior stromal infiltrate. A retrocorneal abscess was present. There was no eyelid edema present. Culture results were negative. Anterior segment inflammation, progression of the corneal edema, and an enlarged ring abscess in the corneal stroma continued. There was complete loss of red reflex and iris detail. The diagnosis was infectious endophthalmitis and anterior chamber and vitreous aspirations were performed. No organisms were seen but a few PMN leukocytes were present in the aspirations. These authors undertook the toxicity studies in rabbits presented in the ocular animal toxicity section under Bentonite. They concluded that the similarity of the findings in animals after injection of Bentonite with the findings in this case report suggested that Bentonite was the responsible agent in the dental assistant's symptoms.

Fuller's Earth

Tonning (1949) reported a man having worked in a Fuller's Earth plant as a young man. The length of employment was estimated at no more than 15 years. He was diagnosed with terminal aspiration pneumonia, pneumoconiosis due to Fuller's Earth exposure, bilateral emphysema, and fibrous pleural adhesions. Lesions differed from typical silicotic lesions of the lungs; no formations of the whorled, acellular collagen typical of silicotic nodules were observed. Isolated cavities in the apices were filled with black sludge and surrounded by vascular and cellular collagen. The dust in the lymph nodes had only stimulated the formation of reticulin fibers. No subpleural nodules were present. At mineralogical analysis, the Fuller's Earth deposits were constituted mainly of Montmorillonite (85.2% to 90%).

Sakula (1961) reported two cases of pneumoconiosis due to Fuller's Earth (Table 24). Mineralogical analysis of the Fuller's Earth established Montmorillonite as the major component.

Kaolin

Lynch, Harrison, and Nagelschmidt (1954) investigated two case studies of men who worked in a Kaolin-processing plant for many years. The lungs of the two persons and chest x-ray films were evaluated. The first case was a 36-year-old man who worked on the plant for 17 years. Chest films were taken at the end of his career and detected lesions of extensive confluent consolidation and nodule formation of advanced pneumoconiosis with infection. Autopsy and microscopic findings included alveolar spaces uniformly expanded, three areas of whorled fibrous tissue, scattered areas of cystic spaces, hilar nodes heavily pigmented, deposits of brownish black particulate matter, a large vessel with recent thrombus, hemorrhage, and necrosis, marked fibrous thickening of the pleura, and dense fibrous scarring of the lymph nodes. The final diagnosis was pneumoconiosis (kaolinosis) with pulmonary thrombosis and infarction of the lungs. The second case study was a 35-year-old man who worked in a Kaolin-processing plant for 21 years. Within his last 3 years, he had dyspnea and a slight cough with small

TABLE 24
Pneumoconiosis cases reportedly linked to exposure to Fuller's Earth (Sakula 1961)

Patient	Symptoms
Male who worked in a Fuller's Earth processing plant for 42 years	Fine to medium miliary mottling of both lungs; sputum examinations were negative for <i>M. tuberculosis</i> ; slowly deteriorating pulmonary function; recurrent bronchitis
Male who worked for 28 years in milling	Chronic cough and sputum; fine miliary mottling throughout both lungs; increasing dyspnea

TABLE 25
Pneumoconiosis cases reportedly linked to exposure to Kaolin (Hale et al. 1956)

Patient	Symptoms	Diagnosis
44-year-old man; worked in a Kaolin mill for 28-years	Cough with thick white sputum; easily dyspneic on slight exertion; well-marked nodulation of silicotic type with coalescence of the nodules in several areas and emphysema	Pneumoconiosis
67-year-old man; worked in china clay bagging for nearly his entire life	Several years of a productive cough; emphysema; massive fibrosis on both sides; no evidence of neoplasm	Pneumoconiosis
44-year-old man; worked in china clay bagging for nearly his entire life	Diffuse nodular mottling with considerable attenuation of the bronchovascular markings	Pneumoconiosis
39-year-old man; worked 14 years with clay	Fine miliary mottling in both lungs; well-marked calcification at the left hilum	Pneumoconiosis
73-year-old man; worked 12 years in open limestone quarries	Small discrete nodular mottling with an increase in the root shadows and the lung markings	Pneumoconiosis
64-year-old man; 43 years loading china clay	Cough and shortness of breath; emphysema; definite nodular mottling	Pneumoconiosis

amounts of dark colored sputum. The sputum was negative for bacteria. Chest films revealed advanced pneumoconiosis with infection, confluent consolidation, nodular infiltration, cavitation, and emphysema. Autopsy and microscopic findings included nodules in the right and middle lobes, pleural spaces were thickened and shaggy, large bulbous emphysematous blebs, a pulmonary artery with organizing thrombus, heavily pigmented hilar lymph nodes, whorled fibrous collagenous tissue, and spaces and walls with macrophages. The final diagnosis was pneumoconiosis (kaolinitis).

Hale et al. (1956) reported six cases of pneumoconiosis due to Kaolin. These are given in Table 25 and not further discussed here.

Butz (1970) reported that a 47-year-old man who was a chronic intravenous drug user died from tetanus. The man had been injecting paregoric, a camphorated opium tincture containing 35 to 46 mg of morphine per 100 ml. Paregoric can be found in proprietary preparations that do not require prescriptions; intravenous drug users often attempt to separate the paregoric from the Kaolin. Often the injection of Kaolin, either through shunts in the lung of an intravenous drug user with obliterative pulmonary arteritis and angiomatoid formations or by extrusion from the arterial lumen and transfer to the pulmonary veins, allows the Kaolin crystals to go into the peripheral circulation. In this patient, numerous skin abscesses were noted on the neck, shoulders, upper extremities, chest, thighs, and lower extremities. In skin sections, the lesions were multiple foreign body granulomata and large birefringent crystals. Adhesions over the pleural surface of the lungs were also noticed. At microscopic examination the lungs had foreign body granulomata within the pulmonary arterioles. Extensive pulmonary edema and masses of pigmented histiocytes filled the alveolar spaces. Extensive periportal fibrosis was seen in the liver. The central nervous system lesions were extremely fine, double refractile particles in nerve bundles entering the anterior roots in the central region.

Herman, Olscamp, and Weisbord (1982), reported a patient with multiple pulmonary Kaolin granulomas. The man had a history of bilateral recurrent pneumothorax. Both pleural spaces were destroyed with a suspension of liquid Kaolin. Recurrent right-sided pneumothorax devolved and reobliteration was again performed. In a follow-up chest radiograph, multiple well-defined peripheral nodules were in both lungs and pathological analysis revealed a bland acellular material surrounded by chronic inflammatory cells. By light microscopy, the particles were consistent with Kaolin. It was presumed that Kaolin entered the lungs through pleuroalveolar or pleurobronchial openings.

Lapenas and Gale (1983) reported that a 35-year-old man who worked at a Kaolin-processing plant for 17 years complained of chest pain and was hospitalized. For the previous 2 years before admittance, the man had packaged dried, processed Kaolin. Chest films revealed diffuse reticulonodular pulmonary infiltrates and a well-defined, noncalcified mass in the upper right lobe. A thoracotomy was performed and an 8 × 12 × 10-cm conglomerate pneumoconiotic lesion containing large amounts of Kaolin was found. X-ray diffraction material from the lesion had peaks corresponding to Kaolinite. The presence of silica was not confirmed by x-ray diffraction.

Lapenas et al. (1984) obtained pulmonary tissue from five Kaolin workers with advanced pneumoconiosis. Chest radiographs detected small irregular shadows and large opacities typical of Kaolin pneumoconiosis. At autopsy, firm, grey-brown nodules and masses were in the parenchyma and in the hilar lymph nodes. Microscopic lesions were extensive pulmonary Kaolinite deposition associated with the formation of peribronchiolar nodules. The nodules were comprised of Kaolinite aggregates transversed by bands of fibrous tissue rather than dense whorled collagen. Kaolin was detected in the lungs. Silica was not detected by either analytical scanning electron microscopy or x-ray diffractometry.

Levin et al. (1996) investigated the death of a 62-year-old man who worked in a cotton textile mill for 43 years. The patient complained of progressive dyspnea and a productive cough. After being admitted to the hospital, a bronchoscopy was performed and no endobronchial lesions were found. A lung biopsy had lesions of severe interstitial fibrosis with bronchioalveolar structures extensively involved in the fibrotic process. Pathological alterations such as bronchiolectasis, interstitial fibrosis with thickening of alveolar septa, mobilization of macrophages, and multinucleated giant cells were identified. Neither ferruginous bodies nor pleural hyaline plaque was identified. Kaolin particles were present with a mean size of $0.88\ \mu\text{m}$. Chrysotile asbestos was also detected, but the majority of particles were Kaolin. The man died as a consequence of respiratory failure despite an aggressive therapy of antibiotics and tuberculosis therapy.

Magnesium Trisilicate

Lee et al. (1993) reported a case of a 30-year-old female with a long-term history of ingesting trisilicate-containing antacids. The patient had repeated attacks of renal colic but the presence of calculi could not be determined by intravenous pyelography nor ureteroscopy. X-ray diffraction did detect a silicate stone. The patient stopped taking trisilicate containing products. The frequency of stone passage decreased and the renal colic was relieved.

Montmorillonite

A 73-year-old Montmorillonite worker developed signs of pneumoconiosis. A chest radiograph was taken 2 years before his death and a bilateral fine reticulonodular shadowing was observed. The man died of acute gastrointestinal hemorrhage from a benign gastric ulcer. A few weeks before his death another chest radiograph indicated a slight increase in the reticulonodular opacities and a mass at the left hilum and apex. At autopsy, numerous soft stellate grey-black dust lesions 4 to 5 mm in diameter that occupied most of the lungs were found. No lesions of progressive massive fibrosis were identified. Also present were lesions of severe emphysema and a 4-cm diameter neoplasm arising from the bronchus of the left upper lobe. At microscopic examination, numerous interstitial collections of dust-laden macrophages were situated around the respiratory bronchioles and along the adjacent alveolar septa. There was a slight degree of fibrosis associated with the dust lesions and the neoplasm was a poorly differentiated adenocarcinoma containing giant cell areas. Mineralogical analysis showed a large amount of calcium Montmorillonite (Gibbs and Pooley 1994).

Zeolite

Casey et al. (1985) reported a patient living in the Nevada desert who developed extensive pleural thickening and interstitial fibrous associated with the pulmonary deposition of Zeolite. An open biopsy of the right lung and pleura was performed on the 52-year-old man. Mycobacterial and fungal cultures were negative. Histopathological evaluation established lesions of chronic

inflammation and fibrosis and presence of many fibrous and nonfibrous particles. The particles were analyzed by SEM and were identified as aluminum silicates. The analytic pattern was characteristic of Zeolites. No asbestos fibers were found and exposure to these fibers was unlikely.

Zirconium Silicate

A nonsmoking 25-year-old woman developed a worsening dry cough and dyspnea after 3.5 years as a tile sorter and glazer. The woman had a history of atopic dermatitis and at age 13 developed pneumonia. An open lung biopsy specimen had lesions of a severe granulomatous interstitial pneumonia with mild fibrosis and numerous very small birefringent crystals around the terminal airways and occasionally in the granulomas. Pulmonary particle analysis established a dust burden almost 100 times the normal. The particles consisted mainly of clay minerals and Zirconium Silicate (Lippo et al. 1993).

SUMMARY

This report provides a review of the safety of Aluminum, Calcium, Lithium Magnesium, Lithium Magnesium Sodium, Magnesium Aluminum, Magnesium, Sodium Magnesium, and Zirconium Silicates, Magnesium Trisilicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite. These ingredients are termed silicates because they contain silicon, oxygen, and one or more metals. Many silicates occur naturally and are mined; yet others are made synthetically.

Typical cosmetic uses of silicates include abrasive, opacifying agent, viscosity-increasing agent, anticaking agent, emulsion stabilizer, binder, and suspending agent. Clay silicates (silicates containing water in their structure) primarily function as adsorbents, opacifiers, and viscosity-increasing agents. Pyrophyllite is also used as a colorant. Current concentrations of use range from as low as 0.01% for Zeolite to a high of 84% for Kaolin. Some ingredients with no uses reported to FDA in 1998 have current concentrations of use reported by the industry, so it is assumed they are in use.

Aluminum Silicate is approved as an indirect food additive in the Code of Federal Regulations (21 CFR 177.2600 and 21 CFR 177.1200). VEEGUM, a tradename for Magnesium Aluminum Silicate, has been designated by the FDA as a raw material with the following number: FD CRMCS no. R0010045 and has an individual Chemical Abstract Registry number, 12199-37-0. According to the European Cosmetic Directive (EU reference no. 391 Annex II), zirconium and its compounds are listed under substances that must not form part of the composition of cosmetic products, with the exception of complexes in Annex III, Part I. IARC has ruled Attapulgit fibers $>5\ \mu\text{m}$ as group 2B, *possibly carcinogenic to humans*, and fibers $<5\ \mu\text{m}$ as group 3, *not classified as to their carcinogenicity to humans* (IARC 1997). Bentonite is considered GRAS as a direct food additive (21 CFR 184.1155). Kaolin is considered GRAS as an indirect

food additive (21 CFR 186.1256). Pyrophyllite is listed as a naturally occurring color additive in the Code of Federal Regulations (21 CFR 73.1400). The natural Zeolites (Clinoptilolite, Phillipsite, Mordenite, Nonfibrous Japanese Zeolite) and synthetic Zeolites *cannot be classified as to their carcinogenicity to humans* (group 3) according to IARC (1997). Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Trisilicate, Attapulgite, Hectorite, and Kaolin are all used in over-the-counter products.

Hectorite and Montmorillonite catalyzed glycine and diglycine oligomerization reactions; oligomers were formed by self-condensation of both purines and pyrimidines in the presence of Montmorillonite treated with Na^+ . Under UV light, adenosine monophosphate molecules were absorbed onto Kaolin and the products were hydrolyzed by phosphodiesterase.

All silicates have the great ability to absorb, especially the clays. Reports describe drugs, bacteria, viruses, and toxins absorbed to clays due to the physical structure of clays and their cationic nature.

No statistically significant absorption of aluminum and elevated levels of silicon were recorded in assayed plasma samples of dogs given Magnesium Trisilicate and Zeolite orally. The urinary excretion of silica was 5.2% in males given 20 g of Magnesium Trisilicate. Ten percent Bentonite in the diets of rats overcame T-2 toxicosis completely. Various Zeolites were added to the diets of pigs. No adverse effects were noted by the supplementation.

A sample of Aluminum Silicate was toxic to pulmonary alveolar macrophages and LDH activity and β -GAL release were increased. Aluminum Silicate had relatively no effect on the hemolysis of rat RBCs. Synthetic Calcium Silicate samples and higher concentrations of Calcium Silicate caused increased hemolysis of human RBCs; a greater fibrous character of Calcium Silicate samples caused increased LDH and β -GAL release. Many clays (Attapulgite, Bentonite, Hectorite, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite) demonstrated cytotoxicity to several macrophage type cell lines and have hemolytic activity towards several species' RBCs. Particle size, fibrogenicity, concentration, and mineral composition had the greatest effect on toxicity. Larger particle size and longer and wider fibers cause more adverse effects. In most of the studies, a dose-dependent effect on cytotoxicity or lysis was observed. Most mineral samples were not 100% pure and many samples already contained toxic dusts or minerals like quartz or cristobalite.

The following are a list of acute oral LD_{50} determinations: Calcium Silicate, 3400 mg/kg in rats; Magnesium Aluminum Silicate, 50000 mg/kg in mice; Zirconium Silicate, >200 g/kg in mice; Hectorite, >5 g/kg in rats; Kaolin, 149 g/kg in rats (death due to bowel obstruction); 15 natural Zeolites, 10 g/kg in rats. In short-term oral toxicity studies, no adverse effects were seen in mice or rabbits dosed up to 5 g/kg Magnesium Aluminum Silicate; beagle dogs and rats fed Aluminum Silicate had no renal lesions. Dogs and rats fed Magnesium Trisilicate for 4 weeks had polydypsia and polyuria, and all dogs had renal

cortical lesions. Guinea pigs had renal lesions after 4 months of drinking Magnesium Trisilicate in their tap water. Rats fed 10% Magnesium Aluminum Silicate had slightly elevated silicon levels of the spleen and dogs and rats fed 10% VEEGUM had no negative responses in 90-day feeding studies. No lesions were found in rats dosed up to 1000 mg/kg for 104 weeks.

The following results are from acute parenteral injection studies. Intratracheal injections of Aluminum Silicate caused lesions in a dose-dependent manner and the intrapleural injections of four different Aluminum Silicate samples all resulted in lesions. One aluminosilicate injection caused three malignant mesotheliomas, one pleural and two peritoneal. No mesotheliomas developed in rats injected intraperitoneally with 25 mg of Calcium Silicate dust. Subcutaneous injection into the oral mucosa and into the back, periosteal injections into periosteal tissue, and intramuscular injections into the thigh of rats and guinea pigs with Zirconium Silicate resulted in mild inflammatory reactions. Attapulgite was injected intraperitoneally, intrapleurally, and intratracheally in various studies. Most studies reported that lesions and mesotheliomas were dependent on fiber length. Samples with a longer length caused greater numbers of mesotheliomas. Subplantar injections of Bentonite caused granulomas. Intratracheal injections of Bentonite and group C *Streptococcus* species caused an 85% mortality compared to a 5% control mortality in mice; another intratracheal injection caused loose reticulin fibrils with no collagen. Kaolin injected with the *Streptococcus* species caused statistically significant but modest mortality in mice. In a series of intrapleural injections, Kaolin was used as a negative control. Heat treated Montmorillonite dosed to rats by means of intratracheal instillation was restricted to alveoli within and adjacent to alveolar ducts. Minor inflammatory reactions, but no lesions, were found in rats given intratracheal injections of Clinoptilolite, and intraperitoneal injections of Mordenite, Synthetic Zeolite 4A, and synthetic Zeolite MS5A (one mesothelioma was seen in rats given MS4A). An intrapleural injection of Nonfibrous Japanese Zeolite caused two mesotheliomas in rats.

Small primary neoplastic lesions were found in two rats exposed to a Calcium Silicate sample in an inhalation chamber. The mass of silicate measured in the lungs ranged from 0.1 to 0.8 mg. Lebrija and Leichester Attapulgite samples caused one peritoneal mesothelioma, one adenocarcinoma, and three bronchoalveolar hyperplasia and two mesotheliomas, one peritoneal mesothelioma, one malignant alveolar tumor and eight bronchoalveolar hyperplasia (inhalation route) in rats, respectively. Both samples contained long fibers. Moderate to extensive respiratory disease was noted in rats chronically exposed to Synthetic Zeolite A by inhalation methods.

The acute dermal LD_{50} was >3.5 g/kg for rabbits exposed to VEEGUM. Magnesium Aluminum Silicate (4%) was a weak primary skin irritant in rabbits and had no cumulative skin irritation in guinea pigs. No gross effects were reported in any of these studies. Sodium Magnesium Silicate (4%) had no primary skin irritation in rabbits and had no cumulative skin irritation in

guinea pigs. Hectorite was nonirritating to the skin of rabbits in a Draize primary skin irritation study.

A 4% solution of Magnesium Aluminum Silicate and a 4% solution of Sodium Magnesium Silicate caused minimal eye irritation in a Draize eye irritation test. Bentonite caused severe iritis after injection into the anterior chamber of the eyes of rabbits. When injected intralamellarily, widespread corneal infiltrates and retrocorneal membranes were recorded. In a primary eye irritation study in rabbits, Hectorite was moderately irritating without washing and practically nonirritating to the eye with a washout. Rats tolerated a single dose of Zeolite A without any adverse reaction in the eye.

Calcium Silicate (250 to 1600 mg/kg) had no discernible effect on nidation or on maternal or fetal survival in rabbits. Magnesium Aluminum Silicate (6000 mg/kg) had neither a teratogenic nor adverse effects on the mouse fetus. Female rats receiving a 20% Kaolin diet exhibited maternal anemia but no significant reduction in birth weight of the pups was recorded. Type A Zeolite produced no adverse effects on the dam, embryo, or fetus in either rats or rabbits at any dose level (74 or 1600 mg/kg). Clinoptilolite had no effect on female rat reproductive performance.

No increase mutation frequencies were seen in the *Salmonella* TA-1530 or G-46 assay and no significant increase in recombinant activity in the *Saccharomyces* D3 assay treated with Calcium Silicate. A subacute dose of 150 mg/kg of Calcium Silicate produced 3% breaks in bone marrow cells arrested in c-metaphase. In a metaphase spread of bone marrow cells, Calcium Silicate produced no significant increase in the number of aberrations compared to controls and in a dominant lethal assay did not induce any dominant lethal mutations. In the *S. typhimurium* LT2 spot test (TA98, TA100, TA1535, TA1537, and TA1538) with or without metabolic activation, Magnesium Aluminum Silicate and Hectorite were found nonmutagenic. In primary hepatocyte cultures, the addition of Attapulgit had no significant unscheduled DNA synthesis (UDS) response or modulated response to AAF (a positive control); Attapulgit at 10 $\mu\text{g}/\text{cm}^2$ caused significant increases in UDS in rat pleural mesothelial cells. Zeolite particles (<10 μm) produced statistically significant increase in the percentage of aberrant metaphases, mostly chromatid breaks.

Applications of 2 g of VEEGUM made to the skin of two humans daily for 1 week caused no effects.

Occupational exposure to mineral dusts has been studied extensively. Fibrosis and pneumoconiosis has been documented in workers involved in the mining and processing of Aluminum Silicate, Calcium Silicate, Zirconium Silicate, Fuller's Earth, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite.

DISCUSSION

The CIR Expert Panel determined that the data provided in this report are sufficient to assess the safety of the tested ingredients: Aluminum Silicate, Calcium Silicate, Magnesium Alu-

minum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite. The Panel did note a concern about inhalation of these ingredients due to reported cases of pneumoconiosis and fibrosis in humans and pulmonary lesions in animals. However, extensive pulmonary damage in humans was the result of direct occupational inhalation of the dusts and lesions seen in animals were affected by particle size, fiber length, and concentration. The Panel recognizes that most of the formulations are not respirable and of the preparations that are respirable, the concentration of the ingredient is very low. Even so, the Panel considered that any spray containing these solids should be formulated to minimize their inhalation.

Note: The cosmetic ingredient, *Talc*, is a hydrated magnesium silicate with the chemical composition of $\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$. Talc occurs in various forms and has a unique crystalline structure which differs from ingredients addressed in this safety assessment. Talc is not included in this report.

CONCLUSION

The CIR Expert Panel concludes that Aluminum Silicate, Calcium Silicate, Magnesium Aluminum Silicate, Magnesium Silicate, Magnesium Trisilicate, Sodium Magnesium Silicate, Zirconium Silicate, Attapulgit, Bentonite, Fuller's Earth, Hectorite, Kaolin, Lithium Magnesium Silicate, Lithium Magnesium Sodium Silicate, Montmorillonite, Pyrophyllite, and Zeolite are safe as used in cosmetic products.

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Final Report on the Safety Assessment of Potassium Silicate, Sodium Metasilicate, and Sodium Silicate¹

Potassium Silicate, Sodium Metasilicate, and Sodium Silicate combine metal cations with silica to form inorganic salts used as corrosion inhibitors in cosmetics. Sodium Metasilicate also functions as a chelating agent and Sodium Silicate as a buffering and pH adjuster. Sodium Metasilicate is currently used in 168 formulations at concentrations ranging from 13% to 18%. Sodium Silicate is currently used in 24 formulations at concentrations ranging from 0.3% to 55%. Potassium Silicate and Sodium Silicate have been reported as being used in industrial cleaners and detergents. Sodium Metasilicate is a GRAS (generally regarded as safe) food ingredient. Aqueous solutions of Sodium Silicate species are a part of a chemical continuum of silicates based on an equilibrium of alkali, water, and silica. pH determines the solubility of silica and, together with concentration, determines the degree of polymerization. Sodium Silicate administered orally is readily absorbed from the alimentary canal and excreted in the urine. The toxicity of these silicates has been related to the molar ratio of $\text{SiO}_2/\text{Na}_2\text{O}$ and the concentration being used. The Sodium Metasilicate acute oral LD_{50} ranged from 847 mg/kg in male rats to 1349.3 mg/kg in female rats and from 770 mg/kg in female mice to 820 mg/kg in male mice. Gross lesions of variable severity were found in the oral cavity, pharynx, esophagus, stomach, larynx, lungs, and kidneys of dogs receiving 0.25 g/kg or more of a commercial detergent containing Sodium Metasilicate; similar lesions were also seen in pigs administered the same detergent and dose. Male rats orally administered 464 mg/kg of a 20% solution containing either 2.0 or 2.4 to 1.0 ratio of sodium oxide showed no signs of toxicity, whereas doses of 1000 and 2150 mg/kg produced gasping, dyspnea, and acute depression. Dogs fed 2.4 g/kg/day of Sodium Silicate for 4 weeks had gross renal lesions but no impairment of renal function. Dermal irritation of Potassium Silicate, Sodium Metasilicate, and Sodium Silicate ranged from negligible to severe, depending on the species tested and the molar ratio and concentration tested. Sodium Metasilicate was negative in the local lymph node assay (LLNA), but a delayed-type hypersensitivity response was observed in mice. Potassium Silicate was nonirritating in two acute eye irritation studies in rabbits. Sodium Metasilicate (42.4% H_2O) was corrosive to the rabbit eye. Sodium Silicate was a severe eye irritant in some eye irritation studies, but was irritating or nonirritating in others. A skin freshener containing Sodium Silicate was nonirritating. Sodium Metasilicate was nonmutagenic in bacterial cells. Rats given Sodium Silicate (600 and 1200 ppm of added silica) in the drinking water in reproductive studies produced a reduced number of offspring: to 67% of controls at 600 ppm and to 80%

of controls at 1200 ppm. Three adult rats injected intratesticularly and subcutaneously with 0.8 mM/kg of Sodium Silicate showed no morphological changes in the testes and no effect on the residual spermatozoa in the ductus deferens. Sodium Metasilicate (37% in a detergent) mixed with water was a severe skin irritant when tested on intact and abraded human skin, but 6%, 7%, and 13% Sodium Silicate were negligible skin irritants to intact and abraded human skin. Sodium Silicate (10% of a 40% aqueous solution) was negative in a repeat-insult predictive patch test in humans. The same aqueous solution of Sodium Silicate was considered a mild irritant under normal use conditions in a study of cumulative irritant properties. The Cosmetic Ingredient Review (CIR) Expert Panel recognized the irritation potential of these ingredients, especially in leave-on products. However, because these ingredients have limited dermal absorption and Sodium Metasilicate is a GRAS direct food substance, the Panel deemed the ingredients safe for use in cosmetic products in the practices of use and concentration described in this safety assessment, when formulated to avoid irritation.

INTRODUCTION

This report reviews the safety of silicate salts as used in cosmetic formulations. Because they are considered to have similar safety profiles, the following silicate salts are reviewed in this assessment: Potassium Silicate (CAS no. 1312-76-1), Sodium Metasilicate (CAS no. 6834-92-0), and Sodium Silicate (CAS no. 1344-09-8).

CHEMISTRY

These ingredients combine metal cations (potassium or sodium) with silica to form inorganic salts. A tabular presentation of chemical descriptions is provided in Table 1.

Physical and Chemical Properties

The properties, synonyms, and specifications are listed in tabular form in Table 2.

According to O'Conner (1961), pH determines the solubility of silica and, together with concentration, determines its degree of polymerization. At about pH 7, silica is only slightly soluble in water. At around pH 12, in a Sodium Metasilicate solution (0.1%), silica is very soluble and exists in monomeric form. At an intermediate pH, Sodium Metasilicate is partially neutralized; that is, it changes ratio and becomes a Sodium Silicate of $1\text{Na}_2\text{O}:\text{XSiO}_2$, where X is greater than unity. Conversely, a Sodium Silicate of the ratio $1\text{Na}_2\text{O}:\text{XSiO}_2$ could be converted to Metasilicate by the addition of alkali.

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TABLE 1
Ingredient descriptions

Ingredient	Description	Reference
Potassium Silicate	SiO ₂ :K ₂ O ratio varies	Budavari (1989)
	Potassium salt of silicic acid	Gottschalck and McEwen (2004)
Sodium Metasilicate	Na ₂ SiO ₃	Gottschalck and McEwen (2004)
	Inorganic salt	Gottschalck and McEwen (2004)
Sodium Silicate	Na ₂ O·xSiO ₂	Lide (1993)
	Sodium salt of silicic acid	Gottschalck and McEwen (2004)

Method of Manufacture

Soluble silicates (Sodium Silicate and Sodium Metasilicate) are manufactured by the reaction of silica sand and sodium carbonate (soda ash) at ~1400°C. Typically, a no. 1 grade of glass sand containing no more than 300 ppm iron and a medium density soda ash are used. Potassium Silicates are manufactured in a similar manner by the reaction of K₂CO₃ and sand (Kirk-Othmer 1982).

Sodium Silicates are either made by the high temperature fusion of silica sand (SiO₂) and soda (Na₂CO₃) at about 1300°C or by a hydrothermal process using silica sand and sodium hy-

droxide as starting materials. Solutions, termed "waterglass," are prepared by the solubilization of lumps of silicate salts in water at elevated temperatures and pressure. The water content of "waterglass" is between 45% and 80%. Powders are prepared by spray- or drum-drying of "waterglass" solutions. The residual water content can be between 0% and 25% (EUCLID 2000).

Impurities

Kirk-Othmer (1982) provided a range of trace elements in a typical Sodium Silicate solution as shown in Table 3. Impurity limits for Arsenic and Lead are shown in Table 2.

TABLE 2
Properties, synonyms, and specifications

<i>Potassium Silicate</i>		
Synonyms	Silicic acid, potassium salt	Gottschalck and McEwen (2004)
Form/description	Yellowish to colorless, translucent to transparent, hygroscopic	Budavari (1989)
Solubility	Insoluble in alcohol, slightly soluble in water	Budavari (1989)
<i>Sodium Metasilicate</i>		
Synonyms	Silicic acid, disodium salt	Gottschalck and McEwen (2004)
	Crystamet, disodium metasilicate, disodium monosilicate, Metso, water glass, sodium metasilicate anhydrous	RTECS (1999)
Form/description	Nonahydrate, efflorescent platelets	Budavari (1989)
Molecular weight	122.08	Budavari (1989)
pH	12 (0.1% solution)	O'Conner (1961)
Density	2.614	Budavari (1989)
Solubility	Insoluble in alcohol, acids, and salt solns.	Budavari (1989)
Melting point	1089°C	CTFA (2000a)
Forms	Anhydrous, pentahydrate, and nonahydrate	21 CFR 184.1769a
Impurity limits	Arsenic (as As) 3 ppm maximum	Nikitakis and McEwen (1990)
	Lead (as Pb) 20 ppm maximum	Nikitakis and McEwen (1990)
<i>Sodium Silicate</i>		
Synonyms	Silicic acid, sodium salt	Gottschalck and McEwen (2004)
	Sodium waterglass, waterglass, soluble glass, sodium silicate glass	EUCLID (2000)
Form/description	Colorless to white or grayish-white, crystal-like clumps or aqueous solutions	Budavari (1989)
pH	Strongly alkaline	Budavari (1989)
Impurity limits	Arsenic (as As) 3 ppm maximum	Nikitakis and McEwen (1990)
(40% solution)	Lead (as Pb) 20 ppm maximum	Nikitakis and McEwen (1990)

TABLE 3
Trace elements in Sodium Silicate (Kirk-Othmer 1982)

Impurity	Measured Values		Impurity	Measured Values	
	Low	High		Low	High
F	6.7 ppm	9.5 ppm	V	Below 0.3 ppm detection limit	0.8 ppm
Cl	130 ppm	1900 ppm	Cr	Below 0.3 ppm detection limit	1.0 ppm
SO ₄	Below 160 ppm detection limit	1700 ppm	Ni	Below 0.3 ppm detection limit	0.3 ppm
N	0.1 ppm	44 ppm	Co	Below 0.3 ppm detection limit	<0.3 ppm
As	Below 1 ppm detection limit	<1 ppm	Zn	Below 0.6 ppm detection limit	2.8 ppm
Hg	Below 0.26 ppb detection limit	2.5 ppb	Cu	Below 0.6 ppm detection limit	1.1 ppm
Pb	0.17 ppm	0.60 ppm	Bi	Below 25 ppm detection limit	<25 ppm
Cd	Below 10 ppb detection limit	21 ppb	Sr	Below 0.2 ppm detection limit	1.5 ppm
Fe	36 ppm	120 ppm	Ba	Below 0.2 ppm detection limit	2.8 ppm
Mg	4 ppm	26 ppm	Mn	0.1 ppm	1.8 ppm
Ca	Below 1 ppm detection limit	76 ppm	Sn	Below 60 ppm detection limit	<60 ppm
Al	50 ppm	220 ppm	Sb	Below 15 ppm detection limit	< 15 ppm
P	Below 18 ppm detection limit	<18 ppm	Se	Below 20 ppm detection limit	<20 ppm

USE

Cosmetic

Potassium Silicate functions as a corrosion inhibitor in cosmetics (Gottschalck and McEwen 2004). Voluntary reports by industry to the Food and Drug Administration (FDA) on product use included use of Potassium Silicate in two formulations as shown in Table 4 (FDA 2001). Industry did not report any concentration of use information for Potassium Silicate.

Sodium Metasilicate functions as a chelating agent and corrosion inhibitor in cosmetic formulations (Gottschalck and McEwen 2004). Of the 191 formulations reported to the FDA, over 80% were used in hair dyes and colors (FDA 2001). Table 4 shows the types of cosmetic formulations in which Sodium Metasilicate is reported to be used and gives current concentrations of use as provided by industry.

In those cases where a current concentration of use is provided, but there are no reports to FDA of use, it should be assumed that the ingredient may be in current use.

Sodium Silicate functions as a buffering agent, corrosion inhibitor, and a pH adjuster (Gottschalck and McEwen 2004). Sodium Silicate was reported to be used in 22 formulations (FDA 2001). Table 4 shows the types of cosmetic formulations in which Sodium Silicate is reported to be used and gives current concentrations of use as provided by industry.

There are no restrictions for the use of these silicate salts in cosmetics in Japan according to the Ministry of Health, Labor, and Welfare (2000) nor in Europe according to the European Economic Community (1999).

Noncosmetic

The principle uses of soluble silicates are in the manufacturing of soaps and detergents. They provide a constant pH value in

the detergent system and aid in the saponification of oils and fats by means of their alkaline nature and buffering ability. Soluble Silicates are also used in water treatment, as an adhesive and fireproof coating additive, as a paper de-inking agent, as an egg preservative, and as a inhibitor of metal corrosion (Kirk-Othmer 1982).

FDA affirmed Sodium Metasilicate as a GRAS (generally regarded as safe) direct food substance (Code of Federal Regulations, 21CFR184.1769a) with no limitation other than current good manufacturing practice. Sodium Metasilicate's uses in foods include processing aid; washing and lye peeling of fruits, vegetables, and nuts; denuding agent in tripe; hog scald agent in removing hair; and a corrosion preventative in canned and bottled water. The Select Committee of the Federation of American Societies for Experimental Biology (FASEB) (1981) concluded: "There is no evidence in the available information on Sodium Metasilicate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used as a food ingredient in a manner now practiced at levels that are now current or might reasonably be expected in the future."

Rhone-Poulenc (1971a) reported Sodium Silicate being used in industrial cleaners and detergents.

Potassium Silicate was reported by Reynolds et al. (1998) as an alternative to sulfur for controlling powdery mildew. Rhone-Poulenc (1971b) reported Potassium Silicate being used in industrial cleaners and detergents.

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

Two groups of four male Sprague-Dawley Cox rats were fasted for 17 to 18 h and then administered Sodium Silicate orally in doses of 40 or 1000 mg/kg body weight (bw). Four control

TABLE 4
Product formulation data

Product category (number of formulations in each category) (FDA 2001)	Formulations containing ingredient (FDA 2001)	Reported range of use concentrations (CTFA 1999, 2000b)
<i>Potassium Silicate</i>		
Noncoloring hair preparations		
Other hair preparations (276)	1	—
Skin care preparations		
Paste masks (mud packs) (269)	1	—
Totals/ranges for Potassium Silicate	2	
<i>Sodium Metasilicate</i>		
Noncoloring hair preparations		
Hair straighteners (63)	1	—
Hair coloring preparations		
Hair dyes and colors (1588)	158	—
Hair lighteners with color (5)	2	14%
Hair bleaches (115)	24	13%–18% (diluted to 7%–14% before use)
Other hair coloring preparations (59)	4	—
Shaving preparations		
Shaving cream (133)	2	—
Totals/ranges for Sodium Metasilicate	191	
<i>Sodium Silicate</i>		
Baby products		
Other baby products (29)	—	0.6%
Eye makeup preparations		
Other eye makeup preparations (151)	1	—
Hair-coloring preparations		
Hair bleaches (115)	7	16%–55% (diluted to 1%–20% before use)
Hair dyes and colors (1572)	—	1%
Other hair coloring preparations (59)	1	35%
Bath preparations		
Bath soap and detergents (405)	2	0.06%–7%
Oral hygiene products		
Dentrifrices (aerosol, liquid, pastes, and powders) (38)	—	0.6%
Shaving preparations		
Shaving cream (133)	6	0.3%–5%
Shaving soap (<4)	—	0.4%
Skin care preparations		
Skin cleansing creams, lotions, liquid, and pads (653)	—	10%
Depilatories (28)	4	2%
Face and neck skin care preparations (304)	1	—
Other skin preparations (692)	—	1%
2001 Totals/ranges for Sodium Silicate	22	0.06%–35%

animals received 10 ml of quartz-distilled water. All suspensions contained <0.5 ppm of silicon and aluminum. Urine samples were collected over an 8-h period and afterwards the remaining urine in the bladder was collected. The concentrations of silicon were measured by induction-coupled RF plasma optical emission spectrometry. Silicon excretion was most rapid during the first 24 h after dosing. After subtracting the control values, the

urinary silicon excretion at 40 and 1000 mg Sodium Silicate/kg was 18.9% and 2.8%, respectively (Benke and Osborn 1979).

In Vitro Assays

Sodium Metasilicate

Neutralized Sodium Metasilicate, at concentrations of up to 0.025 M, inhibited urease and invertase in vitro, but had

little effect on many other enzymes such as pepsin, trypsin, lipase, catalase, or cholinesterase (Kind et al. 1954; Alexander 1968).

Skin² ZK 1350 cultures were used to evaluate skin corrosion and develop a classification of 50 chemicals in a study by Liebsch et al. (1995). Skin² cultures are a three-dimensional human skin model with a stratum corneum grown from neonatal human skin cells. The epidermal side of the cultures was placed onto 15 μ l of Sodium Metasilicate on glass coverslips for 10 s. Phosphate-buffered saline was used to wash the test material residue. Cell viability was assessed using the tetrazolium derivative reduction cytotoxicity assay. The controls were treated with distilled water. In this assay, a corrosive chemical will have a <80% viability rate. A noncorrosive classification corresponds to a >80% viability rate. Sodium Metasilicate had a mean viability ($\pm$ SD) of 65.8 ± 10.4 . The authors classified Sodium Metasilicate as corrosive.

Sodium Silicate

Sodium Silicate was also tested by Liebsch et al. (1995) in the same study as the previous experiment. Two different chemical names were tested, Sodium Silicate A140 and Sodium Silicate H100. Sodium Silicate A140 is classified as group II and Sodium Silicate H100 is classified as non-corrosive according to in vivo UN packing guidelines. The ZK 1350 percent viability mean $\pm$ SD for Sodium Silicate A140 and Sodium Silicate H100 were 82.3 ± 12.0 and 91.5 ± 10.9 , respectively. The corrosivity classification for Sodium Silicate A140 was determined to be non-corrosive, but was noted to be a false negative. Sodium Silicate H100 was classified as non-corrosive. Both chemicals were predicted by the ZK 1350 assay to be non-corrosive according to United Nations (UN) packing guidelines.

ANIMAL TOXICOLOGY

Acute Oral

Sodium Metasilicate

Rhone-Poulenc (1971b) conducted a study in which male Sprague-Dawley rats were administered a 20% solution of Sodium Metasilicate by gastric intubation. Five animals per dose of 464, 1000, 2150, and 4640 mg/kg were used. The animals were observed for 14 days for mortality and signs of toxicity.

All rats given the largest dose died and necropsy was performed on these animals. No apparent signs of toxicity were produced at 464 mg/kg. Animals treated with either ratio at doses of 1000 and 2150 mg/kg had gasping, dyspnea, and acute depression. Signs in groups given 4640 mg/kg included acute depression, nasal discharge, dyspnea, and gasping. All dead rats had gross gastrointestinal hemorrhages with congestion of the kidneys, adrenal glands, liver, lungs, and heart. The acute oral LD₅₀ was 847 mg/kg (Rhone-Poulenc 1971b).

Muggenberg et al. (1974) gave groups of three beagle dogs single doses of 0.1, 0.25, 0.5, 1.0, and 2.5 g/kg of a commercially

available detergent containing Sodium Metasilicate. No details about the percentage of Sodium Metasilicate in the detergent were given.

All dogs that received the highest dose died within 54 h. Gross lesions of variable severity were found in the oral cavity, pharynx, esophagus, stomach, larynx, lungs, and kidneys of all dogs receiving 0.25 g/kg or more. No lesions were found in dogs that received 0.1 g/kg. Microscopic lesions included acute necrosis of the epithelial lining of the digestive tract, necrosis, ulceration and edema of the larynx, edematous lungs, and necrosis of the proximal renal tubules.

In a second experiment, three pigs were given a single dose of 0.25 g/kg of the same detergent used in the dog study. One pig died 95 h after ingestion. Lesions in the pigs were similar to those found in the dogs (Muggenberg et al. 1974).

The Federation of American Societies for Experimental Biology (1981) listed the following LD₅₀ values for Sodium Metasilicate: rat (oral) 1.28 g/kg; rat (oral) 3 g/kg; and mouse (oral) 3 g/kg, and stated that "accidental exposure to strongly alkaline, concentrated solutions of Sodium Metasilicate such as those used in certain common detergent preparations, can produce caustic, irritating effects on contact with the eye, skin, and mucous membranes of the alimentary tract and respiratory system."

Ito et al. (1986) reported the LD₅₀ of Sodium Metasilicate as 1152.8 mg/kg in male rats, 1349.3 mg/kg in female rats, 820 mg/kg in male mice, and 770 mg/kg in female mice. Changes in the animals that survived after peroral administration of large doses in acute studies were mainly bleeding in the stomach and duodenum, and erosion of the small intestine.

Sodium Silicate

A summary of information on Sodium Silicate provided by European companies (EUCLID 2000) included acute oral toxicity data shown in Table 5.

In a study by Rhone-Poulenc (1971b), male Sprague Dawley rats were administered a 20% solution of a 2.0 and 2.4 ratio of Sodium Silicate to 1.0 ratio of sodium oxide by gastric intubation. The 2.0 and 2.4 ratios were corrected for moisture content and tested on an equivalent anhydrous basis. Five animals per dose group at 464, 1000, 2150, and 4640 mg/kg were used. The animals were observed for 14 days for mortality and other signs of toxicity. Necropsy was performed on animals of the largest doses.

In the highest dose group, 4/5 rats of the 2.0 ratio material and 5/5 rats of the 2.4 ratio material died. No apparent signs of toxicity were produced at 464 mg/kg. Animals treated with either ratio at doses of 1000 and 2150 mg/kg had gasping, dyspnea, and acute depression. The highest dose group animals had acute depression, nasal discharge, dyspnea, and gasping. Dead animals had gastrointestinal hemorrhages and congestion of the kidneys, adrenal glands, liver, lungs, and heart. The acute oral LD₅₀ was reported to be 1960 mg/kg in groups receiving the 2.0 ratio material of Sodium Silicate and 2710 mg/kg

TABLE 5
Sodium Silicate oral LD₅₀ values in the rat (EUCLID 2000)

LD ₅₀	Molar ratio/concentration	Remarks
2000–2500 mg/kg	Molar ratio of 1.6 and a concentration of 51%	The acute oral toxicity of alkaline sodium silicates is dependent on the SiO ₂ /Na ₂ O molar ratio, and to a lesser extent on the concentration of dissolved dry matter (due to pH dependence); autopsy results showing acute gastroenteritis, vascular congestion, and mottled livers are consistent with nonspecific causes of death.
1600–8600 mg/kg	Molar ratio of 3.0 and various concentrations	
1500–2200 mg/kg	Molar ratio of 2.0 and concentration of 81%	
1300–2100 mg/kg	Molar ratio of 2.0 and various concentrations	
1600 mg/kg	Molar ratio of 2.0 and concentration of 81%	
7150–10500 mg/kg	Molar ratio of 3.4	Ten male rats of different species were used and the observed range in LD ₅₀ values was due to intraspecies susceptibility. All symptoms of intoxication were reversible and no signs of histopathologic abnormalities were observed 14 days after application of the substance.
>2000 mg/kg	Molar ratio of 3.45 and concentration of 35%	

in groups receiving the 2.4 ratio material (Rhone-Poulenc 1971b).

Short-Term Oral

Sodium Metasilicate

Albino mice (210) and rabbits (20) dosed daily with 200 to 300 mg/kg Sodium Metasilicate for 1 month showed “a cellular proliferation in the internal organs.” No details of number of animals by dose, sex, age, strain, or mortality were reported (Shakhbazyan and Karapetyan 1963).

Schwarz and Milne (1972) found that Sodium Metasilicate (Na₂SiO₃·9H₂O) added to silicon-depleted, chemically defined diets of weanling Fisher 344 rats resulted in 25% to 34% increases in growth rates compared with control animals on silicon-depleted diets. The estimated dose of silicon was about 100 mg/kg/day. Growth retardation and a disturbance in bone formation were reported to be signs of silicon deficiency, presumably as a result of faulty bone matrix formation and inadequate cross-linkage of acid mucopolysaccharides and other connective tissue components.

Sodium Silicate

In a study by Kayongo-Male and Jia (1999), 36 male Sprague-Dawley albino rats were randomly allotted into a two-dietary-treatment experiment. The dietary treatments included a control basal diet consisting of dextrose–egg album in that contained <5.0 ppm Si and a diet supplemented with 500 ppm Si obtained by the addition of Sodium Silicate.

The addition of dietary Si affected rat body-weight changes. Rats on the supplemented diet had slower growth rates than control rats. At the end of 8 weeks, rats on the treated diet weighed

257 g on average compared to 273 g for control rats. Hemoglobin levels were lower ($p < .05$) in treated rats. Plasma Ca content was also lower in treated rats ($p < .05$). Plasma Mg levels were higher ($p < .05$) in control rats. Plasma Cu and P were not affected. The source of Si did not affect ($p < .05$) organ weights or their mineral concentrations except liver Zn concentrations, which were higher in the control group (Kayongo-Male and Jia 1999).

Subchronic Oral

Sodium Metasilicate

In a subchronic study with Sodium Metasilicate in the drinking water of Wistar rats, no specific changes in the high-dose animals were observed. Slight degenerative changes in the epithelium of renal tubules were observed in higher doses. Maximum safety concentrations were 1500 ppm/L/day (792 mg/kg/day) (Ito et al. 1986).

Sodium Silicate

Newberne and Wilson (1970) fed eight female and eight male beagle dog 2.4 g/kg/day of Sodium Silicate in their diets for 4 weeks to study renal damage. Six animals of each sex were used as controls, receiving the same diet without Sodium Silicate. In addition, 15 rats (Charles River CD strain) of each sex were fed the same diet with Sodium Silicate and 15 rats of each sex received the control diet. Animals were killed at the end of 4 weeks and necropsied. Tissues were preserved in formaldehyde for histopathologic examination.

Body weight, feed intake, and urinary specific gravity and blood (protein and glucose) measurements were the same for both test and control dogs and rats. Polydipsia and polyuria were

observed in both the dogs and rats. Gross renal cortical lesions were seen in 8/8 male and 7/8 female dogs. The authors stated that the appearance of the cut surface suggested cortical infarcts. Despite extensive renal damage, impairment of renal function was not detected. No treatment-related lesions were found in the rats (Newberne and Wilson 1970).

Smith et al. (1973) added a Sodium Silicate solution to the drinking water containing 600 and 1200 ppm of added silica and given to groups of six weanling male and six female Sprague-Dawley rats. Growth, nitrogen and phosphorous retention, and reproductive effects were investigated (discussed later in this report). Control groups received no Sodium Silicate in their drinking water. At 4 months of age, the rats of treatment groups were mated. The treated water, 600 ppm, combined with a normal, commercial diet for rats increased body weight gains of the male rats by ~6% over controls but decreased gains of the female rats by ~5% compared to controls. Retention of nitrogen and phosphorous were significantly affected. No apparent effect of the treatment in the drinking water was found on the longevity in rats having started treatment after weaning.

Acute Parenteral

Intraperitoneal injections of a neutralized 2% solution of Sodium Metasilicate (~1200 mg/kg on day 1 and 800 mg/kg on days 2 and 3) into white rats resulted in a 60% decrease in spleen weight and relative enlargement of the kidneys when the animals were examined on the third day. There were microscopic lesions of the lymphatic tissues and cellular damage in parts of the intestinal mucosa (Nanetti 1973).

Dermal Irritation

Potassium Silicate

Potassium Silicate was tested for primary skin irritation according to the Draize Dermal procedure after a 24-h exposure in six rabbits (three male and three female). No dose was indicated. The primary irritation index was 1.83 and the compound was classified as a mild irritant (Rhone-Poulenc 1971a).

A summary of information on Potassium Silicate put together by European companies (EUCLID 2000) included the skin irritation data shown in Table 6.

Sodium Metasilicate

Sodium Metasilicate (42.4% H₂O) was tested for skin irritation according to the Draize Dermal procedure in six rabbits (three male and three female). The results were scored at 8.0 and was classified as a corrosive. The authors stated that the result was expected because the pH of the solution was 12.4 (Rhone-Poulenc 1971b).

A commercial product containing 5% Sodium Metasilicate was tested in acute dermal toxicity studies using male and female white New Zealand rabbits. The dermal LD₅₀ was >200 mg/kg. Necrosis and edema were observed at the treatment site (Rhone-Poulenc 1976).

A Sodium Metasilicate/carbonate granular detergent was applied to intact and abraded skin of rabbits and guinea pigs for 4 h. Skin responses were graded at 4, 24, and 48 h after the patch applications. The detergent contained 37% Sodium Metasilicate. Rabbit skin and guinea pig skin reacted differently as shown in Table 7 (Nixon, Tyson, and Wertz 1975).

Sodium Silicate

Sodium Silicate was tested for primary skin irritation according to the Draize Dermal procedure after 4 and 24 h exposures in rabbits. Both primary irritation indexes for 4 and 24 h were 8.0 and the compound was classified as corrosive (Rhone-Poulenc 1971a).

A 2.0 ratio and 2.4 ratio of Sodium Silicate to 1.0 sodium oxide with 19.5% water was tested for skin irritation according to the Draize Dermal procedure in rabbits. The 2.0 ratio material was scored a 5.9 and was classified as a severe irritant; the 2.4 ratio material was scored a 4.12 and was classified as a moderate irritant. An acute dermal toxicity study utilizing New Zealand white rabbits was also conducted. Both ratio materials of Sodium Silicate were applied to the closely clipped intact abdominal skin and the skin was exposed for 24 h. After the 24 h, the binders were removed and any residual chemical was removed by washing. The animals were observed for 14 days for toxicity. No signs of toxicity were apparent in any of the animals. The 2.0 ratio material produced severe, irreversible erythema and edema at the test site; while the 2.4 ratio material caused more moderate, reversible irritation at the test site. The acute rabbit dermal LD₅₀ was >4640 mg/kg (Rhone-Poulenc 1971b).

TABLE 6
Potassium Silicate: acute dermal irritation in rabbits (EUCLID 2000)

Method	Result	Remarks
OECD Guideline 404 "Acute Dermal Irritation/Corrosion"	Nonirritating	Diluted Potassium Silicate solution Molar ratio = 3.4 Concentration = 8.5–9%
OECD Guideline 404 "Acute Dermal Irritation/Corrosion"	Nonirritating	Diluted Potassium Silicate solution Molar ratio = 3.9 Concentration = 7–7.5%

TABLE 7
Sodium Metasilicate: dermal irritancy (Nixon, Tyson, and Wertz 1975)

Concentration of detergent (w/v aqueous)	Animal species	Mean scores		PII	Tissue destruction		Irritancy judgement
		Intact	Abraded		Intact	Abraded	
50%	Rabbit	>6.8	>8.0	>7.4	5/5	5/5	Corrosive
50%	Guinea pig	0.0	0.6	0.3	0/6	0/6	Negligible

Three detergents containing Sodium Silicate (7% in a high-carbonate detergent, 13% in a low-12 carbonate detergent, and 6% in a phosphate detergent) were applied to intact and abraded skin of rabbits and guinea pigs for four hours. Skin responses were graded at 4, 24, and 48 h after the patch applications. The results from this study are presented in Table 8 (Nixon, Tyson, and Wertz 1975).

In a single insult occlusive patch test, nine rabbits were treated with a skin freshener that contained 10% of a 40% aqueous solution of Sodium Silicate. The compound had a typical weight ratio of $\text{SiO}_2/\text{Na}_2\text{O}$ of 3.25. The skin irritation potential of the test material was nonirritating (Cosmetic, Toiletry, and Fragrance Association [CTFA] 1979a).

Patch tests were performed using three female Hartley guinea pigs. Occlusive patches containing 20% Sodium Silicate were applied to the shaved backs of the three animals. Erythema was detected 48 h later but did not progress to ulceration. Pathological findings at the occlusive patch test site included dyskeratotic cells in the epidermis and polymorphonuclear leukocytic infiltration around the blood vessels (Tanka, Miyachi, and Horio 1982).

A summary of information on Sodium Silicate put together by European companies (EUCLID 2000) included the skin irritation data shown in Table 9.

Immunomodulation

The National Toxicology Program (NTP) (2001) evaluated Sodium Metasilicate as an immunomodulatory agent when applied to female BALB/c mice in a mouse ear swelling test and

local lymph node assay (LLNA) to measure contact hypersensitivity. Concentrations used in the contact hypersensitivity assays were determined by irritancy testing. The minimal irritating concentration was found to be 6% and the maximal nonirritating concentration was 4%. The Sodium Metasilicate concentrations were 0.4%, 2%, and 4% for the sensitization phase, and 6% for the challenge phase. In the LLNA, mice were sensitized to 2%, 4%, and 6% Sodium Metasilicate. 1-Fluoro-2,4-dinitrobenzene (DNFB) was used as a positive control at a concentration of 0.15% for the irritancy test and LLNA, and 0.20% for the swelling test. An evaluation of lymph node subpopulations, cytokine mRNA, and serum immunoglobulin E (IgE) levels was also conducted.

Dermal exposure to (2% to 6%) Sodium Metasilicate did not produce cell proliferation in the draining lymph nodes as measured by the LLNA. However, a delayed-type hypersensitivity (DTH) response was observed when mice were sensitized on the back with 4% Sodium Metasilicate, then challenged on the ear with 6% Sodium Metasilicate. The positive control, DNFB, induced cell proliferation in the draining lymph nodes, and elicited a DTH response. Lymph node subpopulations were also altered by treatment with Sodium Metasilicate. Only B220+Ig+ lymph nodes were shown to increase when the data were presented as a percentage of the total lymph node count. The response was observed at concentrations as low as 4%. An evaluation of the cytokine mRNA revealed an increase in the expression of interferon (IFN)- γ , tumor necrosis factor (TNF)- β , and migration inhibitory factor (MIF) mRNAs. No change in total serum IgE levels was detected (NTP 2001).

TABLE 8
Sodium Silicate: dermal irritancy (Nixon, Tyson, and Wertz 1975)

Detergent type (Sodium Silicate concentration)	Concentration of detergent (w/v aqueous)	Animal species	Mean scores		PII	Tissue destruction		Irritancy judgement
			Intact	Abraded		Intact	Abraded	
High carbonate (7%)	50%	Rabbit	0.9	2.6	1.7	0/6	0/6	Negligible
	50%	Guinea pig	0.0	0.4	0.2	0/6	0/6	Slight
Low carbonate (13%)	50%	Rabbit	0.7	0.8	0.8	0/6	0/6	Slight
	50%	Guinea pig	0.1	1.0	0.5	0/6	0/6	Slight
Phosphate (6%)	50%	Rabbit	1.2	>5.6	>3.4	0/5	2/5	Moderate
	50%	Guinea pig	0.2	1.0	0.6	0/6	0/6	Slight

TABLE 9
Sodium Silicate acute dermal results in rabbits (EUCLID 2000)

Method	Result
Undiluted substance (0.5 ml) applied for 4 h; molar ratio of 3.45; concentration of 35%	Nonirritating
0.5 g substance moistened with physiological saline applied to intact abraded skin for 24 h; molar ratios of 2.9 and 3.2; concentrations of 43%, 36%, and 80%	Irritating; the PII was 3, 3, 0 respectively for 43%, 36%, and 80%
Same application, but molar ratios of 2.4 and 3.2, and concentrations of 44% and 38%	Irritating
Same application, but pH 13.6 material; molar ratio of 1.6; concentration of 52%	Corrosive
0.5 ml solution of pH 12 with a molar ratio of <2	Corrosive
A powder product—2:1 dilution with water; molar ratio was 2; concentration was 66.6%	Nonirritating
Undiluted substance (0.5 ml) applied for 4 h; molar ratio was 3.91; concentration was 28%	Nonirritating
Same application, but molar ratio was 2.83 and concentration was 45%	Slightly irritating
Same application, but molar ratio was 2.09 and concentration was 55%	Moderately irritating
Same application, but molar ratio was 3.3 and concentration was 38%	Slightly irritating
Same application, but molar ratio was 2.09 and concentration was 55%	Slightly Irritating
Same application, but molar ratio was 2.4 and concentration was 40%	Irritating
Same application, but molar ratio was 2 and concentration was 41%	Irritating
The powder was applied dry. The molar ratio was 2	Nonirritating
Molar ratio of 1.6 and concentration of 53.5%	Corrosive
Molar ratio of 3.4 and concentration of 34.5%	Slightly irritating

Ocular Irritation

Potassium Silicate

A summary of information on Potassium Silicate put together by European companies (EUCLID 2000) included the ocular irritation data shown in Table 10.

Sodium Metasilicate

Sodium Metasilicate (42.4% H₂O) was tested in acute ocular irritation studies that were in accordance with the procedure outlined in the Code of Federal Regulations (21CFR191.12.1). Six New Zealand rabbits were exposed to 0.1 ml in one eye; the other eye served as a control. The sample was corrosive to the eye; total destruction of the eye of all the test animals was observed (Rhone-Poulenc, 1971b).

Sodium Silicate

Sodium Silicate ratios (2.0: 1.0 and 2.4:1.0 Na₂O with 19.5% H₂O) were tested in acute ocular irritation studies that were in accordance with the procedure outlined in the Code of Federal Regulations (21CFR191.12.1). Six New Zealand rabbits were

exposed to 0.1 ml in the conjunctival sac of one eye; the other eye served as a control. The 2.0 ratio material produced corneal opacity with scar tissue formation in four of the six rabbits. The remaining two had severe iritis and conjunctivitis. The 2.0 ratio material was classified as corrosive. The 2.4 ratio material produced conjunctivitis, moderate iritis, and two of six test rabbits had slight corneal opacity. Sodium Silicate was classified as a severe ocular irritant (Rhone-Poulenc 1971b).

A skin freshener (10% of a 40% aqueous solution of Sodium Silicate) was tested in a Draize eye irritation study in six rabbits. The compound had a typical weight ratio of SiO₂/Na₂O of 3.25. No eye irritation potential as judged by the Draize classification of eye irritation was demonstrated in this study (CTFA 1979b).

A summary of information on Sodium Silicate put together by European companies (EUCLID 2000) included the ocular irritation data shown in Table 11.

GENOTOXICITY

Sodium Metasilicate

DNA damage and repair assays without metabolic activation were conducted on *Bacillus subtilis* recombination-repair-deficient and wild-type strains. Sodium Metasilicate at concentrations of 0.005–0.5 M was not genotoxic (Kada, Brun, and Marcovich 1960).

Sodium Silicate

Strains B/Sd-4/1,3,4,5 and B/Sd-4/3,4 of *Escheria coli* were used to study the mutagenic action of Sodium Silicate (Demerec, Bertani, and Flint 1951). The streptomycin-dependent bacteria

TABLE 10

Potassium Silicate: ocular irritation in rabbits (EUCLID 2000)

Method	Result
Diluted solution; molar ratio = 3.9; concentration = 7%–7.5%	Nonirritating
Diluted solution; molar ratio = 3.4; concentration = 8.5%–9%	Nonirritating

TABLE 11
Sodium Silicate: Draize ocular irritation in rabbits (EUCLID 2000)

Method	Result	Remark
Molar ratios of 1 and 2; concentrations of 10% and 8%	Irritating	Sodium Silicate solutions of less than 10% are irritating but not highly irritating.
Molar ratios of 2 and 2.9; concentrations of 44% and 43%	Highly irritating	Concentrated solutions of molar ratios >2.9 are severely irritating.
Molar ratio of 3.2; concentration of 36%	Nonirritating	

(Sd-4) were treated with 0.025%, 0.01%, 0.05%, 0.1%, 0.15%, or 0.3% Sodium Silicate for three hours at 37°C. The control suspension was distilled water instead of streptomycin. At the end of treatment, both treated and control suspensions were assayed on streptomycin-agar plates. Samples from the suspensions (0.1 ml) were also plated on streptomycin-free plates, incubated for 6 days, and the frequency of mutants was calculated. Sodium Silicate was nonmutagenic.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Groups of three adult albino rats were injected intratesticularly and subcutaneously with doses of 0.08 mM/kg Sodium Silicate. By the testicular route, the left testis was treated and the right testis served as the control. The rats were killed 2, 7, and 30 days after injection. The testis and the spermatozoa were prepared for microscopic examination. No morphological changes were seen in the testis at anytime after either of the Sodium Silicate injections. No effect on the residual spermatozoa in the ductus deferens was apparent either (Kamboj and Amiya 1964).

As described earlier, Smith et al. (1973) added a Sodium Silicate solution to the drinking water containing 600 and 1200 ppm of added silica and given to groups of six weanling male and six female Sprague-Dawley rats. Control groups received no Sodium Silicate in their drinking water. At 4 months of age, the rats of treatment groups were mated. At 600 ppm and 1200 ppm, the treated water decreased the numbers of offspring born to 67% and 80% of controls, respectively. Also these treatments decreased the numbers of surviving offspring until weaning (3 weeks) to 46% and 24% of the control values.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation

Sodium Metasilicate

A Sodium Metasilicate/carbonate granular detergent was applied to intact and abraded skin of humans for four hours. Each subject afforded eight test sites aligned four on each side of the back about 5 cm from the midline. Sites were vertically spaced 3 cm apart in the area between the scapula and the waist. Erythema and edema were graded 4, 24, and 48 h after the patch applications. Primary irritation indices (PIIs) were calculated by

averaging the scores for all test sites. The detergent contained 37% Sodium Metasilicate and was applied at a concentration of 50% (w/v aqueous). The results from this study are presented in Table 12. The PII was >3.6 and the material was judged to be a severe irritant (Nixon, Tyson, and Wertz 1975).

Clairol (2000a) studied the irritancy of Sodium Metasilicate in a modified soap chamber test. Two hair color kits including a developer, activator, and lightener were tested. Sodium Metasilicate was a component of the activator at a concentration (w/w) of 13.5% in both kits; on-head concentrations were 1.34% (kit 1) and 1.43% (kit 2). The two test patches, a positive-control patch dosed with 2% sodium lauryl sulfate (SLS), and a negative-control patch dosed with deionized water were applied to the lower back of nineteen subjects for approximately 4 h. The test sites were graded for erythema, edema, burning, stinging, and itching approximately 4 h after application (20 min after removal) and approximately 28 h after application (24 h after removal). A separate 24-h 0.75% SLS reactivity patch was applied to the upper back and graded at the 28-h time point only.

No fissuring or scaling was observed over the course of the study. The kit 1 mean erythema + edema grade at 4 h was 1.00 and for 28 h was 0.50. For kit 2, the mean erythema + edema scores at 4 h was 0.95 and for 28 h was 0.53. The positive control had a 28-h erythema + edema grade of 2.92. No adverse events occurred during the course of the study (Clairol 2000a).

In a second modified soap chamber test, Clairol (2000b) tested Sodium Metasilicate to determine the incidence and severity of irritation. Procedures stated in the above study were followed. Twenty-one subjects completed this study. Sodium Metasilicate was a part of the activator in the hair coloring system and concentrations (w/w) were 13.5% in the activator and 2.58% on the head.

No burning or itching was recorded. The mean 6-h and 24-h erythema + edema scores were 1.36 and 0.56, respectively.

TABLE 12
Sodium Metasilicate: human dermal irritancy (Nixon, Tyson, and Wertz 1975)

End point	Intact	Abraded
Mean irritation scores	>3.0	>4.2
Tissue destruction	0/8	1/8

The reactivity control containing 0.75% SLS had a 28-h mean erythema + edema score of 0.89 (Clairol 2000b).

L'Oreal (2000a) assessed 15 bleach formulations in the elbow crease test. Experimental groups comprised 20 to 40 healthy adults. Approximately 0.7 ml of mixed product (developer + activator or developer + base + activator) was applied in the elbow creases on 40 cm² for 50 min without occlusion. The test sites were evaluated for erythema, edema, and vesicles by a trained grader using a 4-point visual scoring system for each parameter. Time points for evaluation were 5 min, 4 h, and 24 h following the removal of the products by rinsing. The Sodium Metasilicate concentrations in the activators and in the product mixtures ranged from 3.4% to 14% and 1% to 7%, respectively.

Under the study conditions, all products induced low grade irritation: almost exclusively mild erythema and only occasionally moderate erythema at 5 min. Observable changes subsided quickly after product removal, leaving slight erythema at 1 h in only a few volunteers. No correlation could be observed between Sodium Metasilicate concentrations and the irritation potential of the product (L'Oreal, 2000a).

L'Oreal (2000b) tested 32 hair bleaches in semiocluded patch tests. Sodium Metasilicate concentrations ranged from 3.4% to 14% in the activators and from 0.75% to 6.8% in mixed products; 0.2 ml of the mixed product were applied under patch tests for 1 h and 15 min on the back. Experimental groups were comprised of 25 healthy adults. Test sites were evaluated for erythema, edema, and vesicles using a 7-point visual scoring system encompassing all the parameters at 30 min and 24 h following the removal of the products by rinsing. Mean irritation scores were calculated for each time point.

Under the study conditions, Sodium Metasilicate produced only mild and transient irritation under exaggerated conditions of application. Irritation scores appeared to be independent of silicate concentration (L'Oreal 2000b).

Sodium Silicate

Nixon, Tyson, and Wertz (1975) applied three detergents containing Sodium Silicate to intact and abraded skin of humans for four hours. One sample contained 7% Sodium Silicate in a high-carbonate detergent, the second contained 13% in a low-carbonate detergent, and the third contained 6% in a phosphate detergent. Eight subjects were tested for each detergent. Each subject afforded eight test sites aligned four on each side of the

back about 5 cm from the midline. Sites were vertically spaced 3 cm apart in the area between the scapula and the waist. Erythema and edema were graded 4, 24, and 48 h after the patch applications. PIIs were calculated by averaging the scores for all test sites.

The authors concluded that each sample had negligible irritancy. The results from this study are presented in Table 13 (Nixon, Tyson, and Wertz 1975).

Hill Top Research, Inc. (1979) conducted a study of cumulative irritant properties of a series of test materials with 10% of a 40% aqueous solution of Sodium Silicate on 12 male and female panelists. The test material was applied to the backs of the panelists in randomized manner. Each sample was reapplied to the same test site on each panelist for the remainder of the study (21 consecutive days) or until the max irritation score was reached. If the max score was reached, the patch was omitted and the patch area was scored for residual irritation for the next three scoring dates.

The test patches were removed by the panelists 23 h after application. The panelists were instructed to take a bath or shower immediately following removal of the patches and to keep the patch areas dry at other times. Approximately 0.3 ml of each sample was applied to each patch. Reactions to the test samples were scored 24 h after application (1 h after patch removal). Scores were classified as following: 0–49 (mild material, no irritation); 50–199 (probably mild in normal use); 200–449 (possibly mild in normal use); 450–580 (experimental cumulative irritant); 581–630 (experimental primary irritant).

The total score calculated for the panelists was 155, classifying the test compound as probably a mild irritant in normal use (Hill Top Research, Inc. 1979).

A skin freshener (10% of a 40% aqueous solution of Sodium Silicate) was evaluated via a 4-day minicumulative irritancy assay. A currently marketed product was used as a mildness frame of reference. Both materials were tested full strength under occlusive patch conditions in 20 humans. The PII for the test product was 0.5 and was 0.88 for the currently marketed product. The test product exhibited acceptable irritancy results and was significantly milder than the reference control (CTFA 1989).

Clairol (2000c) studied the irritancy of Sodium Silicate in a modified soap chamber test. Two hair color kits including a developer, activator, and lightener were tested. Sodium Silicate

TABLE 13
Sodium Silicate: human dermal irritancy (Nixon, Tyson, and Wertz 1975)

Detergent type (Sodium Silicate concentration)	Concentration of detergent (w/v aqueous)	Mean scores			Tissue destruction		Irritancy judgement
		Intact	Abraded	PII	Intact	Abraded	
High carbonate (7%)	50%	0.0	0.0	0.0	0/8	0/8	Negligible
Low carbonate (13%)	50%	0.0	0.2	0.1	0/8	0/8	Negligible
Phosphate (6%)	50%	0.0	0.4	0.3	0/8	0/8	Negligible

was a component of the activator at 35.75% (w/w) with on the head concentrations of 4.26% (kit 1) and 7.61% (kit 2). The two patches listed before, along with a positive-control patch dosed with 2% SLS and a negative-control patch dosed with deionized water, were applied to the lower back of 19 subjects for approximately 4 h. The test sites were graded for erythema, edema, burning, stinging, and itching approximately 4 h after application (20 min after removal) and approximately 28 h after application (24 h after removal). A separate 24-h 0.75% SLS reactivity patch was applied to the upper back and graded at the 28-h time point only.

No fissuring or scaling was observed over the course of the study. The mean erythema + edema scores at 4 and 28 h were 1.24 and 0.45, respectively, for kit 1; the mean erythema + edema scores at 4 and 28 h were 1.26 and 0.53, respectively, for kit 2. The positive control containing 0.75% SLS had a mean 28-h erythema + edema score of 2.92. No adverse events occurred during the course of the study (Clairol 2000c).

In a second modified soap chamber test, Sodium Silicate was tested to determine the incidence and severity of irritation. Procedures stated in the Clairol 2000 study were followed. Twenty-one subjects completed this study. Sodium Silicate was a part of the activator in the hair coloring system and concentrations (% w/w) in the activator and on the head were 35.75 and 2.13, respectively. No burning or itching was recorded. The mean 6-h and 24-h erythema + edema scores were 0.58 and 0.19, respectively. The reactivity control containing 0.75% SLS had a 28-h mean erythema + edema score of 0.89 (Clairol 2000d).

Sodium Silicate was evaluated in an elbow crease test previously described in the clinical dermal irritation section under Sodium Metasilicate (L'Oreal 2000). Sodium Silicate was present in only two activators at concentrations of 10.6% and 29.6% (2.1% and 8.5% respectively, in the product mixture). Under the study conditions, all products induced low-grade irritation: almost exclusively mild erythema and only occasionally moderate erythema at 5 min. Observable changes subsided quickly after product removal, leaving slight erythema at 1 h in only a few volunteers (L'Oreal, 2000c).

Sodium Silicate was evaluated in semiocclusive patch tests previously described in the clinical dermal irritation section under Sodium Metasilicate (L'Oreal 2000b). Sodium Silicate concentrations ranged from 10.6% to 29.6% in the activators and 1.2% to 6.5% in the mixed products. Under the conditions of this study, all products induce only mild and transient irritation under exaggerated conditions of application. Irritation scores appeared to be independent of silicate concentration (L'Oreal 2000d).

Skin Sensitization

To determine its capacity to induce skin irritation and allergic sensitization, 10% of a 40% aqueous solution of Sodium Silicate was used in a repeat-insult predictive patch test. Ten patches were applied to the upper backs of 94 panelists. Five

were placed on the right side and five were placed on the left side. The sample was applied to all panelists for 24 h every Monday, Wednesday, and Friday for 3 consecutive weeks. The samples were applied to the same site each time. The challenge was conducted in week 6 of the study. A single patch was applied to a previously unpatched site. These patches were removed 24 h following application. Reactions were scored 24 and 48 h after removal. Subjects exhibiting challenge patch reactions indicative of possible induced sensitization participated in follow-up testing after 1 week. Within the limits imposed by the sample size and the test procedure itself, the test material did not exhibit any potential for inducing allergic sensitization (CTFA 1979c).

Case Reports

Sodium Metasilicate

Colloidal Sodium Metasilicate, 0.5 L, was orally ingested and led to the patient's death within 1 to 1.5 h. At autopsy, alkali burns were present in the gastric mucosa; and the stomach contained a small amount of liquid with a pH of 11.5. The liquid was chemically analyzed and was found to be condensed "waterglass." At microscopic examination of the lungs, numerous bronchioles and alveoli were filled with amorphous Sodium Metasilicate. Due to the obstruction of the airways, inhibition of alveolar gas diffusion could have been the cause of death. Liquid Sodium Metasilicate solidification occurred in the lungs by means of carbonic acid of expired air. This occurred due to the fact that Sodium Metasilicate starts to solidify at pH 11.3. Gastric secretions had lowered the pH of the Sodium Metasilicate from 12.5 to 11.5 (Sigrist and Flury 1985).

Sodium Silicate

A man who drank 200 ml of a neutralized Sodium Silicate solution (estimated to contain about 100 g of solid Sodium Silicate or more than 1 g/kg) demonstrated prompt vomiting, diarrhea, and gastrointestinal bleeding, and later had albumin, acetone, "sugar," and blood in the urine. The patient recovered even at this dose. The authors noted that such a neutral silicate would be expected to be less corrosive than unneutralized, strongly alkaline Sodium Metasilicate (Eichhorst 1921).

Tanka, Miyachi, and Horio (1982) reported a case involving a 57-year-old man exposed to Sodium Silicate. At first examination, the eruption consisted of lichenified lesions with hyperpigmentation and four ulcers on the dorsum of the left hand. The lesions appeared oval to round and punched out with irregular and elevated margins. Urticarial wheals were not present and axillary lymph nodes were not palpable. A patch test was performed on the flexor surface of the skin using 20% aqueous solution of Sodium Silicate. Within 24 h, macular erythema and papules with itching were noted. A wheal appeared at the application site immediately after the patch was removed at 24 h. The wheal was not seen after a 15-min patch test. Itchy erythema progressed into ulcer formation after 1 week. A scratch test was also performed and resulted in wheal formation after

15 min. A skin biopsy of a lichenified site near an ulcer revealed spongiosis and exocytosis with individual cell keratinization in the upper epidermis. Patchy perivascular cell infiltration of polymorphonuclear leukocytes also was noted. The patch test biopsy specimen had similar lesions.

To further investigate these findings, these authors performed patch tests with 20% Sodium Silicate on the flexor surface of 30 people. After 48 h, positive reactions were noted in 22 of the volunteers. Erythema similar to that of the case study was seen. No ulcers formed. Scratch tests were also performed on the same volunteers with 20% Sodium Silicate. No wheal formation was observed (Tanka, Miyachi, and Horio, 1982).

SUMMARY

This report provides a review of the safety of Potassium Silicate, Sodium Metasilicate, and Sodium Silicate. These ingredients combine metal cations (potassium or sodium) with silica to form inorganic salts.

Aqueous solutions of Sodium Silicate species are a part of a chemical continuum of silicates based on an equilibrium of alkali, water, and silica. pH determines the solubility of silica and, together with concentration, the degree of polymerization.

These ingredients function as corrosion inhibitors in cosmetics; Sodium Metasilicate also functions as a chelating agent and Sodium Silicate as a buffering and pH adjuster. Sodium Metasilicate is currently used in 168 formulations at concentrations ranging from 13% to 18%. Sodium Silicate is currently used in 24 formulations at concentrations ranging from 0.3% to 55%.

Potassium Silicate and Sodium Silicate were reported as being used in industrial cleaners and detergents. Sodium Metasilicate is a GRAS food ingredient.

Sodium Silicate administered orally acts as a mild alkali and was readily absorbed from the alimentary canal and excreted in the urine. Urinary excretion of Sodium Silicate given orally to rats at 40 and 1000 mg/kg was 18.9% and 2.8%, respectively.

The toxicity of these silicates has been related to the molar ratio of $\text{SiO}_2/\text{Na}_2\text{O}$ and the concentration. The acute oral LD_{50} of Sodium Metasilicate ranged from 847 mg/kg in male rats to 1349.3 mg/kg in female rats, and from 770 mg/kg in female mice to 820 mg/kg in male mice. Gross lesions of variable severity were found in the oral cavity, pharynx, esophagus, stomach, larynx, lungs, and kidneys of dogs receiving 0.25 g/kg or more of a commercial detergent containing Sodium Metasilicate. Similar lesions were seen in pigs given the same detergent and dose as in the previous study. Male Sprague-Dawley rats orally administered 464 mg/kg of a 20% solution containing either 2.0 or 2.4 ratio to 1.0 ratio of sodium oxide showed no signs of toxicity, whereas doses of 1000 and 2150 mg/kg produced gasping, dyspnea, and acute depression.

Beagle dogs fed 2.4 g/kg/day of Sodium Silicate for 4 weeks had gross renal lesions but no impairment of renal function. In a oral subchronic study (drinking water containing 600 and 1200 ppm of added silica), there were body weight gains in

male rats, but decreases in female rats. No apparent effect of the treatment in the drinking water was found on the longevity in rats having started treatment after weaning.

Intraperitoneal injections of a neutralized 2% solution of Sodium Metasilicate in white rats resulted in a decrease in spleen weight and relative enlargement of the kidneys.

Dermal irritation of Potassium Silicate, Sodium Metasilicate, and Sodium Silicate ranged from negligible to severe, depending on the species tested and the molar ratio and concentration tested.

Sodium Metasilicate was negative in the local lymph node assay, but a delayed-type hypersensitivity response was observed in mice.

Potassium Silicate was nonirritating in two acute eye irritation studies in rabbits. Sodium Metasilicate (42.4% H_2O) was corrosive to the rabbit eye. Sodium Silicate was a severe eye irritant in acute eye irritation studies. A skin freshener (10% of a 40% aqueous solution) containing Sodium Silicate was nonirritating. Sodium Silicate in another three Draize eye irritation studies was highly irritating, irritating, and nonirritating, respectively.

Sodium Metasilicate was nonmutagenic in a DNA damage and repair assay without metabolic activation using *B. subtilis*. Sodium Silicate was nonmutagenic in studies using *E. coli* stains B/Sd-4/1,3,4,5 and B/Sd-4/3,4.

Rats given Sodium Silicate (600 and 1200 ppm of added silica) in the drinking water in reproductive studies produced a reduced number of offspring; to 67% of controls at 600 ppm and to 80% of controls at 1200 ppm. Three adult rats injected intratesticularly and subcutaneously with 0.8 mM/kg of Sodium Silicate showed no morphological changes in the testes and no effect on the residual spermatozoa in the ductus deferens.

Sodium Metasilicate/carbonate detergent (37% Sodium Metasilicate) mixed 50/50 with water was considered a severe skin irritant when tested on the intact and abraded human skin. Detergents containing 7%, 13%, and 6% Sodium Silicate mixed 50/50 with water, however, were negligible skin irritants to intact and abraded human skin. A 10% of a 40% aqueous solution of Sodium Silicate was negative in a repeat-insult predictive patch test in humans. The same aqueous solution of Sodium Silicate was considered mild under normal use conditions in a study of cumulative irritant properties. Sodium Metasilicate and Sodium Silicate were studied in modified soap chamber tests. No burning or itching was observed and low erythema + edema scores were noted. Sodium Metasilicate and Sodium Silicate, tested in elbow crease studies and semioccluded patch tests, produced low grade and transient irritation.

Colloidal Sodium Metasilicate was fatal to one man and neutralized Sodium Silicate produced vomiting, diarrhea, and gastrointestinal bleeding in another man in separate case reports.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel determined that the data provided in this report are sufficient to

address the safety of the tested ingredient Potassium Silicate, Sodium Metasilicate, and Sodium Silicate. The Panel recognized the irritation potential of these ingredients, especially in leave-on products. However, because these ingredients have limited dermal absorption and Sodium Metasilicate is a GRAS direct food substance, the Panel deemed the ingredients safe as currently used, when formulated to avoid irritation.

CONCLUSION

Based on the available data contained within this report, the CIR Expert Panel concluded that Potassium Silicate, Sodium Metasilicate, and Sodium Silicate are safe for use in cosmetic products in the practices of use and concentration described in this safety assessment, when formulated to avoid irritation.

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POTASSIUM SILICATE, SODIUM METASILICATE AND SILICATE

117

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Final Report of the Cosmetic Ingredient Review Expert Panel

Safety Assessment of Silica and Related Cosmetic Ingredients

September 25, 2009

The 2009 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D. Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D. James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

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ABSTRACT

This is a safety assessment of silica and the related cosmetic ingredients: alumina magnesium metasilicate, aluminum calcium sodium silicate, aluminum iron silicates, hydrated silica, and sodium potassium aluminum silicate. These ingredients are synthetic amorphous silicas and silicates; crystalline silica is not a cosmetic ingredient and is not used in cosmetics. These ingredients are used as bulking agents and for various other functions. The human and animal safety data relevant to the cosmetic use of these ingredients were assessed by the Cosmetic Ingredient Review (CIR) Expert Panel. The Panel concluded that these ingredients are safe as cosmetic ingredients in the practices of use and concentrations as described in this safety assessment when formulated to be non-respirable.

INTRODUCTION

There are 2 categories of silica, crystalline and amorphous. Only the amorphous forms of silica, and more specifically, synthetic amorphous silica and silicates, are used in cosmetics. Accordingly, this safety assessment addresses silica, alumina magnesium metasilicate, aluminum calcium sodium silicate, aluminum iron silicates, hydrated silica, and sodium potassium aluminum silicate.

Crystalline silica is not used in cosmetics.

Synthetic amorphous silica is created by 2 methods, wet and thermal. The wet process creates silica gel and hydrated silica (also known as precipitated silica). The thermal process creates fumed silica, which is also referred to as pyrogenic silica in recent publications. These are the only forms of silica addressed in this safety assessment. The fumed silica is not to be confused with silica fume, which is a crystalline form of silica, not used in cosmetics, and is not considered in this safety assessment. Figure 1 demonstrates the different polymorphs of silica.

In each summary of data, the term for the form of silica used by the author is used. When the type of silica is not made clear the term silica is used.

An earlier safety assessment by the Cosmetic Ingredient Review (CIR) Expert Panel addressed the safety of aluminum silicate, calcium silicate, magnesium aluminum silicate, magnesium silicate, magnesium trisilicate, sodium magnesium silicate, zirconium silicate, attapulgite, bentonite, fuller's earth, hectorite, kaolin, lithium magnesium silicate, lithium magnesium sodium silicate, montmorillonite, potassium silicate, pyrophyllite, sodium metasilicate, sodium silicate, and zeolite. The CIR Expert Panel concluded that these ingredients were "...safe as used in cosmetic products...". The Panel also reviewed potassium silicate, sodium metasilicate, and sodium silicate and concluded that they were "...safe for use in cosmetic products in the practices of use and concentration described in this safety assessment, when formulated to avoid irritation..." (Andersen 2003, 2005).

CHEMISTRY

Definition and Structure

Silica

The CAS No. 7631-86-9 is the general CAS No. which includes all forms of Silicas including amorphous, crystalline, synthetic, and natural forms (United Nations Environmental Programme Chemicals Unit [UNEP] 2004). This safety assessment is limited to amorphous forms of silica.

According to the *International Cosmetic Ingredient Dictionary and Handbook*, silica (CAS Nos. 7631-86-9 [colloidal], 60676-86-0, 112945-52-5 [fumed]) is the inorganic oxide that conforms to the formula SiO_2 . Silica functions as an abrasive, absorbent, anti-caking agent, bulking agent, opacifying agent, and suspending agent - nonsurfactant. Other technical names for Silica are:

- amorphous silica;
- amorphous silicon oxide hydrate;
- silica, amorphous;
- silicon, anhydride;
- silicon dioxide; and
- silicon dioxide, fumed (Gottschalck and Bailey 2008).

The current terminology for “silicon dioxide, fumed” is “pyrogenic silica.”

Alumina Magnesium Metasilicate

Alumina magnesium metasilicate (no CAS No.) is the inorganic compound that conforms generally to the formula: $\text{MgSiO}_3 \cdot \text{Al}_2\text{O}_3$.

Alumina Magnesium metasilicate functions as an absorbent, bulking agent, and a viscosity increasing agent - nonaqueous. Another technical name is magnesium metasilicate/aluminate (Gottschalck and Bailey 2008).

Aluminum Calcium Sodium Silicate

Aluminum Calcium sodium silicate (CAS No. 1344-01-0) is a complex silicate refined from naturally occurring minerals. Aluminum calcium sodium silicate functions as a bulking agent (Gottschalck and Bailey 2008). Other technical names include:

- aluminosilicic acid (unspecified), calcium sodium salt, hydrate;
- aluminosilicic acid, calcium sodium salt;
- calcium sodium aluminosilicate;
- sodium calcium aluminosilicate;
- sodium calcium aluminosilicate, hydrated;
- sodium calcium silicoaluminate; and
- sodium calcium silicoaluminate hydrate (ChemIDplus Lite 2009).

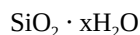
Aluminum Iron Silicates

Aluminum iron silicates (no CAS No.) is a ceramic powder consisting mainly of silicon dioxide, aluminum oxide, and

ferric oxide. Aluminum iron silicates function as abrasives and bulking agents. Another technical name is silica aluminum silicate ceramics (Gottschalck and Bailey 2008).

Hydrated Silica

Hydrated silica (CAS Nos. 1343-98-2 [Silicic Acid]; 10279-57-9; 63231-67-4; 112926-00-8) is the inorganic oxide that conforms generally to the formula



where x varies with the method of production and extent of drying performed on the material. Hydrated silica functions as an abrasive, absorbent, anti-caking agent, bulking agent, opacifying agent, oral care agent, skin-conditioning agent - miscellaneous, and viscosity increasing agent - aqueous. It is also referred to as:

- silicic acid;
- hydrosilicic acid;
- precipitated silica;
- silica gel;
- silica hydrate;
- silicic acid hydrate; and
- silicon dioxide hydrate (Gottschalck and Bailey 2008)
- colloidal silica (Arts et al. 2007).

Sodium Potassium Aluminum Silicate

Sodium Potassium aluminum silicate (CAS No. 12736-96-8 and 66402-68-4) is a complex silicate refined from naturally occurring minerals, or derived synthetically. It functions as a bulking agent (Gottschalck and Bailey 2008).

Amorphous Vs. Crystalline Silica

Silica is a silicon-oxygen tetrahedral unit where a silicon atom is central within 4 oxygen atoms that are shared with adjacent silicon atoms. Various physical forms of silica are caused by differences in the spatial relationships of the tetrahedral that determine physical characteristics. Amorphous silica has an irregular tetrahedral pattern. Crystalline silica is polymorphic where each variety has a characteristic regular 3-dimensional arrangement of the tetrahedral (Heppleston 1969). As would be predicted from these descriptions, crystalline silica has a well-defined x-ray diffraction pattern; whereas amorphous forms of silica do not (Villota and Hawkes 1986).

There are 3 classifications of amorphous silica. Vitreous silica, or fused crystalline silica, is formed by the supercooling of molten silica. It has a low coefficient of thermal expansion, high thermal shock resistance and high ultraviolet transparency. Microamorphous silica is a dense thermally unstable amorphous silica which converts to quartz at high temperatures. Microamorphous silica includes solutions, gels, powders, and porous glasses. This group has the subclasses amorphous silica fibers; microscopic fibers; and microparticulate silica, which includes precipitated and fumed silicas (Villota and Hawkes 1986). Kaewamatawong et al. (2005) divides the microamorphous forms of amorphous silica

into fumed, colloidal, precipitated, diatomaceous earth, gel, and hydrous. The colloidal form ranges in size from 10 micrometers to <10 nanometers.

The different polymorphs of silica are shown in Figure 1. Again, only synthetic amorphous silica forms are used in cosmetics. Crystalline silica forms are not used in cosmetics.

Physical and Chemical Properties

Properties

Silica

The acidity of synthetic amorphous silica is related to the number and reactivity of the silanol groups present on the solid silica surface. Surface silanols ($\text{pK}_a = 7.1$) are more acidic than monosilicic acid ($\text{pK}_a = 9.8$). The acidity increases with the degree of polymerisation (Yates and Healy 1976).

Villota and Hawkes (1986) stated that the surface of silica may be made up of free silanol groups (isolated hydroxyls), hydrogen-bonded silanol groups (hydroxyl groups on adjacent surface silicon atoms) and siloxane groups.

Amorphous silica is capable of rehydroxylating in aqueous systems to form a high ratio of silanol to siloxane groups. Depending on the hydrophobic properties of the solvent, it may form a network-like structure through hydrogen bonding. This gives amorphous silica gelling and thickening abilities in various solvent systems.

Oxygen electron donors of compounds such as ethers, alcohols, and ketones or the nitrogens of amides and amines may interact through hydrogen bonding due to the acid dissociation constant of the silanol groups on the silica surface. Esterification has been reported with an Si-O-C-R structure. A totally dehydrated silica or a fully hydrated silica has little or no adsorption of hydrophobic organocompounds (Villota and Hawkes 1986).

The *Food Chemicals Codex* states that silica is a white, fluffy nongritty powder of extremely fine particle size that is hygroscopic. Silica absorbs moisture from the air in varying amounts (Food and Nutrition Board [FNB] 1996).

Cabot Corporation (2004) stated that silica has thixotropic properties. The particles form a 3-dimensional network in a liquid system which increases viscosity. When shear forces are applied (i.e., stirring), the material flows as a liquid. When the forces cease, the material gels again.

The saturation concentrations for a set of analyzed Silicas ranged from 1.91 to 2.51 mmol/l (European Centre for Ecotoxicology and Toxicology of Chemicals [ECETOC] 2006). Saturation concentration increased with specific surface area. Surface treated, hydrophobic silica had a low solubility compared to hydrophilic silica. This was due to reduced wetting of the surface in aqueous systems. Wetting was increased by alcohol which decreased solubility.

Additional chemical and physical properties of silica are listed in Table 1. Physical properties by Brunauer, Emmett, and Teller (B.E.T.; a rule for the physical adsorption of gas molecules on a solid surface that serves as the basis for the

measurement of the specific surface area of a material) surface areas of 200, 325, or 380 m²/g are shown in Table 2. Note that the properties are the same regardless of B.E.T. in this range.

Particle Size and Form

Silica

Amorphous Silicas are composed of very fine particles (average of 20 µm) which tend to aggregate loosely in the air (Byers and Gage 1961).

Primary particles, or single particles, do not exist in isolation in fumed (pyrogenic) and precipitated silica; only in silica sol (colloidal). Aggregates assemble in chains (fumed) or clusters (precipitated and gel). Agglomerates are assemblies of aggregates, held together by strong physical adhesion forces and not in a dispersible nano size (< 100 nm) (ECETOC 2006; Gray and Muranko 2006).

Methods of Manufacture

All of the cosmetic ingredients in this assessment have mineral sources, but may be synthetically manufactured. Sodium Potassium Aluminum Silicate is refined from naturally occurring minerals or derived synthetically. Aluminum Calcium Sodium Silicate is refined from naturally occurring minerals (Gottschalck and Bailey 2008).

Silica

Amorphous silicas used in cosmetics (silica gel, precipitated silica, and pyrogenic silica in Figure 1) are synthetically produced. A manufacturing process for amorphous pyrogenic silica is shown in Figure 2 (Villota and Hawkes 1986).

Lewinson et al. (1994) stated that silica may be produced by a vapor-phase process producing fumed (pyrogenic) silica or by a wet process producing precipitated silica (silica gel or precipitated silica in Figure 1). Fumed silica is produced in a relatively anhydrous state, whereas precipitated silica contains a larger amount of bound water.

Mean particle size, particle size distribution, and degree of aggregation and/or agglomeration can be determined by adjusting the process parameters (Hurd and Flower 1988).

ECETOC (2006) reports that the thermal synthesis process without liquid water results in the presence of fewer silanol groups on pyrogenic silica (Figure 1).

Silicas manufactured by the wet process (silica gel and precipitated silica) contain between 2% and 10% physically bonded water which can be removed by drying.

Amorphous pyrogenic silica (Figure 1) is manufactured by the hydrolysis of volatile silanes, usually silicon tetrachloride, in the flame of an oxygen-hydrogen burner. The silicon tetrachloride is continuously vaporized, mixed with dry air then hydrogen, and then hydrolysed. The silica is then grown (nucleation, condensation, coagulation) and aggregated.

Precipitated silica and silica gels (Figure 1) are produced from an alkali metal silicate dissolved in water (i.e., water

glass) and an acid, usually sulphuric acid. After the reaction of water glass with the acid, silica is precipitated. The properties of the silica can be influenced by the type of reactor and process parameters. The silica is filtered out and the resulting cake is 15% to 25% silica by weight. The cake is then dried and then milled.

Silica gels (Figure 1) are produced by the neutralization of an aqueous solution of alkali metal silicate with sulphuric acid (gelation). Mixing continues until solidification begins; the gelation conditions dictate particle size in the hydrogel. The gel is then washed of excess salts; this procedure determines specific surface area. The silica gel is now a continuous structure with pores filled with water. The silica may be used as is, or dried. Xerogels are dried until water is still evaporating but the gel no longer shrinks. Aerogels are dried with negligible loss of pore volume.

Silica sols (colloidal silica; Figure 1) are dispersions of silica particles in a liquid, usually water, at 15% to 50%. These are sub-micron particles of silica and the sols flow like water. Silica sols are also produced by hydrolysis of monomeric SiCl_4 in aqueous solution followed by condensation of the original particles. Large particles are produced by hydrolysis of tetraethoxysilane in an alkaline solution of water and alcohol. Silica sols are also produced by redispersion of existing silicas (gels, precipitated or, occasionally pyrogenic). For any process, the dispersed silica particles are stabilized by the addition of KOH, NaOH, NH_3 , or HCl (ECETOC 2006).

There was no information found on the manufacturing processes for the salts of silica in this report.

Analytical Methods

Silica

Surface silanols of silica may be analyzed by Fischer titration; chlorination with thionylchloride (SOCl_2); Zerewitinoff determination with methyl magnesium iodide (CH_3MgI) or methyl lithium (CH_3Li); infrared-spectroscopy; ^{29}Si cross-polarization magic-angle-spinning (CP-MAS) nuclear magnetic resonance (NMR) spectroscopy; titration with caustic soda (NaOH); and thermo-gravimetric analysis (loss on ignition) (ECETOC 2006).

Gas adsorption may be used to determine specific surface area and porosity of pyrogenic silica (ECETOC 2006). Infrared (IR) spectroscopy was used to analyze silanol groups, alkyl groups, and silonol groups. NMR spectroscopy was used to determine relative amounts of mono-, di-, and trialkylsilane groups, cross-linking of alkylsilanes, side chains, ethoxy and methoxy groups, extractable organic compounds, and Si-O-Si bond distribution. Electron spectroscopy was used to analyze composition and chemical state of the surface and concentration gradients and diffusion profiles of silica. Mass spectroscopy was used for trace analysis of the surface of silica. Atomic force microscopy (AFM) was used to analyze morphology and porosity. Gravimetry and titration was used to analyze silanol groups.

Particle size analysis may be done by sieving, cascade impactor, time-of-flight, dynamic light scattering, static light scattering, Fraunhofer diffraction with air disperison, and Fraunhofer diffraction with liquid disperison (ECETOC 2006).

Sayes et al. (2007) stated that the surface area of silica particles may be measured by the B.E.T. method; and size, size distribution, and surface charge by dynamic light-scattering (DLS) spectroscopy.

Impurities

Silica

Cabot Corporation (2004) states that its silica products are >99.8% pure. The moisture content of untreated silica is < 1 wt%. Treated silicas are susceptible to adsorbing chemical vapors.

Pyrogenic silica was reported to be >99.8% pure with Al_2O_3 (< 0.05%), Fe_2O_3 (0.003%), TiO_2 (< 0.03%), Na_2O (<0.0009%), and chlorides as Cl (<0.025%). Precipitated silica and silica gel were reported to be $\geq 95\%$ pure with Na_2O (0.2% to 2.4%), sulphates as SO_3 (0.2% to 3.0%), Fe_2O_3 (<0.05%), and trace oxides (<0.07%) (Roempp 2001).

The composition of colloidal silica was reported to be: SiO_2 ($\geq 30\%$), Na_2O (0.1% to 0.4%), sulfates as NaSO_4 (0.01% to 0.03%), and aluminum oxide as a stabilizer (0.2%) (W.R. Grace & Co. 2003).

UNEP (2004) reported silica to be >95% pure. Possible impurities include: Na_2O (0.2% to 2.1% wt.), sulfates as SO_3 (0.2% to 3.0% wt.), Fe_2O_3 (< 0.05% wt.), and trace oxides (<0.07% wt.). Heavy metal impurities include: antimony (<5 ppm), barium (<50 ppm), chromium (<10 ppm), arsenic (<3 ppm), lead (<10 ppm), mercury (<1 ppm), cadmium (<1ppm), and selenium (<1 ppm).

USE

Cosmetic

According to information supplied to the Food and Drug Administration (FDA 2009) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), silica was used in a total of 3,276 cosmetic products. Use concentrations ranged from 0.0000003 - 44% according to a survey of current use concentrations conducted by the Personal Care Products Council (Council 2008). Hydrated silica is reported to be used in 176 products in the VCRP, at use concentrations of 0.001 - 34% based on the Council survey. Alumina magnesium metasilicate was not reported to be used in any products in the VCRP, but use concentrations between 0.001 and 0.02% were reported in the Council survey. Aluminum calcium sodium silicate was reported to be used in 7 cosmetic products in the VCRP, at use concentrations of 0.4 - 6% in the Council survey. Sodium potassium aluminum silicate was reported to be used in 1 product in the VCRP, with a use concentration of 0.001 - 4% in the Council survey. There were no reported uses in the VCRP or concentration of use reported in the Council survey for Aluminum iron silicates. Available data for the number of uses and use concentrations as a function of cosmetic product type are given in Table 3.

Silica is used in hair color sprays/aerosols. Jensen and O'Brien (1993) reviewed the potential adverse effects of

inhaled aerosols, which depend on the specific chemical species, the concentration, the duration of the exposure, and the site of deposition within the respiratory system.

The aerosol properties associated with the location of deposition in the respiratory system are particle size and density. The parameter most closely associated with this regional deposition is the aerodynamic diameter, d_a , defined as the diameter of a sphere of unit density possessing the same terminal settling velocity as the particle in question. These authors reported a mean aerodynamic diameter of $4.25 \pm 1.5 \mu\text{m}$ for respirable particles that could result in lung exposure (Jensen and O'Brien, 1993).

Bower (1999) reported diameters of anhydrous hair spray particles of 60 - 80 μm and pump hair sprays with particle diameters of $\geq 80 \mu\text{m}$. Johnsen (2004) reported that the mean particle diameter is around 38 μm in a typical aerosol spray. In practice, he stated that aerosols should have at least 99% of particle diameters in the 10 - 110 μm range.

Non-Cosmetic

Silica

Silica is used as a filler in rubber formulations (Byers and Gage 1961).

Silica is used in food preparations as an anticaking agent in dry powders, dispersion agent for dry powders in liquids, antisetling or suspending agent, stabilizer in oil/water emulsions, thickening or thixotropic agent, gelling agent, flavor carrier, extrusion aid, clarification and separation aid, and support matrix for immobilization of enzymes. It is also a general excipient for pharmaceuticals (Villota and Hawkes 1986). It is also used as a defoaming agent, conditioning agent, a chillproofing agent in malt beverages, and a filter aid in foods (FNB 1996). UNEP (2004) states that silica is used in pharmaceuticals as a thickener in pastes and ointments to inhibit the separation of components and maintain flow properties in powder products. It is also used in foods. Silica can function as a carrier for fragrances or flavors. It is used in beer and wine clarification. Silica is used in animal feed as carriers and anticaking agents in vitamins and mineral premixes. Silica is used as reinforcing fillers for many non-staining and colored rubber and silicone products. It is used in "green tires". Silica is also used in paints, lacquers, plastics, and paper. Silica is used as an insecticide by its sorption of the cuticular lipid layer causing dehydration.

The colloidal form of silica is used in fiber, sizing, diazo paper manufacture, cellophane film, ceramics, glass fiber, paints, batteries, foods, and polishing (Kaewamatawong et al. 2005).

Javadzadeh et al. (2007) investigated the use of silica in a drug delivery system.

Aluminum Calcium Sodium Silicate

Aluminum calcium sodium silicate (sodium calcium aluminosilicate, hydrated) is generally recognized as safe (GRAS) in food for use at a level not exceeding 2% in accordance with good manufacturing processes as an anticaking agent (FDA

2007).

Hydrated aluminum calcium sodium silicate is used for countering the effects of aflatoxin (AF) in animal feed (Colvin et al 1989; Kubena et al. 1991; Chestnut et al. 1992; Harvey et al 1994; Smith et al. 1994; Abo-Norag et al 1995; Sarr et al. 1995; Edrington et al 1996; Abdel-Wahhab et al. 1998; Kubena et al. 1998; Ledoux et al. 1998; Mayura et al 1998; Bingham et al. 2004; Sehu et al 2007).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism and Excretion

Oral

Silica and Hydrated Silica

Sauer et al. (1959a,b) orally administered silica (80 mg/dose) in the form of sodium metasilicate, hydrated silica, and silica solution (30%) to guinea pigs (n = 5) in a single dose or in 4 repeated doses every 48 h. Urine and feces were collected in 48-h increments after each dose and analyzed for silica content.

The urinary output of silica, in the form of sodium metasilicate, when orally administered peaked within 48 h and gradually returned to normal after 8 days. When administered 4 times, 48 hours apart, the peak was maintained, but not increased. Forty-eight h after the last dose the concentration of silica in the urine began to return to normal.

The urinary output of silica, in the form of silica solution and precipitated silicic acid, when orally administered peaked within 48 h and gradually returned to normal after 8 days. These peaks were much lower than those of sodium metasilicate. When administered 4 times, 48 hours apart, the silica concentrations behaved similarly to the orally administered silica with a lower peak. When orally administered, 63% of the silica was recovered. The authors suggested that all of the silica in the urine was in the soluble or molybdate reactive form and that the silica particles underwent depolymerization prior to excretion (Sauer et al. 1959a,b).

UNEP (2004) reported an unpublished oral study of silica (1500 mg/kg/d) using female rats (strain and n not specified). Silica was orally administered daily for 30 days. The rats were then killed and necropsied. The silica content in the livers was 1.5 µg, in the kidneys was 6.4 µg, and in the spleen was 5.3 µg. The control values were 1.8, 7.2, and 7.8 µg silica, respectively.

In another unpublished study, female Sprague-Dawley (n not specified) rats were orally administered silica (100 mg/rat; ~500 mg/kg; aqueous suspension) 20 times over 1 month. No clinical signs were observed. The silica content in the liver was 4.2 µg (control value = 1.8 µg), in the spleen was 5.5 µg (7.2 µg), and in the kidneys was 14.2 µg (7.8 µg) (UNEP 2004).

Parenteral**Silica and Hydrated Silica**

Sauer et al. (1959a) also administered silica solution or precipitated silica by intraperitoneal (i.p.) injection to guinea pigs (n = 5; see ORAL section above for details). Urinary silica increased above normal for 16 days. The levels of silica as silicic acid increased above normal for 28 days. Recovery of silica was 48%. The authors suggest that conditions in the peritoneal cavity favor the depolymerization and subsequent excretion of silica.

Intratracheal**Silica and Hydrated Silica**

Byers and Gage (1961) intratracheally injected 3 types of silica (25 mg; 2.5% in 1 ml suspension) into adult albino Wistar rats (n = 100; 50 male, 50 female). Type A1 had a particle size of 19 μm ; type A2 had a particle size of 20 μm but after storage became 60 μm ; and type B had a particle size of 25 μm . Types A1 and A2 were from the same manufacturer. Rats were killed and necropsied at 12, 24, and 52 weeks. The amounts of silica in the tissues are given in Table 4.

The 3 types of silica elicited the same type of response in the lungs between types and sexes. Distribution of silica particles in the lungs was not uniform but aggregated in small areas throughout 1 or both lungs with the occasional large deposit. Silica particles were contained within macrophages, aggregated into foci around terminal and respiratory bronchioles. Some lymphocytes and fibroblasts surrounded these foci and intermingled within the macrophages with reticulin fibers woven through and around the lesion. Where dust deposits were heavy, the structure of the lung segments was completely obliterated by the lesions consisting of a group of macrophages with a few fibroblasts and lymphocytes that were contiguous and distinguished by the peripheral zone of fibroblasts and lymphocytes. These lesions were maximal at 12 weeks and gradually reduced with some contraction resulting in varying degrees of deformity of the lung. The lesions decreased in size and number over time and as function of the amount of silica present in the lungs.

Type B was the most quickly eliminated from the lungs; type A2, the largest particles, were the slowest. Type A2 also had larger lesions and induced a greater amount of fibroblastic proliferation. Lesions in the type B group were initially similar to A1 but the regression of the former was more rapid and final sections showed either a complete resolution or small scattered dust foci with few reticulin fibers. There also remained a few areas of confluent lesions which were small and irregular with a light reticulin network and little retraction. Evidence of infection (mild emphysema, few large abscesses, foci of bronchiectasis, pneumonia and bronchopneumonia) was infrequent and similar to controls.

The authors concluded that the dust from these 3 samples of precipitated silica do not aggregate sufficiently to be entirely retained by the upper respiratory passages and is still detectable in the lungs after 12 months. The lesions are different from those reported to be from quartz. The greater severity of the lesions from types A2 can be attributed to those

surface properties which resulted in its greater tendency to aggregate (Byers and Gage 1961).

Inhalation

Silica and Hydrated Silica

Klosterkötter and Bünemann (1961, 1962) exposed female rats (strain and n not provided) to aerosolized pyrogenic or precipitated silica (concentration and particle size not provided) for up to 6 days. After 3 months of recovery, 73.8% of the inhaled silica had been eliminated from the lungs. Only small amounts of silica were observed in the mediastinal lymph nodes.

In a second similar study, female rats were exposed to aerosolized pyrogenic or precipitated silica (particle size not provided) to study elimination from the lungs. Elimination was only slightly influenced by particle size. The lymphnodes were moderately enlarged with a silica content of < 2% eliminated. Most of the silica was eliminated within 1 to 2 months. Small amounts were detected in the lymph nodes. The precipitated silica, which was less soluble, was more slowly eliminated than the more soluble pyrogenic types (Klosterkötter and Bünemann 1961,1962).

UNEP (2004) reported several unpublished studies. In an unpublished inhalation study of silica (0.050 to 0.055 mg/l; particle size not provided) using female Sprague-Dawley rats (n not provided) the rats were exposed to aerosolized silica for 5 h/d for 5 d/weeks for 1 year. The rats had occurrences of bronchitis, putrid, lung inflammation, and pronounced cell reactions so exposure was reduced to 2 or 3 d/week; the exact time of the change was not provided. Rats in each group were killed and necropsied periodically during treatment and after treatment.

After 6 weeks of treatment, silica was observed in the lungs (0.5 mg) and the mediastinal lymph node (0.02 mg); after 18 weeks these values were 1.2 mg and 0.11 mg; and after 12 months, 1.37 mg and 0.13 mg, respectively. Corresponding to the respiration volume, 1% of the inhaled silica was retained in the lungs. After a recovery period of 5 months, there was 0.160 mg and 0.047 mg silica observed in the lungs and mediastinal lymph node, respectively, a reduction of 88% in the lung and > 50% in the lymph nodes. The increase in lung deposition was rapid at the initial exposure; levels of deposited silica were low from 18 weeks to 12 months of exposure.

In another unpublished inhalation study, female inbred albino rats (strain not specified; n not provided) were exposed to aerosolized silica (dose and particle size not provided) for 40 days. The amount was then increased to 40 to 50 mg/m³ until day 120. A few of the rats were killed and necropsied periodically.

The average 1-day retention value was 28 µg/lung at the lower unspecified concentration. During the first 10 days, a steep linear increase was seen with ~28 µg/day as theoretically expected. Increments then became smaller. The author suggested that elimination increased and that an equilibrium between retention and elimination was established. After 40 exposures, the average 1-day retention value was 59 µg/lung at the high concentration. After 120 exposures, the total deposit

(lung and mediastinal lymph nodes) was 435 $\mu\text{g}/\text{lung}$, equivalent to 7.4 % of the theoretically deposited material (5840 $\mu\text{g}/\text{lung}$, based on the measured 1-day retention); more than 92% of the deposited silica in the alveoli was eliminated during the exposure period. At that time, the mean retention of the lungs was only 300 $\mu\text{g}/\text{lung}$ (~ 69% of the total). The deposition rate in the mediastinal lymph nodes was negligible during the first 40 days, but increased gradually. After 120 exposures, the retention was substantial amounting to 135 μg (~ 31% of the total deposit). A test for the determination of free alveolar cells showed a decrease immediately after a single exposure and 24 hours later an increase of 100% was observed.

In another unpublished inhalation study, aerosolized silica (0.05 mg/l; particle size not provided) was administered for 5 h per day for 3 days to female Sprague-Dawley rats (n not specified). They were observed for up to 3 months. Twenty h after the last exposure, 0.25 mg silica was found in the lungs. After 3 months, the silica content was 0.018 mg. In the lymph node, 0.018 mg silica was found after 1 month and 0.008 mg silica after 3 months.

In an unpublished inhalation study of precipitated and pyrogenic silica (55 mg/m^3 ; particle size not provided), rats (strain and n not specified) were exposed for 5 h to precipitated silica. For the precipitated silica, the mean retention value at 20 h was 0.138 mg/lung. For the pyrogenic silica, the mean retention value was 0.130 mg/lung. For the precipitated silica, the mean silica-content of the lungs after 4 months recovery was 1.022 mg, and 3.113 mg after 12 months. The corresponding values for the mediastinal lymphatic nodes were 0.033 mg and 0.069 mg, respectively. Five months after exposure, the average value for the lungs was only 0.457 mg (87% elimination rate) and 0.052 mg for the mediastinal lymphatic nodes (UNEP 2004).

ECETOC (2006) reported an unpublished study in which rats (strain and n not provided) were exposed to aerosolized hydrophobic silica (50 mg/m^3 ; particle size not provided) for 1 or 3 days. The rats were killed and necropsied after 20 h, 1 month, or 3 months. At 1 month recovery, elimination of silica was 78% (1 day exposure) and 75% (3 days exposure). After 3 months recovery, elimination was 87% and 92%, respectively. There was little silica in the mediastinal lymph nodes.

In another unpublished study, rats (strain and n not provided) were exposed to aerosolized hydrophobic silica (200 mg/m^3 ; particle size not provided) for 5 h/d for 3 days. After 3 months recovery, 81% of the silica was eliminated. Elimination by the lymph nodes was marginal (ECETOC 2006).

Subcutaneous

Pyrogenic Silica

UNEP (2004) reported an unpublished subcutaneous (s.c.) study of a single dose of silica (10 mg) using female Sprague-Dawley rats (n not provided). After 24 hours, 6.89 mg silica was found in the tissue at the application site. After 1 month the amount was decreased to 0.646 mg; after 2 months 0.298 mg was found.

ECETOC (2006) reported an unpublished report where pyrogenic silica (10 mg in water) was subcutaneously injected

in rats (strain and n not specified). It was quickly removed from the injection site. Mean recovery was 6.90 mg at 24 h, 0.65 mg after 1 month, and 0.30 mg after 2 months.

In another study, pyrogenic silica (30, 40, or 50 mg in water) injected subcutaneously in rats was 95% to 97% recovered after 6 weeks (ECETOC 2006).

Cytotoxicity

Pyrogenic and Hydrated Silica

Mammalian Cells

FDA (no date) reported the results of a cytotoxicity test using Chinese hamster V79 cells. There were no effects after 144 h of exposure to silica.

Davies (1981) incubated pyrogenic or hydrated (precipitated) silica or silica gel (13.5, 25 or 50 $\mu\text{g}/\text{cm}^3$) with mouse macrophages for 18 h. Pyrogenic silica and silica gel were cytotoxic to mouse macrophages similarly to crystalline silica (also tested); precipitated silica was less cytotoxic. The author concluded that all 3 silica types were cytotoxic at 13.5 $\mu\text{g}/\text{cm}^3$.

Zimmerman et al. (1986) exposed macrophages and neutrophils from C57BL/6 x DBA/2) F_1 (DBF₁) mice to pyrogenic silica (0, 100, 300, or 500 μg) for 1 to 3 h. Incubation with mid or high concentrations killed 80% to 100% of both types of phagocytes. An additional experiment showed that macrophages and neutrophils incubated with 10 or 30 μg silica were 88% to 99% viable after 1 to 3 h. When incubated in silica, both macrophages and neutrophils were inhibited in their ability to phagocytize sheep red blood cells, less so on neutrophils. The ability of these cells to phagocytize *Listeria monocytogenes* was completely inhibited at 300 μg and above; the inhibition was concentration dependent between 10 and 100 μg . Pre-incubation with silica (100 to 500 μg) also inhibited bactericidal activity of the macrophages and neutrophils; there was no effect on bacterial growth. There was a concentration dependent inhibition of bactericidal activity when the phagocytes were pre-incubated in silica at 10 to 300 μg .

Nyberg et al. (1996) incubated macrophages from male Sprague-Dawley rats with silica particles ($3.2 \pm 0.4 \mu\text{m}$) for 30 min. The number of ingested yeasts were higher than the control silica particles. Silica treated macrophages were similar to resting macrophages.

Pandurangi et al. (1990) incubated sheep blood erythrocytes (1%) with pyrogenic silica (0.02 to 1.0 mg/ml) for 30 min. There was ~85% lysis for all concentrations tested. The authors concluded that the hemolytic activity may be connected to surface free OH group concentration.

Liu et al. (1996) exposed Chinese hamster lung fibroblasts (V79 cells) to silica (0, 20, 40, 80, or 160 $\mu\text{g}/\text{ml}$) for 24 h. Silica was cytotoxic at 80 $\mu\text{g}/\text{ml}$ and no effects were observed at 40 $\mu\text{g}/\text{ml}$.

Mollo et al. (1997) incubated rat pleural mesothelial cells in fluorescein isothiocyanate-labeled silica (17, 33, and 66

µg/ml) for 6 and 24 h. The silica was present in the cytoplasm and concentrated around the nucleus, suggesting particle uptake, at both observation times. There was evidence of silica particles present in internalization vacuoles. The authors suggested that exposure to silica elicited an immediate defense response from cells through release of oxidizing and/or radical annealing agents.

Cha et al. (1999) incubated 2 renal cell lines (S_1 and IMCT) from transgenic mice harboring the SV40 large T antigen gene with various concentrations of silica (particle size 0.5 to 10 µm), or saline (control) for 1 h. The effect of silica (0.6 to 600 µg/ml) on cell injury was examined using trypan blue exclusion. After 1 h, silica-induced injury increased in a concentration-dependent manner in both cell lines. Cell injury increased at 60 µg/ml for S_1 cells and at 6 µg/ml for IMCT cells. At 600 µg/ml, cell injury was $28.0 \pm 1.5\%$ and $47.0 \pm 2.2\%$, respectively. Cell injury was reduced with the addition of the chelator as well as a calcium channel blocker. Cells incubated for 1 h in silica had reduced $[ATP]_i$ in a concentration-dependent manner in both cell lines, but increased $[Ca^{2+}]$. The authors suggested that alteration of intracellular calcium homeostasis by silica is closely related with renal cell injury.

Kim et al. (2002) exposed alveolar macrophages collected from the lungs of Sprague-Dawley rats to silica (0.5 - 10 µm particle size). When macrophages were exposed to silica, cell death occurred in a concentration dependent manner with ~60% viability in cells exposed to 100 to 200 µg/ml which was attenuated by ambroxol. The authors concluded that ambroxol had a depressant effect on the silica-stimulated responses and cell death, which may be due to the inhibition of activation processes, protein kinases, and calcium transport.

Human Cell Lines

O'Reilly et al. (2005) exposed normal human primary fibroblasts to silica (10 to 100 µg/ml) for 24 h to explore the sources of pulmonary inflammation from silica inhalation. Silica exposure induced cyclooxygenase (COX)-2 in a dose dependent manner starting at 10 µg/ml (10-fold more than crystalline silica). COX-1 expression was not affected by silica. Silica was nontoxic to the fibroblasts up to 100 µg/ml. COX-1 mRNA was unchanged by silica exposure. When exposure time was increased to 6 h, expression of COX-1 was induced at all concentrations. Prostaglandin (PG) E_2 was increased in a dose and time dependent manner, ~7 times more potent than crystalline silica. There was no increase in interleukin (IL)-1β expression. Silica exposure increased mPGES expression and was persistent up to 72 h. Silica exposure stimulated production of PGF $_{2\alpha}$ in a dose dependent manner and was 10-fold lower than PGE $_2$. Silica did not induce IL-6, monocyte chemoattractant protein (MCP)-1, or transforming growth factor (TGF)-β production but did strongly induce IL-8 production. The authors suggest that increased production of PGE $_2$ prevents the lung's transient inflammatory response from developing into fibrosis.

Brunner et al. (2006) tested the cytotoxicity of silica. Human mesothelioma MSTO-211H and rodent 3T3 fibroblast

cells were cultured with silica (0 to 15 ppm and 0 to 30 ppm) for 6 and 3 days, respectively. By measuring both the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-conversion and DNA content, there were no effects on the cells by silica.

Sayes et al. (2007) incubated immortalized rat L2 lung epithelial cells, rat lung alveolar macrophages, or both of these cells combined with silica. In L2 cells, silica produced increases in LDH levels at 520 $\mu\text{g}/\text{cm}^2$ of cell culture at 4 h. At 24 and 48 h, LDH levels increased over controls at 5.2, 52, and 520 $\mu\text{g}/\text{cm}^2$ in L2 cells. In alveolar macrophages, silica produced no increase in LDH levels up to 48 h and 5200 $\mu\text{g}/\text{cm}^2$. The 2 cell types were cultured together; silica produced no increase in LDH except for 520 $\mu\text{g}/\text{cm}^2$ at 24 and 48 h.

Silica produced decreases in cellular MTT levels at doses of 5.3 and 52 $\mu\text{g}/\text{cm}^2$ at 4 and 24 h in alveolar macrophages and no effect in L2 cells. In the combined cultures, there were no changes in MTT values due to silica at 4 h but a decrease in MTT levels at 5.2 and 52 $\mu\text{g}/\text{cm}^2$. MIP-2 production did not increase due to L2 cells exposed to silica but did in alveolar macrophages after 24 h. There was no increase in tumor necrosis factor (TNF)- α for either cell type when exposed to silica but levels were increased at 0.52 and 5.2 $\mu\text{g}/\text{cm}^2$ after 24 h. Interleukin (IL)-6 levels were not increased for either type of cell at 24 h, however, when the cells were combined, IL-6 levels were increased at 0.52, 5.2, 52, and 520 $\mu\text{g}/\text{cm}^2$. The authors concluded that there was little correlation between in vivo (see Intratracheal section below) and in vitro results (Sayes et al. 2007).

Bacterial Cells

Several strains of bacteria (0.15 ml bacterial suspension) were exposed to pressed and unpressed high purity silica (0.2 g). Rod-shaped gram-negative strains (*Escherichia coli*, *Bacterium proteus*, *Pseudomonas aeruginosa*, and *Aerobacter aerogenes*) died between 6 h and 3 days in contact with unpressed silica. Gram-positive strains (*Proteus* sp., *Micrococcus pyrogenes aureus*, *Streptococcus faecalis*, *Streptococcus pyrogenes humanus*, *Corynebacterium diphtheriae*, *Candida albicans*, and *Bacillus subtilis*) were somewhat more resistant. Survival of bacteria exposed to unpressed silica was shorter than pressed silica (Keinholz 1970).

Various bacteria were incubated in silica (0.2 g; dilution 1:50,000 for *A. Aerogenes*, *Proteus* sp., *P. aeruginosa*, *E. coli*, and *S. aureus*, and 1:100,000 for *C. albicans* and *B. subtilis*) at 22°C or 37°C. The time until complete mortality was recorded up to 28 days (EC_{100}). ED_{100} ranged from 6 h to 22 days (UNEP 2004).

Sodium Potassium Aluminum Silicate

Alfaro Moreno et al. (1997) incubated thawed Balb 3T3 cells with Mexicali dust (sodium potassium aluminum silicate present as potassium aluminum silicates [98%] and sodium dioxide [2%]; 20, 40, or 80 $\mu\text{g}/\text{ml}$), chrysotile asbestos (40 $\mu\text{g}/\text{ml}$; positive control), or nothing (negative control) for 12 h. The medium was then changed and the cells allowed to incubate for

7 h. The cells were fixed. Between 220 and 280 anaphases for each concentrations were examined blind. Abnormal anaphases were observed in 27.42% of the cases in the low dose group, 29.60% in the mid dose group, and 37.10% in the high dose group. The asbestos induced abnormal anaphases in 34.78% of the cases and 11.62% in the control. The most frequent alterations were multipolar anaphases. An increase in anaphases with retarded chromosomes was observed in the test groups and the positive control. The frequency of anaphase bridges was lower in the treated groups than in the positive control group ($p < .05$). When comparing the mid dose group to the positive control group, there were more lagging chromosomes (23.95% vs 18.20%), fewer anaphase bridges (10.40% vs. 78.80%), and more anaphase bridges (10.40% vs. 1.51%). No changes were observed in the mitotic index of cells exposed to Mexicali dust. The authors concluded that Mexicali dust is capable of inducing anaphasic alterations.

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

Silica

Hazleton Laboratories (1958a) administered a single oral dose of pyrogenic silica (1.00, 2.15, or 3.16 g/kg) to male albino rats ($n = 5$). There were no gross signs of systemic toxicity observed and no mortalities. The LD_{50} was reported to be >3.16 g/kg.

W.R. Grace & Co. (1981) reported that the LD_{50} of pyrogenic silica was > 5.62 g/kg for male rats ($n = 30$). There were no toxic signs or deaths over the 2 weeks observation.

Lewinson et al (1994) orally administered pyrogenic silica (5040, 6350, or 7900 mg/kg in olive oil or 2500 or 5000 mg/kg in peanut oil) to Sprague-Dawley rats ($n = 10$) after fasting. The rats were monitored for 4 weeks, then killed and necropsied. There was no mortality. There were no toxicological signs and the necropsies were unremarkable. The authors concluded that acute dosing with silica is virtually nontoxic by the oral route.

UNEP (2004) and ECETOC (2006) reported several unpublished studies of acute oral toxicity of silica. The results of these studies are summarized in Table 5. ECETOC (2006) reported that there were no signs of toxicity. There were no macroscopic findings at necropsy. Animals died at doses of 10,000 and 20,000 mg/kg. At doses of 5,620 mg/kg and higher, the feces in some animals were white.

Aluminum Calcium Sodium Silicate

Abbés et al. (2006a) orally administered a single dose of hydrated aluminum calcium sodium silicate (400, 600, or 800 mg/kg) to female Balb/c mice ($n = 6$) with or without Zearalenone (ZEN; a mycotoxin produced by fusarium genera; 40

mg/kg). After 48 h, blood samples were collected and the mice killed and examined. ZEN caused reduced total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, total protein, albumin, white blood cell count, immunoglobulin profile (Ig A and Ig G) and T-cell subtypes. ZEN increased uric acid and urea and induced degenerative changes in the spleen tissues. The low dose of hydrated aluminum calcium sodium silicate alone had levels similar to control. The mid and high dose groups had increased cholesterol levels. Hydrated aluminum calcium sodium silicate mitigated the effects of ZEN at all dose levels. No adverse effects were reported for hydrated aluminum calcium sodium silicate alone at any dose.

Abbés et al. (2006b) orally administered a single dose of hydrated aluminum calcium sodium silicate (40 mg/kg or 500 mg/kg) to female Balb/c mice (n = 6) with or without ZEN (40 or 500 mg/kg). The high dose of ZEN is the reported LD₅₀. After 48 h, blood samples were collected and the mice were killed and the kidneys and livers dissected. ZEN increased hematocrit, hemoglobin, white blood cells, lymphocytes, eosinophils, neutrophils, monocytes, and most of the biochemical serum parameters. ZEN reduced platelets and induced degenerative changes in the hepatic and renal tissues. Hydrated aluminum calcium sodium silicate alone had no effect on these parameters.

Parenteral

Silica

Policard and Collet (1954) i.p. injected silica (30, 50, or 100 mg/kg in saline) to Wistar rats and rabbits. At 100 mg/kg, 20% to 30% of the animals died quickly. At 50 mg/kg, all the animals survived.

At necropsy, in the peritoneal cavity, vacuoles were observed in the cytoplasm and the nuclei were fragmented or destroyed; there were areas of damaged cells with normal or slightly altered histocytes at the periphery. Edema was observed that diminished with time. The lymph nodes were enlarged and contained large histocytes in various stages of degeneration. Lymphocytes were less numerous; the medullary sinuses were packed with clear cells. The thymus was atrophied. The spleen was hypertrophied and altered; the malpighian corpuscles were almost gone; the zones of blood sinusoids were disorganized. The liver was enlarged with many fat cells and clusters of cells, mainly histocytes. The adrenals were enlarged (50% to 97%); the lipids in the cortex had a change in distribution; there was a general increase of lipid cells throughout the cortex.

Surviving animals were killed after 8, 20, 30, and 60 days. Peritoneal edema diminished then disappeared over time. Small spherical nodules (1.5 to 2 mm) were observed on the omentum. Mesenteric and tracheobroncheal lymph nodes were 2- to 4-fold larger than controls. Microscopic examination revealed granuloma in the peritoneal lesions. The center was degenerating and there were dead cells and histocytes; the outer edge was made up of histocytes. Connective tissue showed a fibrous reaction. At 20 and 30 days, the center of the granuloma had a few cells and irregular thickened collagen fibers; the

periphery was packed with reticular fibers filling the intercellular spaces and surrounding the cells. The fibrosis gradually increased; after 30 and 60 days there were extensive fibrous areas that were almost acellular; the lymph nodes were similar (Poicard and Collet 1954).

Schepers et al. (1957d) injected silica (10% in saline; 2 ml) into the peritoneal cavity of 2 guinea pigs. Both animals died on day 2 of generalized acute peritoneal inflammatory reaction. The lungs were slightly congested and the spleen was swollen. There was a small amount of fluid and adhesions of the intestines and fibrin deposits on the liver. The remains of the silica were near these reactions.

Kang et al. (1992) intraperitoneally injected female Wistar rats ($n = 5$) with a single dose of pyrogenic silica (0.02, 0.1, and 0.5 g). After 5 days, the rats were killed and necropsied. The control group was injected with saline. No adhesions, ascites, or other intra-abdominal pathology was observed in the control group. The rats in the low, mid, and high dose groups treated with silica had 5 mild, 4 severe, and 4 severe adhesions, respectively. There were no, 4, and 5 occurrences of ascites and deposits of powder adherent to viscera and 5, 4, and 5 rats with ascites in the low, mid, and high dose groups, respectively.

UNEP (2004) reported an unpublished study that concluded that single i.p. injections of ≥ 50 mg silica caused death in rats.

Intravenous

Silica

Swensson et al. (1956) injected amorphous silica (0.01 to 0.1 μm diameter particles), in the form of commercial silica, or ground fused crystalline silica (0.15 to 0.45 μm), 0.05 mg at a time up to 0.1 ml in saline or all at once, into the tail vein of mice until the animals died or were in an unrecoverable condition. The mice survived larger quantities of silica if delivered in smaller doses. The toxicity decreased with increasing particle size. Toxicity of amorphous silica was lower than crystalline silica. The lethal dose of commercial silica ranged from 0.2 ± 0.01 to 0.5 ± 0.02 mg/30 g body weight depending on particle size. The lethal dose of fused silica ranged from 2.1 ± 0.06 to 4.5 ± 0.39 mg/30 g.

In a study described previously, Byers and Gage (1961) injected various amounts of 3 types of silica into rats (strain and n not provided). Most deaths occurred within 2 h. Rats that survived for 24 h recovered fully. The LD_{50} for types A1, A2, and B were 35.2 (confidence interval [CI] 23 to 39), 41.2 (CI 34.5 to 42), and 44.4 (CI 40.5 to 49) mg/kg.

UNEP (2004) reported an unpublished acute i.v. toxicity study of pyrogenic silica using rats. The LD_{50} was 15 mg/kg.

Intratracheal

Pyrogenic and Hydrated Silica

Yuen et al. (1996) intratracheally instilled male Crl:CD BR rats ($n = 3$; 7 to 9 weeks old) with silica particles (10

mg/kg; particle size range 2 to 3.5 μ m). The mice were killed and examined 0.5, 2, and 5 h, and 2 and 10 days after exposure. Neutrophilic inflammation was induced as early as 5 h after exposure. Maximal infiltration of neutrophils into the lungs occurred at 5 to 6 h. The inflammatory response for silica was transient, diminishing at 2 days and back to control levels at 10 days. Within 2 h, chemotactic activity for neutrophils was detected directly in BAL fluids with the influx and appearance of neutrophils into alveolar regions of the lungs. The mRNA expression of 2 known neutrophil chemotactic cytokines in BAL cells, macrophage inflammatory protein-2 (MIP-2) and keratinocyte-derived chemokine (KC), correlated with chemotactic activity and acute pulmonary inflammatory responses. MIP-2 mRNA was expressed prior to detection of chemotactic activity in BAL fluids and was no longer detectable after 2 days. The authors stated that silica produced a potent but transient pulmonary inflammatory response.

Jones et al. (2002) explored the kinetics of lung macrophages by instilling silica (50 mg; 5 μ m particle size) into the right upper lobe of the lungs of New Zealand white rabbits (n = 12). At intervals, the rabbits were re-anesthetized and injected with [^{11} C]R-PK11195 and scanned using positron emission tomography. The rabbits were killed at different times and the lungs examined. All of the rabbits remained healthy throughout the study. Three and 6 days following instillation, the [^{11} C]R-PK11195 was localized to the challenged lung and observed caudally on the contralateral side. On day 1 post-instillation, there were many macrophages containing particles in airspace. On day 5, some particle-bearing macrophages had migrated into the interstitium. There were few neutrophils present. At week 2, most of the particle-bearing macrophages were found in the interstitium and the perivascular lymph vessels. The macrophages did not appear to be highly activated. The silica was removed from the lungs in a highly organized manner and no fibrosis developed.

Kaewamatawong et al. (2005) compared the effects of particle size of colloidal (hydrated) silica on female ICR mice. Ultrafine (14 nm particle size) colloidal silica (120 mg/ml in water) or fine (213 nm) colloidal silica (239 mg/ml in water) were intratracheally administered to the mice (n = 3; 5 control groups, 10 exposure groups). The mice were killed and necropsied at 30 min and 2, 6, 12, and 24 h.

Both types of silica produced bronchiolar degeneration and necrosis, neutrophilic inflammation in alveoli with alveolar type II cell swelling, and particle-laden alveolar macrophage accumulation. Ultrafine silica induced more alveolar hemorrhage, compared to fine silica, from 30 min. There was also more severe bronchiolar epithelial cell necrosis and neutrophil influx in alveoli in the ultrafine-treated mice than in the fine silica-treated mice at 12 and 24 h. Immunolabelling of Laminin in basement membranes of bronchioles and alveoli in the ultrafine silica-treated groups was weaker than the fine silica-treated groups at all time periods. Electron microscopy revealed both types of silica on bronchiolar and alveolar wall surfaces as well as in the cytoplasm of alveolar epithelial cells, alveolar macrophages, and neutrophils. Type I alveolar epithelial cell erosion with basement membrane damage was greater in the ultrafine silica groups than in the fine silica

groups. Bronchiolar epithelial cells in the ultrafine silica groups had more intense vacuolation and necrosis than in the fine silica groups. The authors suggested that ultrafine silica had greater ability to induce lung inflammation and tissue damage than fine silica (Kaewamatawong et al. 2005).

Kaewamatawong et al. (2006) instilled ultrafine colloidal (hydrated) silica (0, 0.3, 3, 10, 30, or 100 μg ; 120 mg/ml in water; 14 nm particle size) into the tracheas of male ICR mice ($n = 10$). After 3 days, the mice were killed and the lungs examined. The total cell counts in broncho alveolar lavage fluid (BALF) were increased for 10, 30 and 100 μg groups. Cell differential analysis of BALF of the 2 highest groups showed increases in numbers of neutrophils and lymphocytes. All exposure groups had increased total protein values in BALF.

In a followup experiment, mice ($n = 8$) were instilled with 50 μl of 30 μg of ultrafine silica of the same particle size. The groups were killed at 1, 3, 7, 15, and 30 days and the lungs examined. There was a transient increase in the total numbers of cells, macrophages, neutrophils, and lymphocytes in BALF. Total numbers of lung cells increased and persisted to day 15 and resolved by day 30. Alveolar macrophages were elevated at day 1 to day 7. Lymphocytes increased until day 7 then returned to control levels by day 30. Total protein in BALF was greater than control at day 1 and returned to control levels by day 15.

On day 1 there were moderate increases of neutrophils sharply demarcated from normal alveoli. There were nodular aggregates of neutrophils and particle-laden alveolar macrophages in some alveolar regions adjacent to the bronchioles. Nodular lesions consisted of neutrophils, active alveolar macrophages, particle-laden alveolar macrophages, and cell debris. At day 3, moderate focal alveolitis was observed at the terminal bronchiolar and alveolar duct regions. Alveolar septal walls were thickened. At day 7, changes were only in the appearance of the aggregated foci consisting of particle-laden alveolar macrophages, lymphocytes, and fibroblasts with occasional collagen fibers. Lesions were located around blood vessels adjacent to terminal bronchioles and alveolar ducts. At day 15, inflammatory signs were decreased and almost or completely resolved. Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) analyses showed an increase of the apoptotic index in lung parenchyma at all time points. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) was detected in lung epithelial cells and activated macrophages and correlated with lung lesions. The authors suggested that small doses of ultrafine colloidal silica caused transient, acute moderate lung inflammation and tissue damage. Oxidative stress and apoptosis may underlie the tissue injury induction (Kaewamatawong et al. 2006).

Sayes et al. (2007) instilled precipitated (hydrated) silica (1 or 5 mg/kg; 1000 to 3000 nm), as well as other particles, intratracheally to male Crl:CD (SK)IGS BR rats ($n = 20$). Controls were administered phosphate-buffered saline (PBS). At 24 h, 1 week, and 1 and 3 months, the BALF of 5 rats/group was analyzed. The number of cells at 24 h was higher than at other time points. Exposure to both doses produced transient and reversible neutrophilic lung inflammation responses at 24 h

which was diminished at 1 week.

Inhalation

Pyrogenic and Hydrated Silica

Lewinson et al. (1994) placed Wistar rats (n = 10; 5 males, 5 females) into an exposure chamber circulating pyrogenic silica (mean exposure 477 mg/m³; lowest reading 342 mg/m³) for 4 h. Approximately 56% of the particles were < 5 µm. The rats were observed for 2 h after exposure then daily for 14 days and periodically weighed. The rats were then killed and necropsied. The rats were restless and had drooping eyelids during exposure. There was no mortality during exposure or during the observation period. Body weights decreased during the first 2 days after exposure then the rats gained weight normally. Necropsies were unremarkable.

Warheit et al (1995) exposed male CD rats (n = 24) to aerosolized precipitated (hydrated) silica (10 and 100 mg/m³) 6 h/d for 3 days followed by recovery periods of 1, 8, 30, and 90 days. The low dose produced a transient inflammatory response which was present 24 h post-exposure and subsided within 8 days. Recovery in the high dose group was similar to low dose group. The author concluded that low concentrations of silica induces a transient inflammatory tissue reaction.

UNEP (2004) reported an unpublished inhalation study (nose only exposure) of precipitated silica (< 5 µm particle size) in Wistar rats (n = 10; 5 male, 5 female). The LC₅₀ was > 0.691 mg/l for 4 h. Clinical signs were restlessness and eye closing. There was no body weight gain in females the first 3 days after exposure and then weight gain was normal. There were no remarkable findings at necropsy after a 14-day observation period.

An unpublished inhalation study of precipitated (hydrated) silica (< 5 µm particle size) used Sprague-Dawley rats (n = 10; 5). The LC₅₀ was > 2.2 mg/l for 1 h. Clinical signs were irritation and dyspnea in most of the rats. One rat died during the 14-day observation period. The editors of the UNEP report considered this study invalid due to methodological deficiencies and short length of exposure.

An unpublished inhalation study (full body exposure) of pyrogenic silica used Sprague-Dawley rats (n = 10). Approximately 84% of the particles were ≤ 3 µm. The LC₅₀ was > 2.08 mg/l for 4 h. Clinical signs were nasal discharge during exposure and crusty eyes and nose, and alopecia during the 14 day observation period. There was no body weight gain in females the first 3 days after exposure and then weight gain was normal. One rat had discolored lungs at necropsy (UNEP 2004).

Unpublished studies of the acute inhalation toxicity of hydrophilic and hydrophobic silica are summarized in Table 6 (ECETOC 2006).

Intramuscular**Silica**

W.R. Grace & Co. (1981) reported a study where silica (200 mg) was implanted into the paravertebral musculature of the lumbar region of rabbits (n = 6). The rabbits were killed and necropsied at 6, 12, and 24 weeks. There were local inflammatory reactions at 6 weeks. There was granulomatous scarring with necrotic muscle fibers and fatty degeneration of local macrophages at the site of implantation.

Dermal**Pyrogenic and Hydrated Silica**

UNEP (2004) reported an unpublished acute dermal toxicity test of precipitated (hydrated) silica using rabbits (n and strain not provided). Silica was applied to intact and abraded skin for 48 h with no effect. The no observed effect level (NOEL) was > 2 g/kg.

In another unpublished study, a single application of 4 different precipitated (hydrated) silica products (in an aqueous paste) was applied to the intact and abraded skin of New Zealand white rabbits (n = 16) under an occlusive patch (length of time not specified). The rabbits were observed for 14 days. There was very slight erythema that disappeared after 2, 4 or 5 days for 3 silica products and no effects by the fourth product (UNEP 2004).

ECETOC (2006) reported several unpublished studies on the acute dermal toxicity of silica to rabbits. Only slight erythema with intact skin and slight erythema and edema with abraded skin were observed. Precipitated silica had an LD₅₀ of > 5 g/kg in 4 studies. Silica gel had an LD₅₀ of > 2 g/kg in 1 study.

Short-Term Toxicity**Oral****Silica**

FDA (no date) reported the results of a dog feeding study of 28 days. There were no effects and the highest no effect level (HNEL) for silica was 800 mg/kg/d. In another study using rats, the oral HNEL was 1 g/kg/d. For doses >2 g/kg/d, the rats had dirty fur, shyness, decreased motor activity, and hemorrhage of the mucous membrane of the eyes and nose. There was a decrease in body weights, feed consumption, hemorrhaging, and cellular atrophy in the liver epithelium.

Silica (8 g/kg/d) was incorporated into the feed of Beagle dogs and CD rats (n not provided) for 4 weeks. The animals were then killed and necropsied. There were no indications of any treatment-related effects observed in either species (Newberne and Wilson 1970).

Silica (0.2%, 1.0%, or 2.5%) was incorporated into the feed of male rats (n = 10) for 28 days. There were no adverse effects or mortality reported. Gross necropsy findings were unremarkable (W.R. Grace & Co. 1981).

Lewinson et al. (1994) administered pyrogenic silica (0, 500, 1000, 2000 mg/kg/d) in the diet of Wistar rats (n = 20; 10 males, 10 females) for 8 weeks. Since the high dose was well tolerated, the dose was increased to 4 g/kg/d after 14 days, to 8 g/kg/d after another 14 days, and finally to 16 g/kg/d. The rats were observed for clinical signs, weighed, and blood sampled before and at the end of the experiment. The rats were killed and necropsied.

Only the 16 g/kg/d dose (~25% of daily feed intake) caused any clinical signs, shyness, dirty fur, reduced activity, cachexia, and hemorrhage in the mucous membranes of the eyes and nose. Two males and 2 females died with severe cachexia in week 8 (days 9 and 13 of the highest dose). This group had pronounced reduction in body weight and decreased feed intake. No changes were observed in hematological parameters. Microscopic evaluation revealed severe atrophy in the epithelium of the liver in the 1 and 16 g/kg dose groups; condensation of the cytoplasm, loss of basophilic structure, and hyperchromatic and contracted nuclei occurred in the liver cells. These findings were observed to a lesser extent in 2 females in the 1 g/kg dose group. There were no effects to the kidneys. There were no treatment-related effects observed in the 500 mg/kg dose group. The authors concluded that lowest observed effect level (LOEL) was 1 g/kg/d and the NOEL was 500 mg/kg/d (Lewinson et al. 1994).

UNEP (2004) and ECETOC (2006) reported unpublished short-term oral toxicity studies of silica. The studies are summarized in Table 7.

Dermal

Silica

ECETOC (2006) reported an unpublished dermal toxicity study of pyrogenic silica (0, 5, and 10 g/kg/d) using albino rabbits (n = 4; 2 male, 2 female). The silica was applied for 18 h/d, 5 d/week for 3 weeks to intact and abraded skin. There were no signs of systemic toxicity. There were no gross or microscopic pathological findings. The silica content of blood, urine, spleen, liver and kidney was similar among groups.

Inhalation

Pyrogenic and Hydrated Silica

Low et al. (1985) and Hemenway et al. (1986) exposed male Fischer 344 rats (n = 45) to aerosolized precipitated (hydrated) silica (30 mg/m³; particle size not provided) for 6 h/d for 8 days. The recovery period was up to 112 days. During exposure, there was an early and transient influx of cells into the lung tissue which returned to normal by day 12. At 5 days post-exposure, the number and differential counts of alveolar lavage-derived cells were similar to controls. The BAL protein, lipid phosphorus, and saturated dipalmitoyl phosphatidyl-choline levels increased immediately after exposure and were normal by day 5 post exposure. There were no differences between controls and treated lungs as to weight, DNA-, protein-, or hydroxyproline-content. The authors concluded that inhaled silica caused an early, transient alveolar inflammatory

response, without producing fibrosis. There was only a mild inflammatory response with no evidence of connective tissue response.

Hemenway et al. (1986) exposed Fischer 344 rats ($n = 15$) to aerosolized silica (concentration unclear; particle size not provided) for 8 days. Three of the rats were killed and necropsied at days 0, 5, 12, 60, and 120 after exposure. There was initial inflammation, predominantly alveolar, which subsided before day 12.

Warheit et al. (1990, 1991) exposed male CD BR rats (n not provided) to aerosolized colloidal (hydrated) silica (10.1, 50.5, and 154 mg/m^3 ; diluted 4:1 with deionized, distilled water; particle size not provided) for 6 h/d, 5 d/week, for 4 weeks followed by a 10 and 94 day recovery period. The controls were unexposed. Lesions were only observed in lungs and associated draining lymph nodes. There was a dose-dependent increase in mean lung weight and lung to body weight ratio after 4 weeks of exposure in the mid and high dose groups. The mean lung to body weight ratio continued to increase in the high dose group 10 days into recovery, but was similar to controls after 3 months. There were dust laden alveolar macrophages, neutrophilic infiltration, and Type II pneumocyte hyperplasia observed in the alveolar duct region of the lungs. Pulmonary lesions progressively decreased in rats examined after the 10 day and 3 month recovery period.

At 3 months post-exposure, most dust laden alveolar macrophages were cleared from the lungs, but small numbers of minute silicotic nodule-like lesions were present in the alveolar ducts and perivascular regions where dust laden alveolar macrophages had aggregated. There was minimal collagen deposition observed in the silicotic nodule-like lesions; the lesions did not increase in size or number over time. The lung clearance half-life was ~ 50 days for the mid and high dose groups. In the high dose group, there was an increase in mean neutrophil count and globulin concentration and a decrease in mean lymphocyte count at the end of the treatment. The increase in mean neutrophil count and decrease in mean lymphocyte count were still present after 3 months of recovery. The tracheal and mediastinal lymph nodes were enlarged with nodular aggregates of dust-laden alveolar macrophages and hyperplastic reticulo-epithelial (RE) cells. The NOAEL was 10.1 mg/m^3 (Warheit et al. 1990,1991).

Reuzel et al. (1991) exposed Wistar rats ($n = 80$; 40 male, 40 female) to pyrogenic silica (17, 44, 164 mg/m^3 ; particle size not provided) in a whole body exposure chamber for 6 h/d, 5 d/week for a total of 14 days. The control was 6 male and 6 female unexposed rats. There was respiratory distress in all groups. One female in the high dose group died. There was decreased body weights and feed consumption in the males in the mid and high dose groups. Hematological measurements were unremarkable. There was increased lung weights in both sexes (47%, 65%, and 86% for the low, mid, and high dose groups) compared to controls. The absolute and relative liver weights were decreased in males, but not females. There were dose-dependent changes in the lungs (i.e., pale, spotted and/or spongy, occasionally irregular surface, alveolar interstitial pneumonia, early granulomata). The mediastinal lymph nodes were enlarged.

The above study was repeated with silica (46, 180, and 668 mg/m³) on Wistar rats (n = 60; 30 males, 30 females). There was respiratory distress in all groups. One male died in the high dose group. There was decreased body weights and feed consumption in the mid and high dose groups. There were increased lung weights in both sexes compared to controls (males 25%, 39%, and 68%; females 34%, 50%, and 86% in the low, mid, and high dose groups, respectively). There were decreased liver weights in all dose groups of the males and the high dose group of the females. The lungs were spotted, swollen, and had irregular surfaces in the high dose groups as well as interstitial pneumonia and early granulomata. There was silica in the mediastinal lymph nodes in the mid and high dose groups and 1 rat in the low dose group. There was accumulation of alveolar macrophages and particulate material in the lungs of males in the mid and high dose group (Reuzel et al 1991).

Lee and Kelly (1993) exposed male Crl:CD(SD)BR rats (n = 25) to aerosolized colloidal (hydrated) silica (0, 10, 50, 150 mg/m³; particle size not provided) for 6 h/day, 5 days/week for 4 weeks. Some of the rats were killed at the end of the exposure period, at 10 days, or 3 months. There were dose dependent lesions observed in the mid and high dose groups but not in the low dose group. Particles were mostly phagocytized by alveolar macrophages in the alveolar duct region and a few free particles were observed in Type I pneumocytes in the alveoli. Particle-laden alveolar macrophages directly penetrated into the bronchiolar interstitium from alveoli and accumulated in bronchus-associated lymphoid tissue, peribronchial, or perivascular interstitium and accumulated in the tracheo-bronchial lymph nodes. Some particle-laden alveolar macrophages in the bronchus-associated lymphoid tissue transmigrated directly into bronchial lumen through the epithelium. The transmigrated particle-laden alveolar macrophages in the tracheo-bronchial lymph nodes were similar to those in the alveoli. They were characterized by slender cytoplasmic processes, phagosomes, myelin figures, cholesterol clefts, and lipid droplets. Migrated particle-laden alveoli macrophages were observed to be necrotic and have released particles in the tracheo-bronchial lymph nodes.

At 3 months, the lungs of the low dose group were normal. The lungs of the mid dose group were normal in appearance, but a small number of tiny nodular aggregates of dust-laden alveoli macrophages and epithelioid cells were observed. One rat had a few silicotic nodules in perivascular regions adjacent to the bronchioles. The high dose group had decreased numbers of particle-laden alveoli macrophages that were sharply circumscribed in the alveoli. Some aggregates of particle-laden alveoli macrophages and epithelioid cells were closely apposed with alveolar walls and transformed into nodular aggregates without any collagen fiber deposition. Three of 10 rats had silicotic nodules in the perivascular region of the bronchioles (Lee and Kelly et al 1993).

UNEP (2004) reported an unpublished short-term inhalation toxicity study using female Wistar rats (n not clear) exposed to aerosolized silica (8 and 40 mg/m³; particle size not provided) for 1 h/d, 5 d/week for up to 3 months. The rats

were killed and necropsied at 7 d and 3 weeks after treatment. There were no macroscopic changes. Histopathologically, there was an occurrence of dust cells in the lungs which decreased during post-exposure. There was no fibrosis of the reticulo-cellular type and normal parenchyma of the lungs. There was no emphysema. A decrease of silica content in the lungs was observed 7 and 48 days after treatment termination. After 3 months, there was almost no silica in the lungs.

ECETOC (2006) reported several unpublished short-term inhalations studies of silica. These studies are summarized in Table 8.

Arts et al. (2007) exposed young adult Wistar (CrI:WI)WU BR rats (n = 20; 10 male and 10 female) to 3 types of aerosolized silica (1, 5, or 25 mg/m³; particle sizes not provided): precipitated (hydrated) silica, silica gel, and pyrogenic silica for 6 h/d for 5 consecutive days followed by a 3-month recovery period. The rats were killed and necropsied. There were no clinical signs during exposure. The effects were limited to 1 day post exposure. Silica levels in the tracheobronchial lymph nodes were below detection limits in all 3 groups. Silica was found in the lungs at day 1 but had cleared by 3 months. All 3 types of silica induced biomarkers of cytotoxicity in BAL fluid, increases in lung and tracheobronchial lymph node weights, and histopathological lung changes in the high dose groups at day 1 post exposure. The mid dose only induced histopathological changes and changes in BAL fluid. The effects of all 3 types of silica, with the exception of slight histopathological lung changes at the higher exposure levels, were reversed during the recovery period. The low dose caused no adverse effects.

Intratracheal

Silica

Schepers et al. (1957d) intratracheally injected rats (n = 10) with silica (5%; 0.25 ml) once per week for 3 weeks. Two rats died after the first injection, 3 died before the third injection. Three rats survived the observation period (length not stated). Pleural effusion was observed in 4 rats, 2 responses were delayed, 1 supervened and the rat survived 220 days. Pulmonary congestion was observed in 3 rats until the ninth month. Tracheobronchial lymph nodes were moderately to markedly enlarged and firm for 5 months. There was abscess formation associated with pneumonitis; accompanying cells were macrophages. Focal granulomatous inflammation was observed in both lungs in rats that survived more than 2 days. Hyperemia of the alveolar walls later resolved. Infiltration of the alveolar walls was mostly by macrophages. Early collagen became profuse in the alveolar walls in relation to focal granulomatous inflammation and cell necrosis.

This experiment was repeated with guinea pigs at double the dose of silica. At 2 weeks, 2 guinea pigs died; the rest survived to be killed and necropsied at intervals. Most of the effects were confined to the lungs; a few consolidated areas were palpable in a few animals. There were multiple foci of atelectasis in the lungs. Tracheobronchial lymph nodes were moderately to markedly enlarged. Cellular phenomena predominated early and resolved with residual fibrotic change.

Granulomatous inflammation was observed in the first month. There was a tendency toward cellular invasion. Slight to moderate atrophic vesicular emphysema was detectable during the second half of the year of observation (Schepers et al. 1957d).

Intravenous

Silica

Schepers et al. (1957d) intravenously injected silica (1% in saline; 5 ml) into rabbits (n = 5) 20 times biweekly. One rabbit died after the second injection and another after half the injections. One died on the 70th day and the last 2 were killed on the 120th and 300th days. There was slight to moderate pleural effusion with pleuritis. The longest surviving rabbits had mediastinal abscesses. The lungs had moderate to marked congestion, which was most severe in the rabbits that died first. There were no foci consolidation but small areas of atelectasis. The lymph nodes were not markedly enlarged. The right ventricles were dilated and hypertrophic, most obviously in the rabbit that died first. The livers were moderately to markedly enlarged, turning pale and firm over time. However, the liver had returned to normal in the rabbit killed on the 300th day. There was some splenomegaly in the 2 rabbits that died during treatment. Atrophy of the spleen increased over time. The size of the kidneys increased over time, to almost double normal size. Histological changes included hyperemia with associated exudate into alveolar spaces, ischemia, dust-filled macrophages in alveolar spaces, distension of the proximal convoluted tubules with fibrosis, and small granulomata that diminished over time. The epithelial cells were well preserved. There was minimal collagenosis. The alveolar walls thickened then became thin.

Subchronic Toxicity

Oral

Pyrogenic and Hydrated Silica

FDA (no date) reported the results of a 90-day rat feeding study of silica. There were no effects and the HNEL was 5 g/kg/d. In another study using rats for 180 days with a 3-week recovery period, the lowest effect level (LEL) was 500 mg/kg/d. There was an increase in adrenal weight in the males. Adrenal weight also increased in the females but only during the recovery period. Histopathology showed an increase in lipid content in the adrenal glands; this resolved during the recovery period.

Hazleton Laboratories (1958c) incorporated pyrogenic silica (1.0%, 3.0%, or 5.0%: 10,000, 30,000, or 50,000 ppm) into the feed of male and female albino weanling rats (n = 30; 15 male, 15 female) for 90 days. Controls were fed the basal diet or 3.0% #1625 Cosmetic Talc. The rats were killed and necropsied at 45 and 90 days. There were no gross signs of toxicity. Growth rates, feed consumption, and survival were similar to controls. The silica content of the liver, kidneys, spleen, blood, and urine was similar to controls. There were no gross, microscopic, nor pathological changes associated with

silica consumption at any dose level.

Silica (50 mg/d) was fed to male and female rats (n = 30) by stomach tube for 3 months. No adverse effects on body weight gain or mortality were observed. The results of pathological examination were similar to controls (W. R. Grace & Co. 1981).

In another experiment, silica (0, 1.0%, 3.0% 5.0%) was incorporated into the feed of male and female rats (n = 30) for 90 days. The positive control group was fed cosmetic talc. There was no systemic toxicity by silica in terms of survival, weight gain, and feed consumption observed. There was no increase in deposition of silica observed in the kidney, livers, spleen, blood, or urine (W. R. Grace & Co. 1981).

Lewinson et al. (1994) orally administered pyrogenic silica (500 mg/kg/day) in the feed of Wistar rats (n = 40; 20 males, 20 females) for 6 months. The animals were then killed and necropsied except for the animals of both sexes which were kept on normal feed for an additional 3 weeks. The animals were observed daily; blood was sampled and the rats weighed periodically.

There were no clinical signs during the treatment period. One male in the treatment group died; a lung infection was observed. Two rats in the control group died with enteritis and cachexia. There were no differences in weights or feed consumption. There were no differences in hematological parameters. Macroscopic evaluation at necropsy was unremarkable. Histopathological examination revealed increased lipid content in the fasciculata and zone fasciculata of the adrenal glands; this condition resolved after the recovery period. No other differences between treated and control animals were observed. The authors concluded that the NOEL was 500 mg/kg/day (Lewinson et al. 1994).

UNEP (2004) reported an unpublished feeding study of precipitated (hydrated) silica (0.5%, 2%, and 6.7%; 300 to 330, 1200 to 1400, and 4000 to 4500 mg/kg/d) using Wistar rats (n = 20; 10 male, 10 female) for 13 weeks. There were no clinical signs; hematological, blood chemistry, and urinary parameters were normal. Feed intake was slightly increased in the high dose females after 4 weeks. Gross and microscopic examinations were unremarkable.

In another unpublished feeding study, precipitated (hydrated) silica gel (3.2% and 10%) was fed to CD-1 rats (n = 24; 12 males, 12 females) for 6 months. Calculated doses were 2170 and 7950 mg/kg/d for the males and 2420 and 8980 mg/kg/d for females. At 6, 13, and 26 weeks, 4 rats of each sex in each group were killed and necropsied and the bone marrow analyzed. There were no treatment-related findings. Behavior was normal. Body weights were not affected. There were no histopathological changes in the kidneys. The NOAEL was 8980 mg/kg/d.

Another unpublished study in which Wistar rats (n = 40; 20 male, 20 female) were fed silica (495 to 497 mg/kg/d) for 6 months resulted in an NOAEL of 497 mg/kg. No further information was provided (UNEP 2004).

ECETOC (2006) reported 2 sub-chronic oral toxicity studies where hydrophobic pyrogenic silica (500 mg/kg/d) was

administered by gavage to Wistar rats (n = 40; 20 male, 20 female) 5 d/week for 6 months. There were no clinical signs nor macroscopic findings at necropsy (ECETOC 2006).

Inhalation

Pyrogenic and Hydrated Silica

Schepers et al. (1957a) placed male and female Wistar rats (n = 25) in inhalation chambers to expose them to pyrogenic silica (average 1.5 mg/ft³ [53 mg/m³]; most measurements ranged from 0.7 to 2.4 mg/ft³ [25 to 85 mg/m³]; particle size not provided). The rats were exposed to aerated silica for 8 h/d then had passive exposure (dust settling) for the remaining 16 h. The exposure was for 5 d/week for 6 months followed by 6 months of recovery. Rats were periodically killed and necropsied. The control group (n = 42) was in normal air and killed and necropsied at 6 and 12 months.

In the test group, 11/25 (44%) died, mostly during the silica exposure. The death rate decreased during the recovery period. The majority of the rats died from pulmonary vascular obstruction and emphysema beginning at the 4th month. Focal pigmentation was conspicuous after 3 months of exposure with profusely scattered small, dark-pink discrete but irregular subpleural foci of reaction. Congestion of the lungs was dominant after 3 months. There was lymph node enlargement after 3 months. There was an incipient tendency toward pulmonary emphysema after 4 months of exposure with lung distension and superficial alveoli dilation. Atelectasis was noted in some rats after 4 to 5 months.

Histological examination revealed invasion of the lymphatic system of the lung by mononuclear macrophages forming clusters of plasma cells and lymphocytes. There was infiltration of large vacuolated cells within the alveolar spaces; the cytoplasm had a foamy appearance with macrophages fused to giant cells. There were large vacuolated cells within the alveolar spaces, with the cytoplasm having foamy appearance, macrophages apparently fused to giant cells. There was progressive nodule formation in the lung parenchyma and peri- and paravascular, in some cases parabronchiolar distribution and accumulation, consisting of central macrophages and surrounding plasma cells, some nodules enveloped by an epithelial layer of cells. Some necrosis was noted in the central zone of the nodules; there was progressive tendency toward fibrosis in the nodules and evidence of progressive emphysematous processes around the nodules.

Average silica load in the lung increased to 1.5 mg/lung after 3 months and remained at that level through exposure. At the end of recovery, the level reduced to 0.3 mg/lung. The authors concluded that the lowest observed adverse effects level (LOAEL) was 53 mg/m³ (Schepers et al. 1957a).

Rats (strain and n not provided) were exposed to aerosolized hydrophilic silica (40 to 50 mg/m³; particle size not provided) for 4 h/d, 5 d/week for 2 to 12 weeks (Klosterkötter 1963). The overall elimination of silica was high without accumulation in the lungs. Equilibrium between retention and elimination was reached quickly. Only 5% to 6% of theoretical deposit of silica was observed after 120 days exposure. There was transfer of silica to the mediastinal lymph

nodes, ~31% of total deposited (1.5% to 2% theoretical deposition). The authors stated that involvement of the lymphatic elimination appeared not to be relevant up to 8 weeks of exposure. The silica particles were able to bypass the lymph nodes and were removed quickly.

Reuzel et al. (1991) reported an unpublished inhalation study of silica (1.3, 5.9, 31 mg/m³; particle size not provided) using Wistar rats (n = 100; 50 male, 50 female). The rats were subjected to full body exposure for 6 h/d, 5 d/week, for 13 weeks. Ten rats of each sex were killed and necropsied at weeks 13, 26, 39, and 52.

There were no mortalities during treatment or recovery. Clinical signs were increased respiration rates in a dose dependent manner and body weight gains were depressed. Red blood cell (RBC) count was increased in males in the high-dose group. In the mid- and high-dose groups, white blood cells (WBC) were elevated in both males and females; the concentration-response relationship was poor. Blood cell counts returned to normal by week 39. Necropsy at 13 weeks revealed swollen and spotted lungs and enlarged mediastinal lymph nodes; the severity was dose dependent. All groups had increased lung weights and collagen content, less so in the low-dose group. All these effects reduced to control levels by the end of the study except for collagen content in males in the mid- and high-dose groups.

After treatment, silica could be detected in the lungs of all the rats in relatively small amounts. In the high-dose group, the average silica amount in the lungs was 0.2 mg. Silica was detected in 1 male in this group in the regional lymph node. At the end of the study, no silica above control levels could be detected in any rat. Microscopic evaluation after treatment revealed accumulation of alveolar macrophages and granular material, cellular debris, polymorphonuclear leukocytes, increased septal cellularity, alveolar bronchialization, focal interstitial fibrosis, cholesterol clefts, and granuloma-like lesions in the lung. The lesions in the lung did not have fibroblastic activity or hyalinization and regressed during recovery. All types of pulmonary lesions were more marked in males than in females. Accumulation of macrophages was observed in the mediastinal lymph node at 13 and 26 weeks. Treatment-related, microscopic changes in the nasal region were occasionally found at week 13 such as focal necrosis and slight atrophy of the olfactory epithelium. Interstitial fibrosis was not noted directly after the exposure period, but was observed for the first time after 13 weeks postexposure, with increasing incidence especially in the high-dose group, and a few in the mid-dose group. There was decreased severity and frequency of the effects until the end of the study. The authors concluded that the NOEL was 1.3 mg/m³.

In a second study, male and female Wistar rats were placed in a whole body inhalation chamber 6 h/d, 5 d/week, for 13 weeks to be exposed to precipitated silica at 35 mg/m³ (particle and agglomerate/aggregage size 1 to ~120 µm). The rats were periodically killed and necropsied over the 52-week recovery period.

Slightly decreased body weight and increased lung and thymus weights were observed. Necropsy revealed swollen and spotted lungs and enlarged mediastinal lymph nodes. Microscopic examination of the lungs revealed accumulation of

alveolar macrophages, intra-alveolar leukocytes, and increased septal cellularity. There was also accumulation of macrophages in the lymph nodes. The collagen content in the lungs was slightly increased. During the recovery period, the effects of silica exposure were mostly gone within 26 weeks. Accumulation of silica and macrophages in the mediastinal lymph nodes were still present at the end of the recovery period (Reuzel et al. 1991).

Johnston et al. (2000) exposed male Fischer 344 rats ($n = 4$) to aerosolized pyrogenic silica ($50.4 \pm 19 \text{ mg/m}^3$; mean diameter $0.81 \text{ }\mu\text{m}$) for 6 h/d, 5d/week, for 13 weeks. The control group was not treated. The silica burden was determined after 6.5 and 13 weeks of exposure and after 3 and 8 months of recovery. The silica load increased quickly during the first 6.5 weeks of exposure (0.76 mg/lung) but less so after 13 weeks (0.88 mg/lung). During recovery, the silica burden disappeared rapidly from lung tissue (15% after 12 weeks; 6% after 32 weeks). BAL showed mean cell numbers in the lavage increased 5- to 15-fold compared to control. The cells comprised $> 50\%$ polymorphonuclear leukocytes (PMN) and some 2% lymphocytes whereas the control lavages only contained $< 1\%$ of either cell type. Protein content and enzyme activities (LDH and glucuronidase) were markedly higher than under control conditions. All BAL markers approached normal levels after 13 weeks recovery in most rats, however, a few had minimal increases.

There was invasion of neutrophils and macrophages into the alveoli after 6.5 weeks but this effect tended to decrease during recovery. Fibrosis was observed in alveolar septa which subsided during recovery. After 13 weeks of exposure, intensely stained TUNEL-positive cells were detected throughout the terminal bronchiolar epithelium and through the parenchyma of the lungs. The authors concluded that aerosolized silica produced transient pulmonary inflammatory response and most biochemical markers return to control levels post exposure (Johnston et al. 2000).

ECETOC (2006) reported an unpublished study where Wistar rats ($n = 20$; 10 males, 10 females) were exposed to hydrophobic pyrogenic silica (0, 0.51, 2.05, and 10.01 mg/m^3 ; particle size not provided) for 6 h/d, 5 d/week for 13 weeks. A group of rats was allowed to recover for 13 weeks before being killed and necropsied. Silica was observed in the lungs in a concentration dependent manner at the end of exposure. Silica was observed in the tracheobronchial lymph nodes in 3 of 5 animals in the high dose group. After recovery, the amount of silica in the lungs was below detection limits in the low dose group and only a small amount was detected in the high dose group.

ECETOC (2006) reported an unpublished study in which Wistar rats ($n = 20$; 10 male, 10 female) were exposed to aerosolized hydrophobic pyrogenic silica (0, 0.51, 2.05, or 10.01 mg/m^3 ; particle size not provided) for 6 h/d, 5d/week for 13 weeks followed by a 13 week recovery. Most effects were in the high-dose group. There was an increase in aspartate-amino-transferase level and alkaline phosphatase activities in males. There was an increase in absolute and relative lung and tracheobronchial lymph node weights. The lungs had a red appearance with white spots. There was an accumulation of alveolar macrophages with few PMN cells accompanied by bronchiolar-alveolar epithelial hyperplasia and interstitial

inflammatory cell infiltrates in lungs. The lung draining mediastinal lymph nodes showed increased histiocytosis and macrophage aggregates in paracortex and/or germinal centers.

Chronic Toxicity

Oral

Pyrogenic and Hydrated Silica

Silica (3.2% or 10%) was incorporated into the feed of rats (n = 24; 12 male, 12 female) for 6 months (W.R. Grace & Co 1981). There were no mortalities. The only clinical sign was discolored stools. Growth and development was normal and feed consumption similar to controls. Necropsy was unremarkable; organ weights, absolute and relative, were similar to controls. Histology and hematology was unremarkable. There were no changes in clinical chemistry.

In another feeding study, rats (n = 24; 12 males, 12 females) were fed silica for 6 months. The low dose males and females consumed an average of 0.78 and 0.55 g silica/week, respectively. The high dose males and females consumed an average of 3.00 and 2.11 g silica/week, respectively. There was no effect with regards to body weight gain, feed consumption, blood chemistry, or urinalysis. There was an increase in the number of leukocytes in the female high dose group and eosinophils in the male high dose group. There was a decrease in glucose concentration and AP activity in the male rats in a dose dependent manner. There was decreased serum calcium concentration in the female rats in a dose dependent manner. There were reduced absolute and reduced liver and prostate weights (W. R. Grace & Co. 1981).

Takizawa et al. (1988) fed precipitated (hydrated) silica gel (1.25, 2.5, or 5% incorporated into feed) to male and female Fischer 344 rats (n = 80; 40 males, 40 females) for 103 weeks. The mean cumulative intake of silica was 143.46, 179.55, and 581.18 g/male rat and 107.25, 205.02, and 435.33 g/female rat for the low, mid and high dose groups, respectively. Survival for all treatment groups was similar to controls. There were no differences between treatment groups and controls with regards to body weight, feed intake, behavior, or in hematological or chemistry parameters. Liver weights in the females in the mid and high dose groups were lower at 12 to 24 months. There were no significant findings at the histopathological examinations.

The above experiment was repeated feeding B6C3F1 mice (n = 80; 40 male, 40 female) for 93 weeks. The mean cumulative intake of silica was 38.45, 79.78, and 160 g/male mouse and 37.02, 72.46, and 157.59 g/female mouse for the low, mid, and high dose groups, respectively. There were no differences in survival between treatment groups and controls. Feed consumption was increased in the mid and high dose groups whereas there was reduced weight increase in the males during weeks 15 through 50 (p < .01) and weeks 30 through 50 for the females (p < .05). There were no remarkable findings with regards to hematology or organ weights. There was no increase in the incidence of tumors (Takizawa et al. 1988).

Inhalation

Pyrogenic and Hydrated Silica

Jötten and Klosterkötter (1951) reported that when rabbits were exposed to aerosolized silica (0.2 to 5.0 μm particle size) there was formation of nodular fibrotic or diffuse fibrotic changes in the lungs. The authors concluded that the concentration of the dissolved silica, the surface forces of the colloidal particles, mechanical and physiochemical conditions were factors in the observed changes.

Schepers et al. (1957a) performed a parallel study (see SUBCHRONIC above for more details) where the Wistar rats ($n = 35$) were exposed to aerosolized precipitated silica (average 1.5 mg/ft^3 [53 mg/m^3]; most measurements ranged 0.7 to 2.4 mg/ft^3 [25 to 85 mg/m^3]; particle size not provided) for 8 h/d, 5 d/week for 12 months. Treatment related deaths were 26/35 (75%). After 6 months of exposure, aggregations of focal pigmentation visible as reddish-tan foci of dust were observed. There was also moderate, well-established generalized emphysema and lymph nodes that were greatly enlarged and firm. The majority of the rats died from pulmonary vascular obstruction and emphysema from the 4th to the 9th month. The authors concluded that high subchronic/chronic exposure to amorphous silica causes severe progressive pulmonary inflammation associated with increased mortality of the animals, primarily through partial obstruction of the pulmonary vasculature combined with pulmonary insufficiency due to emphysema.

Schepers et al. (1957b) exposed male and female albino guinea pigs to aerosolized pyrogenic silica (average concentration 1.5 mg/ft^3 ; ranging 0.7 to 2.4 mg/ft^3 ; 85% between 1 to 10 μm) in 3 experiments. The whole body exposure was for 8 h/d with the remaining 16 h as passive exposure (dust settling). In experiment 1, the guinea pigs ($n = 40$) were exposed to the silica up to 24 months, with a few killed and necropsied every 2 months. In experiment 2, the guinea pigs were exposed for 12 ($n = 15$) or 24 ($n = 18$) months with variable recovery periods up to 12 months. In experiment 3, the guinea pigs ($n = 17$) were exposed for 12 months, followed by a 1 month recovery period, then re-exposure for 8 to 24 h. A control group of 80 guinea pigs were sampled and necropsied from 1 to 36 months.

Two guinea pigs died from non-experimental causes. Overall, the chronic reaction of the lung tissue was established by 4 months of exposure. There was focal pigmentation after 1 month. Lymph nodes were enlarged by 1 month and did not increase over time, including hepatic lymph nodes. There was a tendency for lung emphysema after 4 to 8 months of exposure. Atelectasis was observed histologically.

Histologically, the dominant response was bronchial and peribronchiolar intra-alveolar accumulations of giant cells. At 8 to 12 months there was incipient atrophy of infiltrated alveoli which apparently led to compensatory expansion of adjacent alveoli. There was a combined effect of atelectasis and consolidation around bronchioli, but at the expense of bronchioli distortion. Incipient fibrosis around bronchioli and shrunken alveoli was noted at this stage. There was a marked

tendency toward cuboidal epithelization of atelectactic alveoli by the end of the second year of exposure.

In the lymphoid tissue, medullary hyperplasia with the formation of slight amounts of reticulum was prominent during the second year of exposure. No inflammation, sinus catarrh, nor fibrosis were noted in the lymph nodes.

In the recovery phase after 12 months of exposure, there was progressive recovery beginning almost immediately. There were no macroscopically visible anomalies after 1 year of recovery. Residual sequelae of the tissue reactions were emphysema, mural fibrosis, and bronchiolar and bronchial ectasia stenosis. The authors concluded that chronic exposure to amorphous silica was non-lethal to guinea pigs, but caused significant inflammatory reactions and pulmonary lesions, however, without apparent disability of the animals (Schepers et al. 1957b).

Schepers et al. (1957c) exposed New Zealand white rabbits (n = 10) to aerosolized pyrogenic silica (1.5 mg/ft³, 53 mg/m³; ranging from 0.7 to 2.4 mg/ft³, 25 to 85 mg/m³; 1 to 10 µm) for 8 h/d for 12 months. There was a 6 and 12 month recovery period. There was progressive functional incapacitation and increased hematocrits observed in the majority of the rabbits, possibly due to the combined effect of pulmonary vascular obstruction and emphysema. Blood pressure changes (both increases and decreases) were observed in the majority of the animals which partially recovered with discontinuation of treatment. Essential pulmonary changes included peribronchiolar cellular catarrh, mural cellular infiltration along with deposition of reticulum and some collagen, the formation of peri-vascular cellular nodules, ductal stenosis, and emphysema. During recovery, the cellular reactions and emphysema regressed but minor focal alveolar mural collagen persisted.

Schepers (1959) exposed New Zealand white rabbits (n not clear, ~16) to aerosolized precipitate silica (0, 28, 134, and 360 mg/m³; particle size not provided) for 8 h/d, 5 d/week. The mid and high dose groups were exposed for 9 months, the low dose and the control groups were exposed for 27 months. The rabbits in the mid and high dose became distressed during exposure. Clinical signs were fewer, commenced later, and receded more quickly in the lower concentrations. There was increased body weight gain which corrected when exposure was terminated. The author suggested that this was due to edema. The body weights then decreased. The rabbits had dyspnea and shortness of breath accompanied by cyanosis. Elevated right and left ventricular pressures were concentration and time related.

In the high dose group, emphysema was observed which decreased after termination of treatment. Pulmonary emphysema, vascular stenosis, alveolar cell infiltration, sclerosis, and epithelization granulomatosis, macrophage catarrh were observed. Lesions were observed in the liver, spleen and kidney.

After 6 months of exposure, the cardiac pressure of the low dose group increased steadily. At 24 months, the elevation was 64% over pre-exposure pressure. This effect was partially reversed with termination of treatment (34% after 12 months). The author reported concomitant radiographic changes, electrocardiographic deviations, modification of lung functions, hemolytic changes, anatomical cor pulmonale, congestive cardiac failure, emphysema, and chemical pneumonitis. The

LOAEL was 28 mg/m³ (Schepers 1959).

Schepers (1962) exposed monkeys (*Macacus mulatta*; n = 5) to aerosolized synthetic silica (15 mg/m³; particle size not provided) for up to 12 months. A monkey was killed and necropsied at 3 and 6 months. The remaining monkeys were killed and necropsied after 12 months of exposure. There were 15 untreated control monkeys. Body weight gains decreased and activity decreased during the initial exposures. At 3 months, emphysema was detectable. There was considerable cellular infiltration of the alveoli and alveolar septa was associated with distention of alveoli or accumulation of exudate and macrophages.

After 12 months, the lesions were marked pulmonary emphysema, alveolar wall sclerosis, vascular occlusions, and cor pulmonale. Cor pulmonale was attributed to the emphysema and alveolar wall destruction. Tracheobronchial lymph nodes were slightly enlarged but not fibrotic. The silica content remained low and decreased over time (Schepers 1962).

Klosterkötter (1965) exposed female albino rats (n = 120) to aerosolized pyrogenic silica (~45 mg/m³; particle size not provided) for 4 h/d for up to 1 year followed by a 3 to 8 month recovery period. Some of the rats were killed and necropsied periodically. There were 41/120 deaths. At necropsy, there were small white foci under the pleura, enlarged and discolored lymph nodes with formation of collagen and local necrosis, perivascular and peribronchiolar dust cell granuloma with reticulin and collagen fibers, necrotic cells, desquamative catarrh and thickened alveolar septa.

After the recovery periods, the dust cell granulomas were fewer and reduced in size with only a few dust cells and fibers. The alveolar septa had not completely disappeared. After 3 months, the lymph nodes were enlarged; after 8 months, the size of lymph nodes and the extent of recovery were reduced. The mean silica content of the lungs was 0.32 mg/lung or lymph node (0.6 mg maximum). At the end of exposure, 0.132 mg was found in the mediastinal lymph node (Klosterkötter 1965).

With continued exposure, the cellular reaction decreased and was replaced by degenerative processes (loss of septa with confluence of alveoli), followed by destructive emphysema. Circulatory continuity was extensively impaired by extensive rupture of alveolar septa. Collagen appeared in the alveolar septa.

Groth et al. (1981) exposed male Sprague Dawley rats (n = 80) to aerosolized pyrogenic silica, precipitated silica, or silica gel (15 mg/m³; 6 to 9 mg/m³ respirable $\leq 4.7 \mu\text{m}$). Exposure was for 5.5 to 6 h/day, 5 d/week for up to 12 months. At maximum exposure, a few macrophage aggregates were found in the lungs. Interstitial fibrosis associated with dense collections of mast cells appeared in some of the rats of the control and treatment groups, although there was a trend of a more frequent incidence in those exposed to pyrogenic silica, but a few were observed in some control animals. The authors concluded that the LOAEL was 6 to 9 mg/m³. Macrophage aggregation was less pronounced in rats than in monkeys under these test conditions (see below). Fibrosis was of minor importance as test and control groups were similar.

Another experiment was conducted using male monkeys (*Macaca fascicularis*; n = 10) with exposure to the 3 types of silica for 6 h/d, 5 d/week, 13 or 18 months. The decrease in lung respiratory volume and ventilatory mechanics in the monkeys was more marked in the pyrogenic silica group. Dynamic pulmonary compliance, forced vital capacity, inspiratory capacity, total lung capacity, and forced expiratory flow were decreased. Average flow resistance and closing volume were increased. In the precipitated silica group, lower lung volumes were observed. There were no changes in lung volume parameters, but there were reductions in ventilatory performance and mechanical parameters, dynamic lung compliance, and forced expiratory flow when exposed to silica gel.

Cytoplasmic changes (increases in number of vacuoles) in macrophages in the lungs and tracheal lymph nodes were observed. Large numbers of macrophages and mononuclear cell aggregates (bronchioles, alveolar spaces, venules, arterioles) were observed in the lungs. The frequency and size of the cell aggregates varied with the type of silica (precipitated > pyrogenic > gel). Reticulin fibers were present in the aggregates in all 3 groups. In 6/9 monkeys exposed to pyrogenic silica, collagen in varying quantities was found in 5 to 50% of the aggregates, with signs of early nodular fibrosis. In 3/9 monkeys no or little collagen was present. No collagen fibers were observed in aggregates in the lung of monkeys exposed to silica gel and only very few after exposure to precipitated silica. The authors concluded that early nodular fibrosis indicated that pyrogenic silica is more detrimental than precipitated silica or silica gel. The smaller particle size of pyrogenic silica may be a contributing factor. UNEP (2004) reviewed this study and noted that the monkeys were transported in bags that had contained asbestos and that suspect particles in the lungs were identified as mica and kaolin. Quartz or asbestos fibers could not be ruled out.

In a third study, the authors exposed male Hartley guinea pigs (n = 20) to the 3 types of silica for 5.5 to 6 h/d, 5 d/week for 12 months. A few macrophages containing particles of silica were observed in the lungs and lymph nodes, similar to the findings in rats (see above) (Groth et al. 1981).

Schepers (1981) exposed rabbits (n = 50), rats (n = 84), and guinea pigs (n = 82) to aerosolized precipitated (hydrated) silica (average of 126 mg/m³ (3.57 mg/ft³; particle size not provided) for 8 h/d, 5 d/week, for 12, 15, and 24 months, respectively, followed by a recovery period of up to 12 months. Control groups were not treated. There were no treatment-related differences in mortality between treated and control groups. For the rats, most of the deaths were due to intercurrent infection.

Lung weights increased during exposure but returned to normal during recovery. Particle-phagocytosing macrophages accumulated in alveoli, bronchioles, and lymphoid tissue in all species. Hilar lymph nodes were enlarged, mildly in rats and more evidently in the guinea pigs and rabbits; this disappeared with the termination of treatment. Epithelial proliferation was minimal. Mild deposition of reticulin fibers occurred in alveoli with no evidence of collagen formation. Bronchial and

tracheal epithelia remained intact. No epithelization or pleural changes were observed; no neoplasia occurred.

The emphysema was equally distributed between treated and control groups. It was dominated by the diffuse hypertrophic vesicular distention but apparently did not result from destructive effects on the mucosa or terminal bronchioles and disruption of the continuity of alveolar walls. The author stated that the emphysematous effect in the rats could possibly be due to aging and recurrent epizootic pneumontitis. There was complete reversibility of silica retention and inflammatory responses in guinea pigs within 6 months of recovery. Silicotic processes were completely absent in all species (Schepers 1981).

UNEP (2004) reported several unpublished chronic inhalation toxicity studies of silica. The studies are summarized in Table 9.

ECETOC (2006) reported a series of unpublished studies. Rats (strain and n not provided) were exposed to aerosolized hydrophilic silica (50 to 55 mg/m³; 30 mg/m³ respirable [sic]) for 12 months. Rats were killed and necropsied periodically and after 5 months recovery. At 3 days, there was 0.25 mg silica in the lung and 0.5 mg at 6 weeks. At 12 months, ~1% of the total administered respirable silica was observed in the lungs. Initial accumulation was rapid and dropped off between week 18 and 12 months (0.5 mg at 6 weeks; 1.2 mg at 18 weeks; 1.37 mg at 12 months). At 6 weeks, the mediastinal lymph nodes contained ~ 0.02 mg silica and 0.13 at 12 months. After 5 months of recovery, the silica in the lungs decreased to 0.16 mg/lung (88% reduction) and 0.047 mg/lymph node (> 50% reduction).

Rats (strain and n not provided) were exposed to aerosolized hydrophobic silica (50 mg/m³; particle size not provided) for 5 h/d, 2 d/week for 8 and 12 months. The lungs retained 1.448 mg silica (1.3% of exposure) and 1.759 mg (1.1%), respectively. The lymph nodes retained 0.05 and 0.113 mg, respectively. After 12 month exposure and 1 month recovery, the lungs contained 1.1 mg silica (37.5% elimination) and the lymph nodes contained 0.16 mg. After 3 months recovery, the lungs contained 0.43 mg and the lymph nodes 0.12 mg. After 5 months recovery, the lungs contained 0.41 mg (76.7% elimination) and the lymph nodes 0.13 mg.

Rats (strain and n not provided) were exposed to aerosolized hydrophobic silica (100 mg/m³; particle size not provided) for up to 1 year. Silica content of the lungs and the lymph nodes was 4.33 and 0.132 mg at 3 months, 6.71 and 0.214 mg at 5 months, and 11.46 and 0.378 mg, respectively. After 6 months of recovery, 55.5% of the silica was eliminated from the lungs. Lymph node elimination could not be observed.

Male rats (strain and n not provided) were exposed to aerosolized hydrophobic pyrogenic silica (0, 10, 50, or 150 mg/m³; particle size not provided) for 6 h/d, 5 d/week for 12 months. The low dose had no effect. The mid- and high-dose groups had white foci on the lung surfaces and collections of foamy macrophages within the alveoli. The peribronchial lymph nodes were enlarged.

Male Cynomolgus monkeys (*Macaca fascicularis*; n not provided) were exposed to aerosolized hydrophobic pyrogenic silica (0, 10, 50, or 100 mg/m³; particle size not provided) for 6 h/d, 5 d/week for 12 months. Recovery was 2 or 24 months. The low dose had no effect. The mid- and high-dose groups had interstitial fibrosis, which did not resolve or progress during recovery. Peribronchial lymph nodes were enlarged (ECETOC 2006).

Intratracheal

Silica

Ernst et al. (2002) used female Wistar WU rats (CrI:WI(WU)BR) to test the carcinogenicity of silica after intratracheal instillation in several experiments. The authors also tested the preventive effects of poly-2-vinylpyridine-N-oxide (PVNO) against silica carcinogenicity. Starting at 8 weeks of age, the rats were anesthetized and treated by intratracheal instillation of a particle suspension of silica. In the first experiment, the rats (n = 4) were treated 20 times at 2-week intervals with silica (0.5 mg). A second set was treated 30 times. Two weeks after the last instillation, the rats were killed and the lungs examined. Rats treated with silica had moderate, but transient dyspnea that resolved in 1 to 4 h.

The experiment was repeated with various doses and with the addition of PVNO measuring various parameters, comparing the results to the control saline solution. Silica administered twice at 0.3 mg at 7-day intervals increased lung weights compared to controls. At 1 and 3 mg, there were increased leukocytes, PMNs, lymphocytes, and lung weights. Similar results were observed when administered 3 times. When administered 4 times, there was also an increase in leukocytes and PMNs at 0.3 mg/dose. Rats administered 0.2 mg once had increased leukocytes and PMNs; at 2 mg, there was increased leukocytes, PMNs, and lung weight. PVNO administered with 2 mg of silica reduced the number of leukocytes, PMNs, lymphocytes, and lung weight compared to silica alone.

Using the data from the above experiments, the authors designed experiments lasting 3 and 9 months. The rats treated with silica had decreased body weights of ~5% after 9 months.

The particle-laden alveolar macrophages in the lungs of the silica-treated groups appeared to be generally intact. In the 4-week experiment there was multifocal moderate to severe granulomatous alveolitis characterized by abundant macrophages, fewer fibroblasts, and T-lymphocytes and only a few granulocytes. Over time after instillation of silica, the majority of these inflammatory foci had progressed to “scar-like” interstitial fibrotic granulomas. This process was markedly augmented by additional treatment with PVNO. The authors stated that fibrotic lesions were considered to represent chronic stages of alveolitis induced by silica.

Cells lavaged from the lungs of the rats treated for 9 months had increased reactive nitrogen intermediates, reactive oxygen intermediates and TNF- α than controls when exposed to lipopolysaccharides or Zymosan. The authors concluded that amorphous silica is more toxic than quartz (also tested in this study) but recruitment of leukocytes and PMN

concentration in the lavage seems to be lower and may decrease faster than for quartz. This may be due to amorphous silica's rapid elimination from the lungs. Silica induced inflammation persisted as long as there were repeated instillations.

Lesions in the lungs were characterized by a lack of alveolar lipoproteinosis and relatively low numbers of intra-alveolar macrophages. Most of the macrophages were foamy but not necrotizing. Silica also produced a pronounced but localized interstitial fibrosis (interstitial fibrotic granulomas). The authors suggested that these developed from acute alveolitis observed after a single administration of silica. The authors also suggested that the lesions resulted from acute epithelial damage at the sites of particle deposition with subsequent (granulomatous) inflammation and production of granulation tissue. Silica is not carcinogenic under these test conditions (Ernst et al. 2002).

Ocular Irritation

Precipitated and Hydrated Silica

Hazelton Laboratories (1958a) instilled a single dose of an aqueous solution of pyrogenic silica (3 mg) to the eyes of albino rabbits (n = 3). The eyes were observed at 1, 4, and 24 h. There was mild eye irritation in the form of erythema and vascularization of the lower sclera and nictating membrane, which resolved within 48 h.

W. R. Grace & Co. (1981) reported a Draize test of silica (9 mg) using rabbits (n not provided). The dry material was a mild irritant (score of 2.4) in the unrinsed eyes. The authors suggested that was due to the strongly hydrophilic silica. There was no irritation when the eyes were rinsed or treated with an aqueous suspension.

In another study, silica (10 mg) was instilled into the eye of rabbits and not rinsed or rinsed after 2 or 4 sec. There was faint irritation of the mucous tissues in the eyes not rinsed which resolved after 1 day. There was no irritation in the eyes that were rinsed. When the test was repeated with the same amount of silica in aqueous solution there was no irritation.

Lewinson et al. (1994) instilled pyrogenic or precipitated (hydrated) silica (0.1 g in olive oil) into the eyes of male New Zealand white rabbits (n = 8). After 5 min, the eyes of 5 rabbits in each group were rinsed. After 24 h, the rest of the rabbits' eyes were rinsed. The eyes were examined with a split lamp at 1, 24, 48 and 72 h (24-h exposure only).

The rabbits treated with precipitated silica had slight redness of the conjunctiva at 1 and 24 h in the group rinsed after 5 min and at 1, 24, and 48 h in the group rinsed after 24 h. There were no signs of irritation in the rabbits treated with pyrogenic silica. There were no clinical signs. The authors concluded that precipitated silica was slightly irritating to the eyes of rabbits and pyrogenic silica was not irritating. It is not clear whether the greater water solubility of precipitated silica or the incomplete removal of olive oil caused the difference (Lewinson et al. 1994).

UNEP (2004) reported several unpublished ocular studies. One irritation study of pyrogenic silica (100 mg) used rabbits (n = 3). The silica was instilled without rinsing. There were no signs of irritation up to 96 h after application.

In another ocular irritation study of pyrogenic silica (100 mg) using rabbits (n = 3), the silica was instilled without

rinsing. There were weak irritating effects in the conjunctivae with a redness score of 2/4 in all rabbits at 1 and 2 h, 1 rabbit at 24 h, and non at 72 h. Chemosis and discharge were slight after 1 h. The authors concluded that pyrogenic silica was non-irritating.

In another unpublished study of precipitated (hydrated) silica using rabbits, silica was found to be nonirritating.

In another unpublished study of precipitated silica gel (hydrated; suspended in water), the eyes were unrinsed or rinsed after 2 or 4 sec. The authors concluded that precipitated silica was nonirritating.

In another unpublished study, 4 products of precipitated (hydrated) silica (100 mg) were instilled in the eyes of rabbits. All types had isolated cases of very slight and transient irritating effects on the conjunctiva with a redness score of 1/4. The authors concluded the precipitated silica products were nonirritating.

In another unpublished ocular irritation study, precipitated (hydrated) silica (50% w/v in an aqueous slurry) was nonirritating to rabbits (UNEP 2004).

Unpublished ocular irritation studies are summarized in Table 10 (ECETOC 2006).

Dermal Irritation

Pyrogenic and Hydrated Silica

Hazelton Laboratories (1958b) applied pyrogenic silica (5 or 10 g as a paste in water) to the intact and abraded skin of albino rabbits (n = 4) daily, 5 d/week, for 15 applications. Mild dermal irritation consisting of erythema, atonia, and desquamation was observed for both doses. The abraded skin healed completely.

W. R. Grace & Co. (1981) reported a study where a US Department of Transportation test for skin irritation of silica (assumed at 100%) was performed on rabbits (n = 8) on intact and abraded skin. One rabbit showed very mild reddening of the abraded skin. Silica was determined to be virtually non-irritating.

Lewinson et al. (1994) used a gauze patch to apply pyrogenic silica (0.5 g in olive oil) or precipitated silica (0.5 g in aqueous methylhydroxyethyl cellulose gel 300 P [1%]) to the intact and abraded skin of New Zealand white rabbits (n = 6; 3 male, 3 female) for 24 h. The patch site was scored after removal and 48 h later. The rabbits were observed for clinical signs during exposure and for 14 d after exposure. No irritation was observed for either type of silica on either intact or abraded skin. No effects were observed.

UNEP (2004) reported an unpublished dermal irritation study of pyrogenic silica (0.5 g; 12% suspension/gel in 1% methylhydroxyethyl cellulose in water) using rabbits. The silica was applied under occlusion to the intact (n = 6) and scarified (n = 6) skin of the rabbits for 24 h. There were no signs of irritation under either skin condition. The authors concluded that pyrogenic silica was non-irritating.

In another unpublished dermal irritation study, precipitated (hydrated) silica (0.5 g; 23% suspension/gel in 1%

methylhydroxyethyl cellulose in water) using rabbits, silica was applied under occlusion to the intact (n = 6) and scarified (n = 6) skin of rabbits for 24 h. There were no signs of irritation under either skin condition. The authors concluded that pyrogenic silica was non-irritating.

Another unpublished study reported a dermal irritation study of precipitated (hydrated) silica (0.5 g in 0.5 ml water) on rabbits (n = 3). The test substance was applied to the skin under occlusion for 4 h. There were no irritating effects.

Another unpublished study reported a dermal irritation study of precipitated silica (0.5 g) on rabbits (n = 12). The test substance was applied to the skin under occlusion for 24 h. There were no irritating effects.

Another unpublished study reported a dermal irritation study of precipitated (hydrated) silica (20 mg) on rabbits (n = 8). The test substance was applied to the skin under occlusion for 24 h. There were no irritating effects.

Another unpublished study reported that a patch test of silica was non-irritating to rabbits. No further details were provided.

Another unpublished study reported a dermal irritation study of precipitated (hydrated) silica (190 mg; 17% w/w; ~0.38 g/ml) on rabbits (n = 6). The test substance was applied to the skin under occlusion for 24 h. There was slight erythemas in 4/6 rabbits 0.5 h after removal. There were no irritating effects at 72 h. The authors concluded that precipitated silica was non-irritating.

Another unpublished study reported a dermal irritation study of precipitated (hydrated) silica (33 mg) on rabbits (n = 6). The test substance was applied to the skin under occlusion for 24 h. There was slight erythema at 24 h after removal. The authors concluded that precipitated silica was non-irritating (UNEP 2004).

ECETOC (2004) reported an unpublished dermal toxicity study of pyrogenic silica (0, 5,000, 10,000 mg/kg/d) using albino rabbits (n = 4; 2 male, 2 female). The silica was applied for 18 h/d, 5 d/week for 3 weeks to intact and abraded skin. There were no signs of systemic toxicity. There was no difference in dermal irritation between treated and control (cosmetic talc) groups as both produced mild dermal irritation consisting of erythema, atonia, and desquamation.

Unpublished studies of skin irritation of hydrophilic and hydrophobic silica on rabbits are summarized in Table 11 (ECETOC 2006).

Dermal Sensitization

Hydrated Silica

A guinea pig (n =10) sensitization study of hydrated silica (20% in distilled water) was conducted. No reactions were observed in either the test group or the control group (distilled water; n = 5) (Council 1984).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Pyrogenic Silica

W. R. Grace & Co. (1981) reported a study in which silica (500 mg/d) was fed to male and female rats (n = 40) for 6 months. After 4.5 months, 5 females were mated. There were no adverse effects observed for mortality, body weight gain, hematology, and reproductive performance. Histology of the stomach, intestines, pancreas, liver, and kidneys were similar to controls. Litter size, birth weight, morphology, and development of the offspring were similar to controls.

In another study, pregnant female mice were fed up to 1340 mg/kg silica for 10 days (specific gestation days not provided). There were no effects on nidation or on maternal or fetal survival. Fetal abnormalities were similar to controls.

The same results were reported for rats (up to 1350 mg/kg for 10 days), hamsters (up to 1600 mg/kg for 5 days) and rabbits (up to 1600 mg/kg for 13 days) (W. R. Grace & Co. 1981).

Lewinson et al. (1994) administered pyrogenic silica (500 mg/kg/day) to female Wistar rats (n not provided) in their feed. The female rats were mated with male rats consuming 500 mg/kg/day from the subchronic study (above) at weeks 8 and 17. The rats were weighed periodically, blood sampled monthly (except during pregnancy), and observed daily. The progeny from both matings were examined for abnormalities. At 6 months, the rats were killed and necropsied except for 5 rats which had a 3 week treatment-free period prior to being killed and necropsied.

There were no clinical signs during treatment. Body weights and feed consumption were similar between treatment and control groups. Hematological parameters and organ weights were unremarkable. Reproductive performance was similar between groups. Pathological examination revealed no differences between the groups.

At the first mating, 6 control and 9 treatment dams became pregnant; 7 from each group became pregnant at the second mating. There were no treatment-related effects in litter size, birth weight, physical parameters, or behavior. Development of progeny during lactation was without adverse effects; weight gains were normal. No treatment related effects were found during gross pathology. The authors conclude that the oral NOEL was >500 mg/kg for developmental and reproductive toxicity (Lewinson et al. 1994).

GENOTOXICITY

Mammalian Mutagenicity

Pyrogenic and Hydrated Silica

Liu et al. (1996) performed an in vitro micronucleus test using Chinese hamster lung fibroblasts on silica (20, 40, 80, and 160 $\mu\text{g}/\text{cm}^3$; 0.12, 0.23, 0.46, and 0.93 mg/ml). There was weak, but significant, dose dependent induction of micronuclei at cytotoxic concentrations with the results of the 2 highest dose groups (13.33 ± 1.77 , $p < .05$; 18.00 ± 2.08 , $p <$

.01) being greater than controls (7.67 ± 2.33 ; correlation coefficient 0.96). No clastogenicity was observed in concentrations lower than cytotoxic levels.

Zhong et al. (1997) performed a single-cell gel/Comet assay using Chinese hamster fibroblasts (V79) and human embryonic lung fibroblasts (HEL 299) on silica (68.9 and 137.9 $\mu\text{g}/\text{cm}^2$). There was a dose dependent increase in DNA migration in the gel in both cell types in a similar manner.

UNEP (2004) and ECETOC (2006) reported several unpublished in vitro mutagenicity studies of silica in mammalian cells. Silica was not mutagenic. The studies are summarized in Table 12.

ECETOC (2006) reported several unpublished in vivo mutagenicity studies of silica gel. Silica gel was not mutagenic. The studies are summarized in Table 13.

Microbial Mutagenicity

Silica

Kanematsu et al. (1980) performed a Rec assay and an Ames assay (using *Escherichia coli* TA98, TA100, TA1535, TA1538) on silica. Both assays were negative at 0.001 to 10 M.

Prival et al. (1991) performed an Ames test on synthetic silica (0.033 to 10 mg/plate in dimethylsulfoxide [DMSO]) using *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) and *E. coli* (WP 2) with and without metabolic activation. All results were negative.

Lewinson et al. (1994) exposed *S. typhimurium* (strains TA98, TA100, and TA1535) and *E. coli* (WP2uvrA) to a toluene extract of pyrogenic silica (5 to 1580 $\mu\text{g}/\text{plate}$) with and without metabolic activation. The toluene extract of pyrogenic silica was not mutagenic at any concentration with or without activation. In an additional test, the extract was not mutagenic to *S. typhimurium* TA98 where the epoxide hydrolase inhibitor and glutathione depletor 1,1,1-trichloropropene-2,3-oxide was added to the activation mix to increase sensitivity of the test toward compounds that are activated to mutagenic epoxides.

UNEP (2004) and ECETOC (2006) reported several unpublished mutagenicity studies of silica. There was no evidence of mutagenic activity. The studies are summarized in Table 14.

Mutagenic Inhibition

Hydrated Aluminum Calcium Sodium Silicate

Abdel-Wahhab et al. (1998) incorporated aflatoxin (AF; 2.5 mg/kg feed) with or without hydrated aluminum calcium sodium silicate (0.5%) into the feed of Sprague-Dawley rats ($n = 10$) for 15 days. The rats were killed and bone marrow samples were collected for chromosomal analysis. AF caused structural and numerical aberrations of chromosomes, mainly chromatid breaks and chromatid gaps. Hydrated aluminum calcium sodium silicate decreased these effects for every category

of aberration except polyploidy. Hydrated aluminum calcium sodium silicate alone did not cause an increase in aberrations.

Şişman (2006) incorporated AF B₁ (0, 0.2, 0.5, or 0.8 ppm) with and without hydrated aluminum calcium sodium silicate (5.0 or 10.0 ppm) into the agar feed of adult Oregon-R wild type *Drosophila melanogaster* flies. The flies were then paired and mated and the offspring observed. The low, mid, and high dose of AF caused the retardation of development of the F₁ adults by 1, 2, and 3 days, respectively. Both doses of hydrated aluminum calcium sodium silicate prevented the delayed development. Malformations in the AF treated groups increased from 0.38% (control) to 7.35%, 9.10% and 11.11%, respectively. The low and high dose of hydrated aluminum calcium sodium silicate reduced malformations but not to the levels of the controls. The AF reduced the number of offspring ($p < .05, .01$). Hydrated aluminum calcium sodium silicate mitigated this effect but not to control levels. No ill effects were reported due to hydrated aluminum calcium sodium silicate, only protective effects.

CARCINOGENICITY

Oral

Pyrogenic and Hydrated Silica

In an experiment described earlier by Takizawa et al. (1988), the female mice were fed precipitated (hydrated) silica in feed, the frequency of adenocarcinomas in the lungs was 1/16 (6.25%) for the control and 1/19 (5.3%), 0/20 (0%), and 1/20 (5%) for the low, mid, and high dose groups. In the males, the frequency of adenocarcinomas in the lungs was 1/16 (6.25%) for the control and 2/17 (11.8%), 3/14 (21.4%), and 3/16 (18.8%) for the low, mid, and high dose groups. There was low correlation of hyperplastic nodules/hepatocellular carcinoma/hemangioma/fibrosarcoma in the treatment groups compared to controls. The authors concluded that the non-neoplastic lesions were of no toxicological significance.

Lewinson et al. (1994) orally administered pyrogenic silica to Wistar rats ($n = 40$; 20 males, 20 females) in their feed (100 mg/kg) for 24 months. The rats were weighed before and after treatment. The rats were killed and necropsied. There were no clinical signs observed during the treatment period. The rates of tumors observed in the treated rats were comparable to historical controls. The authors concluded that there were no carcinogenic effects due to pyrogenic silica exposure.

Intratracheal

Pyrogenic Silica

Pott and Roller (2005) intratracheally instilled pyrogenic silica (3 mg in 0.9% PBS; 0.01 to 0.03 μm) into female SPF Wistar rats (HsdCpb:WU) ($n = 40$; 8 to 9 weeks old) 5 times weekly. The rats were then followed until death or the 30th month when they were killed and necropsied. A second group had silica at the same dose instilled 10 times weekly. Controls ($n = 48$) were untreated. In the first group, 37 rats survived the entire experiment, 35 in the second group, and 46/48 in the

control group. The period of time after the first treatment in which 50% of the rats died was 113 and 112 weeks in the first and second groups and 113 weeks in the control group. The percentage of rats with macroscopic lung tumor(s) was 13.5% in the first group, 2.9% in the second group, and 6.5% in the control group. The percentage of rats with macroscopic lung tumor(s) which are probably not a metastasis of other tumors located elsewhere was 8.1% in the first group, none in the second group, and none in the control group. The percentage of rats with benign tumors in the second group was 5.7% and there were none in the control group; this was not analyzed in the first experiment. Neither group had malignant tumors. The percentage of rats with tumors that were metastases of other tumors was 14.3% in the treatment groups 13.0% in the control group.

Inhalation

Hydrated Silica

Campbell (1940) exposed 3 month old mice susceptible to tumors (n = 75) to aerosolized precipitated (hydrated) silica (0.5 g/d; $\leq 5 \mu\text{g}$ particle size) once/h, 6h/d, 5 d/week for a year. The mice were allowed to live out their natural life span up to 917 days from the start of the experiment. Incidence of primary lung tumors was 7.9% in the control group and 21.3% in the treated group in mice living 10 months or longer. There was no obvious fibrosis in the lung tissue; there was fibrotic nodules in the tracheo-bronchial lymph nodes in $> 50\%$ of the mice. The author suggested that most of the silica dust was removed by cilia action through the trachea and also through the lymphatics. Half of the treated mice had overgrowth of the mediastinal connective tissue covering the tracheo-bronchial nodes which occurred in only 10% of the controls. In the treated group, 29.5% had an increase in incidence of overgrowth or hyperplasia of the tracheo-bronchial lymph nodes compared to 14.3% of the controls.

CLINICAL ASSESSMENT OF SAFETY

Oral

Pyrogenic and Hydrated Silica

Worth and Campen (1951) assessed the silica level in the blood of volunteers (n = 264) before and after the oral administration of colloidal silica protein or silica acid, tetraglycol ester (amount not provided). There was a rapid increase of silica blood levels and a rapid elimination in the urine over 8 to 24 h. There was no influence of sex, age, employment, lung disease (dust lung), or other disease.

UNEP (2004) reported an unpublished study of orally administered silica (1250 mg in apple juice) in the form of pyrogenic (n = 6; 5 males, 1 female) and precipitated (hydrated; n = 6; 5 males, 1 female) silica, to volunteers. The solutions were consumed in 2 doses, morning and midday on the same day. The total urine was collected daily and analyzed. During

the 4 days post-treatment, changes of renal silica secretion were not observed. Daily silica increments in urine after ingestion ranged between 7 and 23 mg. For the pyrogenic silica, the individual baseline values of the pre-test phase were very variable and individually different; mean excretion rates ranged from 25 to 87 mg/day. In the post-treatment phase, individual mean excretion rates ranged from 32 to 61 mg/day. For the precipitated silica, the individual baseline values of the pre-test phase were very variable and individually different; mean excretion rates ranged from 16 to 71 mg/day. In the post-treatment phase, individual mean excretion rates ranged from 20 to 81 mg/day. Overall, increases in excretion were not unequivocally detectable. The authors noted that the small apparent increases were in marked contrast to the high dose of 2500 mg silica applied.

In an unpublished study on the effectiveness of silica gel in the treatment of type II hyperlipoproteinemia, 6 adults (3 men, 3 women; 20 to 51 years old) were admitted to a metabolic unit for 3 weeks. Silica was administered with the morning and evening meals starting with an oral dose of 1.0 g/d which increased by 1.0 g/d until 16 g/d was reached. There were no increases in the serum or urinary levels of silica. The number of white and red blood cells and platelets were unaffected. Two subjects had a decrease in serum iron levels, 1 had a decrease in hemoglobin concentration, 2 had a decrease in carotene, 1 had a decrease in serum folate, and 1 had a decrease in vitamin A. Clinical side effects included constipation in half the subjects and an unusual aftertaste in all subjects. One subject had gastritis. The authors concluded that there were no adverse effects of the silica on hepatic or renal function. Silica gel was not absorbed significantly from the intestine (UNEP 2004).

Dermal Irritation and Sensitization

Hydrated Silica

W. R. Grace & Co. (1981) reported a dermal study of a dusting powder that included micronized silica gel (amount not provided) on patients (n = 300). The authors concluded that the powder was non-irritating and non-toxic and could be safely applied to babies, children, and adults; and, from the allergic tests performed, there were little or no sensitizing properties of the powder.

Aqueous sodium lauryl sulfate (25%; ~0.05 ml) was applied to the upper outer arm, volar forearm, or the back of volunteers (n = 27; 18 males, 9 females) under occlusion for 24 h. This was followed by the application of a facial mask containing hydrated silica (0.05 ml; 17%) for 48 h (or 72 h over weekends), under occlusion. This was repeated for 5 inductions. After a 10-day rest, a challenge application was applied on a naive site (upper outer arm, volar forearm, or the back) for 48 h. There was no sensitization observed. The authors concluded that the test material was not likely to cause contact sensitivity under normal use (KGL, Inc. 2003).

Aqueous sodium lauryl sulfate (25%; ~0.05 ml) was applied to the upper outer arm, volar forearm, or the back of volunteers (n = 27; 18 males, 9 females) under occlusion for 24 h. This was followed by the application of a facial powder

containing hydrated silica (21.7436%) made into a 30% aqueous solution for 48 h (or 72 h over weekends), under occlusion. This was repeated for 5 inductions. After a 10-day rest, a challenge application was applied on a naive site (upper outer arm, volar forearm, or the back) for 48 h. There was no sensitization observed. The authors concluded that the test material was not likely to cause contact sensitivity under normal use (KGL, Inc. 2004).

UNEP (2004) reported an unpublished sensitization study of colloidal silica (45%). Patches were applied to volunteers (n = 20; 10 men, 10 women) for 6 days. After 2 weeks, challenge patches were applied for 48 h. Skin under the patches was examined at 1, 2, 3, and 6 days after the first application and on removal of the challenge patch. No skin reactions were observed (UNEP 2004).

Immune Response

Hydrated Silica

Epstein et al. (1963) injected colloidal (hydrated) silica (1 to 4 mg in saline; ~15 μm particle size) subcutaneously 2 to 8 times in volunteers (n = 28). Biopsies were taken from day 1 to 6 months. Granulomatous inflammation was observed within 7 days and persisted for months. The authors suggested that this was a particular type of foreign body response to a fibrogenic agent and not typical epithelioid cell nodules.

Occupational Exposure

Pyrogenic and Hydrated Silica

Volk (1960) studied workers (n = 215) with exposure to silica between 1947 and 1959 using chest x-rays. Exposure ranged from 15 to 100 mg/m^3 , 2 to 6 mg/m^3 , and 3 to 7 mg/m^3 , depending on workstation. Hairline actuation of the interlobar fissures, suggesting slight interlobar pleuritis, was the only remarkable sign. There were no signs of silicosis.

Plunkett and DeWitt (1962) examined 78 workers (aged 21 to 67 years; average 34.23 years) who had been occupationally exposed to precipitated silica from 1941 to 1959. Dust concentrations ranged from 0.35 to 204 mg/m^3 . There was no evidence of silicosis or other pulmonary disease.

Wilson et al. (1979,1981) examined workers (n = 165) exposed to precipitated silica a mean of 8.6 years (44 workers had been exposed a mean of 18 years [10-35 years]). Dust levels varied from <1 to 10 mg/m^3 with some higher intermittent levels. Examination included spirometers, respiratory questionnaires, and chest radiographs. Cough and dyspnea correlated with level/time of smoking and not silica exposure. There were no correlations between yearly change of pulmonary function and dose or time of exposure. The workers with the mean exposure time of 18 years had pulmonary function similar to the rest of the group. There was radiographic evidence of minimal pneumoconiosis that was biased due to prior exposure to limestone. None of the 143 workers with exposure only to silica showed radiographic evidence of pneumoconiosis.

Choudat et al. (1990) examined workers (n = 41) exposed to precipitated silica and compared them to a control group.

The examination included blood gas analysis and chest radiographs. There was a reduction in forced expiratory flow in the exposed group. There was no correlation between the exposure index and pulmonary function. The authors concluded that smoking and exposure to silica synergise to induce small airway disease.

UNEP (2004) reported several unpublished studies of silica exposure. Workers (n = 200) with intensive and regular contact with silica from 1972 to 2000 were evaluated. There was no evidence of skin allergy caused by the silica. There were signs of irritation attributed to the desicative and defatting properties of silica which resulted in skin dryness which could be controlled by regular use of skin-protection ointment.

In another unpublished study, an occupational study of workers (n = 143) exposed to silica from 1959 to 1985 was performed. Exposure ranged from 1 to 34 years. There were complaints of some disorder or exhibition of abnormalities in lung function or histology in 54/143 (36%) of the workers. Dry cough, expectoration or dyspnea was reported in 34/54 of these workers. A total of 42/54 (78%) of these workers had some possible confounding factor (i.e., smoking). Radiological examination did not show any signs of fibrotic disease. Spirometric examination showed obstructive and/or restrictive ventilation disturbances in 24 workers. Most of the adverse findings were associated with confounding factors such as smoking.

In an unpublished occupational exposure study, x-rays were taken of 99 workers who had manufactured silica for various amounts of time. The x-rays revealed no evidence of any occupational disease, including silicosis.

In an unpublished occupational study of workers in precipitated silica factories (1952 to 1981), there was no silicosis in workers employed for 1 to >20 years (mean 13.2 years). There were negative results in hematology, urine analysis, lung functions, and chest x-rays.

In an unpublished study of workers (n = 78) in a factory that manufactured hydrated silica pigment between 1941 and 1959, dust concentrations ranged from 0.35 to 205 mg/m³. No evidence of silicosis or other pulmonary disease was observed. The incidence of illness and injuries were similar to other workers in this plant (UNEP 2004).

ECETOC (2006) reported an unpublished study of workers exposed to pyrogenic (3 factories) or precipitated (2 factories) silica. There were 510 current workers with at least 1 month exposure studied. Complete data sets were collected from 397 workers. Data were also collected from 178 men who were no longer working in the factories. Unexposed workers totaled 210. Chronic bronchitis rates were within expected ranges for all group but somewhat higher in exposed subjects. Differences between factories were absent. The percentage of subjects with obstruction or restriction in breathing were similar between plants. Bronchial hyperresponsiveness was within expected levels. Chest radiographs showed no increased risk of pneumoconiosis in exposed subjects compared to controls. Relevant pleural thickening was not observed. The authors concluded that occupational exposure to silica was not a health risk.

In an unpublished study, 150 workers in a precipitated silica factory were examined by pulmonary function test and X-ray. The workers were exposed for ≥ 6 h/d for at least 5 continuous or discontinuous years. The mean duration was 12.2 years. The control group had been exposed for a maximum of 3 continuous or discontinuous months. The mean ages for the experimental and control groups were 43.1 and 44.3 years, respectively. There were no differences in the distributions and types of dysfunctional measurements observed between exposed and non-exposed groups. There were no differences in the mean percentage of predicted pulmonary function values between exposed and non-exposed groups. None of the X-rays showed signs of pneumoconiosis or fibrosis.

In an unpublished study, 29 workers in a silicone products manufacturing plant were surveyed. Silica exposure ranged from 0.15 to 10 mg/m³ with a mean of 1.7 mg/m³. Ten of 15 workers in the room temperature vulcanizing rubber area complained of upper respiratory tract irritation. In the heat curable rubber compounding area, the potential exposure to silica was greater, some of the workers complained about eye irritation, nausea, headaches, or rashes, none reported upper or lower respiratory problems (ECETOC 2006).

OTHER EVALUATION

The International Agency for Research on Cancer (IARC; 1996) concluded that amorphous silica is not classifiable as to its carcinogenicity to humans based on inadequate evidence in humans and inadequate evidence of increased tumors in animals.

OCCUPATIONAL EXPOSURE LIMITS

OSHA (2004) standard for exposure to amorphous silica is 80 mg/mm³ or 20 mppcf air averaged over an 8-h work shift. NIOSH (2005) recommended that exposure to respirable silica be limited to 6 mg/m³.

SUMMARY

This is a safety assessment of silica, alumina magnesium metasilicate, aluminum calcium sodium silicate, aluminum iron silicates, hydrated silica, and sodium potassium aluminum silicate. Silica is a silicon-oxygen tetrahedra where a silicon atom is central within 4 oxygen atoms that are shared with adjacent silicon atoms. There are many forms of silica. Only the safety of pyrogenic and hydrated amorphous silica and their salts are evaluated in this assessment; crystalline silicas are not included in this assessment.

Free silanol groups on the surface of silica particles influence the adsorption behavior. Silica has thixotropic properties. Amorphous silica does not exist as primary particles, except in solution-based forms, but as aggregates and agglomerates.

Amorphous pyrogenic silica (Figure 1) is manufactured by the hydrolysis of volatile silanes, usually silicon tetrachloride, in the flame of an oxygen-hydrogen burner. Precipitated silica and silica gels are produced from an alkali metal silicate dissolved in water (i.e., water glass) and an acid. Silica sols (colloidal silica) are dispersions of silica particles in a liquid, usually water. Impurities include calcium, sodium, potassium, antimony, barium, chromium, arsenic, lead, mercury, cadmium and selenium.

Analytical methods include colorimetric technique, electron microscopy, ICP-AES, CT-MAS, NMR, IR, BET, DLS, and AFM.

Silica was used in a total of 3,276 cosmetic products. Use concentrations ranged from 0.0000003% to 40%. Hydrated silica was reported to be used in 176 cosmetic products at 0.001% to 34%; aluminum calcium sodium silicate in 7 cosmetic products at 0.4% to 6%; and Sodium potassium aluminum silicate in 1 cosmetic product at 0.001% to 4%. Alumina magnesium metasilicate was not reported to be used by a voluntary FDA survey but was reported to be used at 0.002% to 0.001% in an industry survey. There were no reported uses or concentration of use reported for aluminum iron silicates.

Silica is used in food preparation as an anticaking agent, dispersion agent, suspending agent, and thickening agent. It is a defoaming and conditioning agent in malt beverages. Silica is a thickener in pastes and ointments.

Orally administered silica was mostly excreted through the urine in guinea pigs. Some studies showed accumulation of silica from oral exposure whereas others did not. Silica intraperitoneally injected into guinea pigs was mostly excreted through the urine.

When silica is inhaled by rats, it accumulates in the lungs and lymph nodes initially. The accumulated amount remains at a steady state with continued treatment. When treatment ceases, the silica decreases. When subcutaneously injected into rats, silica is absorbed over a few months.

When silica is inhaled or intratracheally instilled into rats, mice, and rabbits, there is a transient inflammatory response that resolves in days. Incubated macrophages ingested fewer silica particles than *C. albicans* or *S. cerevisiae*. Ultra-fine silica (14 nm) did more damage to the lungs during the inflammation than did fine silica (213 nm).

Silica is not cytotoxic to Chinese hamster V79 cells up to 160 µg/l. Pyrogenic silica and silica gel were cytotoxic to macrophages at 13.5 µg/cm²; precipitated silica was less cytotoxic. Micronuclei were induced in Chinese hamster cells when incubated with 80 and 160 mg/ml. Micronuclei were induced with the presence of silica. There was ~85% lysis of sheep blood erythrocytes incubated with silica. Mesothelial cells incubated in silica were observed to accumulate silica in the

cytoplasm, around the nucleus, and vacuoles. When incubated in pyrogenic silica, both macrophages and neutrophils were inhibited in their ability to phagocytose sheep red blood cells. Silica caused intracellular alteration in calcium homeostasis in renal cells.

Alveolar macrophages exposed to silica had increased protein kinases, NO_x production, and cell death. Human primary fibroblasts exposed to silica produced COX-2 and PGE_2 in a dose dependent manner. COX-1 was not affected. Silica was not cytotoxic to human mesothelioma and rodent fibroblast cells.

Sodium potassium aluminum silicate, in the form of Mexicali dust, induced anaphasic alterations in Balb3T3 cells.

Hydrated aluminum calcium sodium silicate countered the effects of aflatoxin in animal feed.

Silica was reported to have an oral LD_{50} up to $> 40,000 \text{ mg/kg}$ in rats and $> 8,000 \text{ mg/kg}$ in mice.

Orally administered aluminum calcium sodium silicate had no adverse effects up to 800 mg/kg in mice.

The acute dermal NOEL for silica is $> 2,000 \text{ mg/kg}$ for rabbits. When applied as an aqueous paste, there were no adverse effects. LD_{50} was $> 5,000 \text{ mg/kg}$ for precipitated (hydrated) Silcia and $> 2,000 \text{ mg/kg}$ for silica gel on intact and abraded rabbit skin with mild erythma.

Intraperitoneally injected silica was lethal to 20% to 30% of rats and rabbits at 100 mg/kg ; all survived at 50 mg/kg . Peritoneal edema diminished and fibrosis increased over time. Silica injected i.p. was fatal to guinea pigs at 10%. Rats survived 5 days after an i.p. injection of 0.5 g silica, whereas another study reported 50 mg silica to be lethal in rats.

Silica injected in the veins of mice was better tolerated in small doses than in 1 large dose. The lethal dose ranged from 0.2 to 0.5 mg/30 g body weight, depending on particle size. The intravenous LD_{50} of silica was 15 to 44.4 mg/kg in rats, depending on type and source.

The minimum lethal dose of acute intratracheal administration of silica was 1.8 mg/cm^3 in rats. In rats, silica at 30 and 50 mg/kg was fatal to 80% to 90% immediately or within a few hours; these doses were nearly always fatal for rabbits. Silica particles (10 mg/kg ; particle size range 2 to $3.5 \mu\text{m}$) produced potent but transient pulmonary inflammation. Ultrafine silica induced more alveolar hemorrhage, compared to fine silica.

The acute inhalation of silica at 477 mg/m^3 by rats resulted in restlessness, droopy eyelids, lethargy, and dyspnea during treatment. Clinical signs resolved quickly after treatment and necropsies were unremarkable.

Silica at $200 \mu\text{g}$ instilled into the musculature induced local inflammation for up to 6 month with granulomatous scarring with necrotic muscle fibers and fatty degeneration of local macrophages.

Short-term oral doses of silica at $8,000 \text{ mg/kg/d}$ produced no clinical effects in dogs. Short-term oral doses of silica produced no clinical effects for rats; HNELs were up to 1000 mg/kg/d . At $16,000 \text{ mg/kg}$ clinical signs were observed: shyness, dirty fur, reduced activity, cachexia, hemorrhages of mucous membranes of the eyes and nose.

Short-term dermal application of silica to intact and abraded skin resulted in no dermal toxicity in rabbits.

Short-term inhalation of silica up to 668 mg/m³ resulted in respiratory distress during treatment and a short-term inflammation response in the lungs, which resolved quickly when treatment ceased in rats. The lung clearance half-life was ~50 day for 50.5 and 154 mg/m³. The NOAEL was 10.1 mg/m³ in one study. In other studies, the NOEL was 1 and 46 mg/m³.

Intratracheal injections of 5% silica resulted in the death of 3 of 10 rats and 2 of 10 guinea pigs at 10%.

Intravenous injections of 1% silica biweekly for 20 weeks into 5 rabbits resulted in 2 deaths. One more died during recovery. There was pleural effusion with pleuritis, mediastinal abscess formation, and marked congestion of the lungs. Right ventricles were dilated, the livers enlarged, and the spleen atrophied.

The oral subchronic HNEL was 5000 mg/kg/d, the NOEL was 500 mg/kg/d and the lowest effect level was 500 mg/kg/d for rats. There were no clinical signs up to 7950 and 8980 mg/kg/d for male and female rats, respectively. There were no gross findings of toxicity up to 50,000 ppm in feed.

Subchronic inhalation of silica at 53 mg/m³ caused 44% mortality in rats from pulmonary vascular obstruction and emphysema. There was increased respiration rates and decreased weight gain during treatment. Necropsy findings included congestion of the lungs, lymph node enlargement, emphysema, vacuolated cells within alveolar spaces, and increased lung weights and collagen content. There were no mortalities at 31 mg/m³. The LOAEL was 53 mg/m³. The NOEL was 1.3 mg/m³.

Silica incorporated into the feed of rats at up to 10% for 6 months or more produced discolored stool and unremarkable necropsies. Females had increased leukocytes and males had increased eosinophils at 10% in feed. Females had reduced liver weights at 12 and 24 months at 5%. Mice treated with silica in their feed had similar results for up to 103 weeks.

Rabbits chronically exposed to 0.2 and 5.0 µm aerosolized silica had formation of nodular fibrotic or diffuse fibrotic changes in the lungs. Mice bred to be susceptible to tumors exposed to aerosolized silica at 0.5 g/d for a year had increased incidence of lung tumors with no obvious fibrosis of the lung tissue but fibrotic nodules in the tracheo-bronchial lymph nodes. At 53 mg/m³ for a year, treatment related deaths were 75% in rats from pulmonary vascular obstruction and emphysema starting in the 4th month.

Guinea pigs exposed to silica at 1.5 mg/ft³ for up to 24 months had no deaths. A chronic reaction of the lung tissue was established at 4 months and emphysema after 4 to 8 months. Histologically, there was periductal and peribronchiolar intra-alveolar accumulations of the giant cells. In the lymphoid tissue, medullary hyperplasia with the formation of slight

amounts of reticulum was prominent during the second year of exposure. There were no macroscopically visible anomalies after 1 year of recovery.

At 126 mg/m³ of silica for up to 24 months, guinea pigs and rabbits had increased lung weights and particle-phagocytosing macrophages accumulated in alveoli, bronchioles, and lymphoid tissue. There was complete reversibility of silica retention and inflammatory responses in guinea pigs within 6 months of recovery. Silicotic processes were completely absent.

Rabbits exposed to aerosolized silica at 1.5 mg/ft³ for 12 months had progressive functional incapacitation and elevation of hematocrit levels observed in the majority of the rabbits, possibly due to the combined effect of pulmonary vascular obstruction and emphysema. During recovery, the cellular reactions and emphysema regressed but minor focal alveolar mural collagen persisted. In rabbits exposed to 360 mg/m³ for a year, emphysema, pulmonary emphysema, vascular stenosis, alveolar cell infiltration, sclerosis, and epithelization granulomatosis, macrophage catarrh were observed. Lesions were observed in the liver, spleen and kidney. The LOAEL was 28 mg/m³.

Monkeys exposed to aerosolized silica at 15 mg/m³ for 12 months had initial decreased activity and body weight gain. There was emphysema at 3 months and considerable cellular infiltration of the alveoli and alveolar septa associated with distention of alveoli or accumulation of exudate and macrophages. After 12 months, the lesions were marked pulmonary emphysema, alveolar wall sclerosis, vacular occlusions, and cor pulmonale. The silica content remained low and decreased over time. At 50 and 100 mg/m³, there was interstitial fibrosis which did not resolve after 24 months.

When monkeys were exposed to different types of silica, the precipitated (hydrated) silica group had lower lung volumes. There were no changes in lung volume parameters, but in ventilatory performance and mechanical parameters, dynamic lung compliance, and forced expiratory flow when exposed to silica gel. The frequency and size of inflammatory cell aggregates varied with the type of silica.

Rats exposed to aerosolized silica at ~45 mg/m³ for 1 year had 41/120 deaths. There were small white foci under the pleura, enlarged and discolored lymph nodes with formation of collagen and local necrosis, perivascular and peribronchiolar dust cell granuloma with reticulin and collagen fibers, necrotic cells, desquamative catarrh and thickened alveolar septa. After 3 to 8 months recovery, the dust cell granuloma were fewer and reduced in size with only a few dust cells and fibers. The alveolar septa had not completely disappeared and lymph nodes were enlarged.

Rats exposed to aerosolized silica at 15 mg/m³ for 12 months had a few macrophages aggregated in the lungs. The LOAEL was 6 to 9 mg/m³. Rats exposed to aerosolized silica at 50 mg/m³ for 12 months had 1.759 mg silica in the lungs, which decreased to 1.1, 0.43, and 0.41 mg after 1, 3, and 5 months recovery, respectively.

Intratracheal instillation of silica at 0.5 mg in rats every other week for 20 and 30 times resulted in 0.44 mg

silica/lung and associated lymph node.

Silica was a non- to mild ocular irritant in rabbits up to 100 mg.

Silica at 100% was nonirritating to the intact and abraded skin of rabbits.

A guinea pig sensitization study of 20% hydrated silica resulted in no reactions .

No reproductive or teratological effects were observed following the oral administration of silica in rabbits at 1600 mg/kg/d, hamsters at 1600 mg/kg/d, mice 1340 mg/kg/d, and rats up to 1350 mg/kg/d. An oral NOEL of 500 mg/kg/d was reported for rats; an oral NOAEL of 1350 mg/kg/d was also reported.

Silica was not mutagenic using the Rec or Ames test up to 10 M. Silica was not mutagenic in Ames test up to 1580 µg/plate. In a single-cell gel/Comet assay using Chinese hamster fibroblasts, there was an increase in DNA migration in a dose dependent manner. A chromosomal aberration test was negative up to 300 µl/ml without and 1000 µl/ml with metabolic activation. A HGPRT was negative up to 250 µm/ml without and 500 µm/ml with metabolic activation. An unscheduled DNA synthesis test was negative up to 1000 µm/ml.

Silica was not mutagenic to CHO cells, hamster fibroblasts, rat hepatocytes, and human embryonic lung cells. Silica was not mutagenic to mice or rats.

A positive sister chromatid exchange test of AFB₁ showed inhibition by 10⁻⁵ M aluminum calcium sodium silicate. Hydrated aluminum calcium sodium silicate at 0.5% in the feed of rats inhibited the effects of AF.

Oral administration of silica to rats for 24 months was not carcinogenic up to 100 mg/kg/d.

A single intratracheal instillation of 3 mg silica was not carcinogenic. Intratracheal instillation of silica at 0.5 mg twice per week for 30 weeks to rats was not carcinogenic. Silica at 3 mg was not carcinogenic in rats intracheally instilled 5 times weekly for 30 months.

Silica at 0.5 g/d instilled into mice for a year increased the incidence of overgrowth or hyperplasia of the tracheo-bronchial lymph nodes from 14.3% to 29.5%.

The oral lethal dose of pyrogenic silica in humans is 15 g/kg.

Oral ingestion of silica up to 1250 mg resulted in a rapid increase of silica blood levels and a rapid elimination in the urine over 8 to 24 h with no adverse effect reported.

Silica subcutaneously instilled in humans caused granulomatous inflammation within 7 days that persisted for months.

Silica was non-sensitizing at 45%. A powder containing silica up to 45% was non-irritating and non-sensitizing in humans.

Workers in environments with aerosolized silica had few signs of silicosis or pulmonary disease up to 100 mg/m³.

Smoking and exposure to silica synergise to induce small airway disease. Exposure to hydrated silica also had no evidence of silicosis or pulmonary disease. There were signs of dermal irritation due to the desicative and defatting properties of silica.

DISCUSSION

The CIR Expert Panel emphasized that the silica considered in this safety assessment is synthetic amorphous silica (gel, hydrated, and fumed/pyrogenic) and does not include any form of crystalline silica.

The Panel recognizes that there are data gaps regarding use and concentration of these ingredients. However, the overall information available on the types of products in which these ingredients are used and at what concentrations indicate a pattern of use, which was considered by the Expert Panel in assessing safety.

The Panel was concerned about the possibility of iron atoms reaching the lungs if aluminum iron silicates were to be used in a spray. In the absence of inhalation toxicity data, the Panel determined that aluminum iron silicates can be used safely in hair sprays, because the ingredient particle size is not respirable. The Panel reasoned that the particle size of aerosol hair sprays (38 µm) and pump hair sprays (>80 µm) is large compared to respirable particulate sizes (10 µm). The Panel recognizes that most of the formulations are not respirable and of the preparations that are so, the Panel considered that any spray containing these solids should be formulated to minimize their inhalation potential. Aluminum iron silicates is safe as a cosmetic ingredient because the particles for aggregates and agglomerates that are too large to be respirable.

The Panel determined that silicosis was not an issue since crystalline silica is not used in cosmetics.

CONCLUSION

Silica, alumina magnesium metasilicate, aluminum calcium sodium silicate, aluminum iron silicates, hydrated silica, and sodium potassium aluminum silicate are safe as cosmetic ingredients in the practices of use and concentrations as described in this safety assessment.¹

¹Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in the group.

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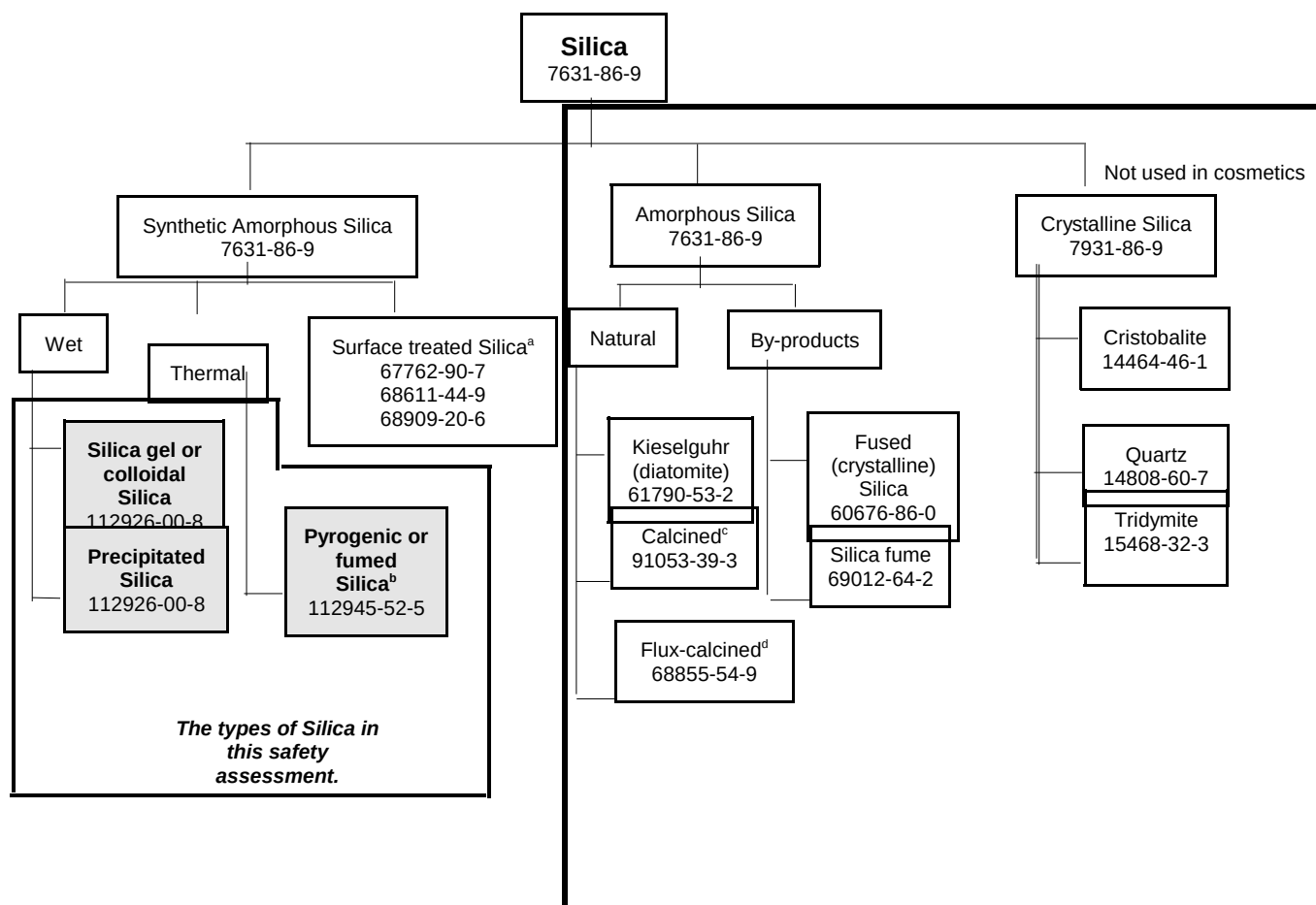


Figure 1. Different polymorphs of silica with CAS Numbers. Shaded boxes represent the types of silica covered in this report. a) All forms of synthetic amorphous silicas can be surface modified either physically or chemically; most common treating agents are organosilicon compounds. b) Pyrogenic silica is also known as fumed silica in English speaking countries and is not to be confused with silica fume, which is crystalline. Pyrogenic is used in recent publications. c) By-product from electrical furnace. d) Partial transformation into cristobalite (after Arts et al. 2007).

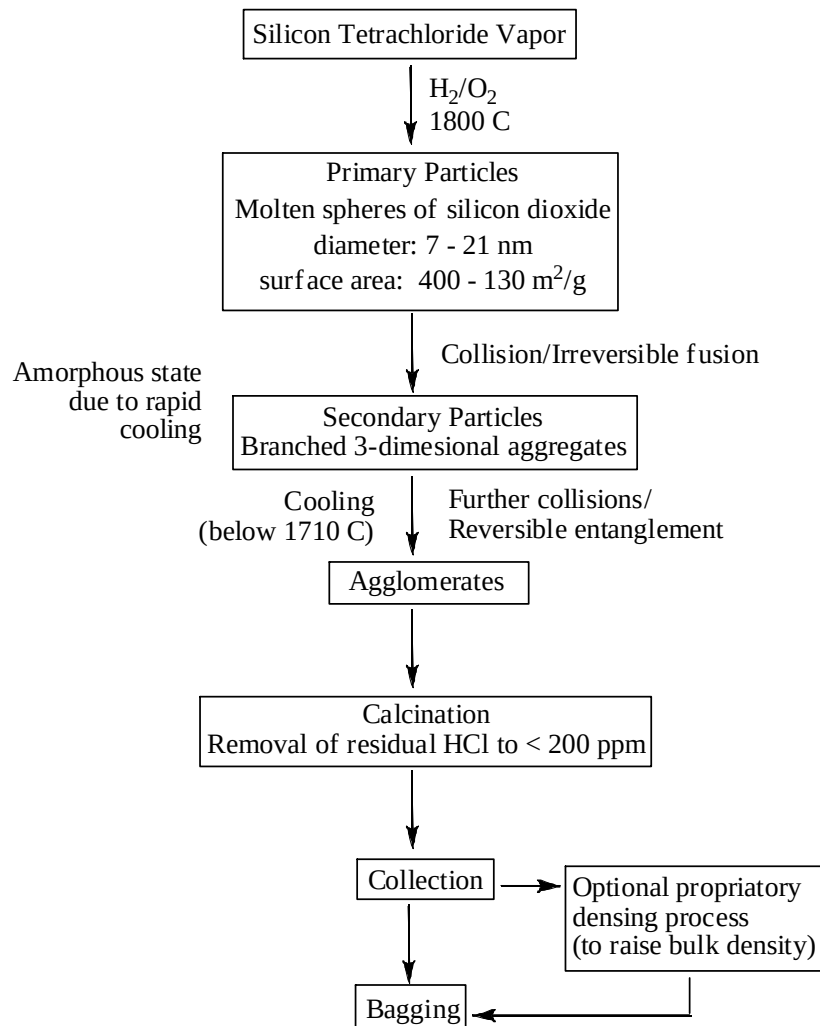


Figure 2. Process for the manufacture of pyrogenic silica (Villota and Hawkes 1986).

Table 1. Physical properties of fumed hydrophobic silica, precipitated hydrophobic silica, silica gel, and silica sol.

Property	Fumed hydrophobic silica	Precipitated hydrophobic silica	Gel ^a	Sol	Reference
Appearance	Fluffy powder	Fluffy powder			Lewinson et al. 1994
	White	White	White	White, milky	Ferch 1976
Molecular weight	60.1				NIOSH 2005
Particle size	1-350 µm				UNEP 2004
pH ^b	3.6-4.5	5-9	3-8	3-5; 8-11	Ferch 1976
	4-9				UNEP 2004
	~3.7 (4% aqueous slurry)				UNEP 2004
Boiling point	4046 °F (2230 °C)				NIOSH 2005
Melting point	3110 °F (1710 °C)				NIOSH 2005
	~1700 °C				UNEP 2004; OSHA 2004
BET surface area (m ² /g)	110-250	100			Lewinson et al. 1994
	50-400	30-500	250-1,000 ^c	50-400	Ferch 1976
Tapped (bulk) density (g/l)	30-250	30-500	500-1,000	n/a ^d	Ferch 1976
	50-320 g/l				UNEP 2004
	40-60 g/l				American International Chemical, Inc. (no date)
Density	~2.2 at 20 °C				UNEP 2004
Specific gravity (g/cm ³)	2.2	1.9-2.2	1.8-2.2	1.0-1.4	Ferch 1976
	2.65				OSHA 2004
Solubility	Insoluble				NIOSH 2005
Water solubility (saturation)	~15-68 mg/l at 20 °C (pH 5.5-6.6)				UNEP 2004
	Insoluble				FNB 1996
Saturation	2.0 mmol/l(120 mg/l)				ECETOC 2006
Moisture (%)	<0.5	3			Lewinson et al. 1994
Stability in water	Stable; ion exchange processes possible				UNEP 2004
Loss on Ignition	1.0% max (2 hr @ 1,000 °C)				UNEP 2004
Loss on drying (% by weight)	< 2.5	5-7	2-6	50-85	Ferch 1976
Loss on heating	1.0% max				American International Chemical, Inc. (no date)
Vapor pressure	~0 mm HG				NIOSH 2005; OSHA 2004
	None				UNEP 2004
Ignition loss (%)	<2	7			Lewinson et al. 1994
	<2	3-14	2-15	50-90	Ferch 1976
Temperature at ignition (°C)	400	400			Lewinson et al. 1994
Decomposition temperature of methyl groups (°C)	300	300			Lewinson et al. 1994
Purity of SiO ₂ (%)	>99.8	>99.5			Lewinson et al. 1994
	> 99.8	> 95	> 95 ^e	15-50	Ferch 1976
Carbon, bound	1	2			Lewinson et al. 1994
Al ₂ O ₃ (%)	<0.05	0.1			Lewinson et al. 1994

Table 1. Physical properties of fumed hydrophobic silica, precipitated hydrophobic silica, silica gel, and silica sol.(*continued*)

Property	Fumed hydrophobic silica	Precipitated hydrophobic silica	Gel ^a	Sol	Reference
Fe ₂ O ₃ (%)	<0.01	0.03			Lewinson et al. 1994
TiO ₂ (%)	<0.03	0.03			Lewinson et al. 1994
HCl (%)	<0.025	<0.025			Lewinson et al. 1994
Dimethyldichlorosilane (%)	<0.1	<0.1			Lewinson et al. 1994
Primary particle size (μm)	0.005-0.05 ^f	0.005-0.1 ^f	0.001-0.01	0.005-0.02	Ferch 1976
Aggregate size (μm)	0.1-1	0.1-1	1-20	n/a	Ferch 1976
Agglomerate size (μm)	1-250	1-250	n/a	n/a	Ferch 1976
Mean pore size (μm)	None	> 0.03	0.0001-1	n/a	Ferch 1976
Pore size distribution	None	Very wide	Narrow	Wide	Ferch 1976
Structure, DBP ^g absorption (ml/100 g)	250-350	80-320	80-350	n/a	Ferch 1976

^a After drying according to DIN 66131 or direct by titration with NaOH solution (Sears 1956).

^b Hydrophilic grades.

^c Porous surface.

^d Not applicable.

^e Dry product, no hydrogel.

^f Primary particles do not normally exist as individual units.

^g Dibutyl phthalate.

Table 2. Physical properties of silica with B.E.T. surface areas of 200, 325, or 380 m²/g (Cabot Corporation 2006a,b,c).

	Value
pH (4% aqueous slurry)	3.7-4.3
325 Mesh residue (44 microns)	0.02% max
Tamped density	50 g/l
Loss on heating	< 1.5%
Specific Gravity	2.2 g/cm ³
Wt./gallon	18.3 lbs
Refractive index	1.46
X-ray form	Amorphous
Assay (% SiO ₂)	>99.8
Oil adsorption	~350 g/100 g oil
Average particle (aggregate) length	0.2-0.3 microns

B.E.T. - a rule for the physical adsorption of gas molecules on a solid surface that serves as the basis for the measurement of the specific surface area of a material

Table 3. Cosmetic product uses and concentrations for silica, hydrated silica, alumina magnesium metasilicate, aluminum calcium sodium silicate, and sodium potassium aluminum silicate.

Product Category (Total products in category - FDA 2008)	2008 uses (FDA 2009)	2008 concentrations (%) (Council 2008)
<i>Silica^a</i>		
Baby products		
Shampoos (55)	-	0.003
Lotions, oils, powders, etc. (132)	1	-
Other (138)	1	10 ^b
Bath products		
Soaps and detergents (1329)	22	0.02-10
Oils, tablets, and salts (257)	18	0.9-2
Capsules (4)	2	-
Other (239)	3	0.02
Eye products		
Eyebrow pencil (147)	42	0.01-6
Eyeliner (684)	89	0.3-19
Shadow (1196)	472	0.001-44
Lotion (177)	46	0.02-4
Makeup remover (131)	4	0.0004
Mascara (463)	135	0.2-10
Other (288)	79	0.04-3
Fragrance products		
Colognes and toilet waters (1288)	-	0.1
Perfumes (569)	6	1
Powders (278)	49	1-10
Sachets (28)	-	-
Other (399)	8	6-18 ^c
Noncoloring hair care products		
Conditioners (1249)	12	0.002
Sprays/aerosol fixatives (371)	-	0.0005
Straighteners (144)	2	3 ^d
Permanent waves (141)	1	-
Rinses (47)	-	0.003
Shampoos (1403)	7	0.02
Tonics, dressings, etc. (1097)	13	0.02-3
Other (716)	16	-
Hair coloring products		
Dyes and colors (2481)	80	0.002-0.3
Tints (58)	1	2
Color sprays/aerosol (8)	5	0.4
Hair lighteners with color (22)	3	-
Bleaches (152)	46	6 ^e
Other (166)	14	1
Makeup		
Blushers (539)	122	2-20
Face powders (613)	198	1-26
Foundations (635)	268	0.01-40
Leg and body paints (29)	2	-
Lipstick (1912)	536	0.01-21
Makeup bases (164)	40	0.5-20
Rouges (99)	35	0.09-3

Table 3. Cosmetic product uses and concentrations for silica, hydrated silica, alumina magnesium metasilicate, aluminum calcium sodium silicate, and sodium potassium aluminum silicate. (*Continued*)

Product Category (Total products in category - FDA 2008)	2008 uses (FDA 2009)	2008 concentrations (%) (Council 2008)
<i>Silica (continued)</i>		
Fixatives (38)	16	0.3-3
Other (406)	107	2-4 ^f
Nail care products		
Basecoats and undercoats (62)	5	5
Creams and lotions (17)	1	2-3
Polish and enamel (419)	70	0.3-9
Other (124)	16	5
Oral hygiene products		
Dentifrices (59)	12	3-16
Other (48)	3	-
Personal hygiene products		
Underarm deodorants (540)	38	0.02-9
Feminine deodorants (21)	1	
Other (514)	27	0.0000003-0.06 ^g
Shaving products		
Aftershave lotions (395)	6	0.2-0.9
Men's talcum (7)	1	-
Preshave lotions (27)	-	5
Shaving cream (162)	1	-
Other (107)	9	0.003
Skin care products		
Cleansing creams, lotions, liquids, and pads (1368)	47	0.002-5
Depilatories (62)	3	1
Face and neck creams, lotions, etc. (1195)	125	0.03-10
Body and hand creams, lotions, etc. (1513)	50	0.02-5 ^h
Foot powders and sprays (48)	7	0.8
Moisturizers (2039)	163	0.008-8
Night creams, lotions, powder and sprays (343)	32	0.01-3
Paste masks/mud packs (418)	22	0.02-6
Fresheners (285)	5	0.00004-3
Other (1244)	97	0.04-11 ⁱ
Suntan products		
Suntan gels, creams, liquids and sprays (156)	8	0.03-2
Indoor tanning preparations (200)	19	-
Other (62)	7	0.6
Total uses/ranges for Silica	3276	0.0000003-44
<i>Hydrated Silica^j</i>		
Bath products		
Soaps and detergents (1329)	13	0.05-4
Oils, tablets, and salts (257)	7	0.4-2
Other (239)	1	4
Eye products		
Eyeliners (684)	1	-
Shadow (1196)	2	-
Lotion (177)	1	0.06-1
Mascara (463)	1	-
Other (288)	3	2

Table 3. Cosmetic product uses and concentrations for silica, hydrated silica, alumina magnesium metasilcate, aluminum calcium sodium silicate, and sodium potassium aluminum silicate. (*Continued*)

Product Category (Total products in category - FDA 2008)	2008 uses (FDA 2009)	2008 concentrations (%) (Council 2008)
<i>Hydrated Silica (continued)</i>		
Fragrance products		
Powders (278)	10	2
Noncoloring hair care products		
Conditioners (1249)	-	0.04
Shampoos (1403)	-	0.05
Tonics, dressings, etc. (1097)	-	2
Hair coloring products		
Bleaches (152)	18	2 ¹
Other (166)	2	-
Makeup		
Blushers (539)	-	-
Face powders (613)	23	4
Foundations (635)	4	3
Makeup bases (164)	1	-
Lipstick (1912)	-	0.003
Other (406)	3	-
Nail care products		
Basecoats and undercoats (62)	6	-
Cuticle softeners (18)	1	-
Creams and lotions (17)	1	-
Polish and enamel (419)	2	1-2
Other (124)	3	-
Oral hygiene products		
Dentifrices (59)	23	7-34
Mouthwashes and breath fresheners (85)	-	-
Other (48)	2	0.2
Personal hygiene products		
Underarm deodorants (540)	-	2
Douches (12)	-	0.03
Feminine deodorants (21)	1	-
Other (514)	3	6
Skin care products		
Cleansing creams, lotions, liquids, and pads (1368)	5	3-17
Depilatories (62)	10	-
Face and neck creams, lotions, etc. (1195)	6	0.09
Body and hand creams, lotions, etc. (1513)	4	0.06-2
Foot powders and sprays (48)	1	-
Moisturizers (2039)	3	1-2
Night creams, lotions, powder and sprays (343)	1	0.04
Paste masks/mud packs (418)	2	0.01-10
Fresheners (285)	4	-
Other (1244)	6	0.001-0.004
Suntan products		
Suntan gels, creams, liquids and sprays (156)	1	0.2-2
Other (62)	1	-
Total uses/ranges for Hydrated Silica	176	0.001-34

Table 3. Cosmetic product uses and concentrations for silica, hydrated silica, alumina magnesium metasilicate, aluminum calcium sodium silicate, and sodium potassium aluminum silicate. (*Continued*)

Product Category (Total products in category - FDA 2008)	2008 uses (FDA 2009)	2008 concentrations (%) (Council 2008)
<i>Alumina Magnesium Metasilicate</i>		
Skin care products		
Face and neck creams, lotions, etc. (1195)	-	0.01
Body and hand creams, lotions, etc. (1513)	-	0.002
Total uses/ranges for Magnesium Aluminum Metasilicate	-	0.002-0.01
<i>Aluminum Calcium Sodium Silicate</i>		
Eye products		
Mascara (463)	-	0.5
Makeup		
Blushers (539)	1	-
Face powders (613)	1	-
Foundations (635)	-	0.4-6
Lipstick (1912)	-	6
Nail care products		
Polish and enamel (419)	4	0.5
Skin care products		
Moisturizers (2039)	1	-
Total uses/ranges for Aluminum Calcium Sodium Silicate	7	0.4-6
<i>Sodium Potassium Aluminum Silicate</i>		
Nail care products		
Basecoats and undercoats (62)	-	4
Polish and enamel (419)	-	0.001
Skin care products		
Paste masks/mud packs (418)	1	-
Total uses/ranges for Sodium Potassium Aluminum Silicate	1	0.001-4

^a Silica; silica, amorphous; silica, fumed; and silicon dioxide, colloidal were listed by the FDA. These data were combined.

^b 10% in a diaper liner.

^c 10% and 18% in a solid perfume

^d 1.5% after dilution.

^e 3% after dilution.

^f 2% in a concealer.

^g 0.006% in a shower gel.

^h 2% in body and hand sprays.

ⁱ 0.6% in a lip moisture cream/ 10% in a foot exfoliant.

^j 1% after dilution.

^k Hydrated silica and silicic acid were listed by the FDA. These data were combined.

^l 6% in a body scrub.

Table 4. Retention of silica (25 mg/rat) in rat tissues after intratracheal injection (Byers and Gage 1961).

Sample (particle size)	Time after injection (weeks)	Lungs (µg/rat)	Liver (µg/rat)	Kidneys (µg/rat)	Spleen (µg/rat)
A1(19 µm)	12	1570	177	33	0
	24	755	20	12.4	1.4
	52	210	61	9.7	0
A2 (20 µm, 60 µm after storage)	12	5150	249	138	0
	24	1550	153	44	19
	52	720	153	56	0
B (25 µm)	12	656	234	34	5.8
	24	324	43	8.9	1.4
	52	108	38	12	0
Average found in normal rat tissue		28	37	11	5

Table 5. Unpublished acute oral toxicity studies of silica reported by UNEP (2004) and ECETOC (2006).

Species (n)	Test substance	Notes	LD ₅₀
Sprague-Dawley rats (20; 10 males, 10 females)	Fumed silica in aqueous solution	There were no clinical signs or pathological observations at necropsy	> 3300 mg/kg
Wistar rats (10)	Precipitated silica	None	> 5110 mg/kg
Sprague-Dawley rats (20; 10 male, 10 female)	Precipitated silica	None	> 5000 mg/kg
Male rats (strain and n unspecified)	Precipitated silica (10 to 5000 mg/kg)	Followed by a 10-day observation period. >100 mg/kg, distended stomachs with bloody patches at the pyloric end were observed at necropsy. At the highest dose, a vascular stomach and reddened intestinal lining were observed. The editors concluded that the test was questionable due to the non-lethality of silica in other studies at similar doses.	470 mg/kg
Male rats (strain and n unspecified)	Precipitated silica in saline	No clinical signs in a 10 day observation period	> 5000 mg/kg
Rats (strain not specified; 10; 5 male, 5 female)	Precipitated silica	No clinical signs	> 5000 mg/kg
Wistar rats (10)	Silica incorporated into a stock diet at a ratio of 1:4 (w/w) for 24 h	Most rats consumed the diet quantitatively. There were no clinical signs or remarkable findings at necropsy. Stool change color to grey with normal consistency but larger than normal pellets.	> 10,000 mg/kg
Male rats (strain and n unspecified)	Precipitated silica in saline	No clinical signs in a 10 day observation period	> 5000 mg/kg
Male Sprague-Dawley rats (30)	Precipitated silica in water	No clinical signs in a 14-day observation period. The stool turned white for 2 days.	> 5620 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Precipitated silica in water	No clinical signs in a 14 day observation period. The stool turned white for 2 days.	> 20,000 mg/kg
Rats (strain and n not specified)	Aqueous colloidal silica (30%)	None	10,000 mg/kg
Rats (strain and n not specified)	Silica	None	40,000 mg/kg
Boltzman rats (5 male)	Hydrophilic fumed silica in 0.5% methylcellulose	None provided.	> 3,160 mg/kg
Sprague-Dawley (10; 5 male, 5 female)	Hydrophilic fumed silica in water	None provided.	> 5,000 mg/kg
Sprague-Dawley (10; 5 male 5 female)	Hydrophilic fumed silica in deionized water	None provided.	> 5,000 mg/kg

Table 5. Unpublished acute oral toxicity studies of Silica reported by UNEP (2004) and ECETOC (2006). *(continued)*

Species (n)	Test substance	Notes	LD ₅₀
Swiss Mice (10 males)	Hydrophilic fumed silica in corn oil	None provided.	> 3,160 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in 1% aqueous gum arabic solution	None provided.	>31,800 mg/kg
Sprague-Dawley rats (20; 10 male, 10 female)	Hydrophilic precipitated silica in Aqueous carboxymethylcellulose	None provided.	5,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in olive oil	None provided.	6,350 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in 10% aqueous arabic gum	None provided.	> 5,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in water	None provided.	> 20,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in water	None provided.	> 10,000 mg/kg
Sprague-Dawley (10; 5 male, 5 female)	Hydrophilic precipitated silica in water	None provided.	> 20,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophilic precipitated silica in water	None provided.	> 20,000 mg/kg
Male Sprague-Dawley rats (n not provided)	Hydrophilic silica gel in saline	None provided.	> 5,000 mg/kg
Male Sprague-Dawley rats (30)	Hydrophilic silica gel in distilled water	None provided.	> 5,620 mg/kg
Sprague-Dawley Rats (10; 5 male, 5 female)	Hydrophilic silica gel in water	24 h observation.	> 31,600 mg/kg
Male rats (strain and n not provided)	Hydrophilic silica sol in aqueous solution (colloidal)	None provided.	> 10,000 mg/kg
Male Sprague-Dawley rats (10)	Hydrophilic silica sol in aqueous solution (colloidal)	None provided.	> 40,000 mg/kg
Sprague-Dawley rats (20; 10 male, 10 female)	Hydrophobic fumed silica in peanut oil	None provided.	> 5,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic fumed silica in corn oil	None provided.	> 5,000 mg/kg
Male Sprague-Dawley (10)	Hydrophobic fumed silica in corn oil	None provided.	> 3,160 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic fumed silica in distilled water	None provided.	Males - 9,200 mg/kg Females - > 10,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic fumed silica in corn oil	None provided.	> 5,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic fumed silica in corn oil	None provided.	> 5,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic fumed silica in corn oil	None provided.	> 5,000 mg/kg
Wistar rats (10; 5 males, 5 females)	Hydrophobic fumed silica in polyethylene glycol 400	None provided.	> 2,000 mg/kg
Sprague-Dawley rats (10; 5 male, 5 female)	Hydrophobic precipitated silica in olive oil	None provided.	> 7,900 mg/kg

Table 6. Acute inhalation toxicity studies of hydrophilic and hydrophobic silica (ECETOC 2006).

Species (n)	Test substance (surface area [m ² /g])	Notes	LC ₅₀ (mg/m ³)
<u>Hydrophilic</u>			
Wistar rats (10; 5 male, 5 female)	Pyrogenic silica (200)	Exposure 4h, nose only. Particle size not provided, 56% of particles < 5 µm. No clinical signs observed and no organ abnormalities at necropsy.	> 139
Male albino rats (strain not specified; 10)	Pyrogenic silica (380)	Exposure for 1 h, nose only. No data on particle size. Vigorous cleansing, hypoactivity, abdominal respiration, gasping nasal exudation, closed eyes. Crust-like material around nose and mouth, fur chalky to touch for 2 days post exposure. Resolved quickly.	>207,000
Albino rats (strain, sex, and n not provided)	Pyrogenic silica (200)	Exposure for 1 h, nose only. No data on particle size.	> 191,300
Sprague-Dawley rats (10; 5 male, 5 female)	Pyrogenic silica (200)	Exposure for 4 h, nose only. Particle size 0.76 5 µm	> 2,080
Wistar rats (10; 5 male, 5 female)	Precipitated silica (190)	Exposure for 4 h, whole body. Particle size not provided, 45% of particles < 5 µm. Some decreased body weight in the females 2 days after exposure which resolved.	> 691
Male Sprague-Dawley rats (10)	Silica gel (not specified)	Exposure for 1 h, nose only. Particle size not provided. 1/10 rats died 2 h after exposure.	> 2,200
Male rats (strain not specified; 2)	Silica sol "solid" (not specified)	Exposure for 4 h, nose only. Particle size not provided.	> 3,100
Male rats (strain not specified; 2)	Silica sol (not specified)	Exposure for 2.5 and 6 h, mist, nose only, at 560 and 520 mg/m ³ . Particle size not provided.	No deaths
Male albino rats (not provided)	Silica sol (not specified)	Exposure for 3.25 h, mist, whole body at 760 mg/m ³ . Particle size not provided.	No deaths
Male albino rats (not provided)	Silica sol (not specified)	Exposure for 4.2 h, mist, whole body at 2,240 and 2500 mg/m ³ . Particle size not provided.	No deaths
Male albino rats (not provided)	Silica sol (not specified)	Exposure for 1.5 h, mist, whole body, at 3,300 mg/m ³ . Particle size not provided.	No deaths
<u>Hydrophobic</u>			
Wistar rats (10; 5 male, 5 female)	Fumed silica (80)	Exposure for 4 h, whole body. Particle size 1.4 - 1.8 µm.	2,863 - 3,730
Rats, strain not specified (10; 5 male, 5 females)	Fumed silica (130)	Exposure for 1 h, whole body. Particle size 0.15 µm.	> 2,280
Wistar rats (10; 5 male, 5 female)	Fumed silica (200)	Exposure for 4 h, whole body with 14 days recovery. Particle size 56% < 5 µm, ≥7.7 µm. Clinical signs transient body weight loss for 2 days. No abnormalities at necropsy.	> 477
SD rats (10; 5 male, 5 female)	Fumed silica (200)	Exposure for 4 h, whole body. Particle size 0.36 µm.	< 4,900
SD rats (10; 5 male, 5 female)	Fumed silica (200)	Exposure for 4 h, whole body. Particle size 0.54 µm. All died at 2,190 mg/m ³ .	< 2,190

Table 6. Acute inhalation toxicity studies of hydrophilic and hydrophobic silica (ECETOC 2006).*(continued)*

Species (n)	Test substance (surface area [m ² /g])	Notes	LC ₅₀ (mg/m ³)
SD rats (10; 5 male, 5 female)	Fumed silica (200)	Exposure for 1 h, whole body. Particle size 0.48 µm.	1,260 - 2,830
Male Wistar rats (10); male Swiss mice (10); male English short hair guinea pigs (10)	Fumed silica (not specified)	Exposure for 6 h, whole body. Particle size not specified. All species preened. Rats hunched. Rats and mice had occasional prostration. No clinical signs in guinea pigs. Consolidation observed in 2/9 guinea pigs. Necropsies unremarkable.	> 250 for all species
Male albino rats (strain not specified; 10)	Fumed silica (not specified)	Exposure for 1 h, whole body. Particle size not specified.	> 3,150
Wistar rats (10; 5 male, 5 female)	Fumed silica (130)	Exposure for 4 h, whole body. Particle size 1.175-1.275 µm with 14 days recovery. At 2,100 mg/m ³ , all rats died within 2.5 h. Clinical signs were few feces; closed eyes; wet, red staining on mouth/nose; labored breathing; respiratory distress; hunched position. Necropsy showed opaqued eyes, enlarged darkened lungs with red areas, white material in nasal turbinates, red areas in intestines. At 540 mg/m ³ , 7/10 died. Clinical signs were closed eyes, red staining of mouth/nose, fur coated with silica, labored breathing, respiratory distress, hunched back. During recover, lethargy, ptoerection, dyspnea, ptosis, few feces, eye crusting/lachrymation, unkempt appearance, anogenital wetness. Necropsy of survivors showed darkened lungs with red and white areas. At 210 mg/m ³ , all rats survived. Clinical signs were closed eyes, labored breathing. Licking inside of mouth, laying on back. During recovery, sporadic instances of few feces, anorexia, chromodacryorrhea, labored breathin, wetness of nose/mout, diarrhea. Transient decreases in body weight. After 14 days recovery, lungs darkened with white and red areas.	450
Wistar rats (10; 5 male, 5 female)	Fumed silica (300)	Exposure for 4 h, whole body. Particle size 0.95-2.15 µm. Results similar to above.	90 - 840 [sic]
SD rats (10; 5 male, 5 female)	Fumed silica (130)	Exposure for 4 h, whole body. Particle size < 0.2 µm. All rats died at 2,530 and 5,300 mg/m ³ . Severe red discoloration of the lungs of rats that died.	1,650
SD rats (10; 5 male, 5 female)	Fumed silica (130)	Exposed for 4 h, nose only. Particle size 7.2-7.7 µm. 4/10 died at 2,200 mg/m ³ . Severe discoloration of the lung in rats that died. Surviving rats had normal lungs except 1 male and 2 females with trace discoloration.	> 2,200
SD rats (10; 5 male, 5 female)	Fumed silica (300)	Exposure for 4 h, whole body. Particle size < 0.1 µm.	90
SD rats (10; 5 male, 5 female)	Fumed silica (300)	Exposure for 4, nose only. Particle size 7-7.1 µm.	500
SD rats (10; 5 male, 5 female)	Fumed silica (300)	Exposure for 4 h; whole body. Particle size < 0.4 µm.	800

Species (n)	Test substance (surface area [m ² /g])	Notes	LC ₅₀ (mg/m ³)
SD rats (10; 5 male, 5 female)	Fumed silica (300)	Exposure for 4 h, nose only. Particle size 6.3-7.7 µm. Author concluded that number of particles (surface area) may be responsible for observed effects.	600
SD rats (6; 3 male, 3 female)	Fumed silica (300)	Exposure for 4 h, whole body. Particle size 83% 1-5 µm, 17% 5-100.	660

Table 7. Short-term oral toxicity studies of silica reported by UNEP (2004) and ECETOC (2006).

Species (n)	Test substance; dose	Notes and results
Sprague-Dawley rats (5)	Precipitated silica gel; 16.5 mg/kg/d (10% w/w in feed), 5.8 g/kg/d for days 1-10 then 24.2 mg/kg/d for days 11-14 (20% in feed)	No observed adverse effects level (NOAEL) ≥24.2 mg/kg. No clinical signs observed.
Female inbred rat (not provided)	Precipitated silica; 1500 mg/kg/d in aqueous solution by gavage daily for 1 month	No clinical signs. Silica content in liver, 1.5 µg; kidney, 6.4 µg; spleen 5.3 µg; 1.8, 7.2, and 7.8 µg in controls, respectively.
Charles river rats (30; 15 male 15 female)	Hydrophilic fumed silica; 0, 1%, 3%, 5% (0, 1,000, 3,000, 5,000 mg/kg) in diet for 13 weeks	No gross signs of systemic toxicity. No effect on growth rate, feed consumption, or survival. No increase in silica content of the liver, kidney, spleen blood, or urine after 45 and 90 days. No gross or microscopic pathological changes.
Female rats (strain and not specified)	Hydrophilic precipitated silica; 0 or 1500 mg/kg by gavage for 4 weeks	No effects to body weight gain, feed consumption, and behavior.
Wistar rats (20; 10 male, 10 female)	Hydrophilic precipitated silica; 0, 0.5%, 2%, or 8% (0, 250, 1,000, 4,000 mg/kg) in diet for 13 weeks	Increased feed intake associated with decreased feed efficiency in the high-dose group; mean absolute and relative weight of the cecum increased in the high-dose group. General condition, behavior, survival, body weights, water intake, and hematological and urinary parameters were not adversely affected. No gross or microscopic pathological abnormalities observed. NOEL = 4,000 mg/kg.
SD rats (10; 5 male, 5 female)	Hydrophilic silica gel; 0 or 10% (0, 16,500 mg/kg) for 2 weeks or 5% (5,800 mg/kg) for days 1-10 then 20% (24,200 mg/kg) for days 11 -14	No gross signs of systemic toxicity. No effect on growth rate, feed consumption, or survival. No gross or microscopic pathological changes.
Male rats (strain not specified; 6)	Hydrophilic silica sol; 7,500 mg/kg in diet, 5 d/week for 2 weeks	All animals lost weight during treatment but gained over the weekend and during recovery period. No effects on organs.
CD rats (n not specified; male and female)	Hydrophilic silica sol; 0 or 800 mg/kg in diet for 4 weeks	No treatment effects in clinical symptoms, urine and blood parameters, necropsy or histopathology.
Beagle dogs (17; 9 male, 8 female)	Hydrophilic silica sol; 0 or 800 mg/kg in diet for 4 weeks	No treatment effects in clinical symptoms, urine and blood parameters, necropsy or histopathology.

Table 7. Short-term oral toxicity studies of silica reported by UNEP (2004) and ECETOC (2006).

Species (n)	Test substance; dose	Notes and results
Wistar rats (10; 5 male, 5 female)	Hydrophobic fumed silica; 0, 500, or 1,000 mg/kg in diet for 5 week or 2,000 mg/kg increased stepwise every 2 weeks to 16,000 mg/kg (25% of daily feed intake) for 8 weeks.	At 2,000 mg/kg, pronounced reduction of body weight associated with reduced feed intake. No changes in biological parameters or macroscopic findings. As the rats reached the highest dose the last 2 weeks, clinical signs were observed: shyness, dirty fur, reduced activity, cachexia, hemorrhages of mucous membranes of the eyes and nose. 2 males and 2 females died in week 8. Microscopic evaluation of the liver showed severe atrophy in the epithelium. NOEL assumed to be 1,000 mg/kg.
Charles River rats (20; 10 male, 10 female)	Fumed silane-treated silica; 1, 2%, 2%, 4% (0, 1,000, 2,000, 4,000 mg/kg) in diet for 13 weeks	No effect on appearance, behavior, growth, survival, clinical studies, or gross pathology. No cytopathological changes. Minimal change in thyroid gland morphology (smaller follicles lined by slightly taller epithelial cells) in males in mid- and high-dose groups.

Table 8. Unpublished short-term inhalation toxicity studies of silica (ECETOC 2006).

Species (n)	Test substance; dose	Notes and results
Male Wistar rats (10)	Hydrophilic fumed silica (0, 1.39, 5.41, 25.3 mg/m ³) for 6 h/d for 5 days. Recovery for 1 or 3 months.	There were no effects in the low-dose group. Mid- and high-dose groups had incidences of pulmonary inflammation after exposure. Effects diminished or disappeared during recovery.
Wistar rats (20; 10 male, 10 female)	Hydrophilic fumed silica (0, 17, 44, 164 mg/m ³) for 6 h/d, 5 d/week for 2 weeks.	Respiratory distress observed in all treatment groups. Reduced body weight gain and feed consumption in the mid- and high-dose groups. Hematological parameters were unremarkable. Increased absolute and relative lung weight in a dose-dependent manner. Lungs of several rats in each test group discolored, spotted, spongy, or irregular on the surface. Lungs increased septal cellularity, alveolar interstitial pneumonia, and early granulomata. Mediastinal lymph nodes of several rats were enlarged. Early granulomata observed in mediastinal lymph nodes in mid- and high-dose groups.
Wistar rats (20; 10 male, 10 female)	Hydrophilic precipitated silica (0, 1.16, 5.39, or 25. mg/m ³) for 6 h/d, 5 d/week. Recovery for 1 and 3 months.	No deaths. No adverse effects in low-dose group. There was a slight decrease in breathing. Incidence of pulmonary inflammation increased in mid- and high-dose groups. Effects lessened or resolved during recovery. NOEL = 1 mg/m ³ .
Wistar rats (20; 10 male, 10 female)	Hydrophilic precipitated silica (0, 46, 180, 668 mg/m ³) 6 h/d, 5 d/week for 2 weeks.	Signs of respiratory distress in all treatment groups. Males in mid- and high-dose groups had reduced body weight and feed consumption. Dose dependent increase in absolute and relative lung weights. In high-dose groups, several rats had spotted and swollen lungs with irregular surface. Males in mid- and high-dose groups had increased septal cellularity, alveolar macrophages and particulates. Some of these changes were present in some mediastinal lymph nodes. Early granulomata in lungs in the high-dose group and mediastinal lymph nodes in the mid- and high-dose groups. NOEL < 46 mg/m ³ .
Male Wistar rats (10)	Hydrophilic silica gel (0, 0.94, 5.13, 25.1 mg/m ³) 6 h/d for 5 days. Recovery for 1 and 3 months.	No effects in low-dose group. Pulmonary inflammation increased in the mid- and high-dose groups. Changes disappeared or lessened during recovery.
CD rat (80; 40 male, 40 female)	Hydrophobic fumed silica (0 or 60 mg/m ³ on day 1 then 30 mg/m ³ thereafter) 6 h/d, 5 d/week for 4 weeks. Recovery for 1, 2, 4, 6, and 12 weeks.	9 male rats died after first day due to acute pulmonary hemorrhage with bronchiolar plugs with emphysema. Active interstitial/alveolar inflammation that changed from diffuse to localized consolidation. After recovery, active inflammation was less prominent but some fibrosis and collagen apparent in the interstitium.
Wistar rats (20; 10 male, 10 female)	Hydrophobic fumed silica (0, 31, 87, or 209 mg/m ³) for 6 h/d, 5 d/week for 2 weeks.	Signs of respiratory distress in all test groups. Body weight and feed consumption reduced in mid- and high-dose groups. Hematological parameters were unremarkable. Increases in absolute and relative lung weights were dose-dependent. Lungs of several rats in all groups were pale, spotted, swollen and spongy. Lungs in all treatment groups had increased cellularity, accumulation of alveolar macrophages, alveolar edema, and early granulomata. NOEL < 31 mg/m ³ .

Table 9. Chronic inhalation studies of silica reported by UNEP (2004).

Species (n)	Test substance; dose	Notes and results
Female, inbred white rats (n = 10)	Precipitated synthetic silica; 55 mg/m ³ for 5 h/d, 5 d/week/ 3, 6, and 12 months. Post exposure 5 months.	At necropsy, some white-grey foci were observed subpleurally. Desquamation of alveolar cells with fine granula after 4 months. After 12 months, peribronchial and intra-alveolar small dust cell foci with few reticulin fibres were found. Small increased cell numbers and fibers were observed. The mediastinal lymph nodes were enlarged and contained dust cells with fine granules. Neither a diffuse nor nodular fibrosis was observed in lungs or lymph nodes. At recovery, effects regressed. Lung weights were normal with a few foci left. There was no significant desquamation. Lymph nodes were slightly enlarged with some dust cells. One day retention value of silica was 0.138 mg/lung. Average silica content was 1.022 mg/lung after 4 months and 3.443 mg after 12 months. Conclusion: The lymphatic system appears to play a minor role in the elimination of silica from the lung. Therefore, there is no evidence for a silicosis or a lymphatic-type pneumoconiosis to develop from exposure to synthetic silica.
Female Sprague-Dawley Rats (n = 150)	Fumed silica; 50 - 55 mg/m ³ , ~30 mg/m ³ respirable for 5 h/d, 5x/week then 2 - 3x/week partway through the experiment for 12 months. Post exposure up to 5 months.	Frequency of exposure was reduced due to fatal cases caused by massive substance-related purulent bronchitis, focal pneumonitis, and massive cellular reactions. After 12 months, ~1% of respirable dust was still retained in the lung. The increase in lung deposition was low from 18 weeks (1.2 mg) to 12 months (1.37 mg) of exposure. Mediastinal lymph nodes contained ~0.13 mg silica after 12 months. After 5 months post exposure, mean silica load was 0.16 mg/lung and 0.047 mg/lymph node, a reduction of 88% in the lung and > 50% in the lymph nodes. Microscopically visible dust foci under pulmonary pleura, mediastinal lymph nodes were moderately enlarged. Interior of alveoli: numerous macrophages accumulated, partially destroyed, associated with deposition of cell debris. Perivascular and peribronchiolar small dust foci of macrophages, associated with mild and moderate formation of connective tissue. Increased collagen formation in alveolar septa. Foci and clusters of phagocytes (partially normal, partially showing decay) and some collagenic fibrosis was observed in the mediastinal lymph nodes. Conclusion: In some cases, silica, at sites of highly concentrated deposits, caused a marked collagenic fibrosis, but without signs of typical silicosis.
Female albino rats (not provided)	Fumed silica; 0.112 mg/l; 5 h/d, 5 d/week for 1 year followed by 4 month recovery.	At 4 months, 1.578 mg were in the lungs and 0.151 in the lymph nodes; at 12 months, 1.820 and 0.430 mg, respectively. At necropsy, white foci in the plasma were observed, the mediastinal lymph node was enlarged. Histological examination revealed desquamative catarrh, sporadic dust modules, and foci with minimal to moderate fibrosis, increased collagen, and sporadic diffuse fibrosis of the alveolar septae and perifocal emphysema. Silicatic nodules were not observed. Lymph nodes: increase of dusted cells and slight to moderate fibrosis, sporadic collagen fibrosis. After recovery, silica content of lungs, 0.92 mg and lymph nodes, 0.814 mg. Necropsy revealed subpleural dust foci and enlarged lymph nodes. Lung weight increased. Microscopically cell desquamation gone whereas there was no improvement in other parameters.
Rabbits (not provided)	Silica; dose not provided; 4-5 h/d, 5 d/week for ~ 3 years followed by 30 to 150 days recovery	No clinical signs during inhalation. Mortality relationship to treatment not clear. Macroscopic examination: emphysema of the lungs. Microscopic evaluation: bronchial and alveolar dequamative catarrh, lymphocytes and leukocytes increased in the alveolas, edema, accumulation of macrophages in the lymph nodes and in the interstitium (perivascular, peribronchial, alveolar septae), granuloma of macrophages, dust cells, some thickening of the alveolar septas. Formation of connective tissue was minimal.

Table 10. Ocular irritation studies of hydrophilic and hydrophobic silica on rabbits (ECETOC 2006).

Type of silica	N	Description	Results
<u>Hydrophilic</u>			
Fumed	8	Instilled 100 mg. Not rinsed (5) or rinsed after 5 min (3)	No signs of irritation.
Fumed	3	Instilled 3 mg	Slight to mild erythema; resolved at 48 h.
Fumed	6	Instilled 3.5 mg	Slight conjunctival erythema or chemosis in some animals at 24, 48, and 72 h. Mean score 0.6 and 0.1, respectively [sic]. Transient opacity in 2 animals at 4 h.
Fumed	9	Instilled 100 mg; not rinsed (6), rinsed (3)	No signs of irritation in washed eyes at 24, 48, and 72 h. Mean score 0.15; very slight conjunctival erythema up to 48 h.
Precipitated	3	Instilled 40 or 100 mg	No signs of irritation at 40 mg. At 100 mg, slight redness at 24, 48, and 72 h; mean score 0.7. Resolved by day 4.
Precipitated	8	Instilled 100 mg; not rinsed (5), rinsed (3)	No signs of irritation.
Precipitated	8	Instilled 0.1 ml of 50% dilution in olive oil; eyes not rinsed (5), rinsed in 5 min (3)	No signs of irritation in rinsed eyes. Very slight erythema (score 1) up to 24 h.
Precipitated	9	Instilled 100 mg; eyes not rinsed (6), rinsed at 4 s (3)	No signs of irritation.
Precipitated	6	Instilled 100 mg, 0.2 ml of 50% slurry	No signs of irritation.
Precipitated	9	Instilled 100 mg; eyes not rinsed (6), rinsed at 4 s (3)	No signs of irritation.
Precipitated	9	Instilled 100 mg; eyes not rinsed (6), rinsed at 4 s (3)	No signs of irritation.
Precipitated	9	Instilled 100 mg; eyes not rinsed (6), rinsed at 4 s (3)	No signs of irritation.
Silica gel	9	Instilled 9 mg; eyes not rinsed (3), rinsed at 2 s (3) or 4 s (3)	No signs of irritation.
<u>Hydrophobic</u>			
Fumed	8	Instilled 100 mg; eyes not rinsed (5), rinsed after 5 min (3)	No signs of irritation.
Fumed	9	Instilled 25 mg; eyes not rinsed (6), rinsed after 30 s (3)	No signs of irritation in washed eyes. 2 unwashed eyes had slight erythema for 24 h. Mean score 0.1 at 24, 48, and 72 h.
Fumed	9	Instilled 3 mg; eyes not rinsed (3), rinsed after 2 s (3) or 4 s (3)	Transient slight to moderate conjunctival erythema at 1 and 4 h. Resolved at 24 h.
Fumed	9	Installed 10 mg; eyes not rinsed (6), rinsed after 30 s (3)	No signs of irritation.
Fumed	9	Installed 10 mg; eyes not rinsed (6), rinsed after 30 s	No signs of irritation.
Fumed	9	Installed ~10-20 mg; eyes not rinsed (6), rinsed after 30 s	No sign of irritation in washed eyes; 2 unwashed eyes had slight erythema for 24 h (mean score 1 at 24, 48, and 72 h).
Fumed	9	Installed 6 mg; eyes not rinsed (3), rinsed after 2 s (3) or 4 s (3)	No signs of irritation.
Fumed	3	Installed 100 mg; eyes not rinsed (6), rinsed at 4 s (3)	No signs of irritation.

Table 11. Rabbit skin irritation studies of hydrophilic and hydrophobic silica (ECETOC 2006).

Type of silica	N	Description	Results
<u>Hydrophilic</u>			
Fumed silica	6	Occlusive patch of 500 mg in 3 ml saline to intact and abraded skin for 24 h.	No signs of irritation to intact skin. Slight erythema on 3 abraded sites
Fumed silica	6	Occlusive patch of 500 mg moistened with saline to intact and abraded skin for 24 h.	Very slight erythema on 1 intact site at 24 h; very slight to well-defined erythema on abraded sites. No sign of erythema at 72 h.
Precipitated silica	3	Occlusive patch of 500 mg to intact skin for 4 h.	No signs of irritation.
Precipitated silica	12	Occlusive patch of 500 mg in methylethyl cellulose to intact and abraded skin for 24 h.	No signs of irritation.
Precipitated silica	6	Occlusive patch of 23 mg to intact and abraded skin for 24 h.	Very slight erythema on 3 abraded sites and 5 intact sites at 24 h.
Precipitated silica	6	Occlusive patch of 190 mg to intact and abraded skin for 24 h.	Very slight erythema on 3 abraded and 4 intact sites at 24 h.
Precipitated silica	12	Occlusive patch of 500 mg in olive oil to intact and abraded skin for 24 h.	No signs of irritation
Precipitated silica	12	Occlusive patch of 500 mg to intact and abraded skin for 24 h.	No signs of irritation.
Precipitated silica	6	Occlusive patch of 500 mg to intact skin for 24 h.	No signs of irritation.
Silica gel	8	Occlusive patch of 20 mg to intact and abraded skin for 24 h.	No signs of irritation.
<u>Hydrophobic</u>			
Fumed silica	12	Occlusive patch of 500 mg in methylethyl cellulose to intact and abraded skin for 24 h.	No signs of irritation.
Fumed silica	6	Occlusive patch of 500 mg moistened with PEG to intact and abraded skin for 24 h.	No signs of irritation.
Fumed silica silane treated	6	Occlusive patch of 500 mg moistened with corn oil to intact and abraded skin for 24 h.	No signs of irritation.
Fumed silica	6	Occlusive patch of 500 mg in 2 ml water to intact and abraded skin for 24 h.	No signs of irritation.
Fumed silica	6	Semi-occlusive application of 500 mg to intact skin for 4 h.	No signs of irritation.
Fumed silica	6	Semi-occlusive application of 500 mg to intact skin for 4 h.	No signs of irritation.
Fumed silica	6	Semi-occlusive application of 500 mg to intact skin for 4 h.	No signs of irritation.
Fumed silica	6	Semi-occlusive application of 500 mg to intact skin for 4 h.	No signs of irritation.
Precipitated silica	12	Occlusive patch of 500 mg in olive oil to intact and abraded skin for 24 h.	No signs of irritation.

Table 12. Unpublished in vitro mutagenicity and chromosomal aberration tests of cultured mammalian cells (UNEP 2004; ECETOC 2006).

Test	Test system	Silica type (concentration)	Results
Chromosomal aberration test, with and without metabolic activation	Chinese hamster ovary (CHO) cells	Fumed silica (19-300 µl/ml without S9, 250-1000 µl/ml with S9)	Negative
Hypoxanthine-guanine phosphoribosyl transferase test (HGPRT)	CHO cells	Fumed silica (10-250 µg/ml without S9, 100-500 µg/ml with S9).	Negative
Unscheduled DNA synthesis	Primary rat hepatocytes	Fumed silica (0.3-1000 µg/ml)	Negative; cytotoxic at 260 - 500 µg/ml
6-Thioguanine resistance	CHO cells	Hydrophilic fumed silica(10-250 µg/ml without S9, 100-500 µg/ml with S9)	No significant mutagenic activity
Chromosome aberration	CHO cells	Hydrophilic fumed silica (38-1,000 µg/ml without S9, 250-1,000 µg/ml with S9)	No clastogenic activity
Unscheduled DNA synthesis	Primary rat hepatocytes	Hydrophilic fumed silica (0.3-1,000 µg/ml with and without S9)	No genotoxic activity
Chromosome aberration	Human embryonic lung cells (Wi-38)	Hydrophilic silica gel (1-1,000 µg/ml without S9)	Clastogenic activity not significant
Clastogenic activity	CHO Cells	Hydrophobic fumed silica (63-500 µg/ml with and without S9)	No clastogenic activity
Clastogenic activity	CHO Cells	Hydrophobic fumed silica (63-500 µg/ml with and without S9)	No clastogenic activity
Clastogenic activity	CHO Cells	Hydrophobic fumed silica (63-500 µg/ml with and without S9)	No clastogenic activity
Clastogenic activity	CHO Cells	Hydrophobic fumed silica (42-333 µg/ml with and without S9)	No clastogenic activity
Chromosomal aberration test, with and without metabolic activation	CHO cells	Fumed silica (19 - 300 µl/ml without S9, 250 - 1000 µl/ml with S9)	Negative
Hypoxanthine-guanine phosphoribosyl transferase test (HGPRT)	CHO cells	Fumed silica (10 - 250 µg/ml without S9, 100 - 500 µg/ml with S9).	Negative

Table 13. Unpublished in vivo mutagenicity studies of silica gel (ECETOC 2006).

Test	Test system	Protocol (dose)	Results
Gene mutation (host mediated)	Mice + <i>S. typhimurium</i> TA1530, G-46 (indicator)	i.p. injection of <i>S. typhimurium</i> cells collected 3 h after last administration (1 or 5 x 1.4-5,000 mg/kg)	No mutagenic activity
Mitotic recombination (host mediated)	Mice (host) + <i>S. cerevisiae</i> D3 (indicator)	i.p. injection of <i>S. cerevisiae</i> cells collected 3 h after last administration (1 or 5 x 1.4-5,000 mg/kg)	No genotoxic activity
Chromosome aberration	Male Sprague-Dawley rat bone marrow	Killed at 6, 24, and 48 h (1 x 1.4-5,000)	Negative
Chromosome aberration	Male Sprague-Dawley rat bone marrow	Killed 6 h after last administration (5 x 1.4-5,000 mg/kg)	Negative
Dominant lethal mutation	Female Sprague-Dawley rat	8 mated, killed 14 days after mating for uterus examination (1 x 1.4-5,000 mg/kg)	Negative
Dominant lethal mutation	Female Sprague-Dawley rat	8 mated, killed 14 days after mating for uterus examination (5 x 1.4-5,000 mg/kg)	Negative

Table 14. Unpublished in vitro mutagenicity studies of silica reported by UNEP (2004) and ECETOC (2006).

Test	Test systems	Silica type (concentration)	Results
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Fumed silica (667 - 10,000 µg/plate)	Negative
Unscheduled DNA synthesis	Primary rat hepatocytes	Fumed silica (0.3 - 1000 µg/ml)	Negative; cytotoxic at 260 - 500 µg/ml
Ames, with metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophilic fumed silica (≤ 10,000 µg/plate)	Negative; not cytotoxic
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophilic fumed silica (≤ 5,000 µg/plate)	Negative; not cytotoxic
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophilic silica gel (≤ 10,000 µg/plate)	Negative; not cytotoxic
Tryptophan reversion, with and without metabolic activation	<i>Escherichia coli</i> WP2	Hydrophilic silica gel (≤ 10,000 µg/plate)	Negative; not cytotoxic
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA1530, G-46)	Hydrophilic silica gel (not provided)	Negative
Forward mutation, without metabolic activation	<i>Saccharomyces cerevisiae</i> (D3)	Hydrophilic silica gel (not provided)	Negative
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1537, TA98)	Hydrophobic fumed silica (1,580 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Tryptophan reversion, with and without metabolic activation	<i>E. coli</i> (WP2)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Ames, with and without metabolic activation	<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537)	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic
Tryptophan reversion, with and without metabolic activation	<i>E. coli</i> (WP2) August 17, 2009	Hydrophobic fumed silica (5,000 µg/plate)	Negative, not cytotoxic

2018 FDA Raw Data

03B - Eyeliner	ALUMINUM SILICATE	1
05E - Rinses (non-coloring)	ALUMINUM SILICATE	1
05F - Shampoos (non-coloring)	ALUMINUM SILICATE	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	ALUMINUM SILICATE	2
06E - Hair Color Sprays (aerosol)	ALUMINUM SILICATE	6
07A - Blushers (all types)	ALUMINUM SILICATE	1
10A - Bath Soaps and Detergents	ALUMINUM SILICATE	2
10E - Other Personal Cleanliness Products	ALUMINUM SILICATE	2
12A - Cleansing	ALUMINUM SILICATE	9
12C - Face and Neck (exc shave)	ALUMINUM SILICATE	22
12D - Body and Hand (exc shave)	ALUMINUM SILICATE	1
12F - Moisturizing	ALUMINUM SILICATE	9
12H - Paste Masks (mud packs)	ALUMINUM SILICATE	7
12I - Skin Fresheners	ALUMINUM SILICATE	1
12J - Other Skin Care Preps	ALUMINUM SILICATE	3
03B - Eyeliner	ATTAPULGITE	1
12H - Paste Masks (mud packs)	ATTAPULGITE	3
01B - Baby Lotions, Oils, Powders, and Creams	BENTONITE	1
02A - Bath Oils, Tablets, and Salts	BENTONITE	1
02D - Other Bath Preparations	BENTONITE	1
03A - Eyebrow Pencil	BENTONITE	1
03B - Eyeliner	BENTONITE	8
03C - Eye Shadow	BENTONITE	3
03D - Eye Lotion	BENTONITE	2
03F - Mascara	BENTONITE	19
03G - Other Eye Makeup Preparations	BENTONITE	5
04C - Powders (dusting and talcum, excluding aftershave talc)	BENTONITE	1
05A - Hair Conditioner	BENTONITE	2
05B - Hair Spray (aerosol fixatives)	BENTONITE	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	BENTONITE	11
05I - Other Hair Preparations	BENTONITE	2
07B - Face Powders	BENTONITE	2
07C - Foundations	BENTONITE	10
07F - Makeup Bases	BENTONITE	2
08E - Nail Polish and Enamel	BENTONITE	20
08F - Nail Polish and Enamel Removers	BENTONITE	1
09A - Dentifrices	BENTONITE	3

09C - Other Oral Hygiene Products	BENTONITE	4
10A - Bath Soaps and Detergents	BENTONITE	6
10B - Deodorants (underarm)	BENTONITE	5
10E - Other Personal Cleanliness Products	BENTONITE	2
11E - Shaving Cream	BENTONITE	1
11F - Shaving Soap	BENTONITE	2
11G - Other Shaving Preparation Products	BENTONITE	1
12A - Cleansing	BENTONITE	23
12C - Face and Neck (exc shave)	BENTONITE	23
12D - Body and Hand (exc shave)	BENTONITE	4
12F - Moisturizing	BENTONITE	20
12G - Night	BENTONITE	2
12H - Paste Masks (mud packs)	BENTONITE	135
12I - Skin Fresheners	BENTONITE	1
12J - Other Skin Care Preps	BENTONITE	12
13A - Suntan Gels, Creams, and Liquids	BENTONITE	1
13C - Other Suntan Preparations	BENTONITE	1
02A - Bath Oils, Tablets, and Salts	CALCIUM SILICATE	6
02C - Bath Capsules	CALCIUM SILICATE	1
02D - Other Bath Preparations	CALCIUM SILICATE	2
03C - Eye Shadow	CALCIUM SILICATE	7
04C - Powders (dusting and talcum, excluding aftershave talc)	CALCIUM SILICATE	44
07A - Blushers (all types)	CALCIUM SILICATE	16
07B - Face Powders	CALCIUM SILICATE	17
07C - Foundations	CALCIUM SILICATE	3
07F - Makeup Bases	CALCIUM SILICATE	1
07I - Other Makeup Preparations	CALCIUM SILICATE	1
08G - Other Manicuring Preparations	CALCIUM SILICATE	1
12A - Cleansing	CALCIUM SILICATE	1
12C - Face and Neck (exc shave)	CALCIUM SILICATE	1
03G - Other Eye Makeup Preparations	FULLER'S EARTH	1
07E - Lipstick	FULLER'S EARTH	1
10A - Bath Soaps and Detergents	FULLER'S EARTH	1
12A - Cleansing	FULLER'S EARTH	3
12C - Face and Neck (exc shave)	FULLER'S EARTH	1
12H - Paste Masks (mud packs)	FULLER'S EARTH	8
03A - Eyebrow Pencil	HECTORITE	2
03B - Eyeliner	HECTORITE	20

03F - Mascara	HECTORITE	11
03G - Other Eye Makeup Preparations	HECTORITE	1
05A - Hair Conditioner	HECTORITE	3
05E - Rinses (non-coloring)	HECTORITE	1
05F - Shampoos (non-coloring)	HECTORITE	2
05G - Tonics, Dressings, and Other Hair Grooming Aids	HECTORITE	1
05I - Other Hair Preparations	HECTORITE	1
06D - Hair Shampoos (coloring)	HECTORITE	1
07A - Blushers (all types)	HECTORITE	3
07B - Face Powders	HECTORITE	3
07C - Foundations	HECTORITE	7
07D - Leg and Body Paints	HECTORITE	1
07I - Other Makeup Preparations	HECTORITE	2
08G - Other Manicuring Preparations	HECTORITE	1
12A - Cleansing	HECTORITE	8
12C - Face and Neck (exc shave)	HECTORITE	3
12D - Body and Hand (exc shave)	HECTORITE	1
12G - Night	HECTORITE	1
12H - Paste Masks (mud packs)	HECTORITE	9
12J - Other Skin Care Preps	HECTORITE	1
01B - Baby Lotions, Oils, Powders, and Creams	KAOLIN	3
02A - Bath Oils, Tablets, and Salts	KAOLIN	9
02D - Other Bath Preparations	KAOLIN	2
03A - Eyebrow Pencil	KAOLIN	20
03B - Eyeliner	KAOLIN	26
03C - Eye Shadow	KAOLIN	356
03D - Eye Lotion	KAOLIN	6
03E - Eye Makeup Remover	KAOLIN	1
03F - Mascara	KAOLIN	62
03G - Other Eye Makeup Preparations	KAOLIN	28
04C - Powders (dusting and talcum, excluding aftershave talc)	KAOLIN	18
05A - Hair Conditioner	KAOLIN	5
05F - Shampoos (non-coloring)	KAOLIN	8
05G - Tonics, Dressings, and Other Hair Grooming Aids	KAOLIN	21
05I - Other Hair Preparations	KAOLIN	6
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	KAOLIN	1
06F - Hair Lighteners with Color	KAOLIN	4
06G - Hair Bleaches	KAOLIN	15

06H - Other Hair Coloring Preparation	KAOLIN	5
07A - Blushers (all types)	KAOLIN	66
07B - Face Powders	KAOLIN	106
07C - Foundations	KAOLIN	62
07D - Leg and Body Paints	KAOLIN	10
07E - Lipstick	KAOLIN	88
07F - Makeup Bases	KAOLIN	9
07G - Rouges	KAOLIN	8
07H - Makeup Fixatives	KAOLIN	6
07I - Other Makeup Preparations	KAOLIN	40
08A - Basecoats and Undercoats	KAOLIN	1
08E - Nail Polish and Enamel	KAOLIN	6
08G - Other Manicuring Preparations	KAOLIN	3
09A - Dentifrices	KAOLIN	1
09B - Mouthwashes and Breath Fresheners	KAOLIN	1
10A - Bath Soaps and Detergents	KAOLIN	22
10B - Deodorants (underarm)	KAOLIN	5
10E - Other Personal Cleanliness Products	KAOLIN	16
11C - Mens Talcum	KAOLIN	1
11E - Shaving Cream	KAOLIN	1
11F - Shaving Soap	KAOLIN	2
11G - Other Shaving Preparation Products	KAOLIN	2
12A - Cleansing	KAOLIN	83
12C - Face and Neck (exc shave)	KAOLIN	67
12D - Body and Hand (exc shave)	KAOLIN	16
12F - Moisturizing	KAOLIN	34
12G - Night	KAOLIN	6
12H - Paste Masks (mud packs)	KAOLIN	432
12I - Skin Fresheners	KAOLIN	4
12J - Other Skin Care Preps	KAOLIN	50
13A - Suntan Gels, Creams, and Liquids	KAOLIN	1
13B - Indoor Tanning Preparations	KAOLIN	1
02A - Bath Oils, Tablets, and Salts	KAOLINITE	1
02C - Bath Capsules	KAOLINITE	2
03C - Eye Shadow	KAOLINITE	16
05F - Shampoos (non-coloring)	KAOLINITE	1
07C - Foundations	KAOLINITE	17
10A - Bath Soaps and Detergents	KAOLINITE	5
12C - Face and Neck (exc shave)	KAOLINITE	1
12F - Moisturizing	KAOLINITE	1

12H - Paste Masks (mud packs)	KAOLINITE	4
07E - Lipstick	LITHIUM MAGNESIUM SILICATE	2
03F - Mascara	LITHIUM MAGNESIUM SODIUM SILICATE	3
03G - Other Eye Makeup Preparations	LITHIUM MAGNESIUM SODIUM SILICATE	2
05G - Tonics, Dressings, and Other Hair Grooming Aids	LITHIUM MAGNESIUM SODIUM SILICATE	3
05I - Other Hair Preparations	LITHIUM MAGNESIUM SODIUM SILICATE	9
07C - Foundations	LITHIUM MAGNESIUM SODIUM SILICATE	3
07D - Leg and Body Paints	LITHIUM MAGNESIUM SODIUM SILICATE	1
07I - Other Makeup Preparations	LITHIUM MAGNESIUM SODIUM SILICATE	1
08E - Nail Polish and Enamel	LITHIUM MAGNESIUM SODIUM SILICATE	10
12A - Cleansing	LITHIUM MAGNESIUM SODIUM SILICATE	1
12B - Depilatories	LITHIUM MAGNESIUM SODIUM SILICATE	17
12C - Face and Neck (exc shave)	LITHIUM MAGNESIUM SODIUM SILICATE	2
12F - Moisturizing	LITHIUM MAGNESIUM SODIUM SILICATE	1
12H - Paste Masks (mud packs)	LITHIUM MAGNESIUM SODIUM SILICATE	3
01B - Baby Lotions, Oils, Powders, and Creams	MAGNESIUM ALUMINUM SILICATE	8
03A - Eyebrow Pencil	MAGNESIUM ALUMINUM SILICATE	20
03B - Eyeliner	MAGNESIUM ALUMINUM SILICATE	49
03C - Eye Shadow	MAGNESIUM ALUMINUM SILICATE	83
03D - Eye Lotion	MAGNESIUM ALUMINUM SILICATE	12
03F - Mascara	MAGNESIUM ALUMINUM SILICATE	28
03G - Other Eye Makeup Preparations	MAGNESIUM ALUMINUM SILICATE	15
04C - Powders (dusting and talcum, excluding aftershave talc)	MAGNESIUM ALUMINUM SILICATE	3
05A - Hair Conditioner	MAGNESIUM ALUMINUM SILICATE	2
05B - Hair Spray (aerosol fixatives)	MAGNESIUM ALUMINUM SILICATE	1
05F - Shampoos (non-coloring)	MAGNESIUM ALUMINUM	3

	SILICATE	
05G - Tonics, Dressings, and Other Hair Grooming Aids	MAGNESIUM ALUMINUM SILICATE	8
05I - Other Hair Preparations	MAGNESIUM ALUMINUM SILICATE	1
06B - Hair Tints	MAGNESIUM ALUMINUM SILICATE	1
06H - Other Hair Coloring Preparation	MAGNESIUM ALUMINUM SILICATE	5
07A - Blushers (all types)	MAGNESIUM ALUMINUM SILICATE	34
07B - Face Powders	MAGNESIUM ALUMINUM SILICATE	27
07C - Foundations	MAGNESIUM ALUMINUM SILICATE	64
07D - Leg and Body Paints	MAGNESIUM ALUMINUM SILICATE	3
07E - Lipstick	MAGNESIUM ALUMINUM SILICATE	3
07F - Makeup Bases	MAGNESIUM ALUMINUM SILICATE	11
07G - Rouges	MAGNESIUM ALUMINUM SILICATE	1
07H - Makeup Fixatives	MAGNESIUM ALUMINUM SILICATE	1
07I - Other Makeup Preparations	MAGNESIUM ALUMINUM SILICATE	23
08E - Nail Polish and Enamel	MAGNESIUM ALUMINUM SILICATE	1
09A - Dentifrices	MAGNESIUM ALUMINUM SILICATE	2
10A - Bath Soaps and Detergents	MAGNESIUM ALUMINUM SILICATE	3
10B - Deodorants (underarm)	MAGNESIUM ALUMINUM SILICATE	8
10E - Other Personal Cleanliness Products	MAGNESIUM ALUMINUM SILICATE	26
11A - Aftershave Lotion	MAGNESIUM ALUMINUM SILICATE	6
11E - Shaving Cream	MAGNESIUM ALUMINUM SILICATE	1
11G - Other Shaving Preparation Products	MAGNESIUM ALUMINUM SILICATE	1
12A - Cleansing	MAGNESIUM ALUMINUM SILICATE	73
12C - Face and Neck (exc shave)	MAGNESIUM ALUMINUM SILICATE	67
12D - Body and Hand (exc shave)	MAGNESIUM ALUMINUM SILICATE	80
12E - Foot Powders and Sprays	MAGNESIUM ALUMINUM SILICATE	1
12F - Moisturizing	MAGNESIUM ALUMINUM SILICATE	100
12G - Night	MAGNESIUM ALUMINUM	16

	SILICATE	
12H - Paste Masks (mud packs)	MAGNESIUM ALUMINUM SILICATE	66
12I - Skin Fresheners	MAGNESIUM ALUMINUM SILICATE	2
12J - Other Skin Care Preps	MAGNESIUM ALUMINUM SILICATE	40
13A - Suntan Gels, Creams, and Liquids	MAGNESIUM ALUMINUM SILICATE	3
13B - Indoor Tanning Preparations	MAGNESIUM ALUMINUM SILICATE	15
03B - Eyeliner	MAGNESIUM SILICATE	8
03C - Eye Shadow	MAGNESIUM SILICATE	14
03G - Other Eye Makeup Preparations	MAGNESIUM SILICATE	2
07A - Blushers (all types)	MAGNESIUM SILICATE	1
07B - Face Powders	MAGNESIUM SILICATE	5
07C - Foundations	MAGNESIUM SILICATE	2
07E - Lipstick	MAGNESIUM SILICATE	14
07I - Other Makeup Preparations	MAGNESIUM SILICATE	16
08E - Nail Polish and Enamel	MAGNESIUM SILICATE	1
12C - Face and Neck (exc shave)	MAGNESIUM SILICATE	2
12D - Body and Hand (exc shave)	MAGNESIUM SILICATE	1
12F - Moisturizing	MAGNESIUM SILICATE	2
12H - Paste Masks (mud packs)	MAGNESIUM SILICATE	2
06G - Hair Bleaches	MAGNESIUM TRISILICATE	1
12B - Depilatories	MAGNESIUM TRISILICATE	16
01B - Baby Lotions, Oils, Powders, and Creams	MONTMORILLONITE	1
02A - Bath Oils, Tablets, and Salts	MONTMORILLONITE	1
03A - Eyebrow Pencil	MONTMORILLONITE	2
03B - Eyeliner	MONTMORILLONITE	3
03C - Eye Shadow	MONTMORILLONITE	18
03E - Eye Makeup Remover	MONTMORILLONITE	3
03F - Mascara	MONTMORILLONITE	10
03G - Other Eye Makeup Preparations	MONTMORILLONITE	3
04C - Powders (dusting and talcum, excluding aftershave talc)	MONTMORILLONITE	1
05F - Shampoos (non-coloring)	MONTMORILLONITE	6
05G - Tonics, Dressings, and Other Hair Grooming Aids	MONTMORILLONITE	1
05I - Other Hair Preparations	MONTMORILLONITE	2
07A - Blushers (all types)	MONTMORILLONITE	6
07B - Face Powders	MONTMORILLONITE	4

07C - Foundations	MONTMORILLONITE	11
07E - Lipstick	MONTMORILLONITE	11
07F - Makeup Bases	MONTMORILLONITE	4
07I - Other Makeup Preparations	MONTMORILLONITE	1
09A - Dentifrices	MONTMORILLONITE	1
09C - Other Oral Hygiene Products	MONTMORILLONITE	1
10A - Bath Soaps and Detergents	MONTMORILLONITE	8
10E - Other Personal Cleanliness Products	MONTMORILLONITE	1
12A - Cleansing	MONTMORILLONITE	20
12C - Face and Neck (exc shave)	MONTMORILLONITE	4
12D - Body and Hand (exc shave)	MONTMORILLONITE	2
12E - Foot Powders and Sprays	MONTMORILLONITE	1
12F - Moisturizing	MONTMORILLONITE	11
12H - Paste Masks (mud packs)	MONTMORILLONITE	35
12J - Other Skin Care Preps	MONTMORILLONITE	5
02C - Bath Capsules	SODIUM MAGNESIUM SILICATE	1
03A - Eyebrow Pencil	SODIUM MAGNESIUM SILICATE	1
03B - Eyeliner	SODIUM MAGNESIUM SILICATE	2
03C - Eye Shadow	SODIUM MAGNESIUM SILICATE	7
03F - Mascara	SODIUM MAGNESIUM SILICATE	1
03G - Other Eye Makeup Preparations	SODIUM MAGNESIUM SILICATE	2
05A - Hair Conditioner	SODIUM MAGNESIUM SILICATE	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	SODIUM MAGNESIUM SILICATE	1
07A - Blushers (all types)	SODIUM MAGNESIUM SILICATE	5
07B - Face Powders	SODIUM MAGNESIUM SILICATE	7
07C - Foundations	SODIUM MAGNESIUM SILICATE	1
07D - Leg and Body Paints	SODIUM MAGNESIUM SILICATE	1
07E - Lipstick	SODIUM MAGNESIUM SILICATE	7
07G - Rouges	SODIUM MAGNESIUM SILICATE	2
07H - Makeup Fixatives	SODIUM MAGNESIUM SILICATE	1
07I - Other Makeup Preparations	SODIUM MAGNESIUM SILICATE	4
09A - Dentifrices	SODIUM MAGNESIUM SILICATE	1

09C - Other Oral Hygiene Products	SODIUM MAGNESIUM SILICATE	1
10A - Bath Soaps and Detergents	SODIUM MAGNESIUM SILICATE	1
10E - Other Personal Cleanliness Products	SODIUM MAGNESIUM SILICATE	2
12A - Cleansing	SODIUM MAGNESIUM SILICATE	5
12B - Depilatories	SODIUM MAGNESIUM SILICATE	8
12C - Face and Neck (exc shave)	SODIUM MAGNESIUM SILICATE	5
12F - Moisturizing	SODIUM MAGNESIUM SILICATE	36
12H - Paste Masks (mud packs)	SODIUM MAGNESIUM SILICATE	35
12J - Other Skin Care Preps	SODIUM MAGNESIUM SILICATE	1
03A - Eyebrow Pencil	ZEOLITE	2
03B - Eyeliner	ZEOLITE	3
03C - Eye Shadow	ZEOLITE	20
05B - Hair Spray (aerosol fixatives)	ZEOLITE	3
05E - Rinses (non-coloring)	ZEOLITE	1
05F - Shampoos (non-coloring)	ZEOLITE	3
05I - Other Hair Preparations	ZEOLITE	1
07A - Blushers (all types)	ZEOLITE	32
07B - Face Powders	ZEOLITE	45
07C - Foundations	ZEOLITE	1
07E - Lipstick	ZEOLITE	3
07I - Other Makeup Preparations	ZEOLITE	4
10A - Bath Soaps and Detergents	ZEOLITE	2
12A - Cleansing	ZEOLITE	7
12C - Face and Neck (exc shave)	ZEOLITE	9
12D - Body and Hand (exc shave)	ZEOLITE	2
12F - Moisturizing	ZEOLITE	14
12H - Paste Masks (mud packs)	ZEOLITE	4
12I - Skin Fresheners	ZEOLITE	1
12H - Paste Masks (mud packs)	POTASSIUM SILICATE	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	SODIUM METASILICATE	1
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	SODIUM METASILICATE	88
06F - Hair Lighteners with Color	SODIUM METASILICATE	6
06G - Hair Bleaches	SODIUM METASILICATE	41

06H - Other Hair Coloring Preparation	SODIUM METASILICATE	1
03G - Other Eye Makeup Preparations	SODIUM SILICATE	4
05G - Tonics, Dressings, and Other Hair Grooming Aids	SODIUM SILICATE	1
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	SODIUM SILICATE	12
06F - Hair Lighteners with Color	SODIUM SILICATE	7
06G - Hair Bleaches	SODIUM SILICATE	33
06H - Other Hair Coloring Preparation	SODIUM SILICATE	2
09A - Dentifrices	SODIUM SILICATE	1
10A - Bath Soaps and Detergents	SODIUM SILICATE	5
10E - Other Personal Cleanliness Products	SODIUM SILICATE	2
11E - Shaving Cream	SODIUM SILICATE	1
12B - Depilatories	SODIUM SILICATE	13
12C - Face and Neck (exc shave)	SODIUM SILICATE	6
12J - Other Skin Care Preps	SODIUM SILICATE	4
03C - Eye Shadow	AMMONIUM SILVER ZINC ALUMINUM SILICATE	13
07A - Blushers (all types)	AMMONIUM SILVER ZINC ALUMINUM SILICATE	2
07B - Face Powders	AMMONIUM SILVER ZINC ALUMINUM SILICATE	1
12H - Paste Masks (mud packs)	AMMONIUM SILVER ZINC ALUMINUM SILICATE	1
05F - Shampoos (non-coloring)	ZINC ZEOLITE	1
03C - Eye Shadow	ALUMINUM CALCIUM SODIUM SILICATE	65
04C - Powders (dusting and talcum, excluding aftershave talc)	ALUMINUM CALCIUM SODIUM SILICATE	2
05I - Other Hair Preparations	ALUMINUM CALCIUM SODIUM SILICATE	1
07A - Blushers (all types)	ALUMINUM CALCIUM SODIUM SILICATE	5
07B - Face Powders	ALUMINUM CALCIUM SODIUM SILICATE	1
07E - Lipstick	ALUMINUM CALCIUM SODIUM SILICATE	125

07I - Other Makeup Preparations	ALUMINUM CALCIUM SODIUM SILICATE	3
08E - Nail Polish and Enamel	ALUMINUM CALCIUM SODIUM SILICATE	31
08G - Other Manicuring Preparations	ALUMINUM CALCIUM SODIUM SILICATE	1
12D - Body and Hand (exc shave)	ALUMINUM CALCIUM SODIUM SILICATE	3
12F - Moisturizing	ALUMINUM CALCIUM SODIUM SILICATE	13
07G - Rouges	ALUMINA MAGNESIUM METASILICATE	1
12C - Face and Neck (exc shave)	ALUMINA MAGNESIUM METASILICATE	1
12H - Paste Masks (mud packs)	ALUMINA MAGNESIUM METASILICATE	1
02A - Bath Oils, Tablets, and Salts	HYDRATED SILICA	7
02D - Other Bath Preparations	HYDRATED SILICA	1
03C - Eye Shadow	HYDRATED SILICA	7
03F - Mascara	HYDRATED SILICA	4
03G - Other Eye Makeup Preparations	HYDRATED SILICA	1
04C - Powders (dusting and talcum, excluding aftershave talc)	HYDRATED SILICA	3
05A - Hair Conditioner	HYDRATED SILICA	1
05F - Shampoos (non-coloring)	HYDRATED SILICA	2
05G - Tonics, Dressings, and Other Hair Grooming Aids	HYDRATED SILICA	1
06F - Hair Lighteners with Color	HYDRATED SILICA	1
06G - Hair Bleaches	HYDRATED SILICA	8

07A - Blushers (all types)	HYDRATED SILICA	2
07B - Face Powders	HYDRATED SILICA	28
07C - Foundations	HYDRATED SILICA	36
07E - Lipstick	HYDRATED SILICA	42
07F - Makeup Bases	HYDRATED SILICA	4
07G - Rouges	HYDRATED SILICA	1
07I - Other Makeup Preparations	HYDRATED SILICA	6
08A - Basecoats and Undercoats	HYDRATED SILICA	2
08E - Nail Polish and Enamel	HYDRATED SILICA	12
08G - Other Manicuring Preparations	HYDRATED SILICA	1
09A - Dentifrices	HYDRATED SILICA	36
09C - Other Oral Hygiene Products	HYDRATED SILICA	4
10A - Bath Soaps and Detergents	HYDRATED SILICA	34
10B - Deodorants (underarm)	HYDRATED SILICA	1
10E - Other Personal Cleanliness Products	HYDRATED SILICA	113
12A - Cleansing	HYDRATED SILICA	15
12B - Depilatories	HYDRATED SILICA	14
12C - Face and Neck (exc shave)	HYDRATED SILICA	5
12D - Body and Hand (exc shave)	HYDRATED SILICA	4
12E - Foot Powders and Sprays	HYDRATED SILICA	1
12F - Moisturizing	HYDRATED SILICA	4
12G - Night	HYDRATED SILICA	2
12H - Paste Masks (mud packs)	HYDRATED SILICA	3
12I - Skin Fresheners	HYDRATED SILICA	3
12J - Other Skin Care Preps	HYDRATED SILICA	22
13A - Suntan Gels, Creams, and Liquids	HYDRATED SILICA	3

01B - Baby Lotions, Oils, Powders, and Creams	SILICA	3
01C - Other Baby Products	SILICA	1
02A - Bath Oils, Tablets, and Salts	SILICA	44
02C - Bath Capsules	SILICA	1
02D - Other Bath Preparations	SILICA	5
03A - Eyebrow Pencil	SILICA	46
03B - Eyeliner	SILICA	217
03C - Eye Shadow	SILICA	1568
03D - Eye Lotion	SILICA	77
03E - Eye Makeup Remover	SILICA	6
03F - Mascara	SILICA	296
03G - Other Eye Makeup Preparations	SILICA	189
04A - Cologne and Toilet waters	SILICA	23
04B - Perfumes	SILICA	10
04C - Powders (dusting and talcum, excluding aftershave talc)	SILICA	65
04E - Other Fragrance Preparation	SILICA	64
05A - Hair Conditioner	SILICA	9
05B - Hair Spray (aerosol fixatives)	SILICA	3
05C - Hair Straighteners	SILICA	3
05F - Shampoos (non-coloring)	SILICA	47
05G - Tonics, Dressings, and Other Hair Grooming Aids	SILICA	31
05I - Other Hair Preparations	SILICA	16
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	SILICA	81
06B - Hair Tints	SILICA	12
06C - Hair Rinses (coloring)	SILICA	1

06E - Hair Color Sprays (aerosol)	SILICA	31
06F - Hair Lighteners with Color	SILICA	8
06G - Hair Bleaches	SILICA	25
06H - Other Hair Coloring Preparation	SILICA	29
07A - Blushers (all types)	SILICA	295
07B - Face Powders	SILICA	437
07C - Foundations	SILICA	436
07D - Leg and Body Paints	SILICA	4
07E - Lipstick	SILICA	1527
07F - Makeup Bases	SILICA	84
07G - Rouges	SILICA	58
07H - Makeup Fixatives	SILICA	9
07I - Other Makeup Preparations	SILICA	336
08A - Basecoats and Undercoats	SILICA	7
08B - Cuticle Softeners	SILICA	2
08C - Nail Creams and Lotions	SILICA	5
08D - Nail Extenders	SILICA	3
08E - Nail Polish and Enamel	SILICA	480
08F - Nail Polish and Enamel Removers	SILICA	1
08G - Other Manicuring Preparations	SILICA	27
09A - Dentifrices	SILICA	28
09C - Other Oral Hygiene Products	SILICA	4
10A - Bath Soaps and Detergents	SILICA	84
10B - Deodorants (underarm)	SILICA	31
10E - Other Personal Cleanliness Products	SILICA	75
11A - Aftershave Lotion	SILICA	21
11E - Shaving Cream	SILICA	11

11G - Other Shaving Preparation Products	SILICA	5
12A - Cleansing	SILICA	78
12B - Depilatories	SILICA	4
12C - Face and Neck (exc shave)	SILICA	278
12D - Body and Hand (exc shave)	SILICA	99
12E - Foot Powders and Sprays	SILICA	3
12F - Moisturizing	SILICA	341
12G - Night	SILICA	54
12H - Paste Masks (mud packs)	SILICA	41
12I - Skin Fresheners	SILICA	8
12J - Other Skin Care Preps	SILICA	130
13A - Suntan Gels, Creams, and Liquids	SILICA	8
13B - Indoor Tanning Preparations	SILICA	26
13C - Other Suntan Preparations	SILICA	4
07C - Foundations	SODIUM POTASSIUM ALUMINUM SILICATE	1
07G - Rouges	SODIUM POTASSIUM ALUMINUM SILICATE	3
07I - Other Makeup Preparations	SODIUM POTASSIUM ALUMINUM SILICATE	1
10E - Other Personal Cleanliness Products	SODIUM POTASSIUM ALUMINUM SILICATE	1
12F - Moisturizing	SODIUM POTASSIUM ALUMINUM SILICATE	5
12H - Paste Masks (mud packs)	SODIUM POTASSIUM ALUMINUM SILICATE	1
03C - Eye Shadow	SILICA, AMORPHOUS	1

03F - Mascara	SILICA, AMORPHOUS	1
07C - Foundations	SILICA, AMORPHOUS	1
09A - Dentifrices	SILICA, AMORPHOUS	1
12C - Face and Neck (exc shave)	SILICA, AMORPHOUS	1
05I - Other Hair Preparations	SILICA, FUMED	2
08E - Nail Polish and Enamel	SILICA, FUMED	11
12D - Body and Hand (exc shave)	SILICA, FUMED	1
01C - Other Baby Products	SILICON DIOXIDE, COLLOIDAL	3
03C - Eye Shadow	SILICON DIOXIDE, COLLOIDAL	2
07B - Face Powders	SILICON DIOXIDE, COLLOIDAL	5
07C - Foundations	SILICON DIOXIDE, COLLOIDAL	1
07E - Lipstick	SILICON DIOXIDE, COLLOIDAL	18
07H - Makeup Fixatives	SILICON DIOXIDE, COLLOIDAL	1
07I - Other Makeup Preparations	SILICON DIOXIDE, COLLOIDAL	1
08D - Nail Extenders	SILICON DIOXIDE, COLLOIDAL	1
08E - Nail Polish and Enamel	SILICON DIOXIDE, COLLOIDAL	12
09A - Dentifrices	SILICON DIOXIDE, COLLOIDAL	5
12F - Moisturizing	SILICON DIOXIDE, COLLOIDAL	1
08G - Other Manicuring Preparations	SILICIC ACID	1
09A - Dentifrices	SILICIC ACID	1
10A - Bath Soaps and Detergents	SILICIC ACID	11
12B - Depilatories	SILICIC ACID	1
12J - Other Skin Care Preps	SILICIC ACID	1



Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 21, 2018

SUBJECT: Concentration of Use by FDA Product Category: Silicates

Concentration of use by FDA Product Category – Silicates*

Attapulgit	Tromethamine Magnesium Aluminum Silicate
Bentonite	Activated Clay
Calcium Silicate	Ammonium Silver Zinc Aluminum Silicate
Fuller's Earth	Aluminum Calcium Magnesium Potassium
Hectorite	Sodium Zinc Silicates
Kaolin	Aluminum Iron Calcium Magnesium Germanium
Lithium Magnesium Silicate	Silicates
Lithium Magnesium Sodium Silicate	Aluminum Iron Calcium Magnesium Zirconium
Magnesium Aluminum Silicate	Silicates
Magnesium Silicate	Sodium Silicate
Magnesium Trisilicate	Sodium Metasilicate
Montmorillonite	Potassium Silicate
Pyrophyllite	Sodium Magnesium Aluminum Silicate
Sodium Magnesium Silicate	Zinc Silicate
Zeolite	Ammonium Silver Silicate
Zirconium Silicate	Gold Zeolite
Sodium Silver Aluminum Silicate	Zinc Zeolite

Ingredient	Product Category	Maximum Concentration of Use
Bentonite	Bath oils, tablets and salts	0.14%
Bentonite	Eye brow pencils	0.25%
Bentonite	Eyeliner	0.25%
Bentonite	Eye lotions	1.5%
Bentonite	Mascaras	2%
Bentonite	Other eye makeup preparations	0.00025%
Bentonite	Hair sprays Pump spray	0.1%
Bentonite	Other hair coloring preparations	17.3%
Bentonite	Face powders	2-2.6%
Bentonite	Makeup bases	0.5%
Bentonite	Nail polish and enamel	0.00008-0.88%
Bentonite	Bath soaps and detergents	0.35%
Bentonite	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.00002-3%
Bentonite	Face and neck products Not spray	0.35-13.7%
Bentonite	Body and hand products Not spray	0.35-14.3%
Bentonite	Paste masks and mud packs	4-29.7%
Bentonite	Other skin care preparations Rinse-off	7-15%
Calcium Silicate	Bath oils, tablets and salts	0.86-1.3%

Calcium Silicate	Eye shadows	1%
Calcium Silicate	Powders (dusting and talcum)	3%
Calcium Silicate	Shampoos (noncoloring)	1.5%
Calcium Silicate	Hair color sprays	0.005%
Calcium Silicate	Blushers	3-5%
Calcium Silicate	Face powders	0.25-5%
Calcium Silicate	Foundations	1.3%
Calcium Silicate	Lipstick	0.00013%
Calcium Silicate	Face and neck products Not spray	4.7-5%
Hectorite	Eye brow pencils	0.3%
Hectorite	Eyeliners	0.1-0.4%
Hectorite	Eye shadows	0.27%
Hectorite	Mascaras	0.5-0.8%
Hectorite	Blushers	3.2%
Hectorite	Nail polish and enamel	0.7%
Hectorite	Skin cleansing (cold cream, cleansing lotions, liquids and pads)	0.5%
Hectorite	Face and neck products Not spray	0.8%
Hectorite	Body and hand products Not spray	1.1%
Hectorite	Paste masks and mud packs	0.5%
Kaolin	Eye brow pencils	0.78-27.2%
Kaolin	Eyeliners	2-16%
Kaolin	Eye shadows	2-15%
Kaolin	Eye lotions	1-3%
Kaolin	Mascaras	7-21.5%
Kaolin	Other eye makeup preparations	0.75%
Kaolin	Hair conditioners	2.5-5%
Kaolin	Shampoos (noncoloring) Aerosol	0.058% 0.0096%
Kaolin	Tonics, dressings and other hair grooming aids	0.15-25%
Kaolin	Other hair preparations (noncoloring)	4.6%
Kaolin	Hair lighteners with color	31%
Kaolin	Hair bleaches	0.5%
Kaolin	Other hair coloring preparations	0.4%
Kaolin	Blushers	1.5-5%
Kaolin	Face powders	2-23.1%
Kaolin	Foundations	0.8-17.4%
Kaolin	Lipstick	0.97-11.9%
Kaolin	Makeup fixatives	13.3%
Kaolin	Other makeup preparations	1%
Kaolin	Basecoats and undercoats (manicuring preparations)	0.0001%

Kaolin	Nail polish and enamel	0.17%
Kaolin	Other manicuring preparations	35.7-53%
Kaolin	Bath soaps and detergents	0.05%
Kaolin	Shaving soap	1.5%
Kaolin	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0005-20.2%
Kaolin	Depilatories	0.25%
Kaolin	Face and neck products Not spray	1-25%
Kaolin	Body and hand products Not spray	3.5-23%
Kaolin	Foot powders and sprays	4%
Kaolin	Paste masks and mud packs	2-33%
Kaolin	Other skin care preparations Rinse-off	35%
Lithium Magnesium Silicate	Tonics, dressings and other hair grooming aids	0.3%
Lithium Magnesium Silicate	Face and neck products Not spray Spray	5% 0.4%
Lithium Magnesium Sodium Silicate	Eyeliners	3%
Lithium Magnesium Sodium Silicate	Eye shadows	4%
Lithium Magnesium Sodium Silicate	Mascaras	1.4%
Lithium Magnesium Sodium Silicate	Other eye makeup preparations	0.0005%
Lithium Magnesium Sodium Silicate	Tonics, dressings and other hair grooming aids	6%
Lithium Magnesium Sodium Silicate	Deodorants Not spray	0.5%
Lithium Magnesium Sodium Silicate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.4%
Lithium Magnesium Sodium Silicate	Body and hand products Not spray	3%
Magnesium Aluminum Silicate	Eye brow pencils	1.7-2%
Magnesium Aluminum Silicate	Eyeliners	0.25-0.4%
Magnesium Aluminum Silicate	Eye shadows	0.25-4.5%
Magnesium Aluminum Silicate	Eye lotions	0.5-1%
Magnesium Aluminum Silicate	Mascaras	0.15-0.5%
Magnesium Aluminum Silicate	Other eye makeup preparations	0.0025-4%
Magnesium Aluminum Silicate	Shampoos (noncoloring)	0.19%
Magnesium Aluminum Silicate	Tonics, dressings and other hair grooming aids	0.5-0.8%
Magnesium Aluminum Silicate	Blushers	0.96-2%
Magnesium Aluminum Silicate	Face powders	1%
Magnesium Aluminum Silicate	Foundations	0.15-2.8%
Magnesium Aluminum Silicate	Makeup bases	2.5%
Magnesium Aluminum Silicate	Makeup fixatives	0.75%

Magnesium Aluminum Silicate	Other makeup preparations	0.0008%
Magnesium Aluminum Silicate	Dentifrices	0.93%
Magnesium Aluminum Silicate	Deodorants Not spray	0.5-0.7%
Magnesium Aluminum Silicate	Aftershave lotions	0.5%
Magnesium Aluminum Silicate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0004-10%
Magnesium Aluminum Silicate	Face and neck products Not spray	0.4-11%
Magnesium Aluminum Silicate	Body and hand products Not spray	0.3-1.6%
Magnesium Aluminum Silicate	Moisturizing products Not spray	0.9%
Magnesium Aluminum Silicate	Night products Not spray	0.6%
Magnesium Aluminum Silicate	Paste masks and mud packs	0.0008-6%
Magnesium Aluminum Silicate	Other skin care preparations Leave-on Rinse-off	1.1% 4.5%
Magnesium Aluminum Silicate	Suntan products Not spray	0.3-0.6%
Magnesium Silicate	Eye brow pencils	12-14.1%
Magnesium Silicate	Eyeliners	4.5-20%
Magnesium Silicate	Eye shadows	3-21.6%
Magnesium Silicate	Foundations	0.001-4.5%
Magnesium Silicate	Lipstick	10%
Magnesium Silicate	Makeup fixatives	2.3%
Magnesium Silicate	Other makeup preparations	19%
Magnesium Silicate	Body and hand products Not spray	1%
Montmorillonite	Eyeliners	2.8%
Montmorillonite	Eye shadows	0.3%
Montmorillonite	Shampoos (noncoloring)	8.2%
Montmorillonite	Blushers (all types)	1%
Montmorillonite	Foundations	0.31%
Montmorillonite	Lipstick	1.7%
Montmorillonite	Bath soaps and detergents	0.5%
Montmorillonite	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	2.5%
Montmorillonite	Face and neck products Not spray	2.5%
Montmorillonite	Body and hand products Not spray	6%
Montmorillonite	Paste masks and mud packs	5%
Montmorillonite	Other skin care preparations	0.05%
Sodium Magnesium Silicate	Paste masks and mud packs	0.2%

Sodium Magnesium Silicate	Other skin care preparations	0.13%
Zeolite	Eyebrow pencils	3.6%
Zeolite	Eyeliners	0.6%
Zeolite	Eye shadows	0.6-1.1%
Zeolite	Eye lotions	0.032%
Zeolite	Hair sprays Aerosol	1%
Zeolite	Tonics, dressings and other hair grooming aids	35.7%
Zeolite	Hair bleaches	5%
Zeolite	Blushers	0.6%
Zeolite	Face powders	0.6-1.1%
Zeolite	Foundations	0.25%
Zeolite	Lipstick	3%
Zeolite	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0043%
Zeolite	Face and neck products Not spray	0.5-1%
Zeolite	Paste masks and mud packs	28-37.8%
Zeolite	Other skin care preparations	0.03-0.5%
Zeolite	Suntan products Not spray	0.25%
Ammonium Silver Zinc Aluminum Silicate	Other skin care preparations Rinse-off	0.001%
Sodium Silicate	Hair dyes and colors	17.2%
Sodium Silicate	Hair lighteners with color	15%
Sodium Silicate	Hair bleaches	35%
Sodium Silicate	Dentifrices	0.44%
Sodium Silicate	Bath soaps and detergents	1.4%
Sodium Silicate	Shaving cream	0.017-1.5%
Sodium Silicate	Depilatories	1.2%
Sodium Metasilicate	Bath oils, tablets and salts	0.1%
Sodium Metasilicate	Hair dyes and colors	5%
Sodium Metasilicate	Hair tints	15%
Sodium Metasilicate	Hair bleaches	5-13%
Sodium Metasilicate	Depilatories	1.2%
Sodium Metasilicate	Other skin care preparations Leave-on	0.001%

*Ingredients included in the title of the table but not found in the title of the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2018
Table prepared May 21, 2018

Kaolin

Health-based recommended occupational exposure limit

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Aan de Minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Kaolin*
Uw kenmerk : DG/MBO/U-932542
Ons kenmerk : U-1523/EP/pg/459-Z55
Bijlagen : 1
Datum : 18 december 2007

Geachte minister,

Graag bied ik u hierbij het advies aan over de beroepsmatige blootstelling aan kaoline. Het maakt deel uit van een uitgebreide reeks, waarin gezondheidkundige advieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Dit advies over kaoline is opgesteld door de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport en aan de minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Hoogachtend,

Prof. dr. J.A. Knottnerus

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Kaolin

Health-based recommended occupational exposure limit

Dutch Expert Committee on Occupational Standards
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2007/12OSH, The Hague, December 18, 2007

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues...” (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature & Food Quality. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.



The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.



The Health Council of the Netherlands is a member of the International Network of Agencies for Health Technology Assessment (INAHTA), an international collaboration of organisations engaged with *health technology assessment*.

This report can be downloaded from www.healthcouncil.nl.

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Contents

Samenvatting 9

Executive summary 13

1 Scope 17

1.1 Background 17

1.2 Committee and procedure 17

1.3 Data 18

2 Identity, properties and monitoring 19

2.1 Chemical identity 19

2.2 Physical and chemical properties 20

2.3 EU Classification and labelling 20

2.4 Validated analytical methods 20

3 Sources 23

4 Exposure 25

4.1 General population 25

4.2 Working population 25

5 Kinetics 29

6	Mechanism of action 31
6.1	Studies in humans 31
6.2	Studies in animals 31
6.3	In vitro studies 31
6.4	Conclusions 32
7	Effects 33
7.1	Observations in humans 33
7.2	Animal experiments 42
7.3	Summary 46
8	Existing guidelines, standards and evaluations 49
8.1	General population 49
8.2	Working population 49
9	Hazard assessment 51
9.1	Assessment of the health risk 51
9.2	Recommendation of the health-based occupational exposure limit 53
9.3	Additional consideration 53
9.4	Groups at extra risk 54
10	Recommendations for research 55
	References 57
	Annexes 63
A	Request for advice 65
B	The committee 67
C	Comments on the public review draft 71
D	Classification of pneumoconiosis 73
E	Occupational exposure to kaolin: case studies 75
F	Occupational exposure to kaolin: epidemiological studies without exposure data 77
G	Occupational exposure to kaolin: epidemiological studies with exposure data 79
H	Abbreviations 88

Samenvatting

Vraagstelling

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in de lucht op de werkplek waaraan werknemers beroepsmatig kunnen worden blootgesteld.

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan kaolinestof. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór mei 2007 zijn verschenen.

Fysische en chemische eigenschappen

Kaoline (CAS 1332-58-7) is een klei-mineraal dat voornamelijk uit kaolinit bestaat, een niet-vezelachtig aluminiumsilicaat. Kaoline bevat daarnaast kleine hoeveelheden van andere mineralen, zoals kwarts, mica en titaniumdioxide. Kaoline is een fijn verdeeld wit tot lichtgeel poeder, met een smaak van aarde. Het wordt gebruikt bij het maken van keramiek en vuurvaste materialen, als vulmiddel in plastics, papier, rubber, verven en lijmen, als coating voor hoogwaardig papier, en als middel tegen diarree.

Monitoring

Blootstelling aan kaoline vindt voornamelijk plaats door de inademing van stofdeeltjes van kaoline. De Amerikaanse instellingen 'National Institute for Occupational Safety and Health' (NIOSH) en 'Occupational Safety and Health Administration' (OSHA) hebben standaardmethoden beschreven voor de kwantitatieve bepaling van respirabel stof. Kaoline wordt aangetoond met behulp van röntgendiffractie of elektronenmicroscopie.

Grenswaarden

Nederland heeft geen grenswaarde voor beroepsmatige blootstelling aan kaolinstof. De Britse 'Health and Safety Executive' (HSE) en de 'American Conference of Governmental Industrial Hygienists' (ACGIH) adviseren een grenswaarde van 2 mg/m³ respirabel kaolinstof. De Amerikaanse instellingen NIOSH en OSHA hanteren een grenswaarde van 5 mg/m³, achtereenvolgens als tijdgewogen gemiddelde over 10 uur en 8 uur.

Daarnaast heeft de ACGIH kaoline geclassificeerd met A4: niet classificeerbaar als kankerverwekkende stof voor de mens.

Kinetiek en toxisch werkingsmechanisme

Het is niet bekend hoe kaolinedeeltjes de longen beschadigen. Wetenschappelijke beschrijvingen van de kinetiek en het toxische werkingsmechanisme van stofdeeltjes van kaoline zijn niet gevonden.

Effecten

Uitgebreide beschrijvingen van casussen en een aantal epidemiologische onderzoeken, hoofdzakelijk uit Groot-Brittannië en de Verenigde Staten, hebben aangetoond dat chronische blootstelling aan kaoline pneumoconiose (stoflong) kan veroorzaken. Pneumoconiose veroorzaakt door kaoline, wordt ook wel kaolinoze genoemd. Pneumoconiose wordt gediagnosticeerd met behulp van röntgenfoto's van de longen. De eenvoudigste vorm van pneumoconiose (ILO categorie 1) gaat nog niet gepaard met een moeizamere ademhaling. De verminderde ademhalingsfunctie wordt voornamelijk gezien bij de gecompliceerde vorm (ILO categorie A-C). De commissie beschouwt echter de eenvoudigste

vorm van pneumoconiose al als een ongewenst effect, overeenkomstig het rapport van de Europese Commissie hierover.¹

Over de hoeveelheid kaoline die gezondheidsproblemen oplevert, is weinig bekend. Wetenschappelijke publicaties met kwantitatieve gegevens over de blootstelling of een dosis-effectrelatie zijn schaars. De beschikbare gegevens bij dieren zijn niet bruikbaar voor het afleiden van een gezondheidskundige advieswaarde. Er is geen onderzoek gevonden dat geschikt is voor de beoordeling van de kankerverwekkendheid van kaoline, en de effecten op de vruchtbaarheid en de ontwikkeling van het nageslacht.

Er is één onderzoek met gegevens over de relatie tussen de cumulatieve dosis en ILO categorie 1 bij werknemers.² Volgens de onderzoekers ontwikkelt de gemiddelde niet-rokende werknemer pneumoconiose ILO categorie 1 op 60-jarige leeftijd na een 40-jarige beroepsmatige blootstelling aan 2,1 mg/m³ respirabel kaolinestof. De betreffende publicatie bevat alleen gegevens over het niveau van de blootstelling waarbij de gemiddelde werknemer pneumoconiose ontwikkelt. Hieruit kan de commissie geen LOAEL of NOAEL afleiden. In deze publicatie ontbreken gegevens over het kwartsgehalte van het kaolinestof waaraan de werknemers waren blootgesteld. De aanwezigheid van kwarts kan het onderscheid tussen kaolinose en silicose (stoflong door blootstelling aan kwarts) bemoeilijken.

Evaluatie en advies

Volgens de commissie is overtuigend aangetoond dat beroepsmatige blootstelling aan kaolinestof schadelijke effecten heeft op de longen en uiteindelijk kan leiden tot pneumoconiose (stoflong).

De commissie is van mening dat beroepsmatige blootstelling aan 2,1 mg/m³ respirabel kaolinestof ongewenst is omdat het een aanzienlijk risico inhoudt op het ontwikkelen van pneumoconiose ILO categorie 1. Uit de beschikbare wetenschappelijke literatuur kan de commissie echter geen NOAEL of LOAEL voor kaolinestof vaststellen. Daarom kan de commissie geen gezondheidskundige advieswaarde afleiden.

Dit advies bevat een aanvullende overweging van de commissie die betrekking heeft op de inademing van respirabel kaolinestof door werknemers.

Executive summary

Scope

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands recommends health-based occupational exposure limits for chemical substances in the air in the workplace. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS).

In this report, the committee discusses the consequences of occupational exposure to kaolin dust. The committee's conclusions are based on scientific papers published before May 2007.

Physical and chemical properties

Kaolin (CAS 1332-58-7) is a clay mineral that mainly consists of kaolinite, a non-fibrous aluminosilicate with small quantities of other minerals, such as quartz, mica and TiO_2 . Kaolin is a fine white to light-yellow powder with an earthy taste. It is used in the manufacture of refractory materials, and ceramics; as a filler in plastics, paper, rubber, paints, and adhesives; as a coating for high quality paper; and as a gastro-intestinal adsorbent.

Monitoring

Exposure to kaolin as respirable dust particles is measured gravimetrically. The National Institute for Occupational Safety and Health (NIOSH) and the US Occupational Safety and Health Administration (OSHA) have described standard methods for the quantitative monitoring of respirable dust. Kaolin is identified by X-ray diffraction and electron microscopy.

Limit values

In the Netherlands, there is no occupational exposure limit for respirable kaolin dust. The UK Health and Safety Executive (HSE) and the American Conference of Governmental Industrial Hygienists (ACGIH) recommend 2 mg/m³ respirable kaolin dust as the occupational exposure limit. The ACGIH threshold limit value (TLV) of 2 mg/m³ respirable kaolin dust is only valid if it contains less than 1% quartz and no asbestos. In addition, the ACGIH has notified kaolin as an A4 substance, meaning that kaolin is not classifiable as a human carcinogen. The US NIOSH recommends a 10h-TWA exposure limit of 5 mg/m³ and the US OSHA uses an 8h-TWA exposure limit of 5 mg/m³.

Kinetics

No adequate studies on the kinetics and the mechanisms of action of kaolin are available.

Effects

Many reports of well-studied cases and a number of cross-sectional studies, mainly in the United Kingdom and the USA, have shown that long-term exposure to kaolin may lead to pneumoconiosis, which has been named kaolinosis. Pneumoconiosis is diagnosed by chest X-ray, and is categorized as simple pneumoconiosis (category 1-3) or complicated pneumoconiosis (category A-C) (annex D). Clinical symptoms and effects on pulmonary function are mainly observed in the complicated form. Although the mildest form of pneumoconiosis ILO category 1 is not yet associated with a decrease of pulmonary function, the committee considers pneumoconiosis category 1 as an undesired health effect, in agreement with the European Committee's report on miner's pneumoconiosis. In this report, pneumoconiosis category 1 is recognized as an occupational disease.¹

Studies with exposure data or dose-effect relationships are scarce. The available animal data are not suitable for the derivation of a health-based recommended occupational exposure limit. Adequate studies on the carcinogenicity or reproduction toxicity of kaolin are not available.

There is one study with human data in which a dose-response relationship was reported for respirable kaolin dust.² According to this study, the average non-smoking worker reaches pneumoconiosis ILO category 1 at the age of 60 after 40 years of occupational exposure to 2.1 mg/m³ respirable kaolin dust. From the data as presented in this publication, the committee can not establish a NOAEL or LOAEL. The publication does not contain information on the quartz content of the respirable dust exposures, the presence of which in kaolin can make it difficult to distinguish kaolinosis from silicosis (pneumoconiosis caused by quartz).

Evaluation and advice

According to the committee, it has convincingly been shown that occupational exposure to kaolin has adverse health effects involving the lungs and with pneumoconiosis as the clinical finding.

It is the committee's opinion that occupational exposure to 2.1 mg/m³ respirable kaolin dust is undesired as it constitutes a substantial risk for the development of pneumoconiosis ILO category 1. However, no studies are available from which the committee can establish a NOAEL or LOAEL for kaolin dust. Therefore, the committee cannot derive a health-based recommended occupational exposure limit for kaolin dust.

In this advice, the committee gives an additional consideration on the inhalatory exposure to respirable kaolin dust in the workplace.

Scope

1.1 Background

At the request of the Minister of Social Affairs and Employment (annex A), the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations on the toxicity of chemical substances used in the workplace. The purpose of these evaluations is to recommend health-based occupational exposure limits for concentrations in the air, provided that the database allows the derivation of such values.

1.2 Committee and procedure

The present document contains the assessment of DECOS, hereafter called the committee, of the health hazard of kaolin. The members of the committee are listed in Annex B.

In 2007 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The committee's recommendations on the health-based occupational exposure limit of kaolin have been based on scientific data which are in the public domain. Data were obtained from the online databases Toxline and Medline/PubMed. The search was performed with "kaolin? OR CAS 1332-58-7" in combination with "toxic? OR epidemio?" The last search was performed in May 2007.

A list of abbreviations is given in Annex H.

Identity, properties and monitoring

2.1 Chemical identity

Kaolin (CAS number 1332-58-7; EEC number 310-127-6; RTECS number GF1670500) is a clay that consists primarily of kaolinite (CAS number 1318-74-7; EEC number 215-286-4), a non-fibrous aluminium silicate with the molecular formula $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$. Kaolin is a fine white to light-yellow powder with an earthy taste.³ It usually contains small amounts of other minerals such as quartz, mica and TiO_2 . The amounts differ depending on the geographic location at which the kaolin is extracted. Kaolin from Cornwall UK (purified Cornish china clay) typically consists of 89% kaolinite, 7% mica and up to 2% quartz⁴, while kaolin from Georgia (USA) typically consists of 98% kaolinite, 1% TiO_2 and <1% quartz.^{5,6} However, some 'kaolin-like' clays may contain substantial amounts of quartz. For example, the kaolin-like clays used in South African pottery contained 23-58% quartz.⁷ In the present risk assessment, exposure to kaolin is defined as exposure to kaolin dust containing $\leq 2\%$ quartz.

Synonyms and trade names for kaolin include Altowhites, Argilla, Bentone, Bilt-Cote, Bolus alba, China clay, Continental, Dixie, Emathlite, Fitrol, Glomax, Hydrite, Hydrated aluminium silicate, Hydrous aluminium silicate, Kaopaous, Kaophills-2, Langford, McNamee, Par clay, Peerless 2, Porcelain clay, and Snow tex.

Kaolin may be used to manufacture refractory ceramic fibres. These fibres are synthetic non-crystalline fibres produced by melting amorphous kaolin and

adding inorganic oxides. Refractory ceramic fibres are excluded from the present assessment.

2.2 Physical and chemical properties

Kaolin is insoluble in water, ether, alcohol, acids and alkaline solutions.³ The particle size of crude kaolin ranges from 0.05 – 150 µm.⁵ Processed kaolin has a particle size of <10 µm, with more than 50% of the particles being <2 µm⁶, that is in the respirable range. Kaolinite has a molecular weight of 258 g/mol, a density of 2.1 – 2.6 g/cm³, and a hardness of 2.3.⁸

Kaolinite particles are disc shaped with a diameter to thickness ratio of about 10:1. They owe their properties to the fact that the silicon atom can bind to oxygen atoms to form extensive thin flat sheets in which each silicon atom is surrounded by four oxygen atoms in a tetrahedral arrangement. These silica sheets can bind to alumina sheets of aluminium oxide (AlO(OH)) in which aluminium is surrounded by an octahedral arrangement of oxygen atoms and hydroxyl groups. The double sheet structure can be stacked in layers held together by hydrogen-bonds. This is the basic structure of kaolinite which is referred to as a 1:1 layer silicate.⁹ The silanol surface groups are slightly acidic and may be negatively charged; the aluminol surface groups are amphoteric or slightly basic and may be positively charged. Flat surfaces of kaolinite thus may reveal uneven distributions of positive and negative charges.

2.3 EU Classification and labelling

No data available.

2.4 Validated analytical methods

Inhalation of dust particles is the major route of human exposure to kaolin. Quantitative methods for measuring respirable dust are gravimetric, and measure the weight of dust collected on a filter from a known volume of air.

The National Institute for Occupational Safety and Health (NIOSH) and the US Occupational Safety and Health Administration (OSHA) have described methods for sampling respirable dust (NIOSH method 0600: particulates not otherwise regulated, respirable) using an appropriate sampler with a polyvinyl chloride or equivalent filter. When sampling respirable dust, the filter is preceded by a Nylon cyclone. Kaolin is identified by X-ray diffraction or electron microscopy.

No validated methods for the determination of the amount of kaolin in biological samples were found.

Sources

Kaolin is a product of the geologic weathering of feldspar, a primary constituent of granite rocks. Deposits usually consist of a soft aggregation of sand, mica and kaolinite. Commercially exploitable deposits are found in several areas around the world. In the United States, the deposits are primarily concentrated in a belt extending from central Georgia to west central South Carolina. In the UK, deposits are found in Cornwall and Devon.^{4,10,11}

Kaolin is removed from the walls of the open pit by washing out the decomposed feldspar with a high pressure water jet, resulting in a kaolin slurry, or by surface strip mining, resulting in moist lumps. Mica and sand are removed from the slurry by differential sedimentation. The refined clay suspension is dried in a coal-fired open pan kiln or by filter press techniques, until the moisture content is about 10%. Then the clay is milled, bagged and loaded for transportation. The moist kaolin lumps are processed by either a 'wet' or a 'dry' method. In the wet method, the kaolin is mixed with water and contaminants are removed by differential sedimentation. Excess water is removed by vacuum filtration and spray drying before the kaolin is shipped as a rehydrated slurry or dry clay. In the dry method contact with water does not occur. The kaolin lumps are sliced, dried and pulverized by air flotation. Sand and other impurities are removed by filtering methods. Then the kaolin is bagged and loaded for transportation.^{6,10,12} The dry process, which generates huge amounts of dust, was generally used before 1940 and has now been largely replaced by the wet process.

The US is the world's largest producer of kaolin with an annual production volume of about 9 million metric tons (1990-2002), of which about 3 million tons are exported. About 175,000 tons are exported to The Netherlands (2002). The UK produces about 3 million tons annually (1990-2002), which is mainly exported. The world production of kaolin in 2004 was 44 million tons, with 53 countries producing more than 1000 metric tons per year.¹³

Kaolin is used in manufacturing refractory materials and ceramics, as a filler in plastics, paper, rubber, paints and adhesives, as a coating for high quality paper, and as a gastro-intestinal adsorbent in the treatment of diarrhea.^{11,14} No specific data are available on the use and origin of kaolin by Dutch industries. Therefore, the committee can not exclude that some of the kaolin used by the Dutch industries contains more than 2% quartz.

Exposure

4.1 General population

Kaolin is a natural component of the soil and may occur widely in ambient air as floating dust¹⁵. Accordingly, exposure of the general population to kaolin is universal, albeit at low concentrations. In case examinations, both ante mortem and post mortem, kaolinite particles have been found in the lungs of men without occupational dust exposure (see WHO report¹⁵). In the vicinity of mines and industrial areas, kaolin is likely to be present at higher concentrations in the air than in non-mining and non-industrial sites. However, there are no measured data available on background concentrations of kaolin nor on the exposure of the general population to kaolin.

4.2 Working population

Processing of kaolin involves considerable exposure to kaolin dust. Exposure was especially significant before the 1960s.¹⁰ After drying, nearly all work stages were carried out in high dust concentrations. Many operational changes have been introduced in the 1970s to improve airborne dust control and to reduce the amount of dust generated in the production process. More effective ventilation and closed technologies have led to a progressive reduction in airborne dust concentrations.^{2,16} Data on the occupational exposure to kaolin in the Netherlands are not available.

Ambient air concentrations of kaolin and personal exposures to kaolin are usually reported as gravimetric dust concentrations. The available data are summarized in Table 4.1.

Three studies on the exposure to kaolin dust from the Georgia kaolin mines have been published.^{11,12,17} A NIOSH study conducted by Sepulveda *et al.* (1983) in 1981 reported that over 50% of the dust fractions (aerodynamic particle size <10 µm) adjacent to pulverizers and to the baghouse were respirable. Other locations sampled exhibited respirable dust fractions of 10-20%. In the mill areas, dust existed of 96% kaolinite and 4% TiO₂. In all job categories (miner, car loader, bin operator, mill operator, baghouse labourer), breathing zone samples (n=44) of respirable dust showed values ≤5 mg/m³. None of the personal respirable dust samples contained quartz. Total dust levels by area showed means above 10 mg/m³ for the loading area, the milling area, and the pulverizing area.¹¹ All samples in this study were full-shift samples.

Altekruse *et al.* (1984) collected personal air samples (n=157) from 1977-1981 at a Georgia kaolin mine where kaolin was still being processed by the dry method. Of the dust particles, 50-80% were in the respirable range. The full-shift respirable dust concentrations were significantly higher in the processing area (2-5 mg/m³) than in the maintenance and mining areas (0-1 mg/m³) (*p*<0.025). Mineral analysis of the dust showed 94-98% kaolinite and 2-6% TiO₂.¹²

The studies described below lack data on the sampling time of exposure measurement. A study by Kennedy *et al.* (1983) at a Georgia kaolin mine reported that all processing facilities were documented by U.S. Mine Safety and Health Administration testing to be below 5 mg/m³ (with <1% quartz) respirable kaolin dust at the time of study (1977-1980). Previous surveys of the same plant had documented maximum levels of 377 mg/m³ in 1951, and 361 mg/m³ in 1960.¹⁷

In the Cornish kaolin industry, several measurements of personal respirable dust concentrations (0.5-4.7 mg/m³) have been reported.^{2,16}

In a Polish study by Szadkowska-Stanczyk *et al.* (2001), 80% of the measurements of kaolin dust within the area of the loaders' work posts exceeded the allowable concentration of 4 mg/m³. Quartz levels in kaolin dust were 3.2-5.1%.¹⁸

In a multi-centre epidemiological study, conducted by the International Agency for Research on Cancer (IARC), the exposure database reported exposure measurements from 4 paper mills. One of the 25 measurements was higher than 10 mg/m³.¹⁹

Table 4.1 Available data on the occupational exposure to kaolin dust.

Exposure group / area	Number and type of samples ^a	Kaolin dust fraction	Average dust concentrations (mg/m ³) ^b	Year of sampling	Country ^{ref}
Miner	10, personal	respirable	~ 0.2 (0.1-0.35) ^c	1981	USA ¹¹
Car loader	3, personal	respirable	~ 1 (0.7-1.2) ^c		
	3, area	total	~ 10 (7-15) ^c		
Bin operator	3, personal	respirable	~ 2 (1.3-2.5) ^c		
	7, area	total	~ 20 (10-30) ^c		
Mill operator	3, personal	respirable	~ 1 (0.8-1.2) ^c		
	8, area	total	~ 20 (10-80) ^c		
Bag house labourer	5, personal	respirable	~ 2 (0.3-5) ^c		
	3, area	total	~ 5 (1.5-9) ^c		
Processing area	14, personal	respirable	~ 3.9	1977	USA ¹²
	9, personal		~ 3.8	1978	
	5, personal		~ 5.3	1980	
	14, personal		~ 1.8	1981	
	68, personal		1.74	1981	
Maintenance area	15, personal		~ 0.9	1980	
	11, personal		~ 0.1	1981	
Mine	12, personal		~ 0.8	1980	
	5, personal		~ 0.1	1981	
	4, personal		0.14	1981	
Processing area	unknown	unknown	Max. value 377	1951	USA ¹⁷
		unknown	Max. value 361	1960	
		respirable	< 5	1977-1980	
Loader/unloader	unknown	respirable	6.1-12.7	1976-1990	Poland ¹⁸
		total	6.7-156.0	1995-1998	
Slurry maker		respirable	0.5-0.9	1976-1990	
		total	4.1-27.5	1995-1998	
Dryers	681, personal	respirable	1.9	1984-1986	UK ¹⁶
Attritor mills	114, personal		2.7		
Calciners	63, personal		2.5		
Slurry plants	69, personal		1.1 ^d		
Tube presses	5, personal		0.5		
Attritor mills		respirable	4.7 (9.32) ^e	1978	UK ²
			2.1 (3.36) ^e	1990	
Dryers	Total of 500 personal and area samples per year		3.5 (5.41) ^e	1978	
			1.7 (2.78) ^e	1990	
Calciners			3.5 (3.93) ^e	1978	
			2.2 (3.25) ^e	1990	
Slurry plants			1.6	1978	
			(mean of 2 samples)		
			1.2 (2.26) ^e	1990	
Paper mills	25, unknown	unknown	0.94 (0-21.5) ^e	1950-1994	International multi-centre study ¹⁹

^a Sampling time was full-shift in references 4 and 39; in the other references sampling time is not given.

^b Results from references 4 and 39 have been estimated from graphs.

^c Average dust concentration together with range shown in parentheses.

^d Assumed average based on regular sampling at an older, dustier plant and a small number of samples from a modern, less dusty plant.

^e Average dust concentration together with upper deciles shown in parentheses.

Kinetics

There are no data available.

Mechanism of action

Many reports of well-studied cases and a number of cross-sectional studies, mainly in the United Kingdom and Georgia, USA, have shown that long-term exposure to kaolin may lead to a pneumoconiosis called kaolinos. Pneumoconiosis is a pulmonary disease caused by the inhalation of inorganic mineral dusts (see Annex D). The toxic mechanism of action through which kaolin induces kaolinos has not been elucidated but reactive hydroxyl radicals have been implicated.

6.1 Studies in humans

No data available.

6.2 Studies in animals

No data available.

6.3 *In vitro* studies

A few studies have examined the hypotheses that kaolin causes pneumoconiosis by catalyzing the generation of reactive oxygen species from chemical substrates supplied by the lung itself^{20,21}, or by distortion and lysis of the cell membrane by non-free radical surface groups²². Using an *in vitro* system, Kennedy *et al.*

(1989)²⁰ showed that kaolin catalyzes the formation of hydroxyl radicals from hydrogen peroxide in the presence of ascorbate as a reducing agent. Lipid peroxidation of membranes was studied with human erythrocyte suspensions. Kaolin (1 mg/ml) significantly increased malondialdehyde content ($p < 0.05$) and caused marked *in vitro* lysis of 50% of the human red blood cells in 1 hour ($p < 0.001$).²⁰

Baser *et al.* (1990)²¹ also showed that kaolin could generate hydroxyl radicals from hydrogen peroxide in an *in vitro* system with ascorbate as a reducing agent. Kaolinite cytotoxicity is suppressed by incubation in the presence of dipalmitoyl phosphatidylcholine, a principle lipid component of pulmonary surfactant.^{22,23} Phagolysosomal enzymatic digestion can hydrolyse and remove the adsorbed surfactant molecules, and so restore kaolin dust cytotoxicity. Mineral specificity of that restoration has been postulated to be the basis for the observed difference in fibrogenic activity between kaolin and quartz.²³

Other studies have shown kaolin-induced cytotoxicity by measuring release of lactate dehydrogenase from macrophages²⁴⁻²⁶, and cultured cells²⁷. Kaolin-induced haemolysis of sheep^{23,28} and human²⁰ erythrocytes has also been reported.

Murphy *et al.* (1993)²⁷ and Banin and Meiri (1990)²⁹ have reported the presence of alumino-silicates in Alzheimer's senile plaques. Banin and Meiri (1990)²⁹ examined possible mechanisms for the interaction of kaolinite with cultured murine neuroblastoma cells. The electrical activity of differentiated cells changed when kaolinite (125 µg/ml) was added. Within minutes, the membrane permeability began to rise, the resting potential depolarized and the ability to maintain action potentials in response to stimulation was lost. Within 30 min, the cells showed clear morphological signs of deterioration. The study concluded that a layout incorporating both negative and positive charges (as on the broken edges of kaolinite), and the non-isodiametrical geometry of the particles may be necessary for the acute neurotoxic interaction. The committee considers these studies on the presence of alumino-silicates in Alzheimer plaques not relevant for the risk assessment.

6.4 Conclusions

The available *in vitro* studies do not allow conclusions on the mechanism of action of kaolin.

Effects

A wide variety of respiratory effects has been reported after exposure to kaolin. Many observations, based on chest X-ray suggest that kaolin workers develop pneumoconiosis. Pneumoconiosis is a pulmonary disease caused by the inhalation of inorganic mineral dusts. These dusts cause irreversible lung lesions (fibrosis) which manifest as parenchymal opacities and pleural abnormalities on chest X-ray films. These films are used to distinguish between simple and complicated pneumoconiosis according to the classification guideline of the International Labour Office (ILO; see Annex D). Kaolin-induced pneumoconiosis is also named kaolinosi. The presence of quartz in kaolin can make it difficult to distinguish kaolinosi from pneumoconiosis caused by quartz (silicosis).

In the epidemiological studies, quantitative exposure data are limited and information on the composition of the kaolin is scarce. Lung function measurements (spirometry) are often used to detect functional changes in the respiratory system and chest X-ray films to detect pathological changes.

7.1 Observations in humans

7.1.1 *Irritation and sensitisation*

Although kaolin is used extensively in cosmetics, no reports have been found on the possible adverse effects of kaolin upon direct skin contact.¹⁵

In a study by Epstein *et al.* (1989)³⁰, a spray of kaolin-containing fluorocarbon aerosols was used as a topical protectant against experimental poison ivy/oak dermatitis. The composition and concentration of the kaolin in the spray was not described. It was stated that none of the compounds used in this study (including kaolin) produced evidence of primary irritation or any unexpected or abnormal systemic responses in any of the 28 healthy volunteers.

Edenfield *et al.* (1960)⁵ mentioned that “exposure of workers to high concentrations of kaolin dust, such as may result from a break in a pipe line or valve, produces little or no irritation of the eyes, nasal or pharyngeal membranes, and no cough or other symptoms of bronchitis”. Since this quote is not substantiated by adequate data, the committee considers this information anecdotal.

7.1.2 *Acute and short-term toxicity*

No data available.

7.1.3 *Long-term toxicity*

Many epidemiological and case studies have been conducted to detect chronic effects in populations of kaolin workers. Most of these studies did not include detailed exposure assessment. Kaolin workers in Georgia, USA, and in Cornwall, UK have been studied most extensively. Case studies are shown in the Table in annex E. Epidemiological studies without exposure data are shown in the Table in annex F. Those with exposure data are given in annex G and described in more detail below.

Epidemiological studies with exposure data

There are five cross-sectional studies on occupational exposure to kaolin dust that include information on exposure levels. Three are from kaolin workers in Georgia, USA, and two are from kaolin workers in the UK.

Kaolin workers, Georgia, USA

In a cross-sectional study (1977-1980), Kennedy *et al.* (1983) surveyed 459 workers for the presence of pneumoconiosis by chest X-ray (International Labour Organization (ILO) classification, 1971) and for respiratory symptoms (dyspnoea, wheezing, cough and sputum).¹⁷ The workers were employed in three Georgia kaolin mining and processing facilities with a mean duration of employment of 12 years. The workers were categorized into the following exposure

groups: production, maintenance, laboratory/mine or administration, in decreasing order of kaolin exposure. In all categories, respirable kaolin concentrations between 1977 and 1980 were $\leq 5 \text{ mg/m}^3$, with $< 1\%$ quartz. It is unclear whether these concentrations are from personal or area measurements, and whether the measurements are full-shift measurements or not.

The prevalence of pneumoconiosis (ILO categories 1-3) was 9.2% ($n=42$), with significantly more black people ($20/147=13.6\%$) than white people ($22/312=7.1\%$) having pneumoconiosis. The distribution of categories of pneumoconiosis did not differ significantly between black and white workers. X-ray films of most cases manifested fine or medium irregular opacities, evenly distributed in the lungs. Eight of the 42 cases showed large opacities representing complicated pneumoconiosis. Pleural thickening was seen in two of the 42 cases. Multivariate analysis showed that the only significant predictors of the X-ray presence of pneumoconiosis were age over 55 years ($p<0.005$) and exposure in the production area over 15 years ($p<0.001$). The X-ray presence of pneumoconiosis did not correlate with respiratory symptoms, smoking history or history of tuberculosis. Pulmonary function was measured by spirometry and expressed as forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). Spirograms of the 34 workers with mild pneumoconiosis were not significantly different from workers without pneumoconiosis. Clinical impairment of pulmonary function did not occur, except for mild, insignificant impairment in advanced cases (dyspnoea on exertion). A reanalysis of the data by Baser *et al.* (1987) showed that kaoline exposure was not independently associated with pneumoconiosis and lung function, and that significant differences in FVC's among production workers existed between the plants studied.³¹

This study did not include retired workers and there was no unexposed control group outside the kaolin industry. Separate data for the different exposure groups were not available. Furthermore, no clear kaolin dust levels were identified (less than 5 mg/m^3 respirable with less than 1% free crystalline silica as documented by U.S. Mine Safety and Health Administration testing; and as high as 377 mg/m^3 and 361 mg/m^3 in processing areas of Plant 3 in 1951 and 1960, respectively). Therefore, no dose-response relationship could be derived.¹⁷ For these reasons, the committee decided not to use this study for the risk assessment.

In 1981, a cross-sectional health study was conducted by Sepulveda *et al.* (1983)¹¹ at a Georgia kaolin mine and mill among 39 current workers and 16 ex-workers, all with over 5 years of exposure, and among 10 current workers with less than 5 years of kaolin exposure. The workers were divided in five job cate-

gories, namely miners, car loaders, bin operators, mill operators and bag-house workers. An external non-kaolin exposed reference group (n=189) for the analysis of pulmonary function included workers from soft drink bottling companies, candy factories, a power tool company, the fabric department of a tire manufacturer, and shipping clerks in a poultry packing plant. Personal respirable dust and area total dust was sampled during full shifts. The airborne dust contained 96% kaolinite, 4% TiO₂ and no quartz. At processing areas, respirable dust fractions (aerodynamic particle size <10 µm) of over 50% were found, while at other locations respirable dust fractions of 10-20% were reported. Total dust levels exceeded 10 mg/m³ in the car loading, bin and mill areas, while in none of the working areas respirable dust exceeded 5 mg/m³.

Based on chest X-ray (ILO classification, 1971), 5 of 39 current workers and 3 of 16 ex-workers with 5 years or more of exposure were identified with pneumoconiosis (prevalence 8/55=15%). These included four cases of simple and four cases of complicated pneumoconiosis. In one current worker with simple pneumoconiosis, previous exposure to asbestos was discovered. Among the current workers with less than 5 years of kaolin exposure (n=8), none had pneumoconiosis. There were no X-ray data for the non-exposed comparison group. Kaolin workers in this study showed significantly lower values ($p<0.05$) for FVC, FEV₁ and peak flow rate than non-kaolin workers, when the values were adjusted for age, height, race, and pack-years of smoking. No relationship between pneumoconiosis and history of smoking or abnormalities of lung function was observed. The size of the subgroups with different job categories was small in this study. Because of the absence of historical air sampling data, this study could not provide reliable estimates of past exposure to kaolin nor a clear relationship between kaolin exposure and pneumoconiosis. Therefore, the committee could not use this study for the risk assessment.

In a cross-sectional study of a Georgia kaolin mine and processing plant, Altek-ruse *et al.* (1984)¹² examined pulmonary function and chest X-ray films from the entire work force of 65 men. In the study, personal air samples (n=157) collected between 1977-1981 were included. Workers were divided into three working area subgroups: production and processing area (continuous dust exposure), mining area (no dust exposure), plant and vehicle maintenance area (intermittent dust exposure). In the dry processing area, 50-80% of the dust was in the respirable range. Between 1977 and 1981, respirable kaolin dust concentrations (containing 94-98% kaolinite, 2-6% TiO₂ and no quartz) were significantly higher ($p<0.025$) in the processing area (2-5 mg/m³) than in the maintenance and mining areas

(≤ 1 mg/m³). The mean concentration of respirable kaolin dust in 1981 was 0.14 mg/m³ in the mining area, and 1.74 mg/m³ in the processing area.

X-ray (ILO classification, 1980) revealed pneumoconiosis in 5 of the 65 workers (prevalence 7.7%), all of whom had worked in the processing area for 7-36 years and all of whom had symptoms of bronchitis. Four of these five had simple, and one had complicated pneumoconiosis. The mean FVC and FEV₁ of the 5 workers with pneumoconiosis was mildly but significantly ($p < 0.05$) reduced compared to the other 19 processing workers. The FVC and FEV₁ showed a small but significant ($p < 0.05$) decline with increasing length of employment at the processing area. However, overall no differences in lung function values (FVC, FEV₁) and lung volumes were reported between working area subgroups. According to the authors, the study indicated that kaolin inhalation alone can lead to pneumoconiosis because of the complete absence of quartz in the dust, and that kaolin pneumoconiosis was associated with significant though mild effects on pulmonary function.¹² Because of the small number of workers included, the committee decided not to use this study for the risk assessment.

Kaolin workers, UK

In 1985, a cross-sectional study by Ogle *et al.* (1989)¹⁶ examined chest X-ray films (ILO classification, revised 1980) of 3,400 current and 289 retired kaolin workers. Pneumoconiosis prevalence was 8.5%, with 271 cases in category 1, 39 in category 2, and 5 in category 3. Of the small opacities observed, 60% were rounded in shape, and 40% were irregular. Pleural thickening was shown in 87 cases, and pleural calcification in 17 cases. Smoking status did not statistically affect the X-ray results. Average personal respirable dust concentrations (1984-1986) ranged from 0.5 – 2.7 mg/m³ depending on location. Information on the sampling time and on the composition of the dust (non-kaolin dust and quartz content) was not given. Employment was categorized into groups with similar dust exposure levels (Table 7.1). The investigators carried out regression analysis yielding regression coefficients (RC) describing the size of the effect of one exposure year on 1000 x Ln (X-ray score). This regression coefficient is a measure of the effect of one exposure year on the X-ray score for the average worker. Employment in attritor mills, china stone mills and as dryers was found to have the greatest effect on the X-ray diagnosed pneumoconiosis classification. Compared to the effects before 1971, the effects post 1971 - with improved working conditions installed in the early seventies - were about 50% lower as reflected by the regression coefficients for all workers in Table 7.1 (fourth column; values printed in bold). When workers in dusty jobs before 1971 were excluded, the

effects post 1971 were about 60-70% lower as reflected by the regression coefficients for the workers excluding those with pre-1971 dust exposure (seventh column, values printed in bold) compared with those for all workers pre-1971 (fourth column, pre-1971). This shows the effect of the improved working conditions since 1971.

Ventilatory capacity (FVC, FEV₁) was independent of employment history, but highly dependent on age and radiological score. The probability of exhibiting symptoms (cough, phlegm, breathlessness, wheeze) was dependent on the level of ventilatory capacity for each of the 3 smoking classes (current, ex- and non-smokers). Age, exposure to dust and X-ray category made no additional contribution.

The study lacks accurate exposure data from before 1978 and an unexposed control group, and therefore conclusions on the exposure-concentration dependent health effects of kaolin exposure are limited. Nevertheless, the investigators found the greatest effect of employment on X-ray category for attritor mill workers and china stone mill workers, and were able to show a major effect of the improved working conditions since the early 1970s. The authors noted that of the post-1971 group, 99 of 123 subjects whose X-ray score was greater than 0 had worked in jobs with little (n=51) or no (n=48) dust exposure. They explained this by the poor quality of the X-ray films (102/123), the higher age (61% being over 50) or by signs of disease other than pneumoconiosis.¹⁶

Table 7.1 Regression coefficients of 1000 x Ln (X-ray film score) on years of exposure.¹⁶

Job classification	Average dust concentration (mg/m ³) ^a	All workers		Workers excluding those with pre-1971 dust exposure	
		Number of subjects ^d	RC (SE) ^e	Number of subjects ^d	RC (SE) ^e
Non-dusty life ^b	not available	3689	6 (0.4)	2925	6 (0.4)
Dryers					
- pre-1971	not available	522	25 (2)	-	-
- post-1971	1.9 (n=681)	997	14 (1)	519	10 (2)
Attritor mills					
- pre-1971	not available	138	44 (4)	-	-
- post-1971	2.7 (n=114)	73	23 (4)	16	12 (11)
Slurry plants	1.1 (n=69) ^c	325	8 (1)	255	7 (1)
China stone mills	not available	37	51 (6)	-	-
Pan kilns	not available	248	13 (2)	-	-

^a Average personal respirable dust concentrations (mg/m³) measured between January 1984 - March 1986, with total number of samples shown in parentheses. Data on sampling time are not available.

^b Defined as the sum of the time before employment, the time after retirement, and the time in jobs with the level of respirable dust < 0.3 mg/m³, summed for all workers.

^c This is an assumed average based on regular sampling at the older, more dusty plant, and a small number of samples at the modern, less dusty plants.

^d Number of subjects at the time of study (1985)

^e RC: regression coefficient describing the size of the effect of one exposure year on 1000 x Ln (X-ray score); SE : standard error

Table 7.2 Regression coefficients of 1000 x Ln (X-ray film score) on years of exposure.²

Job classification	Average dust concentration (mg/m ³) ^a			Number of subjects ^d	RC (SE) ^e
	1978	1990			
Non-dusty life ^b	not available	not available		4112	7.7 (0.4)
Dryers	not available	not available	pre-1971:	456	29.2 (2.2)
	3.5 (5.41)	1.7 (2.78)	post-1971:	1218	13.0 (1.1)
Attritor mills					
- pre-1971	not available	not available	pre-1971:	82	46.9 (6.1)
- post-1971	4.7 (9.32)	2.1 (3.36)	post-1971:	151	21.1 (3.3)
Slurry plants and tube presses	1.6 ^c	1.2 ^c (2.26)		493	8.9 (1.2)
China stone mills	not available	not available		37	30.6 (5.3)
Pan kilns	not available	not available		202	22.7 (2.6)

^a Average personal respirable dust concentrations (mg/m³), with upper deciles shown in parentheses.

^b Defined as the sum of the time before employment, the time after retirement, and the time in jobs with the level of respirable dust < 0.3 mg/m³, summed for all workers.

^c Based on two samples; for slurry plants only

^d Number of subjects at the time of study (1990)

^e RC: regression coefficient describing the size of the effect of one year of exposure on 1000 x Ln (X-ray score); SE: standard error

In a cross-sectional study by Rundle *et al.* (1993)² in 1990, 4112 current and retired UK kaolin workers were studied using an X-ray film survey (ILO classification, 1980), spirometry (FVC, FEV₁), and a questionnaire (occupational and smoking history, respiratory symptoms). The study population was partly identical to that of Ogle *et al.* (1989). Respirable and total dust samples had been regularly taken since 1978 (Table 7.2) and these provided representative dust concentrations for each location from 1978. Dust concentrations before 1978 were based on estimates. No data are presented on the amount of quartz and the amount of non-kaolin dust in the samples. According to the WHO Environmental Health Criteria (no. 231) document on kaolin, UK china clay workers are exposed to kaolin clay containing less than 1.1% quartz.¹⁵ The total occupational respirable dust dose (TODD in mg/m³·years) for each worker was calculated by adding together the average respirable dust concentration (mg/m³) for each year based on his employment in that year. In this study, 60% of the workers had TODD values of 10 mg/m³·years or less, 88% of the workers had values of 30 mg/m³ · years or less, and 1.1% had values greater than 80 mg/m³·years.

Of the 4112 X-ray films, 9.2% showed simple pneumoconiosis category 1, 0.75% showed category 2, and 0.05% showed category 3. Two cases showed complicated pneumoconiosis (large opacities). Of the opacities found, 50% were rounded in shape. X-ray films showed pleural calcification in 9 cases and pleural thickening in 39 cases.

For regression analysis, each X-ray subcategory was transformed in a score drawn from a continuum. In this way, major X-ray category 0 ranged from – 2.718 to 0.754; major X-ray category 1 from 1.162 to 1.920; major X-ray category 2 from 2.061 to 2.713; and major X-ray category 3 from 2.865 to 3.044. With regression analysis of X-ray film score on years of exposure, working in mills, pan kilns and dryers was found to have the greatest effect on X-ray score, which is consistent with the respirable dust concentrations in mills and dryers (Table 7.2). From the results of the regression analysis of X-ray film score on TODD, the authors plotted a graph for an average 60-year old non-smoker, ex-smoker and current smoker, showing the relations between X-ray film category and TODD (Figure 1). The average non-smoking worker would reach X-ray film category 1 at the age of 60 after 85 mg/m³·years of exposure (*e.g.*, working for 40 years at an average dust concentration of 2.1 mg/m³). In the average smoker, the total exposure would need to be 65 mg/m³·years (*e.g.*, working for 40 years at an average dust concentration of 1.6 mg/m³). Regression analysis of lung function values on X-ray film score, age, and smoking class was performed. Regardless of age and smoking class, FEV₁ and FVC decreased with 210 and 356 ml, respectively, with an increase of X-ray film score with one major category.

The study by Rundle *et al.* (1993)² is the only human study available with data on a relationship between total occupational respirable dust dose and major X-ray category. In this study, the TODD to reach ILO category 1 is given for the average worker, only, at the age of 60. This study does not present data on the TODD at which the smallest number of workers develop pneumoconiosis ILO category 1 at the age of 60. No NOAEL or LOAEL can be derived from this type of data presentation. Nevertheless, according to the authors exposure to 2.1 mg/m³ respirable kaolin dust for 40 years causes ILO category 1 in the average worker at the age of 60. This may involve a large number of workers. Like in the study by Ogle *et al.* (1989)¹⁶, the non-exposed group contained workers with pneumoconiosis category ≥ 1 . Of the 413 cases whose median X-ray category was 1 or above, 136 had had no dust exposure, of whom 21 were non-smokers. The authors did not give an explanation for this.

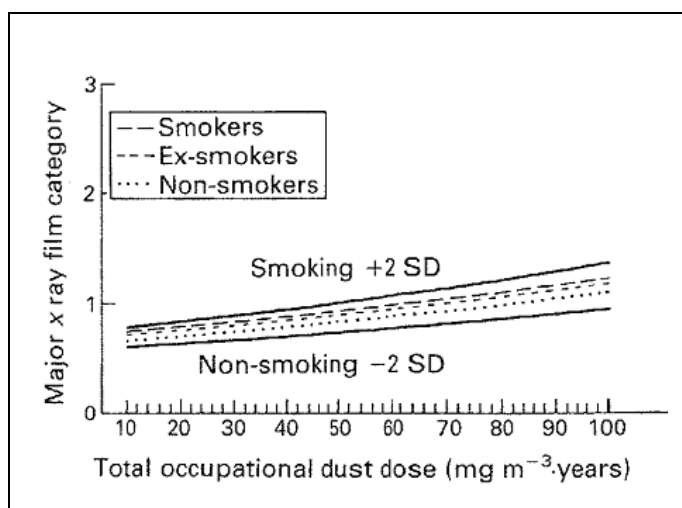


Figure 1. Estimates of X-ray film category for different smoking classes at age 60.²

7.1.4 Carcinogenicity

No adequate studies are available.

Lung tumours have been found incidentally in epidemiological or case studies. Lapenas *et al.* (1984)¹⁴ described a case of widely disseminated intraparenchymous lung carcinoma found at autopsy, next to complicated pneumoconiosis, after kaolin exposure. The authors did not discuss this further. Sheers *et al.* (1964)¹⁰ mentioned one case of carcinoma of the bronchus in a survey of 1394 kaolin workers.

In the study by Szadkowska-Stanczyk *et al.* (2001)¹⁸, 79 cases of lung cancer were investigated in a mortality study among pulp and paper workers. The study was part of a cohort study of 10,575 male and female workers employed for at least one year in a large pulp and paper mill between 1968 and 1990, and observed until 1996. The study had a nested case-control design and for each case, three control subjects were selected and matched according to sex, year of birth, and year of hire. Two cases and one control had been exposed to kaolin dust (concentration unknown). The risk of mortality, adjusted for smoking, from lung cancer for these two cases was six times higher compared to the non-exposed. However, because of the small number of cases, the odds ratio value of 5.64 was not statistically significant (95% confidence interval adjusted for smok-

ing: 0.5-63.4). Subsequent measurements of kaolin dust concentrations in the period 1995-1998 revealed respirable kaolin dust levels in the mill under study, ranging from 6.1 to 12.7 mg/m³ and containing 3.2 to 5.1% free crystalline silica.

7.1.5 *Reproduction toxicity (fertility and development)*

No data available

7.1.6 *Immunological effects*

No data available

7.1.7 *Neurological effects*

No data available.

7.1.8 *In vitro effects*

Kennedy et al. (1989)²⁰ studied lipid peroxidation of membranes in human erythrocytes suspensions. They found that kaolin (1 mg/ml) significantly increased malondialdehyde content ($p < 0.05$), and caused marked in vitro lysis of 50% of the human red blood cells within 1 hour ($p < 0.001$).

7.1.9 *Miscellaneous*

No data available.

7.2 **Animal experiments**

7.2.1 *Irritation and sensitization*

No data available

7.2.2 *Acute toxicity*

A sterilized suspension of 0 or 5 mg/animal Indian kaolin (99% kaolinite, no quartz; particle size $< 5 \mu\text{m}$) in 0.1 ml NaCl (0.15 M) was given once intratracheally to 70 albino Swiss mice of either sex. In this study by Sahu et al. (1978)³², two animals per group were killed periodically at various time points up to 210

days after treatment, and the lungs were examined. Macroscopically, white patches were observed from day 30 onwards, which increased in area, becoming prominent at termination of the experiment on day 210. The tracheobronchial lymph nodes became enlarged from day 90 onwards, and appeared quite prominent at day 210. Microscopically, an inflammatory response involving leucocytes and dust-laden macrophages in alveolar tissue was observed from the first day onwards. Subsequently, fibroblast proliferation increased and became marked at day 120. Lymph nodes contained many small foci of vacuolated macrophages and little reticulin reaction at day 210. The lungs and lymph nodes of the animals in the control group were normal.³²

In a study by Martin *et al.* (1975)³³, ten female Sprague-Dawley rats were given a single intratracheal injection of 50 mg of kaolin per animal (no data available on composition). After three months, an increase in the collagen content was observed, but no increase in the weight of the lungs. Kaolin did not produce lesions of alveolar proteinosis, but most of the kaolin was present in the bronchiolo-vascular lymphatic sheaths. Compared to quartz, which was also tested in this study, kaolin has a considerably less fibrogenic (but not negligible) capacity.³³

Cornish coating grade kaolin, with >98% of the particles in the range of 0.8-6.6 µm, was used as a negative control in intrapleural implantation studies by Wagner *et al.* (1987).³⁴ Forty Fischer F344 rats, 20 of each sex, at about 5 weeks of age, were inoculated intrapleurally with a single injection of kaolin in saline (dose not given). The animals were allowed to live for their natural life span. On death, no mesotheliomas were found.³⁴

7.2.3 Short-term toxicity

In the study by Patterson and Staszak (1977)³⁵, diet containing 0% or 20%* kaolin (Georgia, USA; 97% kaolinite, no quartz) was fed to different groups of four female Sprague-Dawley rats during different time periods before conception and through gestation. The time intervals were: 37 to 68 days, 69 to 95 days, or 96 to 117 days. The authors did not observe significant weight differences between the groups during the first 13 days of gestation. A significant reduction ($p < 0.01$) in all measured maternal blood values (red blood cell count, haematocrit and haemoglobin concentration) was observed in the kaolin group for all three exposure periods compared to the control group.³⁵

* Using standard values for body weight (female rat: 0.35 kg) and daily diet consumption (female rat: 50 g), the 20% dose was recalculated to a daily kaolin concentration of 28.6 g/kg body weight.

Groups of twenty male and twenty female Fischer F344 rats inhaled a dose of 10 mg/m³ kaolin dust for 6 hours/day, 5 days/week, for 12 months. In this study by Wagner *et al.* (1987)³⁴, Cornish coating grade kaolin with >98% of the particles in the range of 0.8-6.6 µm was used. Two rats of each sex were killed at 3, 6, 12 and 24 months. Mean fibrosis gradings of the lungs were 2.8, 2.75, 2.4 and 2.1, respectively (grade 1 normal; grade 2 dust in macrophages; grade 3 early interstitial reaction). After living out their lifespan, the other rats were subjected to a full necropsy and histopathology of the lung, liver, spleen and kidney. In two of these rats, broncho-alveolar hyperplasia was found. Of the 40 rats exposed, 38 rats had no proliferative lesions, nor were any lung carcinomas observed.³⁴

7.2.4 Long-term toxicity and carcinogenicity

In a study by Mossman and Craighead (1982), four to six-week old female Golden Syrian hamsters were implanted with tracheal organ cultures.³⁶ These organ cultures had been exposed in vitro for one hour to 200 mg Georgia kaolin (particle diameter: 3-5 µm), and then maintained in vitro for four weeks. Tumours did not appear in tracheal tissues within 2 years after implantation.

Pott and Roller (2003)³⁷ and Mohr *et al.* (2006)³⁸ found increased lung tumour incidences in female rats after repeated (10 or 20 times) intratracheal instillation of 6 mg kaolin fine (mean particle diameter 2.0 µm) per animal. Tumour formation in rats was also observed after intratracheal instillation of other dusts, including previously considered inert dusts. Lung tumour formation by intratracheal instillation of dusts is supposed to be caused by particle overload which may occur when the volume of particles in the lungs markedly impairs pulmonary clearance mechanisms.^{39,40} Internationally, the relevance of intratracheal instillation is under debate and several investigators consider particle deposition by intratracheal instillation different from particle deposition by chronic inhalation.⁴¹ For kaolin, this view is supported by the lack of epidemiological data showing increased lung tumour incidence in kaolin workers. Lung tumours have only been found incidentally in epidemiological or case studies. Therefore, the committee concludes that there is insufficient epidemiological evidence for carcinogenicity of kaolin dust under occupational exposure conditions.

7.2.5 Mutagenicity and genotoxicity

No data available

7.2.6 *Reproduction toxicity*

In the study by Patterson and Staszak (1977)³⁵ described above, a diet containing 0% or 20% kaolin (Georgia, USA) was fed to different groups of four Sprague-Dawley female rats, at time intervals 37 to 68 days, 69 to 95 days, or 96 to 117 days prior to conception and through gestation. The authors did not observe significant weight differences between the groups during the first 13 days of gestation. All measured maternal blood values (red blood cell count, haematocrit and haemoglobin concentration) were significantly reduced ($p < 0.01$) in the kaolin group compared to the control group for all three time periods. The mean weight of pups from the kaolin diet group was significantly less ($p < 0.01$) than those from the control group (9.4% lower than control). No effects were observed on mean length of pups, litter size, or on morphology of the pups.³⁵

7.2.7 *Immunological effects*

Sluiter et al. (1987)⁴² intraperitoneally injected random-bred female Swiss mice (number of animals unknown) once with kaolin (0.5% w/v in saline; no data available on composition). This evoked a substantial increase in the number of granulocytes in the peripheral blood (6-12 hours after injection), and the peritoneal cavity (6-24 hours after injection).⁴²

Lewis et al. (1976)⁴³ used kaolin to produce a local inflammatory response (oedema) in the rat hind paw in order to screen new anti-inflammatory drugs, and to investigate the inflammatory process. Male Wistar rats (10/group) were administered 50, 100 and 150 mg/ml kaolin (no data available on composition) in 0.9% w/v saline by subplantar injection. Kaolin produced dose-related increases in paw volume.⁴³

7.2.8 *Neurological effects*

Kaolin induction of hydrocephalus in animals (rat, sheep, rhesus monkey) by injecting kaolin into the cisterna magna, is one of the most studied models of congenital hydrocephalus in the literature.^{44,45}

7.2.9 *Miscellaneous*

No data available.

7.3 Summary

The majority of case studies and epidemiological studies have been focussed on pneumoconiosis in kaolin workers. Pneumoconiosis is a pulmonary disease caused by the inhalation of inorganic mineral dusts which cause irreversible lung lesions (fibrosis). Pneumoconiosis is identified by chest X-ray, thereby using the classification guideline from the International Labour Office (see Annex D). Simple pneumoconiosis is defined as small opacities throughout the lung; complicated pneumoconiosis is defined as the presence of large opacities and progressive massive fibrosis in the lung.

In most of the studies, the radiological score was independent of smoking, but dependent on age, time of employment, and type of job. In some studies, a small decrease in lung function (FEV_1 and/or FVC) with increasing radiological score was found. Only in the case of complicated pneumoconiosis, a significant decrease was found. Respiratory symptoms, such as dyspnoea, cough or sputum production, were more frequently reported in cases of complicated pneumoconiosis than in cases of simple pneumoconiosis.

In the greater part of the human studies no data on occupational kaolin exposure levels were given. It is assumed that in the past, especially before 1970, occupational exposure to kaolin at undefined concentrations (but probably under very dusty conditions that no longer exist now) was associated with cases of pneumoconiosis.

In most of the available studies with exposure data, mean respirable concentrations were below 5 mg/m^3 . However, most of the studies presenting health effects, reflect exposures in periods for which measurements were not available, and in which strict control on dust levels was lacking. This makes it difficult to derive long-term dose-response relationships.

The amount of quartz in kaolin may also be an important confounding factor, since occupational exposure to quartz is associated with silicosis, lung cancer, and pulmonary tuberculosis. Purified Cornish china-clay typically contains 2% quartz. Georgia kaolin typically contains < 1% quartz, and is therefore considered as pure kaolin. The presence of quartz in kaolin can make it difficult to distinguish between silicosis and kaolin-induced pneumoconiosis.

Rundle *et al.* (1993)² presented data on a dose-response relationship for kaolin-induced lung effects. However, the amount of non-kaolin dust and the amount of quartz in the dust samples are not described in this study. A total occupational respirable dust dose (TODD) of $65 \text{ mg/m}^3\text{-years}$ (*e.g.*, 40 years exposed to 1.6 mg/m^3) was necessary to reach pneumoconiosis ILO category 1 in an average

smoking male worker, 60 years of age. For an average non-smoking male worker, 60 years of age, a TODD of 85 mg/m³·years (*e.g.*, 40 years exposed to 2.1 mg/m³) was needed to reach ILO category 1. A NOAEL or a LOAEL cannot be established from the data as presented in this study. Regression analysis of lung function values on X-ray film score, age, and smoking class yielded that, regardless of age and smoking class, FEV₁ and FVC decreased 210 and 356 ml, respectively, with an increase of the X-ray film score with one major category. In this study, pneumoconiosis was also identified in non-exposed workers.

Most animal studies are of little relevance in the context of human occupational exposure. In most studies, the purity of kaolin was not established nor its contaminants determined, and limited endpoints were investigated. At 10 mg/m³, fibrosis of the lungs was reported in rats exposed for 12 months. Lung tumour incidence in rats was increased after repeated intratracheal instillation (10 or 20 times 6 mg/animal) of kaolin fine with mean particle diameter of 2.0 µm. Lung tumour formation by intratracheal instillation of dusts is supposed to be caused by particle overload which may occur when the volume of particles in the lungs markedly impairs pulmonary clearance mechanisms.^{39,40} Internationally, the relevance of intratracheal instillation is under debate and several investigators consider particle deposition by intratracheal instillation different from particle deposition by chronic inhalation.⁴¹ For kaolin, this view is supported by the lack of epidemiological data showing increased lung tumour incidence in kaolin workers. Lung tumours have only been found incidentally in epidemiological or case studies. Therefore, the committee concludes that there is insufficient epidemiological evidence for carcinogenicity of kaolin dust under occupational exposure conditions.

No evidence of teratogenic effects was found. Slight decreases in pup weight were seen after exposure of the dams to 20% kaolin in the diet (approximately 28.6 g/kg bw/days) for up to 117 days.

Existing guidelines, standards and evaluations

8.1 General population

For the general population no guidelines exist.

8.2 Working population

The available standards for occupational exposure to kaolin are summarized in Table 8.1. The American Conference of Governmental Industrial Hygienists (ACGIH) has established a TLV (threshold limit value as an 8-hour time-weighted average) of 2 mg/m³ respirable kaolin dust.⁴⁶ In addition, the ACGIH noted kaolin as an A4 substance, meaning that kaolin is not classifiable as a human carcinogen.

Table 8.1 Established limits for occupational exposure to kaolin dust in various countries.

country - organisation	occupational exposure limit (mg/m ³)	dust fraction	time-weighted average	note ^a	ref.
Denmark	2	respirable	8 h	OEL	47
The Netherlands - Ministry of Social Affairs - and Employment					48
Germany - AGS	-				49
- DFG	-				50
UK - HSE	2	respirable	8 h	WEL	51
USA - ACGIH	2 ^b	respirable	8 h	TLV; A4 ^c	46
- NIOSH	10	total	10 h	REL	52
	5	respirable	10 h	REL	
- OSHA	15	total	8 h	PEL	53
	5	respirable	8 h	PEL	
European Union - SCOEL	-				54

^a Abbreviations OEL: occupational exposure limit; PEL: permissible exposure limit; REL: recommended exposure limit; TLV: threshold limit value; WEL: workplace exposure limit;

^b The value is for particulate matter containing no asbestos and < 1% crystalline silica.

^c A4: not classifiable as a human carcinogen because of lack of data.

Hazard assessment

9.1 Assessment of the health risk

Kaolin is a clay that consists primarily of kaolinite, a non-fibrous silicate of aluminium. Crude kaolin has a particle size of 0.05 – 150 µm; the particle size of processed kaolin is less than 10 µm with more than 50% of the particles being less than 2 µm, that is in the respirable range. In this document, the health risk assessment of exposure to kaolin is limited to exposure to kaolin dust containing ≤2% quartz.

Case reports and cross-sectional studies, mainly in the United Kingdom and the USA, have shown that long-term exposure of humans to kaolin dust can lead to kaolin-induced pneumoconiosis, also called kaolinos. Pneumoconiosis is a pulmonary disease caused by the inhalation of inorganic mineral dusts. These dusts cause irreversible lung lesions (fibrosis) which manifest as parenchymal opacities and pleural abnormalities by chest X-ray radiography. Pneumoconiosis is diagnosed by chest X-ray radiography and can be categorized as simple pneumoconiosis (ILO category 1-3) or complicated pneumoconiosis (ILO category A-C) (annex D). The mildest form of pneumoconiosis category 1 is not yet associated with decreased pulmonary function. Clinical symptoms (cough, sputum, dyspnoea) and effects on pulmonary function (decreased FVC and/or FEV₁) are mainly observed in the complicated form. Nevertheless, the committee considers simple pneumoconiosis of ILO category 1 as an undesired health effect because of lung pathology (found in X-ray radiography) which may be the onset of

decreased lung function. This is in line with a report of the European Committee in which miners' pneumoconiosis category 1 is recognized as an occupational disease.¹ It should be noted that the scoring of pneumoconiosis, based on the ILO guidelines (annex D), is a matter of subjective assessment. Due to inter-rater differences in scoring and interpreting of the X-ray films, it can not be ruled out that cases of ILO category 1 have been missed.

Occupational exposure to kaolin has gradually decreased over the years. In the USA, dry processing of kaolin, inducing large amounts of dust, was gradually replaced by the wet method after 1940. After the 1960s, dust control was significantly improved. In the UK, many operational changes have been introduced, mainly in the 1970s, to improve airborne dust control. The older drying processes in pan kilns were phased out in 1971. Several studies from kaolin industries in the USA and the UK, between 1977 and 1990, showed average respirable dust levels below 5 mg/m³.

According to the committee, it has convincingly been shown that the critical effects of kaolin exposure involve the lungs, with pneumoconiosis as the most important clinical observation. In case studies, kaolin particles have been found in lung macrophages present in areas of fibrosis. Epidemiological studies have shown that the radiological category of pneumoconiosis is dependent on age, years of exposure to kaolin, and job type. A few studies have found a correlation between increasing pneumoconiosis category and lung function (decreased FVC and/or FEV₁) or respiratory symptoms (*e.g.*, cough, sputum, dyspnoea), especially for complicated pneumoconiosis.

Studies with quantitative exposure data or dose-effect relationships in humans are scarce. These studies provide information on adverse-effect levels only and not on no-effect levels of kaolin. In some studies also non-exposed, non-smoking subjects had small opacities in the lungs, consistent with simple pneumoconiosis.

Despite the extensiveness of the epidemiological studies, only Rundle *et al.* (1993)² tried to identify a dose-response relationship for kaolin exposure and pneumoconiosis. No data were presented on the amount of quartz and non-kaolin dust in the samples but according to the WHO Environmental Health Criteria 231 report¹⁵, UK china clay workers were exposed to clay containing less than 1.1% quartz. By regression analysis of the logarithm of the X-ray film score on the total occupational respirable dust dose (TODD), Rundle *et al.* (1993) found a relation between the pneumoconiosis category and the personal TODD.² The lower limit of major pneumoconiosis ILO category 1 was reached by the average non-smoking worker at the age of 60 after a TODD of 85 mg/m³-years, that is after being exposed for 40 years to 2.1 mg/m³, and by the average smoking

worker after a TODD of 65 mg/m³·years, that is after exposure for 40 years to 1.6 mg/m³. These TODD levels are for the ‘average worker’ only, and from such levels a NOAEL or a LOAEL cannot be established. Nevertheless, the committee considers exposure to 2.1 mg/m³ respirable kaolin dust for 40 years undesired as ILO category 1 is undesired and the development of ILO category 1 in the average worker may involve a large number of workers.

In the WHO Environmental Health Criteria 231 report¹⁵, an international group of experts estimated that kaoline is at least an order of magnitude less potent than quartz in inducing pneumoconiosis. This conclusion was based on a comparison of the Rundle data with the estimated ranges of silicosis prevalence as a consequence of exposure to quartz: 2-30% of ILO category $\geq 1/1$ silicosis after a 45-year exposure to quartz at 0.05 mg/m³ (*i.e.*, a cumulative exposure to 2.25 mg/m³·years); and 3-90% of ILO category $\geq 1/1$ silicosis after a 45-year exposure to quartz at 0.10 mg/m³ (*i.e.*, a cumulative exposure to 4.5 mg/m³·years). The health-based recommended occupational exposure limit (HBROEL) for respirable quartz is 0.075 mg/m³, as an 8-hour time-weighted average.⁵⁵

Several studies in animals support the respiratory effects observed in humans. Three studies were found that indicated that kaolin has fibrogenic activity and is able to induce an inflammatory response and fibrosis in the lungs of rats and mice. Based on the available studies, it is the committee’s opinion that there is insufficient evidence for carcinogenicity and no evidence for teratogenicity of kaolin, and that kaolin does not need to be classified as a carcinogen or as a substance with reproductive toxicity.

9.2 Recommendation of the health-based occupational exposure limit

The committee concludes that the toxicological database does not contain any studies from which a NOAEL or a LOAEL can be established. Therefore, the database does not allow the derivation and recommendation of a health-based occupational exposure limit for kaolin.

9.3 Additional consideration

Although the mildest form of pneumoconiosis is not yet associated with a decrease of pulmonary function, the committee considers ILO category 1 an undesired health effect in line with the European Committee report on the classification of miners’ pneumoconiosis.¹

The results of the Rundle study² indicate that cumulative exposure to 85 mg/m³-years of respirable kaolin dust is able to induce simple pneumoconiosis ILO category 1 in the average non-smoking worker at the age of 60, that is after 40 years of exposure to 2.1 mg/m³. According to the committee, this exposure level may affect a large number of workers and constitutes an undesired health risk. Therefore, the HBROEL for respirable kaolin dust must be lower than 2.1 mg/m³. On the other hand, kaolin is estimated to be an order of magnitude less potent than quartz in inducing pneumoconiosis. This indicates that the HBROEL for respirable kaolin dust is approximately ten-fold the HBROEL for respirable quartz dust. Taking these considerations into account, it is the committee's opinion that an occupational exposure level of 0.5 mg/m³ for respirable kaolin dust, as an 8-hour time-weighted average concentration, is not associated with pneumoconiosis and might be used as an upper exposure limit for workers.

The committee adds that this consideration is only applicable to kaolin with the particle size distribution and the amount of quartz (typically $\leq 2\%$ w/w)⁴ as present in the UK china clay industry at the time of the measurements.

9.4 Groups at extra risk

Based on the Rundle study², smokers are at higher risk than non-smokers. Another group at higher risk may be workers with decreased lung function or chronic lung disease.

Recommendations for research

The composition including the particle size distribution and the quartz content of kaolin dust in the workplace needs to be defined more accurately in future studies. Accurate exposure data including personal air monitoring and quartz analysis, and objectively obtained effect data should be used to obtain more and better exposure-response relationships. Predisposed workers, like asthmatics, should be distinguished in future studies.

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A	Request for advice
B	The committee
C	Comments on the public review draft
D	Classification of pneumoconiosis
E	Occupational exposure to kaolin: case studies
F	Occupational exposure to kaolin: epidemiological studies without exposure data
G	Occupational exposure to kaolin: epidemiological studies with exposure data
H	Abbreviations

Annexes

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advise the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as

part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the committee is given in Annex B.

The committee

-
- G.J. Mulder, *chairman*
emeritus professor of toxicology, Leiden University, Leiden
 - R.B. Beems
toxicologic pathologist, National Institute for Public Health and the Environment, Bilthoven
 - P.J. Boogaard
toxicologist, Shell International BV, The Hague
 - J.J.A.M. Brokamp, *advisor*
Social and Economic Council, The Hague
 - D.J.J. Heederik
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 - L.A.L.M. Kiemeny
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 - H. van Loveren
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The first draft of this report was prepared in 2005 by R.H.M.M. Schreurs and W.M.L.G. Gubbels-Van Hal from NOTOX BV, 's-Hertogenbosch, The Netherlands, by contract with the Dutch Health Council.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship

will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Comments on the public review draft

A draft of the present report was released in 2007 for public review. The following organisations and persons have commented on the draft report:

- E. González-Fernández, Ministerio de Trabajo y Asuntos Sociales, Spain
- E.L.J. van Hal and J.A.J. Kemps, Koninklijk Verbond van Nederlandse Baksteenfabrikanten, Nederlandse Dakpannencorporatie en Algemene Vereniging voor de Nederlandse Aardewerkindustrie, The Netherlands
- R.T.H. van Welie, Nederlandse Cosmetica Vereniging, The Netherlands
- R.D. Zumwalde, National Institute for Occupational Safety and Health, USA

D

Classification of pneumoconiosis

A series of guidelines on the classification of chest X-ray films (radiographs) for persons with pneumoconiosis has been published by the International Labour Office (ILO) since 1950. The goal of this process was to describe and codify the radiographic abnormalities of pneumoconiosis in a simple, reproducible manner.

In the present manifestation of the ILO system (2002)⁵⁶, the reader is first asked to grade film quality. Parenchymal abnormalities are then divided in small opacities (simple pneumoconiosis) and large opacities (complicated pneumoconiosis). Pleural abnormalities (pleural thickening or pleural calcification) are also assessed.

Small opacities (simple pneumoconiosis) are characterized according to:

- shape and size:
round opacities: p (< 1.5 mm), q (1.5 – 3 mm), r (3 – 10 mm)
irregular opacities: s (< 1.5 mm), t (1.5 – 3 mm), u (3 – 10 mm)
- profusion (frequency):
classification according to a 12-point scale into one of four major categories (0, 1, 2 or 3); if during the reading the major category above or below is seriously considered as an alternative, this is recorded as the second digit.

Normal (category 0)	0/-	0/0	0/1
Mild (category 1)	1/0	1/1	1/2
Moderate (category 2)	2/1	2/2	2/3
Severe (category 3)	3/2	3/3	3/+

- zone:
the lung zones in which the opacities occur: RU (right upper), RM (right middle), RL (right lower), LU (left upper), LM (left middle), LL (left lower)

Large opacities (complicated pneumoconiosis) are classified as:

- **category A:**
greatest diameter exceeding 1 cm and up to 5 cm; or several opacities each greater than 1 cm with the sum of the diameters not exceeding 5 cm;
- **category B:**
one or more large opacities with combined diameter greater than 5 cm but not exceeding the equivalent of the right upper zone;
- **category C:**
one or more large opacities whose combined area exceeds the equivalent of the right upper zone.

E

Occupational exposure to kaolin: case studies

Studied population	Findings	Ref
2 kaolin workers from Georgia, USA a) 23 years in milling area b) 17 years in loading area	Both had large areas of fibrosis in the lungs and kaolinite particles were identified in alveolar macrophages.	5
5 kaolin workers from Georgia, USA age 37-84; 3 had died	Cases had complicated pneumoconiosis, showing small irregular opacities, conglomerate masses and multiple large, well-delineated opacities on the chest radiograph (ILO classification 1980). Respiratory failure was a contributing factor to death in two of the subjects. Particles in the lung were kaolinite.	14
54 Cornish kaolin workers (post mortem 1968-1981) a) china clay group (n=22; 17-55 years of exposure); lung specimens (n=16): ≥90% kaolinite, ≤1.0% quartz; b) miscellaneous group (n=10; 10-52 years of exposure); lung specimens (n=10): 33-91% kaolinite, ≤5% quartz;	Radiographs: available from 26 cases (ILO classification 1971). Smoking histories: unavailable. a) radiographs (n=21) showed simple (n=11) and complicated (n=10) pneumoconiosis; round and irregular radiological opacities and pathologically slight to severe nodular and interstitial fibrosis. Dust concentrations in the lungs: 7.6-289.3 (median 40) mg per gram lung tissue. b) radiographs (n=5) showed 3 cases of simple pneumoconiosis (irregular opacities); pathologically slight to moderate nodular and interstitial fibrosis. Dust concentrations in the lungs: 1.6-28.7 (median 6.5) mg per gram lung tissue. Nodular fibrosis was related to a high quartz content of the lung dust (statistically significant); interstitial fibrosis seemed to be related to a high kaolinite content of lung dust (not statistically significant).	57
2 U.S. kaolin processing workers Both had died at age 35-36 with 17-21 years of employment.	Both had advanced pneumoconiosis, one with pulmonary thrombosis and infarct of the lung; massive nodular fibrosis was observed; one had symptoms of dyspnoea, cough, sputum and ankle oedema.	58
4 Scottish shale miners Age 69-83; 32-49 years of employment; 2 had died.	All cases developed complicated pneumoconiosis, and 2 were found at autopsy also to have peripheral squamous lung cancer. The lung dust in 3 cases was found to contain kaolinite (19-36%), mica (13-16%), and quartz (9-18%).	59
6 workers from a kaolin processing plant in Ayyat, United Arab Republic Age 46-57; 15-25 years of employment.	Chest radiographs (ILO classification 1950) showed simple pneumoconiosis (n=3 category 1, 2 and 3). Two of the three with complicated pneumoconiosis and symptoms (dyspnoea on exertion, cough with sputum) showed progressive massive fibrosis.	60

Occupational exposure to kaolin: epidemiological studies without exposure data

Studied population	Data collected	Findings	Remarks	Ref
1130 current kaolin workers (Georgia, USA)	Chest radiographs, medical and occupational histories, physical examination.	Radiographs showed 44 workers (prevalence 3.9%) with pneumoconiosis. Stage I pneumoconiosis (n=31): fine discrete nodulation in lungs; no symptoms; no progression over 3-12 years. Stage II pneumoconiosis (n=7): fine discrete nodulation in lungs; no symptoms; little tendency to progression. Stage III pneumoconiosis (n=6) nodulation with massive conglomerate fibrosis in the lungs (upper lobes); cough and dyspnea; gradual progression over the years (emphysema). Exposure time > 20 years.	- Data on smoking not available. - Exposure times from workers in stage I and II are unknown.	5
350 current kaolin workers (Georgia, USA)	Chest radiographs (ILO classification 1980), medical and employment records, symptoms and occupational history (questionnaire)	Abnormal radiographs (n=32) were clinically evaluated. Nineteen workers had pulmonary changes consistent with kaolinitis (prevalence 5.4%). Computed tomography of the chest in 12 subjects (exposure time 12-35 years) proved kaolinitis (11 mild, 1 complicated), but without evident pleural abnormalities on radiographs.	- No dry-processing workers included. - Most workers were employed in kaolin products manufacturing, after appearance of strict controls on respiratory dust levels	61
553 current kaolin workers (Cornwall, UK)	Chest radiographs (ILO classification 1959), occupational history, symptoms.	Total kaolinitis prevalence: 9% (48 cases: 36 simple, 12 complicated); < 5 years of exposure: 0% < 15 years of exposure: 4% > 25 years of exposure: 19% men < 25 years of age: 0% men > 65 years of age: 24%	- > 5 years of continuous (millers, baggers, loaders) or intermittent (kiln workers, drymen) exposure to clay dust. - No smoking history available.	10
1676 current kaolin workers (Cornwall, UK)	Chest radiographs (ILO classification 1980), questionnaire (symptoms, smoking history), occupational history, spirometry (FVC, FEV ₁).	Pneumoconiosis: category 0: 77.4% category 1: 17.9% categories 2 and 3: 4.7% Advanced pneumoconiosis was detected in 19 workers (1.1%). FVC showed a small significant reduction with increasing radiological score No relationship between symptoms and past exposure to kaolin was detected.	- Exclusively exposed to kaolin. - No statistical <i>p</i> -values were calculated - Radiological score was unrelated to smoking, but related to the period spent in dusty jobs, and to age - Exposure times unknown.	62
914 workers from a kaolin processing plant in Ayyat, United Arab Republic (1959)	Chest radiographs (ILO classification 1950), occupational history, symptoms.	Pneumoconiosis prevalence: < 10 years (n=264) exposure: 0% 10-15 years (n=133) exposure: 0% 15-20 years (n=326) exposure: 1.2% with symptoms: dyspnoea on exertion, cough with sputum in 2 cases. > 20 years (n=191) exposure: 1% with progressive massive fibrosis and symptoms (cough, sputum and dyspnoea).	Dust from the mill contained 83-86% K ₄ Al ₂ Si ₂ O ₉ and 1-2% quartz. Particles < 3.4 µm	60

G

Occupational exposure to kaolin: epidemiological studies with exposure data

Studied population	Kaolin exposure	Data collected	Findings	Remarks	Ref
459 current kaolin mine and processing workers (Georgia, USA)	Current exposure (1977-1980): Respirable kaolin dust: <5 mg/m ³ (<1% quartz) Mean exposure: 12 years. Exposure in the past: 1951: 377 mg/m ³ 1960: 361 mg/m ³	Chest X-ray films (ILO classification 1971), spirometry (FEV ₁ , FVC), questionnaire (symptoms, smoking history), occupational history.	Simple pneumoconiosis prevalence: Category 0: 90.9% (white 93.0%, black 86.4%) Category 1: 6.3% (white 5.1%, black 8.9%) Category 2: 1.8% (white 1.3%, black 2.7%) Category 3: 1.1% (white 0.7%, black 2.0%) Complicated pneumoconiosis: 1.7% (n=8) In most cases fine or medium irregular opacities. Age >50 years ($p<0.005$) and exposure > 15 years ($p<0.001$) significantly related to pneumoconiosis. Lower FEV ₁ and FVC only in cases of complicated pneumoconiosis. More frequent dyspnea in cases of pneumoconiosis.	No unexposed control group. No exposure data from different job categories. Type of sampling (area or personal) unknown.	17
47 current and 16 former kaolin mine and mill workers (Georgia, USA)	96% kaolinite, 4% TiO ₂ and 0% quartz. <u>Respirable dust</u> (pers.): Mine: 0.1-0.35 mg/m ³ Loader: 0.7-1.2 mg/m ³ Bin: 1.3-2.5 mg/m ³ Mill: 0.8-1.2 mg/m ³ Baghouse: 0.3-5 mg/m ³ <u>Total dust</u> (area): Loader: 7-15 mg/m ³ Bin: 10-30 mg/m ³ Mill: 10-80 mg/m ³ Baghouse: 1.5-9 mg/m ³	Chest X-ray films (ILO classification 1971), spirometry (FEV ₁ , FVC, peak flow rate), questionnaire (symptoms, smoking and occupational history)	Pneumoconiosis prevalence: - Current workers <5 years exposure (n=8): 0% - Current workers ≥5 years exposure (n=39): 4/39 simple (15-39 years exposure) and 1/39 complicated pneumoconiosis (35 years exposure) - Former workers ≥5 years exposure (n=16): 3/16 complicated pneumoconiosis (26-35 years exposure) Small rounded opacities. Seven cases were mill workers, one was miner. All were (ex-)smokers. Abnormal spirometric values in three cases. Reduced spirometric values in kaolin workers compared to controls ($p<0.05$).	No control group data. No data on different exposure groups. No historical exposure data. Small group sizes.	11
189 external controls.					
65 current kaolin mine and processing workers (Georgia, USA)	94-98% kaolinite, 2-6% TiO ₂ and 0% quartz. Respirable dust (pers.): Mine: 0.1-0.8 mg/m ³ Maintenance area: 0.1-0.9 mg/m ³ Processing area: 1.7-5.3 mg/m ³	Chest X-ray films (ILO classification 1980), spirometry (FEV ₁ , FVC), questionnaire (symptoms, smoking and occupational history)	Simple pneumoconiosis: 4/25 processing workers (7-13 years exposure). Complicated pneumoconiosis: 1/25 (36 years exposure). All five had symptoms of bronchitis, and mildly lower spirometric values ($p<0.05$) compared to other processing workers.	No control group. Small group sizes. No historical exposure data.	12

3400 current and 289 former kaolin workers (UK).	Average respirable dust (pers.) (1984-1986): Dryers: 1.9 mg/m ³ Mills: 2.7 mg/m ³ Calciners: 2.5 mg/m ³ Slurry plants: 1.1 mg/m ³ Tube press: 0.5 mg/m ³	Chest X-ray films (ILO classification 1980), spirometry (FEV ₁ , FVC), questionnaire (symptoms, smoking history)	Simple pneumoconiosis prevalence: Category 0: 3374/3689 (91.5%) (age: ≤20-80) Category 1: 271/3689 (7.3%) (age: 21-80) Category 2: 39/3689 (1.1%) (age: 41-80) Category 3: 5/3689 (0.1%) (age: 51-80) Complicated pneumoconiosis: 16/3689 (0.4%) Both rounded and irregular small opacities were observed. Pleural thickening (n=87) and calcification (n=17) was also noted. Ventilatory capacity was related to age and radiological score. The extent of phlegm, cough, wheeze and breathlessness was dependent on the level of ventilatory capacity.	No control group. No historical exposure data. 99/123 subjects with little or no exposure had pneumoconiosis category 1 or higher. Quartz content and non-kaolin dust content unknown.	16
4112 current and former kaolin workers (UK).	Average respirable dust (pers.): (1978) Dryers: 3.5 mg/m ³ Mills: 4.7 mg/m ³ Slurry plants: 1.6 mg/m ³ (1990) Dryers: 1.7 mg/m ³ Mills: 2.1 mg/m ³ Slurry plants: 1.2 mg/m ³	Chest X-ray films (ILO classification 1980), spirometry (FEV ₁ , FVC), questionnaire (symptoms, smoking and occupational history)	Simple pneumoconiosis prevalence: Category 0: 3699/4112 (90%) (age: <20-80) Category 1: 378/4112 (9.2%) (age: 21-80) Category 2: 33/4112 (0.75%) (age: 41-80) Category 3: 2/4112 (0.05%) (age: 51-70) Complicated pneumoconiosis: 2/4112 (0.05%) Both rounded and irregular small opacities were observed. Pleural thickening (n=39) and calcification (n=9) was also noted. Working in dusty jobs had greatest effect on X-ray score. Ventilatory capacity was related to age and radiological score. The incidence of symptoms was related to the level of ventilatory capacity.	No historical exposure data. 136 subjects with no exposure had pneumoconiosis category 1 or higher. Quartz content and non-kaolin dust content unknown.	2

Abbreviations

General terms

<i>bw</i>	body weight
<i>FEV₁</i>	forced expiratory volume at 1 second
<i>FVC</i>	forced vital capacity
<i>HBROEL</i>	health-based recommended occupational exposure limit
<i>LOAEL</i>	lowest observed adverse effect level
<i>NOAEL</i>	no observed adverse effect level
<i>OEL</i>	occupational exposure limit
<i>OES</i>	occupational exposure standard
<i>PEL</i>	permissible exposure limit
<i>REL</i>	Recommended exposure limit
<i>t_{gg}</i>	tijdgewogen gemiddelde
<i>TLV</i>	threshold limit value
<i>TODD</i>	total occupational dust dose
<i>TWA</i>	time-weighted average
<i>WEL</i>	workplace exposure limit

Organisations

<i>ACGIH</i>	American Conference of Governmental Industrial Hygienists
<i>DECOS</i>	Dutch Expert Committee on Occupational Standards
<i>DFG</i>	Deutsche Forschungsgemeinschaft
<i>HSE</i>	Health and Safety Executive
<i>IARC</i>	International Agency for Research on Cancer
<i>ILO</i>	International Labour Office
<i>INRS</i>	Institut National de Recherche et de Sécurité
<i>NIOSH</i>	National Institute for Occupational Safety and Health
<i>OSHA</i>	Occupational Safety and Health Administration
<i>RTECS</i>	Registry of Toxic Effects of Chemical Substances
<i>SER</i>	Social and Economic Council
<i>WHO</i>	World Health Organisation

Statistical terms

<i>RC</i>	regression coefficient
<i>SE</i>	standard error

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme, the International Labour Organization, or the World Health Organization.

Environmental Health Criteria 231

BENTONITE, KAOLIN, AND SELECTED CLAY MINERALS

First draft prepared by Dr Zoltán Adamis, József Fodor National Center for Public Health, National Institute of Chemical Safety, Budapest, Hungary; and Dr Richard B. Williams, US Environmental Protection Agency, Washington, DC, and Regional Office for the Americas of the World Health Organization

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The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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CONTENTS

ENVIRONMENTAL HEALTH CRITERIA FOR BENTONITE, KAOLIN, AND SELECTED CLAY MINERALS

PREAMBLE	viii
ACRONYMS AND ABBREVIATIONS	xvi
1. SUMMARY	1
1.1 Identity, physical and chemical properties, and analytical methods	1
1.2 Sources of human and environmental exposure	2
1.3 Environmental levels and human exposure	2
1.4 Kinetics and metabolism in laboratory animals and humans	3
1.5 Effects on laboratory mammals and <i>in vitro</i> test systems	4
1.6 Effects on humans	5
1.7 Effects on other organisms in the laboratory and field	6
1.8 Evaluation of human health risks and effects on the environment	6
2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, AND ANALYTICAL METHODS	8
2.1 Introduction	8
2.2 Identity	9
2.2.1 Bentonite	9
2.2.2 Kaolin	11
2.2.3 Other clays	12
2.3 Physical and chemical properties	14
2.3.1 Bentonite	14
2.3.2 Kaolin	15
2.3.3 Other clays	15
2.3.4 Surface chemistry	17
2.3.5 Trace elements in clays	18
2.4 Analytical methods	19
2.4.1 Quantitative measurement of dust	19
2.4.2 Identification of phyllosilicates	20

3. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE	23
3.1 Natural occurrence	23
3.1.1 Bentonite	23
3.1.2 Kaolin	23
3.1.3 Other clays	24
3.2 Anthropogenic sources	25
3.2.1 Production levels and sources	25
3.2.1.1 Bentonite	25
3.2.1.2 Kaolin	27
3.2.1.3 Other clays	30
3.2.2 Uses	31
3.2.2.1 Bentonite	31
3.2.2.2 Kaolin	34
3.2.2.3 Other clays	37
4. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE	40
4.1 General population exposure	40
4.1.1 Bentonite	40
4.1.2 Kaolin and other clays	40
4.2 Occupational exposure	42
4.2.1 Bentonite	42
4.2.2 Kaolin	45
4.2.3 Other clays	54
5. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS	56
6. EFFECTS ON LABORATORY MAMMALS AND <i>IN VITRO</i> TEST SYSTEMS	59
6.1 Single exposure	59
6.1.1 Bentonite	59
6.1.2 Kaolin	70
6.1.2.1 Intratracheal administration	70
6.1.2.2 Parenteral administration	77
6.1.3 Other clays	77
6.2 Repeated exposure	78

6.2.1	Bentonite	78
6.2.2	Kaolin	82
6.3	Genotoxicity	83
6.4	Reproductive effects	83
6.5	Administration with other agents	84
6.5.1	Kaolin and microbes and microbe-derived factors	84
6.5.2	Kaolin and quartz	85
6.6	<i>In vitro</i> test systems	85
6.6.1	Bentonite	85
6.6.2	Kaolin	97
6.6.2.1	Haemolysis	97
6.6.2.2	Macrophage studies	108
6.6.2.3	Other tissue cultures and <i>in vitro</i> systems	109
6.6.3	Other clays	110
6.6.4	Relationships between <i>in vivo</i> and <i>in vitro</i> studies	110
6.7	Summary of the effects of quartz	110
7.	EFFECTS ON HUMANS	112
7.1	General population	112
7.2	Occupational exposure	112
7.2.1	Bentonite	112
7.2.2	Kaolin	118
7.2.2.1	Kaolin workers, United Kingdom	119
7.2.2.2	Kaolin workers, USA	128
7.2.2.3	Kaolin workers, other countries	130
7.2.2.4	Brick and tile workers and others	130
7.3	Summary of the effects of quartz	131
8.	EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD	133
9.	EVALUATION OF HUMAN HEALTH RISKS AND EFFECTS ON THE ENVIRONMENT	135
9.1	Evaluation of human health risks	135
9.2	Evaluation of effects on the environment	137

10. CONCLUSIONS AND RECOMMENDATIONS FOR PROTECTION OF HUMAN HEALTH AND THE ENVIRONMENT	138
11. FURTHER RESEARCH	139
12. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES	140
REFERENCES	141
RESUME	160
RESUMEN	168

NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

Environmental Health Criteria

PREAMBLE

Objectives

In 1973 the WHO Environmental Health Criteria Programme was initiated with the following objectives:

- (i) to assess information on the relationship between exposure to environmental pollutants and human health, and to provide guidelines for setting exposure limits;
- (ii) to identify new or potential pollutants;
- (iii) to identify gaps in knowledge concerning the health effects of pollutants;
- (iv) to promote the harmonization of toxicological and epidemiological methods in order to have internationally comparable results.

The first Environmental Health Criteria (EHC) monograph, on mercury, was published in 1976, and since that time an ever-increasing number of assessments of chemicals and of physical effects have been produced. In addition, many EHC monographs have been devoted to evaluating toxicological methodology, e.g., for genetic, neurotoxic, teratogenic and nephrotoxic effects. Other publications have been concerned with epidemiological guidelines, evaluation of short-term tests for carcinogens, biomarkers, effects on the elderly and so forth.

Since its inauguration the EHC Programme has widened its scope, and the importance of environmental effects, in addition to health effects, has been increasingly emphasized in the total evaluation of chemicals.

The original impetus for the Programme came from World Health Assembly resolutions and the recommendations of the 1972 UN Conference on the Human Environment. Subsequently the work became an integral part of the International Programme on Chemical Safety (IPCS), a cooperative programme of UNEP, ILO and WHO. In this manner, with the strong support of the new partners, the importance of occupational health and environmental effects was fully recognized. The EHC monographs have become widely established, used and recognized throughout the world.

The recommendations of the 1992 UN Conference on Environment and Development and the subsequent establishment of the Intergovernmental Forum on Chemical Safety with the priorities for action in the six programme areas of Chapter 19, Agenda 21, all lend further weight to the need for EHC assessments of the risks of chemicals.

Scope

The criteria monographs are intended to provide critical reviews on the effect on human health and the environment of chemicals and of combinations of chemicals and physical and biological agents. As such, they include and review studies that are of direct relevance for the evaluation. However, they do not describe *every* study carried out. Worldwide data are used and are quoted from original studies, not from abstracts or reviews. Both published and unpublished reports are considered, and it is incumbent on the authors to assess all the articles cited in the references. Preference is always given to published data. Unpublished data are used only when relevant published data are absent or when they are pivotal to the risk assessment. A detailed policy statement is available that describes the procedures used for unpublished proprietary data so that this information can be used in the evaluation without compromising its confidential nature (WHO (1990) Revised Guidelines for the Preparation of Environmental Health Criteria Monographs. PCS/90.69, Geneva, World Health Organization).

In the evaluation of human health risks, sound human data, whenever available, are preferred to animal data. Animal and *in vitro* studies provide support and are used mainly to supply evidence missing from human studies. It is mandatory that research on human subjects is conducted in full accord with ethical principles, including the provisions of the Helsinki Declaration.

The EHC monographs are intended to assist national and international authorities in making risk assessments and subsequent risk management decisions. They represent a thorough evaluation of risks and are not, in any sense, recommendations for regulation or standard setting. These latter are the exclusive purview of national and regional governments.

Content

The layout of EHC monographs for chemicals is outlined below.

- Summary — a review of the salient facts and the risk evaluation of the chemical
- Identity — physical and chemical properties, analytical methods
- Sources of exposure
- Environmental transport, distribution and transformation
- Environmental levels and human exposure
- Kinetics and metabolism in laboratory animals and humans
- Effects on laboratory mammals and *in vitro* test systems
- Effects on humans
- Effects on other organisms in the laboratory and field
- Evaluation of human health risks and effects on the environment
- Conclusions and recommendations for protection of human health and the environment
- Further research
- Previous evaluations by international bodies, e.g., IARC, JECFA, JMPR

Selection of chemicals

Since the inception of the EHC Programme, the IPCS has organized meetings of scientists to establish lists of priority chemicals for subsequent evaluation. Such meetings have been held in Ispra, Italy, 1980; Oxford, United Kingdom, 1984; Berlin, Germany, 1987; and North Carolina, USA, 1995. The selection of chemicals has been based on the following criteria: the existence of scientific evidence that the substance presents a hazard to human health and/or the environment; the possible use, persistence, accumulation or degradation of the substance shows that there may be significant human or environmental exposure; the size and nature of populations at risk (both human and other species) and risks for environment; international concern, i.e., the substance is of major interest to several countries; adequate data on the hazards are available.

If an EHC monograph is proposed for a chemical not on the priority list, the IPCS Secretariat consults with the Cooperating Organizations and all the Participating Institutions before embarking on the preparation of the monograph.

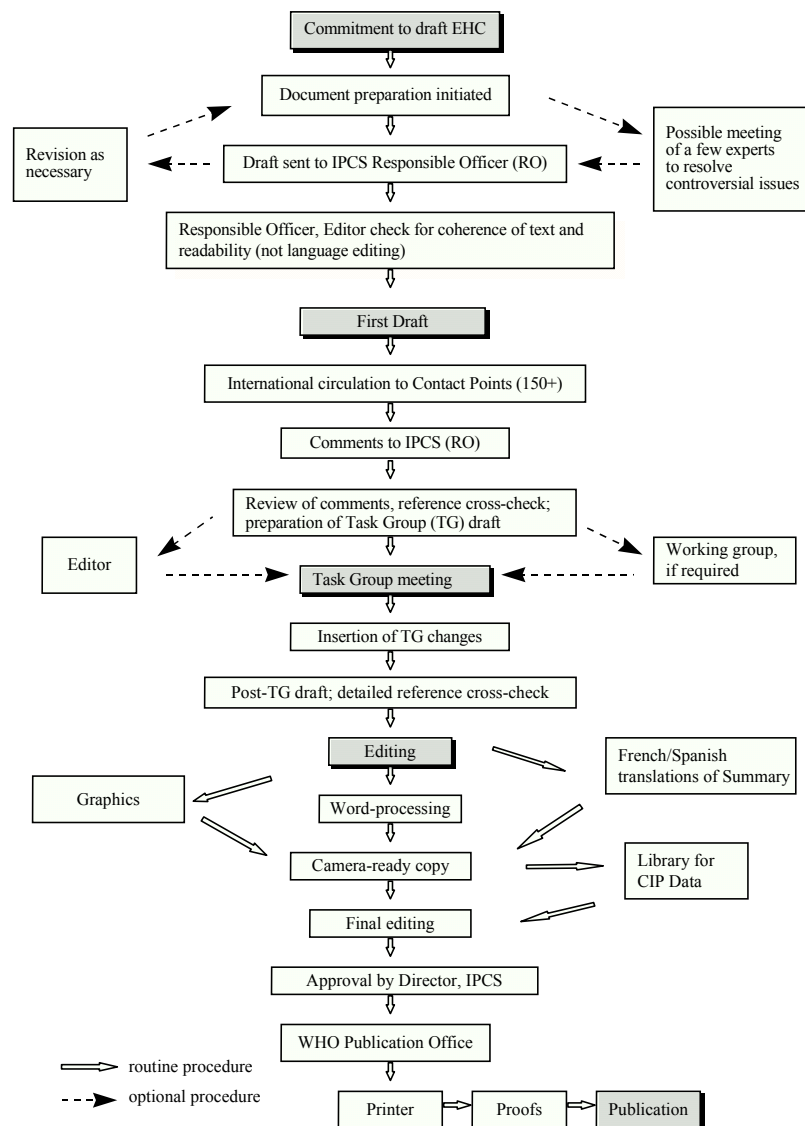
Procedures

The order of procedures that result in the publication of an EHC monograph is shown in the flow chart on p. xii. A designated staff member of IPCS, responsible for the scientific quality of the document, serves as Responsible Officer (RO). The IPCS Editor is responsible for layout and language. The first draft, prepared by consultants or, more usually, staff from an IPCS Participating Institution, is based on extensive literature searches from reference databases such as Medline and Toxline.

The draft document, when received by the RO, may require an initial review by a small panel of experts to determine its scientific quality and objectivity. Once the RO finds the document acceptable as a first draft, it is distributed, in its unedited form, to well over 150 EHC contact points throughout the world who are asked to comment on its completeness and accuracy and, where necessary, provide additional material. The contact points, usually designated by governments, may be Participating Institutions, IPCS Focal Points or individual scientists known for their particular expertise. Generally some four months are allowed before the comments are considered by the RO and author(s). A second draft incorporating comments received and approved by the Director, IPCS, is then distributed to Task Group members, who carry out the peer review, at least six weeks before their meeting.

The Task Group members serve as individual scientists, not as representatives of any organization, government or industry. Their function is to evaluate the accuracy, significance and relevance of the information in the document and to assess the health and environmental risks from exposure to the chemical. A summary and recommendations for further research and improved safety aspects are also required. The composition of the Task Group is dictated by the range of expertise required for the subject of the meeting and by the need for a balanced geographical distribution.

EHC PREPARATION FLOW CHART



The three cooperating organizations of the IPCS recognize the important role played by nongovernmental organizations. Representatives from relevant national and international associations may be invited to join the Task Group as observers. Although observers may provide a valuable contribution to the process, they can speak only at the invitation of the Chairperson. Observers do not participate in the final evaluation of the chemical; this is the sole responsibility of the Task Group members. When the Task Group considers it to be appropriate, it may meet *in camera*.

All individuals who as authors, consultants or advisers participate in the preparation of the EHC monograph must, in addition to serving in their personal capacity as scientists, inform the RO if at any time a conflict of interest, whether actual or potential, could be perceived in their work. They are required to sign a conflict of interest statement. Such a procedure ensures the transparency and probity of the process.

When the Task Group has completed its review and the RO is satisfied as to the scientific correctness and completeness of the document, it then goes for language editing, reference checking and preparation of camera-ready copy. After approval by the Director, IPCS, the monograph is submitted to the WHO Office of Publications for printing. At this time a copy of the final draft is sent to the Chairperson and Rapporteur of the Task Group to check for any errors.

It is accepted that the following criteria should initiate the updating of an EHC monograph: new data are available that would substantially change the evaluation; there is public concern for health or environmental effects of the agent because of greater exposure; an appreciable time period has elapsed since the last evaluation.

All Participating Institutions are informed, through the EHC progress report, of the authors and institutions proposed for the drafting of the documents. A comprehensive file of all comments received on drafts of each EHC monograph is maintained and is available on request. The Chairpersons of Task Groups are briefed before each meeting on their role and responsibility in ensuring that these rules are followed.

WHO TASK GROUP ON ENVIRONMENTAL HEALTH CRITERIA FOR BENTONITE, KAOLIN, AND SELECTED CLAY MINERALS

A WHO Task Group on Environmental Health Criteria for Bentonite, Kaolin, and Selected Clay Minerals met at Bradford University, Bradford, United Kingdom, from 18 to 22 October 2004. The meeting was opened by Professor Jeffrey Lucas on behalf of the University of Bradford and Dr A. Aitio, Programme for the Promotion of Chemical Safety, WHO, on behalf of the IPCS and its three cooperative organizations (UNEP/ILO/WHO). The Task Group reviewed and revised the draft monograph and made an evaluation of the risks for human health and the environment from exposure to bentonite, kaolin, and other clays.

The first draft was prepared by Dr Zoltán Adamis from the József Fodor National Center for Public Health, National Institute of Chemical Safety, Budapest, Hungary, and Dr Richard B. Williams from the US Environmental Protection Agency, Washington, DC, and Regional Office for the Americas of the World Health Organization. The second draft was also prepared by the same authors in collaboration with the secretariat, which incorporated comments received following the circulation of the first draft to the IPCS contact points for Environmental Health Criteria monographs.

Dr A. Aitio was responsible for the overall scientific content of the monograph.

The efforts of all who helped in the preparation and finalization of the monograph are gratefully acknowledged.

* * *

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ACRONYMS AND ABBREVIATIONS

AM	alveolar macrophages
AMAD	activity median aerodynamic diameter
ATP	adenosine triphosphate
CAS	Chemical Abstracts Service
DTA	differential thermal analysis
EDXA	energy-dispersive X-ray analysis
EHC	Environmental Health Criteria monograph
FAO	Food and Agriculture Organization of the United Nations
FEV ₁	forced expiratory volume in 1 s
FVC	forced vital capacity
GRAS	generally regarded as safe
IARC	International Agency for Research on Cancer
ILO	International Labour Organization / International Labour Office
IPCS	International Programme on Chemical Safety
IU	international units
JECFA	Joint Expert Committee on Food Additives and Contaminants
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC ₅₀	median lethal concentration
LD ₅₀	median lethal dose
LDH	lactate dehydrogenase
meq	milliequivalent
MMAD	mass median aerodynamic diameter
mppcf	million particles per cubic foot
NIOSH	National Institute for Occupational Safety and Health (USA)
PAM	pulmonary alveolar macrophages
PMN	polymorphonuclear leukocytes
RO	Responsible Officer
SD	standard deviation
TTC	2,3,5-triphenyltetrazolium chloride
UN	United Nations
UNEP	United Nations Environment Programme
USA	United States of America
WHO	World Health Organization

1. SUMMARY

1.1 Identity, physical and chemical properties, and analytical methods

Bentonite is a rock formed of highly colloidal and plastic clays composed mainly of montmorillonite, a clay mineral of the smectite group, and is produced by *in situ* devitrification of volcanic ash. In addition to montmorillonite, bentonite may contain feldspar, cristobalite, and crystalline quartz. The special properties of bentonite are an ability to form thixotropic gels with water, an ability to absorb large quantities of water, and a high cation exchange capacity. The properties of bentonite are derived from the crystal structure of the smectite group, which is an octahedral alumina sheet between two tetrahedral silica sheets. Variations in interstitial water and exchangeable cations in the interlayer space affect the properties of bentonite and thus the commercial uses of the different types of bentonite. By extension, the term bentonite is applied commercially to any clay with similar properties. Fuller's earth is often a bentonite.

Kaolin or china clay is a mixture of different minerals. Its main component is kaolinite; in addition, it frequently contains quartz, mica, feldspar, illite, and montmorillonite. Kaolinite is made up of tiny sheets of triclinic crystals with pseudohexagonal morphology. It is formed by rock weathering. It has some cation exchange capacity.

The main components of common clay and shale are illite and chlorite. Illite is also a component of ball clays. Illite closely resembles micas, but it has less substitution of aluminium for silicon and/or partial replacement of potassium ions between the unit layers by other cations, such as hydrogen, magnesium, and calcium.

Quantitative measurement of airborne dust containing aluminosilicates is most commonly gravimetric. The methods used for the identification and quantification of aluminosilicates include X-ray diffraction, electron microscopy, energy-dispersive X-ray analysis, differential thermal analysis, electron diffraction, and infrared spectroscopy.

1.2 Sources of human and environmental exposure

Montmorillonite is ubiquitous at low concentrations in soil, in the sediment load of natural waters, and in airborne dust. Biodegradation and bioaccumulation in the food-chain appear minimal, if they occur at all, and abiotic degradation of bentonite into other minerals takes place only on a geological time scale.

Major uses of bentonite include binding foundry sand in moulds; absorbing grease, oil, and animal wastes; pelletizing taconite iron ore; and improving the properties of drilling muds. Speciality uses include serving as an ingredient in ceramics; waterproofing and sealing in civil engineering projects, such as landfill sites and nuclear waste repositories; serving as a filler, stabilizer, or extender in adhesives, paints, cosmetics, and medicines, as a carrier in pesticides and fertilizers, and as a bonding agent in animal feeds; clarifying wine and vegetable oil; and purifying wastewater. The uses of montmorillonite-type Fuller's earth overlap those of bentonite.

Use of kaolin dates back to the third century BC in China. Today it is mined and used in significant quantities for numerous industrial uses. Its most important use is in paper production, where it is used as a coating material. In addition, it is used in great quantities in the paint, rubber, plastic, ceramic, chemical, pharmaceutical, and cosmetics industries.

Common clay and shale, of which illite is often a major component, are used mainly in the manufacture of extruded and other bricks, portland and other cements, concrete blocks and structural concrete, and refractories. Highway surfacing, ceramic tiles, and ceramics and glass are other important uses.

1.3 Environmental levels and human exposure

In view of the widespread distribution of bentonite in nature and its use in an enormous variety of consumer products, general population exposure to low concentrations is ubiquitous.

There is limited information on occupational exposure to bentonite dust in mines, processing plants, and user industries. The highest reported values for total dust and respirable dust concentrations were, respectively, 1430 and 34.9 mg/m³, although most values were below 10 mg/m³ for total dust and below 5 mg/m³ for respirable dust.

Kaolin is a natural component of the soil and occurs widely in ambient air. Kaolin mining and refining involve considerable exposure, and significant exposure is also expected in paper, rubber, and plastic production. Quantitative information on occupational exposure is available for a few countries and industries only. Respirable dust concentrations in kaolin mining and processing are usually below 5 mg/m³.

1.4 Kinetics and metabolism in laboratory animals and humans

No information was available on the kinetics or metabolism of montmorillonite, kaolinite, or illite as they occur in most occupational settings.

Deposition and kinetics of radiolabelled fused montmorillonite after inhalation exposure have been studied in mice, rats, dogs, and humans. Deposition in the nasopharynx increases with particle size and is lower in dogs than in rodents. Tracheobronchial deposition was low and independent of animal species and particle size. Pulmonary deposition was considerably higher in dogs than in rodents and decreased with increasing particle size.

The removal of particles from the lungs took place by solubilization *in situ* and by physical clearance. In dogs, the main mechanism of clearance was solubilization; in rodents, the mechanism of removal was mainly physical transportation. The clearance by mechanical removal was slow, especially in dogs: the half-time was initially 140 days and increased to 6900 days by day 200 post-exposure.

In humans, there was a rapid initial clearance of 8% and 40% of aluminosilicate particles that were, respectively, 1.9 and 6.1 µm in aerodynamic diameter from the lung region over 6 days. Thereafter, 4% and 11% of the two particle sizes were removed following a half-time of 20 days, and the rest with half-times of 330 and 420 days.

Ultrafine particles (<100 nm) have a high deposition in the nasal area; they can penetrate the alveolar/capillary barrier.

1.5 Effects on laboratory mammals and *in vitro* test systems

An important determinant of the toxicity of clays is the content of quartz. The presence of quartz in the clays studied hampers reliable independent estimation of the fibrogenicity of other components of clays.

Single intratracheal injection into rodents of bentonite and montmorillonite with low content of quartz produced dose- and particle size-dependent cytotoxic effects, as well as transient local inflammation, the signs of which included oedema and, consequently, increased lung weight. Single intratracheal exposures of rats to bentonite produced storage foci in the lungs 3–12 months later. After intratracheal exposure of rats to bentonite with a high quartz content, fibrosis was also observed. Bentonite increased the susceptibility of mice to pulmonary infection.

There are limited data on the effects of multiple exposures of experimental animals to montmorillonite or bentonite. Mice maintained on diets containing 10% or 25% bentonite but otherwise adequate to support normal growth displayed slightly reduced growth rates, whereas mice maintained on a similar diet with 50% bentonite showed minimal growth and developed fatty livers and eventually fibrosis of the liver and benign hepatomas (see below).

In vitro studies of the effects of bentonite on a variety of mammalian cell types usually indicated a high degree of cytotoxicity. Concentrations below 1.0 mg/ml of bentonite and montmorillonite particles less than 5 µm in diameter caused membrane damage and even cell lysis, as well as functional changes in several types of cells. The velocity and degree of lysis of sheep erythrocytes were dose dependent.

Kaolin instilled intratracheally produces storage foci, foreign body reaction, and diffuse exudative reaction. After high doses of kaolin (containing 8–65% quartz), fibrosis has been described in some studies, whereas at lower kaolin doses, no fibrosis has been observed in the few available studies.

Information is very limited on the toxicity of illite and non-existent on the toxicity of other components of other commercially important clays. Intratracheally instilled illite with unknown quartz content induced alveolar proteinosis, increased lung weight, and caused collagen synthesis. Illite had limited cytotoxicity towards peritoneal macrophages and was haemolytic *in vitro*.

No adequate studies are available on the carcinogenicity of bentonite. In an inhalation study and in a study using intrapleural injection, kaolin did not induce tumours in rats. No studies are available on the genotoxicity of clays.

Single, very limited studies did not demonstrate developmental toxicity in rats after oral exposure to bentonite or kaolin.

1.6 Effects on humans

General population exposure to low concentrations of montmorillonite and kaolinite, the main components of bentonite and kaolin, respectively, and other clay minerals is ubiquitous. There is no information on the possible effects of such low-level exposure.

Long-term occupational exposures to bentonite dust may cause structural and functional damage to the lungs. However, available data are inadequate to conclusively establish a dose-response relationship or even a cause-and-effect relationship due to limited information on period and intensity of exposure and to confounding factors, such as exposure to silica and tobacco smoke.

Long-term exposure to kaolin causes the development of radiologically diagnosed pneumoconiosis in an exposure-related fashion. Clear-cut deterioration of respiratory function and related symptoms have been reported only in cases with prominent radiological findings. The composition of the clay — i.e., quantity and quality of minerals other than kaolinite — is an important determinant of the effects.

Bentonite, kaolin, and other clays often contain quartz, and exposure to quartz is causally related to silicosis and lung cancer. Statistically significant increases in the incidence of or mortality from chronic bronchitis and pulmonary emphysema have been reported after exposure to quartz.

1.7 Effects on other organisms in the laboratory and field

Bentonite and kaolin have low toxicity to aquatic species, a large number of which have been tested.

1.8 Evaluation of human health risks and effects on the environment

From the limited data available from studies on bentonite-exposed persons, retained montmorillonite appears to effect only mild non-specific tissue changes, which are similar to those that have been described in the spectrum of changes of the “small airways mineral dust disease” (nodular peribronchiolar dust accumulations containing refractile material [montmorillonite] in association with limited interstitial fibrosis). In some of the studies, radiological abnormalities have also been reported.

There exist no reported cases of marked diffuse/nodular pulmonary tissue fibrotic reaction to montmorillonite in the absence of free silica. No quantitative estimates of the potency of bentonite to cause adverse pulmonary effects can be derived.

Long-term exposure to kaolin may lead to a relatively benign pneumoconiosis, known as kaolinosi. Deterioration of lung function has been observed only in cases with prominent radiological alterations. Based on data from china clay workers in the United Kingdom, it can be very roughly estimated that kaolin is at least an order of magnitude less potent than quartz.

Bentonite, kaolin, and other clays often contain quartz, which is known to cause silicosis and lung cancer.

No report on local or systemic adverse effects has been identified from the extensive use of bentonite or kaolin in cosmetics.

Summary

The biological effects of clay minerals are influenced by their mineral composition and particle size. The decreasing rank order of the potencies of quartz, kaolinite, and montmorillonite to produce lung damage is consistent with their known relative active surface areas and surface chemistry.

Bentonite and kaolin have low toxicity towards aquatic organisms.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, AND ANALYTICAL METHODS

2.1 Introduction

Clay is a widely distributed, abundant mineral resource of major industrial importance for an enormous variety of uses (Ampian, 1985). In both value and amount of annual production, it is one of the leading minerals worldwide. In common with many geological terms, the term “clay” is ambiguous and has multiple meanings: a group of fine-grained minerals — i.e., the clay minerals; a particle size (smaller than silt); and a type of rock — i.e., a sedimentary deposit of fine-grained material usually composed largely of clay minerals (Patterson & Murray, 1983; Bates & Jackson, 1987). In the latter definition, clay also includes fine-grained deposits of non-aluminosilicates such as shale and some argillaceous soils.

This Environmental Health Criteria (EHC) monograph deals with the health hazards associated with bentonite, kaolin, and common clay, which are commercially important clay products, as well as the related phyllosilicate minerals montmorillonite, kaolinite, and illite. Fibrous clay minerals, such as sepiolite, attapulgite, and zeolites, are not discussed. For a recent assessment of the health effects of zeolites, see IARC (1997a). As bentonite, kaolin, and common clay may contain varying amounts of silica, a short summary is presented of recent assessments of quartz (IARC, 1997b; IPCS, 2000).

Recently, there has been an increased interest in the toxicity of airborne fine (0.1–2.5 μm) and ultrafine (<0.1 μm) particles. Epidemiological studies have indicated an increase in morbidity and mortality associated with an increase in airborne particulate matter, particularly in the ultrafine size range (Pekkanen et al., 1997; Stölzel et al., 2003). There is little information on how much of total airborne particulate matter is clay dust and what fraction of airborne clay dusts is in the fine or ultrafine mode.

2.2 Identity

2.2.1 Bentonite

The term “bentonite” is ambiguous. As defined by geologists, it is a rock formed of highly colloidal and plastic clays composed mainly of montmorillonite, a clay mineral of the smectite group (Fig. 1), and is produced by *in situ* devitrification of volcanic ash (Parker, 1988). The transformation of ash to bentonite apparently takes place only in water (certainly seawater, probably alkaline lakes, and possibly other fresh water) during or after deposition (Grim, 1968; Patterson & Murray, 1983). Bentonite was named after Fort Benton (Wyoming, USA), the locality where it was first found. In addition to montmorillonite, bentonite may also contain feldspar, biotite, kaolinite, illite, cristobalite, pyroxene, zircon, and crystalline quartz (Parkes, 1982).

By extension, the term bentonite is applied commercially to any plastic, colloidal, and swelling clay regardless of its geological origin. Such clays are ordinarily composed largely of minerals of the montmorillonite group.

Bentonite is a rock or a clay base industrial material. It is therefore a mixture of minerals. No “molecular” formula can be given.

The Chemical Abstracts Service (CAS) registry number for bentonite is 1302-78-9.

Synonyms and trade names used to designate bentonite include Albagel Premium USP 4444, Bentonite magma, Bentonite 2073, Bentopharm, CI 77004, E558, HI-Gel, HI-Jel, Imvite I.G.B.A., Magbond, mineral sopa, Montmorillonite, Panther creek bentonite, soap clay, Southern bentonite, taylorite, Tixoton, Veegum HS, Volclay, Volclay Bentonite BC, and Wilkinite (CIREP, 2003; RTECS, 2003a).

The term “montmorillonite” is also ambiguous and is used both for a group of related clay minerals and for a specific member of that group (Bates & Jackson, 1987). For the former use, smectite is more appropriate (a group of clay minerals that includes montmorillonite, saponite, sauconite, beidellite, nontronite, etc.) (Fig. 1).

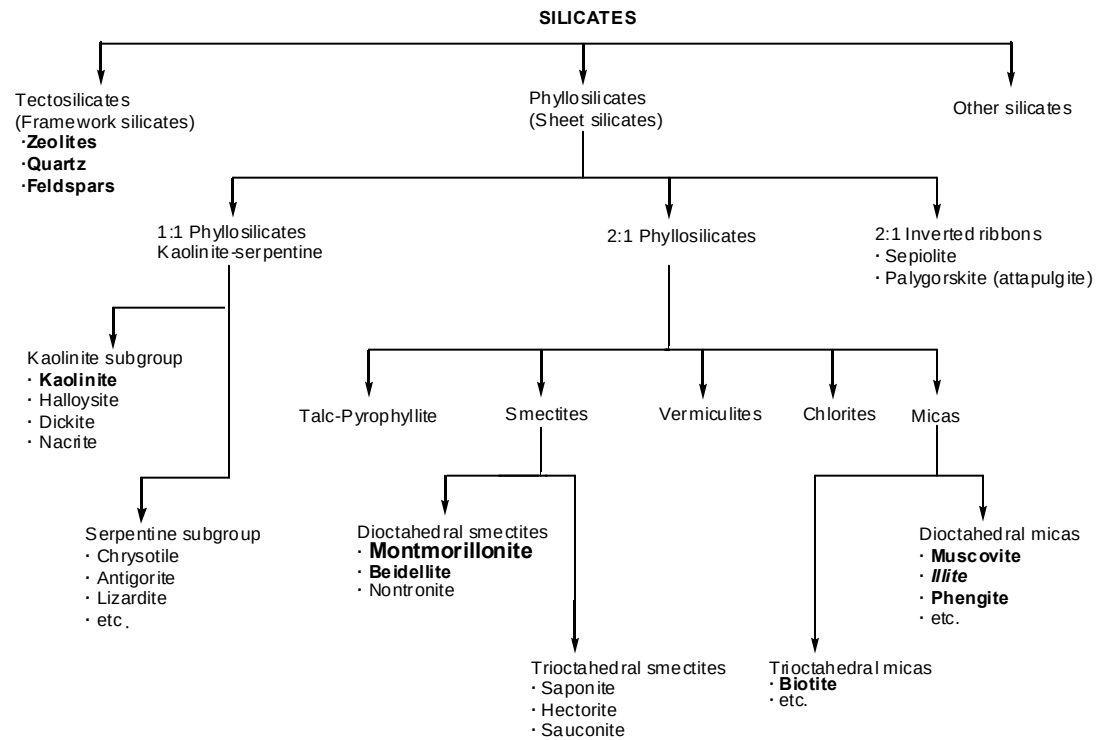


Fig. 1. Classification of silicates (Bailey, 1980b; Rieder et al., 1998). Minerals that can be frequently found in bentonite or kaolin are in bold; the main components are in large typeface. *Illite* is a component of common soil and sediments and is classified as a mica.

The basic crystal structure of smectites is an octahedral alumina sheet between two tetrahedral silica sheets. Atoms in these sheets common to both layers are oxygens. These three-layer units are stacked one above another with oxygens in neighbouring layers adjacent to each other. This produces a weak bond, allowing water and other polar molecules to enter between layers and induce an expansion of the mineral structure. In the tetrahedral coordination, silicon may be substituted by aluminium and possibly phosphorus; in the octahedral coordination, aluminium may be substituted by magnesium, iron, lithium, chromium, zinc, or nickel. Differences in the substitutions within the lattice in terms of position and elemental composition give rise to the various montmorillonite clay minerals: montmorillonite, nontronite, saponite, hectorite, sauconite, beidellite, volkhonskoite, pimelite, and griffithite.

The molecular formula for montmorillonite is usually given as $(M^+_x \cdot nH_2O) (Al_{2-y}Mg_x)Si_4O_{10}(OH)_2$, where $M^+ = Na^+, K^+, Mg^{2+}$, or Ca^{2+} (Brindley & Brown, 1980). Ideally, $x = 0.33$. The CAS registry number for montmorillonite is 1318-93-0.

Fuller's earth is loosely defined by its properties and commercial use and not by its mineralogy and molecular structure (Grim, 1968; Patterson & Murray, 1983; Ampian, 1985; Bates & Jackson, 1987; Hosterman & Patterson, 1992). The term is derived from the initial usage of certain clays in bleaching, degreasing, and fulling (shrinking) woollen fabrics. Grim (1968) defines Fuller's earth as "any natural earthy material which will decolorize mineral or vegetable oils to a sufficient extent to be of economic importance." The term encompasses not only some bentonites but also some kaolinites and some silts with minimal clay content. In the USA, the term Fuller's earth is used to mean palygorskite, a fibrous magnesium–aluminium silicate mineral close to sepiolite (IARC, 1997c). Data on production and other aspects of Fuller's earth often do not distinguish between bentonite and non-bentonite types.

2.2.2 Kaolin

The name "kaolin" is derived from the word Kau-Ling, or high ridge, the name given to a hill near Jau-chau Fu, China, where kaolin was first mined (Sepulveda et al., 1983). Kaolin, commonly referred to as china clay, is a clay that contains 10–95% of the mineral kaolinite and usually consists mainly of kaolinite (85–95%). In addition to kaolinite, kaolin usually contains quartz and mica and also, less fre-

quently, feldspar, illite, montmorillonite, ilmenite, anastase, haematite, bauxite, zircon, rutile, kyanite, sillimanite, graphite, attapulgite, and halloysite. Some clays used for purposes similar to those for which kaolin is used may contain substantial amounts of quartz: “kaolin-like” clays used in South African pottery contained 23–58% quartz and, as the other major constituent, 20–36% kaolinite (Rees et al., 1992).

The CAS registry number for kaolin is 1332-58-7.

Synonyms and trade names for kaolin include Altowhites, Argilla, Bentone, Bolbus alba, China clay, CI 77004, Emathlite, Fitrol, Fitrol desiccite 25, Glomax, Hydrite, Kaopaous, Kaophills-2, Kolite, Langford, McNamee, Parclay, Pigment white 19, Porcelain clay, Snow tex, terra alba, and white bole (CIREP, 2003; RTECS, 2003b).

The structure of kaolinite is a tetrahedral silica sheet alternating with an octahedral alumina sheet. These sheets are arranged so that the tips of the silica tetrahedrons and the adjacent layers of the octahedral sheet form a common layer (Grim, 1968). In the layer common to the octahedral and tetrahedral groups, two-thirds of the oxygen atoms are shared by the silicon and aluminium, and then they become O instead of OH. The charges within the structural unit are balanced. Analyses of many samples of kaolinite minerals have shown that there is very little substitution in the lattice (Grim, 1968). The molecular formula that is common for the kaolinite group (kaolinite, nacrite, dickite) is $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ (Grim, 1968).

The CAS registry number for kaolinite is 1318-74-7.

2.2.3 Other clays

In addition to bentonite (and Fuller’s earth) and kaolin, the main types of commercial clays are ball clay, common clay and shale, and fire clay. Ball clay consists primarily of kaolinite, with minor amounts of illite, chlorite, smectite minerals, quartz, and organic materials. Common clay and shale contain illite and chlorite as major components, while fire clay comprises mainly kaolinite, halloysite, and/or diaspore (Virta, 2002).

Illite is a name for a group of mica-like clay minerals proposed by R.E. Grim, R.H. Bray, and W.F. Bradley in 1937 to honour the US state of Illinois, a source of illite and a state that had supported research on clay (Grim, 1968; Bates & Jackson, 1987). Illite clay

minerals show substantially no expanding lattice characteristics and are characterized by intense 1.0-nm 001 and 0.33-nm 003 peaks that are not modified by glycerol or ethylene glycol solvation, potassium saturation, or heating to 550 °C (Fanning et al., 1989). Grim (1968) stated that illite includes both trioctahedral (biotite) and dioctahedral (muscovite) types of crystallization. The US Geological Survey report entitled *A Laboratory Manual for X-Ray Powder Diffraction*, however, limits illite to minerals with a dioctahedral, muscovite structure (USGS, 2001). Velde (1995) separated mica-like minerals such as illite from true micas on the basis that the former have almost exclusively potassium as the interlayer ion and a charge imbalance always slightly less than 1.0 per unit cell. (This value for the charge imbalance is much below the 1.3–1.5 given by Grim [1968].) In addition, true micas usually have a greater grain size than the mica-like clay minerals (i.e., >2 µm) and commonly are found in rocks that have been subjected to elevated temperatures.

The Association Internationale Pour l'Étude des Argiles Nomenclature Committee (Rieder et al., 1998) defines illite as a serial name for dioctahedral interlayer-deficient mica with composition $K_{0.65}(Al_2)(Si_{3.35}Al_{0.65})O_{10}(OH)_2$. The aluminium in octahedral positions can be partially substituted by Mg^{2+} , Fe^{2+} , and Fe^{3+} .

The CAS registry number of illite is 1217-36-03 (Grim, 1968). Synonyms of illite include hydromica, illidromica, and glimmerton.

The chemical composition of illite deposits from Malaysia is summarized in Fan & Aw (1989) and from the USA and Germany in Grim (1968) and Gaudette et al. (1966). The water content of Malaysian illite ranged from 19% to 26%, and that of the illites described by Grim (1968), from 6% to 12%. The density of illite is 2.6–2.9 g/cm³ (Grim, 1968; Tamás, 1982). Gaudette et al. (1966) mentioned that illites from Illinois, Oklahoma, and Wisconsin, USA, were light green or light to dark grey clays. Illite mined in Füzérradvány, Hungary, is described as greasy, clay-like, and of high plasticity (Tamás, 1982). Its colour is determined by its iron content and may be white, yellow, lilac, or reddish brown.

2.3 Physical and chemical properties

2.3.1 Bentonite

Bentonite feels greasy and soap-like to the touch (Bates & Jackson, 1987). Freshly exposed bentonite is white to pale green or blue and, with exposure, darkens in time to yellow, red, or brown (Parker, 1988). The special properties of bentonite are an ability to form thixotropic gels with water, an ability to absorb large quantities of water with an accompanying increase in volume of as much as 12–15 times its dry bulk, and a high cation exchange capacity.

Substitutions of silicon by cations produce an excess of negative charges in the lattice, which is balanced by cations (Na^+ , K^+ , Mg^{2+} , Ca^{2+}) in the interlayer space. These cations are exchangeable due to their loose binding and, together with broken bonds (approximately 20% of exchange capacity), give montmorillonite a rather high (about 100 meq/100 g) cation exchange capacity, which is little affected by particle size. This cation exchange capacity allows the mineral to bind not only inorganic cations such as caesium but also organic cations such as the herbicides diquat, paraquat (Weber et al., 1965), and s-triazines (Weber, 1970), and even bio-organic particles such as rheoviruses (Lipson & Stotzky, 1983) and proteins (Potter & Stollerman, 1961), which appear to act as cations. Variation in exchangeable cations affects the maximum amount of water uptake and swelling. These are greatest with sodium and least with potassium and magnesium.

Interstitial water held in the clay mineral lattice is an additional major factor controlling the plastic, bonding, compaction, suspension, and other properties of montmorillonite-group clay minerals. Within each crystal, the water layer appears to be an integral number of molecules in thickness. Physical characteristics of bentonite are affected by whether the montmorillonite composing it has water layers of uniform thickness or whether it is a mixture of hydrates with water layers of more than one thickness. Loss of absorbed water from between the silicate sheets takes place at relatively low temperatures (100–200 °C). Loss of structural water (i.e., the hydroxyls) begins at 450–500 °C and is complete at 600–750 °C. Further heating to 800–900 °C disintegrates the crystal lattice and produces a variety of phases, such as mullite, cristobalite, and cordierite, depending on initial composition and structure. The ability of montmorillonite to rapidly take up water and expand is lost after heating to a critical temperature, which ranges

from 105 to 390 °C, depending on the composition of the exchangeable cations. The ability to take up water affects the utilization and commercial value of bentonite (Grim, 1968; Parker, 1988).

Montmorillonite clay minerals occur as minute particles, which, under electron microscopy, appear as aggregates of irregular or hexagonal flakes or, less commonly, of thin laths (Grim, 1968). Differences in substitution affect and in some cases control morphology.

2.3.2 Kaolin

Kaolinite, the main constituent of kaolin, is formed by rock weathering. It is white, greyish-white, or slightly coloured. It is made up of tiny, thin, pseudohexagonal, flexible sheets of triclinic crystal with a diameter of 0.2–12 µm. It has a density of 2.1–2.6 g/cm³. The cation exchange capacity of kaolinite is considerably less than that of montmorillonite, in the order of 2–10 meq/100 g, depending on the particle size, but the rate of the exchange reaction is rapid, almost instantaneous (Grim, 1968). Kaolinite adsorbs small molecular substances such as lecithin, quinoline, paraquat, and diquat, but also proteins, polyacrylonitrile, bacteria, and viruses (McLaren et al., 1958; Mortensen, 1961; Weber et al., 1965; Steel & Anderson, 1972; Wallace et al., 1975; Adamis & Timár, 1980; Schiffenbauer & Stotzky, 1982; Lipson & Stotzky, 1983). The adsorbed material can be easily removed from the particles because adsorption is limited to the surface of the particles (planes, edges), unlike the case with montmorillonite, where the adsorbed molecules are also bound between the layers (Weber et al., 1965).

Upon heating, kaolinite starts to lose water at approximately 400 °C, and the dehydration approaches completeness at approximately 525 °C (Grim, 1968). The dehydration depends on the particle size and crystallinity.

2.3.3 Other clays

Illite, together with chlorite, is the main component of common clay and shale. It is also an important impurity in limestone, which can affect the properties and thus the value of the stone for construction and other purposes (Carr et al., 1994). Despite the widespread occurrence of illite in nature, large deposits of high purity are quite rare (as discussed in section 3.2.1.3).

Illite usually occurs as very small (0.1–2 μm), poorly defined flakes commonly grouped into irregular aggregates. Lath-shaped and ribbon-shaped illite particles up to 30 μm in length and 0.1–0.3 μm in width have also been described (Srodon & Eberl, 1984), but their existence is controversial. Velde (1985) stated unqualifiedly that these so-called filamentous illites are mixed-layer structures. Srodon & Eberl (1984), however, drawing on the same references plus their own data, concluded that these filaments in some cases are mixed-layer structures but in other cases are composed only of illite, and they further supported their view with scanning electron microscopic photographs of lath-shaped crystals of what they identified as illite.

The special properties of illite are derived from its molecular structure. The balancing cation is mainly or entirely potassium, and charge deficiency from substitutions is at least twice that of smectites (i.e., 1.3–1.5 per unit cell layer) and is mainly in the silica sheet and close to the surface of the unit layer rather than in the octahedral layer as in smectites (Grim, 1968). These differences from smectites produce a structure in which interlayer balancing cations are not easily exchanged and the unit layers are relatively fixed in position and do not permit polar ions such as water to readily enter between them and produce expansion.

Illite reacts with both inorganic and organic ions and has a cation exchange capacity of 10–40 meq/100 g, a value intermediate between those of montmorillonite and kaolinite (Grim, 1968). Ion exchange capacity is reduced by heating. The potassium in the interlayer space is “fixed” to a considerable degree, making it not readily available to plants, a matter of importance in soil science and agriculture. A portion of the interlayer potassium, however, can be slowly leached, leading to the degradation of the illite. Such degradation, however, can be reversed by the addition of potassium. Wilken & Wirth (1986) stated that fithian illite from Illinois, USA, adsorbed hexachlorobenzene suspended in distilled water with a sorption partition coefficient of 2200–2600 and that more than half of this adsorbed hexachlorobenzene could be desorbed by further contact with distilled water. However, the fithian illite used in the experiment had a composition of 30% quartz, 19% feldspar, 11% kaolinite, 1% organic carbon, and 40% illite, making it impossible to know how much of the measured adsorption could be ascribed to illite.

The dehydration and other changes in illite with heating have been studied by several investigators, with inconsistent results (Grim, 1968). Some of the inconsistency in findings may result from differences in the period at which samples were held at a given temperature, since dehydration is a function of both time and temperature (Roy, 1949). It is also probable that small differences in particle size, crystal structure, and molecular composition among samples of what were ostensibly the same mineral contributed to the inconsistencies. Dehydration takes place either smoothly or in steps between about 100 and 800 or 850 °C for both biotite and muscovite illites. Loss of structure by the various illite minerals occurs between about 850 and 1000 °C.

2.3.4 Surface chemistry

Chemical properties of the surfaces of silicates and, in particular, clay minerals are strongly dependent on their mineral structure. The basic unit of montmorillonite crystals is an extended layer composed of an octahedral alumina sheet (O) between two tetrahedral silica sheets (T), forming a TOT unit (Bailey, 1980a). The stacks of TOT layers produce the montmorillonite crystals. Isomorphic substitutions in the octahedral sheet (few tetrahedral substitutions are observed in montmorillonite) create an excess of negative structural charge that is delocalized in the lattice. Cations located between two consecutive layers contribute to compensate the structural charge and to keep the layers bound. These cations can easily be exchanged, since they are retained by electrostatic attractions. The surface area associated with the basal surfaces of the extended TOT units is known as the interlayer surface when it corresponds to consecutive layers or as the external surface when it corresponds to the external basal surfaces of a crystal. The external and interlayer surface area represents approximately 95% of the total surface area of montmorillonite. On the other hand, the periodic structure of the montmorillonite crystals is interrupted at the edges, where the broken bonds compensate their charge by the specific adsorption of protons and water molecules (Schindler & Stumm, 1987; Stumm & Wollast, 1990; Stumm, 1997). This interruption of the periodic structure confers to the edge surface an amphoteric character — i.e., a pH-dependent surface charge and the capacity to react specifically with cations, anions, and molecules (organic and inorganic), forming chemical bonds.

The kaolinite layers are formed only by a tetrahedral sheet of silica and an octahedral sheet of alumina, which contain almost no isomorphic substitutions (Bailey, 1980a). Hydroxyls of the octahedral sheet bind to oxygens of the tetrahedral sheet of the consecutive layer in the kaolinite crystal. The structural charge of the crystal is non-existent, and no molecules or cations are present in the interlayer space. The surface area of the kaolinite is thus reduced to external surface area and to edge surface area. The edge surface area is similar in nature and properties to the one observed in montmorillonite and represents approximately 20% of the total surface area. However, two types of external surfaces are defined for kaolinite: one associated with the outermost tetrahedral sheet and the other associated with the outermost octahedral sheet. The reactivity of the external octahedral sheet is due to the hydroxyl groups present, which i) can produce a pH-dependent surface charge by protonation and deprotonation reactions and ii) can react specifically with other molecules (Huertas et al., 1998).

Quartz is the most relevant accessory mineral in bentonite and kaolin. Its structure is a tridimensional framework of silicate tetrahedra arranged sharing corners. In contrast with montmorillonite and kaolinite, quartz surface area is defined only as an external surface. Broken bonds, which compensate charge by adsorption of protons or water molecules, are found in all the crystal phases.

The “active” surface area is close to 100% in quartz, 20% in kaolinite, and 5% in montmorillonite. This may be related to the observed toxicity of these materials (see chapter 6).

2.3.5 Trace elements in clays

Knowledge of mechanisms controlling the distribution of trace elements in clays is scarce and contradictory, in spite of the many investigations carried out on the geochemical behaviour of some trace elements used in geological reconstruction as “geochemical indicators” (Fiore et al., 2003). It is well known that clays contain trace elements that literature indicate as toxic and/or micronutrients (i.e., antimony, arsenic, cadmium, cobalt, copper, lead, mercury, nickel, selenium, tellurium, thallium, zinc) whose concentrations are widely variable, depending on their geological history. These trace elements may be in the clay (or accessory) mineral structure as well as adsorbed on clay particles, which play the most important role in controlling their distribution and abundance. Chemical elements in crystalline positions

are usually “locked,” whereas those adsorbed may be mobilized and transferred to leaching solutions.

For information on the leaching and bioavailability of clay components, see chapter 5.

2.4 Analytical methods

2.4.1 Quantitative measurement of dust

Inhaled dust appears to be the major mode of human exposure to aluminosilicates, and a general review of methodology for dust sampling and analysis is presented in Degueldre (1983). Quantitative methods for the measurement of total dust and respirable dust are described in the *NIOSH Manual of Analytical Methods* (Eller & Cassinelli, 1994), in Part 40 of the US government’s Code of Federal Regulations (US EPA, 1996a), and in *Mine Safety and Health Administration Handbook No. PH90-IV-4* (US Department of Labor, 1990). All of these methods are gravimetric and measure the weight of dust collected on a filter from a known volume of air.

Although the basic principles behind these methods are simple, accurate and precise results demand sensitive equipment and attention to detail. The National Institute for Occupational Safety and Health (NIOSH) method for total dust requires an appropriate sampler with a polyvinyl chloride or equivalent filter with a pore size of 2–5 μm , a pump with a well characterized, uniform rate of air flow of 1.5–2.0 litres/min, and a microbalance capable of weighing to 0.01 mg, as well as a vacuum desiccator, a static neutralizer, and a chamber with constant temperature and humidity for storing the filters. Guidance for optimizing performance of the 10-mm cyclone sampler is provided by Bartley & Breuer (1982). Briant & Moss (1984) recommended use of a conducting, graphite-filled nylon cyclone to avoid developing an electrostatic charge on the cyclone, which may reduce the measured value for respirable dust below the actual value. Significant errors can also be introduced by air leakage in the filter assembly, the accumulation of static electrical charge on the filter, and many other factors.

Another quantitative measurement of dusts is the determination of particle number. The toxicity of particles has been shown to be related more to surface than to mass. The smaller the particle, the larger the surface area to mass ratio.

2.4.2 Identification of phyllosilicates

There is no single or simple procedure for the positive identification of montmorillonite-group or other aluminosilicates or for their quantification in dust and other samples. The application of several methods may be necessary for even approximate identification and rough quantification. These methods include X-ray diffraction, electron microscopy, energy-dispersive X-ray analysis, differential thermal analysis, and infrared spectroscopy. In the past, chemical methods based on differences in resistance of various clay minerals to chemical attack, the so-called “rational methods of analysis,” were used.

X-ray powder diffraction analysis is the basic technique for clay mineral analysis (Moore & Reynolds, 1989). After preliminary removal of sand, clay is separated from silt by centrifugation or sedimentation from suspensions. X-ray diffraction patterns are obtained for air-dried samples and, in the case of oriented aggregates, also for samples treated with ethylene glycol vapour or heated to 350 and 550 °C. Diffraction patterns are compared with standards (Grim, 1968; Thorez, 1975; Brindley & Brown, 1980; JCPDS, 1981) for identification of minerals. Comparisons are complicated, however, by variations in diffraction patterns arising from differences in amounts of absorbed water, by the presence of imperfections in the crystal lattice structure of the minerals, and by mixed-layer structures formed by interstratification of minerals within a single particle (Grim, 1968). Approximate quantification of mineral abundance in samples containing several minerals is possible, although subject to a variety of complications and errors (Starkey et al., 1984; Salt, 1985).

Transmission electron microscopy is valuable for identifying aluminosilicates with a distinctive morphology (Starkey et al., 1984). Particles dispersed on a plastic film can be observed directly by transmission microscopy or shadowed to increase contrast by evaporating a heavy metal onto specimens prior to examination. Grim (1968) and Starkey et al. (1984) provide an entry into the extensive literature on this subject. Many aluminosilicates (e.g., montmorillonite, which occurs as broad mosaic sheets decomposing into minute flakes) lack a distinctive morphology and cannot be identified by this technique.

Energy-dispersive X-ray analysis (EDXA) — also referred to as energy-dispersive X-ray microanalysis, X-ray microanalysis, electron microscopic microanalysis, and energy-dispersive X-ray spectrometry — and electron diffraction may permit the rapid identification of

individual clay mineral particles (Sahle et al., 1990; Lee, 1993) and have been applied particularly to the identification of inhaled particles sampled via bronchoalveolar lavage or from lung specimens (Johnson et al., 1986; Costabel et al., 1990; Monsó et al., 1991, 1997; Chariot et al., 1992; Bernstein et al., 1994; Dufresne et al., 1994). EDXA requires a scanning or transmission electron microscope equipped with an energy-dispersive X-ray spectrometer and appropriate mathematical tools for analysing the resulting spectra. EDXA identifies and quantifies elements above atomic number 8. Since the basic classification of clay minerals is based on structural formula and the atomic composition is similar for many different clay minerals, EDXA cannot provide secure identification except by comparison with standards previously identified by other means. Application of EDXA without appropriate standards is likely to generate significant errors (Newbury et al., 1995). In practice, EDXA is ordinarily combined with conventional transmission electron microscopy to first visualize a particle. Probe size is then adjusted downward so that only the selected particle is analysed. The best results are obtained by operating the micro-analysis in scanning transmission mode.

Differential thermal analysis (DTA) is based on temperature differences between the sample and a thermally inert material during heating or cooling and is most useful for mineral identification in samples composed mainly or entirely of a single clay mineral (Grim & Rowland, 1944; Mackenzie, 1970; Smykatz-Kloss, 1974). The sample and a control, a thermally inert material (e.g., aluminium oxide), are heated in separate crucibles in a well regulated furnace. During heating, the change in temperature of the clay sample will be modified by endothermic and exothermic reactions. Identification is done by comparing the temperature difference trace with standard curves (Grim & Rowland, 1944; Mackenzie, 1970; Smykatz-Kloss, 1974). DTA is not without difficulties, since results are influenced by furnace atmosphere, heat conductance of sample and crucible, type of thermocouple, rate of heating, grain size of sample, and many other factors (Smykatz-Kloss, 1974). Identification of component minerals in mixtures and quantification of mixtures by use of DTA are difficult and often may be impossible due to overlapping DTA curves (Grim, 1947; Smykatz-Kloss, 1974).

Many aluminosilicates can be identified by distinctive infrared absorption spectra. Absorption is normally measured over a range of frequencies (Grim, 1968), and the resulting spectrum compared with published standards (Van der Marel & Beutelspacher, 1976; Ferraro,

1982). Satisfactory measurements require appropriate mounting of specimens and minimizing of scattering and reflection. The latter is accomplished by using particles smaller than the minimum wavelength (Grim, 1968).

Montmorillonite can be distinguished from beidellite, nontronite, and saponite by its irreversible collapse of the structure after Li saturation and heat treatment. Li migration to the octahedral charge converts montmorillonite to a non-expandable structure upon treatment with water, glycol, or ethylene glycol. The procedure is known as the Greene-Kelly test (Greene-Kelly, 1955). Other smectites, such as beidellite and saponite, should expand to give the characteristic 1.77-nm reflection upon solvation with ethylene glycol.

3. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

3.1 Natural occurrence

3.1.1 *Bentonite*

Bentonite derived from ash falls tends to be in beds of uniform thickness (from a few millimetres to 15 m) and extensive over large areas (Parker, 1988). Bentonite from ash falls and other sources occurs worldwide in strata spanning a broad range of ages, but is most abundant in Cretaceous or younger rocks.

Bentonite is a widely distributed material. Accordingly, its major component, montmorillonite, occurs abundantly as dust at and near surface deposits of bentonite and is dispersed widely by air and moving water. Montmorillonite is thus ubiquitous in low concentrations worldwide in soil, in the sediment load of natural waters, and in airborne dust. Biodegradation appears minimal, if it occurs at all, and there is no evidence of or reason to suspect accumulation in the food-chain. Abiotic degradation of bentonite into other minerals takes place only on a geological time scale (Parker, 1988).

3.1.2 *Kaolin*

Kaolin and the clay mineral kaolinite are natural components of the soil and occur widely in ambient air as floating dust. Kaolinite is formed mainly by decomposition of feldspars (potassium feldspars), granite, and aluminium silicates. It is also not uncommon to find kaolin deposited together with other minerals (illite, bentonite). The process of kaolin formation is called kaolinization (Grim, 1968).

Kaolinite formation occurs in three ways:

- crumbling and transformation of rocks due to the effects of climatic factors (Zettlitz type);
- transformation of rocks due to hydrothermal effects (Cornwall type); and
- formation by climatic and hydrothermal effects (mixed type).

The type of clay mineral formed during the decay of rocks containing aluminium silicates is influenced by the climate, the aluminium/silicon ratio, and pH. Conditions conducive for kaolinite formation are strong dissolution of Ca^{2+} , Mg^{2+} , and K^{+} ions and the presence of H^{+} ions (pH 4–5) (Parker, 1988).

Kaolinite quarries can be categorized according to the geo-historical age of the parent rock:

- Precambrian (Ukraine, Spain, Czech and Slovak republics)
- Postcambrian (Cornwall, England, and Ural Mountains, Russia)
- Palaeovolcanite (Meissen, Germany)
- Neovolcanite (Tokaj Mountain, Hungary).

Kaolinite can also be categorized according to whether it remained at the place of formation or was transported (Parkes, 1982; Kuzvart, 1984):

- *primary*: lateritic types formed mainly under tropical climatic conditions (South America, Africa, Australia); and
- *secondary*: transported by different forces, water, wind (Georgia, USA).

Owing to the different ways in which kaolin can form, several kinds of minerals may occur in natural kaolins. For example, the kaolin of Cornwall, England, contains 10–40% kaolinite; the rest is made of quartz, mica, and feldspar. The kaolin of Georgia, USA, contains 85–95% kaolinite, as well as quartz, muscovite, and feldspar (Patterson & Murray, 1975).

3.1.3 Other clays

Illite is widely distributed in nature, abundant, and often the dominant clay mineral in soil, terrestrial deposits, sedimentary rocks, freshwater sediments, and most deep-sea clays (Grim, 1968). It is also an important impurity in limestone, affecting the properties and thus the value of the stone for construction and other purposes (Carr et al., 1994). Despite the widespread occurrence of illite in nature, large deposits of high purity are quite rare.

Illite, together with smectites, is formed by weathering of acid igneous rock containing considerable quantities of potassium and magnesium under conditions that permit the potash and magnesia to

remain in the weathering environment after breakdown of the parent material (Grim, 1968). Deer et al. (1975) and Srodon & Eberl (1984) identified many potential sources of illite, including weathering of silicates (primarily feldspar), alteration of other clay minerals, and degradation of muscovite. Righi & Meunier (1995) concluded that illite can also be degraded by weathering into illite–montmorillonite (smectite) mixed-layer minerals.

3.2 Anthropogenic sources

3.2.1 Production levels and sources

3.2.1.1 Bentonite

Bentonite (Table 1) and bentonite Fuller's earth (Table 2) are mined worldwide. The USA is the major producer of bentonite Fuller's earth (Table 2). Approximately 90% of world bentonite production is concentrated in 13 countries: the USA, Greece, the Commonwealth of Independent States, Turkey, Germany, Italy, Japan, Mexico, Ukraine, Bulgaria, Czech Republic, South Africa, and Australia (Table 1). The USA, Greece, and the Commonwealth of Independent States account for roughly 55% of the annual world production of 10 million tonnes. Wyoming produces the bulk of bentonite mined in the USA (Ampian, 1985). In 2002, the bulk of US production was used domestically, and only a small fraction, 11%, was exported worldwide (Virta, 2002). In addition to the mining of natural deposits, small amounts of bentonite, mainly hectorite, are produced synthetically in both Europe and the USA for use as a catalyst.

Most bentonite is mined by stripping methods from open pits after removing any overburden, although underground methods are used in a few places, such as the Combe Hay district in the United Kingdom (Patterson & Murray, 1983). Since deposits are often not uniform in composition, bentonite from a single pit may be separated into several stockpiles, which subsequently are blended to obtain the desired composition. Bentonite is usually processed by breaking large pieces into smaller fragments, drying at low to moderate temperatures to remove water and other volatiles without altering the molecular structure of the

EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

Table 1. Bentonite production in selected countries^{a-d}

Country	Bentonite production (kilotonnes) ^e			
	1991	1995	1999	2002 ^f
Algeria ^g	26	17	15	27 ^h
Argentina	108	111	129	89 ^h
Australia ^{f,g}	35	35	180	200 ^g
Brazil, beneficiated	130	150	275	165
Bulgaria	NA	126	243	250
Commonwealth of Independent States ^{f,i}	2 400	1 300	700	750
Croatia	XX	7	8	11
Cyprus	59	50	139	125
Czech Republic	XX	54	160	250
Egypt ^f	6 ^h	2 ^h	50	50
Germany ^f	583 ^h	529 ^h	500 ^h	500
Greece ^f	600 ^h	1 115 ^h	950 ^h	950
Hungary	18	23	16	30
Iran ⁱ	40	55	65	80
Italy	402	591	500	500
Japan	554	478	428	400
Macedonia ^f	XX	30	30	30
Mexico	145	73	209	400
Morocco	9	29	37	66 ^h
Pakistan	5	6	15	28
Peru	15	27	20	18
Poland	35	6	5 ^k	30 ^k
Romania	150 ^f	42	20	15
South Africa ⁱ	65	71	50	218 ^h
Spain ^f	150	173	150 ^h	150
Turkey	124	602	900	600
Turkmenistan ^f	XX	50	50	50
Ukraine ^f	XX	NA	300	300
USA	3 430	3 820	4 070	3 970 ^h
Total	9 360	9 800	10 400	10 300

Table 1 (Contd)

- ^a From USGS (1997); Virta (2001, 2002).
- ^b Figures are rounded to the nearest 1000 tonnes and may not add to totals shown. Listed are countries producing >10 000 tonnes each (approximately 0.1% of the world total production) in 2002.
- ^c Tables include data available through August 21, 2003.
- ^d In addition to the countries listed, Canada and China are believed to produce bentonite, but output is not reported, and available information is inadequate to make reliable estimates of output levels.
- ^e NA = not available; XX = not applicable.
- ^f Figures are estimated, unless otherwise indicated (see footnote h).
- ^g Includes bentonitic clays.
- ^h Reported figure.
- ⁱ Does not include Armenia, Georgia, or Turkmenistan for the year 1995 or Armenia, Georgia, Turkmenistan, or Ukraine for the years 1999 and 2002.
- ^j Year beginning March 21 of that stated
- ^k Montmorillonite-type bleaching clay.
- ^l May include other clays.

bentonite, and grinding to the desired size. The desired size is generally 200 mesh (US standard sieve size) or finer, which is equivalent to particle diameters of less than 70 µm. A coarser granular material is also produced for kitty litter applications. Processing may also include beneficiation, which may involve removing sand and other impurities as well as modifying the type of exchangeable ions in the crystal lattice. Some of the calcium bentonite produced in Texas, USA, is, for example, treated with sodium hydroxide to replace calcium with sodium in the montmorillonite and make the resulting product more suitable for use in drilling mud (Hosterman & Patterson, 1992). An organic-clad bentonite is produced for speciality purposes (paint, speciality greases, etc.) by replacing the inorganic exchangeable cations in the montmorillonite with an alkyl ammonium organic cation and is marketed under trade names such as Bentone and Nikkagel.

3.2.1.2 Kaolin

Large quantities of kaolin are mined and traded internationally. Virta (2002) lists 55 countries with a production of more than 1000 tonnes per year. Production figures for each country that produced >0.1% (>43 000 tonnes) of the estimated world production of kaolin in 2002 are given in Table 3. The People's Republic of China was estimated to produce 1.9, 2, and 2.12 million tonnes in 2000, 2001, and 2002 (Lines, 2003). The estimated annual production capacity of kaolin in China was 3.2–3.4 million tonnes in 2002–2003, out of which washed kaolin was only 700 000 tonnes (Ma & Tang, 2002; Moore, 2003).

EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

Table 2. World bentonite Fuller's earth production^{a,b}

Country	Fuller's earth production (kilotonnes)			
	1991	1995	1999	2002 ^c
Algeria	5	5 ^c	3	4 ^d
Argentina ^c	2	2	2	2
Australia (attapulgit)	15 ^c	15 ^c	6	6
Germany (unprocessed) ^c	708 ^d	600	500	500
Italy ^c	23 ^d	34 ^d	30	30
Mexico	41	16	48	150
Morocco (smectite)	38	15	22	42 ^d
Pakistan	22	13	16	15
Senegal (attapulgit)	129	120	136	176 ^d
South Africa (attapulgit) ^c	8	8	7	8
Spain (attapulgit) ^c	73	94	90	90
United Kingdom ^{c,e}	189 ^d	132	140	140
USA ^f	2740	2640	2560	2730
Total	3990	3690	3560	3890

^a From USGS (1997); Virta (2001, 2002).

^b Figures are rounded to the nearest 1000 tonnes. Table excludes former and current centrally planned economy countries, some of which may produce Fuller's earth, but for which no data are available. In addition to the countries listed, France, India, Iran, Japan, and Turkey reportedly have produced Fuller's earth in the past and may continue to do so, but no data are available.

^c Figures are estimated, unless otherwise indicated (see footnote d).

^d Reported figure.

^e Saleable product.

^f Sold or used by producers.

Kaolin is usually removed from the mines in large moist lumps, and the initial process of refining involves chiefly a change in the physical state. Two main methods have been used for the refining of the natural clay: the air flotation or dry process, and the wet process. In the dry process, the clay is dried, pulverized, and then carried by currents of hot air into classifying chambers, from which it emerges as a stream of finely powdered kaolin. This process, which generates huge amounts of dust, was generally used prior to 1940 and has now been largely replaced by the wet process. In this process, the crude clay is mixed with water and vigorously agitated, and the individual kaolin particles are thus separated from each other and suspended in water. Water is then removed using different procedures (Edenfield, 1960).

Sources of Human and Environmental Exposure

Table 3. Kaolin production in selected countries^{a,b}

Country	Kaolin production (kilotonnes)			
	1991	1995	1999	2002 ^c
Australia (includes ball clay) ^c	190	210	200	230
Austria (marketable) ^c	72	57	50	50
Belgium ^c	260 ^d	300	300	300
Brazil (beneficiated)	746	1 067	1 517	1 820
Bulgaria	106	115 ^c	98	100
Colombia (includes common clay)	1 984 ^c	7 300	8 000 ^d	8 500 ^c
Czech Republic	XX ^e	2 800	5 183	5 500
Egypt	193	293	290	300
France (marketable)	344	345	325	300
Germany	684	1 925	1 800	1 800
Greece	189	69	60	60
India (processed and saleable crude)	741	713	670	710
Iran	150	266	837	800
Kazakhstan ^c	XX	XX	70	70
Korea, Republic of	1 755	2 792	1 858	2 381 ^d
Malaysia	187	211	214	258 ^d
Mexico	167	222	490	680
Nigeria ^c	1	105 ^d	110 ^d	110
Pakistan	45	31	65	50
Paraguay	74 ^c	66 ^c	67	67
Poland (washed)	44	53	89	142
Portugal ^c	150 ^d	180	175	175
Russia (concentrate)	XX	50	41	45
Serbia and Montenegro (crude and washed)	XX	62	43 ^c	70 ^c
South Africa	134	147	122	91 ^d
Spain (marketable, crude and washed) ^f	538 ^c	316	320 ^c	350 ^c
Thailand (beneficiated)	256	461	113	165
Turkey	187	490	450	600
Ukraine	XX	950	222	225
United Kingdom (sales) ^g	2 911	2 586	2 303	2 400

EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

Table 3 (Contd)

Country	Kaolin production (kilotonnes)			
	1991	1995	1999	2002 ^c
USA ^h	9 570	9 480	9 160	8 010 ^d
USSR ⁱ	9 000	XX	XX	XX
Uzbekistan ^c	XX	5 500	5 500	5 500
Vietnam ^c	1	1	398	600
Total	32 600	37 600	41 500	43 200

^a From USGS (1997); Virta (1997, 2002).

^b Figures are rounded to the nearest 1000 tonnes and may not add to totals shown. Table reports data available through August 21, 2003. Countries producing more than 430 000 tonnes (approximately 0.1% of the world total production) have been listed. In addition to the countries listed, China is known to have produced and Morocco and Suriname may also have produced kaolin, but information was considered inadequate to make reliable estimates of output levels (USGS, 1997; Virta, 1997, 2002).

^c Figures are estimated, unless otherwise indicated (see footnote d).

^d Reported figure.

^e XX = Not applicable.

^f Includes crude and washed kaolin and refractory clays not further described.

^g Dry weight.

^h Kaolin sold or used by producers.

ⁱ Dissolved in December 1991. This commodity is believed to be produced mainly in Ukraine and Uzbekistan.

Because of the varying composition of raw kaolin and different uses, raw kaolin generally requires processing (flotation, sedimentation, baking, etc.) to acquire characteristics suited to specific industrial uses.

3.2.1.3 Other clays

Illite is often an important or dominant component of common clay (Murray, 1994). Common clay is produced worldwide and is a major industrial mineral. In the USA, common clay and shale are produced in 41 states. In 2002, this production was 23 million tonnes and had a value of \$148 million (Virta, 2002).

Illite occurs in commercially valuable purity and quantity in Malaysia (Bidor area of Perak), the USA (Illinois), the United Kingdom (South Wales), and Hungary (Tokaj Mountain in the north-eastern part of the country) (Grim, 1968; Fan & Aw, 1989). The most valuable ore is pure white and contains minimal iron. The commercial utility and thus the value of illite deposits may be reduced by lack of homogeneity in mineral content. There is no information on the annual production of relatively pure illite worldwide or by country.

3.2.2 Uses

3.2.2.1 Bentonite

Bentonite has many applications to a broad range of industrial and other activities. Usage of domestic production in the USA in 1995, 1999, and 2002 is summarized in Table 4. Major domestic uses, which include binding foundry sand (i.e., in moulds for castings), absorbing grease, oil, and animal wastes, pelletizing taconite iron ore, and improving the properties of many drilling muds, were 79% of the total. Most of the bentonite (79%) exported from the USA was used in foundry sand and drilling mud. Speciality uses of bentonite include serving as an ingredient for ceramics; waterproofing and sealing in civil engineering projects (e.g., blocking seepage loss from landfill sites, nuclear waste repositories, irrigation ditches, treatment ponds, and the like); serving as a filler, stabilizer, or extender in adhesives, paints, cosmetics, medicines, and other products, as a carrier in pesticides and fertilizers, and as a bonding agent in animal feeds; clarifying wine and vegetable oil; and purifying wastewater (Patterson & Murray, 1983; Kuzvart, 1984; Hosterman & Patterson, 1992; Hanchar et al., 2004). Small amounts of bentonite are also used as a catalyst in the refining of petroleum.

The use of sodium bentonite in hazardous waste containment utilizes a number of its special properties (Jepson, 1984). Its swelling ability makes it an effective soil sealant, since, by swelling within the interstices of the soil with which it is mixed, bentonite plugs the voids in the soil, creating a barrier of very low permeability. Swelling ability is aided by the possibility of a very small average particle size, allowing bentonite to plug even the smallest of voids. The high cation exchange capacity enhances the retention of wastes, especially heavy

EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

Table 4. Usage of bentonite produced in the USA^a

Use	Bentonite usage (kilotonnes) ^{b,c}		
	1995	1999	2002
Domestic			
Absorbents: Pet waste	574	1100	899
Absorbents: Other	88.4	W	W
Adhesives	W	2.17	2.1
Animal feed	97.8	49.9	42.4
Ceramics (except refractories) ^d	W	W	W
Drilling mud	627	733	762
Filler and extender applications ^e	69.9	47.1	45.7
Filtering, clarifying, decolorizing	W	91.6	127
Foundry sand	745	876	762
Pelletizing (iron ore) ^f	646	611	536
Miscellaneous refractories and kiln furniture	214	W	W
Miscellaneous ^g	94.9	81.8	117
Waterproofing and sealing	228	270	269
Total	3390	3870	3560
Exports			
Drilling mud	86.5	66.7	59.2
Foundry sand	256	238	244
Other ^h	89.1	124	106
Total	431	428	408
Grand total	3820	4290	3970

^a From USGS (1997); Virta (1999, 2002).

^b Data are rounded to no more than three significant figures and may not add to the totals shown.

^c W = Withheld to avoid disclosing company proprietary data; included with "Miscellaneous."

^d Includes catalysts and pottery.

^e Includes asphalt tiles, cosmetics, ink, medical, miscellaneous filler and extender applications, paint, paperfilling, pesticides and related products, pharmaceuticals, and plastics.

^f Excludes shipments to Canada.

^g Includes waterproofing seals, chemical manufacturing, filtering and clarifying oils, heavy clay products, lightweight aggregate, water treatment and filtering, and other unknown uses.

^h Includes absorbents, ceramics, fillers and extenders, filtering and clarifying oils, miscellaneous refractories, pelletizing refractories, waterproofing and sealing, and other unknown uses.

metals. In addition, a mixture of sodium bentonite and soil forms a tough, flexible mastic that is highly durable and not easily ruptured.

The ability of bentonite to bind cationic metals and certain pesticides has been applied experimentally to detoxifying victims of paraquat poisoning (Meredith & Vale, 1987) and to reducing the transfer of radiocaesium to milk and other animal-derived foods (Giese, 1989; Unsworth et al., 1989). Although effective in reducing the mortality of experimentally poisoned rats, there was no evidence that lavage with bentonite decreased human mortality following accidental ingestion of paraquat. Inclusion of bentonite in the diet of cattle reduced the transfer of radiocaesium into milk, but bentonite proved far less effective than cyanoferrates such as Prussian blue and thus was not the treatment of choice. In addition to these examples, there is an extensive literature documenting the ability of bentonite to adsorb these and other toxics. None of this research, however, appears to have led to major new uses for bentonite.

The chemical composition of bentonite affects its usage (Patterson & Murray, 1983; Kuzvart, 1984; Hosterman & Patterson, 1992). High-swelling bentonite, in which sodium is usually the dominant exchangeable ion, is preferred for drilling muds, pelletizing iron ore, and sealing and waterproofing, whereas low-swelling calcium bentonite is preferred for filtering, clarifying, and absorbing and for serving as a filler, stabilizer, extender, carrier, bonding agent, or catalyst. Both types are used as a foundry sand bond. Sodium bentonites provide good dry strength in moulds, whereas calcium bentonites provide good “green” (condition prior to drying) strength.

Bentonite is used in a large number of different cosmetic products, such as paste masks, skin care and cleansing preparations, eyeliners, foundations, and others. In 1998, bentonite was reported to be used in 78 different cosmetics in the USA, usually at concentrations between 1% and 10%, but reaching 80% in some paste masks (CIREP, 2003). Bentonite has been approved for use as a “Generally regarded as safe” (GRAS) food additive in the USA (US FDA, 2004).

The uses of montmorillonite-type Fuller’s earth overlap those of bentonite (Hosterman & Patterson, 1992) (Table 5). Major uses include serving as an adsorbent for oil, grease, and animal waste and as a carrier for pesticides and fertilizers. Minor uses are filtering, clarifying, and decolorizing and serving as filler in paints, adhesives, and pharmaceuticals.

EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

Table 5. Fuller's earth sold or used by producers in the USA, by use^a

Use	Amount sold or used (kilotonnes) ^{b,c}		
	1995	1999	2002
Oil and grease absorbent	285	275	409
Pet waste absorbent	1530	1580	1580
Animal feed	72.7	82.9	81.5
Fertilizers	50.8	137	139
Fillers, extenders, binders ^d	75	63.9	58.8
Filtering, clarifying, decolorizing animal, mineral, vegetable oils, greases	9.07	W	63.3
Pesticides and related products	302	67.8	102
Miscellaneous ^e	130	245	264
Exports ^f	161	114	30.9
Total	2640	2560	2730

^a From USGS (1997); Virta (1999, 2002).

^b Data are rounded to no more than three significant digits and may not add to the totals shown.

^c W = Withheld to avoid disclosing company proprietary data; included with "Miscellaneous."

^d Includes adhesives, asphalt emulsions and tiles, gypsum products, medical applications, pharmaceuticals, cosmetics, paint, plastics, textiles, and other unknown uses.

^e Includes catalysts (oil refining), electrical porcelain, drilling mud, roofing granules, chemical manufacturing, floor and wall tile, portland cement, refractories, and other unknown uses.

^f Includes absorbents, drilling mud, fillers, extenders and binders, floor and wall tiles, mineral oils and greases, and other unknown uses.

3.2.2.2 Kaolin

In China, east of King-te-Chen city, kaolin was used for making porcelain in the third century BC.

In Europe, kaolin was used for making earthenware in about 5000 BC, but the method of making porcelain was unknown until about 1710. After that time, porcelain production spread first to Europe and then to the whole world (Kuzvart, 1984).

Like bentonite and common clay, kaolin is an important industrial mineral that has an enormous variety of uses. Uses of kaolin mined in the USA for the years 1995, 1999, and 2002 are summarized in Table 6. In all three years, about two-thirds of the total production was used

domestically and the remainder exported. Use of kaolin as a coating for paper accounted for almost half of the total domestic consumption and for roughly 80% of the exported kaolin. Widespread use of kaolin-coated papers in the manufacture of cigarettes (Wynder & Hoffman, 1967) may expose smokers to kaolinite particles by inhalation. Other important uses of kaolin were as a filler in the production of paint, paper, and rubber, as a component of fibreglass and mineral wool, as a landfill liner, and as a catalyst in oil and gas refining. The usage historically associated with kaolin, manufacture of porcelain and china-ware, accounted for less than 1% of the domestic consumption in the USA. In China, 80–85% of the total production in 2003 was used for ceramics, 5% for paper, 3% for rubber, and 2% for paint. In India, 290 000 tonnes were used for ceramics, 84 000 tonnes for paints, 68 000 tonnes for paper/paperboard, 29 000 tonnes for detergents, and 27 500 tonnes for rubber (Moore, 2003).

Kaolinite has a number of properties relevant to medicine. It is an excellent adsorbent and will adsorb not only lipids and proteins (Wallace et al., 1975; Adamis & Timár, 1980) but also viruses and bacteria (Steel & Anderson, 1972; Schiffenbauer & Stotzky, 1982; Lipson & Stotzky, 1983). It can be used to induce aggregation of platelets (Cronberg & Caen, 1971), to initiate coagulation of plasma by activation of factor XII (Walsh, 1972), and to remove non-specific haemagglutinin inhibitors from serum (Haukenes & Aasen, 1972; Inouye & Kono, 1972). Kaolin is used in medical therapy as a local and gastrointestinal adsorbent (Kaopectate, bolus alba).

Kaolin is used in a large number of different cosmetic products, such as eyeshadows, blushers, face powders, “powders,” mascaras, foundations, makeup bases, and others. In 1998, kaolin was reported to be used in 509 different cosmetics in the USA, usually at concentrations between 5% and 30%, but reaching 84% in some paste masks (CIREP, 2003). Medical, pharmaceutical, and cosmetic uses, however, accounted for roughly 0.01% of the total US consumption of kaolin (Table 6).

EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

Table 6. Kaolin sold or used by producers in the USA, by use^a

Use	Amount sold or used (kilotonnes) ^{b,c}		
	1995	1999	2002
Domestic			
Ceramics			
- Catalyst (oil and gas refining)	93.2	208	210
- Electrical porcelain	7.6	12.7	8.3
- Fine china and dinnerware	26.4	23.5	27.4
- Floor and wall tile	38.3	39.8	63.1
- Pottery	20.6	11.2	13.4
- Roofing granules	24.9	43.2	36.5
- Sanitary ware	67.9	75.6	85.2
- Miscellaneous	152	26.3	W
Chemical manufacture	130	23.2	31.6
Civil engineering	W	W	W
Fibreglass, mineral wool	402	329	288
Fillers, extenders, binders			
- Adhesive	71.6	81.5	67.4
- Fertilizer	W	W	3.55
- Medical, pharmaceutical, cosmetic	W	W	0.754
- Paint	270	288	298
- Paper coating	2800	3000	2540
- Paper filling	853	791	450
- Pesticide	11.2	13.1	W
- Plastic	39.5	39.7	49.7
- Rubber	194	222	177
- Miscellaneous	156	115	107
Heavy clay products			
- Brick, common and face	230	126	70.9
- Portland cement	W	54.2	W
Refractories			904 ^d
- Firebrick, block and shapes	26.8	13.8	
- Grog and calcines	190	135	

Sources of Human and Environmental Exposure

Table 6 (Contd)

Use	Amount sold or used (kilotonnes) ^{b,c}		
	1995	1999	2002
- High-alumina brick and specialties, kiln furniture	885	W	
- Foundry sand, mortar, cement, miscellaneous refractories	145	621	
Miscellaneous applications	138	430	91.6
Total	6970	6720	5520
Exports			
Ceramics	187	210	203
Paint	67.7	88.1	85
Paper coating	2040	1970	2040
Paper filling	145	110	93.9
Rubber	36.3	45.7	50.7
Miscellaneous ^e	165.9	23.8	19
Total	2510	2440	2490
Grand total	9480	9160	8010

^a From USGS (1997); Virta (2001, 2002).

^b Data are rounded to no more than three significant digits and may not add to the totals shown.

^c W = Withheld to avoid disclosing company proprietary data; included with "Miscellaneous" or "Miscellaneous applications."

^d Includes firebrick (blocks and shapes), grogs and calcines, high-alumina brick and specialties, kiln furniture, and miscellaneous refractories.

^e Includes 145 000 tonnes of foundry sand, mortar, cement, and miscellaneous refractories in 1995.

3.2.2.3 Other clays

Common clay and shale, of which illite is often a major component, are used mainly in the manufacture of extruded and other bricks, portland and other cements, concrete blocks and structural concrete, and refractories. Highway surfacing, ceramic tiles, and ceramics and glass are other important uses (Virta, 2002; see Table 7). The relatively pure illite mined in Hungary, Malaysia, and the USA has a variety of uses. The illite produced in Malaysia is mainly exported to Japan, where it is mostly used to coat welding rods because of its fluxing properties. It is also used as a filler in sponge rubber and latex foam (Fan & Aw, 1989). Illite increases the plasticity and workability of the porcelain mass and produces a superior end product by filling the gaps in the kaolin crystals. Use in cosmetics requires a light-

EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

coloured illite with minimal iron. Fan & Aw (1988) suggested that illite could also serve as a less expensive substitute for feldspar in ceramics.

Table 7. Common clay and shale sold or used by producers in the USA, by use^{a,b}

Use	Amount sold or used (kilotonnes) ^{c,d}		
	1995	1999	2002
Ceramics and glass ^e	139	181	174
Civil engineering and sealing	180	34.8	W
Floor and wall tile			
- Ceramic	301	400	387
- Other ^f	73	W	115
Heavy clay products			
- Brick, extruded	11 200	12 000	11 300
- Brick, other	1 640	1 800	1 500
- Drain tile and sewer pipe	136	27	39
- Flowerpots	48.6	W	46
- Flue linings	59.8	58.9	47.7
- Structural tile	21.5	22.7	W
- Other ^g	503	160	110
Lightweight aggregate			
- Concrete block	2 530	2 430	2 370
- Highway surfacing	248	317	364
- Structural concrete	869	929	908
- Miscellaneous ^h	521	259	361
Portland and other cements	6 400	5 010	3 950
Refractories ⁱ	459	785	795
Miscellaneous ^j	268	429	556
Total	25 600	24 800	23 000

^a From USGS (1997); Virta (1999, 2002).

^b Excludes Puerto Rico.

^c Data are rounded to no more than three significant digits and may not add to the totals shown.

^d W = Withheld to avoid disclosing company proprietary data; included with "Other" or "Miscellaneous."

^e Includes pottery and roofing granules.

Sources of Human and Environmental Exposure

Table 7 (Contd)

- ^f Includes quarry tile and miscellaneous floor and wall tiles.
- ^g Includes flower pots, roofing tile, sewer pipe, structural tile, and miscellaneous clay products.
- ^h Includes miscellaneous lightweight aggregates.
- ⁱ Includes firebrick, blocks and shapes, grogs and calcines, mortar and cement, plugs, taps, wads, and miscellaneous refractories.
- ^j Includes asphalt emulsions, exports, miscellaneous civil engineering and sealings, miscellaneous fillers, extenders and binders, pelletizing (iron ore), wallboard, and other unknown uses.

4. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

4.1 General population exposure

4.1.1 *Bentonite*

Bentonite is widely distributed in natural materials that commonly occur as fine particles readily dispersed by air circulation and moving water. Bentonite is also widely used in an enormous variety of consumer products. Accordingly, general population exposure to low concentrations of bentonite must be widespread, if not universal. However, while Churg (1983) identified small amounts of kaolinite and illite in the lungs of a group of subjects drawn from the general population (see section 4.1.2), he did not detect montmorillonite. Similarly, while Dumortier et al. (1989) reported illite and kaolinite as ubiquitous in bronchoalveolar lavage (see section 4.1.2), they did not identify any particle as bentonite.

4.1.2 *Kaolin and other clays*

Kaolin and other clays are natural components of the soil and occur widely in ambient air as floating dust. Accordingly, exposure of the general population to them must be universal, albeit at low concentrations. In the vicinity of mines and industrial projects, kaolinite is likely to be present at high concentrations in air; no data are available, however.

The occurrence of mineral fibres in lung specimens from 20 persons who had no occupational dust exposure was determined on autopsy. Thirteen different minerals, among which were kaolinite and illite, were identified in the lungs of patients; 71% of the particles were fibres. There was no correlation between the number or type of fibres and age, sex, or smoking (Churg, 1983).

In a further study, Churg & Wiggs (1985) studied the relationship between mineral particles (and fibres) in the lungs (from autopsy) and lung cancer in men without occupational mineral exposure. The average number of mineral particles in the lungs of cancer patients was $525 \times 10^6/\text{g}$ dry lung, and in the referents, $261 \times 10^6/\text{g}$ dry lung. Kaolinite, talc, mica, feldspar, and crystalline quartz comprised the

majority of particles in both groups. Approximately 90% of the particles were smaller than 2 μm , and 60% were smaller than 1 μm .

Dumortier et al. (1989) similarly reported kaolinite, illite, and mica in most of 51 subjects sampled via bronchoalveolar lavage. These subjects were all occupationally exposed to various industrial dusts, but not including kaolin or other clays to any great extent. The presence of illite in human lungs is also not a recent phenomenon, since it was found in the lungs of a 5300-year-old male human cadaver that had been well preserved by glacial ice (Pabst & Hofer, 1998).

Paoletti et al. (1987) identified 17 different mineral types in the lung parenchyma of 10 deceased subjects resident in an urban area and with no occupational dust exposure. Approximately 70% of the minerals consisted of phyllosilicates, in particular micas (10 cases), clays (kaolin and pyrophyllite, 10 cases), and chlorites (6 cases).

Samples of nine brands of tobacco and alveolar macrophages from cigarette smokers contained particles identified as kaolinite by energy-dispersive X-ray spectrometry (Brody & Craighead, 1975).

Kaolinite and mica particles were identified in the macrophages of the bronchoalveolar lavage fluid of 18 and 12, respectively, of the total of 22 patients for whom pulmonary lavage was performed. The frequency of these particles was higher among smokers and ex-smokers than among non-smokers (Johnson et al., 1986).

Mastin et al. (1986) determined the inorganic particles in the lungs of persons not occupationally exposed. Quartz, talc, and kaolinite could be detected in the lungs of practically all subjects (number of lungs examined: 48).

Kalliomaäki et al. (1989) detected several different mineral types in the lungs from 11 unselected autopsy cases, including quartz, feldspar, kaolinite, and mica, reflecting the Finnish bedrock. Kaolinite particles constituted $10 \pm 5\%$ of the mineral particles in the lungs. More mineral particles were found in the lungs of non-smokers, but the differences were not statistically significant. Similarly, Yamada and co-workers (1997) also detected (fibrous) talc, mica, silica, chlorite, and kaolinite

particles in the lungs of female lung cancer patients and referents from urban and rural areas in Japan.

4.2 Occupational exposure

Occupational exposure to clay dusts has been studied in many industries. However, numerical figures are usually given on total dust or respirable dust only, without analysis of the components of the dust. When information is given on individual components, it is mostly on silica. Exposure to silica in different trades has recently been extensively summarized (IARC, 1997b; IPCS, 2000) and will not be reviewed here. Clay particles have been detected in the lung tissue of bronchoalveolar lavage fluid of various worker groups (Churg, 1983; Churg & Wiggs, 1985; Johnson et al., 1986; Wagner et al., 1986; Dumortier et al., 1989; Dufresne et al., 1994; Gibbs & Pooley, 1994; Sébastien et al., 1994; Monsó et al., 1997). Exposure to clay dusts can also occur in small, family-run pottery shops — and may be considerably higher than in large workshops. Such exposure will not be considered in this review because of a lack of available information.

4.2.1 Bentonite

The information on occupational exposure to bentonite dust in mines, processing plants, and user industries (foundries) is summarized in Table 8. These data reveal a wide range of values for both total and respirable bentonite dust and indicate that very high total dust concentrations can be present in industrial settings involving both the production and utilization of bentonite. The highest values for total dust concentrations were reported by Melkonjan et al. (1981) in their study of bentonite mining and processing in Bulgaria. Only one of the eight types of locations they sampled, inside dump truck cabs during loading, had values consistently below 10 mg/m³. Their highest reported values, 1150 and 1430 mg/m³, and highest averages, 504 and 749 mg/m³, obtained in the vicinity of packing and loading operations, reveal an extraordinary level of dustiness. These highest values are more than an order of magnitude greater than the highest values reported in any of the other studies summarized in Table 8. In the 1970s in foundries in Germany (Orth & Nisi, 1980), the dust concentrations in air were mostly below older observations for bentonite plants in the USA (McNally & Trostler, 1941; Phibbs et al., 1971), as well as far below contemporary observations from bentonite plants and

Table 8. Occupational exposure to bentonite dust

Location/date of observations	No. of observations / no. of facilities ^a	Dust concentration ^b (mg/m ³)		No. of observations of facilities with respirable dust >10 mg/m ³	No. of observations of quartz >1%	Reference
		Range of observations	Average/range of facilities			
Fuller's earth plant, USA (Illinois) / 1934	5 / 1	1–19 ^c	6	1	? ^d	McNally & Trostler (1941)
Bentonite plants, USA (Wyoming) / 1950–1967	17 / 4	1–92 ^c	11–60	10	17	Phibbs et al. (1971)
Older iron foundry, Germany / pre-1980 ^e	6 / 1	0.39–2.9 t	1.7	0	?	Orth & Nisi (1980)
	6 / 1	0.08–0.76 Rt	0.31	0	?	
	6 / 1	3.7–12.3 i	6.6	1	?	
	2 / 1	1.9–4.0 Ri	3.0	0	?	
Modern iron foundry, Germany / pre-1980 ^e	5 / 1	0.56–2.8 t	1.6	0	?	Orth & Nisi (1980)
	5 / 1	0.15–0.43 Rt	0.23	0	?	
	4 / 1	1.7–7.9 i	4.8	0	?	
	2 / 1	0.81–3.2 Ri	2.0	0	?	
Bentonite mines and processing plants, Bulgaria / pre-1981	220 / ?	1.6–1430.0	333	Many	0	Melkonjan et al. (1981)

Table 8 (Contd)

Location/date of observations	No. of observations / no. of facilities ^a	Dust concentration ^b (mg/m ³)		No. of observations >10 mg/m ³ total or >5 mg/m ³ respirable	No. of observations of quartz > 1%	Reference
		Range of observations	Average/range of facilities			
Non-ferrous foundry, USA / pre-1987	13 / 1	0.77–27.9	6.65	3	At least 7	Que-Hee (1989)
Mines and processing plants, USA / 1992–1998	251 / 16	0.20–1.94 R	0.66	0	13	US Mine Safety and Health Administration, personal communication (1999)
		0.00–6.07 mainly R	0.06–2.18	1	85	
Mines and processing plants, Turkey, 1996–1999	24 / 11	2.6–11.2 R	?	At least 1	?	Turkish Ministry of Labor & Social Security, personal communication, 1999
Foundries, Turkey / 1990–1998	207 / 70	0.05–33.9 R	?	At least 8	?	

^a Facilities may include mines, processing plants, and user industries.

^b Values are for total dust unless indicated as respirable dust by R. i = impact sampler; t = continuous filter tape sampler using beta ray absorption; R = respirable dust.

^c Original data in mppcf (million particles per cubic foot), converted by the approximate relationship 30 mppcf = 10 mg/m³.

^d ? = no information.

^e Authors believe that results obtained with the impact sampler (i) and total dust values from the tape sampler are flawed and underestimate the true values.

mines in Bulgaria (Melkonjan et al., 1981). In general, however, these data for total dust concentrations represent too limited a sampling to detect much in the way of regional, temporal, or other trends. Orth & Nisi (1980), however, concluded that their study of two German iron foundries demonstrated that change from the older, partially mechanized methods to modern, fully automatic, boxless moulding resulted in a reduction in dustiness. This may be true; however, in view of their problems in measurement methodology, the small differences between the two foundries in average dustiness, and the fact that only one foundry of each type was sampled, the conclusion seems premature.

Values for respirable bentonite dust are generally far below those for total bentonite dust (Table 8). However, values as high as 33.9 mg/m³ have been reported in a foundry (Turkish Ministry of Labor & Social Security, personal communication, 1999). None of the observations from the two German foundries (Orth & Nisi, 1980) and only 1 of the 251 recent observations from the USA (US Mine Safety and Health Administration, personal communication, 1999) exceeded 5 mg/m³, whereas the Turkish data for respirable dust in foundries suggest that this value is exceeded more frequently. Silica, which is widely viewed as the major hazard in many industrial dusts (Phibbs et al., 1971; Oudiz et al., 1983; Oxman et al., 1993), was a significant component of bentonite dust, especially in foundries.

4.2.2 Kaolin

Stobbe et al. (1986) analysed mine dusts of West Virginia, USA. Respirable dust samples collected in three locations in the mines contained 64% illite, 21% calcite, 8.5% kaolinite, and 6.7% quartz on average.

Processing of china clay involves considerable exposure (Tables 9 and 10). Exposure was especially significant before the 1960s (Sheers, 1964). Following drying, nearly all work stages were carried out in high dust concentrations (conveyor belts, bagging, storage in bulk). At present, ventilation is much more effective, and closed technologies are also widely used.

Lesser et al. (1978) analysed the free silica content of the airborne dust from a kaolin mill in Georgia and another from South Carolina and noted that while in most of the (total dust) samples free silica was

Table 9. Dust exposure in the kaolin industry

Time of sampling	Exposure group	No. and type of samples	Parameter measured	Result, average (mg/m ³)	Reference
1981	Miner	10; personal	Respirable dust	~0.2 (0.1–0.35) ^a	Sepulveda et al. (1983)
	Car loader	3; personal	Respirable dust	~1 (0.7–1.2) ^a	
	Car loader	3; area	Total dust	~10 (7–15) ^a	
	Bin operator	3; personal	Respirable dust	~2 (1.3–2.5) ^a	
	Bin operator	7; area	Total dust	~20 (10–30) ^a	
	Mill operator	3; personal	Respirable dust	~1 (0.8–1.2) ^a	
	Mill operator	8; area	Total dust	~20 (10–80) ^a	
	Baghouse labourer	5; personal	Respirable dust	~2 (0.3–5) ^a	
	Baghouse labourer	3; area	Total dust	~5 (1.5–9) ^a	
	Kaolin mining and processing		Respirable kaolin dust	Maximal value 377	
1951				Maximal value 361	Kennedy et al. (1983)
1960				All measurements <5	
1979					

Table 9 (Contd)

Time of sampling	Exposure group	No. and type of samples	Parameter measured	Result, average (mg/m ³)	Reference
1977	Kaolin processing	14; personal	Respirable dust	3.9 ^{b,c}	Altekruse et al. (1984)
1978		9; personal		3.8 ^{b,c}	
1980		5; personal		5.3 ^{b,c}	
1981		14; personal		1.8 ^{b,c}	
1981	Maintenance area	68; personal		1.74 ^b	
1980		15; personal		0.9 ^{b,c}	
1981		11; personal		0.1 ^{b,c}	
1981		12; personal		0.8 ^{b,c}	
1981	Kaolin mine	5; personal		0.1 ^{b,c}	
1980		4; personal		0.14 ^b	
1990	"UK China Clay industry, potentially dusty occupations"	8000, both breathing zone and area sampling	Respirable dust	1.6	Comyns et al. (1994)
1978	Attritor mill	Altogether 500 personal samples annually for the industry	Respirable dust	4.7 (9.32) ^d	Rundle et al. (1993)
1990				2.1 (3.36) ^d	
1978	Dryers			3.5 (5.41) ^d	
1990				1.7 (2.78) ^d	

Table 9 (Contd)

Time of sampling	Exposure group	No. and type of samples	Parameter measured	Result, average (mg/m ³)	Reference
1978	Calciners			3.5 (3.93) ^d	Rundle et al. (1993)
1990				2.23 (3.25) ^d	
1978	Slurry plants			1.6 ^e	
1990				1.2 (2.26) ^d	
1984–1986	Attritor mill	114; personal	Respirable dust	2.7	Ogle et al. (1989)
	Dryers	681; personal		1.9	
	Calciners	63; personal		2.5	
	Slurry plants	69; personal		1.1	
	Tube presses	5; personal		0.5	

^a Average (range), estimated from graphical presentation.^b Mean concentration.^c Estimated from a graph.^d Mean (9th decile).^e Mean, based on two samples.

Table 10. Dust exposure in brick, pipe, and tile industries

Time of sampling	Exposure group	No. and type of samples	Parameter measured	Dust concentration, mean (range) ^a (mg/m ³)	Quartz concentration, mean (range) ^a (mg/m ³)	Reference
1990–1991	Office, canteen	18; personal	Respirable dust	0.4 (0.3–1.3)	0.04 (0.02–0.13)	Love et al. (1999)
	Supervisors	336; personal		0.6 (0.3–1.6)	0.05 (0.02–0.18)	
	Quarries, moulders	301; personal		1.0 (0.4–1.6)	0.11 (0.04–0.28)	
	Brick cutters	10; personal		1.0 (0.3–1.4)	0.12 (0.04–0.21)	
	Mixed (fitters, fork truck drivers)	164; personal		1.2 (0.4–5.9)	0.07 (0.02–0.15)	
	Packers, kiln brick layers	202; personal		1.5 (0.3–2.3)	0.11 (0.02–0.21)	
	Forklift drivers in kilns or driers	46; personal		1.6 (0.3–2.4)	0.13 (0.04–0.22)	
	Pan mill operators	83; personal		1.7 (0.4–5.8)	0.18 (0.04–0.75)	
	Labourers	172; personal		1.7 (0.3–4.8)	0.15 (0.03–0.38)	
	Sand users (blasters, applicators)	47; personal		2.3 (0.4–4.4)	0.23 (0.03–0.36)	
	Clean-up squad	25; personal		6.9 (1.9–7.8)	0.27 (0.05–0.33)	
	Kiln demolition	3; personal		10 ^b	0.62	

Table 10 (Contd)

Time of sampling	Exposure group	No. and type of samples	Parameter measured	Dust concentration, mean (range) ^a (mg/m ³)	Quartz concentration, mean (range) (mg/m ³) ^a	Reference
1985 ^c	Low dust exposure: offsetter, fireman, drier operator	35; personal	Respirable dust	1.02 SD 0.41	Silica 0.7% (mean)	Myers et al. (1989a)
	Medium dust exposure: setter, clamp packer, packer, preparation plant, hyster operator, rock face machine	29; personal		2.18 SD 1.42	Silica 3.9% (mean)	
	High dust exposure: drawer kiln cleaner, maintenance worker, downdraught kilns	22; personal		6.71 SD 4.80	Silica 3.2% (mean)	
	Hoisting winch, feeder and circulatory crusher	28	Respirable dust	4.3 (mean)	6.1%	
1979–1980 ^c			Total dust	9.3 (0.7–50.4)	18.4%	Wiecek et al. (1983)
	Press receipt of green brick	22	Respirable dust	1.9 (mean)	5.8%	
			Total dust	4.2 (0.8–12.3)	10.8%	
	Backing off and setting the green brick in the furnace	28	Respirable dust	6.3 (mean)	3.7%	
			Total dust	19.0 (0.8–61.6)	5.8%	

Table 10 (Contd)

Time of sampling	Exposure group	No. and type of samples	Parameter measured	Dust concentration, mean (range) ^a (mg/m ³)	Quartz concentration, mean (range) (mg/m ³) ^a	Reference
1970–1971	Boring the ready-made product from the furnace	26	Respirable dust	33.9 (mean)	2.42%	Rajhans & Budlovsky (1972)
			Total dust	118.9 (7.3–418.0)	4.7%	
	Furnace and disposal	22	Respirable dust	41.8 (mean)	2.88%	
			Total dust	197.9 (44.0–636.0)	4.6%	
	Brick plant; shale as raw material; dusty operations	9	Respirable dust	2.39 (mean)	15% free respirable silica	
		16		3 (mean)	20%	
		5		3 (mean)	20%	
		5		2.6 (mean)	19%	
	Brick plant; shale and clay as raw materials; dusty operations	6		2.24 (mean)	21%	
		6		4.26 (mean)	14.7%	
ca. 1948 ^d		5		1.5 (mean)	7.5%	Keatinge & Potter (1949)
	Brick plant; clay as raw material; dusty operations	5		1.2 (mean)	12.5%	
		8		1.05 (mean)	10%	
	Brick plant, different tasks	9	Respirable dust ^e	2.8 (0.3–5.7)		
		19	Total dust	143 (3.5–250)		

Table 10 (Contd)

Time of sampling	Exposure group	No. and type of samples	Parameter measured	Dust concentration, mean (range) ^a (mg/m ³)	Quartz concentration, mean (range) (mg/m ³) ^a	Reference
1973	Sanitary ware manufacture	15	Respirable dust	0.42–0.54 (range)	28–39% quartz	Rees et al. (1992)
1974	Sanitary ware manufacture	24		0.02–0.69 (range)	4.19	
1986	Sanitary ware manufacture	43		0.8–4.2 (range)	10.60	
1989	Sanitary ware manufacture	9		0.38–2.1 (range)	8.28	
1974	Tile manufacture	24		0.35–8.41 (range)	9–29	
1989–1990	Wall tile and small bathroom fitting production		Respirable dust			
	- Slip production	4		2.8 (1.5–6.1) ^f	0.27 (mean)	
	- Tile dust production	3		1.2 (0.5–2.1) ^f	–	
	- Bathroom fittings	9		1.2 (0.2–6.6) ^f	0.17–0.4 (range)	
	- Tile making	9		1.4 (0.7–2.8) ^f	0.16–0.19 (range)	
	- Biscuit firing and sorting	5		0.9 (0.7–4.2) ^f	0.13 (mean)	
	- Tile glazing and glost sorting	6		1.9 (0.9–3.0) ^f	0.06 (mean)	
	- Bathroom fitting glazing	2		2.1 (0.9–3.3) ^f	0.07 (mean)	

Table 10 (Contd)

^a Unless otherwise noted.;

^bOne site only.;

^c Brick workers;

^dSampling time not given in the paper, assumed from the year of publication.

^e Below 5.5 μm .;

^fMedian (range).

not detectable, in some specimens (three baggers and a bulk loader) quartz was present at approximately 1%. They also said that in all but one of the plants, some baggers and loaders were exposed to about 1% free silica (no numbers provided).

Significant exposure is expected also in paper, rubber, and plastic production. When producing insulator materials, quartzite and feldspar exposure may occur (Parkes, 1982). Sahle et al. (1990) carried out measurements of airborne dust in a Swedish paper mill. Total dust exposure at the plant was generally less than 3 mg/m³. The respirable fraction of the total dust varied from 15% to 70%. The inorganic dust was 36% of the total dust, and 10–15% of the total fibres were inorganic. The respirable dust at the plant contained kaolinite fibres, wollastonite, talc and other silicates, and cellulose fibres.

Dust conditions in power stations were studied in Poland by Wojtczak et al. (1989). In 14 power stations, average respirable dust and total dust concentrations ranged from 0.45 to 6.95 mg/m³ and from 1.55 to 85.0 mg/m³, respectively. The mean content of free crystalline quartz was less than 10%. At the workplace where ash dust was also encountered, the presence of respirable fibre at concentrations below 0.2 fibre/cm³ was measured. Alpha-quartz and mullite were found in all ash samples. Occasionally, kaolinite and orthoclase were also found. Only at 5 power stations out of 14 was the mean respirable dust concentration below 1 mg/m³.

In ceramic factories, workers are exposed to mixed dust. Analysis of the workplace respirable dust of five Hungarian ceramic factories demonstrated the presence of kaolinite (19–69%) and crystalline quartz (7–30%). X-ray amorphous material amounted to 7–76%, nearly half of which was amorphous silica (Adamis & Krass, 1991).

4.2.3 Other clays

There is some relevant information on exposure to illite dust in coal mining and in the manufacture of bricks, but none concerning the mining and processing of relatively pure deposits of illite. Wiecek et al. (1983), in their study of six brick manufacturing plants in Poland, reported illite in workplace floating dust prior to the firing of the bricks. In a sample of 27 dusts from European coal mines, illite was present in most and comprised as much as 55% of the fine material (particles <1.65 µm in mean geometric diameter) in one sample group (Bruch & Rosmanith, 1985). Illite comprised 11% of the total dust and

46% of the mineral dust (i.e., excluding coal dust) in a sample from a coal mine in Saar, Germany, and was also present in the lungs of most and possibly all of a sample of 13 Saar coal miners (Leiteritz et al., 1967). Stobbe et al. (1986) reported that in dust samples collected in nine locations in a bituminous coal mine in West Virginia, USA, illite represented 49–80% of the mineral dust and averaged 64%. In another study of a West Virginia coal mine, illite ranged from 3 to 89% of the mineral dust and averaged 52% (Grayson & Peng, 1986). In addition, observations in Dumortier et al. (1989) discussed above in section 4.1.2 indicated that even in industrial workers not specifically exposed to clay mineral dusts, illite can form a significant fraction of the particles retained by the lungs.

Data from the various studies summarized above suggest that workers in some occupations are frequently exposed to illite particles, which may comprise a large or major portion of certain industrial workplace dusts.

5. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Clays are not distinct chemicals, but are complex mixtures with usually a large component of specific clay minerals, such as montmorillonite, kaolinite, or illite. No information was available on the kinetics or metabolism of montmorillonite, kaolinite, or illite as they occur in most occupational settings. However, Snipes and co-workers (1983a, 1983b) and Bailey et al. (1982) studied the kinetics of fused aluminosilicate particles, prepared from montmorillonite by heating, in mice, rats, dogs, and humans after inhalation exposure. The method consisted of exchange by radiolabel (^{85}Sr , ^{88}Y , or ^{134}Cs) of cations normally present in montmorillonite clay, aerosolization, and heating at 1100 °C to yield spherical particles in which the radiolabel was incorporated in the lattice and very resistant to leaching. The polydisperse aerosol could then be segregated to different monodisperse fractions as needed and the specific fractions resuspended in an aqueous medium and administered to the experimental animals or human volunteers by inhalation.

The initial deposition of particles in the nasopharynx was lower in dogs than in mice or rats and increased with increasing activity median aerodynamic diameter (AMAD) (Table 11), whereas the tracheobronchial deposition was low and independent of animal species and particle size. The pulmonary deposition was remarkably higher in dogs than in mice and rats and decreased with increasing particle size; the change with size was especially marked in rats and mice.

The removal of particles from the lungs took place by solubilization *in situ* (leading to label distribution in the blood and carcass and excretion in the urine) and by physical clearance. For dogs, the overall clearance was slow, and the major mechanism of removal was solubilization. Rats and mice removed the label from the lungs rapidly, and the mechanism was mainly mechanical removal. The mechanical removal in rats and mice was mostly via the gastrointestinal tract, while in dogs it was mainly to the lung-associated lymph nodes. The mechanical clearance was independent of the particle size, while

Kinetics and Metabolism in Laboratory Animals and Humans

solubilization was faster for smaller particles. The initial half-time for the mechanical clearance was 140 days for dogs, which increased by 200 days post-exposure to 6900 days. In rats and mice, the initial half-time for mechanical clearance was 35 days and increased to 690 days in rats and 460 days in mice by approximately 500 days post-exposure.

Table 11. Deposition of ^{134}Cs -labelled aluminosilicate particles in dogs, rats, and mice after inhalation exposure^a

Animal species	Particle size		Percentage of initial body burden deposited		
	AMAD ^b (μm)	Geometric diameter (μm)	Naso-pharynx	Tracheo-bronchial	Pulmonary
Dog	0.7	0.35	30	5	60
	1.5	0.90	40	5	50
	2.8	1.8	40	5	50
	Poly-disperse ^c	–	40	5	50
Rat	0.7	0.35	55	5	20
	1.5	0.90	62	5	13
	2.8	1.8	67	5	8
	Poly-disperse ^c	–	63	5	12
Mouse	0.7	0.35	45	5	30
	1.5	0.90	62	5	13
	2.8	1.8	67	5	8
	Poly-disperse ^c	–	62	5	13

^a From Snipes et al. (1983a).

^b Activity median aerodynamic diameter.

^c AMAD and geometric standard deviation approximately 1.5–3 μm and 1.5–2.0, respectively.

Radiolabel from fused aluminosilicate particles, instilled in the apical lung lobe in dogs, was transferred mainly to the lateral tracheo-bronchial lymph nodes; after instillation to the cardiac or diaphragmatic lung lobes, radiolabel was mainly in the middle tracheobronchial lymph nodes (Snipes et al., 1983b). More recent studies indicate that ultrafine particles (<100 nm) have a high deposition in the nasal area of rats (Gerde et al., 1991) and humans (Cheng et al., 1988). Ultrafine particles can penetrate the alveolar/capillary barrier (Nemmar et al., 2004).

After inhalation exposure of seven volunteers to monodisperse fused aluminosilicate particles (1.9 or 6.1 μm aerodynamic diameter), there was a rapid initial clearance from the lung region of 8% and 40% of the two particle sizes, respectively, over 6 days, which was considered to represent deposition in the conducting airways. Of the remaining activity, 4% of the smaller and 11% of the larger particles cleared initially with a half-time of about 20 days, and the rest with half-times of 330 and 420 days. Taking into account the *in situ* dissolution (estimated to be 0.001 and 0.0005/day for the two particle sizes), the mechanical clearance half-time during the slow clearance stage is approximately 600 days and is independent of the particle size.

Two limited studies have addressed the leaching and bioavailability of metals from clays (Mascolo et al., 2004; Wiles et al., 2004). No significant differences were observed in the contents of aluminium, antimony, barium, bromine, caesium, calcium, cerium, chromium, cobalt, copper, dysprosium, europium, hafnium, iron, lanthanum, lutetium, magnesium, manganese, neodymium, nickel, samarium, scandium, selenium, sodium, strontium, sulfur, tantalum, tellurium, terbium, thorium, titanium, uranium, vanadium, ytterbium, zinc, or zirconium in the brain, kidney, liver, or tibia from pregnant SD rats dosed with 2% sodium montmorillonite or calcium montmorillonite clay compared with animals fed the basal diet. The main element components of the clays were aluminium (10%), iron (3%), and magnesium (0.5%) (as well as sodium in the sodium montmorillonite, 1%), with small amounts (usually less than 0.1%) of barium, caesium, manganese, strontium, zinc, and zirconium. The authors concluded that at this dietary level, the clays did not liberate significant amounts of trace elements (Wiles et al., 2004). Another study suggested that when rats were given 450 mg/kg of body weight of (three different varieties of) bentonite by gastric lavage for 6 days, contents of arsenic, cadmium, mercury, selenium, stibium, and tellurium in different organs were higher than in animals given normal diet (Mascolo et al., 2004). Because of limited reporting, the results are difficult to interpret.

6. EFFECTS ON LABORATORY MAMMALS AND *IN VITRO* TEST SYSTEMS

Clays are not distinct chemicals, but are complex mixtures with usually a large component of specific clay minerals, such as montmorillonite, kaolinite, or illite. They may also contain entities with well characterized adverse effects on health, such as quartz. In studies on the effects of clays or clay minerals on laboratory animals and even in *in vitro* tests, it is often not known what it was precisely that was studied and the impurities present in the material used. Thus, the results must be interpreted carefully. In this chapter, an effort is made to describe the material studied, but many of the studies, especially the older ones, give few details.

6.1 Single exposure

6.1.1 Bentonite

Data on single exposures of experimental animals to bentonite via intratracheal and intradermal administration, summarized in Table 12, indicate a variety of harmful effects. Bentonite has also been administered to animals by the intravenous and intramuscular routes (Boros & Warren, 1970, 1973), but these studies were not destined for, and cannot be used for, the assessment of health risks from bentonite.

Intratracheal injection of moderate amounts (0.16–0.27 g/kg of body weight) of finely powdered (where specified, mean diameter $<5\ \mu\text{m}$) bentonite into rodents evoked responses indicative of cytotoxic effects, as well as rapid and/or long-lasting irritation of or damage to the lungs. Gross effects on the lungs of rats administered 0.19 g/kg of body weight included inflammation and oedema within 12 h, bronchopneumonia by day 1, and a doubling of wet weight by day 3, followed by a gradual decline to a value still significantly above the initial weight by day 90 (Tátrai et al., 1983). The histology of the inflammation included large numbers of neutrophil leukocytes plus some macrophages and lymphocytes at 12 h, mainly eosinophil and necrobiotic leukocytes by day 1, and collapse of the reticular network by day 3. Some bentonite particles were held within the macrophages and leukocytes at day 3.

Table 12. Effects of single exposure to bentonite (montmorillonite) in mammals

Species	Sex ^a	Dose (g/kg of body weight) ^b and dosing regimen / period of observation	Findings ^c	Reference
Intratracheal				
Rat (strain not identified)	M	Dose not specified, ^d diameter mainly <5 µm / 365 days	Three dusts with no or low concentrations of quartz and cristobalite gave rise only to storage foci; a fourth dust with the highest concentration of quartz (65%) produced productive fibrosis.	Timár et al. (1966)
Rat (strain not identified)	n.s.	40 mg ^e (0.16), diameter <5 µm / 365 days	Storage foci in lung after an unspecified period not greater than 365 days.	Timár et al. (1979)
Rat (strain not identified)	n.s.	60 mg ^f (0.24), particle size not specified / 6 months	At 6 months, storage foci in lung.	Timár et al. (1980)
Rat (CFY LATI-Gödöllő)		60 mg ^g (0.24), particle size 0–2 µm; lung pathology studied after 3 or 10 months	At 3 and 10 months, sporadic storage foci of macrophage-derived cells; loose reticular network around the foci; no mitotic figures in the cells of the foci; no significant increase in mitotic figures in septal cells.	Ungváry et al. (1983)
Rat (CFY LATI-Gödöllő)	M	40 mg ^h (0.19), diameter <2 µm / 90 days	Lung weight nearly doubled at day 3; at day 90, decreased from weight at day 3, but still significantly above initial weight. Histology: Within 12 h, inflammation, perivascular oedema, and incipient bronchopneumonia; inflammatory reaction mainly neutrophil leukocytes plus some macrophages and lymphocytes. At day 1, bronchopneumonia, necrobiotic leukocytes, and eosinophil leukocytes. Reticular network collapsed by day 3. Some	Tátrai et al. (1983)

Table 12 (Contd)

Species	Sex ^a	Dose (g/kg of body weight) ^b and dosing regimen / period of observation	Findings ^c	Reference
Rat (CFY LATI-Göddölö)	M	60 mg ^l (0.22), diameter <5 µm / 12 months	bentonite particles held within macrophages and leukocytes at day 3. At day 90, bentonite in dust storage foci and foci surrounded by dense reticular network.	Tátrai et al. (1985)
Rat (Sprague-Dawley)	M	5–45 mg modified montmorillonite (0.017–0.15), median diameter 0.84 µm / 90 days	Acid phosphatase activity increased at day 3, but not at 1 month or 12 months; fibrosis not classifiable at day 3 and Belt-King grade 1 at 1 month and 12 months.	Schreider et al. (1985)
Rat (Sprague-Dawley CFY)	M	10 mg ^l (0.027), diameter <5 µm, median diameter 1–2 µm / 60 days	Through 90 days, no change in behaviour, body weight, or lung weight; at 90 days, gross lesions in lung; increased number of PAM; most instilled particles in alveoli and alveolar ducts and within PAM; mild fibrosis.	Adamis & Krass (1991)
Rat (SD)	M	10 mg of a kaolin containing 86% kaolinite, 4% quartz, 5% illite, and 5% amorphous silica	No effect on soluble protein or LDH in lavage from lung in samples taken on days 15, 30, and 60.	Adamis et al. (1998)

Bronchopulmonary lavage after 14, 30, and 90 days, number of cells, activities, LDH activity, protein, and phospholipid content of supernatant measured. Kaolinite caused a statistically significant increase in the protein content at 14 days, which returned to control level thereafter. No changes were observed in the other parameters studied.

Table 12 (Contd)

Species	Sex ^a	Dose (g/kg of body weight) ^b and dosing regimen / period of observation	Findings ^c	Reference
Rat (Alderley Park Strain 1 SPF)	n.s.	0.5 mg ^k (0.0012), average diameter 3 µm / 7 days	In lavage from lung, large and rapid increase in PMN and LDH with maximum at day 2 and declining to somewhat above baseline at day 7; soluble protein was similar except for peak at day 1; AM showed increase by day 7.	Sykes et al. (1982)
	n.s.	5 mg ^k (0.012), average diameter 3 µm / 100 days	In lavage from lung, large and rapid increase in PMN to same level as with 0.5 mg dose, with peak at day 1 and a gradual decline to baseline by day 49; LDH and soluble protein also increased sharply, with a peak at day 1 and a subsequent decline, but had not returned to baseline value by day 100; AM increased sharply, with a peak at day 7, and declined slightly by day 100.	
Rat (Sprague- Dawley CFY)	M	60 mg ^l (0.27), diameter <7 µm, mainly 1–2 µm / 90 days	In lavage from lung, phospholipid and acid phosphatase activity elevated by day 3; phospholipid remained elevated at day 90 and acid phosphatase at day 30 (not reported after day 30).	Adamis et al. (1985)
Rat (Sprague- Dawley CFY)	M	60 mg ^l (0.22), diameter <2 µm / 12 months	At 3 months in lavage from lung, phospholipid and hydroxyproline somewhat elevated; relative abundance of phospholipid fractions unchanged.	Adamis et al. (1986)

Table 12 (Contd)

Species	Sex ^a	Dose (g/kg of body weight) ^b and dosing regimen / period of observation	Findings ^c	Reference
Mouse (strain not identified), 90–120 per group	n.s.	1, 10, or 100 µg bentonite ^k (5×10^{-3} , 5×10^{-4} , or 5×10^{-5}), diameter 0.2–1.1 µm for 80% of the particles, median 0.4 µm injected intratracheally, followed by aerosolized group C <i>Streptococcus</i> sp.: mortality over 15 days recorded	Excess mortality 85% over controls from <i>Streptococcus</i> infection at instilled dose of 100 µg; 43% excess mortality with 10 µg; no significant change at 1 µg.	Hatch et al. (1985)
Intradermal				
Guinea-pig (out- bred albino)	n.s.	10 mg ^k (0.014), diameter 79– 90 µm, injected into dorsal skin; some animals sensitized by injection of Freund adjuvant prior to injection of bentonite / 30 days	Sensitized and control animals showed identical responses; chronic inflammatory response with ulceration and maximum induration at 7–10 days. Histology: At day 2, vascular dilation and some infiltration of monocytes and PMN; by day 5, fairly dense macrophage infiltration between collagen bundles and some phagocytosis of bentonite particles; the lesion continued to evolve into a loose, disorganized mass of mainly multinuclear, phago- cytic cells, which by day 30 had phagocytized most of the bentonite; starting by day 10, active fibrosis ultimately walled off the lesion and subdivided infiltrating cells into clumps.	Browett et al. (1980)

Table 12 (Contd)

Species	Sex ^a	Dose (g/kg of body weight) ^b and dosing regimen / period of observation	Findings ^c	Reference
Rat (Wistar)	M	0.05 ml 5% bentonite gel ^l (0.01) injected under plantar surface of left paw / 42 days	Pronounced oedema in paw appeared within 7 h.	Marek (1981)
Rat (Wistar SPF)	M	0.05 ml 5% bentonite gel ^m (0.01) injected under plantar surface of left hind paw / 42 days	Pronounced oedema in paw within 1 h, continued to enlarge slowly for 1 week, then regressed slowly but was still evident at 6 weeks. Histology: Marked increase in PMN and macrophages peaking at about 30 h; PMN declines to baseline by 4 weeks; macrophages remained elevated through 6 weeks.	Marek & Blaha (1982)
Rat (Sprague- Dawley)	M	100 mg ⁿ (0.44), diameter <2 µm in 5-cm incision / 8 days	Incision failed to close; marked inflammation, development of foreign body granulomata, and collagen synthesis, compared with control incisions.	Cohen et al. (1976)
Guinea-pig ^o	n.s.	Dose not specified, ^p injected on both sides of back / 20 months	At autopsy (8 and 20 months), bentonite found encapsulated as a compact mass beneath skin.	Campbell & Gloyne (1942)
Guinea-pig	n.s.	5 mg ^q (0.01) in each of two incisions following a sub- infective dose (3200 cells) of <i>Staphylococcus aureus</i>	Infection established in 80–100% of montmorillonite- contaminated incisions (compared with about 0% in controls).	Rodeheaver et al. (1974)

Table 12 (Contd)

- ^a M = male; n.s. = sex not specified.
- ^b Value in parentheses is approximate dose in g/kg of body weight, estimated from the mean of the range of experimental animal weights (if given in the reference). Otherwise, an average weight of 250 g was assumed for rats, 20 g for mice, and 500 g for guinea-pigs.
- ^c Abbreviations used: AM = alveolar macrophages; LDH = lactate dehydrogenase; PAM = pulmonary alveolar macrophages; PMN = polymorphonuclear leukocytes.
- ^d Four mixed dusts were used; composition as weight per cent was estimated by microscopic examination: i) quartz 65%, montmorillonite 15%, kaolinite 10%, feldspar 10%; ii) quartz 30%, montmorillonite 55%, kaolinite 18%, feldspar 7%; iii) quartz 15%, cristobalite 30%, montmorillonite 45%, calcite 6%, feldspar 4%; iv) montmorillonite 98%, kaolinite 2%.
- ^e Bentonite (76% montmorillonite, 3% quartz, 18% cristobalite, 3% feldspar).
- ^f Bentonite (composition not given); vehicle for injection not given.
- ^g Bentonite (montmorillonite 45%, quartz 15%, cristobalite 30%, calcite 6%, feldspar 4%) in 1 ml physiological sodium chloride containing 40 000 IU penicillin.
- ^h Bentonite (73% montmorillonite, 18% cristobalite, 3% quartz, 3% feldspar) and 40 000 IU penicillin injected in saline.
- ⁱ Montmorillonite (composition not given) treated to remove biologically active cations (Ca^{2+} , Mg^{2+}) and any organic matter; heated to 750 °C to collapse the lattice and inactivate any traces of silica; injected in physiological saline.
- ^j Bentonite (73% montmorillonite, 18% cristobalite [incorporated in aluminium silicate], 3% crystalline quartz, 3% feldspar) injected in sterile isotonic saline.
- ^k Bentonite (composition not given) injected in saline solution.
- ^l Composition not given.
- ^m Composition not given; rats received simultaneously an injection of 0.05 ml of 10% kaolin suspension in right paw.
- ⁿ Sterile montmorillonite (composition not given).
- ^o Two individuals.
- ^p Bentonite Fuller's earth (85% montmorillonite, <1% quartz) in saline.
- ^q Colloidal montmorillonite (particle size not specified) saturated with calcium, magnesium, or potassium (the specific cation had little effect on the results).

Changes in gross morphology and histology were accompanied by changes in the chemistry of lung lavage fluid. These included elevated levels of phospholipid (Adamis et al., 1985) and acid phosphatase (Adamis et al., 1985; Tátrai et al., 1985) by day 3. The increase in phospholipid was not, however, accompanied by any change in the relative abundance of phospholipid fractions (Adamis et al., 1986). Phospholipid was still somewhat elevated 3 months after exposure (Adamis et al., 1985, 1986). Tátrai et al. (1985) reported acid phosphatase back to baseline level at 1 and 12 months after exposure, whereas Adamis et al. (1985) found it still elevated at day 30. Hydroxyproline was slightly elevated 3 months after exposure (Adamis et al., 1986).

The effects of smaller intratracheal doses of bentonite on rats are uncertain. Adamis & Krass (1991) reported that 0.027 g/kg of body weight had no effect on protein or lactate dehydrogenase (LDH) in lavage from the lung 15–60 days after dosage, whereas Sykes et al. (1982), also using lavage from the lungs, noted a variety of effects indicating irritation and damage immediately following doses of 0.012 and 0.0012 g/kg of body weight. These effects included large and rapid increases in soluble protein, LDH, and polymorphonuclear leukocytes, with peaks at day 1 or day 2 followed by a decline to somewhat above baseline by day 7. Alveolar macrophages were increased by day 7. The findings in these two studies need not be contradictory, since the observations of Sykes et al. (1982) permit the possibility that elevated values for LDH and protein were present following dosage in the experiment of Adamis & Krass (1991), but declined back to baseline prior to their first sample on day 15.

The changes in gross morphology, white cell abundances, and biochemistry described above appear to form a generally coherent picture. Swelling and oedema stem from changes at the cellular level. Henderson et al. (1978a, 1978b) found increased LDH and protein in the lavage-sensitive indicators of lung damage that preceded visible mechanical damage and impairment of gas exchange. Increased LDH was ascribed to damage to cell membranes and leakage from lung cells, whereas increased protein was ascribed to increased vascular permeability. The latter conclusion is supported by analyses by Sykes et al. (1982), which reported serum albumin as the major constituent of the recovered protein. The increases in polymorphonuclear leukocytes may be triggered by phagocytosis of bentonite particles by alveolar macrophages (Terashima et al., 1997).

Single intratracheal exposures of rats to bentonite produced storage foci in the lungs 3–12 months later (Timár et al., 1979, 1980; Tátrai et al., 1983; Ungváry et al., 1983); even 12 months after exposure, fibrosis had not progressed beyond Belt-King grade 1 (Tátrai et al., 1985). Macroscopically, the foci were visible in fixed material as greyish-white masses up to the size of a “pin prick or a poppy seed” — i.e., approximately 1 mm (Timár et al., 1966). The structure of these storage foci was described in detail by Timár et al. (1966) using slides stained with haematoxylin-eosin, Van Gieson’s solution, and Foot’s silver impregnation. Timár et al. (1966: 5–6) reported that “The foci contained large cells with foamy protoplasm, and [with] nuclei often located at the edge of the cells. In the foam cells no lipids could be demonstrated by Sudan staining. PAS [*para*-aminosalicylic acid] reaction was intensely positive, showing that substances of carbohydrate nature are accumulated in the foci. In polarized light a great mass of intracellularly located doubly refractive fine grainy substance was to be seen. This characteristic alteration was to be observed in the lungs of the animals killed the 40th day after the application of the dust, and the microscopic findings in the tissues had not changed essentially up to the end of the experiment [365 days]. In the foci, sometimes a poorly developed reticular network of loose structure occurred too, but collagen fibres did not develop. The silver impregnated preparations showed in the area of the foci the precipitation of a greyish-black homogeneous substance between the fibres.”

By ultrastructural analysis, Ungváry et al. (1983) augmented the above description, noting that whereas the cytoplasm of the peripheral cells of the foci was foamy, the closely packed cells of the centre with dislocated nuclei, which formed the bulk of the foci, had dark, granulated cytoplasm. No cell division was observed among cells of the foci 3 or 10 months after exposure. The cytoplasm of the dark, electron-dense cells contained a small number of mitochondria and occasionally other organelles plus a large amount of lamellar electron-dense material very similar in appearance to high phospholipid-containing breakdown products of intracellular origin. This material, however, by means of X-ray microanalysis, was demonstrated to be bentonite, which was swollen, presumably due to its montmorillonite content in direct contact with the cellular fluid. “The width of the electron dense and electronlucent layers depends on the degree of the swelling; the crystalline and the swollen ends of bentonite are spread out like cards” (Ungváry et al., 1983: 213). Cells of the foci with light foamy cytoplasm also contained bentonite, but in much smaller amounts. Both the above findings and the enzyme histochemical information on the

foci (Ungváry et al., 1983) suggested that the metabolism of the foci is low. Ungváry et al. (1983) concluded that clearance of bentonite stored in foci from the lung is relatively slow and reflects the fact that cells of the foci are designed for long-term storage. The authors speculated that absence of toxic effects in the storage cells indicated that these cells have modified the surface characteristics of bentonite and coated the particles with protective molecules absorbed onto the bentonite.

Timár et al. (1966) provided evidence that the above cytotoxic responses leading to storage foci stem from montmorillonite and not from quartz or other impurities in the bentonite by comparing the effects of four mixed dusts containing varying proportions of montmorillonite, quartz, cristobalite, and other minerals. The composition of these dusts, essentially synthetic bentonites, is summarized in footnote d of Table 12. Dusts containing 55–98% montmorillonite and i) no quartz or cristobalite, ii) 30% quartz, or iii) 15% quartz and 30% cristobalite induced storage foci and no progressive fibrosis in rats following intratracheal administration. Only the dust with 15% montmorillonite and 65% quartz induced fibrosis. The authors ascribed the failure of the dust containing 30% quartz to produce productive fibrosis to a protective effect of the 55% montmorillonite, which they suggested may coat the surface of the quartz particles and thus inhibit the normal cytotoxic effects of the quartz. They further suggested a similar protective effect of montmorillonite against the cytotoxic effects of cristobalite. Havrankova & Skoda (1993) also reported that bentonite exerted a protective effect on rats against the toxicity of quartz dust. Observations by Schreider et al. (1985) supported the importance of molecular properties (crystalline structure and possibly exchangeable cations) in regulating the toxic effects of montmorillonite. They reported that an intratracheal dose (0.15 g/kg of body weight) of montmorillonite, washed to remove organic matter and biologically active cations and heated to 750 °C to collapse the crystal lattice and inactivate any traces of silica, induced not only increased pulmonary alveolar macrophages but also gross lesions in the lung and mild fibrosis at 90 days. This mild fibrosis, however, was not defined in terms of Belt-King grades.

In addition to directly damaging the lung, bentonite had a potential, even at low doses, for increasing susceptibility to pulmonary infection. Hatch et al. (1985) followed single intratracheal injections of 5, 0.5, or 0.05 mg/kg of body weight into mice with aerosolized Group C *Streptococcus* sp. (Table 12). The lowest dose did not significantly elevate mortality from *Streptococcus* infection over controls, whereas the highest dose produced 85% excess mortality and the intermediate dose 43% excess mortality over controls. Earlier studies by Hatch et al. (1981) demonstrated a close correlation between the effects on susceptibility to infection following dosing by sustained inhalation and by administering the same amount of test substance in a single intratracheal injection.

The mechanism by which bentonite increases susceptibility is unknown. Oberson et al. (1993), however, postulated that enzyme inhibition by bentonite may be a factor, based on their *in vitro* observation that a low concentration of bentonite can totally suppress the activity of human leukocyte elastase, an enzyme important in the destruction of microorganisms phagocytized by neutrophils.

In most experiments, bentonite also displayed local toxic effects after intra- or subdermal injection (Table 12). Subdermal injection of 10 mg/kg of body weight as 5% bentonite gel into the plantar of rats produced pronounced oedema within an hour (Marek, 1981; Marek & Blaha, 1982). The swelling continued to enlarge for a week and then slowly regressed, but it was still evident 6 weeks after injection. The oedema was accompanied by marked increases in macrophages and polymorphonuclear leukocytes, which peaked at 30 h. The leukocytes declined back to the baseline after 4 weeks, whereas the macrophages were still elevated at 6 weeks. Subdermally implanting 440 mg of sterile montmorillonite per kg of body weight into incisions in the backs of rats produced marked inflammation accompanied by the development of foreign body granulomata and collagen synthesis (Cohen et al., 1976). Incisions with montmorillonite failed to close during the 8-day duration of the experiment.

The granulomatous inflammation resulting from a moderate intradermal injection of relatively coarse (diameter 79–90 μm) bentonite into the skin of guinea-pigs was followed by Browett et al. (1980) both visually and with light and electron microscopy. The injection induced a chronic inflammatory response with ulceration and maximum induration at 7–10 days. By day 2, there was vascular dilation and some infiltration of monocytes and polymorphonuclear leukocytes. The

macrophage infiltration rapidly increased in density and by the termination of observations, day 30, had become mainly multinuclear cells, had phagocytized most of the bentonite particles, and had evolved into a loose, disorganized mass. This mass was walled off from adjoining tissues by active fibrosis, which also subdivided the cells into clumps.

In a limited experiment involving two guinea-pigs and subdermal injection of bentonite Fuller's earth, the bentonite was found encapsulated as a compact mass beneath the skin at autopsy (Campbell & Gloyne, 1942). However, no adverse reaction to the injected bentonite was reported. McNally & Trostler (1941) also reported no adverse reactions following intraperitoneal injection of an unspecified dose of bentonite Fuller's earth into flank muscles of rabbits, other than adhesions in one animal.

As in the case of the lungs, montmorillonite also increased the susceptibility of subdermal tissue to infection (Rodeheaver et al., 1974). A dose of 0.01 g of sterile montmorillonite per kg of body weight in each of two experimental wounds in guinea-pigs 5 min after a subinfective dose of *Staphylococcus aureus* established infections in 80–100% of the incisions. Three separate experiments, which were run using montmorillonite saturated with calcium, magnesium, or potassium, yielded similar results. Almost none of the control incisions that received only *S. aureus* became infected.

6.1.2 Kaolin

6.1.2.1 Intratracheal administration

In the landmark paper in which intratracheal instillation as a means of studying the effects of dust on lungs was described, no collagen production was detected in the lung of a guinea-pig 336 days after the administration (whereas fibrosis was observed after similar injection of quartz) (Kettle & Hilton, 1932). Similarly, intratracheal instillation of kaolin from South Wales or untreated or ignited Cornish kaolin did not induce fibrosis in rats (King et al., 1948) (Table 13).

Table 13. Effects of intratracheal instillation of kaolin and illite on the respiratory tract

Species / gender ^a / number	Treatment	Findings	Reference
Guinea-pig / 2	Intratracheal instillation of an unstated amount of kaolin, follow-up for 14 and 336 days	No collagen production in 336 days; in animals treated similarly with quartz, fibrosis observed.	Kettle & Hilton (1932)
Rat / 12	Intratracheal instillation of a single dose of commercial acid-washed kaolin containing 8% hydrated free silica and 12% mica; two animals killed between days 3 and 6; the rest kept for life (up until 8 months)	Grade 2–3 fibrosis observed in rats after 8 months (grade 1 = minimal reticulon fibrosis, 4 = maximal fibrosis, as induced by quartz).	Belt & King (1945)
Rat / 6 or 10 per group	A single intratracheal instillation (50–60 mg) of washed South Wales kaolin, untreated Cornish kaolin, ignited Cornish kaolin, quartz; follow-up up to 6 months	South Wales kaolin (10 rats): Up to 60 days, no fibrous reaction; thereafter, local reticulonosis, no fibrosis or emphysema. Cornish kaolin (10): Eight animals died within 10 days; the remaining two showed no fibrous reaction. Ignited Cornish kaolin (6): Only four rats available for study; their survival was 14, 28, 73, and 140 days; in the last-mentioned, there was local reticulonosis. Quartz (6): Severe nodular silicosis in all five animals available for study; survival 68, 121, 130, 207, and 240 days.	King et al. (1948)

Table 13 (Contd)

Species / gender ^a / number	Treatment	Findings	Reference
Rat	Intratracheal administration of kaolin, kaolin baked for 1 h at 900 or 1200 °C	Active phagocytosis, local storage of the dust without reticular fibres, and nodules were observed. In the case of heat-treated kaolin samples, the reaction was somewhat stronger, although fibrosis reached grade 1 in only a few cases (Belt-King scale). Histological signs resembling silicosis did not develop.	Schmidt & Luchtrath (1958)
Rat / 25 per group	A single intratracheal administration of kaolin containing kaolinite and quartz in ratios 82/18, 70/30, or 35/65; follow-up to 1 year	Foreign body reaction with the two samples containing 82 or 70% kaolinite, productive fibrosis with the sample containing 65% quartz.	Timár et al. (1966)
Rat / 10–15 per group	Single intratracheal instillation of kaolin (sericite and quartz as main impurities, 1% quartz), particle size <5 µm; histological analysis of lungs after 4 months	Cellular lesions, some loose reticulin, with either no collagen or a few collagen fibres.	Goldstein & Rendall (1969)
Rat / 10 per group	Intratracheal instillation of 50 mg non-specified kaolin, 3-month follow-up	Amount of collagen / amount of dust in lung after kaolin exposure 3 times higher than in titanium dioxide-treated control and 2.4 times more than in coal-treated animals, but only 15% of that in the quartz-treated animals.	Martin et al. (1977)

Table 13 (Contd)

Species / gender ^a / number	Treatment	Findings	Reference
Rat, strain not specified / number and gender not specified	40 mg of illite clay F, nominal composition 100% illite (diameter <2 or 2–5 µm), kaolin S (82% kaolinite, 18% quartz; diameter <3 µm), or kaolin Sz (95% kaolinite, 5% quartz; diameter <2 or 2–5 µm), instilled to rats; animals killed after 5, 15, 40, and 365 days and histological analysis performed	Illite F and kaolin Sz <2 µm caused "storage foci," kaolin S, "foreign body" reaction, and kaolin Sz 2–5 µm, mainly storage foci, rarely foreign body reaction.	Timár et al. (1979)
Rat, strain not specified / 10	60 mg of illite (not described) instilled intratracheally and followed for 6 months; lung weight, lipid, phospholipid, and hydroxyproline were analysed, and histological and histochemical studies on collagen performed	"Storage foci" observed in lungs of illite-treated rats.	Timár et al. (1980)
Rat, Sprague-Dawley / female / 10 per group	30 or 50 ^b mg non-specified kaolin or illite clay injected intratracheally, followed for 3 and 12 months	Kaolin increased the lung weight 9 and 4 mg and collagen formed <26 and <7 mg/mg of injected dust at 3 and 12 months (all normalized to quartz = 100). Illite clay induced alveolar proteinosis and thus increased the lung weight 17 and 6 mg, and collagen formed <26 and 11 mg/mg of injected dust at 3 and 12 months (all normalized to quartz = 100).	Le Bouffant et al. (1980)

Table 13 (Contd)

Species / gender ^a / number	Treatment	Findings	Reference
Rat, Sprague-Dawley / female / 10 per group	30 or 50 ^b mg non-specified kaolin or illite clay injected intratracheally, followed for 3 and 12 months	Correlation to haemolysis (rat erythrocytes) and release of LDH ^c and alkaline phosphatase from rabbit alveolar macrophages (see Table 17) weak.	Le Bouffant et al. (1980)
Rat, Fischer F344 / 10 per group	5 mg kaolin (non-specified), MMAD ^d 2.1 µm, instilled intratracheally, killed 1 day, 3 days, 7 days, 3 months, and 6 months later	Acute inflammatory reaction on day 1; thereafter, a slight interstitial cell thickening. At 3 and 6 months, lungs were normal.	Vallyathan et al. (1988)
Rat / male / 5 per group	Intratracheal injection of 10 mg of a kaolin containing 67% kaolinite and 23% quartz; bronchopulmonary lavage after 15 days, LDH activity and protein content and, after 15, 30, and 60 days, phospholipid content of supernatant measured	Kaolin did not induce significant LDH, protein, or phospholipid leakage to the supernate fraction. No change in the LDH or protein leakage was observed after similar exposure to eight other minerals, including two quartz samples. The quartz samples, however, increased the phospholipid content at 30 and 60 days.	Adams & Krass (1991)
Rat, SD / male	Intratracheal injection of 10 mg of a kaolin containing 86% kaolinite, 4% quartz, 5% illite, and 5% amorphous silica; bronchopulmonary lavage after 14, 30, and 90 days, number of cells, activities, LDH activity, protein and phospholipid content of supernatant measured	Kaolinite caused a statistically significant increase in the protein content at 14 days, which returned to control level thereafter. No changes were observed in the other parameters studied.	Adams et al. (1998)

Table 13 (Contd)

Species / gender ^a / number	Treatment	Findings	Reference
Mouse / 70 per group	Intratracheal instillation of 5 mg of kaolin, 91% >3.62 µm in diameter; follow-up 210 days	Fibroblast reaction from 60 days post-exposure, prominent from day 120. Grade 2 fibrosis by day 210 (Belt-King scale).	Sahu et al. (1978)
Rat, Wistar / female / 20 per group	Kaolinite 1 (K1) contained 2% muscovite; K2, 1% quartz and 9% muscovite; K3, <1% quartz and anatase and 1% muscovite; and K4, 1% quartz and anatase and 2% muscovite; mean value for volume distribution was 3.6 and 2.6 µm for K3 and K4, not analysed for K1 or K2; instilled once intratracheally at 50 mg/kg of body weight; autopsy after 7 months; lung weight, histology, amount of dust, hydroxyproline, and total lipid content analysed; two samples of quartz also investigated, dose given 5 mg/kg of body weight	All kaolinite samples fibrogenic. Absolute amount of hydroxyproline roughly similar after exposure to kaolinites and quartz, hydroxyproline / retained dust in kaolinite-treated animals 1/10 that in quartz-treated animals. Absolute increase of lung weight one-sixth and of total lipids roughly 10% of that in quartz-treated animals.	Rosmanith et al. (1989)
Rats, SD(SD)BR / male / 6 per group	Exposed by intratracheal instillation to 50 mg dust from the town Mexicali (see above), mean diameter 3.2 µm; lung analysis after 30 days	Multifocal interstitial lung disease. Mononuclear cell accumulation and presence of collagen fibres.	Osornio-Vargas et al. (1991)

^a Where available.^b Composition or particle size not given.^c LDH = lactate dehydrogenase.^d MMAD = mass median aerodynamic diameter.

After the instillation of a single dose of commercial acid-washed kaolin containing 8% hydrated free silica and 12% mica, grade 2–3 fibrosis was observed in rats after 8 months (grade 1 = minimal reticulin fibrosis, grade 4 = maximal fibrosis, as induced by quartz) (Belt & King, 1945) (Table 13).

Following intratracheal dust treatment in rats, the histological reaction was found to depend on the composition of the dust (Tímár et al., 1966). Foreign body reaction as an effect of kaolinite was observed in all cases where the crystalline quartz content of the dust was less than or equal to 30%. The sample containing 65% quartz and 35% kaolinite caused progressive fibrosis. In addition to the composition, particle size also played a role in the development of the tissue reaction. Kaolin samples containing particles less than 2 µm caused storage foci (Tímár et al., 1979), while the kaolin samples containing bigger particles (particle size between 2 and 5 µm) caused mainly storage foci but also, to a smaller extent, foreign body reaction (Table 13).

Goldstein & Rendall (1969) observed cellular reaction with minimal fibrosis: some loose reticulin with either no collagen in some animals or a few collagen fibres in other rats 4 months after an intratracheal instillation of kaolin (Table 13).

Martin et al. (1977) observed an increase in the collagen content in the lungs of rats 3 months after an intratracheal instillation of non-specified kaolin; the reaction was considerably weaker than after a similar treatment with quartz (Table 13).

Sahu et al. (1978) described the development of grade 2 fibrosis in mice after 7 months of exposure to non-specified kaolin (Table 13).

Rosmanith et al. (1989) compared the fibrogenicity of four kaolinite samples with that of quartz in rats using intratracheal instillation. The fibrogenicity of kaolinites, as measured by the increase in hydroxyproline content in relation to the amount of dust retained, was approximately 1/10 that of quartz, but the inflammatory reaction was considerably less (Table 13).

No significant LDH, protein, or phospholipid leakage to the supernate fraction was observed in bronchoalveolar fluid 15–60 days after an intratracheal instillation of kaolin to rats. In this system, effects of quartz became apparent towards the end of the observation period (Adamis & Krass, 1991) (Table 13).

6.1.2.2 Parenteral administration

Policard & Collet (1954) demonstrated the development of reticulin fibres in rats 1–3 months after intraperitoneal administration of kaolin (90% <2 µm in diameter). The dust sample contained 1.2% free silica, an amount that the authors considered not to cause the effects observed.

Intraperitoneal administration of kaolinite (<3 µm or ~10 µm particle size; no quartz detectable with X-ray analysis) was fibrogenic in mice; the smaller particle size was just as active as quartz and led to fibrosis in 35 days, whereas fibrosis became evident only after 200 days for the larger size particles (Rüttner et al., 1952).

Intraperitoneally instilled kaolin and kaolin baked for 1 h at 900 or 1200 °C caused marked fibrosis. The degree of the reaction was only slightly smaller than that caused by quartz (Schmidt & Luchtrath, 1958).

In a study of a large number of organic and inorganic particulates, kaolin (composition not specified, particle size 0.25–25 µm) injected intraperitoneally into rats produced a granulomatous reaction at 1 and 3 months, but no fibrosis. *In vitro*, it had low toxicity: it killed less than 2% of peritoneal macrophages and approximately 4% of alveolar macrophages (Styles & Wilson, 1973). For the group of some 20 particulate materials, the authors considered that there was a good correlation between toxicity to macrophages and fibrogenicity after intraperitoneal injection.

6.1.3 Other clays

Three and 12 months after an intratracheal instillation of illite clay with unknown quartz content, alveolar proteinosis and increased lung weight and collagen synthesis were observed in rat lungs (Table 13).

6.2 Repeated exposure

6.2.1 Bentonite

A case of profound hypochromic anaemia and mild hypokalaemia has been reported in a cat that habitually consumed large amounts of cat litter stated to contain 99% bentonite. The clinical status was dramatically improved by a transfusion and intravenous hydration. A similar episode was seen in the same cat a month later, when the use of the same litter material was resumed (Hornfeldt & Westfall, 1996). The causality is uncertain, as anaemia causes pica in cats (McDonough, 1997).

Bentonite has also been administered to animals using intravenous and intramuscular routes (Boros & Warren, 1970, 1973), but these studies were not destined for, and cannot be used for, the assessment of health risks from bentonite.

No long-term studies on the effects of bentonite in experimental animals using inhalation exposure are available.

In a study by Wilson (1953b, 1954), the reports of which provide only limited details, mice were fed bentonite (undefined) at 10, 25, and 50% of their diet (Table 14). The total numbers of mice in different treatment groups were not given, but of the 12 mice given 50% bentonite that survived 200 days or more, 11 developed tumours that the authors call “benign hepatomas” (without histological description). Eight of these mice had also been given thorotrast (known to be a liver carcinogen in mice, rats, and humans; IARC, 2001) before starting the diet study. Nine animals of the control group survived more than 200 days, and one developed hepatoma. In historical controls, no hepatomas were observed in 18 animals that had been given thorotrast and had lived >215 days; nor were hepatomas detected in 24 non-treated mice that had survived 484–575 days. Nine animals given low-protein, choline-deficient diet also survived more than 200 days, and three developed hepatomas. Development of hepatomas in these strains of mice on low-protein, choline-deficient diet had also been reported by the authors in an earlier study (Wilson, 1951).

Mice maintained on the diet with 50% bentonite also showed minimal growth and developed fatty livers and fibrosis of the liver

Table 14. Effects of repeated exposure to clays in experimental animals

Species / gender / number	Dose / study details / period of experiment	Findings	Reference
Bentonite			
Mouse, three inbred strains ^a :	Non-specified bentonite, at 10, 25, or 50% of the diet, and non-specified montmorillonite, at 50% of the diet, fed to mice for 60 days; body weight gain and liver histology studied	Fifty per cent bentonite and montmorillonite caused a 70–80% decrease in weight gain; 10% and 20% bentonite caused a smaller decrease in weight gain; at the high dose of bentonite, fatty liver developed.	Wilson (1953a)
2 females and 2 males per group in bentonite studies			
2 mice per group in montmorillonite and modified-diet studies		Barium sulfate or cellulose at 50% of the diet did not lead to significant weight gain reduction; the effect was not due to smaller food intake. When choline or casein was added to the diet, the growth rate was partially restored. When choline or casein was added to the diet containing 50% bentonite, the growth rate was partially restored and liver morphology remained normal.	

Table 14 (Contd)

Species / gender / number	Dose / study details / period of experiment	Findings	Reference
Mouse, three inbred strains / number and gender not indicated	Non-specified bentonite, at 10, 25, or 50% of the diet, fed to mice for an unspecified time (>200 days); body weight gain and liver histology studied; for liver imaging, some of the mice were given thorotrast (amount not given) prior to starting the dietary experiment	With 10% and 25%, slightly reduced growth, with 50%, minimal growth, fatty and fibrotic liver. At the highest dose, "benign hepatomas" in 9 of 12 mice that survived >200 days. Eight of the mice with tumours had also received thorotrast. Of the control mice, 9 survived >200 days, and 1 developed hepatoma. Twenty-four historical controls that had survived 484–575 days were studied for hepatic tumours, and none was detected. Of historical controls with thorotrast injections, 18 had survived 216–511 days; no hepatomas were observed. Of 9 mice given low-protein, choline-deficient diet that survived >200 days, 3 developed hepatoma.	Wilson (1953b, 1954)
Kaolin			
Guinea-pig / number and gender not given	Inhalation exposure to china clay "as supplied to the potteries" at unspecified concentration 2 h/day every day, every second day, twice weekly, or weekly; follow-up until 6 months	Until 3 months after the exposure, pulmonary reaction was slight, limited to mild alveolar proliferation. Thereafter, thickening of the alveolar walls, emphysema, and patchy bronchopneumonia with capillary congestion and massive eosinophil infiltration occurred. By 6 months, plaque formation and capillary bronchitis were observed.	Carleton (1924)

Table 14 (Contd)

Species / gender / number	Dose / study details / period of experiment	Findings	Reference
Clay dust			
Rat, SD(SD)BR / male / 6 per group	Exposed by inhalation to dust from the town Mexicali, containing illite, calcium montmorillonite, kaolinite, alpha-quartz (23%), and small amounts of feldspars, calcite, volcanic glass; diameter approximately 10 μ m, exposure 12 mg/m ³ for 3 h, tissue analysis after 0 h, 24 h, 8 days, 1 month	Accumulation of mononuclear cells and macrophages by 24 h in the airways and alveolar spaces; disappearance of particles and cellular changes by 8 days.	Osornio-Vargas et al. (1991)

^a Inbred strains maintained in the author's laboratory for investigation of physiology and pathology of mouse liver.

(Table 14). Mice maintained on diets containing 10 or 25% bentonite displayed slightly reduced growth rates and no hepatic tumours (Wilson, 1953b, 1954).

In a separate, 60-day study (Wilson, 1953a), the addition of choline or casein to the 50% bentonite diet was reported to partially prevent the development of fatty liver and cirrhosis and permitted an improved but still subnormal growth rate. The author concluded that bentonite binds choline and other nutrients so that they cannot be absorbed into the body, and this deficiency then induces liver damage and hepatomas (Wilson, 1951, 1953a, 1953b, 1954). Because of the mixed exposure, limited numbers of animals, and limited reporting, these studies cannot be used to assess the carcinogenicity of bentonite.

6.2.2 Kaolin

Carleton (1924) studied the effects of kaolin (see Table 14) by inhalation in guinea-pigs. Until 3 months after the exposure, mild alveolar proliferation only was observed. Thereafter, patchy bronchopneumonia occurred, with massive eosinophil infiltration. By 6 months, plaque formation and capillary bronchitis were observed.

Wagner and co-workers (1987) studied the carcinogenicity of palygorskite and attapulgite in an inhalation study and used “coating grade” kaolin as a negative control: 20 male and 20 female Fischer rats were exposed by inhalation (10 mg/m³, 91.4% of the particles <4.6 µm in diameter) for 6 h/day, 5 days/week, until they died. However, at 3, 6, and 12 months, four rats were killed from each group for ancillary studies (leaving only 28 animals for the carcinogenicity study). At the end of the study, full autopsy and microscopic analysis of the lungs, liver, kidney, spleen, and other organs were performed. In two kaolin-exposed rats, bronchoalveolar hyperplasia but no benign or malignant tumours of the lungs or pleura were observed. However, the number of tumours in the positive control group (crocidolite treatment at the same exposure level) was also low (one adenocarcinoma only). The mean fibrosis grading in the rats at interim sacrifices and at the end of the experiment was between 2.1 and 2.8 on a scale of 1–8 (1 being normal; 2, dust in macrophages; 3, early interstitial reaction; 4, first signs of fibrosis; 5, 6, 7, increasing fibrosis; 8, severe fibrosis).

As another part of the study on the carcinogenicity of mineral fibres, Wagner et al. (1987) injected a single dose (amount not given) of kaolin intrapleurally into 20 male and 20 female Fischer rats and followed the animals until moribund or dead (survival of animals in different groups not given). None of the kaolin-treated rats developed mesothelioma, whereas 34 of 40 of those given crocidolite did.

Mossman & Craighead (1982) treated cultured tracheas from hamsters with Georgia kaolin (composition not indicated; diameter 3–5 µm) and kaolin coated with 3-methylcholanthrene, implanted the tracheas after 4 weeks into syngeneic hamsters, and followed the animals until moribund at 105–110 weeks. Animals treated with kaolin did not develop tumours, but a high incidence of pulmonary tumours, often fatal, was observed in animals treated with kaolin coated with 3-methylcholanthrene. Animals treated with 3-methylcholanthrene-coated haematite or carbon particles also developed a similar spectrum of tumours (carcinomas, sarcomas, undifferentiated tumours).

6.3 Genotoxicity

No information was available on the genotoxicity of clays or clay minerals. Information on quartz has been summarized by IARC (1997) and IPCS (2001). Quartz did not test positively in standard bacterial mutagenesis assays. Results of genotoxicity studies of quartz conflict, and a direct genotoxic effect for quartz has not been confirmed or ruled out (IPCS, 2001).

6.4 Reproductive effects

In a study that provided limited details, Sprague-Dawley rats were administered calcium or sodium montmorillonite orally on pregnancy days 1–15. No effects were observed on the litter weight, implantation rates, or resorptions (Wiles et al., 2004) (Table 15).

Thirty-six Sprague-Dawley rats were fed a control diet, 20% kaolin (air-floated Georgia kaolin) diet, and iron-supplemented 20% kaolin diet 37–117 days before mating and during pregnancy (Patterson & Staszak, 1977). Dams receiving kaolin diet developed anaemia, whereas anaemia was not observed in dams receiving iron supplementation. The birth weight of the pups of the dams receiving kaolin was 9% smaller than that of the control dams ($P < 0.01$); again, iron

supplementation prevented this decrease. There was no effect on the litter size or macroscopic malformations (Table 15).

Table 15. Developmental effects of bentonite (montmorillonite) in rats

Strain, number	Dose / period of experiment	Findings	Reference
Sprague-Dawley	2% calcium montmorillonite or sodium montmorillonite in the diet + daily gavage with 1 ml of 0.25% aqueous clay suspension on pregnancy days 1–15	No effect on maternal weight or maternal organ weights, litter weight, embryonic implantations, or resorptions.	Wiles et al. (2004)
Sprague-Dawley, 36	20% Georgia kaolin (crystal composition not given) in the diet 37–117 days before mating and during pregnancy	Maternal anaemia and reduction of pup birth weight observed; both reversed by iron supplementation. No terata observed, but details on the analysis not provided.	Patterson & Staszak (1977)

6.5 Administration with other agents

6.5.1 Kaolin and microbes and microbe-derived factors

In the study described in section 6.1.1 on bentonite (see Table 12), Hatch et al. (1985) also studied the effect of kaolin on infection resistance in mice. Intratracheal instillation of 100 µg kaolin (not further defined) caused a slightly (40%) and statistically not significantly increased mortality upon challenge by exposure to aerosolized group C streptococci by inhalation.

Intratracheal instillation of kaolin “prepared to the specification of the Society of Leather Trades” with atypical mycobacteria (not *Mycobacterium tuberculosis*) induced fibrotic reaction in the lungs of guinea-pigs (Byers & King, 1959). No control experiment with kaolin alone was performed.

Attygalle et al. (1954) studied the combined effects of dust samples characterized as mainly crystalline, to a small extent amorphous, aluminium silicate containing 77% kaolinite, 12% mica, and 8% quartz together with autoclaved *M. tuberculosis*. Kaolin samples alone did not cause macroscopic changes in rats or guinea-pigs.

Microscopically, a diffuse exudative reaction developed with giant cells containing dust particles after 30 days. The cells were prone to form groups. Noduli, however, were not found. Fine reticulin fibres were found in the cells. The lesions showed no progress and, after 500 days, were still of minimal grade 1. An avirulent strain (BCG) alone also produced a network of fine reticulin fibres, but no progressive fibrosis. In contrast, kaolin and BCG together produced, after 180–270 days, reactions of grade 2–3, with thickening of the alveolar walls and collagen fibre bundles. Thereafter, the lesions showed regression and, after 500 days, were of grade 1 only (reticulin fibre network with mononuclear cells).

Similarly, when kaolin (75 mg, not specified qualitatively) was injected into guinea-pigs intratracheally together with *Candida albicans*, thick reticulin fibres and collagenous fibrosis ensued; acute inflammatory reaction followed by formation of thick reticulin fibres were observed after kaolin alone (Zaidi et al., 1981).

6.5.2 Kaolin and quartz

Schmidt & Luchtrath (1958) studied the effects of intratracheal administration of a 2:3 mixture of kaolin and quartz. Kaolin alone did not cause significant reaction in the lung. The mixture of kaolin and quartz caused significant changes in the lung; after 9 months, the fibrosis was grade 5. (Using pure quartz, the development of silicosis was somewhat quicker, but after 9 months, the difference compared with the kaolin–quartz mixture was insignificant.)

6.6 In vitro test systems

6.6.1 Bentonite

Experimental protocols in the studies summarized in Table 16 appear fairly uniform; cell suspensions were incubated with bentonite or montmorillonite for (where specified) 30 min to 24 h, and the cells were tested for physical integrity or functional ability. Cell types included rat peritoneal macrophages, rabbit and rat alveolar macrophages, a macrophage-like cell line, other white cells, human and other erythrocytes, rodent neural cells, hamster tracheal epithelial cells, and

Table 16. Effects of exposure to bentonite *in vitro*

System / species / gender	Dose (mg/ml) ^a / treatment	Findings ^b	References
Peritoneal macrophages			
Rat (Sprague-Dawley CFY)	Bentonite (73% montmorillonite, 3% quartz, 18% cristobalite; diameter not given) incubated with the cells for 24 h either as such or after dry milling for 32 h	Dry milling decreased the methylene blue adsorption to a third and the inhibition of TTC reduction by 75%.	Juhasz et al. (1978)
Rat (Sprague-Dawley CFY male)	Bentonite ^{c,d} (<5 µm diameter, median 1–2 µm) incubated in cell suspension for 24 h	More cytotoxic than quartz, other mineral dusts, or ceramic dusts as measured by decrease in TTC reduction activity.	Adamis & Timár (1976); Adamis & Krass (1991)
	Bentonite ^c 0.3 mg/ml (<5 µm diameter, median 1–2 µm) incubated in cell suspension for 3 h	Inert as measured by release of LDH into medium.	
	Bentonite ^{d,e} (<2 µm diameter) incubated in cell suspension for 3 h and 24 h	As cytotoxic as quartz in 3-h incubation and more cytotoxic than quartz in 24-h incubation as measured by decrease in TTC reduction activity.	Adamis & Timár (1978)
	Bentonite ^e 0.09, 0.27, 0.45 mg/ml (<2 µm diameter) incubated in cell suspension for 3 h	Intracellular and total (intracellular + medium) LDH activity decreased with increasing dose.	
	Bentonite ^{d,e} (<2 µm diameter) incubated in cell suspension for 24 h	More cytotoxic than quartz or other mineral dusts as measured by decrease in TTC reduction activity.	Adamis & Timár (1980)

Table 16 (Contd)

System / species / gender	Dose (mg/ml) ^a / treatment	Findings ^b	References
Rat (Sprague-Dawley CFY male)	Bentonite ^{de} (<2 µm diameter) incubated in cell suspension for 3, 4, and 24 h	After 3-h incubation with higher dose (not specified), intracellular LDH activity reduced, but no increase in LDH activity detected in medium; with concentration equal to one-third of higher dose, no reduction in intracellular LDH activity and no increase in LDH activity in medium after 4 h; after 24-h incubation with this lower dose, intracellular LDH activity reduced and LDH activity in medium increased.	Adamis & Timár (1980)
	Bentonite ^{df} with 3% and 34% quartz (<7 µm diameter, mode 1–2 µm) incubated ^g in cell suspension	Similar results with both bentonites; very cytotoxic as measured by decrease in TTC reduction activity; no loss of intracellular LDH to the medium detected.	Adamis et al. (1985)
	Bentonite ^{cd} (mode diameter 1–2 µm) incubated ^g in cell suspension	Very cytotoxic as measured by decrease in TTC reduction activity; no loss of intracellular LDH to the medium detected.	
Alveolar macrophages			
Rabbit (New Zealand)	Bentonite ^e 1.0 mg/ml (0.4 µm median diameter) incubated in cell suspension for 20 h	Viability index (trypan blue exclusion) lowered by 65% ($P < 0.05$) and cellular ATP (luciferin–luciferase reaction) by 92% ($P < 0.05$).	Hatch et al. (1985)

Table 16 (Contd)

System / species / gender	Dose (mg/ml) ^a / treatment	Findings ^b	References
Rabbit (New Zealand)	Montmorillonite ^e 0.5 mg/ml (<5 µm diameter) incubated in cell suspension for 24 h	Complete (100%) haemolysis.	Daniel & Le Bouffant (1980)
Rat (strain not specified)	Bentonite ^h 1.0 mg/ml (<5 µm diameter, mode 2.7 µm) incubated in cell suspension for 2 h	Small increase above control in β-glucuronidase detected in medium; LDH and β-N-acetylglucosaminidase detected in medium less than control.	Vallyathan et al. (1988)
Neutrophils			
Human (multiple donors)	Montmorillonite ⁱ 10 mg/ml incubated ^d in cell suspension	Rapid and complete lysis, with most of LDH released from cells adsorbed onto the montmorillonite; pretreatment of montmorillonite with 1% human albumin for 30 min blocked adsorption without affecting lysis; pretreatment with 10% human albumin blocked both adsorption and lysis.	Dougherty et al. (1985)
Human phagocytic cells from one donor	Montmorillonite STx-1, 1% quartz and 10% cristobalite, particle size 2.5 µm median volume diameter	Kaolinite at concentrations in the order of 1 mg/ml induced luminol-dependent chemiluminescence as an expression of generation of reactive oxygen species in both monocytes and neutrophils when opsonized or when not opsonized.	Gormley et al. (1985)

Table 16 (Contd)

System / species / gender	Dose (mg/ml) ^a / treatment	Findings ^b	References
Leukocytes			
Human	Sterile montmorillonite ^e saturated with magnesium 5.3 mg/ml (<2 µm diameter) incubated in cell suspension for 30 min, then suspension inoculated with <i>Staphylococcus aureus</i> or <i>Pseudomonas aeruginosa</i>	Leukocytes lost all capacity to phagocytize and kill <i>S. aureus</i> and <i>P. aeruginosa</i> .	Haury et al. (1977)
Erythrocytes			
Rat (strain not specified)	Montmorillonite ^e 1.0 mg/ml (<5 µm diameter) incubated ^g in cell suspension for 1 h	Complete (100%) haemolysis.	Daniel & Le Bouffant (1980)
Sheep (citrated blood)	Bentonite ^e (median diameter 5 µm) incubated in cell suspension	Speed of haemolysis dependent on concentration of bentonite; 100% within 5 min at 0.2 mg/ml; approximately 12% within 30 min at 0.01 mg/ml.	Ottery & Gormley (1978)
Sheep	Bentonite ^h at four concentrations; 0.1–0.7 mg/ml (<5 µm diameter, mode 2.7 µm), incubated in cell suspension for 50 min	Most haemolytic of mineral dusts tested; linear, dose-dependent response; about 7% haemolysis with 0.1 mg/ml; 84% with 0.74 mg/ml.	Vallyathan et al. (1988)

Table 16 (Contd)

System / species / gender	Dose (mg/ml) ^a / treatment	Findings ^b	References
Sheep (citratd blood, single donor)	Montmorillonite ^k at six concentrations, ^l 0.005–0.25 mg/ml (70% <1 µm diameter), incubated in cell suspension for 1 h	More haemolytic than silica or chrysotile; clear dose–response relationship; 5% haemolysis with 0.005 mg/ml; 95% with 0.25 mg/ml.	Woodworth et al. (1982)
Cow (erythrocytes washed with buffered saline)	Montmorillonite ^e at several concentrations (<50 µm diameter) incubated in cell suspension for 1 h	A concentration of 0.006 mg/ml induced 50% haemolysis of erythrocytes; particles 0.2–2 µm in diameter were most active; particles >2 µm diameter had little or no haemolytic activity; reduction of surface charge and cation exchange capacity by coating particles with an aluminium-hydroxy polymer largely eliminated haemolytic capacity.	Oscarson et al. (1986)
Human (citratd blood)	Bentonite ^c 1.0 mg/ml (<5 µm diameter; median 1–2 µm) incubated in cell suspension for 24 h	Almost complete (95%) haemolysis.	Adamis & Krass (1991)

Table 16 (Contd)

System / species / gender	Dose (mg/ml) ^a / treatment	Findings ^b	References
Human (multiple donors)	Montmorillonite ^l 0.6–20 mg/ml incubated ^g in cell suspension	Haemolysis 30% from incubation with an unspecified concentration of montmorillonite; 20% haemolysis using montmorillonite pretreated with 1% human albumin; pre-treatment of montmorillonite with 10% human albumin virtually blocked haemolysis.	Dougherty et al. (1985)
Human	Bentonites, ^m calcium montmorillonite, ^e montmorillonite ^e + quartz (<5 µm diameter) incubated in cell suspension for 1 h	Calculated concentration causing 50% haemolysis = 1.0 mg/ml for bentonite (a), ^m 1.66 mg/ml for bentonite (b), ^m 5.0 mg/ml for calcium montmorillonite, and 0.8 mg/ml for montmorillonite + quartz; haemolytic activity proportionate to surface area of mineral powder; haemolytic activity of minerals largely lost after heating to over 500 °C.	Mányai et al. (1969)
Human	Bentonites, ^m calcium montmorillonite, ^e montmorillonite ^e + quartz (<5 µm diameter) incubated in cell suspension for 1 h	Maximum haemolysis between pH 7 and pH 8, and much diminished below pH 7.	Mányai et al. (1969)

Table 16 (Contd)

System / species / gender	Dose (mg/ml) ^a / treatment	Findings ^b	References
Neural cells			
Neuroblastoma (N1E-115) cells with differentiation induced by dimethylsulfoxide	Montmorillonite ^d 0.1–1.0 mg/ml incubated briefly in cell suspension	Within minutes, resting potential depolarized and ability to maintain action potentials in response to stimulation was lost; within 30 min, severe morphological deterioration of cells.	Banin & Meiri (1990)
Neuronal (primary cells from 11- to 13-day-old mouse embryos)	Montmorillonite ^d and bentonite ^d 0.1 mg/ml incubated in cell suspension for 1 h	Clay particles rapidly collected on plasma membrane; after 1 h, cells largely lysed by montmorillonite and completely lysed by bentonite.	Murphy et al. (1993a)
Neuroblastoma (N1E-115 cells)	Montmorillonite ^d and bentonite ^d 0.1 mg/ml incubated in cell suspension for 18 h	Clay particles rapidly collected on plasma membrane; after 18 h, cells unharmed by montmorillonite or by bentonite.	Murphy et al. (1993a)
Neuroblastoma (N1E-115) cells and oligodendroglial (ROC-1) cells	Montmorillonite ^e and bentonite ^e 0.01, 0.03, 0.1 mg/ml (mainly 1–2 μ m diameter) incubated in cell suspension for 1, 6, or 24 h	No lysis of cells or alteration of LDH activity in medium; slight release of fatty acids (mainly saturated) from cells; montmorillonite significantly decreased viability (assessed by trypan blue exclusion) of N1E-115 cells after 24 h, whereas bentonite had no effect.	Murphy et al. (1993b)

Table 16 (Contd)

System / species / gender	Dose (mg/ml) ^a / treatment	Findings ^b	References
Other cell types			
Tracheal epithelial (cloned cell line from Syrian hamster, strain 87.20) in log growth phase	Montmorillonite ^k at four concentrations, 0.003–0.1 mg/ml ^r (70% <1 µm diameter), incubated in cell suspension for 24 h	Cells phagocytized clay particles; dose-dependent damage to plasma membrane as evidenced by loss of ⁵¹ Cr from cells; loss of ⁵¹ Cr after 24 h ranged from approximately 12% with 0.003 mg/ml to 58% with 0.1 mg/ml.	Woodworth et al. (1982)
Human umbilical vein endothelial cells	Montmorillonite ^e and bentonite ^e 0.01, 0.03, 0.1 mg/ml (mainly 1–2 µm diameter) incubated in cell suspension for 1, 6, or 24 h	Both montmorillonite and bentonite associated with plasma membrane within 1 h; after 24 h, both caused lysis of cells, a large dose-dependent release of fatty acids (mainly saturated) from cells, and a significant increase in LDH activity in the medium.	Murphy et al. (1993b)
Macrophage-like cell line P338D ₁	Three montmorillonites (Saz-1, Swy-1, STx-1) from Source Clays repository, particle sizes 2.5–3.1 µm; Saz-1 with no cristobalite or quartz, Swy-1 with 5% quartz, and STx-1 with 10% cristobalite and 1% quartz	Cell viability at 20 µg/ml 79% for Saz-1, 47% for Swy-1, and 21% for STx-1.	Gormley & Addison (1983)

Table 16 (Contd)

System / species / gender	Dose (mg/ml) ^a / treatment	Findings ^b	References
Artificial organelles			
Liposomes (artificial phospholipid membrane vesicles ^o 0.1–2 µm diameter) entrapping dissolved chromate (CrO ₄ ²⁻)	Montmorillonite ^k at five concentrations, ^p 0.1–10 mg/ml (70% <1 µm diameter), incubated in cell suspension for 1 h	Dose-dependent loss of chromate from vesicles; loss of chromate after 1 h (in excess of spontaneous rate) ranged from about 6% with 0.1 mg/ml to about 44% with 10 mg/ml; spontaneous rate was 4–6%.	Woodworth et al. (1982)
^a Dose recalculated as necessary to mg/ml. ^b ATP = adenosine triphosphate; LDH = lactate dehydrogenase; TTC = 2,3,5-triphenyltetrazolium chloride. ^c 73% montmorillonite, 18% cristobalite (incorporated into montmorillonite), 3% quartz, 3% feldspar. ^d Dose not specified. ^e Composition not specified. ^f Composition not given except for quartz. ^g Time not specified. ^h Bentonite described as 90% pure with 0.8% silicon. ⁱ Composition and particle size not specified. ^j 1.0, 2.4, 5.0, and 7.4 mg/ml. ^k >99% pure; <1% feldspar, biotite, and gypsum. ^l 0.005, 0.010, 0.025, 0.05, 0.1, and 0.25 mg/ml. ^m One bentonite (a) described as predominantly montmorillonite with small amounts of kaolinite and quartz; the other (b) as montmorillonite 25%, illite 50%, quartz 25%. ⁿ 0.003, 0.01, 0.03, and 0.1 mg/ml. ^o Prepared from dipalmitoyl phosphatidylcholine, sphingomyelin, cholesterol, and dicetylphosphate. ^p 0.1, 0.3, 1, 3, and 10 mg/ml.			

human umbilical vein endothelial cells. The results usually indicated a high degree of cytotoxicity. Concentrations below 1.0 mg/ml of bentonite and montmorillonite particles less than 5 μm in diameter caused lysis of human neutrophils, many types of erythrocytes, mouse embryo neuronal cells, and human umbilical vein endothelial cells. The velocity and degree of lysis of sheep erythrocytes were dose dependent. Using cow erythrocytes and montmorillonite, Oscarson et al. (1986) found that particles 0.2–2.0 μm in diameter were most active and that particles greater than 2.0 μm in diameter showed little or no haemolytic activity. The ability of low concentrations of these particles to lyse mammalian cells, although widespread, was not universal, since Murphy et al. (1993a, 1993b) reported no lysis of neuroblastoma cells or oligodendroglial cells incubated in 0.1 mg montmorillonite or bentonite/ml for 18 or 24 h.

Lytic activity appeared to stem from surface properties of the particles, since it was blocked by heating montmorillonite and bentonite to over 500 °C, which altered surface characteristics (Mányai et al., 1969), or by pretreatment of montmorillonite with 10% albumin, which coated the particles and thus altered surface characteristics (Dougherty et al., 1985). Mányai et al. (1969) also reported that the haemolytic activity of bentonite and montmorillonite was proportional to the surface areas of the mineral powder.

Other indications of damage to the physical integrity of cells from montmorillonite or bentonite particles were a dose-dependent loss of ^{51}Cr from hamster tracheal epithelial cells (Woodworth et al., 1982), a dose-dependent loss of fatty acids from human umbilical vein endothelial cells (Murphy et al., 1993b), and elevated loss of β -glucuronidase from rat alveolar macrophages (Vallyathan et al., 1988). Montmorillonite also caused a dose-dependent loss of chromate from artificial liposomes (Woodworth et al., 1982) and suppressed the activity of the enzyme human elastase.

Loss of LDH from cells is a sensitive measure of damage to cell membranes. Several authors, however, reported less loss of LDH to the supernatant in cell cultures incubated with bentonite or montmorillonite than in the controls (Table 16). Dougherty et al. (1985) explained this paradox by demonstrating that although LDH was greatly diminished in quantity in cells incubated with montmorillonite, LDH lost from cells was largely adsorbed on the montmorillonite particles and thus did not appear in the supernatant. Pretreatment of the montmorillonite with 1% human albumin blocked this adsorption

without affecting lysis. These observations may also explain the low value of another enzyme used to measure cell membrane damage, β -*N*-acetylglucosaminidase, in the supernatant from suspensions of rat alveolar macrophages incubated with bentonite (Vallyathan et al., 1988).

In addition to the lytic and membrane damage effects described above, bentonite and montmorillonite also usually exerted other cytotoxic effects. Oberson et al. (1993) noted that montmorillonite particles totally suppressed hydrolysis of elastin by human leukocyte elastase. Adamis and his co-workers (Adamis & Timár, 1976, 1978, 1980; Adamis et al., 1985; Adamis & Krass, 1991) found that bentonite particles were highly cytotoxic to rat peritoneal macrophages as measured by a decrease in 2,3,5-triphenyltetrazolium chloride (TTC) reduction activity (Table 16). Hatch et al. (1985) reported that incubation of rabbit alveolar macrophages with bentonite greatly lowered the viability of the cells as well as cellular ATP. Murphy et al. (1993b) similarly found that montmorillonite significantly decreased the viability of neuroblastoma cells (assessed by trypan blue exclusion), but surprisingly that bentonite had no effect on these cells as measured by this test. The particle size of both minerals, mainly 1–2 μ m diameter, was appropriate to produce adverse effects; the composition of the minerals, however, was not specified.

Functional effects of montmorillonite particles on mammalian cells were also reported. Neuroblastoma cells (with differentiation induced by exposure to dimethylsulfoxide) briefly exposed to up to 1.0 mg/ml had their resting potentials depolarized and lost the ability to maintain action potentials in response to stimulation (Banin & Meiri, 1990). Human leukocytes incubated with sterile, magnesium-saturated montmorillonite at a concentration of 5.3 mg/ml lost all capacity to phagocytize *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Haury et al., 1977). Exposure of hamster tracheal epithelial cells to montmorillonite, however, did not prevent these cells from phagocytizing the clay particles (Woodworth et al., 1982).

6.6.2 Kaolin

6.6.2.1 Haemolysis

Different kaolin samples were found to have a significant haemolysing effect on red cells (Table 17). Mányai et al. (1970) investigated a water-washed Hungarian kaolin sample. The non-heat-treated samples had a haemolytic effect. The sample pretreated by heating at 200 or 350 °C for 90 min had a somewhat stronger haemolytic effect on the red cells than the original sample.

The heat treatment caused the loss of water adsorbed to the surface. Samples pretreated by heating at 500 or 650 °C lost the structurally bound water, and the haemolytic activity was practically abolished. Undoubtedly, the presence of hydroxyl groups is important with respect to the interaction between the red cell and the crystal. Heat treatment of kaolin at 800 or 950 °C resulted in the loss of the crystal structure and the formation of mullite and cristobalite and restoration of haemolytic activity. Mányai et al. (1970) established that the haemolytic activity of the kaolin samples is determined by the crystal structure and hydration of the surface. Studies by Adamis & Krass (1991) showed that the haemolytic activities of kaolin samples coming from different sources and used in the ceramic industry were significantly different. Narang et al. (1977) found that the haemolytic activity of the kaolin sample increased with the amount of silicic acid that could be dissolved from the particles.

Oscarson et al. (1981) determined the haemolytic activity of montmorillonite, kaolinite, quartz, and several fibrous silicates. Montmorillonite had the highest haemolytic activity, followed in decreasing order by quartz, palygorskite, chrysotile, and kaolinite. Polyvinylpyridine-*N*-oxide inhibited the haemolysis caused by montmorillonite and kaolinite. If the incubation medium contained sucrose instead of physiological saline, no haemolysis was observed with the dusts. These results indicate that the hydrogen bonding and ionic interactions are important in the haemolytic action of the silicate surface on the erythrocyte membrane.

Robertson et al. (1982) studied the effect of nitrous oxide on the cytotoxicity of dusts. Kaolinite adsorbed nitrous oxide; although the

Table 17. Toxicity of kaolin and illite clay *in vitro*

System / species / gender	Dose (mg/ml) / treatment ^a	Findings ^a	References
Peritoneal macrophages			
Rat (Sprague-Dawley CFY)	Two types of kaolin (90% kaolinite, 4% quartz; or 93% kaolinite, 4% quartz; diameter not given) incubated with cells for 24 h either as such or after dry milling for 32 h	Dry milling decreased the methylene blue adsorption to a third and the inhibition of TTC reduction by one-half to two-thirds.	Juhasz et al. (1978)
Mouse (Swiss T.O.)	Kaolin (non-specified), 100 µg/ml, incubation for 18 h	Approximately 25% release of LDH and β-glucuronidase release with native kaolin; one-half to two-thirds of the activity lost upon calcination.	Brown et al. (1980)
Rat (Sprague-Dawley CFY), male	Six different kaolins, kaolinite content 51–95%, quartz content 5–20%; 1.0 mg/ml (<5 µm diameter, median 1–2 µm) incubated in cell suspension for 1 h	All samples considered cytotoxic based on TTC reduction, except one (30%), which had 71% kaolinite and 22% quartz. All considered inert based on small LDH release, except the one with the highest quartz concentration (29%); the kaolinite concentration in this specimen was 67%.	Adamis & Timár (1976); Timár et al. (1979, 1980); Adamis et al., (1986); Adamis & Krass (1991)
	One sample of an illite clay (28% illite, 28% quartz); 1.0 mg/ml (<5 µm diameter, median 1–2 µm) incubated in cell suspension for 1 h	Cytotoxic based on TTC reduction, but not cytotoxic based on small LDH release.	

Table 17 (Contd)

System / species / gender	Dose (mg/ml) / treatment ^a	Findings ^a	References
Rat (Sprague-Dawley CFY), male	Four different kaolins (from Hungary, silica content 4, 5, 18, and 30%, <5 µm diameter, otherwise not specified) incubated in cell suspension for 24 h; similar experiment with a non-specified illite clay	Three out of four kaolins and illite considered cytotoxic based on TTC reduction; no relationship between quartz concentration and cytotoxicity. The clays studied did not induce release of LDH, but illite decreased intracellular LDH activity.	Adamis & Timár (1980); Adamis et al. (1985)
Mouse (T.O.), female	Culture of unstimulated macrophages with Cornwall kaolinite (not specified) for 18 h	Kaolinite induced LDH release from macrophages; this was prevented by polyvinylpyrrolidone-N-oxide.	Davies & Preece (1983)
Mouse (T.O.), female	Cornwall kaolin (98% kaolinite, 2% mica), 98% <5 µm in diameter, incubated with cells at 40 µg/ml for 18 h	Kaolinite induced a 70% LDH release to the medium; the release was partly prevented by treatment of kaolin with polyvinylpyrrolidone-N-oxide and fully prevented by additional treatment with polyacrylic acid.	Davies (1983)
Rat (Wistar, SPF), both sexes	Kaolin (composition not specified, diameter 0.2–25 µm), 0.5 mg/10 ⁶ cells incubated for 2 h	Of all cells with particles, 0.6% and 1.6% dead cells with particles at 1 and 2 h (lowest toxicity group of three).	Styles & Wilson (1973)
Mouse	Fifteen respirable dust samples from kaolin drying and calcining plants in England (kaolinite content 84–96%, mica 3–6%, quartz 1%, feldspar 0–7%), a sample of Cornish kaolin (K1, 98% kaolinite, no quartz or feldspar, 2% mica), and a sample of Georgia kaolin (K2, 99% kaolinite, no	All dust samples were cytotoxic. The quartz content could not explain the cytotoxicity. The kaolinite samples showed a dose-dependent cytotoxicity, which could not be explained by their content of ancillary materials. Polyacrylic acid treatment of kaolin has only a	Davies et al. (1984)

Table 17 (Contd)

System / species / gender	Dose (mg/ml) / treatment ^a	Findings ^a	References
	quartz, mica, or feldspar, and reference quartz DQ12, mica, gibbsite, and titanium dioxide as controls; incubation for 18 h with macrophages, LDH release measured	small effect on its cytotoxicity, indicating that the positive charge at the edge of the mineral (blocked by acrylic acid) is not a major determinant of the toxicity.	
Alveolar macrophages			
Rat (Wistar, SPF), both sexes	Kaolin (composition not specified, diameter 0.2–25 µm), 0.5 mg/10 ⁶ cells incubated for 2 h	Of all cells with particles, 3.7% and 4.2% dead cells with particles at 1 and 2 h (lowest toxicity group of three).	Styles & Wilson (1973)
Rabbit (New Zealand)	Kaolinite (>99% pure), >99% respirable size, incubated with cells at 0.25–2.5 mg/ml	Kaolinite caused an inhibition of amino acid incorporation into protein in a dose-dependent manner, 65% inhibition at 1 mg/ml. Inhibition reversed by addition of serum.	Low et al. (1980)
Guinea-pig	Kaolin (non-specified), 100 µg/ml, incubation for 18 h	Approximately 30% release of LDH and β-glucuronidase release with native kaolin; >90% of the activity lost upon calcination.	Brown et al. (1980)
Rabbit (New Zealand)	Kaolin (unspecified) and illite clay (unspecified) (<5 µm diameter), 0.5 mg/ml incubated in cell suspension for 24 h	Kaolin induced a 15.3% release of LDH and a 7% release of alkaline phosphatase. Illite clay induced a 2% release of LDH and a 1.3% release of alkaline phosphatase. For quartz, the figures were 51% and 16%.	Daniel & Le Bouffant (1980)

Table 17 (Contd)

System / species / gender	Dose (mg/ml) / treatment ^a	Findings ^a	References
Rat (Sprague-Dawley), male	Georgia kaolin (≥96% kaolinite, no quartz, >95% >5 µm in diameter), incubated with cells for 1 h at 0.1–1 mg/litre	Kaolin induced a dose-dependent release of LDH, β-glucuronidase, and β-N-acetylglucosaminidase of 60–80%. The effect was largely (9–15% release) abolished by lecithin.	Wallace et al (1985)
Rat (strain not specified)	Kaolin, 1.0 mg/ml (<5 µm diameter, MMAD 2.1 µm) incubated in cell suspension for 2 h	Kaolin induced an 80% release of LDH and a 60% release of β-glucuronidase and β-N-acetylglucosaminidase, being most cytotoxic of all minerals studied, quartz included.	Vallyathan et al. (1988)
Rat (Wistar), male	Alveolar macrophages incubated with Mexicali dust (see Table 13); LDH release measured	Concentration- and time-dependent release of LDH, which reacted 50% at 0.5 mg/ml and was much more pronounced than with quartz.	Osorio-Vargas et al. (1991)
Leukocytes			
Human phagocytic cells from one donor	Well crystallized standard kaolinite KGa-1, no quartz, cristobalite, or mica, particle size 3.2 µm median volume diameter	Kaolinite at concentrations of approximately 1 mg/ml induced luminol-dependent chemiluminescence as an expression of generation of reactive oxygen species in both monocytes and neutrophils when opsonized and when not opsonized.	Gormley et al. (1985)

Table 17 (Contd)

System / species / gender	Dose (mg/ml) / treatment ^a	Findings ^a	References
Erythrocytes			
Human washed erythrocytes	Erythrocytes incubated with Hungarian water-cleaned kaolin (composition not indicated; <5 µm in diameter), as such or after heat treatment at 290–900 °C for 90 min	Kaolin was strongly haemolytic; heating for 90 min to 200 or 350 °C increased, but heating to 500 or 650 °C practically abolished, the haemolytic potency. Kaolin heated at 800 or 950 °C was at least as potent a haemolyser as non-treated kaolin.	Mányai et al. (1970)
Sheep, plasma-free erythrocytes	Kaolin (source and composition unspecified), <5 µm in diameter, incubated with cells at 1 mg/ml for 2 h	Kaolin caused 40% haemolysis; acid and alkali treatments of the clay decreased its haemolytic potency.	Narang et al. (1977)
Rat (strain not specified)	Kaolin (unspecified) and illite clay (unspecified) (<5 µm in diameter) 1.0 mg/ml incubated in cell suspension for 1 h	Kaolin caused 98% haemolysis, illite 24% (quartz 48%).	Daniel & Le Bouffant (1980); Le Bouffant et al. (1980)
Rabbit	Kaolin (non-specified), incubation for 50 min	Twenty per cent haemolysis caused by 1.3 mg kaolin in a total volume of 4 ml; 11.6 mg of calcined kaolin was needed for the same effect.	Brown et al. (1980)
Sheep (citratd blood)	Kaolin (median volume diameter 4.7 µm) incubated in cell suspension	Haemolysis 60% in 30 min; haemolytic potency similar to that of quartz.	Ottery & Gornley (1978)
Sheep	Kaolin, 1.0 mg/ml (<5 µm diameter, MMAD 2.1 µm) at five concentrations, 0.1–1 mg/ml, incubated in cell suspension for 50 min	Linear, dose-dependent haemolysis; about 20% haemolysis with 0.5 mg/ml; 35% with 1 mg/ml; approximately 2 times as potent as quartz.	Vallyathan et al. (1988)

Table 17 (Contd)

System / species / gender	Dose (mg/ml) / treatment ^a	Findings ^a	References
Sheep (citratd blood, single donor)	Kaolinite 90% <2 µm in diameter at six concentrations, 0.005–0.25 mg/ml, incubated in cell suspension for 1 h	More haemolytic per mg than silica or talc; 20% haemolysis with 0.25 mg/ml; 95% with 25 mg/ml.	Woodworth et al. (1982)
Sheep	Georgia kaolin (≥96% kaolinite, no quartz, >95% >5 µm in diameter), incubated with cells for 1 h	Kaolin induced a dose-dependent haemolysis, which reached 42% at 1 mg/ml. The effect was completely abolished by lecithin.	Wallace et al. (1985)
Bovine erythrocytes washed with buffered saline	South Carolina kaolinite (composition not specified) incubated in cell suspension for 1 h	A concentration of 0.6 mg/ml induced 50% haemolysis of erythrocytes. Polyvinylpyridine- <i>N</i> -oxide, a hydrogen-bonding material, partly inhibited the haemolysis.	Oscarson et al. (1981)
Bovine erythrocytes washed with buffered saline	South Carolina kaolinite (composition not specified) at several concentrations of different particle sizes incubated in cell suspension for 1 h	A concentration of 0.6 mg/ml induced 50% haemolysis of erythrocytes. Of the minerals studied, kaolinite was least haemolytic, the potency being 1/20 that of silica. Particles of 0.2–2 µm diameter were most active; particles <0.2 µm or >20 µm diameter had no or little haemolytic activity; reduction of surface charge and cation exchange capacity by coating particles with an aluminium-hydroxy polymer largely eliminated haemolytic capacity.	Oscarson et al. (1986)

Table 17 (Contd)

System / species / gender	Dose (mg/ml) / treatment ^a	Findings ^a	References
Human (citratred blood)	Six different kaolins, kaolinite content 51–95%, quartz content 5–20%; 1.0 mg/ml (<5 µm diameter, median 1–2 µm) incubated in cell suspension for 1 h	Haemolysis 60–90% for all samples except one (30%), which had 71% kaolinite and 22% quartz.	Adamis & Krass (1991)
	One sample of an illite clay (28% illite, 28% quartz); 1.0 mg/ml (<5 µm diameter, median 1–2 µm) incubated in cell suspension for 1 h	Haemolysis 95%.	
Human	One "bentonite," containing 50% illite, 25% montmorillonite, 25% quartz; two undefined illite clays, one kaolinite with dickite and nakrite as main components and quartz as a minor component, one kaolin with kaolinite as the main component, and two unspecified kaolins ground in a ball mill to diameter <5 µm, incubated in cell suspension for 1 h	Fifty per cent haemolysis caused by 1.5–4 mg/ml kaolinites (0.06–0.115 m ² /ml); and 1.0–4.0 mg/ml (0.039–0.12 m ² /ml) illites. Haemolytic activity roughly proportionate to surface area of mineral powder; haemolytic activity largely lost after heating to over 500 °C.	Mányai et al. (1969)
	Dust from the town Mexicali (see Table 14)	Concentration of 2 mg/ml produced a 95 ± 3% haemolysis in 1 h; the haemolysis was stronger than with quartz.	
Human red blood cells from normal donors			Osornio-Vargas et al. (1991)

Table 17 (Contd)

System / species / gender	Dose (mg/ml) / treatment ^a	Findings ^a	References
Neural cells			
Neuroblastoma (N1E-115) cells with differentiation induced by dimethylsulfoxide	Standard kaolinite KGa-1 0.1–1.0 mg/ml incubated in cell suspension	Within minutes, resting potential depolarized and ability to maintain action potentials in response to stimulation was lost; within 30 min, severe morphological deterioration of cells.	Banin & Meiri (1990)
Neuroblastoma (N1E-115) cells and oligodendroglial (ROC-1) cells	South Carolina kaolinite (non-specified, mainly 1–2 µm diameter) incubated at 0.1 mg/ml in cell suspension for 24 h	No alteration of LDH activity in medium for either cell type; no decrease of the viability (assessed by trypan blue exclusion) of N1E-115 cells after 24 h.	Murphy et al. (1993b)
Other cell types and <i>in vitro</i> systems			
Tracheal epithelial (cloned cell line from Syrian hamster, strain 87.20) in log growth phase in monolayer	Kaolinite 90% <2 µm in diameter at four concentrations, 0.003–0.1 mg/ml, incubated in cell suspension for 24 h	Cells phagocytized clay particles; dose-dependent damage to plasma membrane as evidenced by loss of ⁵¹ Cr from cells; loss of ⁵¹ Cr after 24 h approximately 40% with 0.1 mg/ml, twice that of quartz.	Woodworth et al. (1982)
Human umbilical vein endothelial cells	South Carolina kaolinite (non-specified, mainly 1–2 µm diameter) incubated at 0.1 mg/ml in cell suspension for 24 h	Kaolinite induced a statistically significant 50% increase in LDH activity in the medium and killed 90% of the cells in 24 h.	Murphy et al. (1993b)

Table 17 (Contd)

System / species / gender	Dose (mg/ml) / treatment ^a	Findings ^a	References
Macrophage-like cell line P338D ₁	Three kaolinites, 1 with "high crystallinity," 1 with "medium crystallinity," and 1 with "low crystallinity," diameter <5 µm, incubated at 80 µg/ml for 48 h	Kaolinites caused a 78–91% decrease in the viability of the cells and induced leakage of LDH and β-N-acetylglucosaminidase. Adsorption of nitrous oxide on the minerals slightly decreased the effect on viability.	Robertson et al. (1982)
V79-4 Chinese hamster lung cell line	Non-specified kaolin incubated with the cells for 6–7 days	LD ₅₀ 20 mg/ml for kaolin, which was the most toxic of the 21 particulate and fibrous materials tested.	Brown et al. (1980)
Macrophage-like cell line P338D ₁	Thirty respirable dust specimens from coal mines in United Kingdom; cytotoxicity index developed from effects on trypan blue exclusion, release of LDH, glucosaminidase, and lactic acid production	A positive correlation between ash content and cytotoxicity of the dusts. In dusts with >90% coal, there was also a correlation between kaolin + mica content and cytotoxicity.	Gormley et al. (1979)
Macrophage-like cell line P338D ₁	Two kaolinites (KGa-1, KGa-2) from Source Clays repository, particle sizes 3.2 and 3.9 µm, with no cristobalite or quartz, incubated for 48 h	Cell viability not changed at 20 µg/ml and 60–70% at 80 µg/ml.	Gormley & Addison (1983)
Isolated human leukocyte elastase	Cornwall kaolinite and four different illite clays (composition and particle size not specified), 5 µg/ml or 20 µg/ml	Kaolinite (5 µg/ml) caused 90% inhibition of the enzyme, illites (20 µg/ml), 10–53% inhibition.	Oberson et al. (1996)

Table 17 (Contd)

System / species / gender	Dose (mg/ml) / treatment ^a	Findings ^a	References
Artificial organelles			
Liposomes (artificial phospholipid membrane vesicles ^b 0.1–2 µm diameter) entrapping dissolved chromate (CrO ₄ ²⁻)	Kaolinite, 90% <2 µm in diameter, at five concentrations, 0.1–10 mg/ml, incubated in cell suspension for 1 h	Dose-dependent loss of chromate from vesicles; loss of chromate after 1 h (in excess of spontaneous rate) approximately 20% with 10 mg/ml; spontaneous rate was 4–6%.	Woodworth et al. (1982)

^a LD₅₀ = median lethal dose; LDH = lactate dehydrogenase; MMAD = mass median aerodynamic diameter; TTC = 2,3,5-triphenyltetrazolium chloride.

^b Prepared from dipalmitoyl phosphatidylcholine, sphingomyelin, cholesterol, and dicetylphosphate.

adsorption was small, it caused a significant decrease in the cytotoxicity of the dust.

6.6.2.2 Macrophage studies

Styles & Wilson (1973) studied the effect of a large number of mineral dusts and polymers on the alveolar and peritoneal macrophages of rat. The kaolin sample did not damage the macrophages. Five out of six kaolin samples decreased TTC reduction by peritoneal macrophages, but only one of them also induced a release of LDH to the medium (Adamis & Timar, 1976, 1986; Timar et al., 1979, 1980; Adamis et al., 1986; Adamis & Krass, 1991) (Table 17). The surface cationic groups may play a role in the cytotoxic effect of aluminium silicates (kaolin, illite, bentonite). This is suggested by the observed decrease in the cytotoxic effect of aluminium silicate by adsorption of paraquat cations (Adamis & Tímár, 1976). The cytotoxic effect increases with increasing adsorption capacity of the kaolin sample (Adamis & Tímár, 1980; Adamis et al., 1985). This relationship has also been observed in connection with methylene blue, paraquat cation, serum protein, serum lipid, and serum phospholipid adsorption. After dry grinding the kaolin sample in an oscillating mill for 32 h, the adsorption and, at the same time, the macrophage cytotoxic effect decreased (Juhasz et al., 1978). The above indicates the importance of the nature of the crystal surface and the magnitude of the adsorption capacity on the sample's cytotoxicity.

Quartz, asbestos, and kaolinite increased the elastase secretion of peritoneal macrophages. The increase, however, was not as great as that observed after phagocytosis of latex particles. With alveolar macrophages, a difference was also found between the inert latex and the dust samples. This difference probably reflects the cytotoxicity of the dust. Kaolinite has a cytotoxic effect on both peritoneal and alveolar macrophages (White & Kuhn, 1980).

Davies (1983) and Davies et al. (1984) studied the cytotoxicity of Cornwall (United Kingdom) and Georgian (USA) kaolinite on mouse peritoneal macrophages. The Cornwall sample contained 98% kaolinite and 2% mica, and 98% of the particles were smaller than 5 µm in diameter; the Georgia kaolin contained 99% kaolinite and no quartz, mica, or feldspar. Both caused cytoplasmic LDH liberation from macrophages, and the cytotoxicity was apparently caused by kaolinite, not the other dust components. After polyvinylpyridine-*N*-oxide adsorption, the cytotoxic effect of kaolinite specimens from Cornwall

decreased significantly (Davies & Preece, 1983; Davies et al., 1984). Davies et al. (1984) concluded that the amorphous silica-rich gel coating the kaolinite particles was probably responsible for the cytotoxic effect of kaolinite. The flake-like morphology of kaolinite is unlikely to play a role.

Low et al. (1980), studying rabbit alveolar macrophage cells, found that the presence of kaolinite inhibited the incorporation of amino acids into proteins through the non-competitive inhibition of amino acid transport.

Brown et al. (1980) compared different macrophage experimental systems. Kaolin proved to be cytotoxic; after thermal treatment (calcined kaolin), however, the damaging effect decreased significantly.

6.6.2.3 *Other tissue cultures and in vitro systems*

Gormley et al. (1979) studied the cytotoxicity of 30 respirable mine dust specimens with varying compositions from the United Kingdom using a macrophage-like P388 D1 cell line. Positive correlation was found between cytotoxicity and the kaolin and mica (combined) content of dusts containing >90% coal, but not in dusts containing less coal. In the study described in Table 16 on bentonite, Gormley & Addison (1983) studied the cytotoxicity of two standard kaolinites, KGa-1 and KGa-2, with particle sizes of 3.2 and 3.9 μm , which do not contain quartz or cristobalite. They showed very little effect on cell viability at 20 $\mu\text{g/ml}$; even at 80 $\mu\text{g/ml}$, the cell viability remained at 60% or more.

Kaolinite treated with hydrochloric acid did not increase the liberation of LDH from macrophages. The activity of quartz samples was considerably increased by treatment with hydrochloric acid (Kriegseis et al., 1987).

Bruch & Rosmanith (1985) found that mixed dusts containing kaolinite, illite, and quartz inhibit TTC reduction and induce liberation of LDH from guinea-pig alveolar macrophages; no apparent relationship emerged between the toxicity and dust composition.

6.6.3 Other clays

Illite inhibited TTC reduction but caused only a small LDH release from peritoneal macrophages and was haemolytic to human erythrocytes *in vitro* (Table 17).

6.6.4 Relationships between *in vivo* and *in vitro* studies

Tímár et al. (1980) did not find any direct relationship between the *in vitro* cytotoxicity of mineral dusts and their *in vivo* fibrogenicity. Mixed dusts containing 10–30% quartz that were inert in *in vitro* experiments were found to cause fibrosis *in vivo* after a long exposure time. Le Bouffant et al. (1980) reached the same conclusion based on *in vitro* (haemolysis, macrophage) and *in vivo* animal experiments (lung weight, collagen content after 3 and 12 months). On the other hand, of several particulate organic and inorganic materials, asbestos and polyurethane foam dust were the most toxic to alveolar and peritoneal macrophages and induced the most pronounced fibrosis in rat peritoneum; most of the materials studied neither were cytotoxic *in vitro* nor caused peritoneal fibrosis (Styles & Wilson, 1973).

6.7 Summary of the effects of quartz¹

Quartz dust induces cellular inflammation *in vivo*. Short-term experimental studies of rats have found that intratracheal instillation of quartz particles leads to the formation of discrete silicotic nodules in rats, mice, and hamsters. Inhalation of aerosolized quartz particles impairs alveolar macrophage clearance functions and leads to progressive lesions and pneumonitis. Oxidative stress (i.e., increased formation of hydroxyl radicals, reactive oxygen species, or reactive nitrogen species) has been observed in rats after intratracheal instillation or inhalation of quartz. Many experimental *in vitro* studies have found that the surface characteristics of the crystalline silica particle influence its fibrogenic activity and a number of features related to its cytotoxicity. Although many potential contributory mechanisms have been described in the literature, the mechanisms responsible for cellular damage by quartz particles are complex and not completely understood.

¹ This section has been taken directly from IPCS (2000), pages 4–5.

Effects on Laboratory Mammals and In Vitro Test Systems

Long-term inhalation studies of rats and mice have shown that quartz particles produce cellular proliferation, nodule formation, suppressed immune function, and alveolar proteinosis. Experimental studies of rats reported the occurrence of adenocarcinomas and squamous cell carcinomas after the inhalation or intratracheal instillation of quartz. Pulmonary tumours were not observed in experiments with hamsters or mice. Adequate dose-response data (e.g., no-adverse-effect or lowest-adverse-effect levels) for rats or other rodents are not available because few multiple-dose carcinogenicity studies have been performed.

Quartz did not test positively in standard bacterial mutagenesis assays. Results of genotoxicity studies of quartz conflict, and a direct genotoxic effect for quartz has not been confirmed or ruled out.

In experimental studies of particles, results may vary depending on the test material, particle size of the material, concentration administered, and species tested. The experiments with quartz particles involved specimens from various sources, using various doses, particle sizes, and species, which could have affected the observations.

Data on the reproductive and developmental effects of quartz in laboratory animals are not available.

7. EFFECTS ON HUMANS

7.1 General population

General population exposure to low concentrations of clay minerals is universal (see section 4.1). However, no studies are available on the health effects of clays in the general population.

7.2 Occupational exposure

There are important limitations in the data on the health effects of clays in humans. Most studies are case reports or case series (bentonite) or cross-sectional in design (kaolin). Smoking data are not available in most of the studies. In many studies, exposure characterization, both qualitatively and quantitatively, is incomplete.

7.2.1 Bentonite

Data on the effects of occupational exposure to bentonite are summarized in Table 18.

Five workers exposed to bentonite or Fuller's earth (Campbell & Gloyne, 1942; Tønning, 1949; Sakula, 1961; Gibbs & Pooley, 1994) for periods ranging from less than 15 years to more than 40 years developed dyspnoea, in some cases accompanied by cough and sputum, and pneumoconiosis. The general characteristics of the pneumoconiosis of the above five workers, as revealed on autopsy, included emphysema, lesions, numerous firm black patches and nodules 1–20 mm in diameter, dilated air sacs, adhesions, isolated cavities filled with "black sludge," and other evidence of physical deterioration. Gibbs & Pooley (1994) reported that the accumulated dust amounted to 128 mg/g of dry lung (analysed by transmission electron microscopy). There was very little or no fibrosis of the type associated with silicosis. Microscopic examination of lung tissue revealed that the nodules were aggregates of fine brown pigment lying free in tissue spaces or contained in macrophages held in a fine reticulum. The pigment was confirmed as Fuller's earth by microscopic examination and, in the studies of Sakula (1961) and Gibbs & Pooley (1994), by X-ray diffraction. Authors were unanimous that the pathology was markedly

Table 18. Case reports and case series on effects of occupational exposure to bentonite^a

Mineral	Exposure	Effects	References
Fuller's earth ^b ; montmorillonite 85–90%, silica trace	1 worker, 38 years to dust from processing (milling)	Increasing dyspnoea and cough prior to abrupt death after illness at age 56; no tuberculosis; autopsy: pulmonary fibrosis, soft adhesions, and black fibrotic patches, but no silicotic nodules; bronchiole had thickened walls with collagenous fibres containing dust particles.	Campbell & Gloyne (1942)
Fuller's earth ^c montmorillonite ^d	2 workers, 10 and 14 years to high but unmeasured concentrations of dust from grinding, drying, and bagging 3 workers, 10 months to 3 years to dust	Radiological evidence of damage to lungs, including nodules and thickened areas; respiratory problems.	Gattner (1955)
Fuller's earth, ^e calcium mont- morillonite, 0.8% quartz	3 workers, 0.5–15 years to dust 1 worker, >40 years to dust from processing (drying and bagging); also smoked cigarettes	Radiological evidence for possible slight damage to lungs. No radiological evidence for damage to lungs. Shortness of breath; bilateral, fine reticulonodular shadowing in X-ray; autopsy (after haemorrhage from gastric ulcer): lungs — pneumoconiosis; emphysema; soft, grey black, stellate lesions 4–5 mm across; dust (montmorillonite)-laden macrophages around bronchioles; 128 mg mineral dust/g dry tissue analysed by transmission electron microscopy.	Gibbs & Pooley (1994)

Table 18 (Contd)

Mineral	Exposure	Effects	References
Fuller's earth, ^d montmorillonite with some quartz and amorphous silica	49 workers; average dust concentrations 18, 32, 56, 120, and 390 mg/m ³ in different parts of the plant 5 years prior to medical study, and apparently higher prior to these measurements	Twenty-eight workers with exposures of 3.5–16 years had significant radiological evidence of damage to lungs. Duration of employment was 5.6, 7.7, 10, and 16 years for the workers whose pulmonary damage was classified as 1, 2, 3, or 4 on a scale of 1–4. The number of workers in these categories was 21, 18, 8, and 2.	McNally & Trostler (1941)
Fuller's earth ^b	5 workers, 4–39 years to dust working in grinding and sieving of Fuller's earth	Exposure 4, 5, or 19 years, no radiological evidence of damage to lungs; 35 and 39 years, radiological evidence of damage to lungs; at autopsy, one of the latter two showed advanced pneumoconiosis with dark nodules and extensive patchy fibrosis, but no evidence of silicotic nodulation or massive fibrosis.	Middleton (1940)
Bentonite	180 workers in a bentonite mine and 73 and 39 workers in two bentonite refining factories; bentonite contained 30% free silicon dioxide as vitreous silica and β -cristobalite; particle size was 54%, 62%, and 95% $\leq 1 \mu\text{m}$ in diameter	Prevalence of silicosis in the examined groups was 6% in the mine, 35.5% in factory A with high exposure, and 12.8 in factory B. Pulmonary effects were linked more to intensity than to duration of exposure in different sections of the refinery.	Rombola & Guardascione (1955)

Table 18 (Contd)

Mineral	Exposure	Effects	References
Bentonite ^f with 5–11% silica	32 workers, for varying periods to dust from processing; dust concentration at four plants 2–275 mppcf ^g (average of 17 samples, 76 mppcf), no other known exposure to silica dust	Fourteen workers showed radiological evidence of silicosis, ranging from minimal to advanced.	Phibbs et al. (1971)
	1 worker for 10 years to dust; no other known exposure to silica dust	Death at age 72, apparently from advanced silicosis.	
	2 workers, ages 62 and 67, for 10 years to dust; no other known exposure to silica dust	Radiological evidence of silicosis; 67-year-old had lung nodules 1–3 cm in diameter, with high silica content (4.6 mg/1.5 g tissue) and chronic granulomatous inflammation.	
	All the workers (exact number not specified) in a bentonite processing mill, for varying periods to varying concentrations of dust	Eight (all regular cigarette smokers) showed radiological evidence of pulmonary fibrosis ranging from scattered nodules (1 person) and fine nodules (1 person) to progressive massive fibrosis (3 persons).	
Fuller's earth ^b	1 worker for 42 years to dust at a high but unmeasured concentration	Pneumoconiosis; chest radiographs showed fine to medium mottling of lungs; no evidence of tuberculosis; died at age 67 (no autopsy).	Sakula (1961)

Table 18 (Contd)

Mineral	Exposure	Effects	References
Fuller's earth ^b	1 worker for 35 years to dust at a high but unmeasured concentration	Pneumoconiosis; chronic cough and sputum with increasing dyspnoea; chest radiographs showed fine mottling of lungs; no evidence of tuberculosis; autopsy after death at age 65 showed lungs emphysematous with many 1- to 2-mm round, black nodules containing montmorillonite and possibly other mineral matter derived from montmorillonite.	Sakula (1961)
Fuller's earth ^b	1 worker for an unknown period (thought to be less than 15 years) to dust	Death from pneumonia at age 79 (about 50 years after last exposure); autopsy revealed pneumoconiosis, emphysema, and numerous small, black, firm nodules 5–7.5 mm in diameter in the lungs; nodules were aggregates of a fine brown pigment consisting mainly of macrophages containing dust particles composed mainly of Fuller's earth; no evidence of fibrosis.	Tonning (1949)

^a All cases of pulmonary exposure described below apparently involved only male workers.

^b Nutfield district, Surrey, United Kingdom.

^c Sachsen-Anhalt, Germany; in part of the exposure period for workers with long exposures, 5–20% quartz sand was added to the Fuller's earth during processing.

^d Illinois, USA.

^e Bath, United Kingdom.

^f Wyoming, USA.

^g mppcf = million particles per cubic foot; 30 mppcf equals approximately 10 mg/m³.

different from that present in silicosis. McNally & Trostler (1941) summarized the observations of Middleton (1940), who reported pathology similar to the above in a worker exposed to Fuller's earth dust for 35 or 39 years.

The observations of Tønning (1949) support the conclusions of Ungváry et al. (1983) that clearance of bentonite stored in foci from the lung is relatively slow. Bentonite stored in nodules in the lungs that were noted on autopsy stemmed from exposure to dust more than 50 years previous to death at age 79.

Three of the studies summarized in Table 18 (Middleton, 1940; Gattner, 1955; Sakula, 1961) reported radiological evidence, not supported by autopsy, of significant damage to lungs following exposures to bentonite or montmorillonite dust of 10–42 years. Gattner (1955) also reported radiological evidence of possible slight damage to lungs in three workers following 1–3 years of exposure and, in three other workers, no radiological evidence of damage following 0.5–15 years of exposure. Dust concentrations were described as high by Sakula (1961), but were not measured in any of these studies. McNally & Trostler (1941) reported significant radiological evidence of lung damage in 28 workers exposed to bentonite dust for 3.5–16 years, but severity of damage was not correlated with period of exposure or the age of the individual. Twenty-one co-workers with exposures of 1.75–16 years, however, displayed no radiological evidence of damage. The authors ascribed this lack of correlation between damage and period of exposure to the fact that dust concentrations varied widely within the plant (Table 18) and that the men worked at different jobs during the course of their employment and thus were exposed to varying concentrations of dust, which overwhelmed any effects from total period of employment. No attempt was made in any of the above studies to approximately reconstruct actual exposures, as has been done, for example, with silica exposure (Rice et al., 1984). Mikhailova-Docheva et al. (1986), however, reported that damage to the lungs increased with increasing exposure to bentonite dust (containing 1% quartz), although radiological data on Bulgarian workers presented in their paper appeared too limited to permit any final conclusions to be drawn. Their data concurred with those of McNally & Trostler (1941) on the absence of radiological evidence of lung damage in many exposed workers. The data also supported the reasonable assumption of greater damage from higher concentrations of dust. Bulgarian processing workers involved in grinding and packaging bentonite and exposed to 20–40 mg/m³ dust displayed in general more severe lung

damage than production workers involved in removing bentonite from open pit mines and exposed to 1–5 mg/m³ dust.

Observations by Phibbs et al. (1971) demonstrate that exposure to bentonite dust containing 5–11% silica can lead to silicosis (Table 18). Exposure to this bentonite dust for 10 years with no other known exposure to silica dust led to advanced silicosis in three workers and to death, apparently from silicosis, in one of these three. All showed radiological evidence of silicosis, and the presence of silica in the lungs was confirmed by biopsy in one case. In a sample of 32 workers exposed for varying periods to dust from processing, 14 showed radiological evidence of silicosis (Phibbs et al., 1971). These observations suggest that the beneficial effects ascribed to bentonite by Timár et al. (1966) and by Havrankova & Skoda (1993) in protecting rat lungs from silicosis may not be present in humans.

The role of silica in inducing pneumoconiosis in all of the above studies on bentonite and Fuller's earth workers remains uncertain. All of the mineral deposits contained at least trace amounts of silica, and, in the case of the workers examined by Gattner (1955), some were exposed during a period in which quartz sand was added to the Fuller's earth in processing.

The effects of the confounding factor tobacco smoking on the progressive damage to workers' lungs from inhaling bentonite dust remain unknown. Two of the papers cited in Table 18, Phibbs et al. (1971) and Gibbs & Pooley (1994), mention that some of the afflicted workers smoked cigarettes, but other papers provide no information on this matter. In view of the ability of cigarette smoke to aggravate the development of pneumoconiosis in asbestos miners (Blanc & Gamsu, 1989), dental technicians (Choudat, 1994), and coal miners (Sadler & Roy, 1990), level of smoking may be an important factor in determining the degree of lung damage from bentonite exposure.

7.2.2 Kaolin

Many case reports and case series have suggested that exposure to kaolin causes pneumoconiosis (Lemaistre, 1894; Hlava, 1897; Middleton, 1936; Perry, 1947; Thomas, 1952; Lynch & McIver, 1954; Hale et al., 1956; Edenfield, 1960; Warraki & Herant, 1963; Sheers, 1964; Vallyathan et al., 1982; Levin et al., 1996). In several cases, however, it was not clear whether kaolinite and quartz or quartz alone

was responsible for the resulting pneumoconiosis (Gudjonsson & Jacobsen, 1934; Sundius et al., 1936).

7.2.2.1 Kaolin workers, United Kingdom

In England, a number of papers have dealt with the effects of kaolin originating from Cornwall mines (Table 19). Hale et al. (1956) performed medical and radiological examinations of those occupationally exposed to kaolin and reported in detail on six workers who had been working in the drying and bagging of kaolin. All had radiological pneumoconiosis, and two were further studied in autopsy. In one case, characteristic silicotic-type nodulation together with progressive tuberculosis were found. Large quantities of kaolinite and amorphous quartz were found in the lung. In another case, large quantities of pure kaolinite (as much as 20–40 g) were found in the lung without tuberculosis but with severe fibrosis. The disease was like the pneumoconiosis of coal miners and differed from classic silicosis. In the upper part of the lung, greyish or blue-greyish massive confluent lesions were described, which were not as hard on palpation as the silicotic conglomerates. Hale and co-workers (1956) also studied the lung of a kaolin worker from Georgia, USA, and observed that the dust in the lungs consisted entirely of kaolinite; no trace of quartz could be seen.

In a cross-sectional study of 533 Cornish china clay workers, there was an exposure time-dependent increase in the prevalence of radiologically diagnosed kaolinitis, from 4% in those with less than 15 years of exposure to 19% among those with more than 25 years of exposure (Sheers, 1964). Among 526 workers with less than 5 years of exposure, no kaolinitis was observed. Among the workers who were more heavily exposed (milling and bagging), the prevalence was 6% for those with a work history of 5–15 years and 23% for those with an exposure of more than 15 years. Confluent lesions were found in 12 workers, and 30 workers had International Labour Office (ILO) categories 2–3 lesions (ILO, 1959). There was little evidence of disability related to kaolinitis; only one worker with massive fibrosis had become disabled and changed to a lighter job.

Table 19. Effects of occupational exposure to kaolin and other clays on health

Study design, studied population	Exposure measurement	Exposure	Findings ^a	References
Cross-sectional; 533 Cornish china clay workers	Occupational history from records for everyone. Millers, baggers, loaders considered to be continuously exposed, kiln workers and drymen intermittently exposed	Exposure to china clay; industrial hygiene expertise used to group occupations based on the intensity of exposure; no quantitative data or qualitative assessment of the dust	Exposure time-dependent increase in the prevalence of radiologically diagnosed kaolinosis, from 4% in those with less than 15 years of exposure to 19% among those with more than 25 years of exposure. Among 526 workers with less than 5 years of exposure, no kaolinosis was observed. Among the workers who were more heavily exposed (milling and bagging), the prevalence was 6% for those with a work history of 5–15 years and 23% for those with an exposure of >15 years. Confluent lesions were found in 12 workers, and 30 workers had ILO categories 2–3 lesions. There was little evidence of disability related to kaolinosis; only one worker with massive fibrosis had become disabled and changed to a lighter job.	Sheers (1964)
Cross-sectional; 1728 Cornish china clay workers in 1977	Occupational group and history of work in each group for all workers	Exposure to china clay; industrial hygiene expertise used to group occupations based on the intensity of exposure; no quantitative data or qualitative assessment of the dust	77.4% of workers in pneumoconiosis category 0, 17.9% in category 1, 4.7% in category 2 or 3. Advanced pneumoconiosis in 19 workers. Every dusty job contributed to the amount of simple pneumoconiosis. Smoking unrelated to radiographic appearance. Vital capacity deteriorated with advancing pneumoconiosis; for “FEV ₁ ” the association was not statistically significant. Subjective symptoms not related to past exposure as assessed by years worked in different jobs.	Oldham (1983)

Table 19 (Contd)

Study design, studied population	Exposure measurement	Exposure	Findings ^a	References
Cross-sectional; 3831 employees and 336 pensioners in china clay industry in United Kingdom in 1985	Analysis by job classification	Average exposures at the time of the study 0.5–2.7 mg/m ³ (see Table 9)	3374 workers in pneumoconiosis category 0, 271 in category 1, 39 in category 2, and 5 in category 3. Employment in mills had strongest effect on pneumoconiosis category, followed by dryers (in keeping with the exposure levels). In kilns, the exposure was also high, but pneumoconiosis was not equally prevalent; kaolin there is no longer crystalline. Work in mills or as dryer before 1971 had twice the effect on pneumoconiosis prevalence as work there after 1971. Ventilatory capacity related to radiological status, no consistent independent relation with occupational history was observed. Respiratory symptoms were related to ventilatory function.	Ogle et al. (1989)
Cross-sectional; 4401 current and retired china clay workers in United Kingdom in 1990	Dust measurements from 1978, estimated exposure before 1978; detailed occupational history for each participant	Average respirable dust exposure 1.2–4.7 mg/m ³ (see Table 9)	Small opacity profusion related to work dustiness and total occupational dust dose; to reach category 1 by age 60, the estimated total dose for non-smokers was 85 mg/m ³ ·years, for smokers, 65 mg/m ³ ·years. The major determinant of respiratory symptoms was smoking; total dust exposure had a minor effect.	Rundle et al. (1993)

Table 19 (Contd)

Study design, studied population	Exposure measurement	Exposure	Findings ^a	References
Cross-sectional; 4401 current and retired china clay workers in United Kingdom in 1990	Dust measurements from 1978, estimated exposure before 1978; detailed occupational history for each participant	Average respirable dust exposure 1.2–4.7 mg/m ³ (see Table 9)	Univariate analysis showed relationships between lung function and age, X-ray score, smoking class, occupational history, and total occupational dust dose. In multiple regression analysis, when the effects of age, X-ray score, and smoking class had been accounted for, there was no independent additional effect from total occupational dust dose or occupational history.	Comyns et al. (1994)
Cross-sectional; 39 current and 16 ex-kaolin workers at a kaolin mine and mill in Georgia, USA	Respirable and total dust analysed at the time of study (see Table 9)	Dust composed of 96% kaolinite, 4% titanium dioxide, no free silica, no asbestiform fibres; average respirable dust in all job categories ≤ 2 mg/m ³	Of current and ex-workers with ≥ 5 years of exposure ($n = 55$), 4 had simple pneumoconiosis and 4 complicated pneumoconiosis. Mean adjusted FVC, FEV ₁ , peak flow lower ($P < 0.05$) among kaolin workers than among 189 non-kaolin-exposed referents.	Sepulveda et al. (1983)
Cross-sectional; 459 workers in three Georgia kaolin mining and processing facilities with ≥ 1 year work history; mean	Details on measurements not given	At the time of study, US Mine Safety and Health Administration documented exposure to kaolin dust < 5 mg/m ³ with less than 1% free silica; survey	417 workers had pneumoconiosis category 0, 29 category 1, 8 category 2, and 5 category 3. Of the blacks, 13.6%, and of the whites, 7.6% had pneumoconiosis. Pneumoconiosis was significantly related to age, > 15 years of exposure, and greatest dust exposure. Complicated pneumoconiosis (large opacities) related to decreased respiratory function,	Kennedy et al. (1983)

Table 19 (Contd)

Study design, studied population	Exposure measurement	Exposure	Findings ^a	References
duration of employment 12 years		in one of the plants in 1951 and 1960 showed kaolin dust concentrations of 377 and 361 mg/m ³ ; in 1951, the raw material had 0.25% free silica	but otherwise there was no correlation between pneumoconiosis and respiratory function.	
Cross-sectional; all 65 men employed in a Georgia kaolin mine studied	During 5-year period, 157 measurements of respirable dust (see Table 9)	Dust composed of 94–98% kaolinite and 2–6% anastase (TiO ₂); no asbestiform fibres of crystalline silica; mean respir- able dust in process- ing area 1.74 mg/m ³ , 0.14 mg/m ³ in the mine	Five of the workers had radiological pneumoconiosis. All had worked in the processing area. For the whole group, FVC and FEV ₁ were within the normal range, but they were lower for the workers with pneumoconiosis. FVC and FEV ₁ decreased with years of employment in the processing area. Pneumoconiosis was not related to smoking.	Altekruse et al. (1984)
Cross-sectional; 2379 current kaolin workers in Georgia, USA	No measure- ments; occupa- tional title as proxy	The authors state that the free silica expo- sure is negligible because of washing out of impurities in the process	4.4% prevalence of category ≥1 simple pneumo- coniosis, 0.89% prevalence of complicated pneumoconiosis; 7.1% of white and 19% of black dry processors and 4.1% of white and 9.1% of white wet processors had pneumoconiosis. FEV ₁ was <80% of the expected among 7.5, 12.8, and 33.3% and FVC was <80% among 8.0, 10.5, and 33.3% of those with normal chest radiograph and those with simple and complicated pneumoconiosis. Similarly, among	Morgan et al. (1988)

Table 19 (Contd)

Study design, studied population	Exposure measurement	Exposure	Findings ^a	References
Cross-sectional: workers of two Georgia, USA, kaolin plants	Reanalysis of data from two of the three plants studied by Kennedy et al. (1983); no measurements		lifelong non-smokers, the frequency of lowered FEV ₁ and FVC was elevated among those with complicated pneumoconiosis.	Baser et al. (1989)
Cross-sectional; 914 workers in earthenware industry, United Arab Republic	No quantitative measurements	83–86% of respirable dust potassium aluminium silicate, 1– 2% free silica	19/162 and 21/223 workers in the two plants had pneumoconiosis. The adjusted prevalence of pneumoconiosis increased 1.1% for each year in production. Workers in plant 1 had a 2.7 times higher prevalence than workers in plant 2. In plant 1, 15– 20% of production had been calcined kaolin, while plant 2 had produced hydrous clay only.	Warraki & Herant (1963)

Table 19 (Contd)

Study design, studied population	Exposure measurement	Exposure	Findings ^a	References
Cross-sectional, 11 workers in the bagging section of a kaolin refinery in Sri Lanka, average age 35.2 years, duration of employment 3–9 years (average 6 years)	No measurements available	Kaolinite content of kaolin >99%, 76.8% <3 µm in diameter	No radiological abnormalities observed. The absence of pneumoconiosis most likely due to short duration of employment and young age of the workers studied.	Uragoda & Fernando (1974)
Cross-sectional; 18 factories in heavy clay industry in United Kingdom; 1934 current employees	1465 personal dust samples collected at the time of the study and analysed for respirable dust and quartz	Cumulative exposure <40 mg/m ³ .years respirable dust for 94% and <4 mg/m ³ .years quartz for 93%; for details, see Table 10	1.4% had radiographic small opacities in ILO category ≥1/0, 0.4% had small opacities in ILO category ≥2/1. Risk dependent on lifetime exposure to quartz and respirable dust. Anamnestic chronic bronchitis, wheezing, and breathlessness related to dust exposure. Dust and quartz exposure strongly correlated.	Love et al. (1999)

Table 19 (Contd)

Study design, studied population	Exposure measurement	Exposure	Findings ^a	References
Cross-sectional; 268 current brick workers in South Africa	97 respirable, 78 total dust, and 29 silica analyses from three of the five participating factories at the time of the study	Mean respirable dust and total dust expo- sure 2.22 and 15.6 mg/m ³ ; mean free silica 2.1%; for exposure in different groups, see Table 10	4.2% had profusion score $\geq 1/0$; profusion score significantly related to cumulative respirable dust exposure. Anamnestic respiratory symptoms and FVC and FEV ₁ significantly related to exposure to respirable dust.	Myers & Cornell (1989); Myers et al. (1989b)

^a FEV = forced expiratory volume; FEV₁ = forced expiratory volume in 1 s; FVC = forced vital capacity; ILO = International Labour Organization.

In a further questionnaire, a radiological and lung function study among 1676 workers of the largest china clay producers in Cornwall, advanced pneumoconiosis was observed in 19 workers, all employed in dusty workplaces, and pneumoconiosis category 2 or 3 was reported in 79 workers. Vital capacity showed a significant reduction with increasing pneumoconiosis. No symptoms related to years worked in different jobs were observed (Oldham, 1983).

Wagner et al. (1986) examined the lungs from 62 workers who had died between 1968 and 1981, had been employed in kaolin production, and had been referred to the pneumoconiosis unit of the Medical Research Council of the United Kingdom as suspected of having kaolin-induced lung damage. In lung specimens from 16 of the workers, $\geq 90\%$ of the lung dust was kaolinite, $\leq 1.1\%$ quartz, and $\leq 1.0\%$ feldspar; none of these workers had worked with china stone. Among the 16 workers in whose lung specimens there was $< 90\%$ kaolinite, $\geq 0.9\%$ quartz, and $\geq 1.1\%$ feldspar, 12 had worked with china stone. Nodular fibrosis appeared to be related to a high quartz content of the dust recovered from the lung, whereas among those with a high content of kaolinite in the lungs, interstitial fibrosis was observed.

In a cross-sectional study of employees and retirees from the United Kingdom china clay industry, 271 of 3374 workers had ILO pneumoconiosis category 1, 39 category 2, and 5 category 3 (ILO, 1981). Employment in dusty operations was accompanied by the highest risk of pneumoconiosis; however, in kilns where exposure was also high, pneumoconiosis was less frequent. As the authors propose, the likely explanation is that in the kiln department, kaolinite is no longer crystalline. Ventilatory capacity was related to the radiological status of the lungs (Ogle et al., 1989).

In a further follow-up of the United Kingdom china clay workers, small opacity profusion was related to work dustiness and estimated total occupational dust dose. The major determinant of respiratory symptoms was smoking; total dust exposure had only a minor effect (Rundle et al., 1993). In a further analysis of the same workforce, univariate analysis demonstrated a relationship between lung function and age, X-ray score, smoking class, occupational history, and total occupational dust dose. In multiple regression analysis, total occupational dust dose and occupational history were not independent additional risk factors for decreased lung function (Comyns et al., 1994).

7.2.2.2 Kaolin workers, USA

Kaolin workers in Georgia have been studied extensively (Table 19). Edenfield (1960) found pneumoconiosis in 44 (3.9%) of 1130 persons working with kaolin. All these had worked for a number of years in the loading area or some other area of the plant that in earlier years had been very dusty. Only 2 had worked in the kaolin industry less than 10 years, and 19 had been employed for more than 20 years. All of those classed as having severe pneumoconiosis (stage III) had worked more than 20 years, and all had worked in heavy dust in the car loading and bagging areas. Thirty-one of the 44 had a stage I pneumoconiosis; they had no symptoms or signs of respiratory dysfunction. Similarly, the seven workers with stage II pneumoconiosis exhibited no respiratory symptoms. The six cases with stage III pneumoconiosis also had emphysema, had complaints of cough and dyspnoea, and had been placed in jobs requiring minimal activity.

Lapenas & Gale (1983) found diffuse reticulonodular lung infiltration and a nodule in the upper lung lobe in a 35-year-old worker of a Georgia kaolin processing factory who had been occupationally exposed to kaolin aerosol for 17 years. Exploratory thoracotomy revealed an 8 × 12 × 10 cm conglomerate pneumoconiotic lesion containing large amounts of kaolinite. Quartz could not be demonstrated by scanning electron microscopy or X-ray diffraction.

Lapenas et al. (1984) performed a pathological study on biopsy or autopsy specimens from five patients admitted to the Medical Center Hospital of central Georgia between 1976 and 1981, who were estimated to represent some of the most advanced cases of kaolin pneumoconiosis seen in that hospital. Respiratory failure was a contributing factor to death in two of the three autopsy cases. Chest X-ray demonstrated small irregular shadows and large obscure patches typical of kaolin pneumoconiosis. Histological examinations revealed significant kaolinite deposits and peribronchial nodules. The nodules differed from those in silicotic patients and consisted mainly of kaolinite aggregates traversed by fibrous tissue trabecules. The presence of kaolinite in the lungs was confirmed by mineralogical examinations, while quartz could not be demonstrated.

Sepulveda et al. (1983) examined 39 current and 16 ex-workers of a Georgia kaolin mine and mill. Average respirable dust levels were approximately 0.2 mg/m^3 in the mine and between 1 and 2 mg/m^3 for other work stations. Samples of respirable dust contained 96% kaolinite, 4% titanium dioxide, and no silica. Pneumoconiosis was found in 15% of the workers and ex-workers with 5 years or more of exposure. Kaolin workers had a decreased forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), and mean adjusted peak flow when the values were adjusted for age, height, race, and pack-years of smoking ($P < 0.05$).

Kennedy et al. (1983) examined 459 kaolinite workers from central Georgia with a mean duration of employment of 12 years. Pneumoconiosis occurred in 9.2% of the workers and, among employees older than 54 years, was associated with high dust exposure. Among black workers with "complicated pneumoconiosis," the FVC was 81.6%, indicating borderline restriction, and the FEV_1/FVC was significantly lowered. The raw material from the mine contained 0.25% free crystalline silica. At the time of the study, the airborne concentrations of kaolin dust were below 5 mg/m^3 and the dust contained less than 1% free silica, but dust concentrations up to 377 mg/m^3 had been recorded in the past.

In a cross-sectional study of a Georgia kaolin mine and processing plant, Altekruse et al. (1984) examined the pulmonary function and lung radiography of all 65 employees. The respirable dust concentration at the time of the study was 0.14 mg/m^3 in the mine area and 1.74 mg/m^3 in the kaolinite processing area, but had been higher earlier (see Table 9). About 94–98% of the particles were kaolinite and 2–6% anatase (titanium dioxide). Asbestiform fibres and crystalline silica were not present in the samples. Five workers, all of whom worked in the kaolinite processing area with highest exposure, showed radiological evidence of pneumoconiosis; they had worked for the company for 7–36 years. There was a slight, exposure duration-dependent decrease in the FVC and FEV_1 among the workers ($P < 0.05$), those with radiological pneumoconiosis having lower lung function than the others.

In a cross-sectional study of pulmonary function and radiology among workers of 12 kaolin companies in Georgia (Morgan et al., 1988), an increased prevalence of pneumoconiosis and decreased lung function (FEV_1 , but not FVC) were observed among the workers working with calcined clay. Among workers with more than a 3-year

tenure, the adjusted prevalence of simple and complicated pneumoconiosis was 3.2% and 0.63%, respectively. Smoking did not explain the presence of decreased FEV₁, nor was there a relationship between pneumoconiosis and lung function. The authors stated that the workers were not exposed to quartz.

7.2.2.3 *Kaolin workers, other countries*

Warraki & Herant (1963) examined radiologically 914 china clay workers of Ayyat, United Arab Republic; 264 of them had been exposed for less than 10 years, 133 for 10–15 years, 326 for 15–20 years, and 191 for more than 20 years. Pneumoconiosis was diagnosed in six workers, all with exposures longer than 15 years. In two cases, confluent masses were found in the lungs; during a 2.5-year follow-up, one of these died with cor pulmonale. No measurements of dust were reported; a sample of airborne dust contained 1–2% free silica (Table 19).

Uragoda & Fernando (1974) examined workers in kaolinite processing in Sri Lanka. No individuals working with wet clay were examined because their risk of disease from dust exposure was considered low. Among the 11 persons working with dry materials (sacking and weighing), X-rays showed no sign of disease. The most frequent complaints were skin irritation and dermatitis, probably due to the tropical climate (high temperature and humidity). Lack of pneumoconiosis was to be expected because of the short exposure time (average 6 years, range 3–9 years).

7.2.2.4 *Brick and tile workers and others*

Lesser et al. (1978) described nine cases of disabling pneumoconiosis in workers who had worked in the Missouri, USA, fire brick industry. The chest X-ray and lung tissue analysis (from two cases) were consistent with silicosis.

In an extensive cross-sectional study of the respiratory health of brick workers in South Africa (Myers & Cornell, 1989; Myers et al., 1989b), 4.2% of the workforce studied had a radiological profusion score $\geq 1/0$, and it was significantly related to cumulative respirable dust exposure. Similarly, anamnestic respiratory symptoms and respiratory function measurements (FVC and FEV₁) were significantly related to exposure to respirable dust (Table 19).

Love et al. (1999) performed a cross-sectional study on the respiratory health of workers in the heavy clay industry in the United Kingdom and analysed the results with relation to dust exposure at the time of the study. Of the workforce studied, 1.4% had radiographic small opacities in the ILO category $\geq 1/0$, and 0.4% had radiographic small opacities in the ILO category $\geq 2/1$ (ILO, 1981). The risk of radiological abnormality and of anamnestic chronic bronchitis, wheezing, and breathlessness increased with estimated lifetime exposure to respirable dust and quartz. The exposures to dust and quartz correlated strongly with each other (Table 19).

Seaton et al. (1981) described findings for four pneumoconiosis cases referred to the Pneumoconiosis Medical Board of the United Kingdom for disablement benefit and who for practically all their working life had worked in shale mining. Pneumoconiosis was identified in two cases at autopsy, in one by lobectomy, and in one by X-ray. Two of the patients were found at autopsy also to have peripheral squamous lung cancer. The clinical and histological picture of the disease mainly resembled the pneumoconiosis of coal miners and kaolin workers. The lung dust was analysed for three of the cases and was found to contain kaolinite (19–36%), mica (13–16%), and quartz (9–18%).

7.3 Summary of the effects of quartz¹

There are many epidemiological studies of occupational cohorts exposed to respirable quartz dust. Silicosis, lung cancer, and pulmonary tuberculosis are associated with occupational exposure to quartz dust. IARC [the International Agency for Research on Cancer] classified inhaled crystalline silica (quartz or cristobalite) from occupational sources as a Group 1 carcinogen based on sufficient evidence of carcinogenicity in humans and experimental animals; “in making the overall evaluation, the Working Group noted that carcinogenicity in humans was not detected in all industrial circumstances studied. Carcinogenicity may be dependent on inherent characteristics of the crystalline silica or on external factors affecting its biological activity or distribution of its polymorphs” (IARC, 1997b).

¹ This section is taken directly from IPCS (2000), page 5.

Statistically significant increases in deaths or cases of bronchitis, emphysema, chronic obstructive pulmonary disease, autoimmune-related diseases (i.e., scleroderma, rheumatoid arthritis, systemic lupus erythematosus), and renal diseases have been reported.

Silicosis is the critical effect for hazard identification and exposure–response assessment. There are sufficient epidemiological data to allow the risk of silicosis to be quantitatively estimated, but not to permit accurate estimations of risks for other health effects mentioned above.

The risk estimates for silicosis prevalence for a working lifetime of exposure to respirable quartz dust concentrations of about 0.05 or 0.10 mg/m³ in the occupational environment vary widely (i.e., 2–90%).¹ Regarding exposure to ambient quartz in the general environment, a benchmark dose analysis predicted that the silicosis risk for a continuous 70-year lifetime exposure to 0.008 mg/m³ (estimated high crystalline silica concentration in US metropolitan areas) is less than 3% for healthy individuals not compromised by other respiratory diseases or conditions and for ambient environment (US EPA, 1996b). The silicosis risk for persons with respiratory diseases exposed to ambient quartz in the general environment was not evaluated.

¹ For the development of silicosis, it has been estimated that 45-year exposure to 0.05 mg/m³ quartz leads to a prevalence of 2–30% of ILO category ≥1/1 silicosis; for an exposure of 0.10 mg/m³, the predicted range of the prevalence of ILO category ≥1/1 silicosis is 3–90%.

8. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

Information on environmental effects associated with bentonite, kaolin, or other clays is limited.

In laboratory experiments utilizing both genetically engineered microorganisms and their normal counterparts, increasing concentrations of montmorillonite in the soil enhanced the rate of nitrification (Jones et al., 1991).

The 96-h LC₅₀ for rainbow trout (*Oncorhynchus mykiss*) of Wyoming bentonite, used as a viscosifier in drilling fluids, was 19 g/litre (Sprague & Logan, 1979).

A commercial viscosifier described as finely ground sodium bentonite¹ did not kill hardhead catfish (*Galeichthys [Arius] felis*), striped mullet (*Mugil cephalus*), pigfish (*Orthopristis chrysoptera*), sheepshead (*Archosargus probatocephalus*), silver perch (*Bairdiella chrysoura*), red drum (*Sciaenops ocellatus*), Atlantic croaker (*Micropogonias undulatus*), pinfish (*Lagodon rhomboides*), southern kingfish (*Menticirrhus americanus*), black drum (*Pogonias cromis*), crevalle jack (*Caranx hippos*), spot (*Leiostomus xanthurus*), Atlantic spadefish (*Chaetodipterus faber*), planehead filefish (*Stephanolepis hispidus*), Atlantic midshipman (*Nautopaedium [Porichthys] porosissimus*), gulf killifish (*Fundulus grandis*), sheepshead minnow (*Cyprinodon variegatus*), striped burrfish (*Chilomycterus schoepfi*), puffer (*Sphoeroides* spp., unspecified), searobins (*Prionotus* spp., unspecified), coneshell (*Penaeus* spp., unspecified), blue crab (*Callinectes sapidus*), or shell oyster (*Ostrea virginica*) at the highest concentration tested, 7.5 g/litre, within 24 h. One spotted seatrout (*Cynoscion nebulosus*) (number tested not given) died at 6 g/litre in 22 h (Daugherty, 1951).

The 24- and 48-h LC₅₀ values for kaolinite toxicity to the water flea (*Daphnia pulex*) were >1.1 g/litre (Lee, 1976).

¹ http://www.baroididp.com/baroid_idp_prod/baroid_idp_prod_datasheets.asp

Georgia kaolin caused <10% mortality of sea urchin (*Strongylocentrotus purpuratus*), Japanese clam (*Tapes japonica*), hermit crab (*Pagurus hirsutiussculus*), isopod (*Sphaeroma pentodon*), mud snail (*Nassarius obsoletus*), blue mussel (*Mytilus edulis*), and tunicates (*Molgula manhattensis* and *Styela montereyensis*) within 5–12 days. The 200-h LC₁₀ values for coast mussel (*Mytilus californianus*), black-spotted bay shrimp (*Crangon nigromaculata*), migrant prawn (*Palaemon macrodactylus*), dungeness crab (*Cancer magister*), and the polychaete *Neanthes succinea* were 26, 16, 24, 10, and 9 g/litre, respectively. The 100-h LC₁₀ values for the tunicate *Ascidia ceratodes*, amphipod *Anisogammarus confervicolus*, and shiner perch (*Cymatogaster aggregata*) were 7, 38, and 1 g/litre, respectively (McFarland & Peddicord, 1980).

No effect on the hatching success or egg development rate of four marine fish species — red seabream (*Pagrus major*), black porgy (*Acanthopagrus schlegeli*), striped knifefish (*Oplegnathus fasciatus*), and threeline grunt (*Parapristipoma trilineatum*) — was observed at kaolinite concentrations up to 10 g/litre for 24 h. Larvae were more sensitive to kaolinite: the 12-h LC₅₀ values were 170 and 710 mg/litre for *P. trilineatum* and *O. fasciatus*, respectively; mortality was also observed for *P. major* at concentrations of 1000 mg/litre and above (Isono et al., 1998).

In a study on the effects of drilling fluid components on soils and plants, Miller et al. (1980) dosed natural soil (pH 6, with 8.1% organic matter and 34.7% moisture content) with bentonite. No effect was observed on the growth of beans (*Phaseolus vulgaris*) or corn (*Zea mays*) at a concentration of 135 g/1.6 kg soil.

In a series of 100 necropsies among 11 mammalian and eight avian species in the San Diego Zoo, California, USA, interstitial fibrosis was observed in 20% of the mammals studied. In birds, granulomas around tertiary bronchi, but no fibrosis, were observed. Electron and X-ray diffraction showed that the minerals present in the lungs were muscovite mica and illite (Brambilla et al., 1979). As the authors noted, the finding does not infer causality between clays present in the lungs and pulmonary effects.

9. EVALUATION OF HUMAN HEALTH RISKS AND EFFECTS ON THE ENVIRONMENT

9.1 Evaluation of human health risks

Bentonite, kaolin, and other clay materials vary remarkably in composition, depending on the source and process of manufacture. This is a major obstacle for any universal hazard characterization for all these products.

Several elements and other chemicals may be adsorbed to clays because of their cation exchange capacity and, if released, may exert toxic effects.

One of the important components of all clays is crystalline silica, the amount of which varies greatly. Crystalline silica causes various lung diseases, including silicosis and lung cancer. The content of crystalline silica will often be the decisive factor in clay-induced adverse health effects. For quartz-induced lung cancer, no reliable quantitative risk estimates can be made. For quantitative estimates of quartz-induced silicosis, see below and chapter 7.

There are no studies on the possible adverse effects of clay minerals upon direct skin contact; however, both bentonite and kaolin are used extensively in cosmetics, and the absence of reports of adverse effects indicates that these clays pose no important health hazards via the dermal route.

There are no studies in humans of the effects of long-term oral exposure to clays (or clays contaminated with toxic chemicals). No adequate studies on the carcinogenicity of bentonite were available. One limited study on kaolin in experimental animals did not demonstrate important adverse long-term toxicity or carcinogenicity.

No data are available on the genotoxicity of clay materials *in vitro* or *in vivo* (except for quartz). No information is available on the developmental toxicity of clay materials or their effects on fertility in humans; one very limited study on the developmental toxicity of bentonite and another on kaolin did not indicate adverse effects on the progeny.

Information on the toxicity of bentonite by the inhalation route in humans is limited to case reports and case series, in most of which both qualitative information and quantitative information on exposure are very limited and confounding factors, such as exposure to silica and tobacco smoke, have not been considered.

From the limited data available from studies on bentonite-exposed persons, retained montmorillonite appears to effect only mild non-specific tissue changes, which are similar to those that have been described in the spectrum of changes of the “small airways mineral dust disease” (nodular peribronchiolar dust accumulations containing refractile material [montmorillonite] in association with limited interstitial fibrosis). In some of the studies, radiological abnormalities have also been reported.

There exist no reported cases of marked diffuse/nodular pulmonary tissue fibrotic reaction to montmorillonite in the absence of free silica.

Bentonite is toxic to a variety of mammalian cells *in vitro*. When introduced to tissues, it produces transient, local inflammation. Bentonite increases susceptibility to bacterial infection in mice. No such effect has been reported in humans exposed to bentonite dust.

Many reports of well studied cases and a substantial number of cross-sectional studies, mainly in the United Kingdom and Georgia, USA, have clearly demonstrated that long-term exposure to kaolin may lead to a pneumoconiosis, which has been named kaolinosis. This disease develops even in the absence of exposure to silica, as demonstrated by the absence of silica in the dust and in lung tissue specimens. The disease is usually mild. Symptoms and lowered pulmonary function are observed only when the radiological changes are prominent.

A quantitative universal estimate of the risk of kaolinosis cannot be made with the data available. However, based on data from United Kingdom china clay workers (exposed to clay containing <1.1% quartz), it has been estimated that to reach the ILO profusion category $\geq 1/1$ by age 60, the estimated cumulative exposure to kaolin for non-smokers is 85 mg/m³·years, and for smokers, 65 mg/m³·years (Rundle et al., 1993). For comparison, IPCS (2000) estimated that 45-year exposure to quartz at 0.05 mg/m³ (i.e., a cumulative exposure of

Evaluation of Human Health Risks, Effects on the Environment

2.25 mg/m³·years) leads to a prevalence of 2–30% of ILO category $\geq 1/1$ silicosis; for an exposure of 0.10 mg/m³ (4.5 mg/m³·years), the predicted range of the prevalence of ILO category $\geq 1/1$ silicosis is 3–90%.

It can thus be very roughly estimated that kaolin is at least an order of magnitude less potent than quartz.

Kaolin is toxic to a variety of mammalian cells *in vitro*, and it produces transient inflammation in the lungs of experimental animals after intratracheal instillation.

Illite shows some cytotoxicity in *in vitro* studies and limited pulmonary toxicity in experimental animals after intratracheal instillation.

The decreasing rank order of the potencies of quartz, kaolinite, and montmorillonite to produce lung damage is consistent with their known relative “active” surface areas and surface chemistry.

9.2 Evaluation of effects on the environment

Bentonite and kaolin have low toxicity to aquatic species, a large number of which have been tested.

There is no reason to believe that the mining or processing of bentonite, kaolin, and other clays poses significant toxicological dangers to the environment. However, physical disturbance to the land, excessive stream sedimentation, and similar destructive processes resulting from the large-scale mining and processing of clays, like any large-scale mining operation, have a potential for significant environmental damage.

10. CONCLUSIONS AND RECOMMENDATIONS FOR PROTECTION OF HUMAN HEALTH AND THE ENVIRONMENT

Kaolin produces a specific pneumoconiosis, known as kaolinosi. Its fibrogenic potential is considered to be at least an order of magnitude less than that of quartz. Specific exposure limits should be set, and kaolin should not be considered an inert (nuisance) dust.

With regard to bentonite, a comparable montmorillonite pneumoconiosis has not been consistently reported. Based on its surface chemistry, lack of fibrogenicity in experimental systems, and limited human findings, inhaled bentonite is likely to be less dangerous to humans than kaolin.

In order to decrease adverse health effects from occupational exposure to clays, the Task Group recommends that the responsible risk managers:

- set limits for occupational exposure to clay materials, taking into consideration the quartz content;
- enforce and ascertain compliance with the limits by regular exposure monitoring;
- prepare guidelines to ensure good workplace practice;
- disseminate information on the hazards to exposed workers in an appropriate form;
- institute appropriate medical monitoring programmes to protect populations at risk (including pre-employment and periodical medical examinations, including, where appropriate, chest X-ray); and
- oblige clay producers to declare the content of hazardous components, such as quartz, of the saleable products.

For the protection of the general public, the Task Group recommends that when clays are used for medical and cosmetic purposes, not only the total content but also the mobility and bioavailability of potentially toxic substances in the products be established.

11. FURTHER RESEARCH

In order to improve the assessment of risks from exposure to clays, the Task Group recommends research in the following areas:

- contribution of clay mineral particles in the possible health effects of the fine and ultrafine particles in ambient air;
- longitudinal studies among occupationally exposed people with mineralogical analysis of the particles and the particle distribution in the air and, where possible, in the lung tissues of the deceased;
- studies on long-term effects and carcinogenicity in animals of pure minerals with defined particle size distribution by inhalation;
- experimental studies on inhaled clay minerals and interactions and possible synergism with tobacco smoke;
- genotoxicity studies *in vitro* and *in vivo*;
- studies on the possible uptake and kinetics of clay components after exposure via the gastrointestinal tract and the lungs;
- kinetics of adsorption to and release from clays of toxicants and nutrients;
- mechanistic studies for the identification of methods to predict the fibrogenicity of particulate matter from short-term studies; and
- potential use of different imaging methods in the determination of preclinical (early) stages of clay-induced pulmonary disease.

12. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES

Bentonite was evaluated by the Joint FAO/WHO Expert Committee on Food Additives in 1976, but no acceptable daily intake was allocated (JECFA, 1976).

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EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

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EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

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EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

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EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

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EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

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EHC 231: Bentonite, Kaolin, and Selected Clay Minerals

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RESUME

1. Identité, propriétés physiques et chimiques et méthodes d'analyse

La bentonite est une roche composée d'argiles fortement colloïdales et plastiques, principalement de la montmorillonite, un minéral argileux du groupe de la smectite. Elle est produite par dévitrification *in situ* de cendre volcanique. Outre la montmorillonite, la bentonite peut renfermer du feldspath, de la cristobalite et du quartz cristallin. Entre autres propriétés spécifiques, la bentonite présente une capacité à former des gels thixotropes avec l'eau et à absorber de grandes quantités d'eau, ainsi qu'une forte capacité d'échange de cations. Les propriétés de la bentonite découlent de la structure cristalline des minéraux du groupe de la smectite, qui se présente sous la forme d'un feuillet d'octaèdres d'alumine, placé entre deux feuillets de tétraèdres de silice. La quantité d'eau interstitielle et la nature des cations échangeables présents dans l'espace intercouches influent sur les propriétés de la bentonite et ainsi sur les usages industriels des différents types de bentonite. Par extension, le terme de bentonite s'applique dans l'industrie à toute argile présentant des propriétés similaires. La terre de Fuller est souvent une bentonite.

Le kaolin ou argile de Chine est un mélange de différents minéraux. Son principal composant est la kaolinite. Il contient souvent en outre du quartz, du mica, du feldspath, de l'illite et de la montmorillonite. La kaolinite est constituée de minuscules feuillets de cristaux tricliniques, dotés d'une morphologie pseudo-hexagonale. Elle se forme par altération météorique des roches. Elle possède une certaine capacité d'échange de cations.

Les principaux composants de l'argile commune et de l'argile litée sont l'illite et la chlorite. L'illite est aussi un composant des argiles plastiques pour céramiques. Il existe une ressemblance étroite entre l'illite et les micas, mais dans la première, le nombre d'atomes d'aluminium remplacés par des atomes de silicium et/ou le remplacement partiel des ions potassium entre les couches unitaires par d'autres cations, tels que l'hydrogène, le magnésium ou le calcium, sont moindres.

Les mesures quantitatives portant sur les poussières en suspension dans l'air contenant des aluminosilicates utilisent le plus souvent des méthodes gravimétriques. Pour identifier et quantifier les aluminosilicates, on recourt notamment à la diffraction de rayons X, à la microscopie électronique, à l'analyse par rayons X à dispersion d'énergie, à l'analyse thermique différentielle, à la diffraction d'électrons et à la spectroscopie infrarouge.

2. Sources d'exposition humaine et environnementale

La montmorillonite est partout présente à faible concentration dans le sol, la charge sédimentaire des eaux naturelles et les poussières en suspension dans l'air. Sa biodégradation et sa bioaccumulation dans la chaîne alimentaire semblent minimales, à supposer même qu'elles se produisent, et la dégradation abiotique de la bentonite présente dans d'autres minéraux s'effectue seulement à l'échelle des temps géologiques.

Parmi les principaux usages de la bentonite, on peut mentionner : la liaison du sable de fonderie dans les moules, l'absorption des graisses, des huiles et des déchets animaux, la préparation des boulettes de taconite (minerai de fer) et l'amélioration des propriétés des boues de forage. Les usages spéciaux comprennent l'utilisation comme ingrédient dans les céramiques, l'imperméabilisation et l'étanchéité dans les projets de génie civil tels que les sites de décharge et les dépôts de déchets radioactifs, l'utilisation comme charge, stabilisant ou diluant dans les adhésifs, les peintures, les cosmétiques et les médicaments, comme vecteurs dans les pesticides et les engrais et comme liant dans les aliments pour animaux, la clarification du vin et des huiles végétale, ainsi que la purification des eaux usées. Les usages de la terre de Fuller de type montmorillonite recoupent ceux de la bentonite.

L'utilisation du kaolin remonte au troisième siècle avant Jésus-Christ en Chine. Aujourd'hui, le kaolin est extrait des mines et employé en quantités importantes en vue d'un grand nombre d'usages industriels. Son application la plus importante est la production du papier, dans laquelle il sert de matériau d'enduction. On l'utilise en outre en grandes quantités dans les secteurs du caoutchouc, des matières plastiques, des céramiques, des produits chimiques, des produits pharmaceutiques et des cosmétiques.

On utilise principalement l'argile commune et l'argile litée, dont l'illite est souvent un composant majeur, dans la fabrication de briques extrudées et autres, de ciments portland et autres, de blocs de béton et de béton de structure et de produits réfractaires. Les revêtements routiers, les tuiles en céramique, ainsi que les céramiques et les verres, constituent d'autres usages importants.

3. Concentrations dans l'environnement et exposition humaine

Compte tenu de la diffusion étendue de la bentonite dans la nature et de son utilisation dans une très grande variété de produits de consommation, la population générale est exposée partout à de faibles concentrations de ce matériau.

On dispose de données limitées sur l'exposition professionnelle à la poussière de bentonite dans les mines, les usines de traitement et les industries utilisatrices. Les valeurs maximales rapportées pour les concentrations de poussières totales et inhalables sont respectivement de 1430 mg/m³ et de 34,9 mg/m³, bien que la plupart des valeurs soient inférieures à 10 mg/m³ pour les poussières totales et à 5 mg/m³ pour les poussières inhalables.

Le kaolin est un composant naturel du sol et se rencontre souvent dans l'air ambiant. L'extraction minière et le raffinage du kaolin impliquent une exposition considérable et on s'attend également à ce que la production du papier, du caoutchouc et des matières plastiques s'accompagne d'une exposition importante. On ne dispose d'informations quantitatives sur l'exposition professionnelle que pour un petit nombre de pays et d'industries. Les concentrations de poussières inhalables dans l'extraction minière et le traitement du kaolin sont habituellement inférieures à 5 mg/m³.

4. Cinétique et métabolisme chez les animaux de laboratoires et les êtres humains

On ne dispose d'aucune donnée sur la cinétique ou le métabolisme de la montmorillonite, de la kaolinite ou de l'illite dans les conditions où l'on rencontre ces matériaux dans la plupart des milieux de travail.

On a étudié le dépôt et la cinétique de montmorillonite fondue radiomarquée chez la souris, le rat, le chien et l'homme après une exposition par inhalation. Le dépôt dans le nasopharynx augmente avec la taille de particule et prend une ampleur moindre chez le chien que chez les rongeurs. Le dépôt trachéobronchique est faible et indépendant de l'espèce animale et de la taille de particule. Le dépôt pulmonaire est considérablement plus important chez le chien que chez les rongeurs et diminue lorsque la taille de particule augmente.

L'élimination des particules des poumons s'effectue par solubilisation *in situ* et par clairance physique. Chez le chien, le principal mécanisme de clairance est la solubilisation. Chez les rongeurs, le premier mécanisme d'élimination est le transport physique. La clairance par élimination mécanique est lente, en particulier chez le chien. La demi-vie est initialement de 140 jours et passe à 6900 jours au 200^e jour après l'exposition.

Chez l'homme, on observe, au bout de 6 jours, une rapide clairance initiale à partir de la région pulmonaire de 8 % pour les particules d'aluminosilicate ayant un diamètre aérodynamique de 1,9 μm et de 40 % pour celles dont le diamètre aérodynamique est de 6,1 μm . Puis, 4 % et 11 % respectivement des particules de l'une et l'autre tailles sont éliminées après une demi-vie de 20 jours, les particules restantes étant évacuées avec des demi-vies de 330 jours et de 420 jours respectivement.

Une forte proportion des particules ultrafines ($< 100 \text{ nm}$) se dépose dans la région nasale. Ces particules peuvent franchir la barrière alvéolaire/capillaire.

5. Effets sur les mammifères de laboratoire et dans les systèmes de test *in vitro*

La toxicité des argiles est largement déterminée par leur teneur en quartz. La présence de quartz dans les argiles étudiées s'oppose à l'établissement d'une estimation indépendante fiable de la fibrogénicité des autres composants de ces argiles.

Chez les rongeurs, une injection intratrachéale unique de bentonite et de montmorillonite présentant une faible teneur en quartz a déclenché des effets cytotoxiques dépendants de la dose et de la taille de particule, ainsi qu'une inflammation locale transitoire, se manifestant notamment par des oedèmes et donc par une augmentation du poids des poumons. Chez le rat, des expositions intratrachéales uniques à la bentonite ont donné lieu à la constitution de foyers de stockage dans les poumons 3 à 12 mois plus tard. Après l'exposition intratrachéale de rats à de la bentonite à forte teneur en quartz, on a également observé des fibroses. La bentonite accroît la sensibilité des souris aux infections pulmonaires.

On dispose de données limitées sur les effets d'expositions multiples à la montmorillonite ou à la bentonite sur les animaux de laboratoire. Des souris maintenues à un régime alimentaire contenant 10 ou 25 % de bentonite, mais par ailleurs conçu pour soutenir une croissance normale, ont présenté des vitesses de croissance légèrement réduites, tandis que pour d'autres souris recevant un régime similaire, mais renfermant 50 % de bentonite, la croissance était minimale, avec apparition d'une stéatose hépatique et finalement d'une fibrose du foie et d'hépatomes bénins (voir ci-après).

Les études *in vitro* des effets de la bentonite sur divers types de cellules de mammifère indiquent habituellement une forte cytotoxicité. Des concentrations de bentonite inférieures à 1,0 mg/ml et des particules de montmorillonite de diamètre inférieur à 5 µm provoquent une détérioration de la membrane et même une lyse cellulaire, ainsi des modifications fonctionnelles dans plusieurs types de cellules. La vitesse et le degré de lyse des érythrocytes ovins se révèlent dose-dépendants.

L'instillation intratrachéale de kaolin donne des foyers de stockage, une réaction à un corps étranger et une réaction exsudative diffuse. Après l'administration de doses élevées de kaolin (contenant 8 à 65 % de quartz), certaines études ont décrit l'apparition de fibroses, tandis

que pour des doses plus faibles de kaolin, les quelques études disponibles n'ont pas relevé de fibrose.

Les données sur la toxicité de l'illite sont très limitées et inexistantes sur celle des autres composants des autres argiles importantes pour l'industrie. L'instillation intratrachéale d'illite ayant une teneur en quartz inconnue a induit une protéinose alvéolaire, augmenté le poids des poumons et provoqué la synthèse de collagène. L'illite présente une cytotoxicité limitée à l'égard des macrophages péritonéaux et une action hémolytique *in vitro*.

On ne dispose d'aucune étude satisfaisante sur la cancérogénicité de la bentonite. Dans le cadre d'une étude par inhalation et dans celui d'une étude avec injection intrapleurale, le kaolin n'a pas induit de tumeurs chez le rat. On ne dispose d'aucune étude sur la génotoxicité des argiles.

Des études très limitées et isolées n'ont pas mis en évidence de toxicité pour le développement chez le rat après une exposition orale à la bentonite ou au kaolin.

6. Effets sur l'homme

La population générale est partout exposée à de faibles concentrations de montmorillonite et de kaolinite, les principaux composants respectivement de la bentonite et du kaolin, et d'autres minéraux argileux. Il n'existe pas de données sur les effets potentiels de telles expositions de faible intensité.

Les expositions professionnelles à long terme à la poussière de bentonite peuvent provoquer des lésions structurales et fonctionnelles des poumons. Cependant, les données disponibles sont insuffisantes pour établir incontestablement une relation dose-réponse ou même une relation de cause à effet en raison du manque d'informations sur la période sur laquelle s'est déroulée l'exposition et sur l'intensité de cette dernière, et de l'existence de facteurs de confusion, tels que l'exposition à la silice et à la fumée de tabac.

L'exposition à long terme au kaolin entraîne le développement de pneumoconioses diagnostiquées radiologiquement en relation avec l'exposition. On n'a signalé des détériorations nettes de la fonction respiratoire et des symptômes associés que dans les cas présentant des anomalies radiologiques importantes. La composition de l'argile, à savoir la quantité et la nature des minéraux qu'elle contient en dehors de la kaolinite, est un déterminant majeur des effets.

La bentonite, le kaolin et autres argiles contiennent souvent du quartz et il existe une relation causale entre l'exposition au quartz et la silicose et le cancer du poumon. On a rapporté des augmentations statistiquement significatives de l'incidence de la bronchite chronique et de l'emphysème pulmonaire, ou de la mortalité due à ces maladies après des expositions au quartz.

7. Effets sur d'autres organismes en laboratoire et sur le terrain

La bentonite et le kaolin présentent une faible toxicité pour les espèces aquatiques, dont un grand nombre ont été soumis à des essais.

8. Evaluation des risques pour l'homme et des effets sur l'environnement

D'après les données limitées issues des études portant sur des personnes exposées à la bentonite, la montmorillonite retenue ne semble provoquer que des modifications tissulaires non spécifiques sans gravité, similaires à celles décrites dans le spectre de modifications induites par la « maladie des petites voies aériennes causée par des poussières minérales » (accumulations périlobulaires et nodulaires de poussières contenant un matériau réfractaire [montmorillonite], associées à une fibrose interstitielle limitée). Certaines études rapportent des anomalies radiologiques.

Aucun cas de réaction fibrotique diffuse ou nodulaire marquée des tissus pulmonaires à la montmorillonite en l'absence de silice libre n'a été signalé. On ne peut déduire aucune estimation quantitative de la capacité de la bentonite à provoquer des effets pulmonaires indésirables.

L'exposition à long terme au kaolin peut entraîner une pneumoconiose relativement bénigne, connue sous le nom de kaolinose. On n'a observé de détérioration de la fonction pulmonaire que dans les cas présentant des modifications radiologiques notables. D'après les données relatives aux travailleurs en contact avec l'argile de Chine au Royaume Uni, on peut estimer très grossièrement que le pouvoir toxique du kaolin est inférieur d'un ordre de grandeur au moins à celui du quartz.

La bentonite, le kaolin et d'autres argiles contiennent du quartz, dont on sait qu'il provoque des silicoses et des cancers du poumon.

On n'a identifié aucun signalement d'effets indésirables, locaux ou systémiques, résultant de l'usage étendu de la bentonite ou du kaolin dans les produits cosmétiques.

Les effets biologiques des minéraux argileux dépendent de leur composition minérale et de leur taille de particule. Le classement décroissant des capacités du quartz, de la kaolinite et de la montmorillonite à provoquer des lésions pulmonaires est cohérent avec ce que l'on sait des surfaces spécifiques actives de ces matériaux et de leur chimie de surface.

La bentonite et le kaolin présentent une faible toxicité à l'égard des organismes aquatiques.

RESUMEN

1. Identidad, propiedades físicas y químicas y métodos analíticos

La bentonita es una roca formada por arcillas muy coloidales y plásticas cuyo componente fundamental es la montmorillonita, mineral arcilloso del grupo de la esmectita, que se produce por la desvitrificación *in situ* de la ceniza volcánica. Además de la montmorillonita, la bentonita puede contener feldespato, cristobalita y cuarzo cristalino. Entre las propiedades especiales de la bentonita cabe mencionar su capacidad para formar geles tixotrópicos con el agua y para absorber grandes cantidades de ésta y una elevada capacidad de intercambio de cationes. Las propiedades de la bentonita se derivan de la estructura cristalina del grupo de la esmectita, consistente en una lámina de alúmina octaédrica situada entre otras dos de silicio tetraédrico. Las variaciones del agua intersticial y los cationes intercambiables del espacio interlamilar influyen en las propiedades de la bentonita y, por consiguiente, en las aplicaciones comerciales de sus distintos tipos. Por extensión, el término bentonita se aplica comercialmente a cualquier arcilla con propiedades semejantes. La tierra de fuller es con frecuencia una bentonita.

El caolín o arcilla caolínica es una mezcla de minerales diferentes. Su principal componente es la caolinita; con frecuencia contiene además cuarzo, mica, feldespato, ilita y montmorillonita. La caolinita está formada por capas diminutas de cristales triclinicos de morfología pseudohexagonal. Se forma por la meteorización de las rocas y tiene cierta capacidad de intercambio de cationes.

Los principales componentes de la arcilla común y la pizarra son la ilita y la clorita. La ilita es también un componente de las arcillas de alfarero. Es muy parecida a las micas, pero tiene un grado de sustitución menor de silicio por aluminio y/o una sustitución parcial de iones de potasio entre las capas unitarias por otros cationes, como hidrógeno, magnesio y calcio.

La medición cuantitativa del polvo suspendido en el aire que contiene silicatos de aluminio se suele realizar normalmente mediante análisis gravimétrico. Los métodos utilizados para la identificación y cuantificación de los silicatos de aluminio son la difracción por rayos X, la microscopía electrónica, el análisis de energía dispersiva de rayos X, el análisis térmico diferencial, la difracción electrónica y la espectroscopia de infrarrojos.

2. Fuentes de exposición humana y ambiental

La montmorillonita suele estar presente en concentraciones bajas en el suelo, en los sedimentos de las aguas naturales y en el polvo suspendido en el aire. La biodegradación y la bioacumulación en la cadena alimentaria parecen ser mínimas, o nulas, y la degradación abiótica de la bentonita en otros minerales sólo se produce a una escala de tiempo geológica.

Las principales aplicaciones de la bentonita son la aglutinación de la arena de fundición en los moldes; la absorción de grasa, aceite y desechos de animales; la nodulización del mineral de hierro de la taconita; y la mejora de las propiedades de los lodos de perforación. Entre sus aplicaciones especiales cabe mencionar su empleo como ingrediente de la cerámica; en la impermeabilización y el sellado en proyectos de ingeniería civil, por ejemplo de vertederos y depósitos de desechos nucleares; como relleno, estabilizador o diluyente de adhesivos, pinturas, cosméticos y medicinas, como excipiente en plaguicidas y fertilizantes y como aglutinante en los piensos; como clarificador del vino y el aceite vegetal; y como purificador de aguas residuales. Las aplicaciones de la tierra de fuller del tipo de la montmorillonita se superponen con las de la bentonita.

El uso del caolín se remonta al siglo III a.C. en China. En la actualidad se extrae y utiliza en cantidades significativas para numerosos usos industriales. Su aplicación más importante es la producción de papel como material de revestimiento. Además, se usa en grandes cantidades en las industrias de la pintura, el caucho, el plástico, la cerámica, los productos químicos, los productos farmacéuticos y la cosmética.

La arcilla y el esquisto bituminoso, de los que a menudo la illita es uno de los componentes principales, se utilizan sobre todo en la fabricación ladrillos (por extrusión o de otros tipos), cemento portland o de otros tipos, bloques y estructuras de hormigón, y material refractario. Encuentran también aplicaciones importantes en el revestimiento de carreteras, las baldosas cerámicas y en cerámicas y vidrios.

3. Niveles ambientales y exposición humana

Habida cuenta de la amplia distribución de la bentonita en la naturaleza y de su utilización en una enorme variedad de productos de consumo, la exposición de la población general a bajas concentraciones es ubicua.

La información relativa a la exposición ocupacional al polvo de bentonita en las minas, las instalaciones de elaboración y las industrias que la utilizan es limitada. Los valores máximos notificados para las concentraciones de polvo total y polvo respirable fueron, respectivamente, de 1430 y 34,9 mg/m³, aunque la mayor parte fueron inferiores a 10 mg/m³ para el polvo total y a 5 mg/m³ para el polvo respirable.

El caolín es un componente natural del suelo y se encuentra ampliamente distribuido en el aire del medio ambiente. Su extracción y refinado conlleva una exposición considerable, que también es previsible en la producción de papel, caucho y plástico. Sólo se dispone de información cuantitativa sobre la exposición ocupacional para un pequeño número de países y de industrias. Las concentraciones de polvo respirable en la extracción y elaboración del caolín suelen ser inferiores a 5 mg/m³.

4. Cinética y metabolismo en animales de laboratorio y en el ser humano

No se obtuvo información sobre la cinética o el metabolismo de la montmorillonita, la caolinita o la illita tal como se encuentran en la mayoría de los lugares de trabajo.

Se ha estudiado la deposición y la cinética de la montmorillonita fundida radiomarcada en ratones, ratas, perros y personas tras su exposición por inhalación. La deposición nasofaríngea aumenta con el tamaño de las partículas y es menor en los perros que en los roedores. La deposición traqueobronquial fue escasa e independiente de la

especie animal y del tamaño de las partículas. La deposición pulmonar fue considerablemente más alta en los perros que en los roedores y disminuía al aumentar el tamaño de las partículas.

La eliminación de las partículas de los pulmones tuvo lugar mediante solubilización *in situ* y arrastre físico. En los perros, el mecanismo principal de eliminación fue la solubilización y en los roedores el transporte físico. La eliminación por medios mecánicos fue lenta, especialmente en los perros: la semivida fue inicialmente de 140 días y aumentó a 6900 días a partir del día 200 después de la exposición.

En las personas se registró una eliminación inicial rápida del 8% y el 40% de partículas de silicato de aluminio con un diámetro aerodinámico de 1,9 y 6,1 μm , respectivamente, de la región pulmonar durante seis días. A continuación, se eliminaron el 4% y el 11% de los dos tamaños de partículas tras una semivida de 20 días y el resto con semividas de 330 y 420 días.

Las partículas ultrafinas (<100 nm) tienen una deposición alta en la zona nasal y pueden superar la barrera alveolar/capilar.

5. Efectos en mamíferos de laboratorio y en sistemas de ensayo *in vitro*

Un factor determinante importante de la toxicidad de las arcillas es el contenido en cuarzo. La presencia de cuarzo en las arcillas estudiadas dificulta la realización de una estimación independiente fidedigna de la fibrogenicidad de otros componentes.

La inyección intratraqueal única en roedores de bentonita y montmorillonita con un contenido bajo de cuarzo produjo efectos citotóxicos dependientes de la dosis y el tamaño de las partículas, así como inflamación local transitoria, cuyos signos incluían el edema y, en consecuencia, un aumento de peso del pulmón. Las exposiciones intratraqueales únicas de ratas a la bentonita produjeron focos de acumulación en los pulmones 3-12 meses más tarde. Tras la exposición intratraqueal de ratas a bentonita con un contenido elevado de cuarzo también se observó fibrosis. La bentonita aumentó la susceptibilidad de los ratones a la infección pulmonar.

Son limitados los datos sobre los efectos de exposiciones múltiples de animales experimentales a la montmorillonita o la bentonita. Los ratones que recibieron alimentos con un 10% o un 25% de bentonita, pero por lo demás adecuados para un crecimiento normal, presentaron tasas de crecimiento ligeramente reducidas, mientras que en los ratones alimentados de manera semejante, pero con un 50% de bentonita, se registró un crecimiento mínimo y la formación de hígados grasos y en último término de fibrosis del hígado y hepatomas benignos (véase *infra*).

Los estudios *in vitro* de los efectos de la bentonita en diversos tipos de células de mamíferos normalmente pusieron de manifiesto un alto grado de citotoxicidad. Las concentraciones inferiores a 1,0 mg/ml de partículas de bentonita y montmorillonita con un diámetro inferior a 5 µm produjeron daños en las membranas, e incluso lisis celular, así como cambios funcionales en varios tipos de células. La velocidad y el grado de la lisis de los eritrocitos de oveja eran dependientes de la dosis.

El caolín instilado por vía intratraqueal produce focos de acumulación, reacción ante cuerpos extraños y reacción exudativa difusa. En algunos estudios se ha descrito fibrosis tras la administración de dosis elevadas de caolín (con un 8-65% de cuarzo), mientras que, con la administración de dosis más bajas, no se ha observado fibrosis en los pocos estudios disponibles.

La información sobre la toxicidad de la ilita es muy limitada y es nula sobre la de otros componentes de otras arcillas comercialmente importantes. La instilación intratraqueal de ilita con un contenido desconocido de cuarzo indujo proteinosis alveolar, aumento del peso de los pulmones y síntesis de colágeno. La ilita tuvo una citotoxicidad limitada para los macrófagos peritoneales y actividad hemolítica *in vitro*.

No se dispone de estudios adecuados sobre la carcinogenicidad de la bentonita. En un estudio de inhalación y en otro de inyección intrapleural, el caolín no indujo tumores en las ratas. No hay estudios sobre la genotoxicidad de las arcillas.

En estudios únicos muy limitados en ratas no se demostró la toxicidad en el desarrollo tras la exposición oral a la bentonita o el caolín.

6. Efectos en el ser humano

La exposición de la población general a concentraciones bajas de montmorillonita y caolinita, que son los principales componentes de la bentonita y el caolín, respectivamente, y de otros minerales arcillosos es ubicua. No hay información sobre los posibles efectos de dicha exposición a concentraciones bajas.

Las exposiciones ocupacionales prolongadas al polvo de bentonita pueden provocar daños estructurales y funcionales en los pulmones. Sin embargo, los datos disponibles son insuficientes para establecer de manera concluyente una relación dosis-respuesta o incluso relaciones de causa y efecto, debido a la escasa información sobre el tiempo y la intensidad de la exposición y a factores de confusión, tales como la exposición al silicio y al humo del tabaco.

La exposición prolongada al caolín provoca la aparición de neumoconiosis diagnosticada radiológicamente, relacionada con la exposición. Solamente se han notificado un deterioro claramente definido de la función respiratoria y síntomas conexos en casos con resultados radiológicos importantes. La composición de la arcilla, es decir, la cantidad y calidad de los minerales distintos de la caolinita, es un factor determinante importante de los efectos.

La bentonita, el caolín y otras arcillas contienen con frecuencia cuarzo y la exposición al cuarzo tiene una relación causal con la silicosis y el cáncer de pulmón. Se han notificado aumentos estadísticamente significativos de la incidencia de bronquitis crónica y enfisema pulmonar o de mortalidad derivada de estas patologías tras la exposición al cuarzo.

7. Efectos en otros organismos en el laboratorio y en el medio ambiente

La bentonita y el caolín tienen una toxicidad baja para las especies acuáticas, habiéndose sometido a prueba un gran número de ellas.

8. Evaluación del riesgo para la salud humana y de los efectos en el medio ambiente

Según los limitados datos disponibles de estudios sobre personas expuestas a la bentonita, la montmorillonita retenida sólo parece inducir cambios tisulares no específicos ligeros, que son semejantes a los descritos en el espectro de cambios de la “enfermedad de los conductos aéreos pequeños debida al polvo mineral” (acumulaciones nodulares de polvo con material refringente [montmorillonita] en la zona peribronquiolar, junto con fibrosis intersticial limitada). En algunos de los estudios se han notificado asimismo anomalías radiológicas.

No hay casos notificados de reacción fibrótica difusa/nodular acentuada del tejido pulmonar a la montmorillonita en ausencia de silicio libre. No se pueden derivar estimaciones cuantitativas de la capacidad de la bentonita para provocar efectos adversos en los pulmones.

La exposición prolongada al caolín puede dar lugar a una neumoconiosis relativamente benigna, conocida como caolinosis. Se ha observado un deterioro de la función pulmonar sólo en los casos en que había alteraciones radiológicas importantes. Basándose en los datos obtenidos de trabajadores del caolín en el Reino Unido, se puede estimar de manera muy aproximada que su potencia es como mínimo de un orden de magnitud más bajo que la del cuarzo.

La bentonita, el caolín y otras arcillas contienen con frecuencia cuarzo, componente capaz de provocar silicosis y cáncer de pulmón.

No se han encontrado informes sobre los efectos adversos locales o sistémicos debidos al uso frecuente de bentonita o caolín en los cosméticos.

Los efectos biológicos de los minerales arcillosos dependen de su composición mineral y del tamaño de las partículas. El orden decreciente de la potencia del cuarzo, la caolinita y la montmorillonita para producir daño en los pulmones está en consonancia con sus zonas relativas de superficie activa y con la química de la superficie.

La bentonita y el caolín tienen una toxicidad baja para los organismos acuáticos.

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Scientific Committee on Consumer Safety

SCCS

OPINION

on the safety of aluminium in cosmetic products

- Submission III -



The SCCS adopted this document
by written procedure on 6 May 2022

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Members of the Working Group are acknowledged for their valuable contribution to this Opinion. The members of the Working Group are:

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1. ABSTRACT

The SCCS concludes the following:

1. *In light of the new data provided, does the SCCS consider Aluminium compounds safe when used in cosmetic products other than deodorants, antiperspirants, lipsticks and toothpastes? In the event that the estimated exposure to Aluminium from cosmetic products is found to be of concern, SCCS is asked to recommend safe concentration limits for each category.*

The SCCS considers that aluminium compounds are safe when used

- a. in non-sprayable product categories at the maximum levels indicated in Table 4; and
- b. in sprayable antiperspirant products, provided that the maximum percentage of particles with 10 µm diameter does not exceed 20% of the total aerosolised particles

The SCCS could not assess safety of aluminium compounds in sprayable applications other than antiperspirant. These applications (including eau de toilette, eau de parfum and hair spray) are therefore excluded from this Opinion.

2. *Does the SCCS have any further scientific concerns regarding the use of relevant Aluminium compounds in cosmetic products taking into account the newly submitted information on aggregate exposure to Aluminium from cosmetics, medicines (e.g. antacids) and food intake?*

As aluminium does not belong to substances classified as CMR 1A and B, only exposure from cosmetic uses was considered in this safety assessment.

However, in a scenario provided by the Applicant, where exposure from non-cosmetic sources of aluminium (food and pharmaceuticals) was aggregated with exposure from cosmetics, food contributed in a similar order of magnitude as cosmetics. The aggregate exposure to aluminum from cosmetic and non-cosmetic sources may therefore exceed safe limits.

Keywords: SCCS, revision, scientific opinion, aluminium, submission III, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on the safety of aluminium in cosmetic products - Submission III, preliminary version of 6 May 2022, SCCS/1644/22

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In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide Opinions on questions concerning health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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TABLE OF CONTENTS

ACKNOWLEDGMENTS	2
1. ABSTRACT	3
2. MANDATE FROM THE EUROPEAN COMMISSION	6
3. OPINION.....	8
3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS	8
3.2 EXPOSURE ASSESSMENT & TOXICOKINETICS	8
3.2.1 Function and uses	8
3.2.2 Dermal / percutaneous absorption	10
3.2.3 Other studies on toxicokinetics	10
3.2.4 Calculation of SED/LED	11
3.3 TOXICOLOGICAL EVALUATION.....	40
3.4 SAFETY EVALUATION (including calculation of the MoS)	43
3.5 DISCUSSION	45
4. CONCLUSION	47
5. MINORITY OPINION	47
6. REFERENCES	48

2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Aluminium (Al) and Al compounds are used in a variety of cosmetic products, predominantly in deodorants, antiperspirants, lipsticks and toothpastes. Several Al compounds are regulated in different entries of the Cosmetics Regulation (EC) No 1223/2009¹.

In 2013, a risk assessment issued by the Norwegian Scientific Committee for Food Safety reported that cosmetic products, and in particular antiperspirants, constitute a significantly larger contribution to the total systemic Al exposure compared to diet at least for the Norwegian population that was used in the study. As a result, SCCS was mandated to evaluate the possible risk for human health arising from the presence of Al in cosmetics. The assessment was based on products and aluminium compounds that contributed to the highest consumer's exposure, namely antiperspirants/deodorants, toothpastes and lipsticks. In its Opinion SCCS/1525/14, the SCCS concluded that, due to the lack of adequate data on dermal penetration, the requested risk assessment could not be performed². In 2016, industry submitted a new safety dossier to address the dermal penetration and the fate of Al (after skin application) based on a human exposure study. At its plenary meeting on 3 March 2020, the SCCS adopted its final Opinion SCCS/1613/19³ and in March 2021 an addendum to this Opinion was published⁴.

In the addendum, the SCCS concluded that the use of aluminium compounds is safe at the following equivalent aluminium concentrations up to:

- 6.25% in non-spray deodorants or non-spray antiperspirants
- 10.60% in spray deodorants or spray antiperspirants
- 2.65% in toothpaste, and
- 14% in lipstick

In addition, the SCCS stated "*...the systemic exposure to aluminium via daily applications of cosmetic products does not add significantly to the systemic body burden of aluminium from other sources. Exposure to aluminium may also occur from sources other than cosmetic products, and a major source of aluminium in the population is the diet. This assessment has not taken into account the daily dietary intake of aluminium*".

Following the discussion at the Cosmetics Working Group held on 25 June 2020 and in light of the comments received on the use of Al compounds in a variety of products other than deodorants, antiperspirants, lipsticks and toothpastes, the Commission considered opportune to request from industry to submit additional information on the 'other product categories' and on the aggregate exposure not only from cosmetics.

In March 2021, industry submitted a dossier focusing on the aggregate exposure to aluminium concerning the European population when considering the use of cosmetics and personal care products, medicines (e.g. antacids) and dietary intake.

The current request is based on the dossier submission III by the applicant in order to demonstrate the safe use of aluminium in product categories other than deodorants, antiperspirants, lipsticks and toothpastes, as well as concerning the aggregate exposure. The current submission includes in particular additional data and considerations on the MoS calculation and aggregate exposure from cosmetics, medicines and food intake.

¹ Annex III (entry 50 - Aluminium Zirconium Chloride Hydroxide, and entries 189, 190, 192 - hair dyes), in Annex IV (entries 117, 118, 119, 150 - colorants) and Annex VI (entry 27a - coating for UV-filter).

² "Aluminium is a known systemic toxicant at high doses. The SCCS is of the opinion that due to the lack of adequate data on dermal penetration to estimate the internal dose of aluminium following cosmetic uses, risk assessment cannot be performed. Therefore, internal exposure to aluminium after skin application should be determined using a human exposure study under use conditions" (Opinion SCCS/1525/14).

³ SCCS (Scientific Committee on Consumer Safety), Opinion on the safety of aluminium in cosmetic products, preliminary version of 30-31 October 2019, final version of 03-04 March 2020, SCCS/1613/19, [link](#).

⁴ https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_248.pdf

1
2
3 **Terms of reference**
4

- 5 1. *In light of the new data provided, does the SCCS consider Aluminium compounds*
6 *safe when used in cosmetic products other than deodorants, antiperspirants, lipsticks*
7 *and toothpastes? In the event that the estimated exposure to Aluminium from*
8 *cosmetic products is found to be of concern, SCCS is asked to recommend safe*
9 *concentration limits for each category.*
- 10 2. *Does the SCCS have any further scientific concerns regarding the use of relevant*
11 *Aluminium compounds in cosmetic products taking into account the newly submitted*
12 *information on aggregate exposure to Aluminium from cosmetics, medicines (e.g.*
13 *antacids) and food intake?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

Physicochemical properties of aluminium compounds that can generally be used as cosmetic ingredients have been summarised in Annex I of the previous SCCS Opinion (SCCS/1613/19). The new submission presents an industry survey that indicates the substances and amounts that are actually used. The INCI names and CAS numbers of these substances are given in Tables 2 and 3 of this Opinion. The respective physicochemical properties of the substances can be retrieved from Annex 1 of SCCS/1613/19.

3.2 EXPOSURE ASSESSMENT & TOXICOKINETICS

3.2.1 Function and uses

Taken from the previous Opinion (SCCS/1613/19):

Antiperspirants

Aluminium salts in antiperspirants, such as aluminium chlorohydrate, form insoluble aluminium hydroxide polymer gel plugs within sweat ducts to temporarily prevent sweat reaching the surface of the skin. These substances are soluble at very low pH in the formulation; however, once applied on the skin they form chemically inert complexes with basic components of sweat and skin. The relatively high molecular weight of the compounds, low 'Log P' and positive charge limit the potential for skin penetration through the *stratum corneum*. Moreover, absorption across the skin is further minimised by the formation of protein complexes in the outermost layers of the *stratum corneum* (Hostynek, 2003). These chemical properties limit the systemic delivery of aluminium via the intake skin.

Lipsticks

Aluminium colloidal colorant 'lakes' are mainly used in lipsticks. Colloidal colourants are prepared under aqueous conditions by reaction between aluminium oxide and the organic pigments in order to make them insoluble. Aluminium oxide is usually freshly prepared by reaction of aluminium sulphate or aluminium chloride with sodium carbonate or sodium bicarbonate or aqueous ammonia. Due to the complex molecular structures and high molecular weights of organic lakes, the aluminium represents only a small part of the weight of the raw material of which the extractable (bioaccessible) part will represent only a fraction.

Toothpastes

Insoluble minerals are used in toothpastes mainly to act as mild abrasives and to provide shine/gloss benefit through the polishing of the enamel. They are also used to improve rheology in striped toothpastes. Toothpastes may also contain aluminium colloidal colourant "lakes" and pigments.

New submission:

In reply to a request of the European Commission regarding Al-content of 'other cosmetic products' than antiperspirants, lipsticks and toothpastes, the Applicant has submitted a report that includes the findings of a market survey on Al-containing products.

According to the Applicant, this company survey inquired in seven large multi-national companies for the total volume of products sold in Europe in 2016 (and toothpaste data from one company from 2020), whether or not the products included an aluminium containing ingredient, and the concentration values of aluminium and its compounds in the product formulations. The inclusion criteria were ingredients where aluminium comprised at least 0.01% of the material, and formulations where the aluminium containing ingredient was present in at least 0.01% in the formulation. Regarding the tonnage data, the criteria were products where at least 10 kg were placed on the market in 2016 within the EEC+5 region.

In total, around 5300 formulations were identified that contained Al-compounds as ingredients. A total of 51 different Al-compounds were identified. The reason why Al-compounds are used in these different product categories is because, like e.g. Aluminium Starch Octenylsuccinate, they may absorb oil, sweat or odour. Furthermore, they may function as pigments and thickening agents (<https://cosmeticsinfo.org/aluminum>). Table 1 lists the number of reported formulations for different product categories.

Table 1: Number of unique formulations containing one or more Al-ingredients per product category

	Product as labelled in the model	Number of unique formulas
1	AfterShave	12
2	BarSoap	7
3	BodyLotion	102
4	BodySpray	3
5	DeoRollOn – Gel	37
6	DeoRollOn – RollOn	358
7	DeoRollOn – Stick	145
8	DeoRollOn – Wipes	0
9	DeoSpray – Anti-Perspirant	435
10	DeoSpray – Pump	27
11	EaudeParfum, EaudeToilette	3
12	EyeLiner	217
13	EyeShadow	728
14	FaceMoisturizer	378
15	HairSpray	6
16	HairStyling	45
17	HandCream	16
18	Lipstick	1346
19	LiquidHandSoap	0
20	LiquiMakeupFoundation	979
21	MakeupRemover	33
22	Mascara	60
23	Mouthwash	0
24	RinseoffConditioner	93
25	Shampoo	181
26	Showergel	13
27	Toothpaste	68

Representativeness

According to the Applicant, the survey on occurrence and amount of Al-ingredients used in cosmetic products was conducted with the AI-consortium.

According to the Applicant, the AI consortium members represent approximately 40% of the EU market share based on retail value (source: Euromonitor 2016, data made available to the SCCS) and include some of the highest market shares. Of the total of the more than 389 companies represented, 370 (95%) have below 1% market share and 343 (88%) below 0.1% of the market share. The mean market share is below 0.2% for the non-consortium members. Therefore, the consortium believes that this AI Consortium data is representative of the EU marketplace. In addition, when comparing to subsequent years, the market share of the consortium members remains stable and within the same range as 2016.

SCCS comments

Based on the data presented, the SCCS considers the presented survey as largely representative for the aluminium-containing ingredients and aluminium-containing products on the European market.

3.2.2 Dermal / percutaneous absorption

The data related to dermal/percutaneous absorption were assessed and commented upon by the SCCS in the previous Opinion on Aluminum (SCCS/1613/19). Below is a brief summary.

Dermal absorption was calculated from an exposure study with 6 female volunteers after application of 0.75 g antiperspirant per axilla on 100 cm², respectively. The antiperspirant contained Aluminum-Chlorhydrate which had been doped with radioactive ²⁶Al and the volunteers were biomonitoring for ²⁶Al in 24h-urine (TNO, 2019). This yielded a skin absorption value of 0.00052%. Combined with the Al found in the feces in the same study (0.0014%), this yields an overall percentage of bioavailable Al of 0.00192%.

SCCS comment

The respective dermal absorption percentage of 0.00192% was used in the presented exposure calculation. This is considered a valid absorption value. Due to occlusion and formulation characteristics of antiperspirants, the dermal absorption for antiperspirants is considered to be a conservative value that is also valid for the other cosmetic products addressed in this opinion.

3.2.3 Other studies on toxicokinetics

Toxicokinetic studies were reviewed in SCCS/1613/19, and were summarised as follows:

Oral absorption: EFSA (2008) concluded that a value of 0.3% oral bioavailability was appropriate to use in human risk assessment for soluble aluminium in drinking water (i.e. without food) and 0.1% with food.

Lung absorption: Taken together, all available data suggest that absorption of aluminium from lung deposits in the blood is low. For the purposes of lung exposure modelling and risk assessment, a conservative value for aluminium uptake by the lung is 3% (Jones & Bennett, 1986; DeVoto & Yokel, 1994).

SCCS comments

The SCCS considers that oral bioavailability of 0.1% is an appropriate value for use in risk assessment for indirect ingestion after removal from the upper respiratory tract by the mucociliary elevator.

Regarding inhalation, as reported in the SCCS/1613/19, a conservative value for aluminium uptake by the lung of 3% is an appropriate value for use in risk assessment. From the upper respiratory tract, no data on bioavailability is available, but since mucociliary clearance will lead to expectorate or swallowing of the Al trapped in that part of the body, intestinal resorption values of 0.1 or 0.3% can be used (EFSA, 2008).

3.2.4 Calculation of SED/LED

The applicant has submitted a comprehensive report on single product and aggregate exposure to Al via cosmetics, as well as an aggregate assessment of Al in cosmetics, medicines (antacids) and food. The assessment is based on a survey among the European industry that included 7 large producers.

3.2.4.1 Concentrations in cosmetics

According to the Applicant, the aluminium concentration data reported in this section were collected in the company survey. Six companies provided comprehensive data on Al use across a range of cosmetic products for the year 2016. An additional company provided comprehensive data on Al use in toothpastes for the year 2020. The seven companies participating in the project identified 51 different Al-ingredients that were present in cosmetic products sold in the European market.

The results of the survey showed that 21 Al-ingredients were uniquely reported by a single company, thus exhibiting a unique aluminium concentration data point, provided by that company. These single data points, listed in Table 2, were used as constants in the exposure model, meaning that these 21 Al-ingredients always had the same concentration of aluminium as reported by the company using it.

According to the Applicant, 30 additional Al-ingredients were reportedly used by more than one company and, as the individual companies use different raw materials, multiple concentration values were reported for these raw materials. Table 3 reports the summary statistics of the aluminium concentrations reported for these Al-ingredients. Concentration values were reported as a list of single values or as ranges. Ranges were treated by the exposure model as uniform distributions and the model randomly selected a single value from the distribution whenever it was required.

Table 2: Al ingredients with one single concentration reported (one company)

#	INCI NAME	CAS #	Aluminium Concentration in Ingredient
1	ALUMINA /SYNTHETIC RUBY POWDER	1344-28-1 / 1333-84-2	52.93%
2	ALUMINIUM HYDROXIDE SULPHATE	1332-73-6 / 21645-51-2 / 8011-94-7	19.26%
3	ALUMINIUM LACTATE	18917-91-4	9.17%
4	ALUMINIUM ZIRCONIUM PENTACHLOROHYDRATE	57158-29-9 / 173762-83-9	8.40%
5	ALUMINUM TRISTEARATE	637-12-7	3.08%
6	ALUMINUM BENZOATE	555-32-8	6.91%
7	ALUMINUM CALCIUM SODIUM SILICATE	1344-01-0	14.81%
8	ALUMINUM DIMYRISTATE	56639-51-1	5.41%
9	ALUMINUM DISTEARATE	300-92-5	4.42%
10	ALUMINUM POWDER	7429-90-5	100.00%
11	ALUMINUM SILICATE	1327-36-2	24.73%
12	CARMINE (COCHINEAL LAKE)	1390-65-4	21.50%
13	KAOLIN	1332-58-7	5.23%
14	Montmorillonite	10043-67-1	7.80%
15	RED 21 LAKE	15086-94-9 / 548-26-5 / 15876-39-8	6.20%
16	RED 22 LAKE	548-26-5	12.80%
17	RED 40 LAKE	68583-95-9 / 25956-17-6	24.70%
18	RED 7 LAKE	5281-04-9	22.60%
19	SAPPHIRE POWDER	1317-82-4	52.90%
20	SODIUM POTASSIUM ALUMINUM SILICATE	12736-96-8	11.75%
21	TOURMALINE	1317-93-7	20.70%

Table 3: Al ingredients with a range of aluminum concentrations reported in industry survey (several companies)

#	INCI NAME	CAS #	Aluminium Concentration in Ingredient			
			Min	Max	Mean	# of reported concentrations
1	ALUMINA	1344-28-1	0.35%	0.69%	0.52%	2
2	ALUMINIUM, ZINC, MAGNESIUM AND CALCIUM STEARATES	7047-84-9	3.40%	3.70%	3.55%	2 (2 uniforms)
3	ALUMINUM CHLORIDE	7446-70-0 / 7784-13-6	10.00%	12.00%	11.09%	2 (1 uniform)
4	ALUMINUM CHLOROHYDRATE	1327-41-9	12.20%	30.90%	21.30%	14 (5 uniforms)
5	ALUMINUM HYDROXIDE	21645-51-2	1.19%	34.60%	11.57%	4
6	ALUMINUM SESQUICHLOROXYDRATE/ DIALUMINIUM CHLORIDE PENTAHYDROXIDE?	12042-91-0	11.50%	25.50%	18.48%	4 (2 uniforms)
7	ALUMINUM STARCH OCTENYLSUCCINATE	9087-61-0	0.16%	50.00%	27.85%	8 (1 uniform)
8	ALUMINUM STEARATE	7047-84-9	3.00%	7.83%	5.42%	2
9	ALUMINUM ZIRCONIUM OCTACHLOROXYDRATE	57158-29-9 / 98106-55-9	10.21%	10.21%	10.21%	2
10	ALUMINUM ZIRCONIUM TETRACHLOROXYDRATE GLY	134910-86-4	14.50%	15.50%	15.33%	3 (1 uniform)
11	ALUMINIUM ZIRCONIUM TRICHLOROXYDREX GLY	134375-99-8	15.48%	17.80%	16.64%	2
12	BENTONITE	1302-78-9	9.00%	10.80%	9.90%	2
13	BLUE 1 LAKE/ ACID BLUE 9 ALUMINUM LAKE	68921-42-6	0.02%	31.50%	15.63%	4
14	CALCIUM ALUMINUM BOROSILICATE/ GLASS	65997-17-3	1.79%	13.20%	4.16%	5
15	MAGNESIUM ALUMINUM SILICATE	1327-43-1 / 12511-31-8	7.00%	18.82%	11.61%	3

Opinion on the safety of aluminium in cosmetic products - Submission III

#	INCI NAME	CAS #	Aluminium Concentration in Ingredient			
			Min	Max	Mean	# of reported concentrations
16	MAGNESIUM ALUMINUM SILICATE (ARGILLA)	12199-37-0	1.40%	21.00%	8.40%	3 (2 uniforms)
17	MICA	12001-26-2	0.04%	21.20%	11.72%	59 (6 uniforms)
18	PERLITE	93763-70-3	7.40%	8.20%	7.80%	2
19	POTASSIUM ALUM	10043-67-1 / 7784-24-9	3.95%	6.25%	5.10%	2
20	POTASSIUM ALUMINIUM SULPHATE	10043-24-9 / 7784-24-9	5.68%	10.44%	8.06%	2
21	PUMICE	1332-09-8	6.60%	7.14%	6.78%	3
22	RED 26 LAKE	18472-87-2	24.70%	29.68%	27.53%	4
23	RED 30 LAKE	2379-74-0	20.76%	41.40%	27.99%	3
24	RED 33 LAKE	3567-66-6	30.10%	31.60%	30.85%	2
25	SILICA/KAOLIN	1332-58-7	19.30%	23.80%	21.33%	3
26	SYNTHETIC FLUORPHLOGOPITE	12003-38-2	4.62%	6.41%	5.52%	2
27	TITANIUM DIOXIDE COATED WITH ALUMINIUM OXIDE	1344-28-1	1.56%	26.50%	7.34%	5
28	ULTRAMARINES	101357-30-6	13.00%	16.70%	15.47%	3
29	YELLOW 5 LAKE	12225-21-7	23.00%	39.70%	30.10%	5
30	YELLOW 6 LAKE FD&C	12227-60-0 / 15790-07-5 / 2783-94-0 / 174514-58-0	0.47%	24.50%	12.49%	2

By combining % Al in the ingredient with % of Al-ingredient used in the product, the Applicant derived distributions for the concentration values for each product category included in the exposure assessment (see Table 4) and for the product categories not included in the exposure assessment (see Table 5).

Table 4: Min, max and mean values of the reported concentrations of aluminium in each product type and category included in the exposure assessment.

	Product as labelled in the model	# of Al-ingredients identified across the product types	Aluminium concentrations (%) in products		
			Min	Max	Mean
1	AfterShave	1	0.00%	2.15%	0.59%
2	BarSoap	2	0.00%	0.07%	0.01%
3	BodyLotion	10	0.00%	3.81%	0.21%
4	BodySpray	1	1.10%	1.18%	1.12%
5	DeoRollOn – Gel	7	0.00%	6.18%	1.70%
6	DeoRollOn – RollOn	12	0.00%	5.63%	1.45%
7	DeoRollOn – Stick	9	0.00%	7.73%	1.05%
8	DeoRollOn – Wipes	0	0.00%	0.00%	0.00%
9	DeoSpray – Anti-Perspirant	8	0.00%	3.24%	0.32%
10	DeoSpray – Pump	5	0.00%	4.88%	1.17%
11	EaudeParfum, EaudeToilette	2	0.01%	0.05%	0.03%
12	EyeShadow	19	0.11%	43.31%	6.24%
13	EyeLiner	16	0.01%	15.76%	2.28%
14	FaceMoisturizer	19	0.00%	10.59%	0.69%
15	HairSpray	1	0.00%	0.00%	0.00%
16	HairStyling	7	0.00%	1.19%	0.10%
17	HandCream	2	0.00%	0.86%	0.30%
18	Lipstick	19	0.00%	14.62%	1.60%
19	LiquidHandSoap	0	0.00%	0.00%	0.00%
20	LiquMakeupFoundation	17	0.01%	6.59%	0.43%
21	MakeupRemover	14	0.00%	10.59%	0.62%
22	Mascara	11	0.00%	3.13%	0.53%
23	Mouthwash	0	0.00%	0.00%	0.00%
24	RinseoffConditioner	6	0.00%	7.14%	0.12%
25	Shampoo	7	0.00%	7.14%	0.45%
26	Showergel	7	0.00%	0.89%	0.13%
27	Toothpaste	6	0.00%	3.18%	0.07%

Table 5: Min, max and mean values of the reported concentrations of aluminium in each product type and category that is NOT included in the exposure calculations.

Product Type	# of Al-ingredients included in the product type	Aluminium concentrations (%) in products		
		Min	Max	Mean
Other products with and without AP	3	0.00001%	2.794%	0.832%
Shaving products	1	0.09410%	0.094%	0.094%
Nail varnish	11	0.00223%	3.618%	0.166%
Eye contour products	7	0.00007%	1.290%	0.153%
Sun cream/lotion	10	0.00209%	8.403%	0.467%
Sun cream/lotion pump spray	2	0.33208%	0.332%	0.332%
Hair Color and Perms	2	0.00006%	0.002%	0.000%
Lip care products: Lip balm	3	0.00001%	0.606%	0.119%
Other products	17	0.01278%	15.887%	3.943%
Other products - Decoloration	2	0.00668%	4.760%	0.818%
Other products - oxidative coloration	2	0.01837%	0.120%	0.054%
Talc	1	0.01330%	0.013%	0.013%

Also, data on occurrence of Al in cosmetics was derived for product categories included in the assessment (see Table 6) and those not included in the exposure assessment (Table 7).

Table 6: Product categories that can potentially contain Al and were included in the exposure assessment: Quantities sold in Europe in 2016 and relative occurrence of aluminium-containing products

	Product as labelled in the model	Quantity of all products sold (tonnes)	Quantity of products with aluminium sold (tonnes)	Occurrence
1	AfterShave	2,379.73	88.15	3.70%
2	BarSoap	42,259.45	983.45	2.33%
3	BodyLotion	75,047.33	21,872.97	29.15%
4	BodySpray	20,025.64	62.60	0.31%
5	DeoRollOn – Gel	975.33	959.83	98.41%
6	DeoRollOn – RollOn	16,980.30	15,751.75	92.76%
7	DeoRollOn – Stick	3,848.38	2,764.84	71.84%
8	DeoRollOn – Wipes	0.02	-	0.00%
9	DeoSpray – Anti-Perspirant	56,680.32	48,360.40	85.32%
10	DeoSpray – Pump	1,738.97	735.26	42.28%
11	EaudeParfum, EaudeToilette	3,927.52	0.31	0.01%
12	EyeLiner	69.79	41.82	59.92%
13	EyeShadow	116.49	116.28	99.82%
14	FaceMoisturizer	21,060.00	5,353.81	25.42%
15	HairSpray	40,048.88	19.79	0.05%
16	HairStyling	41,859.15	1,293.27	3.09%
17	HandCream	4,685.26	569.42	12.15%
18	Lipstick	298.02	261.30	87.68%
19	LiquidHandSoap	29,321.13	-	0.00%
20	LiquMakeupFoundation	2,516.76	2,284.24	90.76%
21	MakeupRemover	56,284.66	501.96	0.89%
22	Mascara	821.92	132.81	16.16%
23	Mouthwash	10,555.08	-	0.00%
24	RinseoffConditioner	148,701.36	8,470.45	5.70%
25	Shampoo	428,111.81	67,911.38	15.86%
26	Showergel	352,998.42	1,038.42	0.29%
27	Toothpaste*	76,634.45	19,632.27	25.62%

* One company shared Toothpaste data from 2020 and not 2016.

Table 7: Product categories that can potentially contain Al and were **NOT** included in the exposure assessment: Quantities sold in Europe in 2016 and relative occurrence of aluminium-containing products

Product type	Quantity of all products sold (tons)	Quantity of products sold (tons) with aluminium	Occurrence
Other products with and without AP	207.29	143.63	69.29%
Body hair removal products	36.43	0	0.00%
Shaving products	35,184.80	0.57	0.00%
Eye liner & Eye pencil	69.79	41.82	59.92%
Nail varnish	613.27	190.09	31.00%
Eye contour products	242.23	99.33	41.01%
Sun cream/lotion	11,690.92	3398.42	29.07%
Sun cream/lotion aerosol spray	1,733.24	0	0.00%
Sun cream/lotion pump spray	5,454.00	55.12	1.01%
Hair Color and Perms	30,299.49	987.11	3.26%
Lip care products: Lip balm	493.72	124.44	25.20%
Other products	3,697.65	206.20	5.58%
Other products - Decoloration	8,763.56	850.52	9.71%
Other products - oxidative coloration	23,888.04	1661.23	6.95%
Talc	296.38	18.49	6.24%
Toners/astringents	1,970.74	0	0.00%

Additional information provided by the Applicant upon request (05.11.2021)

Upon request of the SCCS, the Applicant has stated that the beach products (i.e., sunscreen lotion) and other product categories listed in Tables 5 and 7 were not part of the Creme model because they are only used periodically. Importantly, the additional exposure from the excluded cosmetic product categories listed in Tables 5 and 7 are a minor contribution to the aggregate and total daily exposure to aluminium. The Applicant has further provided a deterministic exposure assessment for sunscreens (Table 8):

Table 8: Daily exposure estimate of aluminium. (Deterministic exposure calculations for sunscreen products combined with probabilistic assessment)

	Maximum / Day	Mean / Day
Aluminium in Product	8.40%	0.47%
Quantity of Product Per Day (g)	18	18
Dermal Absorption from SCCS Opinion	0.00192%	0.00192%
Systemic Exposure (µg/person)	29.04	1.61
Systemic Exposure (µg/kg bw/day)	0.48	0.03
MOS	375	6692
Proportion of PTWI	27%	1%
Probabilistic exposure All Sources P95 from Creme Report (µg/kg bw/day)	0.1631	0.1631
Total systemic body burden: Sunscreen + Probabilistic (µg/kg bw/day)	0.6471	0.1900
MOS (total systemic body burden)	278	947

SCCS comment

The SCCS understands that the 'Al-concentrations' of Al-containing cosmetics ingredients mentioned in Tables 2 and 3 represent the Al-contents in terms of the stoichiometric ratios of Al in these ingredients.

It is unclear to the SCCS why a range of Al-contents ('Al-concentrations') in the Al-ingredient for some of the Al-ingredients may result from the defined stoichiometry of the chemical substance (e.g. for potassium aluminium sulphate in Table 3). However, the SCCS has calculated the Al-contents based on stoichiometry and found that the Al-content in the maximal value was not underestimated by using the ranges provided by the Applicant. Therefore, risk assessment based on exposure calculations with these maximum values is considered valid.

Some products had been excluded from the aggregate exposure assessment (see Tables 5, 7) without explanation in the submitted report. Upon request, the Applicant has submitted the explanation and an additional deterministic exposure assessment of sunscreens. The assessment using the maximum Al level possible is considered valid by the SCCS.

The SCCS considers that sunscreen products should be included in the assessment, because on a seasonal basis they are used regularly, even daily, and because the high amounts applied may represent a considerable source for exposure. Furthermore, the presence

probability of aluminium in sunscreens (i.e. the fraction of products that contain the substance) is 30%, which cannot be considered negligible.

Some of the products that have not been considered may also be used regularly (shaving products, eyeliner, eye contour products and lip care products). However, for these products the tonnage of Al-containing products sold is very low, and therefore their contribution to dermal exposure can be considered as negligible.

3.2.4.2 Frequency of use and co-use

The frequency of application and co-use data for cosmetic products are available from Kantar Worldpanel, except for Mascara, Eye Shadow, Eye Liner and Makeup Remover. Kantar data on product usage frequency (at the resolution of one hour) were collected from consumer product consumption surveys from 2007 and 2008 for France, Germany, Spain, and the United Kingdom. In the case of Mascara, Eye Shadow, Eye Liner and Makeup Remover, frequency of use data is available from two studies (Bremmer, 2006a; Biesterbos, 2013). The Biesterbos study was based on 516 Dutch subjects (302 females, 210 males).

3.2.4.3 Amount per use

According to the Applicant, amount per use data comes from the Colipa studies (for Deo Roll-On (Gel, Roll-On, Stick), Deo Spray (Anti-Perspirant, Pump), cosmetics and toothpaste)) and additional data from the CTFA studies for certain cosmetics. The following is a set of abbreviations used here for the data sources mentioned above:

- COLIPA1: "European consumer exposure to cosmetic products, a framework for conducting population exposure assessments." (Hall et al., 2007)
- COLIPA2: "European consumer exposure to cosmetic products, a framework for conducting population exposure assessments Part 2." (Hall et al., 2010)
- CTFA1: "Exposure data for cosmetic products: lipstick, body lotion, and face cream." (Loretz L., et al., 2005)
- CTFA2 "Exposure data for personal care products: hairspray, spray perfume, liquid foundation, shampoo, body wash, and solid antiperspirant. (Loretz et al., 2006)
- CTFA3 "Exposure data for cosmetic products: facial cleanser, hair conditioner, and eye shadow." (Loretz et al., 2008)

The amounts for Bar Soap products were taken from the publication of Comiskey et al. (2017), who report the amounts used for showering and washing hands. For conservative reasons, the exposure model was based upon the amounts of Bar Soap products used for showering as they are significantly higher than the amounts related to hand washing. The amounts for Liquid Hand Soap were derived from Ficheux et al. (2016), which is a study about the amount per use of cosmetic products consumed at home by the French adult, child and baby populations.

The amounts of Deo Spray products reported in COLIPA 1 only apply to pressurised (propellant-driven) sprays, since the amount used/day includes propellants. For Deo Spray-Pump products, lower values should be applied; in accordance with the consortium, it was decided to use the amounts of the DeoRollOn products as a proxy.

The amounts for Mascara, Eye liner and Make-up remover products come from Biesterbos et al. (2012).

3.2.4.4 Retention and bioavailability factors

Users of cosmetic products may be exposed to aluminium via three different routes: the dermal route, which is applicable to all cosmetics, the inhalation route, which is applicable to spray products only, and the oral route, which is applicable to lipsticks and toothpastes only. These routes require three different sets of bioavailability and retention factors.

In the exposure report by the Applicant, dermal retention factors for personal care products vary depending on the product type with leave-on, non-spray products (e.g. body lotion) set at 100% and rinse-off products (e.g. Shampoo) conservatively set at 1% by default. The dermal retention factors for Toothpaste and Mouthwash are set at 5% and 10%, respectively; these are the values set by the SCCS guidelines (SCCS 2012-2018).

According to the Applicant, the dermal retention factor for DeoSpray products is based on findings from the literature, where it has been shown that, of the amount of spray that is directed to the skin, only 23.5% actually lands on the skin (Steiling et al., 2012). The dermal retention factor for HairSpray is set at 10%, which means that 10% of sprayed product remains on the scalp (Api et al., 2008, SCCS 2012-2018). According to Comiskey et al. (2017), 80% of EauDeToilette and EauDeParfum are retained on the skin.

A single aluminium percutaneous absorption factor of 0.00192% is assumed for all products applied to the skin. This is the dermal absorption percentage derived in the most recent SCCS opinion on aluminium (SCCS 2020).

Bioavailability factors are the proportion of a substance which enters the circulation when it comes into contact with the body and are used to estimate the internal exposure from the external exposure. For toothpaste, mouthwash, and lipsticks, which may be partly or totally ingested, the model used an oral bioavailability value of aluminium of 0.1%, as per food and beverages. The amount of foods and antacids absorbed by the GI tract depends on the oral circulation through the GI tract. EFSA (2008) reports an oral bioavailability value of approximately 0.3% for Al in drinking water and less than 0.1% for Al in food and beverages.

Table 9 presents a summary of the used penetration, retention and bioavailability factors.

Table 9: Summary of assessment factors used in the exposure assessment

Product	Dermal retention factor	Dermal penetration factor	Adjusted Inhalation factor	Lung Bioavailability Factor (RF ^a)	Upper airways factor (NRF ^b)	Ingestion factor	Oral bioavailability factor
Food and beverages						100%	0.1%
Water						100%	0.3%
Antacids						100%	0.1%
AfterShave	100%	0.00192%					
BarSoap	1%	0.00192%					
BodyLotion	100%	0.00192%					
BodySpray	100%	0.00192%					
DeoRollOn – Stick	100%	0.00192%					
DeoRollOn – RollOn	100%	0.00192%					
DeoRollOn – Gel	100%	0.00192%					
DeoRollOn – Wipes	100%	0.00192%					
DeoSpray – Anti-Perspirant	23.5%	0.00192%	0.892%	3% (20%)	0.1% (80%)		
DeoSpray – Pump	23.5%	0.00192%	0.892%	3% (2%)	0.1% (98%)		
EauDeParfum EauDeToilette	80%	0.00192%	0.234%	3% (2%)	0.1% (98%)		
EyeShadow	100%	0.00192%					
Face Moisturizer	100%	0.00192%					
HairSpray	10%	0.00192%	1.047%	3% (20%)	0.1% (80%)		
HairStyling	10%	0.00192%					
HandCream	100%	0.00192%					
Lipstick	0%	0.00192%				100%	0.1%
LiquidHandSoap	1%	0.00192%					
LiquiMakeupFoundation	100%	0.00192%					
Mascara	100%	0.00192%					
Mouthwash	10%	0.00192%				10%	0.1%
RinseoffConditioner	1%	0.00192%					
Shampoo	1%	0.00192%					
Showergel	1%	0.00192%					
Toothpaste	5%	0.00192%				5%	0.1%
EyeLiner	100%	0.00192%					
MakeupRemover	1%	0.00192%					

^a Respirable Fraction^b Non-Respirable Fraction

SCCS comments

The Applicant cites Steiling et al. 2012 to support a dermal retention of 23.5% to be used for reducing inhalation exposure. The cited value is the P90 for dermal load from an experimental study, which could be used as reasonable worst case for dermal exposure. However, subtracting an upper percentile value for dermal load from the total available amount will not result in a conservative value for inhalation and therefore cannot be used for deriving an adjustment factor for inhalation. Hence, the SCCS considers the "adjusted inhalation factors" for DeoSpray-antiperspirant and DeoSpray-pump not valid.

Regarding Eau de Toilette, Eau de Parfum and HairSpray, Comiskey et al, 2017 cite an internal report that is not available to the SCCS. Therefore, the 80% dermal retention factor cannot be evaluated. Hence, the SCCS considers also the "adjusted inhalation factors" for Eau de Toilette, Eau de Parfum and HairSpray not valid.

3.2.4.5 Inhalation exposure

According to the Applicant, the exposure model developed by Creme Global, utilised in the exposure study, requires several inputs. Among them, there is the output obtained from a separate computational model, called the 2-box model, that returns the fraction of aluminium inhaled by the consumer of a spray product. Since higher tier experimental data

on each individual spray product were not available, estimates of the contribution from lung exposure rely on conservative computational model predictions.

According to the Applicant, the inhalation route is applicable only to spray products: DeoSpray – Anti-Perspirant, DeoSpray-Pump, EAUdeParfum, EAUdeToilette, HairSpray. Cosmetic spray products (HairSpray, DeoSpray, EAUdeToilette and EAUdeParfum) are directed to the skin and may also be incidentally inhaled. After dermal exposure, a percentage of the product remains on the skin once applied (dermal retention factor). For inhalation exposure, a percentage of the amount of product that is released is available to be inhaled; this percentage is calculated as 100% minus the dermal retention factor. An 'inhalation factor' is proposed to calculate the percentage of chemical released into the atmosphere that is available for inhalation. The inhalation factor depends on the product and is based on calculations using the 2-box model. The dermal retention factors and the inhalation factors are shown in Table 10.

Table 10: Dermal retention and inhalation factors

Product	Dermal Retention Factor (DRF)	% Available for Inhalation (1-DRF)	Inhalation Factor (IF) ^d	Adjusted Inhalation Factor (IF x (1- DRF))
DeoSpray, Anti-Perspirant and Pump	23.5% ^a	76.5%	1.167%	0.892%
HairSpray	10% ^b	90%	1.163%	1.047%
EAUdeToilette, EAUdeParfum	80% ^c	20%	1.171%	0.234%

^a Steiling et al., 2012

^b Api et al., 2008

^c Comiskey et al., 2017

^d Calculated via 2-box model

According to the Applicant, The 'adjusted inhalation factor' is a multiplication of the 'inhalation factor' (calculated by the 2-box model) and the '% available for inhalation' for a specific product. As an example, we know that for DeoSpray 76.5% of the product that is released from the can is available for inhalation. The Inhalation Factor for DeoSpray (as calculated by the 2-box model) is 1.167%, which means that 1.167% of the product that is released into a room may be inhaled by the user. And as only 76.5% of it is available for inhalation, this results in an Adjusted Inhalation Factor of 0.892% for DeoSpray (i.e. 0.892% of the total amount of product released from the can may be inhaled by the user).

Some aerosol spray products, called in the model "DeoSpray – Anti-Perspirant", can be classified as "compressed". This describes a technology which reduces the quantity of propellant in the can and, proportionately, reduces the spray rate to ensure equivalent product release (and hence consumer exposure) compared to standard dilute aerosols over the same duration of spray (Unilever 2015). The cosmetic companies participating in the company survey reported whether their aerosol spray products are compressed and, if so, the size of the spray rate reduction compared to standard dilute aerosols. Given that the Creme model uses exposure data for standard aerosols, the higher concentration of aluminium in the compressed product would lead to an artificially high modelled consumer exposure. In order to account for the reduced spray rate in the Creme model, the percentage of spray rate reduction is used by the exposure model as a multiplier of the aluminium concentrations to adjust exposure for compressed products. The percentages of spray rate reduction reported in the company survey are in the range 53-68%.

For aluminium, a lung bioavailability value of 3% is reported (DeVoto & Yokel, 1994), which has been considered in this 2-box model. It is generally considered that the cut-off value for droplets/particles to reach the deep lung, and be available for absorption, is <10 µm (Steiling et al, 2014). This is known as the 'respirable fraction'. Determination of what

proportion of a sprayed product is considered as the respirable fraction is based on particle size measurements. Industry data demonstrates that for aerosol spray product, approximately 20% of the spray particles are $<10\ \mu\text{m}$ (unpublished industry data). The remaining 80%, known as the non-respirable fraction (or inhalable fraction) is likely deposited in the upper respiratory tract (nasopharyngeal and tracheobronchial region). For this fraction, mucociliary clearance leads to expectoration or swallowing of the aluminium trapped in the upper respiratory tract, and hence the oral absorption value of 0.1% can be applied (EFSA, 2008).

For the calculation of inhalation exposure, the Applicant used a 2-box model based on the work of Nicas & Mark (2014) and Sahmel et al. (2009).

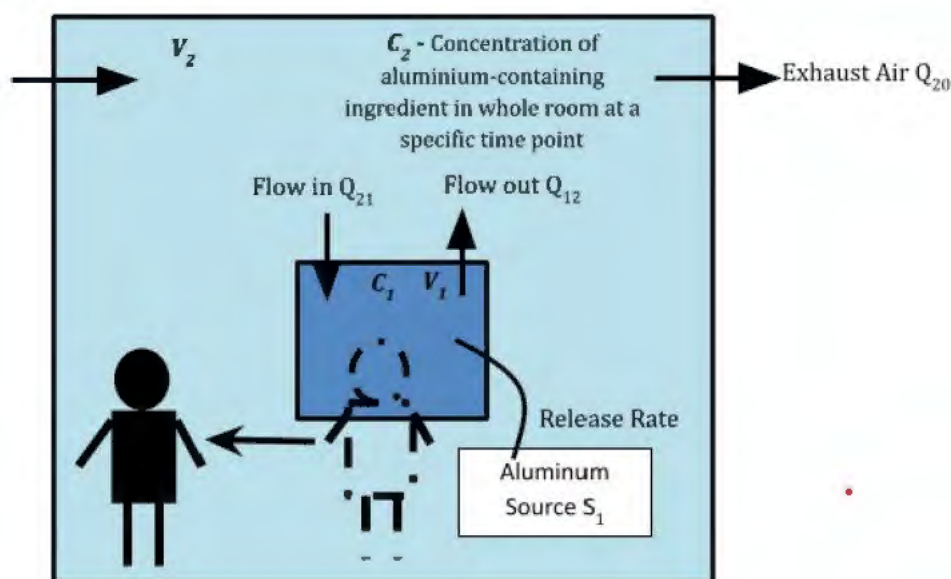


Figure 4: Two-box model described in terms of relevant parameters, with description of parameters (inset).

Where:

- C_1 – aluminium concentration in air in box 1
- C_2 – aluminium concentration in air in box 2
- S_1 – source of aluminium used in box 1
- Q_{12} – airflow from box 1 to box 2 (m^3/min)
- Q_{21} – airflow from box 2 to box 1 (m^3/min)
- Q_{02} – airflow from external world to box 2 (m^3/min) (see Section 3.2.)
- Q_{20} – airflow from box 2 to external world (m^3/min)
- V_1 – volume of box 1 (m^3)
- V_2 – volume of box 2 (m^3)

1 Mathematically, the mass balance can be described by:

$$\text{Zone 1 : } V_1 (dC_1/dt) = S_1 + Q_{21}C_2(t) + Q_{01}C_0 - (Q_{12} + Q_{10}) C_1(t)$$

$$\text{Zone 2 : } V_2 (dC_2/dt) = S_2 + Q_{12}C_1(t) + Q_{02}C_0(t) - (Q_{21} + Q_{20}) C_2(t)$$

2
3
4 The following parameter values were used for calculating the inhalation factor for input into
5 the Crème model.

6
7 Table 11: Parameters used for calculating the inhalation factor for input into the Crème
8 model
9

Parameter	Value
Zone 1 volume (V1)	2 m ³ ^e
Time in zone 1 (t1)	2 min ^e
Zone 2 volume (V2)	10 m ³ (bathroom) ^{b,e}
Time in zone 2 (t2)	10 min ^e
Air Flow (Zone 1 -> Zone 2)	7.24 m ³ /min ^c
Air Flow (Zone 2 -> Outside)	0.1 m ³ /min ^c
Human inhalation rate (IR)	13.1 L/min ^d
Emission Duration	0.17 min (DeoSpray) ^a , 0.24 min (HairSpray) ^a , 0.08 min (EaudeParfum, EaudeToilette) ^a

^a Bremmer et al., 2006a

^b Bremmer et al., 2006b

^c ECHA, 2016

^d Salem & Katz, (2006) & Finley et al. (1994)

^e Rothe et al., 2011

10
11
12 According to the Applicant, the 2-box model requires also as input the quantity of
13 aluminium which is released into the air each minute. This value is calculated by multiplying
14 the product's spray rate (mg/min), the emission duration (min) and aluminium
15 concentration (%) in the product. These three parameters are also used to calculate the
16 total amount of aluminium released (TAR). The TAR is used to estimate the inhalation factor
17 which corresponds to the fraction of TAR that is inhaled during the exposure event, i.e. the
18 model divides the total exposure by the TAR. In doing so, the model cancels out the effect
19 of product's spray rate and aluminium concentration on the result; these two parameters
20 can be varied but the inhalation rate remains unchanged. However, dividing the total
21 exposure by the TAR does not completely cancel out the effect of the emission duration. The
22 longer the length of the emission duration, the more product is released into the air and is
23 available for inhalation by the user before the larger product particles, under the effect of
24 gravity, drop towards the floor, exit the area surrounding the user's head, and are no longer
25 available for inhalation. The 2-box model can calculate the exposure per unit of bodyweight
26 by dividing the total exposure by the bodyweight. However, the model is used here to

estimate the fraction of product that is typically inhaled by an adult, regardless of their bodyweight. The bodyweight was considered in the Crème model.

Table 12 contains the list of the inhalation exposure inputs used to estimate the inhalation exposure to the spray products, together with the references to the scientific literature. All these factors are applied to the exposure levels as multipliers, as explained in detail in this section.

Inhalation Factor	DeoSpray – Anti-Perspirant	DeoSpray – Pump	EaudeParfum, EaudeToilette	HairSpray
Adjusted Inhalation factor	0.892%	0.892%	0.234%	1.047%
Respirable fraction (<10 µm) (RF) ^d	20%	2%	2%	20%
Lung Bioavailability Factor for Aluminium ^a	3%	3%	3%	3%
Non-respirable fraction (NRF), calculated as 1-RF	80%	98%	98%	80%
Oral bioavailability (to account for Al from upper airways ^b)	0.1%	0.1%	0.1%	0.1%
Compression factor ^c	53-68%	NA	NA	NA

^a DeVoto & Yokel, 1994

^b EFSA, 2008

^c Values submitted in the company survey

^d Based on particle size measurements (unpublished company data).

In the following, the step-wise approach used by the Applicant is described:

Step 1: the model selects each diary and retrieve the list of cosmetic products used each day. For example, the diary of subject #1 on the first day report the use of an “DeoSpray – Anti-Perspirant” deodorant and of body lotion.

Step 2: the model randomly selects one of the formulas used in “DeoSpray – Anti-Perspirant” deodorants reported by the companies. The amount sold of each formula is used as a statistical weight to determine the frequency with which each formula is selected. Let’s assume that the model selects formula F7, that was reported by company A, which contains 10% of MAGNESIUM ALUMINUM SILICATE (ARGILLA) and 15% of ALUMINUM CHLOROHYDRATE.

Step 3: the model goes in the ingredient list of company A and retrieves the aluminium concentrations related to the two aluminium ingredients used in formula F7. In case the company reported a range of aluminium concentrations, the model randomly selects a number within the range. Let’s assume in this case the concentration 2% of aluminium concentration MAGNESIUM ALUMINUM SILICATE (ARGILLA) and 12.7% to ALUMINUM CHLOROHYDRATE

Step 4: the model calculates the total aluminium concentration in formula F7 as the sum of the products between the concentration of the aluminium ingredients in the formula times the concentration of aluminium in each ingredient, as shown the following equation:

$$\text{Total-Al-concentration } (C) = 10\% * 2\% + 15\% * 12.7\% = 2.105\%$$

Step 5: the model calculates the exposure level of aluminium by entering the total aluminium concentration in equation (1). Let’s assume that the selected “DeoSpray – Anti-Perspirant” deodorant is a compressed one with compression factor equal to 53%, the used amount is 1.43 g (or 1,430 mg), which is the applied daily amount of spray deodorant recommended as default value in exposure assessments by the SCCS 10th notes of guidance (SCCS 2018), and the bodyweight of the subject is 60 kg. Then the resulting exposure level is calculated as

$$E\left(\frac{mg}{kg}\right) = \frac{1430(mg) \times 0.892\% \times 53\% \times 2.105\%}{60kg} = 0.002372$$

Step 6: the exposure level E calculated in the previous step is then split into its respirable and non-respirable components. The respirable component is equal to E multiplied times the respirable fraction RF and the lung bioavailability factors BL which are equal to 20% and 3%, respectively (see Table 25).

$$E_{respirable}\left(\frac{mg}{kg}\right) = E \times RF \times BL = 1.42E - 05$$

The non-respirable component is instead equal to E multiplied by the non-respirable fraction NF and the upper respiratory tract bioavailability factor BUA which are equal to 80% and 0.3%, respectively (see Table 25).

$$E_{non-respirable}(\frac{mg}{kg}) = E \times NF \times BUA = 5.69 E - 06$$

Step 7: the model repeats the above steps for each consumption event of the subject where a “DeoSpray – Anti-Perspirant” deodorant has been used. Then calculate the total respirable and non-respirable exposure levels and divide them by 7 (this is the number of days recorded in each diary) to calculate the average daily exposure.

The aluminium concentration for each product type is assigned at subject level, i.e. the model assumes that the subject uses the same product over the week hence the concentration does not change. For example, if a subject reported the usage of “DeoSpray – Anti-Perspirant” deodorant in three events, the model assigns the same aluminium concentration to all the three events.

The model analyses the exposure to aluminium as a single chemical, thus removing the dependence on source of the ingredient. Information about the exposure to each aluminium ingredient is stored by the model but not analysed.

SCCS comments

In the calculations of inhalation exposure, a very high airflow from zone 1 to zone 2 has been assumed, without support by literature (the cited ECHA, 2016, does not give the respective value, neither can it be found in the references cited therein). However, based on the SCCS Notes of Guidance (11th Revision, SCCS/1628/21), a conservative approach is warranted, with the assumption of no ventilation. This means that the inhalation factor used in the submission dossier is too low.

No reference is given for the used compression factor in step 5.

The Applicant cites unpublished industry data for the assumption that approximately 20% of the spray particles are $<10 \mu m$ for aerosol spray products. This data is not available to the SCCS and therefore cannot be evaluated.

The procedure for deriving the factor “available for inhalation” is questionable, since the experiments used to derive the dermal absorption rate aimed at deriving a worst case for dermal exposure. As explained in the comments on bioavailability, subtracting a worst-case dermal exposure from the total 100% does not result in conservative estimates for inhalation. A conservative estimate for inhalation would e.g. result from subtracting a lower estimate for dermal exposure. In consequence, the SCCS does not accept the respective factor “available for inhalation” (1-DRF) proposed in Table 10.

3.2.4.6 Exposure calculations and Scenarios for cosmetics

According to the Applicant, a tiered approach that starts from a deterministic model up to a fully probabilistic model was used to assess the exposure to aluminium contained in personal care products, foods and medications (antacids). The aim was to estimate the total amount of aluminium to which humans are exposed from all these various sources. This amount is otherwise known as aggregate exposure.

The Creme Global model was used as the main tool to assess aggregate exposure to aluminium. The Creme tool was run first in a deterministic tier 1 mode, which simply summed the P95 exposure values for the individual products to obtain the aggregate exposure, and then in a Tier 2, probabilistic mode, to estimate the impact of the two approaches on the exposure levels related to cosmetic products.

The Creme Global model is based on the following equation for calculating the daily chemical exposure for a group of cosmetic products i and a single subject j :

$$E_{ij} = \frac{F_{ij} \times A_{ij} \times X_i \times C_{ij}}{7 \times BW_j} \quad (1)$$

Where each term on the right-hand side can be a fixed estimate or drawn from a distribution and each term is understood as follows:

E_{ij} is the systemic/internal exposure in subject j to aluminium from product group I [$\mu\text{g}/\text{kg}/\text{day}$]

F_{ij} is the frequency of applications/consumption events by subject j of product group I [day⁻¹] during the seven-day Kantar survey.

A_{ij} is the amount used by subject j per application/consumption event of product group i [μg]

X_i represents the exposure factors, reported in Section 3.9, for product group i [%] multiplied one by each other. These are independent of the subject.

C_{ij} is the concentration of aluminium in product group i [unitless] assigned to subject j , BW_j is the consumer's bodyweight [kg]

A group of products is one for which all included products are considered equivalent. For example, various brands and colours of lipstick, etc. are assessed together under the product group lipstick.

Aggregate aluminium exposure for each individual subject is estimated by

$$SE_j = \sum_{i=1}^n E_{ij} \quad (2)$$

Where:

SE_{ij} is the total (or aggregated) systemic/internal exposure to aluminium from all products [$\mu\text{g}/\text{kg}/\text{day}$] for subject j

E_{ij} is the systemic/internal exposure to aluminium from product group i [$\mu\text{g}/\text{kg}/\text{day}$] for subject j

The population-level exposure can then be calculated from the individual subject-level exposures. The population can be defined in two ways. The first is the Total Population, i.e. all subjects in the survey, and the other is the Exposed Population, i.e. only those consumers who are exposed to aluminium. Given that there are two potential populations that can be used to characterise exposure, an immediate question posed is to determine which one is most appropriate. One guiding principle is that for aggregate exposure resulting from multiple sources that are used by most of the population (e.g. all categories of cosmetics and personal care products or most foods in the diet), then exposure is well represented by the Total Population. However, if exposure is due to a small number of products or is due to an infrequently occurring substance, then the Exposed Population is likely the more appropriate set to use. This is so that there is not inappropriate dilution of exposure statistics by the inclusion of large number of zero values in their calculation.

Then the population daily exposure (PE) can be written as:

$$PE = Stat(E_1, E_2, \dots, E_N) \quad (4)$$

where E_j is the daily exposure for individual j , and the $Stat()$ function is typically something like the 95th percentile.

The main differences in the values of the model parameters that were used in the three scenarios are summarized in Table 18\$.

Table 18\$: Summary of the differences in value of the main model parameters used in the three scenarios

Table 18: Summary of the differences in value of the main model parameters used in the three scenarios.

	Product as labelled in the model	Conservative, screening analysis based on single value estimates at P95 for cosmetics		Probabilistic model		
		Scenario 1 Max Concentration	Scenario 1 Occurrence	Scenarios 2 & 3 Concentration	Scenario 2 Occurrence	Scenario 3 Occurrence
1	AfterShave	2.150%	100%	Distribution (see Table 6)	100%	30%
2	BarSoap	0.070%				35%
3	BodyLotion	3.810%				30%
4	BodySpray	1.180%				5%
5	DeoRollOn – Stick	7.730%				75%
6	DeoRollOn – RollOn	5.630%				95%
7	DeoRollOn – Gel	6.180%				100%
8	DeoRollOn – Wipes	0.000%				80%
9	DeoSpray – AP	3.240%				90%
10	DeoSpray – Pump	4.876%				100%
11	EaudeParfum, EaudeToilette	0.050%				40%
12	EyeShadow	43.310%				100%
13	FaceMoisturizer	10.590%				35%
14	HairSpray	0.000%				5%
15	HairStyling	1.190%				15%
16	HandCream	0.860%				30%
17	Lipstick	14.620%				90%
18	LiquidHandSoap	0.000%				50%
19	LiquMakeupFoundation	6.590%				95%
20	Mascara	3.130%				35%
21	Mouthwash	0.000%				60%
22	RinseoffConditioner	7.140%				35%
23	Shampoo	7.140%				40%
24	Showergel	0.890%				50%
25	Toothpaste	3.18%				55%
26	EyeLiner	15.760%				60%
27	MakeupRemover	10.590%				25%
28	Food and drinking water	see Table 10	100%			100%
29	Antacids	see Table 12	100%			100%

Deterministic calculation for Scenario 1:

Based on point estimates of each parameter of the model, the exposure was calculated by multiplying the factors as in equation (1) above for each product individually; however, the calculation cannot be carried out at subject level but only at population level.

The point estimates of the different factors are worst-case estimates (upper percentiles of the value distributions). Creme Global, in accordance with the Aluminium Consortium, decided to use as default values for the model parameters:

the maximum concentration value of aluminium in each product category

- the 95th percentiles of the value distributions retrieved from the above-mentioned surveys and databases for all the other parameters.
- set the aluminium occurrence equal to 100% for each product category.

1 To calculate the systemic exposure in a deterministic model, a basic method is to simply
2 sum up the population exposures from the individual products as:
3

$$SE = Stat(E_1) + Stat(E_2) + \dots + Stat(E_M) \quad (5)$$

4
5
6 where the Stat(E_i) terms are the population exposure statistics for a single product E_i. The
7 expectation here was that the sum of these statistics would provide a good estimate of the
8 corresponding statistic for the aggregate statistic for the systemic exposure. However, for
9 most statistics, there is little mathematical reason to suggest that this summation would be
10 a good estimate.
11

12
13 The model analysed the exposure to aluminium as a chemical from each formulation, by
14 taking into consideration all ingredients which might contribute aluminium to the
15 formulation, thus removing the dependence on the source of the ingredient. Information
16 about the exposure to each Al-ingredient was stored by the model but not analysed.
17 Equation (1) was used to estimate the dermal and oral exposure levels.

18 The oral route is applicable to lipsticks and toothpaste products which can be ingested. In
19 this case, the Xi factor corresponds to the oral bioavailability factor.
20
21

Table 13: Summary statistics of the exposure to Al from the use of cosmetics per route of exposure for the three different scenarios provided by the Applicant

Route	Statistics	Exposed Population			Total Population		
		Scenario 1	Scenario 2	Scenario 3	Scenario 1	Scenario 2	Scenario 3
Dermal	Count	104780	104780	101364	104836	104836	104836
	Mean*	0.01657	0.002565	0.001727	0.01656	0.002562	0.001663
	P50*	0.01004	0.001087	0.0004282	0.01003	0.001085	0.0003614
	P95*	0.0529	0.0102	0.007553	0.05283	0.01019	0.007370
Ingestion	Count	95760	95760	61064	104836	104836	104836
	Mean*	0.03174	0.0006820	0.0006595	0.02861	0.0006146	0.0003831
	P50*	0.02617	0.0002215	0.0001798	0.02342	0.0001711	2.65e-05
	P95*	0.07635	0.002450	0.002386	0.07404	0.002314	0.001617
Inhalation Non-Respirable	Count	73328	73328	48984	104836	104836	104836
	Mean*	0.002787	0.000932	0.001324	0.00192	0.0006423	0.0005856
	P50*	5.4e-06	3.80e-06	0.0002713	2.3e-06	1.1e-06	0.0
	P95*	0.013054	0.004840	0.005737	0.01089	0.003906	0.003656
Inhalation Respirable	Count	73328	73328	48984	104836	104836	104836
	Mean*	0.019	0.006355	0.008920	0.013084	0.004383	0.003944
	P50*	3.4e-06	6.14e-06	0.0007796	1.43e-06	8.0e-07	0.0
	P95*	0.09735	0.03445	0.04123	0.0804	0.02746	0.02528
Total Systemic (Combined Oral, Dermal & Inhalation)	Count	104780	104780	101413	104836	104836	104836
	Mean*	0.06024	0.00821	0.006820	0.06018	0.008200	0.006577
	P50*	0.04486	0.002665	0.001384	0.0448	0.002659	0.001219
	P95*	0.1671	0.03647	0.03317	0.1671	0.03647	0.03238

* Units for Mean, P50 and P95 are µg/kg bw/day.

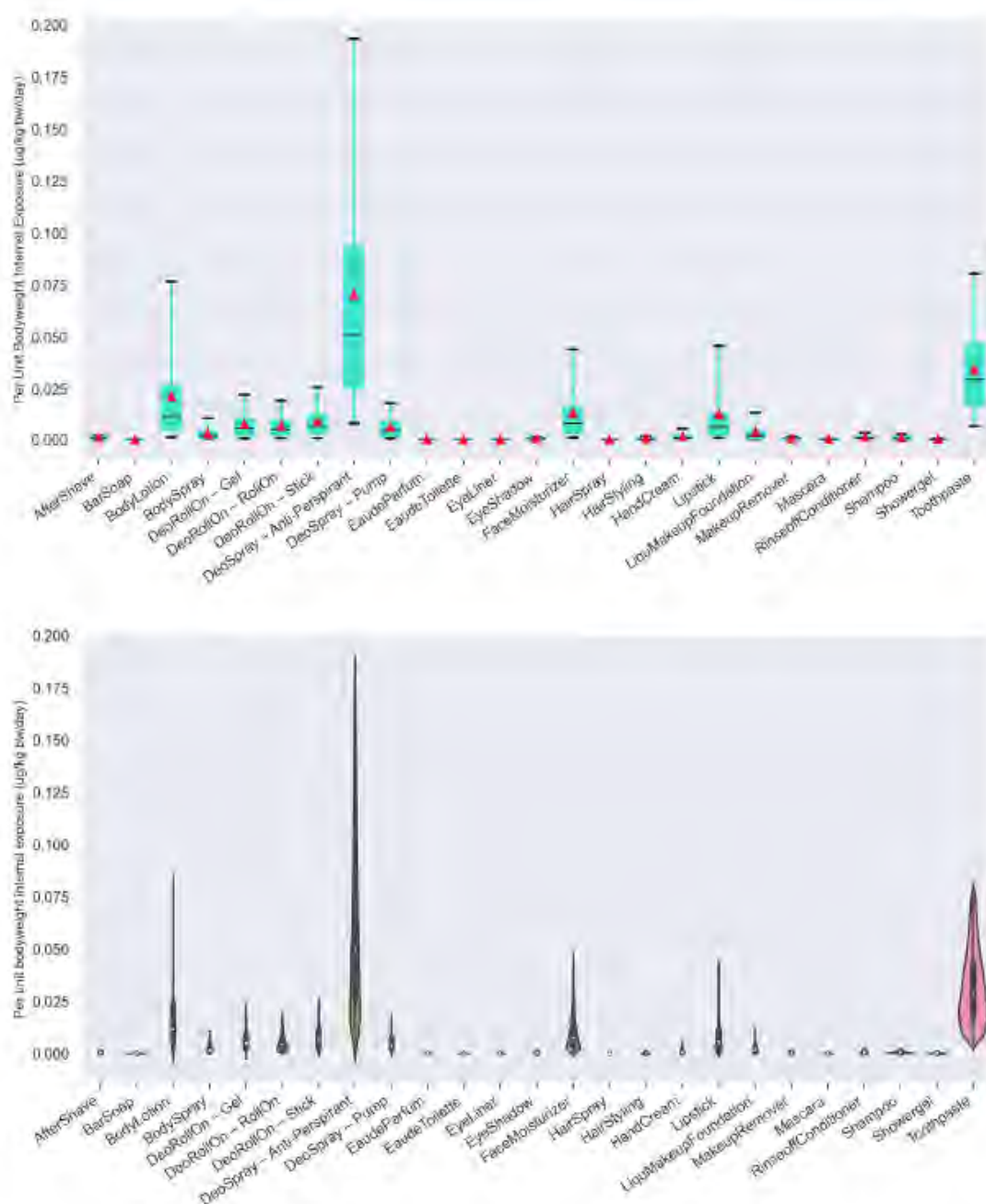


Figure 1: Distribution of internal exposure levels per cosmetic product, scenario 1

In a previous dossier (Cosmetics Europe, 2016), the Applicant had provided a deterministic 2Box-Model calculation for the systemic body burden of aluminium via the lung. When aluminium chlorohydrate was used in spray/aerosol antiperspirants, a 2-box exposure model was used. The 2-Box model calculation was performed using the input parameters in Table 14.

Table 14: Parameter values used for the calculation of lung exposure in the 2-Box model (Cosmetics Europe, 2016)

		Reference
Distribution volume at time point 1 (V1)	2 m ³	Rothe <i>et al.</i> , 2011
Exposure time 1 (t1)	2 min	Rothe <i>et al.</i> , 2011
Distribution volume at time point 2 (V2)	10 m ³	Rothe <i>et al.</i> , 2011
Exposure time 2 (t2)	10 min	Rothe <i>et al.</i> , 2011
Human breath minute volume (BR)	13.1 l/min	Finley <i>et al.</i> , 1994; Salem & Katz, 2006
Mean human body weight (BW)	60 kg	SCCS (2015), 9th notes of guidance
Airborne Fraction (AF)*	76.5%	Steiling <i>et al.</i> , 2012
Respirable Fraction (<10 µm) (RF)	20%	based on particle size measurements (unpublished company data) The cut-off value for droplets/particles reaching the deeper lung is set as <10 µm - SCCS (2015), 9th notes of guidance
Non-Respirable fraction (NRF)	80%	The inhaled Al that is trapped in the upper respiratory tract (and does not reach the deeper lung) is also considered
Lung deposition rate	20%	Lippmann (1977)
Gas exchange factor	75%	Rothe <i>et al.</i> , 2011
Concentration of Al in spray formulation incl. propellant (C)	2.86%	Maximum value reported by industry
Amount sprayed per day (A)	6.1 g	Hall <i>et al.</i> , 2007 (6.1 g is the 90 th percentile value of daily deo/ap spray usage (including the propellant and solvent))
Bioavailability of Al from alveoli	3%	DeVoto & Yokel, 1994
Bioavailability of Al from upper airways	0.3%	EFSA J (754), 1-34, 2008 (typical value for oral bioavailability)

* It should be noted that ACH is not a volatile substance and the fraction that is present on the skin would not become volatile and be available for inhalation.

Calculation of the substance amount relevant for exposure (EA)

The amount of Al that will be available for inhalation after spraying is calculated as follows:

$$EA [g] = A [g] \times C [\%] \times AF [\%] \times RF [\%] / NRF [\%]$$

The Applicant further calculated the systemic exposure dose for aluminium upon inhalation as follows:

For the respirable fraction:

$$EA [g] = A [g] \times C [\%] \times AF [\%] \times RF [\%]$$

$$EA_{resp} = 6.1 \text{ g} \times 0.0286 \times 0.765 \times 0.2 = 0.02669 \text{ g}$$

For the non-respirable fraction:

$$EA [g] = A [g] \times C [\%] \times AF [\%] \times NRF [\%]$$

EA non-resp = $6.1 \text{ g} \times 0.0286 \times 0.765 \times 0.8 = 0.10677 \text{ g}$

Calculation of the Exposure Dose (ED)

The inhaled amounts (IM) at time point 1 and 2 (IM 1/2, 2/10 min) are calculated with the following formula:

$$\text{IM } 1/2 \text{ [mg]} = (\text{EA [mg]} / \text{V}1/2 \text{ [l]}) \times \text{BR [l/min]} \times \text{t}1/2 \text{ [min]}$$

For the respirable fraction:

$$\text{first 2 minutes: IM 1 [mg]} = (26.69 / 2000) \times 13.1 \times 2 = 0.350$$

$$\text{following 10 minutes: IM 2 [mg]} = (26.69 / 10000) \times 13.1 \times 10 = 0.350$$

For the non-respirable fraction:

$$\text{first 2 minutes: IM 1 [mg]} = (106.77 / 2000) \times 13.1 \times 2 = 1.399$$

$$\text{following 10 minutes: IM 2 [mg]} = (106.77 / 10000) \times 13.1 \times 10 = 1.399$$

The ED is then calculated as follows:

$$\text{ED(inhal)} \text{ [mg/kg bw/d]} = (\text{IM 1} + \text{IM 2 mg}) / \text{BW [kg]}$$

$$\text{ED(resp)} \text{ [mg/kg bw/d]} = (0.350 + 0.350) / 60 = 0.01167$$

$$\text{ED(non-resp)} \text{ [mg/kg bw/d]} = (1.399 + 1.399) / 60 = 0.04663$$

$$\text{ED(total)} \text{ [mg/kg bw/d]} = 0.01167 + 0.04663 = 0.0583$$

Thus, the total amount of Al exposure via inhalation within 12 minutes after spraying of an antiperspirant spray in a 10 m³ room according to this calculation is 0.0583 mg/kg bw/day.

Calculation of the Deposited Dose (DD)

Of the inhaled amounts of Al, only part will actually be deposited in the respiratory tract. Mean lung deposition is considered to be 20% of the respirable dose (Lippmann, 1977), whereas the remaining 80% will be available in the upper airways. Deposition in the upper respiratory tract amounts to approximately 75%, whereas the remaining 25% will be exhaled (Rothe et al., 2011, European Commission, 1996).

The deposited amounts of Al in the lung and in the upper respiratory tract are calculated as follows:

$$\text{DD(lung)} = \text{ED(resp)} \times 0.2$$

$$\text{DD(upper tract)} = (\text{ED(resp)} \times 0.8 \times 0.75) + (\text{ED(non-resp)} \times 0.75)$$

$$\text{DD(lung)} \text{ [}\mu\text{g/kg bw/d]} = 11.67 \times 0.2 = 2.334$$

$$\text{DD(upper tract)} \text{ [}\mu\text{g/kg bw/d]} = (11.67 \times 0.8 \times 0.75) + (46.63 \times 0.75) = 41.975$$

Calculation of the systemic exposure dose (SED) of aluminium upon inhalation

In order to estimate the systemically available dose of aluminium upon inhalation, bioavailability of aluminium from the deep lung (alveoli) and from the upper respiratory tract have to be taken into account separately. DeVoto & Yokel reported a lung bioavailability of aluminium of 3% (DeVoto & Yokel, 1994).

From the upper respiratory tract, no data on bioavailability is available, but since mucociliary clearance will lead to expectorate or swallowing of the Al trapped in that part, intestinal resorption values can be used (0.3%, from EFSA, 2008).

Table 15: Calculation of respirable and non-respirable fraction using the 2-Box model

	Modelled deposition [µg/kg bw/d]	Systemically available (3% lung, 0.3% upper airways) SED [µg/kg bw/d]
Deep lung	2.334	0.070
Upper respiratory tract	41.975	0.126
Total	44.309	0.196

According to the above calculation in Table 15, the total systemically available dose of aluminium through use of an antiperspirant spray would be 0.196 µg/kg bw/d (inhalation route only).

SCCS comment

Of the presented scenarios, only Scenario 1 can be accepted according to the SCCS Notes of guidance, because Scenarios 2 and 3 use distributions for the concentrations in products and not the maximal levels. Scenario 3 in addition uses occurrence data that can be change over time.

Since the inhalation adjustment factors used for deriving the inhalation estimates are not considered valid by the SCCS, the exposure estimates for products that are relevant for inhalation (i.e. DeoSpray-Antiperspirant, DeoSpray-Pump, EAUdeParfum, EAUdeToilette, HairSpray) are considered not valid, either.

The deterministic 2Box-model calculation provided with the 2016-Dossier by the Applicant is in principle valid but uses an adjustment factor for inhalation based on Steiling et al., 2012 that is not considered appropriate by the SCCS.

3.2.4.7 Aggregate exposure with food and antacids

According to the Applicant, concentrations of aluminium in food were defined based on the 2006 UK Total Diet Study (Rose, et al., 2010) which outlines aluminium concentration levels in 20 food groups, based on measurements. The 20 food groups were defined from the British Total Diet Study for Monitoring Food Constituents from 1981 (Peattie, 1983). Drinking water is the only food group not studied in Peattie, 1983. The aluminium concentration value of drinking water has been retrieved from the EU Drinking Water Directive 98/83/EC of 3 November 1985, which set a concentration limit of 0.2 mg/L. The WHO Drinking Water Guidelines (WHO, 2011) explain that it is reasonable to expect municipal drinking water treatment plants to contain between 0.1-0.2 mg/L. Estimates of aluminium in foods are for the element itself rather than an Al-ingredient, so a breakdown by Al-ingredient was not required.

According to the Applicant, the concentrations of aluminium in antacids are drawn from Micromedex data (Webb, 2000) and individual product websites. The Al-amount in one dosing ranges from 48.5 – 207 mg for different products.

Frequency of use and co-use in food was taken from the Dutch (DNFCS), Irish (NSIFCS) and UK (NDNS) national dietary surveys. The NSIFCS and NDNS surveys are 7-day semi-weighted food diaries and the DNFCS comprises 2 non-consecutive 24-hour diaries. Co-use of foods is automatically taken care of in the food consumption diaries.

Frequency of use data for antacids was not identified for European consumers and so US data was used as a proxy. Frequency of use data of antacids comes from a 30-day weighted US National Health and Nutrition Examination Survey (NHANES) 2007-2008 survey on supplement use. The results are based on a representative sample of 10,537 people in the US. Antacids are independent of all other products because the model does not assume any correlation between the consumption of antacids and the consumption of foods and cosmetics. Also, not all the subjects were considered as antacid-consumers by the model; only those subjects randomly matched with antacid-consumer subjects from the NHANES survey were considered as such. Hence, it is useful to contrast exposure estimates for the general population, which models less than 5% of consumers using antacids, and the 'exposed population' which assumes that antacid use is 100%.

The data of aluminium concentrations in food and drinking water are presented for aluminium as a standalone chemical not bounded to any specific compound, while antacids may contain different Al-ingredients. To make sure that the results obtained from all the different sources are comparable, the analyses must be carried out on the final concentrations of aluminium in every media.

The calculation of the exposure levels related to foods and antacids follows the same process previously described for cosmetics. The only difference is in the equation to calculate the exposure levels; exposure from foods and antacids must take in account other factors so equation (1) is to be substituted with two proper equations.

The aluminium exposure levels from food are calculated as

$$E_{ij} = \frac{F_{ij} \times A_{ij} \times X \times C_{ij}}{n \times BW} \quad (6)$$

Where each term on the right-hand side can be a fixed estimate or drawn from a distribution

and each term is understood as follows:

E_{ij} is the systemic/internal exposure to aluminium from food group i [$\mu\text{g}/\text{kg}/\text{day}$]

F_{ij} is the frequency of consumption events of food group i [day⁻¹] during the survey of length n days

A_{ij} is the amount consumed at each consumption event of food group i [μg]

X is the oral bioavailability factor [%]

C_{ij} is the concentration of aluminium in food group i [unitless]

BW is the consumer's bodyweight [kg]

A food group is one of those listed in Table 10.

The aluminium exposure levels from antacids are calculated as:

$$E_{ij} = \frac{F_{ij} \times A_{ij} \times X \times C_{ij} \times P_{ij}}{30 \times BW} \quad (7)$$

Where each term on the right-hand side can be a fixed estimate or drawn from a distribution and each term is understood as follows:

E_{ij} is the systemic/internal exposure to aluminium from antacid of brand i [$\mu\text{g}/\text{kg}/\text{day}$]

F_{ij} is the frequency of consumption events of antacid of brand i [day⁻¹] during the survey of length 30 days

A_{ij} is the recommended serving size of antacid of brand i [μg]

X is the oral bioavailability factor [%]

P_{ij} is the occurrence of aluminium in antacid of brand i [unitless]

Cij is the concentration of aluminium in antacid i [unitless]

BW is the consumer's bodyweight [kg]

Table 16: Summary statistics of the internal exposure to Al per route for Scenario 1

Route	Statistics	Exposed Population				
		Ingestion	Dermal	Inhalation Respirable	Inhalation Non-Respirable	Total
All Ingested Al**	Count	104836	0	0	0	104836
	Mean*	0.1175	0.0000	0.0000	0.0000	0.1175
	P95*	0.1966	0.0000	0.0000	0.0000	0.1966
Antacids	Count	4264	0	0	0	4264
	Mean*	0.2341	0.0000	0.0000	0.0000	0.2341
	P95*	1.0391	0.0000	0.0000	0.0000	1.0391
Cosmetics	Count	95760	104780	73328	73328	104780
	Mean*	0.03174	0.01657	0.01899	0.002786	0.06023
	P95*	0.07636	0.05288	0.09733	0.01306	0.16715
Food	Count	104836	0	0	0	104836
	Mean*	0.0749	0.0000	0.0000	0.0000	0.0749
	P95*	0.1253	0.0000	0.0000	0.0000	0.1253

* Units for Mean and P95 are µg/kg bw/day.

** "All Ingested Al" is the combination of exposure from food, from antacids and from ingested cosmetics.

Conclusions by the Applicant:

- Food is the principal overall source of exposure to aluminium for the majority of the population.
- The population cohort taking antacids have the highest exposure to aluminium.
- Probabilistic estimates (Scenario 2 and 3) indicate that cosmetics contribute no more than 7-9% of mean aggregate exposures to aluminium, and 20-23% of P95 aggregate exposures, suggesting cosmetics are a minor source of aggregate consumer exposure.
- While the output of this exposure estimate suggests that inhalation exposure (primarily of aerosol deodorants) appears to be the main route of exposure to aluminium from cosmetics, the 2-Box model methodology is very conservative and overestimates inhalation exposure. There is scope to further refine this estimate with experimental measurements if needed.
- Overall, the margin of safety relative to the point of departure identified by the SCCS is sufficiently protective (4900 for Scenario 2, P95).
- In the context of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) tolerable weekly intake (TWI) of 2 mg/kg bw/week, which is equivalent to an internal systemic exposure of 1.8 µg/kg bw/day, total consumer exposure represents a small contribution to the tolerable intake, with cosmetics making a minor contribution of less than 0.5% (mean) and 2% (95th percentile) of the tolerable intake.

SCCS comment

The SCCS notes that food may contribute to the aggregate exposure to aluminium in the same order of magnitude as cosmetics, when deterministic estimates are compared across the sectors food, pharmaceuticals and cosmetics. Pharmaceuticals such as antacids may contribute to a higher amount but are consumed by only a small fraction of the population under different risk-benefit considerations.

3.3 TOXICOLOGICAL EVALUATION

The data related to toxicological evaluation were assessed and commented upon by the SCCS in the previous Opinion (SCCS/1613/19). Only SCCS' comments and main conclusions are included in this section.

General toxicity

The toxicological evaluation is focused on the toxicity of aluminium compounds relevant to the risk assessment of cosmetic ingredients containing aluminium. There is an extensive body of literature on the health effects and toxicity of aluminium; a number of extensive reviews and authoritative evaluations were published before 2014 (WHO IPCS 1997; Krewski et al., 2007; ATSDR, 2008; EFSA, 2008; FAO/WHO JECFA 2007; Environment Canada & Health Canada 2010; AFSSAPS 2011; FAO/WHO JECFA, 2012; VKM 2013; Willhite et al., 2014). For the 2017 SCHEER Opinion on aluminium in toys, a literature search covering the period from 01/01/2008 until 31/01/2017 was performed. The evaluation by JECFA (2011) was based on new data which included a developmental toxicity study specifically evaluating neurobehavioural endpoints (Poirier et al., 2011). The LOAELs identified in these studies were consistent with the body of data reviewed previously by other committees; however, the oral developmental toxicity study in rats provided a suitable and robust NOAEL for risk assessment (30 mg/kg bw/day). By applying the standard uncertainty factor of 100 to this NOAEL and considering the bioavailability of aluminium citrate, the JECFA considered it appropriate to revise the PTWI (provisional tolerable weekly intake) upward to 2 mg/kg bw/week. This new data by the JECFA Committee therefore supersedes its earlier Opinions in 2008 and does not contradict the 2008 EFSA Opinion. The SCCS agrees on the NOAEL of 30 mg/kg bw/day used by JECFA for risk assessment.

Irritation/sensitisation

Local dermal effects have been observed when aluminium compounds (10% [w/v] chloride, nitrate) have been applied to the skin of mice, rabbits and pigs over five-day periods (once per day) including epidermal damage, hyperkeratosis, acanthosis and microabscesses (Lansdown, 1973). In this study, these effects were not observed with aluminium acetate, hydroxide or chlorohydrate compounds (SCCS/1626/20 Final version Addendum to the scientific opinion SCCS/1613/19 on the safety of aluminium in cosmetic products (lipstick) - Submission II).

Aluminium compounds are widely used in antiperspirants without acute harmful effects to the skin. Some people, however, may be unusually sensitive to topically-applied aluminium compounds. Skin irritation has been reported in human subjects following the application of aluminium chloride hexahydrate in ethanol used in a high-dose (20% ACH) formulation for the treatment of axillary or palmar hyperhidrosis (excessive sweating) (Ellis and Scurr, 1979; Goh, 1990; Reisfeld & Berliner, 2008) and after use of a crystal deodorant containing alum (Gallego et al., 1999).

Although some high-strength antiperspirants used in hyperhidrosis treatments, using aluminium chloride, have been associated with irritation of the axilla, the long history of cosmetic antiperspirant use would suggest that irritation of the axilla is uncommon. There are several examples of cosmetic product formulations that include raw materials that are irritant in isolation, yet acceptable amongst consumers (e.g. surfactants, menthol).

With respect to skin sensitisation, the SCCS agrees that the available animal studies show that aluminium compounds used in antiperspirants are not skin sensitising. There is limited evidence that aluminium compounds can cause contact allergy in humans. However, taking into account the widespread use of these compounds, the SCCS considers this to be a rare phenomenon.

Mutagenicity/Genotoxicity

The most commonly reported mode of genotoxic action is induction of oxidative stress by aluminium ions. The other suggested MoA is inhibition by Al ions of proteins involved in mitotic spindle function. Hence, an existence of a threshold mechanism for Al ions can be assumed. Considering all the data, the SCCS is of the opinion that under the scenarios of dermal exposure in cosmetics, aluminium is not likely to pose a risk of genotoxic effects. The SCCS is aware of the request addressed by ECHA for a combined *in vivo* mammalian erythrocyte micronucleus test and an *in vivo* mammalian Comet assay with additional specific investigation on oxidative DNA damage in rats by oral route, using aluminium sulphate.

Carcinogenicity

Carcinogenicity studies in animals have been reviewed by the SCCS and were summarised in the Annex of the previous Opinion ((SCCS/1525/14, Revision of 18 June 2014). There was no indication of carcinogenicity at high dietary doses (up to 850 mg Al/kg bw/day) in animal studies, and the SCCS considers that carcinogenicity is not expected at exposure levels that are achieved via cosmetic use.

The new submission comprised only a new exposure assessment, not a new toxicological evaluation. Therefore, only the study selected previously for the safety assessment (Poirier et al, 2011) is reported here in detail, all other studies are summarised and discussed in preceding Opinions, notably in SCCS/1613/19. Only the final SCCS statement taken from discussion of the previous Opinion is reported here for transparency.

Fertility and reproduction toxicity

Poirier et al., 2011, reported a 12-month neuro-developmental toxicity study of aluminium citrate. The study in Sprague-Dawley rats was conducted according to a double-blind, vehicle-controlled randomised design by exposing offspring to aluminium citrate in-utero, through lactation, and then via drinking water post-weaning. The study was conducted according to Good Laboratory Practice (GLP) and was conducted to distinguish between cumulative neurodegenerative and cognitive changes from aberrant neural development alterations. Three dose levels were used: 30, 100, 300 mg Al/kg bw/day, in addition to control groups that received either water or a sodium citrate solution (27.2 g/L) compared to 27.2 g sodium citrate/L in the control group. Aluminium citrate was selected for the study since it is the most soluble and bioavailable aluminium salt. It is also the salt which is likely to be formed readily in the body when absorbed aluminium reacts with endogenous citrate. Pregnant dams (n=20 per group) were exposed to aluminium citrate from gestational day 6 through lactation, and then the offspring (n = 80 per group) were exposed post-weaning until postnatal day 364.

Aluminium citrate was generally well tolerated in the dams at all doses, except the high dose (300 mg Al/kg bw/day) where diarrhoea occurred in 8 of the treated dams. In high-

1 dosed pups, the main toxic effects were observed in the urinary tract (damage and the
2 formation of calculi (chalky secretions blocking the urinary tract)), resulting in high
3 mortality in the male offspring (see Table 3 below). This caused a differential response in
4 female and male pups. High-dose males were euthanised on study day 98 because of
5 excessive clinical signs (including weight loss, diarrhoea, mild dehydration and poor hair
6 coat).

7
8 In the same study, Poirier also evaluated the relative distribution of aluminium following
9 repeated oral administration of various aluminium salts. Sprague–Dawley rats (n= 5 per sex
10 per group) were orally gavaged with formulations of aluminium citrate, sulphate, nitrate,
11 chloride and hydroxide, each delivering a dosage of 30 mg/kg body weight aluminium.
12 Control animals were similarly dosed with deionised water. Animals were dosed daily for
13 either 7 days or 14 days, followed by blood and organ collection. The distribution and
14 concentrations of aluminium present in different tissues and organs, were measured by
15 ICPMass Spectrometry. From this analysis, concentrations in the blood were much lower
16 than those that distributed heterogeneously into other tissues and organs, in both females
17 and males. The authors state that 'of the few significant differences, concentrations of
18 aluminium were highest for the aluminium citrate treatment.' The authors further conclude
19 from their data that 'bioavailability of the three Al salts (chloride, sulfate and nitrate) and
20 the Al hydroxide looks much lower than that of the Al citrate'.

21
22 However, as ^{26}Al was not used as a tracer, it is not possible to know the absolute oral
23 bioavailability of the administered dose.
24

SCCS comment

Based on the results of this neurodevelopmental toxicity study, the SCCS derives a NOAEL of 30 mg/kg bw/day, which will be used for MoS calculation. This is in line with SCHEER (2017), where the same NOAEL from the same study was used to derive migration limits for Al in toys.

Furthermore, on the basis of available information on solubility (Annex 1 of SCCS/1613/19), the SCCS agrees that aluminium citrate can be regarded as the most bioavailable of the Al salts assessed in this Opinion. Therefore, the derived NOAEL can be regarded as a worst case for all aluminium salts discussed in this Opinion.

Human data

The SCCS considers that aluminium is a known neurotoxicant in animals. Circumstantial evidence has linked this metal with several neurodegenerative disorders, like Alzheimer's disease (Miu and Benga, 2006; Percy et al., 2011), Parkinson's diseases (Oyanagi, 2005) and other chronic neurodegenerative diseases (Bondy, 2010), but no causal relationship has yet been proven.

3.4 SAFETY EVALUATION (including calculation of the MoS)

The new submission comprised only a new exposure assessment, not a new toxicological evaluation. Therefore, the toxicological assessment, selected NOAEL and point of departure derived in SCCS/1613/19 remain valid.

Based on the results of the neurodevelopmental toxicity study on rats with aluminium citrate (Poirier et al., 2011), the SCCS derived a NOAEL of 30 mg aluminium citrate/kg bw/day. After adjustment for the rat oral bioavailability (0.6%) of aluminium citrate (Poirier et al., 2011, Zhou et al., 2008), the systemic exposure at the NOAEL is estimated to be **180 µg Al/kg bw/day**. This value is used as a point of departure for the safety assessment and MoS calculation.

The provided exposure report included an aggregate exposure assessment of all Cosmetics categories containing aluminium at maximum levels (Scenario 1). The respective aggregate estimate is considered valid as aggregate value for all assessed product categories, for which the main exposure routes are dermal and oral exposure. The deterministic assessment to aluminium in sunscreens needs to be added to this aggregate assessment.

Some assumptions regarding inhalation exposure used in the aggregate assessment are considered as not valid for a conservative exposure calculation by the SCCS. Therefore, the exposure calculations cannot be used for the product categories for which inhalation exposure is relevant (i.e. deodorant spray-antiperspirant, deodorant spray-pump, Eau de Toilette, Eau de Parfum, hairspray).

For deodorant spray-antiperspirant, calculations and experimental values had been submitted by the Applicant before (SCCS/1613/19). Upon new evaluation by the SCCS, the experimental values from the study by Meech *et al.* (2011) cannot be accepted for risk assessment, because the ventilation rates used were too high. In the 2Box-model-calculation, a conservative approach had been followed with the assumption of no ventilation, so that these can on principle be used for risk assessment. This calculation, however, was also based on adjusting inhalation exposure with a factor based on P90 for dermal load from Steiling et al., 2014. Since this factor enters linearly into the calculations, adjustment is easily possible, so that the SCCS corrected for that and took the resulting

estimate over to the aggregate exposure calculation. Under the assumption that deodorant spray-antiperspirant results in equal or higher exposure than deodorant spray-pump, and that only one of them is used at a time, these calculations also represent a conservative assessment for deodorant spray-pump.

Table 17: Adjustment of the value for systemically available aluminium from deodorant spray-antiperspirant

	Systemically available inhal. ($\mu\text{g/kg bw/day}$)	Reference
2-Box model, 76.5% availability	0.196	Dossier 2016
2-Box model, 100% availability	0.256	adjusted value

For the other product categories relevant for inhalation (Eau de Toilette, Eau de Parfum, hairspray) no valid inhalation exposure assessment is available. Therefore, these product categories are excluded from the opinion.

Table 18: Calculation of aggregate exposure and MoS for aluminium-containing cosmetics with the exception of Eau de Toilette, Eau de Parfum, hairspray

Product type	Systemic Exposure (internal dose) $\mu\text{g Al/kg bw/day}$	Remarks	MOS based on POD of $180 \mu\text{g Al/kg bw/day}$
Dermal from exposure report	0.053	P95, exposed population, Table 13	3403
Dermal sunscreen, deterministic	0.484	Deterministic calculation, max. content, NoG	372
Oral from exposure report	0.076	P95, exposed population, Table 13	2358
Inhalation from antiperspirant sprays/aerosols	0.256	Adjusted from 2-Box calculation	703
Aggregation over routes	0.869		207

SCCS conclusion

Under the assumption that approximately 20% of the spray particles are $<10 \mu\text{m}$ for aerosol spray products, the SCCS considers that Al is safe for use in antiperspirant products (spray and non-spray) and all other product categories, except for spray applications other than antiperspirant.

3.5 DISCUSSION

Physicochemical properties

Physicochemical properties of aluminium compounds that can generally be used as cosmetic ingredients have been summarised in Annex I of the previous SCCS Opinion (SCCS/1613/19). The new submission presents an industry survey that indicates the substances that are actually used and in what amounts. The INCI names and CAS numbers of these substances are given in Tables 2 and 3 of this Opinion. The respective physicochemical properties of the substances can be retrieved from Annex 1 of SCCS/1613/19.

On the basis of available information on solubility (Annex 1 of SCCS/1613/19), the SCCS agrees that aluminium citrate can be regarded as the most bioavailable of the Al salts assessed in this Opinion.

Exposure assessment & Toxicokinetics

The SCCS understands that the 'Al-concentrations' of Al-containing cosmetics ingredients mentioned in Tables 2 and 3 represent the Al-contents in terms of the stoichiometric ratios of Al in these ingredients.

It is unclear to the SCCS why a range of Al-contents ('Al-concentrations') in the Al-ingredient for some of the Al-ingredients may result from the defined stoichiometry of the chemical substance (e.g. for potassium aluminium sulphate in Table 3). However, the SCCS has calculated the Al-contents based on stoichiometry and found no underestimation of Al-content in the upper bound (max value). Therefore, risk assessment based on exposure calculations with these maximum values is considered valid.

Some products had been excluded from the aggregate exposure assessment (see Tables 5, 7) without explanation in the submitted report. Upon request, the Applicant has submitted the explanation and an additional deterministic assessment of sunscreens. The assessment using the maximum Al level possible is considered valid by the SCCS.

The SCCS considers that sunscreen products should be included in the assessment, because on a seasonal basis they are used regularly, even daily, and due to the high amount applied may present a large exposure source. Furthermore, the presence probability of aluminium in sunscreens is 30%, which cannot be considered negligible.

Some of the products that have not been considered may also be used regularly (shaving products, eyeliner, eye contour products and lip care products). However, for these products the tonnage of Al-containing products sold is very low, and therefore their contribution to dermal exposure can be considered as negligible.

Scenario 1 can be accepted according to the SCCS Notes of guidance, but not Scenarios 2 and 3, because these use distributions for the concentrations in products and not the maximal levels. Scenario 3 in addition uses occurrence data that can be specific in time.

Since the inhalation adjustment factors used for deriving the inhalation estimates are not considered valid by the SCCS, the exposure estimates for products that are relevant for inhalation (i.e. deodorant spray-antiperspirant, deodorant spray-pump, Eau de Parfum, Eau de Toilette, hairspray) are considered not valid, either.

The deterministic 2Box-model calculation provided with the 2016-Dossier by the Applicant is on principle valid but uses an adjustment factor for inhalation based on Steiling et al., 2012

1 that is not considered appropriate by the SCCS. Therefore, the respective inhalation
2 exposure was recalculated by the SCCS.

3
4 The SCCS notes that food may contribute to the aggregate exposure to aluminium in the
5 same order of magnitude as cosmetics, when deterministic estimates are compared across
6 the sectors food, pharmaceuticals and cosmetics. Pharmaceuticals such as antacids may
7 contribute more but are consumed by only a small fraction of the population under different
8 risk-benefit considerations.

9
10 ***Toxicological Evaluation***

11
12 Based on the results of the neurodevelopmental toxicity study, the SCCS derives a NOAEL
13 of 30 mg/kg bw/day, which will be used for MoS calculation. This is in line with SCHEER
14 (2017), where the same NOAEL from the same study was used to derive migration limits for
15 Al in toys.
16
17

4. CONCLUSION

1. *In light of the new data provided, does the SCCS consider Aluminium compounds safe when used in cosmetic products other than deodorants, antiperspirants, lipsticks and toothpastes? In the event that the estimated exposure to Aluminium from cosmetic products is found to be of concern, SCCS is asked to recommend safe concentration limits for each category.*

The SCCS considers that aluminium compounds are safe when used

- in non-sprayable product categories at the maximum levels indicated in Table 4; and
- in sprayable antiperspirant products, provided that the maximum percentage of particles with 10 µm diameter does not exceed 20% of the total aerosolised particles

The SCCS could not assess safety of aluminium compounds in sprayable applications other than antiperspirant. These applications (including eau de toilette, eau de parfum and hair spray) are therefore excluded from this Opinion.

2. *Does the SCCS have any further scientific concerns regarding the use of relevant Aluminium compounds in cosmetic products taking into account the newly submitted information on aggregate exposure to Aluminium from cosmetics, medicines (e.g. antacids) and food intake?*

As aluminium does not belong to substances classified as CMR 1A and B, only exposure from cosmetic uses was considered in this safety assessment. However, in a scenario provided by the Applicant, where exposure from non-cosmetic sources of aluminium (food and pharmaceuticals) was aggregated with exposure from cosmetics, food contributed in a similar order of magnitude as cosmetics. The aggregate exposure to aluminum from cosmetic and non-cosmetic sources may therefore exceed safe limits.

5. MINORITY OPINION

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6. REFERENCES

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7. GLOSSARY OF TERMS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181.

8. LIST OF ABBREVIATIONS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181.

Amended Safety Assessment of Naturally-Sourced Clays as Used in Cosmetics

Status: Final Amended Report
Release Date: March 31, 2023
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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. Previous Panel members involved in this assessment: Ronald C. Shank, Ph.D. and Daniel C. Liebler, PhD. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

ABBREVIATIONS

16HBE	human bronchial epithelial cell line 16HBE14o-
AICIS	Australian Industrial Chemicals Introduction Scheme
BAL	bronchoalveolar lavage
CCK-8	cell counting kit-8
cfu	colony forming units
cGMPs	current good manufacturing practices
CHO	Chinese hamster ovary
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
ECVAM	European Centre for the Validation of Alternative Methods
EU	European Union
FDA	Food and Drug Administration
β-GLUC	β-glucuronidase
GRAS	generally recognized as safe
HRIPT	human repeated insult patch test
IARC	International Agency for Research on Cancer
ICP-OES	inductively coupled plasma-optical emission spectrometry
IL-1	interleukin-1
LDH	lactate dehydrogenase
MDGF	macrophages-derived growth factor
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
mU	milli units
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OSHA	Occupational Safety and Health Administration
OTC	over-the-counter
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered saline
PEL	permissible exposure limit
PM _{2.5}	particulate matter with aerodynamic equivalent diameter of 2.5 µm or less
PMN	polymorphonuclear
REL	recommended exposure limit
t ₅₀	50% decrease of tissue viability
TG	test guideline
UDS	unscheduled DNA synthesis
UV	ultraviolet light
VCRP	Voluntary Cosmetic Registration Program
wINCI <i>Dictionary</i>	web-based International Cosmetic Ingredient Dictionary and Handbook

ABSTRACT

The Expert Panel for Cosmetic Safety (Panel) assessed the safety of 8 naturally-sourced clay ingredients, of which 6 were previously reviewed, as used in cosmetic formulations. All of these ingredients are reported to function in cosmetics as absorbents and bulking agents; other cosmetic functions are also reported. The Panel reviewed all relevant data and concluded that Kaolin is safe in cosmetics in the present practices of use and concentration described in this safety assessment. The remaining 7 naturally-sourced clay ingredients are safe in cosmetics in the present practices of use and concentration, with the exception that the available data are insufficient to make a determination that these ingredients are safe in products that may be incidentally inhaled.

INTRODUCTION

The Expert Panel for Cosmetic Ingredient Safety (Panel) previously reviewed the safety of 6 naturally-sourced clay ingredients in a report that was published in 2003.¹ At that time, the Panel concluded that these ingredients are safe as used in cosmetic products. In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 yr, and it has been at least 15 yr since this assessment has been issued. This report has been reopened to reassess the safety of the 6 clays included in that original report, and also includes 2 additional ingredients, i.e., Clay and Illite. In total, this report assesses the safety of 8 naturally-sourced clay ingredients (listed below) as used in cosmetics. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*; see Table 1), all of these ingredients are reported to function in cosmetics as absorbents and bulking agents; other cosmetic functions are also reported.²

Attapulgite*
Bentonite*
Clay
Fuller's Earth*

Hectorite*
Illite
Kaolin*
Montmorillonite*

**Previously reviewed by the Panel.*

The Panel has also reviewed related ingredients. In a report that was finalized in 2019, the Panel concluded that synthetically-manufactured amorphous silica and hydrated silica are safe in the present practices of use and concentration when formulated to be non-irritating.³ In 2021, the Panel concluded that silicate ingredients, including aluminum silicate and magnesium aluminum silicate, are safe in cosmetics in the present practices of use and concentration when formulated to be non-irritating, with the exception that the data are insufficient to make a determination that naturally-sourced (i.e., mined) silicate ingredients are safe for use in products that may be incidentally inhaled.⁴ (The reports on these ingredients are available on the Cosmetic Ingredient Review (CIR) website (<https://www.cir-safety.org/ingredients>). Although the clay ingredients comprise silica and/or silicates, silicates, synthetically-manufactured amorphous silica, and hydrated silica are neither part of this safety assessment, nor are data from those reports included in this assessment.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (<https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites>; <https://www.cir-safety.org/supplementaldoc/cir-report-format-outline>). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Excerpts from the summaries of the previous report on clay ingredients are disseminated throughout the text of this review document, as appropriate, and are identified by *italicized text*. (This information is not included in the tables or the summary section.)

CHEMISTRY

Definition and Structure

The definitions of Clay (CAS No. 53801-44-8) and the other clay ingredients included in this review are provided in Table 1.² These inorganic oxide ingredients, comprising in part silicon dioxide, are solids derived from naturally occurring minerals.

Clays in general have atomic lattices consisting of two structural units.¹ One unit consists of two sheets of closely packed oxygens or hydroxyls. Aluminum, iron, or magnesium atoms are embedded within these sheets in octahedral coordination, so that they are equidistant from the oxygen or hydroxyl groups. The second unit is composed of silica tetrahedrons. Assuming there are no distortions in each tetrahedron, a silicon atom is equidistant from four oxygens or hydroxyls, if needed to balance the structure, arranged in the form of a tetrahedron with a silicon atom in the center. The silica tetrahedral groups are arranged in a hexagonal network, which is repeated infinitely to form a sheet of composition $\text{Si}_4\text{O}_6(\text{OH})_4$. The tips of the tetrahedrons all point in the same direction and the bases are all in the same plane. Substantial distortion of these units occurs in order to fit into determined unit-cell dimensions of minerals.

Figure 1 depicts the general structure of clay ingredients. Clays are composed of magnesium, lithium, aluminum, and/or iron silicate sheets with various exchangeable cations. These sheet-like structures are in sharp contrast to the hexagonal crystalline structure of crystalline silica (e.g., quartz).

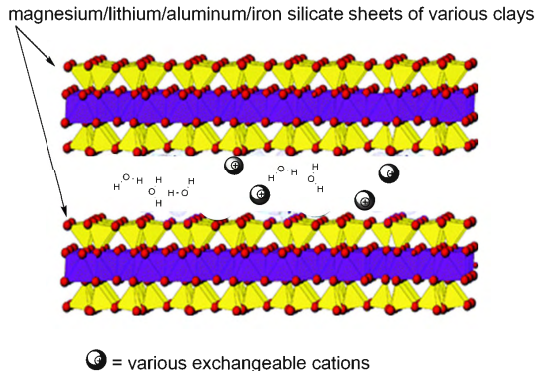


Figure 1. General structure of clay ingredients. CIR Staff

Attapulgite

The structurally important element is the amphibole double silica chain oriented with its long direction parallel to the *c*-axis.¹ Attapulgite consists of double silica chains situated parallel to the *c*-axis with the chains linked together through oxygens at their longitudinal edges. Tetrahedral apexes in successive chains point in the opposite direction. The linked chains form a kind of double-ribbed sheet with two rows of tetrahedral apexes at alternate intervals in the top and bottom of the sheets. The ribbed sheets are arranged so that the apex oxygens of successive sheets point together and are held together by aluminum and/or magnesium in octahedral coordination between the apex oxygens of successive sheets. Chains of water molecules run parallel to the *c*-axis and fill the interstices between the amphibole chains. Aluminum substitutions for silicon are considered probable.

Bentonite, Hectorite, and Montmorillonite

Bentonite, Hectorite, and Montmorillonite (also known as smectites) units comprise of two silica tetrahedral sheets with a central alumina octahedral sheet.¹ All tetrahedral tips point in the same direction and toward the center of the unit. The tips of the tetrahedrons of each silica sheet and one of the hydroxyl layers of the octahedral sheet form a common layer. As in Kaolin, the atoms common to both the tetrahedral and octahedral layer are O instead of OH. These layers are continuous in the *a* and *b* directions and are stacked one above the other in the *c* direction. As a consequence, O layers in the units become adjacent and a very weak bond is created with the possibility of cleavage. The preeminent feature of these clay ingredients is the ability of water and organic molecules to enter between unit layers and expand in the *c* direction. Expansion properties are reversible; however, the structure is completely collapsed by removal of interlayer polar molecules. Most of these clay ingredients have substitutions within their lattices: aluminum or phosphorous for silicon in the tetrahedral coordination and/or magnesium, iron, zinc, nickel, lithium, etc. for aluminum in the octahedral sheet.

Illite

Illite is a non-expanding, clay-sized, dioctahedral mineral that is considered part of the mica group.^{5,6} It is a layered aluminosilicate, known as a phyllosilicate. Its basic unit is a layer composed of two inward-pointing silica tetragonal sheets with a central alumina octahedral sheet. Poorly hydrated potassium cations occupy the space between the sequence of layers, which prevents swelling or the expansion of the layers. Illite is very similar structurally to common mica (muscovite), with slightly more silicon, magnesium, iron, and water, and slightly less tetrahedral aluminum and interlayer potassium.

Kaolin

Kaolin's structure is composed of a single silica tetrahedral sheet and a single alumina octahedral sheet combined in a unit so that the tips of the silica tetrahedrons and one of the layers of the octahedral sheet form a common layer.¹ All the tips of the silica tetrahedrons point in the same direction and toward the center of the unit made by the silica and octahedral sheets. Composite octahedral-tetrahedral layers are formed due to the similarity between the sheets *a* and *b* dimensions. The common layer between the octahedral and tetrahedral groups consists of two-thirds of shared atoms between silicon and aluminum that become O instead of OH. Analyses of Kaolin have shown there is little substitution within the lattice. In a small percentage of cases, iron and/or titanium has replaced aluminum. This has only been seen in the relatively poor crystalline varieties of Kaolin.

Chemical Properties

Chemical properties for clay ingredients are described in Table 2.^{1,7-9} Clay ingredients are generally described as insoluble in water.

A supplier has reported the particle size distribution at D₅₀ for Bentonite and Kaolin to be 61.1 and 3.1 µm, respectively.¹⁰ The mean particle sizes for 5 different Hectorite products were 19.9 - 25.4 µm, and the particle ranges for these products were 2.9 - 131.7 µm.¹¹ Another supplier provided specifications for a Clay raw material containing 75% Illite, 19% Kaolin, and 6% Montmorillonite (see Clay in Table 2); however, there is no information therein to indicate the particle size of resultant final cosmetic formulations.⁹

Method of Manufacture

Attapulgite

Attapulgite is produced through an opencast mining technique, stripping layers with heavy machinery.¹ The clay is then transported to a processing plant where crushing, drying, classification, and pulverizing take place. High-heat drying to remove water may occur to enhance absorbent qualities. Attapulgite is mined in 10 countries: Australia, China, France, India, Russia, Senegal, South Africa, Spain, Turkey, and the US.

Bentonite

Large deposits of Bentonite have been discovered in Canada, China, France, Germany, Great Britain, Greece, Hungary, Italy, Japan, Mexico, New Zealand, North Africa, Poland, South Africa, the post-Soviet states, and the US.¹ The mine ore of Bentonite is processed to remove grit and non-swelling materials.

A supplier has reported that Bentonite is mined mineral.¹² The material is then washed, filtered, dried, treated, and tested for quality.

Clay

A supplier has reported that Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) is naturally sourced, mechanically refined, and not chemically processed.⁹ A dehydration process is used to eliminate bacteria prior to sorting through induction.

Illite

Illite is formed by the weathering of silicates (feldspar), by the alteration of other clay minerals, or during the degradation of muscovite.^{5,6} The formation of Illite is generally favored by alkaline conditions and by high concentrations of aluminum and potassium. Deposits of Illite are widely distributed globally: it is commonly found in soil and argillaceous sedimentary rocks, as well as in some low-grade metamorphic rocks.^{5,6,8}

Kaolin

Deposits of Kaolin have been found in England, the US, France, the Czech Republic and Slovakia, Germany, and Japan.¹ Kaolin is extracted from kaolinized granite by washing it out with powerful water hoses. The clay stream is then pumped to the separation plant where sand and mica are removed. The purified clay is filtered when wet and then dried. The very fine powder is formed by milling.

Composition/Impurities

Attapulgite

Attapulgite commonly is found with smectites, amorphous silica, chert, and other minerals.¹ A typical mineral composition of Attapulgite is approximately 55% silicon dioxide, 10% aluminum oxide, 3.5% iron (III) oxide, 10.5% magnesium oxide, 0.5% potassium oxide, and 20% water.

Bentonite

The principal constituent of Bentonite is Montmorillonite.¹ However, other minerals such as Illite, kaolinite, and nonargillaceous detrital minerals can be present. Most bentonites appear relatively pure and other mineral contributions rarely exceed 10%. Cristobalite is often present. Montmorillonite compositions frequently vary either in its lattice structure or in the exchangeable ions present. A typical mineral composition of Bentonite is approximately 60% silicon dioxide, 20% aluminum oxide, 3% iron (II) oxide, 1.5% magnesium oxide, 0.6% calcium oxide, 0.6% potassium oxide, and 21% sodium oxide.

According to the *Food Chemicals Codex*, Bentonite is composed of natural smectite clays consisting primarily of colloidal hydrated aluminum silicates of the Montmorillonite or Hectorite type of minerals with varying quantities of alkalis, alkaline earths, and iron.⁷ Food-grade Bentonite should contain no more than 5 mg/kg arsenic, no more than 15 mg/kg lead, and no more than 1000 colony-forming units (cfu)/g aerobic microbes. Bentonite should be negative for *Escherichia coli* in 25 g.

In particle size analysis of Bentonite, 90% of the particles were smaller than 68 µm, 50% were smaller than 5µm, and 10% were smaller than 2 µm.¹³ No particles were smaller than 0.9 µm. A second analysis of Bentonite showed that 90% of the particles were smaller than 25 µm, 50% were smaller than 6.5 µm, and 10% were smaller than 1.6 µm.¹⁴ No particles were smaller than 0.5 µm.

Clay

A supplier has reported that Clay contains 75% Illite, 19% Kaolin, and 6% Montmorillonite and does not contain quartz.⁹ Trace heavy metals may be present: < 0.5 ppm antimony, < 17 ppm arsenic, < 0.5 ppm cadmium, < 7 ppm cobalt, < 0.5 ppm tin, < 0.05 ppm mercury, < 20 ppm nickel, and < 20 ppm lead were detected using inductively coupled plasma-optical emission spectrometry (ICP-OES). Bacterial, yeast, and mold content were below the threshold of detection. Dioxin and polychlorinated biphenyl “results are near zero.”

Fuller’s Earth

Principal deposits of Fuller’s Earth include Montmorillonite, Bentonite, Attapulgite, and sepiolite.¹

Hectorite

Principal impurities of Hectorite include calcite, dolomite, silica crystals, and grit.¹ A typical mineral composition of Hectorite is approximately 56% silicon dioxide, 0.1% aluminum oxide, 0.03% iron (III) oxide, 25% magnesium oxide, 0.1% potassium oxide, 3% sodium oxide, 1% lithium dioxide, 6% fluorine, and 12% water.

According to some suppliers of Hectorite products (97 - 100% pure), crystalline silica (also described as quartz and/or CAS No. 14808-6-7) may be an impurity.^{15,16} Quantities of crystalline silica were reported to be 1 - 3%.

Illite

The sheets of Illite are composed of silicon, magnesium, iron, aluminum, potassium, and water.⁶ In analysis of 0.5 kg samples of 3 clay products containing Illite, the major element composition (as mean concentration) comprises silicon (23.02%), aluminum (8.80%), calcium (4.55%), iron (4.22%), potassium (3.19%), titanium (0.48%), sulfur (0.21%), phosphorus (0.10%), and manganese (0.041%).¹⁷ Trace element impurities (as mean concentration) were identified as barium (426.70 mg/kg), rubidium (253.7 mg/kg), strontium (227.07 mg/kg), zinc (100.97 mg/kg), cesium (65.93 mg/kg), nickel (35.37 mg/kg), neodymium (30.23 mg/kg), lanthanum (28.00 mg/kg), lead (26.77 mg/kg), copper (25.07 mg/kg), arsenic (16.33 mg/kg), thorium (8.83 mg/kg), uranium (3.78 mg/kg), and bromine (< 1 mg/kg). Bulk composition analysis indicated the samples contained calcite and quartz.

Kaolin

Quartz, mica, and feldspar are often found associated with Kaolin as the crude mineral and are often removed through screening and elutriation.¹ Potentially pathogenic organisms were absent. The bacteria present were mostly gram-positive aerobic spore-formers. A typical mineral composition of Kaolin (reported as kaolinite) is approximately 45% silicon dioxide, 39% aluminum oxide, 0.8% iron (III) oxide, 0.08% magnesium oxide, 0.08% calcium oxide, 0.1% potassium oxide, 0.7% sodium oxide, 0.2% titanium (IV) oxide, and 14% water.

According to the *Food Chemicals Codex*, Kaolin is a purified clay consisting mainly of alumina, silica, and water.⁷ Food-grade Kaolin should contain no more than 3 mg/kg arsenic and no more than 10 mg/kg lead.

According to some suppliers of Kaolin products (up to 100% pure), crystalline silica (described as quartz, free respirable silica, and/or CAS No. 14808-6-7) may be an impurity.^{18,19} Quantities of crystalline silica were reported to be ≤ 2%.

Montmorillonite

A typical mineral composition of Montmorillonite is approximately 51% silicon dioxide, 20% aluminum oxide, 0.8% iron (III) oxide, 3% magnesium oxide, 2% calcium oxide, 0.1% potassium oxide, 0.04% sodium oxide, 0.1% zinc oxide, and 23% water.¹

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA’s Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of

cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP survey data, Kaolin has the most reported uses in cosmetic products, with a total of 787; the majority of uses are in leave-on formulations (Table 3).²⁰ Bentonite has the second most reported uses in cosmetic products, with a total of 221; half are reported in leave-on formulations. The frequencies of use for both of these ingredients have greatly changed since the original safety assessment was finalized; in 1998, Kaolin was reported to have 509 uses, and Bentonite was reported to have 94.¹ The results of concentration of use surveys conducted by the Council in 2022 indicate Kaolin has the highest maximum concentration of use in leave-on formulations; it is used at up to 53.2% in manicuring preparations.²¹ For leave-on dermal preparations, specifically, Kaolin also has the highest reported maximum concentration of use, at 16% in face and neck products, and Bentonite has the next highest, at 8% in face and neck preparations. According to the original safety assessment, the maximum leave-on use concentration in 1999 for Kaolin was 100% in skin care preparations; the maximum leave-on use concentration for Bentonite was 8% in makeup foundations.¹

Clay ingredients may be used in products that can be incidentally ingested; for example, Kaolin is used in lipstick (at up to 14.5%).²¹ Additionally, clay ingredients have been reported to be used in products that may come into contact with the eyes and mucous membranes; for example, Kaolin is used at up to 8.5% in eye shadows and at up to 5% in bath soaps and detergents.

Moreover, clay ingredients are used in cosmetic formulations that could possibly be inhaled; for example, Bentonite is reported to be used at 0.9% in spray suntan products and Kaolin is reported to be used at up to 15% in face powders.²¹ In practice, as stated in the Panel's respiratory exposure resource document (<https://www.cir-safety.org/cir-findings>), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

While no data have been submitted from the cosmetics industry indicating that these clay ingredients are used in nanoform in cosmetic formulation, a report from a nanotechnology research company provides statistical data showing that nanoclays can be used as cosmetic additives for lipsticks, eyeliners, and toothpaste, functioning for rheology modification, viscosity control, thixotropic effect, as well as increased stability and pigment dispersibility.²²

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients, and without consumer habits and practices data or particle size data related to this use technology, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

In regulations regarding cosmetic products in the European Union (EU), no restrictions were listed for Attapulgit, Clay, Fuller's Earth, Hectorite, Illite, or Montmorillonite.²³ Bentonite and Kaolin are listed in Annex IV-Allowed Colorants under CI 77004 with the chemical name of "natural hydrated aluminum silicate...containing calcium, magnesium or iron carbonate, ferric hydroxide, quartz-sand, mica, etc. as impurities;" the remaining clay ingredients named in this report are not restricted from cosmetic use in any way. Note, these ingredients are not approved as colorants in the US.² Bentonite, Hectorite, and Kaolin were included in the scientific opinion by the EU Scientific Committee on Consumer Safety (SCCS) on the safety of aluminum in cosmetic products.²⁴ The SCCS concluded that the use of aluminum compounds is safe at the following equivalent aluminum concentrations up to 6.25% in non-spray deodorants/antiperspirants, 10.60% in spray deodorants/antiperspirants, 2.65% in toothpaste, and 14% in lipstick.

According to the Australian Industrial Chemicals Introduction Scheme (AICIS), the following ingredients are Tier I chemicals (not considered to pose an unreasonable risk to the health of workers and public health): Bentonite, Fuller's Earth, Kaolin, and Montmorillonite.²⁵ Attapulgit is a Tier II chemical (requires risk management measures to be instituted for safe use for human health). Hectorite is listed as a chemical unlikely to require further regulation to manage risks to human health.

Non-Cosmetic

Based on the properties of broad surface area, rich porosity, diverse morphology, good adsorption performance, and high ion exchange capacity, nanoclays have been widely applied in many fields, such as drug delivery systems,²⁶ food and beverage packaging,²⁷ paper manufacturing,²⁸ and constructional material.²⁹

Attapulgit

Attapulgit is reported to be used in absorbents, pesticides, oil and petroleum treatment, and as a filler in many products.¹

Bentonite

Bentonite is reported to be used in foundry sand bonding, bleaching clay in oil refining and decolorizers, filtering agents, water impedance, animal feed, pharmaceuticals, paint, plasticity increasers, and iron-ore pelletizing.¹

Bentonite is generally recognized as safe (GRAS) as a direct food additive for humans (21 CFR§184.1155) and animals (21 CFR§582.1155). Bentonite is also GRAS as an indirect food additive in adhesives and components of coatings (21 CFR§175.105), in paper and paperboard components (as a colorant only, 21 CFR§176.170), in adjuvants as a colorant for polymers (21 CFR§178.3297),

Clay

Clay (natural) is GRAS as an indirect food additive in polymers (cellophane; 21 CFR§177.1200).

Fuller's Earth

Fuller's Earth is reported to be used as a military decontaminant for removal of hazardous materials from the skin.³⁰

Hectorite

Hectorite has been approved for use in internally and externally applied products, as well as dentifrices and externally approved pharmaceuticals.¹

Hectorite is reported to be used as drug-delivery system in anticancer therapy because of its biocompatibility, mechanical strength, and natural availability.³¹

Illite

Illite has been studied for use in environmental remediation of contaminated soils and water as an adsorbent.³²⁻³⁵ It also has been studied for use in veterinary applications, such as dietary supplements for swine³⁶ and topical therapeutic treatment in equine injuries.³⁷

Kaolin

Kaolin is reported to be used in the paper industry to fill and coat the surface of paper, as a filler in rubber and plastics, paint extender, ceramics manufacture, ink, adhesives, insecticides, medicines, food additives, bleaching, adsorbents, cement, fertilizers, crayons, pencils, detergents, porcelain enamels, paste, foundries, linoleum, floor tiles, and textiles.¹ It has been classified by the National Formulary as a tablet and/or capsule diluent.

Kaolin clay is GRAS as an indirect food additive with no limitation other than current good manufacturing practice (21 CFR§186.1256). It is used in the manufacture of paper and paperboard that contact food. Kaolin (colloidal) is an approved over-the-counter (OTC) drug as an antidiarrheal active ingredient (21 CFR§335.10), an anorectal active ingredient (21 CFR§346.14), and a skin protectant active ingredient (from 4% to 20%; 21 CFR§347.10). Kaolin is used as a digestive aid (21 CFR§310.545); however, the data are currently inadequate to establish general recognition of the safety and effectiveness of this ingredient for this specified use. Kaolin is exempted from the requirement of a tolerance for pesticide residues when used on or in food commodities to aid in the control of insects, fungi, and bacteria (food/feed use; 40 CFR§180.1180).

Kaolin minerals (specifically kaolinite) have been studied for use in environmental remediation of contaminated soils and water.^{34,35} This clay material has also been studied for use in veterinary applications, such as dietary supplements for swine³⁶ and topical therapeutic treatment in equine injuries.³⁷

Montmorillonite

Montmorillonite is reported to be used for food packaging and in paper manufacturing.^{27,28} It also has been studied for use in environmental remediation of contaminated soils and water^{34,35} and in veterinary applications, such as use in dietary supplements for swine.³⁶

TOXICOKINETIC STUDIES

Absorption, Distribution, Metabolism, and Excretion (ADME)

Clay

In ex vitro bioavailability studies using human skin models, the ability of transcutaneous passage of heavy metals (vanadium, lead, arsenic, barium, nickel, chromium, and aluminum) in 3 clay pastes was analyzed.³⁸ The clay pastes were white Montmorillonite, Kaolin, and Clay (composed of 75% Illite, 19% Kaolin, and 6% Montmorillonite). Approximately 150 g of each product were tested with human skin samples in Franz cells and incubated for 24 h. The tested products, the diffusion liquids, and the storage liquids were then analyzed for metal content (details not provided). No detectable quantities of heavy metals were found in the diffusion or storage liquids. It was determined that the traces of heavy metal in the clay pastes did not penetrate cutaneous tissue.

Kaolin

In a dietary study, a group of 10 male Sprague-Dawley rats were fed a control diet plus 0.5 ml 20% Kaolin – 1% pectin for 48 h.¹ Stool samples were collected 72 h later and analyzed for volume, sodium, potassium, and fat content. The results were a 103% increase in sodium, a 184% increase in potassium, and fat excretion remained at baseline.

Montmorillonite

Polydisperse and monodisperse [¹³⁴Cs]-fused Montmorillonite suspensions were administered to groups of 40 rats and mice and to 120 beagle dogs by a multiport nose-only inhalation exposure system.¹ Aerosol concentrations ranged from 0.1 to 0.001 mg of fused Montmorillonite/l of air. Equal numbers of male and female rats and mice and 74 male and 46 female dogs were utilized. Exposure times for rats and mice ranged from 25 to 45 min and for dogs 15 to 50 min. All animals were whole-body counted for the labeled particles. Five rats and 5 mice from each group were killed 4 h after exposure. The remaining rats and mice were killed at various times after exposure. Tissues from rats and mice were collected on post-exposure days 2, 4, 8, 16, 32, 64, 128, 256, 365, 512, 730, and 850. Tissues and excreta from the dogs were also collected on the same schedule, but also at 4, 5, 7, and 9 yr after inhalation exposure. Two dogs were scheduled for termination at times ranging from 4 h to 9 yr. All animals were necropsied and tissues from lungs, lung-associated lymph nodes, gastrointestinal tract, spleen, kidneys, abdominal lymph nodes, blood, skeleton, muscle, and skin were prepared for analysis of [¹³⁴Cs]-exposure. The mass of material deposited into the lungs of rats and mice was ~0.01 to 0.1 mg and for dogs was ~1 to 10 mg. The mass of Montmorillonite for all three species was < 0.1 mg/g of lung. Clearance of the initial ¹³⁴Cs occurred by dissolution and mechanical clearance. Mechanical clearance from the nasopharynx was rapid, and the clearance rate was decreased to a negligible value for all three species within a few days. Most initial deposits cleared via the gastrointestinal tract. Long-term mechanical clearance from the pulmonary region occurred at a constant rate for all species. Solubilization was the primary factor in long-term lung clearance for most particles inhaled by dogs and mechanical clearance was dominant in rats and mice. Most of the long-term clearance of deposited particles went to lung-associated lymph nodes in dogs and occurred at a slower rate as compared to rats and mice. Rats and mice had a rapid clearance from the pulmonary region, where most of the mechanical clearance occurred via the gastrointestinal tract. Long-term clearance of the particles in dogs occurred at 3500-d half-time in the lymph nodes and 6900-d half-time clearance in the gastrointestinal tract. The transport rate of the particles in the dog was 0.0002/d of the lung burden. The long-term biological clearance half-term day was 690 d for rats and 490 d for mice. The lymph node accumulation process was modeled by a short-term process that became negligible after a few days.

Radiolabeled, [¹³⁴Cs]-fused Montmorillonite particles were instilled into specific lung lobes or injected intraperitoneally into 32 beagle dogs.¹ Necropsy was performed 34, 182, and 365 d later. Specific sites of instillation included right apical lobe, right cardiac lobe, right diaphragmatic lobe, right intermediate lobe, left apical lobe, left diaphragmatic lobe, and intraperitoneal. Initial burdens in the peritoneal cavity or the lungs ranged from 0.50 to 14 µCi for 29 dogs and from 42 to 64 µCi lung burdens for the other 3 dogs. Effective translocation half-time of lung instillations was 390 d. The accumulation rate of [¹³⁴Cs]-fused particles in the lymph nodes was 0.03% per day. Individual lung lobes cleared particles to 1 - 2 lymph nodes, and specific lymph nodes accumulated particles from 1 - 3 lung lobes. Lymph nodes that collected particles from the lung included the left mediastinal node and the left, left-middle, right, and right-middle tracheobronchial lymph nodes. The destination for translocated particles was primarily the nodes proximate to the tracheal bifurcation. Particles injected into the peritoneal cavity were translocated mainly to mesenteric lymph nodes and left and right sternal lymph nodes. A small percentage of particles went to the left tracheobronchial lymph node.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Clay

In an acute dermal toxicity study performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 402, rats received 2000 mg/kg bw of Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) on clipped skin.³⁸ The test material was moistened with 0.2 ml distilled water. A control group received only distilled water. The rats were observed for 14 d. No mortality or clinical signs of toxicity were observed. The LD₅₀ was greater than 2000 mg/kg. No further details were provided.

Oral

Clay

In an acute oral study performed in accordance with OECD TG 423, rats were exposed to a single dose of 2000 mg/kg bw of Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) in distilled water.³⁸ No mortality or clinical signs of toxicity were observed. Visceral examination did not reveal any lesions of pathological significance except uterine distension in one rat, which was not considered to be treatment-related. The LD₅₀ was greater than 2000 mg/kg bw. No further details were provided.

Hectorite

Five male and 5 female Sprague-Dawley rats were administered a single dose of 5 g/kg of Hectorite by gavage.¹ None of the animals died; the acute oral LD₅₀ was > 5.0 g/kg bw.

Kaolin

In an acute oral study, 120 rats were fed doses of Kaolin ranging from 100 to 210 g/kg.¹ Fourteen rats were controls. Kaolin was inert and non-static except for the danger of bowel obstruction resulting in perforation. The clinical signs were listlessness, anorexia, oliguria, hypothermia, and dyspnea. These were pathological reactions from overdistention of the alimentary canal by an inert solid. The number of fatalities and the incidence and advance of bowel obstruction along the small intestine were dose-related. The dose that killed 50% of the rats by bowel obstruction was 149 g/kg.

Inhalation

Clay

In an acute inhalation study performed in accordance with OECD TG 403, rats were exposed to 3.856 mg/l air of Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite).³⁸ A control group was exposed to air passed through inert bed material (iron grit). Both groups of rats were exposed for 4 h and then observed for 14 d. No mortality or clinical signs of toxicity were observed. The LC₅₀ was greater than 3.856 mg/l. No further details were provided.

Illite, Montmorillonite, Kaolin

The inhalation toxicity of an environmental dust sample containing Illite, Montmorillonite, Kaolin and α -quartz was determined in a group of 6 male CD(SD)BR rats exposed to an aerosol of the dust (12 mg/m³, ~ 10 μ m mean diameter) for 3 h in open cages.³⁹ The dust was composed of approximately 75% of the 3 clay aluminum-silicates and approximately 20% α -quartz. A control group of 6 male rats were exposed to room air only. Animals were killed at recovery periods of 0 h, 24 h, 8 d, and 30 d. Lung tissues underwent microscopic and histopathological examination. Rats that were exposed to the dust exhibited preferential particle deposition at the first alveolar duct bifurcations after the terminal bronchiole immediately after the 3 h exposure. No extracellular particles were observed in the recovery periods after this point, in either treated or control animals. The average number of particles observed at the first bifurcation after the 3 h exposure (recovery time 0 h) was 4.6 ± 1.0 particles per bifurcation. Histological sections showed prominent first bifurcation characterized by accumulation of mononuclear cells 24 h after exposure. The presence of macrophages with ingested aluminum silicate particles were observed. Macrophage migration to the bifurcations was observed to a lesser degree immediately after exposure. At 24 h, $87 \pm 12\%$ of the first bifurcation contained a significantly increased number of macrophages. After 8 and 30 d, particles and alveolar macrophages were not significantly elevated and histology was back to normal.

Parenteral

Illite, Montmorillonite, Kaolin

In a study of the environmental dust sample described above, 6 male albino Wistar rats were instilled with 50 mg of the dust (3.2 μ m mean diameter) in 300 μ l of sterile phosphate-buffered saline (PBS).³⁹ The dust particles were injected into the trachea. Control animals (groups of 6) received either 300 μ l of PBS, 50 mg of carbonyl iron in 300 μ l PBS, or 50 mg of α -quartz in 300 μ l PBS. The animals were killed 30 d later. Tissues, particularly the lungs and trachea, underwent microscopic and histopathological examination. Multifocal interstitial lung disease was observed using light microscopy. Mononuclear cell infiltrates, composed of macrophages and lymphocytes located mainly around the small airways and alveolar walls, were identified during examination of all lung sections. No nodules or granulomata were observed. Collagen fibers were observed in the interstitial lesions. The α -quartz instillation resulted in multiple silicotic nodules, iron instillation did not produce any interstitial lesions (some alveolar macrophages with intracellular iron spheres were identified), and no alterations were observed in the animals that received PBS alone.

Short-Term Toxicity Studies

Parenteral

Kaolin

Nano-sized Kaolin (primary particle size 4.8 μ m) was instilled intratracheally in groups of 4 male *gpt* delta transgenic mice (a strain that allows for analysis of deletion mutations) as either a single dose of 0.2 mg/animal or multiple doses of 0.2 mg/animal/wk for 4 consecutive instillations.⁴⁰ Control mice received solvent alone intratracheally. The mice were killed at 12 wk after instillation (for a single dose) or 8 wk after the last instillation (for multiple doses). Tissues, particularly the lungs and kidneys, underwent histopathological examination. Kaolin-phagocytized alveolar macrophages were found, diffusely distributed in the lungs. Focal granulomatous formation, with or without phagocytized alveolar macrophages, were also frequently observed in the lungs of mice that received multiple instillations. Similar observations were made in the mice that received a single instillation, but with a slight degree of particle accumulation and granuloma formation in the lungs. No abnormalities were observed in the kidneys.

Subchronic Toxicity Studies

Oral

Montmorillonite

In a 90-d feed study, 10 male Wistar rats received a Montmorillonite clay (40 mg/kg/d; modified with hexadecyltrimethylammonium bromide) in the diet.⁴¹ Another 10 male rats received only standard diet as control. During the treatment period, clinical signs, body weight, and feed and water consumption were recorded weekly. At the end of the treatment period, the rats were fasted for 18 h before being killed. Histopathological examinations were performed, and liver, kidneys, lungs, spleen, brain, testes, gastrointestinal tract, and heart were weighed. Blood samples were obtained for analysis. No rats died during the treatment period and no remarkable clinical signs were observed. Body weight gains and feed and water consumption were comparable to controls. No significant changes were noted in clinical biochemistry, organ weights, or in the histopathological examinations when compared to controls.

Parenteral

Kaolin

Toxicity of some of the minerals present in coal-mine dust was examined in groups of 10 SPF Sprague-Dawley rats.¹ The rats were exposed over a period of 3 mo to 50 mg/animal intratracheal instillations of Kaolin. The following assessments were made: weight of the fresh lungs; macroscopic and microscopic lesions in the lungs; amount of collagen and dust present in the lungs; and calculation of the toxicity index from the amount of collagen formed per mg of dust. The weight of fresh lungs subjected to Kaolin was 1.76 g. Collagen formed per lung was 23.9 mg. The dust per lung was 30.2 mg and the collagen/dust ratio was 0.79. Microscopic examinations of the lungs showed no alveolar proteinosis but Kaolin was detected in the bronchovascular lymphoid sheaths. No information regarding nonexposed lungs was presented. The opinion of the investigators was that exposure to Kaolin results in "pulmonary toxicity" and possesses "fibrogenic capacity."

Chronic Toxicity Studies

Oral

Montmorillonite

The potential toxicity of a naturally-occurring calcium Montmorillonite clay, an anti-caking agent in animal feed, was studied using groups of 10 male and 10 female Sprague-Dawley rats for 28 wk.⁴² The rats received the test material at 0, 0.25, 0.5, 1.0, or 2.0% w/w in their feed. Rats were observed daily for clinical signs and deaths. Feed consumption was recorded daily for the first month and then every fourth day. Body weights were measured weekly. At the treatment end, final body weights were recorded and blood was drawn for analysis. After the rats were killed, histopathological examinations were performed, and select organs were weighed. Total feed consumption, cumulative feed consumption, body weight, total body weight gain, and relative organ weights were not affected in either sex at any dose tested. No differences in relative organ weights or gross or histopathological changes compared to controls were observed. Non-dose-dependent significant changes were observed in mean corpuscular hemoglobin, serum calcium, serum vitamin A, and serum iron.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Kaolin

Groups of 12 Sprague-Dawley female rats were fed a control diet, 20% Kaolin diet, or iron-supplemented 20% Kaolin diet.¹ The diets were fed for 37 to 86 d, 69 to 85 d, and 96 to 117 d prior to fertilization. These same diets were fed for the duration of the gestation period. The animals fed the 20% Kaolin diet had significant reductions in hemoglobin, hematocrit, and red blood cell numbers, indicating maternal anemia. Significant reduction in the birth weight of the pups was observed. Animals fed the iron-supplemented diet maintained their hematocrit, hemoglobin, and red blood cell levels.

In a study of 12 fetal lambs and 6 fetal rhesus monkeys, sterile suspensions of 2% Kaolin in saline were injected into the cisterna magna.¹ Fetal lambs received 1 to 3 ml of Kaolin and fetal monkeys received 0.5 to 1.0 ml of Kaolin. After injection, the fetuses were replaced into the uterus. Prenatal ultrasound monitoring was used to document the progression of fetal ventriculomegaly. Cesarean sections were scheduled for gestation days 140 to 145 for the sheep and gestation days 160 to 165 for the monkeys. Newborn animals with gross head enlargement were killed 2 h after birth and necropsy was performed. Brains were sectioned for gross and microscopic examination. Five lambs and 1 monkey underwent ventriculoamniotic shunting at 120 d after gestation. Ventricular dilatation was apparent at 1 wk following Kaolin injections. The cerebral mantle was markedly thinned, with relative preservation of the cortex and severe attenuation of the white matter. The average cortical thickness of the cingulate gyrus in the Kaolin-injected sheep was 716 μ m compared to 1225 μ m in control animals. The corpus callosum was an average of 125 μ m in thickness in the sheep compared to 475 μ m in control animals. Microscopic examination found the cortical neurons well preserved and contained the complexity and density of neural processes. A mild-to-moderate fibrotic reaction and inflammatory cell response along the basal meninges was apparent. A large number of macrophages containing Kaolin infiltrated the subarachnoid space. In 5 fetuses, Kaolin was injected mistakenly into either the epidural tissues superficial to the cisterna magna or into the cervical musculature. None of these fetuses had hydrocephalus at birth.

GENOTOXICITY STUDIES

In vitro and in vivo genotoxicity studies summarized here are described in Table 4.

Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite; 5000 µg) was not mutagenic in an Ames test, with or without metabolic activation.³⁸ Unmodified Montmorillonite clay (at up to 125 µg/ml) and one type of cation-exchanged (hexadecyltrimethyl-ammonium) montmorillonite clay (at up to 250 µg/ml) also were not mutagenic in an Ames test with or without metabolic activation, but significant increases in revertant colonies were observed in one strain with metabolic activation in 2 other cation-exchanged montmorillonite clays.⁴³ No mutagenic activity was observed in a *Salmonella*/microsome assay with and without metabolic activation when tested in Montmorillonite and cation-exchanged montmorillonite in both nano- and non-nano-sized material at up to 141 µg/ml and up to 14.1 µg/plate, respectively.⁴⁴ However, the cation-exchanged montmorillonite material (both nano- and non-nano-sized, at up to 226 µg/ml and 170 µg/ml, respectively) in this study was genotoxic in a concentration-related manner in a Comet assay with Caco-2 cells. The natural clay was not considered genotoxic. Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cell cultures when tested at up to 5000 µg/ml, with or without metabolic activation.³⁸ Micronucleus induction was observed in a dose-dependent manner to micro- and nano-sized Kaolin in CHO AA8 and primary normal human diploid epidermal keratinocytes and fibroblasts, with fine particles having a higher genotoxic potency than coarse particles.⁴⁵ A 4-fold increased frequency of micronucleated cells was observed in human lung cancer A549 cells following exposure to nano-sized Kaolin.⁴⁰ Statistically significant increases in the frequency of micronuclei were induced by Montmorillonite clay at 62.5 µg/ml in a cytokinesis block micronucleus cytome assay in human hepatoma cell lines, but this effect was not observed at a concentration of 31.25 µg/ml or lower.⁴⁶ No effects in nucleoplasmic bridges or nuclear buds were observed at any concentration in this study. In an in vitro micronucleus assay and kinetochore analysis using human lung fibroblasts, the genotoxic potential of Bentonite at up to 15 µg/cm² was determined to be generally low, but could be altered by the content of quartz and available transition metals.⁴⁷ In an in vitro Comet assay with micro- and nano-sized Kaolin in CHO AA8 and primary normal human diploid epidermal keratinocytes and fibroblasts, the test materials promoted DNA damage in a dose-dependent manner, and the particles that were 200 nm had a higher DNA-damaging potency than those that were 4.8 µm.⁴⁵

In an in vivo Comet assay with nano-sized Kaolin intratracheally instilled in mice, DNA damage was induced with 0.2 mg/mouse, but not with 0.05 mg/mouse, after a 3-h exposure. No difference in induction was observed after the 24 h exposure compared to the 3 h exposure.⁴⁰ Increased *gpt* and *Spi*⁻ mutant frequencies were observed in the lungs of the mice following intratracheal instillation with either single or multiple doses of 0.2 mg nano-sized Kaolin. A mutation spectra analysis showed > 60% of G:C to C:G transversion occurred in the *gpt* genes. In another Comet assay, rats were given 2 oral doses of up to 1000 mg/kg bw cation-exchanged montmorillonite clay.⁴⁸ There was no statistically significant difference in % tail DNA between the negative controls and the different treatment groups for any of the cells (liver, kidneys, colon) tested.

Attapulgit

In 2 studies looking at unscheduled DNA synthesis (UDS) in rat hepatocytes, Attapulgit did not cause a significant increase in DNA-specific activity at up to 10 µg/ml with no cytotoxicity.¹ However, in another UDS study using rat pleural mesothelial cells, Attapulgit tested at 2, 4, or 10 µg/cm² produced a significant increase in UDS and inhibited cell growth at 10 µg/cm². Attapulgit did not induce point mutations in a third DNA study.

Hectorite

In the Salmonella typhimurium LT2 spot test (TA98, TA100, TA1535, TA1537, and TA1538) with or without metabolic activation, Hectorite was found to be non-mutagenic.¹ In primary hepatocyte cultures, the addition of Attapulgit at 10 µg/cm² caused significant increases in UDS in rat pleural mesothelial cells.

CARCINOGENICITY STUDIES

The International Agency for Research on Cancer (IARC) has determined there is inadequate evidence in humans for the carcinogenicity of Attapulgit (IARC uses the mineralogical term “palygorskite” for Attapulgit).⁴⁹ Further, IARC has determined there is insufficient evidence in experimental animals for the carcinogenicity of short Attapulgit fibers (< 5 µm); however, there is sufficient evidence in experimental animals for the carcinogenicity of long Attapulgit fibers (> 5 µm). Overall, long Attapulgit fibers (> 5 µm) are possibly carcinogenic to humans (Group 2B) and short Attapulgit fibers (< 5 µm) cannot be classified as to their carcinogenicity to humans (Group 3).

Attapulgit (palygorskite fibers > 5 µm in length) is listed by California Proposition 65 as a carcinogen.⁵⁰

Inhalation

Attapulgit

In a rat inhalation study, groups of 40 (20 male and 20 female) SPF Fischer rats were exposed to samples of Attapulgit dust mined in Lebrija or Leicester in inhalation chambers at a concentration of 10 mg/m³ for 6 h/d for 5 d/wk until they were killed.¹ Negative and positive control groups received Kaolin and crocidolite, respectively, at 10 mg/m³. Four

animals were killed at 3, 6, and 12 mo, and the remaining rats were allowed to live their life span. All animals were subject to necropsy; the lungs, liver, spleen, kidneys, and other relevant organs were examined microscopically. At microscopic examination, 1 peritoneal mesothelioma, 1 adenocarcinoma, and 3 bronchoalveolar hyperplasia were found in rats treated with Lebrija Attapulgit. Thirty-five rats had no proliferative changes. In rats treated with Leicester Attapulgit, proliferative lesions observed included 2 mesothelioma, 1 peritoneal mesothelioma, 1 malignant alveolar neoplasm, 2 benign alveolar neoplasms, and 8 bronchoalveolar hyperplasias. Twenty-seven rats had no proliferative lesions. Rats exposed to the negative-control Kaolin had 2 bronchoalveolar hyperplasias. Rats in the positive-control crocidolite group had 1 adenocarcinoma and 3 bronchoalveolar hyperplasias.

Kaolin

Kaolin was reported to be the negative control in the above rat inhalation study.¹ The rats received 10 mg/m³ Kaolin for 6 h/d for 5 d/wk. Two bronchoalveolar hyperplasias were reported.

Parenteral

Attapulgit

In an intratracheal study, groups of 5 rats received a single instillation of Attapulgit at 1, 5, and 10 mg.¹ One month after treatment, bronchoalveolar lavage and microscopic examination of the lungs were performed. The average length of the fibers was 0.8 μ m, and 100% of the fibers were less than 3 μ m. Every test animal had type A lesions, which are characterized by an accumulation of inflammatory cells, mostly macrophages, and epithelioid cells around fiber deposits. These inflammatory cells form a compact cellular infiltrate at the periphery of the deposits and some are focally dispersed throughout the alveolar region. The bronchoalveolar lavage (BAL) had mostly macrophages and a small number of neutrophils at 5- and 10-mg doses. At the 5-mg dose, 3.6% of the cells were lymphocytes.

Two groups of 30 to 50 female Osbourne-Mendel rats received a single direct application to the left pleural surface by open thoracotomy of 40 mg of 1 of 2 Attapulgit samples.¹ The samples were 90% pure with quartz being the other component. One dose consisted of fibers > 4 μ m and the other contained no fibers > 4 μ m. The rats were killed at the end of 2 yr. Pleural sarcomas were seen in 2/29 rats. The incidences of pleural sarcomas in the untreated groups were 3/491 and 17/615 of the rats receiving the pleural implants of Attapulgit. Of rats receiving the positive control, crocidolite, 14/29 developed pleural mesotheliomas.

Attapulgit (20 mg/ml of 0.9% sodium chloride) was injected into the pleural cavities of 36 Sprague-Dawley rats.¹ The median fiber length was 0.77 μ m. Two control groups, untreated and saline-injected, were utilized. Necropsy was performed after the rats died or killed when moribund. No mesothelial neoplasms were found in either controls or in rats treated with Attapulgit. Survival times between the Attapulgit-treated group and the controls were not statistically different.

Attapulgit was injected intrapleurally as a single dose of 0.5, 2, 4, 8, 16, or 32 mg into 6 groups of 25 Fischer 344 rats.¹ Nearly all the fibers were < 1 μ m in length. Mesotheliomas were present in 2/140 treated rats compared to 1/79 incidences in control groups. The median life span was 839 days for Attapulgit-treated animals and 729 days for nontreated animals.

In another intrapleural study, injections of 20 mg of different Attapulgit fiber samples in 1 ml of saline were given to 2-mo-old Sprague-Dawley rats.¹ The control group received only a saline injection. All rats were allowed to live full life span. The mean length of Attapulgit fibers in this experiment was 0.77 μ m. The number of groups were not reported; however, 36 rats were reported to comprise each group. Pulmonary and thoracic neoplasms were fixed and processed for histopathological examination. The survival time of the treated groups (788 $\pm$ 155 days) was very similar to that of the control groups (809 $\pm$ 110 days). The incidence of mesothelioma was 0% for control groups and treated groups. The researchers concluded Attapulgit was not carcinogenic in this study.

Samples of Attapulgit from Lebrija, Torrejon, and Leicester were injected intrapleurally as a single injection in groups of 20 male and 20 female SPF Fischer rats.¹ Concentrations were not reported; however, fiber length was reported as < 2 μ m, for Lebrija Attapulgit, at most 0.54% > 6 μ m for Torrejon Attapulgit, and 19% > 6 μ m for Leicester Attapulgit. Kaolin and saline were used as negative controls, and crocidolite was used as a positive control. The animals were allowed to live their life span but were killed if they appeared distressed. Upon death, necropsy and microscopic examination of tissue were performed. Dust extraction was obtained from granulomas removed from the diaphragm or mediastinal tissue. Mesotheliomas were reported in 2, 14, 30, and 34 rats for Lebrija Attapulgit, Torrejon Attapulgit, Leicester Attapulgit, and crocidolite, respectively. In the negative controls, no mesotheliomas were reported for the Kaolin and 1 mesothelioma was reported for the saline group. Lebrija Attapulgit dust extracted from the lung had fibers < 2 μ m. Material examined from Torrejon Attapulgit was fibrous and had fiber length up to 8 μ m. Leicester Attapulgit fibers from extracted lungs were up to 25 μ m. The investigators considered these fibers to be tumorigenic.

Three samples of 25 mg of Attapulgit dust were injected intraperitoneally into 40 Wistar rats.¹ Electron microscopy of the sample revealed 37.5% of fibers < 2 μ m long and 70.0% < 5 μ m. All animals were observed until they died either

spontaneously or were killed. Saline was injected into 80 control animals. The time required to produce the first tumor in the rats was 257 days and the tumor incidence rate was 65%.

In a carcinogenicity study conducted with 3 samples of Attapulgit labeled Georgia, Lebrija, and Morimoiro, female Wistar rats (112, 115, and 114 per sample type, respectively) were injected intraperitoneally.¹ Each sample was injected 1/wk for 9 wk at 60 mg per injection. Fiber dimensions for each of the samples Morimoiro, Georgia, and Lebrija were as follows: <50% fiber length was 0.7, 0.5, and 0.8 μm , respectively and <50% fiber diameter of 0.07, 0.07, and 0.04 μm , respectively. Some rats died spontaneously or others in poor health were killed. Surviving animals were killed 2.5 yr after treatment for necropsy. At necropsy, neoplasms or organs with suspected neoplasm tissue were fixed for microscopic examination. The percentage of rats with tumors were 3.5%, 3.5%, and 3.6% for the Morimoiro, Lebrija, and Georgia samples, respectively. These 3 samples were determined to be noncarcinogenic.

In another experiment by the same investigators, a fourth sample of Attapulgit from Caceres was tested in 30 rats.¹ Intraperitoneal injections of 2, 4, and 4 mg were administered consecutively for 3 wk. The fiber length and diameter of this sample were <50% 1.3 and 0.07 μm , respectively. Animals in poor health were killed. Surviving animals were killed 2.5 years after treatment for necropsy. At postmortem examination, parts of neoplasms or organs with suspected neoplasm tissue were fixed for microscopic examination. Forty percent of the rats had tumors. The results were considered moderate in relation to the dose.

Montmorillonite

Heat-treated Montmorillonite in doses of 5, 15, and 45 mg was given to groups of 4 Sprague-Dawley rats by intratracheal instillation.¹ Following a 3-mo postexposure period, the animals were killed and tissues were subjected to microscopic examination. The Montmorillonite particles were mainly restricted to alveoli within and adjacent to alveolar ducts regardless of dose. Most particles were contained within small to moderate numbers of pulmonary alveolar macrophages. However, some particles were free in alveoli. Adjacent alveoli septae were mildly thickened. Interstitial fibrosis was present in all groups. At the 5- and 15-mg doses, fibrosis was mild to moderate, multifocal, and loose, meaning less collagen. The 45-mg dose produced dense fibrosis. Macrophages contained clay particles and lymphocytes were present in the lesions. Occasionally giant multinucleate cells were seen.

OTHER RELEVANT STUDIES

Adsorption

Clay ingredients are reported to adsorb various chemicals, molecules, and microorganisms.¹ These compounds include, but are not limited to, strychnine, quinine, atropine, ampicillin, amoxycillin, Agrobacterium radiobacter, Escherichia coli, Serratia marcescens, Bacillus species, bacterial endotoxins and enterotoxins, and aflatoxins.

Cytotoxicity

Numerous studies with various cell lines on the cytotoxic and hemolytic effects of clay ingredients have been reported.¹ Results varied and may have been dependent on different factors, including mineral composition of the test materials.

Illite and Montmorillonite

The protective effect of Illite and Montmorillonite (up to 1 mg/ml each) on alterations in cell viability and epithelial barrier function induced by mycotoxins was evaluated using Caco-2 cells in a colorimetric 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and a lactate dehydrogenase (LDH) assay.⁵¹ Both clays provided protection against mycotoxin effects. Aflatoxin B1- and fumonisin B1-induced cytotoxicity were completely abolished by Illite. Decreases in the gene expression of specific claudin isoforms and the reduction of trans-epithelial electrical resistance of cell monolayers (an indicator of the epithelial barrier integrity) induced by mycotoxins were reversed by Illite. Montmorillonite also provided protection against mycotoxin effects, but at a lesser degree.

Illite, Montmorillonite, Kaolin

The cytotoxicity of an environmental dust sample (dust samples collected from a Mexican city) comprising approximately 75% Illite, Montmorillonite, and Kaolin, and approximately 20% α -quartz was studied in alveolar macrophages obtained from male albino Wistar rats.³⁹ LDH release was used as an indicator of cell membrane disruption. The alveolar macrophages were incubated with and without the dust (3.2 μm mean diameter) for up to 2 h. LDH was measured in the supernatant at 0, 1, and 2 h, with controls run in parallel. Rat alveolar macrophages incubated with the dust released increasing amount of LDH into the medium as a function of time. Significant levels above control values (2.2 ± 2.6 LDH U/l) were observed by 1 h (19.5 ± 2.6 LDH U/l) and 2 h (29.5 ± 4.0 LDH U/li) of incubation.

The hemolytic activity of the dust was also investigated by incubating different particle concentrations (0.1, 0.5, 1.0, 1.5, and 2.0 mg/ml) with a 0.6% suspension of human red blood cells obtained from normal donors. Cell-particle suspensions were incubated at 37°C in PBS for 1 h under moderate agitation. The results indicated the dust was highly hemolytic. An amount of 2 mg/ml produced $95 \pm 3\%$ hemolysis. The effect was observed starting at 1 mg/ml in a dose-related manner.

Kaolin

In a study examining the toxic mechanisms of typical fine particulate air pollution (PM_{2.5}), human bronchial epithelial (16HBE) cells were treated with nano-scale Kaolin at concentrations of 40 to 240 µg/ml.⁵² The particle size information was not available; however, the authors stated the nano-scale Kaolin utilized in the study was to imitate Kaolin in atmospheric fine particles (PM_{2.5}). Cytotoxicity results of the cell counting kit-8 (CCK-8) assay showed the 16HBE cells had high viability after exposure to 40 µg/ml nano-sized Kaolin, but cell viability decreased significantly at doses greater than 80 µg/ml. A lactate dehydrogenase assay indicated that nano-sized Kaolin caused membrane disruption in a dose-dependent manner.

Hemostatic Response

Bentonite

The ability for Bentonite (2/3 weight) and a zeolite (type not specified; 1/3 weight) to act as a hemostatic agent was studied in 12 male Sprague-Dawley rats.⁵³ Another 12 rats served as controls. Approximately 8 g of the material was applied on wounded skin. Wounds were circular, full-thickness and 2 cm in diameter; skin samples were excised and evaluated stereologically after scarification. On days 12 and 21, 6 rats from the test group and 6 rats from the control group were killed. At day 12 termination, a reduction in the length density of the blood vessels (31%) and diameter of the large and small vessels (38% and 16%, respectively) was observed in the rats that received the test material. At day 21 termination, volume density of both the dermis and collagen bundles was reduced by 25% in the treated rats when compared to the controls. The researchers concluded the hemostatic agent containing Bentonite may cause vasoconstriction and inhibition of neoangiogenesis.

Other Parenteral Studies

Attapulgit

In an intratracheal study, groups of 5 rats received a single instillation of Attapulgit at 1, 5, and 10 mg.¹ One month after treatment, BAL and microscopic examination of the lungs were performed. The average length of the fibers was 0.8 µm, and 100% of the fibers were less than 3 µm. Every test animal had type A lesions. Type A lesions are characterized by an accumulation of inflammatory cells mostly macrophages, and epithelioid cells around fiber deposits. These inflammatory cells form a compact cellular infiltrate at the periphery of the deposits and some are focally dispersed throughout the alveolar region. The BAL had mostly macrophages and a small number of neutrophils at 5 and 10 mg doses. At the 5 mg dose, 3.6% of the cells were lymphocytes.

Groups of 5 male Wistar rats received 1, 5, or 10 mg of Attapulgit by transtracheal injection to examine alveolar macrophage production of interleukin-1 (IL-1) and macrophages-derived growth factor (MDGF) from fibroblasts.¹ Saline and chrysotile B asbestos were used as controls. At 1 mo, Attapulgit produced granulomas and the chrysotile B produced fibrosis. At 8 mo, the granulomatous reactions had either resolved or were greatly diminished, whereas the fibrosis persisted. Cells obtained by BAL included multinucleated giant macrophages in animals treated with Attapulgit, but not in those treated with chrysotile B. Enhanced production of IL-1 was seen in all treated groups. MDGF production was only seen in animals with lung fibrosis.

Attapulgit with a mean fiber length of 0.8 µm and diameter of 0.02 µm was delivered to the lungs of sheep by bronchoscopic cannulation.¹ The tracheal lobe of 16 sheep was subjected to a single exposure of 100 mg of Attapulgit in 100 ml of saline. A BAL was conducted at 2, 12, 24, 40, and 60 d, and necropsy was conducted on day 60. Total BAL cells, macrophages, and neutrophils, fibronectin content, and LDH and β-glucuronidase (β-GLUC) activity were examined. Nine samples of the tracheal lobe of the lung were obtained each time for microscopic examination. The controls were saline-exposed sheep and had no changes in BAL or pulmonary morphology. The total BAL cells/ml and subpopulations increased significantly above control numbers at days 12, 24, and 40 but returned to control levels by day 60. Albumin and procollagen III did not differ from controls, whereas fibronectin, LDH, and β-GLUC activities were significantly above the controls. Microscopic examination revealed infiltrates that were predominantly alveolar and peribronchial lesions. Macrophagic alveolitis with minimal airway distortion was seen. Three sheep had lesions of peribronchiolar alveolitis.

Bentonite

The ability of Bentonite to increase susceptibility to bacterial pneumonia was studied in mice.¹ The animals were injected intratracheally with 1, 10, or 100 µg Bentonite. In vivo bacterial-infectivity screening assays were conducted by exposing the animals to aerosolized Group C Streptococcus species. The severity of infection was calculated by recording the deaths of the mice over a 15-d period. Control animals were exposed to titanium dioxide. At the 100 µg dose, Bentonite increased the infectivity of the bacteria. Mortality was 85%. Even at 10 µg, Bentonite caused increased animal mortality (43.3%). Control dusts at 100 µg produced only a 5% mortality.

The effects of Bentonite dust in rats was analyzed in a 2-part intratracheal study.¹ A 0.5 mg dose of Bentonite with a mean size of 0.3 µm was instilled. Control animals were injected with sterile saline and titanium dioxide. Animals were killed at 1, 2, 6, 24, and 48 h and 4 and 7 d after instillation. Bronchopulmonary lavage was carried out and alveolar

macrophages and polymorphonuclear leukocytes were recovered. The activity of LDH and protein content of the lavage fluid were also determined. In the first experiment, a rapid influx of polymorphonuclear (PMN) leukocytes was detected at 6 h. PMN leukocyte response peaked at approximately 19×10^6 cells after instillation and started declining more slowly up to 4 d. At 7 d, the polymorphonuclear leukocyte numbers were 2.5×10^6 . The greatest increase in the numbers of alveolar macrophages recovered occurred at 4 and 7 d. The mean diameter of macrophages increased from 11.0 to 12.5 μm over the first 48 h after instillation. The mean diameter decreased at 4 and 7 d. LDH activity at 24 h was maintained at 40 milli units (mU)/ml and then increased (73 mU/ml) with the influx of polymorphonuclear leukocytes into the lungs after 48 h. Protein concentration was calculated at 500 $\mu\text{g}/\text{cm}^3$ for the first 24 h and was maintained for 48 h.

In the second experiment, after instillation of 5 mg of Bentonite, the animals were killed at 1, 7, 49, and 100 d.¹ In addition to the above measured parameters, peroxidase and lysozyme activity were also measured. A large number of polymorphonuclear leukocytes were recovered at day 1. However, the severity of the response did not differ significantly from the 0.5 mg dose. By 7 d, the numbers had decreased and were similar to control values. A significant decrease in the number of alveolar macrophages compared to controls was observed at 24 h after instillation. This decrease was followed by a sharp increase that exceeded control values by 7 d. Total number estimates were similar to those of the first experiment. LDH activity and protein concentration from Bentonite and titanium dioxide were very similar. The initial rise at day 1 following administration was short-lived. Peroxidase activity was minimal. Lysozyme activity rose sharply between 1 and 7 d, but returned to control values at 49 and 100 d.

In an intratracheal study, a single dose of 40 mg of Bentonite suspended in 1 ml of physiological saline containing 40,000 IU of crystalline penicillin was administered to male CFY rats.¹ The Bentonite composition consisted of 73% Montmorillonite, 18% cristobalite, 3% quartz, 3% feldspar, and 3% other minerals. Particle sizes were $< 2 \mu\text{m}$. The control group received 1 ml of physiological saline containing 40,000 IU of crystalline penicillin. Animals were killed 12, 24, 48, or 72 h or 90 d after exposure. Body and lung weights of the rats were measured. The right lung was fixed and sectioned for microscopic examination. The lipids and phospholipids were analyzed in the left lung. The body weights of the rats were moderately decreased and the lung weight increased 72 h after Bentonite exposure. After 90 d, the lung weight was only slightly greater than that of the control animals. Upon microscopic examination at 12 h, Bentonite exposure had resulted in a nonspecific inflammation of mostly neutrophils with perivascular edema, alveolitis, and incipient bronchopneumonia. A small number of macrophages and lymphocytes were detected. Dust particles were observed in the leukocytes and macrophages or extracellularly in the alveoli. After 24 h, bronchopneumonia was present after coalescence of the inflammatory foci; the pneumonia then became necrotizing and desquamative. Necrotic neutrophilic leukocytes and eosinophil leukocytes were observed. The reticular network collapsed between 48 and 72 h. After 90 d of exposure, Bentonite caused storage focal tissue reaction (large foamy cells with pale cytoplasm). Closely-packed cells with dark cytoplasm and nuclei were located at the periphery. After 12 and 24 h, the amount of lipids and phospholipids in the lungs was not altered. However, between 48 and 72 h, the lipid and phospholipid content increased but distribution remained the same. After 90 d, the value was the same as seen at 72 h.

In another study by the same research group, male CFY rats were given a single intratracheal dose of 60 mg of Bentonite in 1 ml of physiological saline containing 40,000 IU crystalline penicillin.¹ Bentonite particle size was less than 5 μm . Control groups received 1 ml physiological saline containing 40,000 IU penicillin. Animals were killed at the end of 72 h, weeks 2 and 4, and months 3, 6, and 12. The acid phosphatase activity and the progression of fibrosis were determined. The lungs were processed for microscopic examination and fibrosis determined by Belt and King's classification. Acid phosphatase activity was increased at 72 h and had returned to normal by the first month. Loose reticulin fibrils, but no collagen, were observed after months 1 - 12.

Bentonite dust was administered intratracheally as a single 60-mg dose to Sprague-Dawley rats.¹ The animals were killed 3, 6, and 12 mo after exposure. The right lung was studied microscopically and the lipids, phospholipids, and hydroxyproline values were determined. Significantly greater phospholipid values compared to controls were observed. Among the phospholipid fractions, the greatest quantitative increase was seen in phosphatidylcholine (more than twice the control) and the smallest increase was seen in phosphatidylethanolamine (less than 1.6 times). After 6 and 12 mo, the values were similar. Lung lipids had a greater range of values than did the phospholipids (no details given). The wet weight of the lung in grams increased in 5% to 10% Bentonite-treated rats compared to controls at month 3. No difference was detected at 6 and 12 mo. Hydroxyproline content of treated rats (mg/g lung wet weight) was very similar to controls at 3, 6, and 12 mo.

Subplantar injections of 0.05 ml of a 5% solution of Bentonite were given to male Wistar rats.¹ The rats either received Bentonite injections in both hind paws at an interval of 24 h, or their left paw was injected with Bentonite and their right paw injected with 0.05 ml of a 10% solution of Kaolin (control). Subcutaneous Bentonite granulomas were produced on the left side, both dorsally and ventrally. Simultaneously, Kaolin granulomas were produced on the right side analogous to the Bentonite injection. Sodium salicylate and prednisone suppressed the Bentonite edema during the first 24 h. The presence of mononuclear cells was confirmed.

Kaolin

The ability of Kaolin to increase susceptibility to bacterial pneumonia was studied in mice.¹ The animals were injected intratracheally with 100 µg Kaolin. In vivo bacterial infectivity screening assays were conducted by exposing the animals to aerosolized Group C Streptococcus species. The severity of infection was calculated by recording the deaths of the mice over a 15-d period. Control animals were exposed to titanium dioxide. A 100-µg dose of Kaolin caused statistically significant but modest (< 50%) increased death due to infection by a large dose. Mortality was calculated at 38.9%. Control dusts at 100 µg produced only a 5% increase in mortality.

DERMAL IRRITATION AND SENSITIZATION

In vitro, animal, and human dermal irritation, sensitization, and photoallergy studies are summarized in Table 5.

A formulation containing 38% Montmorillonite was predicted to be non-irritating in an EpiDerm™ skin model when tested neat.⁵⁴ Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) was not irritating to rabbit skin when tested at 500 mg in distilled water.³⁸ A formulation containing 1.75% Bentonite was not irritating to 25 human subjects in a 14-d cumulative irritation assay, nor was a mud mask containing 8% Bentonite irritating in a single-insult occlusive patch test in 19 subjects.^{55,56} No visible irritation was observed in a 4-wk clinical use test (50 subjects) of a facial cleanser containing 2% Bentonite and 2% Kaolin; however, some subjects reported perceived discomfort and/or irritation.⁵⁷

A formulation containing 38% Montmorillonite was predicted to be non-sensitizing in a KeratinoSens™ assay.⁵⁴ No sensitization was observed in guinea pig studies of 50% Hectorite (Buehler test, details not provided) or of Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite; intradermal induction at 5%; no further information on dosing for topical induction or challenge provided).^{11,38} Dermal sensitization was not reported in human repeated insult patch tests (HRIPTs) with a foot mask containing 3.5% Bentonite (102 subjects), a clay mask containing 3.8% Bentonite (108 subjects), or in a face cream containing 7.5% Bentonite (52 subjects).⁵⁸⁻⁶⁰ No sensitization was observed in HRIPTs with a lip product containing 14.5% Kaolin (54 subjects) or a clay mask containing 40% Kaolin (51 subjects); however, one subject in a study of a clay mask containing 14.5% Kaolin (103 subjects) had moderate erythema progressing to erythema and edema with papules through the induction and challenge phase.⁶¹⁻⁶³ A sunscreen with 1.75% Bentonite was not a photosensitizer in 23 human subjects.⁶⁴

Hectorite

A primary irritation study patterned after the Draize method was conducted using 6 white rabbits.¹ Either a 0.5 ml or a 0.5 g sample of Hectorite was applied to two sites, one on abraded skin, and the other on intact skin of the backs of the rabbits. The test sites were occluded for 24 h. At the end of the 24 h, the binders were removed and the sites were gently wiped clean. One-half hour later, the sites were examined and scored for erythema and edema. The sites were examined again at 72 h. The average score was 0.0. Hectorite was nonirritating to the skin of rabbits.

OCULAR IRRITATION STUDIES

Animal

Clay

In an ocular irritation study performed in accordance with OECD TG 405, 100 mg of Clay (75% Illite, 16% Kaolin, and 9% Montmorillonite) was instilled into one eye of rabbits.³⁸ The other eye served as a control and was instilled with 0.1 ml normal saline. No adverse effects were noted following treatment up to 72 h after instillation. The test material was considered to be non-irritating to rabbit eyes. No further details were provided.

Hectorite

In a primary eye irritation study using 9 New Zealand white rabbits, a 0.1 ml liquid or semisolid (100 mg of the solid) sample was instilled into the one eye of each rabbit.¹ The eyes of 6 rabbits were not rinsed, and the eyes of 3 rabbits were rinsed approximately 4 s. All untreated eyes served as controls. The eyes were then examined with sodium fluorescein and an ultraviolet lamp at 24, 48, and 72 h and at 7 d. The mean score at 24 h was 2.0. All subsequent scores were 0.0. The test sample was considered moderately irritating to rabbit eyes without rinsing and practically nonirritating to the eyes with rinsing 4 s after instillation.

Kaolin

The potential for ocular irritation from a clay mask with 14.5% Kaolin was investigated in a tissue equivalent assay with EpiOcular™ cultures.⁶⁵ The EpiOcular™ human cell constructs were exposed to 100 µl test material under standard culture conditions for 4, 8, 16, and 24 h. Tissue viabilities were then examined by MTT assay. The duration of exposure resulting in a 50% decrease of tissue viability (t₅₀) was calculated to be 5.2 h (at 4 h, tissue viability was 63.4%). The positive control yielded expected results. As residual test article may bind to the tissue and result in a false MTT reduction signal, a freeze-killed tissue control was used, and calculations were performed to correct for the amount of MTT reduced directly by the test article residues in the tissues. The clay mask with 14.5% Kaolin was predicted not to be an ocular irritant in this assay.

CLINICAL STUDIES

In a study of total pulmonary non-asbestos mineral content in lung tissue from 20 individuals with no occupational dust exposure, Attapulgite and Kaolin were identified in 12 individuals.¹ No correlations were made between numbers or types of fibers and age, sex, or smoking. Approximately 8400 out of 106,000 fibers (7.9%) were identified as Attapulgite and approximately 3500 out of 106,000 fibers (3.3%) were identified as Kaolin. Mineralogical analysis found that 100% of the Attapulgite fibers and 94% of the Kaolin fibers were 1 - 4.9 μm in length.

Oral

Montmorillonite

The effect of oral ingestion of Montmorillonite on protection against the adverse effects of the ingestion of aflatoxins were studied in 23 male and 27 female human subjects.⁶⁶ The subjects received 1.5 g/d or 3.0 g/d in capsules. A total of 9 capsules were ingested over a 2-wk period. The study was randomized and double-blinded. Blood and urine samples were collected before and after the study. Mild gastrointestinal effects were reported with no statistical significance found between the treatment groups. No significant differences in hematology, liver and kidney function, or electrolytes were reported in either group.

Case Reports

Bentonite

Several case studies involving Bentonite workers have been reported.¹ Some milling plants had dangerous concentrations of silica that ranged from 2 to 10 times the safe maximal concentration according to the US Bureau of Mines. Silicotuberculosis developed in four patients studied.

Fuller's Earth

A patient that reported working no more than 15 yr in a Fuller's Earth plant as a young man was diagnosed with terminal aspiration pneumonia, pneumoconiosis due to Fuller's Earth exposure, bilateral emphysema, and fibrous pleural adhesions.¹ The lesions differed from typical silicotic lesions of the lungs; no formations of the whorled, acellular collagen typical of silicotic nodules were observed. Isolated cavities in the apices were filled with black sludge and surrounded by vascular and cellular collagen. The dust in the lymph nodes had only stimulated the formation of reticulin fibers. No subpleural nodules were present. At mineralogical analysis, the Fuller's Earth deposits were constituted mainly of Montmorillonite (85.2% to 90%).

Two additional cases of pneumoconiosis in employees that worked in processing or milling Fuller's Earth for at least 28 yr were reported.¹

Kaolin

A patient was reported with multiple pulmonary Kaolin granulomas.¹ The man had a history of bilateral recurrent pneumothorax. Both pleural spaces were destroyed with a suspension of liquid Kaolin. Recurrent right-sided pneumothorax devolved and reobliteration was again performed. In a follow-up chest radiograph, multiple well-defined peripheral nodules were in both lungs and pathological analysis revealed a bland acellular material surrounded by chronic inflammatory cells. By light microscopy, the particles were consistent with Kaolin. It was presumed that Kaolin entered the lungs through pleuroalveolar or pleurobronchial openings.

In another investigation, the death of a 62-yr-old man who worked in a cotton textile mill for 43 yr was reported.¹ The patient complained of progressive dyspnea and a productive cough. After being admitted to the hospital, a bronchoscopy was performed and no endobronchial lesions were found. A lung biopsy had lesions of severe interstitial fibrosis with bronchioalveolar structures extensively involved in the fibrotic process. Pathological alterations such as bronchiectasis, interstitial fibrosis with thickening of alveolar septa, mobilization of macrophages, and multinucleated giant cells were identified. Neither ferruginous bodies nor pleural hyaline plaque was identified. Kaolin particles were present with a mean size of 0.88 μm . Chrysotile asbestos was also detected, but the majority of particles were Kaolin. The man died as a consequence of respiratory failure despite an aggressive therapy of antibiotics and tuberculosis therapy.

The lungs and chest x-ray films were evaluated in a pair of case studies of men who worked in a Kaolin-processing plant for many years.¹ The first case was a 36-yr-old man who worked on the plant for 17 yr. Chest films were taken at the end of his career and detected lesions of extensive confluent consolidation and nodule formation of advanced pneumoconiosis with infection. Autopsy and microscopic findings included alveolar spaces uniformly expanded, three areas of whorled fibrous tissue, scattered areas of cystic spaces, hilar nodes heavily pigmented, deposits of brownish black particulate matter, a large vessel with recent thrombus, hemorrhage, and necrosis, marked fibrous thickening of the pleura, and dense fibrous scarring of the lymph nodes. The final diagnosis was pneumoconiosis (kaolinosi) with pulmonary thrombosis and infarction of the lungs. The second case study was a 35-yr-old man who worked in the Kaolin-processing plant for 21 yr. Within his last 3 yr, he had dyspnea and a slight cough with small amounts of dark colored sputum. The sputum was negative for bacteria. Chest films revealed advanced pneumoconiosis with infection, confluent consolidation, nodular infiltration, cavitation, and emphysema. Autopsy and microscopic findings included nodules in the right and middle

lobes, pleural spaces were thickened and shaggy, large bulbous emphysematous blebs, a pulmonary artery with organizing thrombus, heavily pigmented hilar lymph nodes, whorled fibrous collagenous tissue, and spaces and walls with macrophages. The final diagnosis was pneumoconiosis (kaolinosis).

A 35-yr-old man who worked at a Kaolin-processing plant for 17 yr presented with chest pain and was hospitalized.¹ For the previous 2 yr before admittance, the man had packaged dried, processed Kaolin. Chest films revealed diffuse reticulonodular pulmonary infiltrates and a well-defined, noncalcified mass in the upper right lobe. A thoracotomy was performed and an 8 cm x 12 cm x 10 cm conglomerate pneumoconiotic lesion containing large amounts of Kaolin was found. X-ray diffraction material from the lesion had peaks corresponding to Kaolinite. The presence of silica was not confirmed by x-ray diffraction.

Pulmonary tissue was obtained from 5 Kaolin workers with advanced pneumoconiosis.¹ Chest radiographs detected small irregular shadows and large opacities typical of Kaolin pneumoconiosis. At autopsy, firm, grey-brown nodules and masses were in the parenchyma and in the hilar lymph nodes. Microscopic lesions were extensive pulmonary Kaolinite deposition associated with the formation of peribronchiolar nodules. The nodules were composed of Kaolinite aggregates transversed by bands of fibrous tissue rather than dense whorled collagen. Kaolin was detected in the lungs. Silica was not detected by either analytical scanning electron microscopy or x-ray diffractometry.

Six additional cases of pneumoconiosis in employees of 12 yr in Kaolin processing or milling facilities were reported.¹

Montmorillonite

A 73-yr-old Montmorillonite worker developed signs of pneumoconiosis, but subsequently died of acute gastrointestinal hemorrhage from a benign gastric ulcer.¹ A chest radiograph taken 2 yr before his death showed a bilateral fine reticulonodular shadowing, while another radiograph taken a few weeks before his death indicated a slight increase in the reticulonodular opacities and a mass at the left hilum and apex. At autopsy, numerous soft stellate grey-black dust lesions 4 - 5 mm in diameter were observed occupying most of the lungs. No lesions of progressive massive fibrosis were identified. Also present were lesions of severe emphysema and a 4 cm diameter neoplasm arising from the bronchus of the left upper lobe. At microscopic examination, numerous interstitial collections of dust-laden macrophages were situated around the respiratory bronchioles and along the adjacent alveolar septa. A slight degree of fibrosis associated with the dust lesions was observed, and the neoplasm was a poorly differentiated adenocarcinoma containing giant cell areas. Mineralogical analysis showed a large amount of calcium Montmorillonite.

OCCUPATIONAL EXPOSURE

Attapulgit

A cohort of 2302 men employed for at least 1 mo between January 1, 1940 and December 31, 1975 at an Attapulgit mining and milling facility was studied.¹ A significant deficit of mortality from nonmalignant respiratory disease was observed based on age, calendar year, and rates. A marked deficit of nonmalignant respiratory disease was seen regardless of presumed dust exposure level, induction-latency period, or duration of employment. A statistically significant excess of mortality from lung cancer was observed among whites, but a deficit occurred among nonwhites. Lung-cancer risk in either race was not altered substantially with presumed dust exposure level, induction-latency period, or duration employed, with one exception—those employed for at least 5 yr in high-exposure level jobs. An increased mortality was observed for gastric cancer (6 observed) and a deficit due to nonmalignant respiratory disease was observed (9 observed).

Kaolin

A study was performed on the prevalence of ventilatory impairment, chest symptoms, and radiographic abnormalities in over 2000 Kaolin workers representing over 95% of the employees in the industry at the time.¹ Of the participants, 19% admitted having a cough. Of those participants with a cough, 17% had an abnormal forced expiratory volume and 14% had an abnormal vital capacity. Of those without a cough, 5.5% had an abnormal forced expiratory volume and 7% had an abnormal vital capacity. Also, 18% of the participants admitted to chronic sputum production. Of those with sputum production, 16% had abnormal forced expiratory volume, and 12.5% had abnormal vital capacity. Of those without sputum production, 6% had an abnormal forced expiratory volume, and 7.5% had an abnormal vital capacity. About 30% of the participants complained of shortness of breath, 3.1% of the cases were classified as severe. Wheezing was reported by 29% of the subjects. Satisfactory chest films for 2069 of the subjects were available for examination. Radiographic findings of 90 subjects revealed simple pneumoconiosis. Eighteen subjects (0.89%) had complicated pneumoconiosis. Of men with either case of pneumoconiosis, 51.1% were dry processors, compared to 6.3% of the men who worked in wet processing. Of the nonsmoking participants (549), 542 and 537 men had a satisfactory forced expiratory volume and forced vital capacity, respectively, in addition to an acceptable chest radiograph. Of these nonsmoking workers, 516 were studied for dust exposure and pulmonary function. Among the nonsmokers with no pneumoconiosis, those persons working in calcined clay had a greater prevalence of lung function abnormalities. This group had a significant increase in the risk of having an abnormal forced expiratory volume but tended to have less incidences of pneumoconiosis. In short, ventilatory impairment was related to the presence of complicated pneumoconiosis, employment in clay calcining, and cigarette smoking. Also work in dry processing was associated with a greater risk of developing pneumoconiosis.

The lungs of 62 recently deceased men between the years of 1968 to 1981 were taken for an assessment of the severity of lung disease.¹ Fifty-four of the 62 men worked with Kaolin or related kaolinized mineral stone. All the test subjects were employed in the mining industry. Chest radiographs were available for 39 of the 62 cases. Sections of lung tissue were examined microscopically for nodular and interstitial fibrosis and an overall grade ranging from 0 (none) to 3 (severe). Samples from 42 cases were analyzed for mineral content by x-ray diffraction and lung-dust concentrations. Radiographic lesions included 13 cases of progressive massive fibrosis and 22 cases of simple pneumoconiosis. Only four cases had no evidence of any disease. Nodular opacities tended to reflect a high quartz content, whereas high-Kaolin lung content had interstitial changes and irregular radiological changes. An increasing quartz concentration appears to be related to nodular fibrosis. The degree of interstitial fibrosis appeared to be more related to dust lung concentrations, although these results failed to reach statistical significance.

The Occupational Safety and Health Administration (OSHA) lists the following permissible exposure limit (PEL) for 8 h work shifts for Kaolin: total dust - 15 mg/m³ and respirable fraction - 5 mg/m³.^{67,68} The National Institute for Occupational Safety and Health (NIOSH) lists the following recommended exposure limit (REL) for up to 10 h time weighted average for Kaolin: total dust - 10 mg/m³ and respirable fraction - 5 mg/m³.

Bentonite

In a toxicological and occupational epidemiological review of Bentonite, the authors concluded Bentonite is probably not more toxic than any other inert insoluble dusts.⁶⁹ However, because some forms may contain variable amounts of respirable crystalline silica, prudent management and adherence to occupational exposure limits is appropriate.

SUMMARY

This report assesses the safety of 8 clay ingredients as used in cosmetics. All of these ingredients are reported to function as absorbents and bulking agents; other cosmetic functions are also reported. The Panel previously reviewed the safety of Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, and Montmorillonite in a report that was published in 2003. In that report, the Panel concluded that these ingredients were safe as used in cosmetic ingredients. In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 yr, and it has been at least 15 yr since this assessment has been issued.

According to 2023 VCRP survey data, Kaolin has the most reported uses in cosmetic products, with a total of 787; the majority of uses are in leave-on formulations. Bentonite has the second most reported uses in cosmetic products, with a total of 221; a little more than half are reported in leave-on formulations. The frequencies of use for both of these ingredients have greatly changed since the original safety assessment was finalized; in 1998, Kaolin was reported to have 509 uses and Bentonite was reported to have 94. The results of concentration of use surveys conducted by the Council in 2022 indicate Kaolin also has the highest maximum concentration of use in leave-on formulations; it is used at up to 53.2% in manicuring preparations. For leave-on dermal preparations, specifically, Kaolin also has the highest reported maximum concentration of use, at 16% in face and neck products, and Bentonite has the next highest, at 8% in face and neck preparations. According to the original safety assessment, the maximum leave-on use concentration in 1999 for Kaolin was 100% in skin care preparations; the maximum leave-on use concentration for Bentonite was 8% in makeup foundations.

Several of these clay ingredients are GRAS as direct and/or indirect food additives. Kaolin is also an approved OTC drug.

Ex vitro bioavailability studies were performed using human skin models. Trace heavy metals in 3 clay pastes (white Montmorillonite, Kaolin, and Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite)) did not penetrate cutaneous tissue.

In acute dermal and oral toxicity studies in rats, Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) had an LD₅₀ greater than 2000 mg/kg. The same product was tested in an acute inhalation study in rats and had an LC₅₀ greater than 3.856 mg/l. Inhalation studies in rats with an environmental dust sample composed of approximately 75% of Illite, Montmorillonite, and Kaolin and approximately 20% α -quartz demonstrated that the majority of particles were deposited at the first alveolar duct bifurcations, and at 24-h later, numerous particles had been ingested by alveolar macrophages. Rats instilled intratracheally with the same dust developed a multifocal interstitial lung disease.

Nano-sized Kaolin (primary particle size 4.8 μ m) instilled intratracheally in mice (single and multiple (4) instillations) produced diffuse alveolar macrophages containing Kaolin in the lungs. Focal granulomatous formation, with or without alveolar macrophages containing Kaolin, were also frequently observed in the lungs of mice that received multiple instillations. Similar observations were made in mice that received a single instillation, but with a slight degree of particle accumulation and granuloma formation in the lungs. No abnormalities were observed in the kidneys.

In a 90-d oral study in male rats, a modified Montmorillonite clay at 40 mg/kg/d did not cause any deaths during treatment, and no significant changes were noted in clinical biochemistry, organ weights, or in histopathological examinations when compared to controls. As an anti-caking agent in animal feed, calcium Montmorillonite clay produced non-dose-dependent significant changes in mean corpuscular hemoglobin, serum calcium, serum vitamin A, and serum iron when tested at up to 2.0% w/w in rats in a dietary study; however, no adverse effects were noted in feed consumption, body weight, organ weights, or in gross or histopathological exams.

Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite; 5000 µg) was not mutagenic in an Ames test, with or without metabolic activation. Unmodified Montmorillonite clay (at up to 125 µg/ml) and one type of cation-exchanged Montmorillonite clay (at up to 250 µg/ml) also were not mutagenic in an Ames test with or without metabolic activation, but significant increases in revertant colonies were observed in one strain with metabolic activation in 2 other cation-exchanged Montmorillonite clays. No mutagenic activity was observed in a *Salmonella*/microsome assay with and without metabolic activation when tested in Montmorillonite and cation-exchanged montmorillonite in both nano- and non-nano-sized material at up to 141 µg/ml and up to 14.1 µg/plate, respectively. However, the cation-exchanged Montmorillonite material (both nano- and non-nano-sized, at up to 226 µg/ml and 170 µg/ml, respectively) in this study was genotoxic in a concentration-related manner in a Comet assay with Caco-2 cells. Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cell cultures when tested at up to 5000 µg/ml, with or without metabolic activation. Micronucleus induction was observed in a dose-dependent manner to micro- and nano-sized Kaolin in CHO AA8 and primary normal human diploid epidermal keratinocytes and fibroblasts, with fine particles having a higher genotoxic potency than coarse particles. A 4-fold increased frequency of micronucleated cells was observed in human lung cancer A549 cells following exposure to nano-sized Kaolin. Statistically significant increases in the frequency of micronuclei were induced by Montmorillonite clay at 62.5 µg/ml in a cytokinesis block micronucleus cytome assay in human hepatoma cell lines, but this effect was not observed at a concentration of 31.25 µg/ml or lower. No effects in nucleoplasmic bridges or nuclear buds were observed at any concentration in this study. In an in vitro micronucleus assay and kinetochore analysis using human lung fibroblasts, the genotoxic potential of Bentonite at up to 15 µg/cm² was determined to be generally low, but could be altered by the content of quartz and available transition metals. In an in vitro Comet assay with micro- and nano-sized Kaolin in CHO AA8 and primary normal human diploid epidermal keratinocytes and fibroblasts, the test materials promoted DNA damage in a dose-dependent manner, with greater DNA-damaging potency in the nano-sized Kaolin than in the micro-sized Kaolin.

In an in vivo Comet assay with nano-sized Kaolin intratracheally instilled in mice, DNA damage was induced at 0.2 mg/mouse but not at 0.05 mg/mouse after 3 h exposure. No difference in induction was observed after 24 h exposure compared to the 3 h exposure. Increased *gpt* and Spi- mutant frequencies were observed in the lungs of the mice following intratracheal instillation with either single or multiple doses of 0.2 mg nano-sized Kaolin. A mutation spectra analysis showed > 60% of G:C to C:G transversion occurred in the *gpt* genes. In another Comet assay, rats were given 2 oral doses of up to 1000 mg/kg bw cation-exchanged montmorillonite clay. There was no statistically significant difference in % tail DNA between the negative controls and the different treatment groups for any of the cells (liver, kidneys, colon) tested.

IARC has determined there is inadequate evidence in humans for the carcinogenicity of Attapulgit. Further, IARC has determined there is insufficient evidence in experimental animals for the carcinogenicity of short Attapulgit fibers (< 5 µm); however, there is sufficient evidence in experimental animals for the carcinogenicity of long Attapulgit fibers (> 5 µm). Overall, long Attapulgit fibers (> 5 µm) are possibly carcinogenic to humans (Group 2B) and short Attapulgit fibers (< 5 µm) cannot be classified as to its carcinogenicity to humans (Group 3). Attapulgit (palygorskite fibers > 5 µm in length) is listed by California Proposition 65 as a carcinogen.

The ability for Bentonite (2/3 weight) and a zeolite (type not specified; 1/3 weight) to act as a hemostatic agent was studied in 12 male Sprague-Dawley rats. This hemostatic agent may cause vasoconstriction and inhibition of neoangiogenesis. Illite and Montmorillonite were observed to have protective effects on cytotoxicity induced by mycotoxins in MTT and LDH assays. An environmental dust sample composed of approximately 75% of Illite, Montmorillonite, and Kaolin and approximately 20% α-quartz induced LDH release from alveolar macrophages of rats, and showed hemolytic effects on human red blood cells. Cytotoxicity results of the CCK-8 assay showed the 16HBE cells had high viability after exposure to 40 µg/ml nano-sized Kaolin, but cell viability decreased significantly at doses greater than 80 µg/ml. A LDH assay indicated that nano-sized Kaolin caused membrane disruption in a dose-dependent manner.

A formulation containing 38% Montmorillonite was predicted to be non-irritating in an EpiDerm™ skin model when tested neat. Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) was not irritating to rabbit skin when tested at 500 mg in distilled water. A formulation containing 1.75% Bentonite was not irritating to 25 human subjects in a 14-d cumulative irritation assay, nor was a mud mask containing 8% Bentonite irritating in a single-insult patch test in 19 subjects. No visible irritation was observed in a 4-wk clinical use test (50 subjects) of a facial cleanser containing 2% Bentonite and 2% Kaolin; however, some subjects reported perceived discomfort and/ or irritation.

A formulation containing 38% Montmorillonite was predicted to be non-sensitizing in a KeratinoSens™ assay. No sensitization was observed in guinea pig studies of 50% Hectorite (further dosing information not provided) or Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite; intradermal induction at 5%, no further information on dosing for topical induction or challenge provided). Dermal sensitization was not reported in HRIPTs with a foot mask containing 3.5% Bentonite (102 subjects), a clay mask containing 3.8% Bentonite (108 subjects), or in a face cream containing 7.5% Bentonite (52 subjects). No sensitization was observed in HRIPTs with a lip product containing 14.5% Kaolin (54 subjects) or a clay mask containing 40% Kaolin (51 subjects); however, one subject in a study of a clay mask containing 14.5% Kaolin (103 subjects) had moderate erythema progressing to erythema and edema with papules through the induction and challenge phase. A sunscreen with 1.75% Bentonite was not a photosensitizer in 23 human subjects.

A clay mask with 14.5% Kaolin was predicted to not be an ocular irritant in a tissue equivalent assay with EpiOcular™. In an ocular irritation study in rabbits, Clay (75% Illite, 19% Kaolin, and 6% Montmorillonite) produced no adverse effects and was considered to be non-irritating.

Nine capsules containing Montmorillonite (up to 3.0 g/d) were administered to 50 human subjects over a 2-wk period. Only mild gastrointestinal effects were reported.

OSHA lists the following PEL for 8-h work shifts for Kaolin: total dust - 15 mg/m³ and respirable fraction - 5 mg/m³. NIOSH lists the following REL for up to 10-h time weighted average for Kaolin: total dust - 10 mg/m³ and respirable fraction - 5 mg/m³. In a toxicological and epidemiological review of Bentonite, the authors concluded Bentonite is probably not more toxic than any other particulate. However, because some forms may contain variable amounts of respirable crystalline Silica, prudent management and adherence to occupational exposure limits is appropriate.

DISCUSSION

In 2003, the Panel published a final report that included Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, and Montmorillonite, and concluded that the ingredients named in that report were safe as used in cosmetic products. In accordance with its Procedures, the Panel re-evaluates the conclusions of previously-issued reports approximately every 15 years, and when appropriate, additional ingredients are included in the resulting re-review. Accordingly, the Panel re-reviewed the safety of these 6 clays, with the addition of 2 related ingredients (Clay and Illite), and concluded that the available data are sufficient to determine that Kaolin is safe in cosmetics in the present practices of use and concentration as described in this safety assessment. Furthermore, the Panel concluded that the remaining 7 naturally-sourced clay ingredients are safe in cosmetics in the present practices of use and concentration, except for those products that may be incidentally inhaled, for which the available data are insufficient.

The Panel expressed concern regarding heavy metals that may be present in these ingredients. Heavy metals associated with clay ingredients did not penetrate the skin. The Panel stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit these impurities in cosmetic formulations.

The Panel was made aware that nanoforms of clay ingredients could potentially be used in cosmetic formulations, including those that could result in incidental ingestion (e.g., lipstick and toothpaste; categories of sprayable products were not reported based on current available data). However, use of nanoform ingredients is unlikely to translate into nanoparticle form within final formulations under in-use conditions (or under in-use exposure scenarios). In these formulations, low concentrations of use (e.g., maximum reported use concentration of Kaolin in lipstick is 14.5%) would limit exposure, and in addition, processing would be expected to result in much larger particle sizes (by, for example, agglomeration) in the consumer product.

Additionally, some naturally-sourced clay ingredients were reported to be used in spray and powder products that could possibly be inhaled. For example, Bentonite is reported to be used at 0.9% in spray suntan products and Kaolin is reported to be used at up to 15% in face powders. For Kaolin, the data available from inhalation studies, including acute, chronic, and carcinogenicity data, suggest little potential for adverse respiratory effects at relevant doses for this naturally-sourced clay ingredient. These data have mitigated the concern of the use of Kaolin in cosmetic products which may be incidentally inhaled.

Conversely, the data are insufficient to determine the safety of the remaining 7 naturally-sourced clays for use in formulations which may be incidentally inhaled. The Panel noted that Bentonite and Hectorite may contain crystalline silica (cristobalite), which is a human carcinogen, as an impurity. Furthermore, the Panel noted that Attapulgite with long fibers (> 5 µm) is possibly carcinogenic to humans and animals. The additional data needed to determine safety of these 7 ingredients for such use are composition and impurities data, especially quantification of crystalline silica, and negative repeated-dose inhalation data on naturally-sourced clay ingredients.

The Panel's respiratory exposure resource document (<https://www.cir-safety.org/cir-findings>) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Therefore, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Kaolin is safe in cosmetics in the present practices of use and concentration described in this safety assessment. The Panel also concluded that the following 7 ingredients are safe in cosmetics in the present practice of use and concentration described in this safety assessment, with the exception that the available data are insufficient to make a determination of safety for these ingredients in products that may be incidentally inhaled.

Attapulgite
Bentonite
Clay
Fuller's Earth

Hectorite
Illite
Montmorillonite

TABLES

Table 1. Definitions and reported cosmetic functions of the ingredients in this safety assessment.²

Ingredient, CAS No.	Definition	Reported Functions
Attapulgit 12174-11-7 1337-76-4	Attapulgit is a variety of Fuller's Earth found typically near Attapulgas, Georgia. It is characterized by having a chain structure rather than the usual sheet structure of other clay minerals.	Abrasives; Absorbents; Bulking Agents; Opacifying Agents; Viscosity Increasing Agents - Aqueous
Bentonite 1302-78-9	Bentonite is a native hydrated colloidal aluminum silicate clay.	Absorbents; Bulking Agents; Dispersing Agents - Nonsurfactant; Emulsion Stabilizers; Opacifying Agents; Viscosity Increasing Agents - Aqueous
Clay 53801-44-8	Clay is a group of phyllosilicate minerals produced by the chemical and physical weathering of rock. It consists chiefly of varying amounts of hydrated silica and alumina, and is characterized by a particle size of less than 2 micrometers.	Absorbents; Binders; Bulking Agents; Skin-Conditioning Agents - Misc.; Viscosity Increasing Agents - Aqueous
Fuller's Earth 8031-18-3	Fuller's Earth is a non-plastic variety of kaolin containing an aluminum magnesium silicate.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents
Hectorite 12173-47-6 68084-71-9	Hectorite is one of the montmorillonite minerals that are the principal constituents of bentonite clay.	Absorbents; Bulking Agents; Dispersing Agents - Nonsurfactant; Opacifying Agents; Viscosity Increasing Agents - Aqueous
Illite 12173-60-3	Illite refers to a group of clay sized micas that have a higher lattice water content and lower potassium content than mica.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents
Kaolin 1332-58-7	Kaolin is a native hydrated aluminum silicate with an approximate composition of $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$.	Abrasives; Absorbents; Anticaking Agents; Bulking Agents; Opacifying Agents; Skin Protectants; Slip Modifiers
Montmorillonite 1318-93-0	Montmorillonite is a complex aluminum/magnesium silicate clay.	Abrasives; Absorbents; Bulking Agents; Emulsion Stabilizers; Opacifying Agents; Viscosity Increasing Agents - Aqueous

Table 2. Chemical properties

Property	Value	Reference
<i>Attapulgite</i>		
Physical Form	White, gray, or transparent, dull, elongated, lath-shaped crystals in bundles that comprise thin sheets of minute interlaced fibers; surface is protonated and hydrated	1
Chemical Formula	$\text{Mg}(\text{Al}_{0.5-1}\text{Fe}_{0-0.5})\text{Si}_4\text{O}_{10}(\text{OH}) \cdot 4\text{H}_2\text{O}$	1
Density (g/ml)	2.2	1
Solubility	Insoluble in water	1
Particle Size/Length (μm)	< 5	1
<i>Bentonite</i>		
Physical Form	Crystalline, claylike material, available as an odorless, pale buff or cream to grayish-colored fine powder, which is free from grit; dioctahedral	1
Chemical Formula	$\text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 \cdot 2\text{H}_2\text{O}$	1
Formula Weight (Da)	359.16	1
Solubility	Insoluble in water, alcohol, fixed oils, glycerin, dilute acid, and alkali solutions	1,7
Particle Size/Length (μm)	Mainly 50-150 with some 1-2	1
<i>Clay</i>		
Physical Form	Beige green powder	9
pH	Between 8 and 9	9
Grain size	Variation 1: 80% < 5 μm ; 100% < 10 μm Variation 2: 90% < 20 μm ; 100% < 40 μm Variation 3: 90% < 77 μm ; 100% < 100 μm Variation 4: 90% < 750 μm ; 100% < 1100 μm	9
<i>Fuller's Earth</i>		
Physical Form	Non-plastic variety of Kaolin; sheet structure	1
<i>Hectorite</i>		
Physical Form	Translucent colorless mineral when mined and turns white when dried; tridecahedral	1
Chemical Formula	$\text{Na}_{0.33}(\text{Mg}_{2.67}\text{Li}_{0.33})\text{Si}_4\text{O}_{10}(\text{OH})_2$	1
Specific Gravity (g/ml)	2.65	1
<i>Illite</i>		
Physical Form	Gray-white to silvery-white, greenish-gray claylike material; waxy, greasy, earthy, or dull luster; dioctahedral	8
Chemical Formula	$\text{K}_{0.65}\text{Al}_{2.0}[\text{Al}_{0.65}\text{Si}_{3.35}\text{O}_{10}](\text{OH})_2$	8
Specific Gravity (g/ml)	2.79 - 2.80	8
<i>Kaolin</i>		
Physical Form	White or yellowish white, earthy mass or white powder; unctuous when moist	1
Chemical Formula	$\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$	1
Formula Weight (Da)	258.2	1
Solubility	Insoluble in water, alcohol, dilute acids, and alkali solutions	1,7
<i>Montmorillonite</i>		
Chemical Formula	$\text{R}^{+}_{0.33}(\text{Al}, \text{Mg})_2\text{Si}_4\text{O}_{10}(\text{OH})_2$, where $\text{R}^{+} = \text{Na}^{+}, \text{K}^{+}, \text{Mg}^{2+}$ or Ca^{2+}	1

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
	Attapulgite				Bentonite			
Totals	1	10	NR	8	221	94	0.00025-17.3	0.5-80
summarized by likely duration and exposure*								
Duration of Use								
Leave-On	1	5	NR	8	110	37	0.00025-8	0.8-8
Rinse-Off	NR	5	NR	8	98	57	0.22-17.3	0.5-80
Diluted for (Bath) Use	NR	NR	NR	NR	13	NR	NR	5
Exposure Type**								
Eye Area	1	NR	NR	NR	20	8	0.00025-4.5	0.8-5
Incidental Ingestion	NR	NR	NR	NR	8	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	8 ^b	14 ^a ; 40 ^b	5 ^a ; 7 ^b	0.9	1-3 ^a ; 2-5 ^b
Incidental Inhalation-Powder	NR	5	NR	8 ^b	2; 1 ^c ; 40 ^b	7 ^b	8 ^c	2-5 ^b
Dermal Contact	1	10	NR	8	185	88	0.00025-10	0.5-80
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	6	4	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	17.3	NR
Nail	NR	NR	NR	NR	12	1	2.8	1
Mucous Membrane	NR	NR	NR	NR	29	3	0.22-4.7	0.5-5
Baby Products	NR	NR	NR	NR	3	NR	NR	NR
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams					1	NR	NR	NR
Other Baby Products					2	NR	NR	NR
Bath Preparations (diluted for use)								
Bath Oils, Tablets, and Salts					11	NR	NR	5
Bath Capsules								
Other Bath Preparations					2	NR	NR	NR
Eye Makeup Preparations								
Eyebrow Pencil								
Eyeliners					4	6	0.00025	5
Eye Shadow					2	NR	4.5	NR
Eye Lotion					2	NR	NR	NR
Eye Makeup Remover					1	NR	NR	NR
Mascara					10	1	1.5	0.8
Other Eye Makeup Preparations	1	NR	NR	NR	1	1	NR	NR
Fragrance Preparations								
Powders (dusting/talcum, excl aftershave talc)	NR	5	NR	NR				
Hair Preparations (non-coloring)								
Hair Conditioner					NR	1	NR	NR
Hair Sprays (aerosol fixatives)								
Hair Straighteners					NR	3	NR	NR
Rinses (non-coloring)								
Shampoos (non-coloring)								
Tonics, Dressings, and Other Hair Grooming Aids					4	NR	NR	NR
Wave Sets								
Other Hair Preparations					2	NR	NR	NR
Hair Coloring Preparations								
Hair Tints								
Hair Shampoos (coloring)								
Hair Lighteners with Color								
Hair Bleaches					NR	NR	17.3	NR
Makeup Preparations								
Blushers (all types)					2	NR	4	NR
Face Powders					2	NR	NR	NR
Foundations						5	NR	2 – 8
Leg and Body Paints								
Lipstick					1			
Makeup Bases					1	3	NR	1
Rouges								
Makeup Fixatives								
Other Makeup Preparations					1	NR	0.6	NR
Manicuring Preparations (Nail)								
Basecoats and Undercoats								
Cuticle Softeners					NR	1	NR	1
Nail Polish and Enamel					11	NR	NR	NR
Nail Polish and Enamel Removers								
Other Manicuring Preparations					1	NR	2.8	NR

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
Oral Hygiene Products								
Dentifrices					4	NR	NR	NR
Other Oral Hygiene Products					3	NR	NR	NR
Personal Cleanliness Products								
Bath Soaps and Detergents					6	1	0.22 - 4.7	0.5
Deodorants (underarm)								
Other Personal Cleanliness Products					2	2	NR	NR
Shaving Preparations								
Beard Softeners								
Mens Talcum								
Preshave Lotions (all types)					1	NR	NR	NR
Shaving Cream								
Shaving Soap								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing					15	6	1.3 - 7	NR
Depilatories								
Face and Neck (exc shave)					38	1	8	2 - 5
Body and Hand (exc shave)	NR	NR	NR	8	2	6	NR	2 - 5
Moisturizing					9	2	NR	3
Night					NR	1	NR	NR
Paste Masks (mud packs)	NR	5	NR	8	66	44	1.5 - 10	12 - 80
Skin Fresheners					1	1	NR	NR
Other Skin Care Preparations					13	8	NR	NR
Suntan Preparations								
Suntan Gels, Creams, and Liquids					NR	1	2.5 (not spray) 0.9 (spray)	NR
Other Suntan Preparations					NR	NR	NR	1
	Clay				Fuller's Earth			
Totals	53	NA	4.5	NA	8	3	NR	NR
summarized by likely duration and exposure*								
Duration of Use								
Leave-On	37	NA	NR	NA	3	1	NR	NR
Rinse-Off	14	NA	4.5	NA	5	2	NR	NR
Diluted for (Bath) Use	2	NA	NR	NA	NR	NR	NR	NR
Exposure Type**								
Eye Area	12	NA	NR	NA	NR	NR	NR	NR
Incidental Ingestion	8	NA	NR	NA	NR	NR	NR	NR
Incidental Inhalation-Spray	1 ^a ; 11 ^b	NA	NR	NA	1 ^a ; 1 ^b	NR	NR	NR
Incidental Inhalation-Powder	1; 11 ^b	NA	NR	NA	1 ^b	NR	NR	NR
Dermal Contact	38	NA	4.5	NA	7	3	NR	NR
Deodorant (underarm)	NR	NA	NR	NA	NR	NR	NR	NR
Hair - Non-Coloring	4	NA	NR	NA	1	NR	NR	NR
Hair-Coloring	NR	NA	NR	NA	NR	NR	NR	NR
Nail	NR	NA	NR	NA	NR	NR	NR	NR
Mucous Membrane	14	NA	NR	NA	1	NR	NR	NR
Baby Products	NR	NA	NR	NA	NR	NR	NR	NR
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams								
Other Baby Products								
Bath Preparations (diluted for use)								
Bath Oils, Tablets, and Salts	2	NA	NR	NA				
Bath Capsules								
Other Bath Preparations								
Eye Makeup Preparations								
Eyebrow Pencil	1	NA	NR	NA				
Eyeliner								
Eye Shadow	8	NA	NR	NA				
Eye Lotion								
Eye Makeup Remover								
Mascara	3	NA	NR	NA				
Other Eye Makeup Preparations								
Fragrance Preparations								
Powders (dusting/talcum, excl aftershave talc)								
Hair Preparations (non-coloring)								
Hair Conditioner	1	NA	NR	NA				
Hair Sprays (aerosol fixatives)								

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
Hair Straighteners								
Rinses (non-coloring)								
Shampoos (non-coloring)	2	NA	NR	NA				
Tonics, Dressings, and Other Hair Grooming Aids					1	NR	NR	NR
Wave Sets								
Other Hair Preparations	1	NA	NR	NA				
Hair Coloring Preparations								
Hair Tints								
Hair Shampoos (coloring)								
Hair Lighteners with Color								
Hair Bleaches								
Makeup Preparations								
Blushers (all types)	3	NA	NR	NA				
Face Powders	1	NA	NR	NA				
Foundations	1	NA	NR	NA				
Leg and Body Paints								
Lipstick	6	NA	NR	NA				
Makeup Bases	1	NA	NR	NA				
Rouges								
Makeup Fixatives								
Other Makeup Preparations								
Manicuring Preparations (Nail)								
Basecoats and Undercoats								
Cuticle Softeners								
Nail Polish and Enamel								
Nail Polish and Enamel Removers								
Other Manicuring Preparations								
Oral Hygiene Products								
Dentifrices								
Other Oral Hygiene Products	2	NA	NR	NA				
Personal Cleanliness Products								
Bath Soaps and Detergents	4	NA	NR	NA	1	NR	NR	NR
Deodorants (underarm)								
Other Personal Cleanliness Products								
Shaving Preparations								
Beard Softeners								
Mens Talcum								
Preshave Lotions (all types)								
Shaving Cream								
Shaving Soap								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing	2	NA	4.5	NA				
Depilatories								
Face and Neck (exc shave)	10	NA	NR	NA	1	NR	NR	NR
Body and Hand (exc shave)	1	NA	NR	NA				
Moisturizing	1	NA	NR	NA				
Night								
Paste Masks (mud packs)	3	NA	NR	NA	4	2	NR	NR
Skin Fresheners								
Other Skin Care Preparations					1	1	NR	NR
Suntan Preparations								
Suntan Gels, Creams, and Liquids								
Other Suntan Preparations								

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
	Hectorite				Illite			
Totals	45	18	0.057-1.5	0.4-100	57	NA	0.0015-3.8	NA
summarized by likely duration and exposure*								
Duration of Use								
<i>Leave-On</i>	31	10	0.057-1.5	0.7-15	29	NA	0.0015-0.025	NA
<i>Rinse-Off</i>	14	8	0.1-0.13	0.4-100	26	NA	0.34-3.8	NA
<i>Diluted for (Bath) Use</i>	NR	NR	NR	NR	2	NA	NR	NA
Exposure Type**								
Eye Area	15	4	0.057	0.7	NR	NA	NR	NA
Incidental Ingestion	NR	NR	NR	NR	1	NA	NR	NA
Incidental Inhalation-Spray	1 ^a ; 8 ^b	1 ^a	NR	8 ^b	3 ^a ; 18 ^b	NA	0.25 ^a	NA
Incidental Inhalation-Powder	1; 8 ^b ; 1 ^c	NR	0.1 ^c	8 ^b	3; 18 ^b	NA	NR	NA
Dermal Contact	26	10	0.057-1.5	0.4-100	53	NA	0.0015-3.8	NA
Deodorant (underarm)	NR	1 ^a	NR	0.7 ^a	NR	NA	NR	NA
Hair - Non-Coloring	6	NR	0.1-0.13	1	3	NA	0.25	NA
Hair-Coloring	1	5	0.1	NR	NR	NA	NR	NA
Nail	1	2	NR	NR	NR	NA	NR	NA
Mucous Membrane	NR	1	NR	NR	3	NA	0.34	NA
Baby Products	1	NR	NR	NR	NR	NA	0.0015	NA
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams	1	NR	NR	NR				
Other Baby Products					NR	NA	0.0015	NA
Bath Preparations (diluted for use)								
Bath Oils, Tablets, and Salts					1	NA	NR	NA
Bath Capsules								
Other Bath Preparations					1	NA	NR	NA
Eye Makeup Preparations								
Eyebrow Pencil								
Eyeliner	3	3	0.057	NR				
Eye Shadow	1	NR	NR	NR				
Eye Lotion								
Eye Makeup Remover								
Mascara	11	1	NR	0.7				
Other Eye Makeup Preparations								
Fragrance Preparations								
Powders (dusting/talcum, excl aftershave talc)								
Hair Preparations (non-coloring)								
Hair Conditioner	3	NR	0.13	NR				
Hair Sprays (aerosol fixatives)								
Hair Straighteners								
Rinses (non-coloring)	1	NR	NR	NR				
Shampoos (non-coloring)	1	NR	0.1	1	1	NA	NR	NA
Tonics, Dressings, and Other Hair Grooming Aids	NR	NR	0.13 (not spray)	NR	2	NA	0.025	NA
Wave Sets								
Other Hair Preparations	1	NR	NR	NR				
Hair Coloring Preparations								
Hair Tints								
Hair Shampoos (coloring)	1	NR	0.1	NR				
Hair Lighteners with Color								
Hair Bleaches	NR	5	NR	NR				
Makeup Preparations								
Blushers (all types)								
Face Powders	1	NR	NR	NR	2	NA	NR	NA
Foundations	NR	NR	1.5	15	1	NA	NR	NA
Leg and Body Paints								
Lipstick								
Makeup Bases	2	NR	NR	NR				
Rouges								
Makeup Fixatives								
Other Makeup Preparations	NR	1	NR	NR				
Manicuring Preparations (Nail)								
Basecoats and Undercoats	NR	1	NR	NR				
Cuticle Softeners								
Nail Polish and Enamel	NR	1	NR	NR				
Nail Polish and Enamel Removers								
Other Manicuring Preparations	1	NR	NR	NR				

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
Oral Hygiene Products								
Dentifrices	NR	NR	NR	NR	1	NA	NR	NA
Other Oral Hygiene Products								
Personal Cleanliness Products								
Bath Soaps and Detergents					1	NA	0.34	NA
Deodorants (underarm)	NR	1	NR	0.7				
Other Personal Cleanliness Products	NR	1	NR	NR				
Shaving Preparations								
Beard Softeners								
Mens Talcum								
Preshave Lotions (all types)								
Shaving Cream								
Shaving Soap								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing	5	NR	NR	100	6	NA	3.8	NA
Depilatories								
Face and Neck (exc shave)	8	NR	0.1 (not spray)	NR	18	NA	NR	NA
Body and Hand (exc shave)	NR	NR	NR	8				
Moisturizing					2	NA	NR	NA
Night	1	NR	NR	NR				
Paste Masks (mud packs)	3	2	NR	0.4 - 8	17	NA	1 - 2	NA
Skin Fresheners								
Other Skin Care Preparations	1	1	NR	NR	6	NA	NR	NA
Suntan Preparations								
Suntan Gels, Creams, and Liquids	NR	1	NR	NR				
Other Suntan Preparations								
	Kaolin				Montmorillonite			
Totals	787[†]	509	0.0005-53.2	0.01-100	54	NR	0.1-3	NR
summarized by likely duration and exposure*								
Duration of Use								
Leave-On	502	501	0.0015-53.2	2-100	20	NR	1.2-2	NR
Rinse-Off	262	7	0.0005-33	0.01-84	32	NR	0.1-3	NR
Diluted for (Bath) Use	23	1	NR	NR	2	NR	NR	NR
Exposure Type**								
Eye Area	89	231	0.001-8.5	3-48	2	NR	NR	NR
Incidental Ingestion	135	6	0.0053-14.5	12-30	1	NR	NR	NR
Incidental Inhalation-Spray	1; 35 ^a ; 90 ^b	NR	0.0015-25 ^a	2-25 ^a ; 3 ^b	2 ^a ; 10 ^b	NR	NR	NR
Incidental Inhalation-Powder	38; 90 ^b ; 1 ^c	98	0.01-15; 0.11-16 ^c	5-30; 3 ^b	1 ^c ; 10 ^b	NR	1.2-2 ^c	NR
Dermal Contact	562	466	0.001-33	0.01-100	48	NR	0.1-3	NR
Deodorant (underarm)	6 ^a	NR	2.6	NR	NR	NR	NR	NR
Hair - Non-Coloring	46	5	0.0005-26	4-15	5	NR	NR	NR
Hair-Coloring	8	1	5-19.5	NR	NR	NR	NR	NR
Nail	11	NR	0.023-53.2	53-54	NR	NR	NR	NR
Mucous Membrane	182	8	0.0053-14.5	3-30	8	NR	NR	NR
Baby Products	1	NR	0.0085	NR	1	NR	NR	NR
as reported by product category								
Baby Products								
Baby Lotions/Oils/Powders/Creams	1	NR	NR	NR	1			
Other Baby Products	NR	NR	0.0085	NR				
Bath Preparations (diluted for use)								
Bath Oils, Tablets, and Salts	15	NR	NR	NR	1	NR	NR	NR
Bath Capsules								
Other Bath Preparations	8	1	NR	NR	1	NR	NR	NR
Eye Makeup Preparations								
Eyebrow Pencil	3	5	4.3	15 - 17	1	NR	NR	NR
Eyeliner	6	9	0.82 - 2	25 - 48				
Eye Shadow	42	171	0.35 - 8.5	3 - 29				
Eye Lotion	1	NR	NR	NR	NR	NR	NR	NR
Eye Makeup Remover	NR	NR	0.001	NR	1	NR	NR	NR
Mascara	25	31	0.5 - 4.7	8 - 18				
Other Eye Makeup Preparations	12	15	NR	20				
Fragrance Preparations								
Powders (dusting/talcum, excl aftershave talc)	NR	40	NR	5				
Hair Preparations (non-coloring)								
Hair Conditioner	6	5	0.0005 - 26	4	1	NR	NR	NR
Hair Spray (aerosol fixatives)	1	NR	NR	NR				
Hair Straighteners								

Table 3. Frequency (2023/1998) and concentration (2022/1999) of use according to likely duration and exposure and by product category.^{1,20,21}

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2023	1998	2022	1999	2023	1998	2022	1999
Rinses (non-coloring)								
Shampoos (non-coloring)	14	NR	0.058 - 0.14	NR	1	NR	NR	NR
Tonics, Dressings, and Other Hair Grooming Aids	14	NR	0.0015 - 25	15				
Wave Sets	1	NR	NR	NR				
Other Hair Preparations	10	1	0.1	5	3	NR	NR	NR
Hair Coloring Preparations								
Hair Tints	NR	NR	5	NR				
Hair Shampoos (coloring)								
Hair Lighteners with Color	2	NR	19.3	NR				
Hair Bleaches	6	NR	19.5	NR				
Makeup Preparations								
Blushers (all types)	10	72	0.05 - 15	14 - 20				
Face Powders	38	58	0.01 - 15	30				
Foundations	29 [‡]	45	0.5 - 6	6 - 36				
Leg and Body Paints	1	NR	NR	NR				
Lipstick	118	6	0.0053 - 14.5	12 - 30				
Makeup Bases	2	24	1	7 - 25				
Rouges	3	2	NR	NR				
Makeup Fixatives	NR	3	NR	NR				
Other Makeup Preparations	11	20	2 - 4	10 - 24	1	NR	NR	NR
Manicuring Preparations (Nail)								
Basecoats and Undercoats	1	NR	NR	NR				
Cuticle Softeners								
Nail Polish and Enamel	9	NR	0.023	NR				
Nail Polish and Enamel Removers								
Other Manicuring Preparations	1	NR	35.5 - 53.2	53 - 54				
Oral Hygiene Products								
Dentifrices	16	NR	NR	NR	1	NR	NR	NR
Other Oral Hygiene Products	1							
Personal Cleanliness Products								
Bath Soaps and Detergents	15 [‡]	1	1 - 5	3	2	NR	NR	NR
Deodorants (underarm)	6	NR	2.6 (not spray)	NR				
Other Personal Cleanliness Products	9	NR	NR	NR	3	NR	NR	NR
Shaving Preparations								
Beard Softeners	1	NR	NR	NR				
Mens Talcum								
Preshave Lotions (all types)								
Shaving Cream	NR	NR	0.25	NR				
Shaving Soap								
Other Shaving Preparations								
Skin Care Preparations								
Cleansing	72	NR	3.4 - 20	0.01	8	NR	0.3	NR
Depilatories	NR	NR	4	NR				
Face and Neck (exc shave)	76	NR	0.11 - 16 (not spray)	3	7	NR	1.2 - 2 (not spray)	NR
Body and Hand (exc shave)	14	NR	3.3 - 12 (not spray)	NR	3	NR	NR	NR
Moisturizing	18	NR	0.2 - 1 (not spray)	25	2	NR	NR	NR
Night	2	NR	NR	NR				
Paste Masks (mud packs)	120 [‡]	NR	5 - 33	12 - 84	15	NR	0.1-3	NR
Skin Fresheners	1	NR	0.25	2				
Other Skin Care Preparations	46 [‡]	NR	10 - 20.4	3 - 100	2	NR	NR	NR
Suntan Preparations								
Suntan Gels, Creams, and Liquids	NR	NR	NR	25				
Other Suntan Preparations								

NR – not reported

NA – not applicable

*likely duration and exposure is derived based on product category (see Use Categorization <https://www.cir-safety.org/cir-findings>)

**Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

^a It is possible these products are sprays, but it is not specified whether the reported uses are sprays.^b Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories^c It is possible these products are powders, but it is not specified whether the reported uses are powders.[‡] Includes entries for Kaolinite from the VCRP database.

Table 4. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
IN VITRO						
Bentonite particles with α -quartz content (up to 6%) and with different chemical modifications (acid, alkaline, organic, untreated); gypsum and quartz were negative and positive controls	1 to 15 $\mu\text{g}/\text{cm}^2$	isotonic NaCl solution	Human lung fibroblasts (IMR90)	Micronucleus assay and kinetochore analysis; treated cells incubated for 36, 48, or 72 h	In acidic sample, formation of micronuclei was only slightly increased after exposure to samples with a quartz content of 4% - 5% for 36 h (15 $\mu\text{g}/\text{cm}^2$), 48 h (5 $\mu\text{g}/\text{cm}^2$), and 72 h (1 $\mu\text{g}/\text{cm}^2$); and with a quartz content of 1% for 72 h (1 $\mu\text{g}/\text{cm}^2$). In the alkaline sample, the formation of micronuclei was only slightly increased after exposure to sample with a quartz content of 5% for 48 h and 72 h (15 $\mu\text{g}/\text{cm}^2$). Untreated and organic activated Bentonite particles did not show genotoxic effects; Bentonite particles with a quartz content of < 1% were in negative in the micronucleus assay. Statistically significant reductions in kinetochore-positive micronuclei were not observed with the Bentonite samples	47
Clay (75% Illite, 16% Kaolin, and 9% Montmorillonite)	5000 μg	Not reported	<i>S. typhimurium</i> strains TA98, TA100, TA102, TA1535, TA1537	Ames test in accordance with OECD TG 471; with and without metabolic activation	Not mutagenic with or without metabolic activation	38
Clay (75% Illite, 16% Kaolin, and 9% Montmorillonite)	156.25, 312.5, 625, 1250, 2500, or 5000 $\mu\text{g}/\text{ml}$	culture medium	CHO - K1 cell cultures	Mammalian chromosome aberration test in accordance with OECD TG 473; with and without metabolic activation	Test material did not induce chromosomal aberrations, with or without metabolic activation	38
Kaolin; particle size 4.8 μm and 200 nm	0.2 - 200 $\mu\text{g}/\text{ml}$	particles suspended in saline containing 0.05% Tween 80; suspensions then mixed with culture medium supplemented with 10% fetal bovine serum prior to treating cells	CHO AA8 cells; primary normal human diploid epidermal keratinocytes and fibroblasts	Micronucleus assay; cells were incubated with test materials for 6 h; mitomycin C was positive control	Micronucleus induction in a dose-dependent manner, with frequencies of micronucleated cells increased 3- to 4-fold at 200 $\mu\text{g}/\text{ml}$ in all cell types; fine particles of Kaolin had higher genotoxic potency than coarse particles, with no significant differences detected among the 3 cell types	45
Kaolin; primary particle size 4.8 μm	0.02 - 200 $\mu\text{g}/\text{ml}$	particles suspended in saline containing 0.05% Tween 80; suspensions then mixed with culture medium supplemented with 10% fetal bovine serum prior to treating cells	Human lung cancer A549 cells	Micronucleus assay; cells were incubated with test materials for 6 h	A 4-fold increased frequency of micronucleated cells was observed	40

Table 4. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
Kaolin; particle size 4.8 μm and 200 nm	0.2 - 200 $\mu\text{g/ml}$	particles suspended in saline containing 0.05% Tween 80; suspensions then mixed with culture medium supplemented with 10% fetal bovine serum prior to treating cells	CHO AA8 cells; primary normal human diploid epidermal keratinocytes and fibroblasts	Comet assay; 1-h treatment	Kaolin particles promoted DNA damage in a dose-dependent manner; %tail DNA was increased 8- to 20-fold by exposure to the particles at 200 $\mu\text{g/ml}$ for all cells tested; 200 nm particles had a higher DNA-damaging potency than the 4.8 μm particles, while no significant difference was detected among three cell types	⁴⁵
Montmorillonite-based clay minerals (1 unmodified clay and 3 cation-exchanged clays)	unmodified clay tested at up to 125 $\mu\text{g/ml}$; modified clays tested at up to 250 $\mu\text{g/ml}$	MilliQ water	<i>S. typhimurium</i> strains TA97A, TA98, TA100, TA102, TA104	Ames test; with and without metabolic activation	No significant increases in revertant colonies observed in the unmodified clay (tested at up to 125 $\mu\text{g/ml}$) or in one of the modified clays (tested at up to 250 $\mu\text{g/ml}$); however, significant increases in revertant colonies observed in S9 in strain TA98 in the other two modified clays (tested at up to 8 $\mu\text{g/ml}$ and 125 $\mu\text{g/ml}$, respectively); no changes observed for the same strain without S9 or in the rest of the strains for these 2 modified clays	⁴³
Montmorillonite, natural and organo-modified	0-14.1 $\mu\text{g/plate}$ for unfiltered material; 0-141 $\mu\text{g/ml}$ for filtered material (particles larger than nano-range removed)	MilliQ water	<i>S. typhimurium</i> TA98 and TA100	<i>Salmonella</i> /microsome assay; with and without metabolic activation; suspensions were tested both filtered (removing particle larger than nano-range) and unfiltered material	No mutagenic activity observed with or without metabolic activation	⁴⁴
Montmorillonite, natural and cation-exchanged	56.5, 85, 113, or 170 $\mu\text{g/ml}$ for unfiltered material; up to 226 $\mu\text{g/ml}$ for filtered material (particles larger than nano-range removed)	culture medium	Caco-2 cells	Comet assay; suspensions were tested both filtered (removing particle larger than nano-range) and unfiltered material	Unfiltered and filtered modified Montmorillonite was genotoxic in a concentration-related manner, with statistical significance at the 2 highest concentrations tested for each, when compared to negative controls; no genotoxic effects were observed in the unfiltered and unfiltered natural Montmorillonite	⁴⁴
Unmodified Montmorillonite clay	15.65, 31.25, or 62.5 $\mu\text{g/ml}$	serum-free medium supplemented with B27 (no further details on supplement)	Human hepatoma cell lines (HepG2)	Cytokinesis block micronucleus cytome assay; cells were incubated with test material for 4 or 24 h; positive controls were benzo(a)pyrene and etoposide	Test material induced statistically significant increases ($p < 0.0001$) only in the frequency of micronuclei in 1000 binucleated cells at the highest concentration tested; no effects observed in nucleoplasmic bridges or nuclear buds; positive controls yielded expected results	⁴⁶

Table 4. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
IN VIVO						
Kaolin; primary particle size 4.8 μ m (major peak average 357.6 $\pm$ 199.4 nm)	Single dose of 0.05 or 0.2 mg/animal	particles suspended in saline containing 0.05% Tween 80; particles agglomeration in suspensions observed (low-density tabular structures with rectangular or hexagonal shape were observed)	Groups of 5 male C57BL/6J mice	Comet assay; mice were intratracheally instilled with particles; negative control received solvent; 3 h after instillation, mice were killed and lungs were removed for analysis; additional exposure time also examined (24 h)	DNA damage was induced at 0.2 mg/mouse (up to 2 - 3-fold), but not at 0.05 mg/mouse, after 3 h exposure; DNA damage induced at 24 h did not differ from 3 h exposure	40
Kaolin; primary particle size 4.8 μ m (major peak average 357.6 $\pm$ 199.4 nm)	Single dose or multiple doses (4x) of 0.2 mg/animal	particles suspended in saline containing 0.05% Tween 80; particles agglomeration in suspensions observed (low-density tabular structures with rectangular or hexagonal shape were observed)	Groups of 10 male <i>gpt</i> delta transgenic mice	<i>gpt</i> mutagenesis assay; mice were intratracheally instilled with particles; negative control received solvent; mice killed at 12 (single dose) or 8 (multiple doses) wk; lungs and kidneys removed for analysis	Increased <i>gpt</i> and <i>Spi</i> ⁻ mutant frequencies observed in the lungs of the mice; mutation spectra analysis showed > 60% of the base substitution occurred in the <i>gpt</i> genes	40
cation-exchanged Montmorillonite	0, 250, 500, or 1000 mg/kg bw; 1000 mg/kg bw in range finding study	Water or cell-culture medium	Groups of 3 male and 3 female Wistar Hannover Galas rats; 2 rats of each sex in a range finding study	Comet assay; rats received 2 single doses of test material by gavage 24 h apart; 3 h after dosing, rats were killed and examined macroscopically, and liver, kidneys, and colon were dissected and analyzed for DNA damage; water or cell-culture medium was negative control and ethylmethanesulfonate was positive control	No statistically significant difference in % tail DNA between the negative controls and the different treatment groups for any of the organs tested for DNA damage; positive control yielded expected results; all rats survived during treatment period with no clinical signs of abnormalities	48

Table 5. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION						
IN VITRO						
Formulation containing 38% Montmorillonite	25 µl calcium and magnesium free Dulbecco's phosphate buffered saline	Tested neat; 25 mg	EpiDerm™ tissues	EpiDerm™ skin model in accordance with OECD TG 439	Not an irritant; mean viability = 105.7%	54
ANIMAL						
Clay (75% Illite, 16% Kaolin, and 9% Montmorillonite)	0.5 ml distilled water	500 mg	rabbits (no further details)	Acute dermal irritation study in accordance with OECD TG 404; patches were applied to intact skin with gauze patch for 4 h; after exposure period, residual test substance was removed with cotton soaked in distilled water; skin reactions scored at 1, 24, 48, and 72 h post-patch removal	Not irritating; mean dermal irritation scores for erythema and edema were 0.0	38
HUMAN						
Formulation containing 1.75% Bentonite	none	0.05 ml	25 subjects	14-d cumulative irritation assay under occlusive patches; test sites on the back or upper arm and patches were 15 mm diameter disks; positive control site received 0.05 ml of 0.25% sodium lauryl sulfate and negative control site was a plain patch	Irritation potential was negligible; no adverse events reported	55
Mud mask containing 8% Bentonite	none	As supplied	19 subjects	Single-insult occlusive patch test	No irritation; primary irritation index = 0.0	56
Facial cleanser containing 2% Bentonite and 2% Kaolin	none	As supplied	50 subjects	4-wk clinical use test; single-blind baseline controlled monadic design; subjects instructed to use test material daily in the morning as a facial cleanser, twice weekly in the evening as an exfoliating scrub, twice weekly in the evening as a purifying mask, and 3 times weekly in the evening as a facial cleanser	No visible irritation; however, 8 subjects reported perceived discomfort and/or irritation, including burning/stinging and/or redness/dryness while using the test material; 6/8 subject responses were not considered related to the use of the test material and 2/8 subject responses were not sufficient intensity to warrant discontinuation of product	57
SENSITIZATION						
IN VITRO						
Formulation containing 38% Montmorillonite	Cell culture medium	Not reported	KeratiNoSens™ cells	KeratiNoSens™ assay (validated by the European Centre for the Validation of Alternative Methods (ECVAM)); 12 concentrations in 3 repetitions, in 3 replicates (details not reported); luciferase induction and cellular viability determined after 48 h incubation time	Not a sensitizer; test material weakly toxic to cells	54
ANIMAL						
Clay (75% Illite, 16% Kaolin, and 9% Montmorillonite)	Distilled water	5% (w/v) for intradermal injection; concentration details not provided for topical induction or challenge; topical dose was 100 mg test material in 0.2 ml vehicle	10 guinea pigs/sex	Skin sensitization study in accordance with OECD TG 406; no further details provided	Negative for sensitization; no positive skin responses at 24 or 48 h post-patch removal at challenge; no clinical signs during treatment	38
50% Hectorite	Not reported	Not reported	Guinea pigs; number/sex not provided	Buehler test; no further details provided	Non-sensitizing	11

Table 5. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
HUMAN						
Foot mask containing 3.5% Bentonite	As supplied	0.2 g	102 subjects	HRIPT under occlusive patches; induction patch applied on the back for 9 total applications; 10-15 d non-treatment period followed by challenge patch applied to naïve site and scored at 48 h and 72 h post-application; patch was ~ 2 cm ²	No dermal sensitization; no adverse events reported	⁶⁰
Clay mask containing 3.8% Bentonite	As supplied	0.1 - 0.15 g	108 subjects; 25% with self-perceived sensitive skin	HRIPT under semi-occlusive patches; induction patch applied on the back for 9 total applications; after a 2-wk non-treatment period, challenge patch applied to naïve site and scored at 24 h and 72 h post-application; patch was ~ 2 cm ² and contained ~ 25 - 38 mg/cm ² test material	No dermal irritation or sensitization	⁵⁸
Face cream containing 7.5% Bentonite	As supplied	0.1 - 0.15 g; volatilized for 30-90 min prior to application	52 subjects	HRIPT under occlusive patches; induction patch applied on the back for 9 total applications; after a 2-wk non-treatment period, challenge patch applied to naïve site and scored at 24 h and 72 h post-application; no further details on patches provided	No dermal irritation or sensitization	⁵⁹
Lip product containing 14.5% Kaolin	As supplied	0.1 - 0.15 g	54 subjects	HRIPT under occlusive patches; induction patch applied on the back for 9 total applications; after a 2-wk non-treatment period, challenge patch applied to naïve site and score at 24 h and 72 h post-application; patch contained ~ 25 - 38 mg/cm ² test material	No dermal irritation or sensitization	⁶¹
Clay mask containing 14.5% Kaolin	Neat	0.1 ml	103 subjects	HRIPT under occlusive patches; induction patch applied on the back or upper arm ;after a 10-15 d non-treatment period, challenge patch applied to naïve site and scored at 48 and 72 h post-application; material was applied to a 2 x 2 cm ² Webril pad	Not sensitizing; moderate erythema at the first induction in one subject mildly progressed through the induction period to erythema and edema with papules; no other adverse events reported during study	⁶³
Clay mask containing 40% Kaolin	As supplied	0.1 - 0.15 g	51 subjects	HRIPT under occlusive patches; induction patch applied on the back for 9 total applications; after a 2-wk non-treatment period, challenge patch applied to naïve site and scored at 24 h and 72 h post-application; no further details on patches provided	No dermal irritation or sensitization	⁶²
PHOTOSENSITIZATION						
HUMAN						
Sunscreen containing 1.75% Bentonite	As supplied	0.2 ml	23 subjects	Photoallergy test under occlusive patch; on day 1, subjects received test material on 2 sites on the back for 24 h, after which, subjects received a total of 6 applications (twice weekly) on the back; 24 h after application, one site was irradiated with UVB + UVA with full Xenon lamp spectrum, other site was non-irradiated control; after a non-treatment period of 10-17 d, challenge patches on naïve sites were placed and one site was irradiated with 6 J/cm ² UVA and ½ the minimal erythema dose of UVB; sites were evaluated at 24, 48, and 72 h after irradiation	Not a photosensitizer	⁶⁴

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.11

Revision Date 20.06.2023

Print Date 21.11.2023

GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Kaolin

Product Number : K7375

Brand : Sigma-Aldrich

REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.

CAS-No. : 1332-58-7

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science Sp.z.o.o.
Szelągowska 30
PL-61-626 POZNAN

Telephone : +48 61 8290-100

Fax : +48 61 8290-120

E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +(48)-223988029 (CHEMTREC) 112
(numer alarmowy)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.

2.2 Label elements

Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.



2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Ecological information:

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

Toxicological information:

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms : Hydrated aluminum silicate
Bolus
Aluminum silicate hydroxide

CAS-No. : 1332-58-7

EC-No. : 310-194-1

No components need to be disclosed according to the applicable regulations.

SECTION 4: First aid measures

4.1 Description of first-aid measures

If inhaled

After inhalation: fresh air.

In case of skin contact

In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact

After eye contact: rinse out with plenty of water. Remove contact lenses.

If swallowed

After swallowing: make victim drink water (two glasses at most). Consult doctor if feeling unwell.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available



SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

Water Foam Carbon dioxide (CO₂) Dry powder

Unsuitable extinguishing media

For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Aluminum oxide

silicon oxides

Combustible.

Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters

In the event of fire, wear self-contained breathing apparatus.

5.4 Further information

Suppress (knock down) gases/vapors/mists with a water spray jet.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Avoid inhalation of dusts. Evacuate the danger area, observe emergency procedures, consult an expert.

For personal protection see section 8.

6.2 Environmental precautions

No special precautionary measures necessary.

6.3 Methods and materials for containment and cleaning up

Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions

Tightly closed. Dry.

Storage class

Storage class (TRGS 510): 11: Combustible Solids

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated



SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Full contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0,11 mm

Break through time: 480 min

Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the EC approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Respiratory protection

required when dusts are generated.

Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.

Recommended Filter type: Filter type P1

The entrepreneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.



Control of environmental exposure
No special precautionary measures necessary.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a) Physical state	solid
b) Color	No data available
c) Odor	No data available
d) Melting point/freezing point	No data available
e) Initial boiling point and boiling range	No data available
f) Flammability (solid, gas)	No data available
g) Upper/lower flammability or explosive limits	No data available
h) Flash point	No data available
i) Autoignition temperature	No data available
j) Decomposition temperature	No data available
k) pH	No data available
l) Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
m) Water solubility	No data available
n) Partition coefficient: n-octanol/water	Not applicable for inorganic substances
o) Vapor pressure	No data available
p) Density	No data available
Relative density	No data available
q) Relative vapor density	No data available
r) Particle characteristics	No data available
s) Explosive properties	No data available
t) Oxidizing properties	none



9.2 Other safety information

No data available

SECTION 10: Stability and reactivity

10.1 Reactivity

The following applies in general to flammable organic substances and mixtures: in correspondingly fine distribution, when whirled up a dust explosion potential may generally be assumed.

10.2 Chemical stability

The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

no information available

10.5 Incompatible materials

Strong oxidizing agents

10.6 Hazardous decomposition products

In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity

Oral: No data available

Inhalation: No data available

Dermal: No data available

Skin corrosion/irritation

No data available

Serious eye damage/eye irritation

No data available

Respiratory or skin sensitization

No data available

Germ cell mutagenicity

No data available

Carcinogenicity

No data available

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available



Aspiration hazard

No data available

11.2 Additional Information**Endocrine disrupting properties****Product:**

Assessment

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

RTECS: GF1670500

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information**12.1 Toxicity**

No data available

12.2 Persistence and degradability

The methods for determining biodegradability are not applicable to inorganic substances.

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties**Product:**

Assessment

: The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects

No ecological problems are to be expected when the product is handled and used with due care and attention.



SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product

See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

SECTION 14: Transport information

14.1 UN number

ADR/RID: -

IMDG: -

IATA: -

14.2 UN proper shipping name

ADR/RID: Not dangerous goods

IMDG: Not dangerous goods

IATA: Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: -

IMDG: -

IATA: -

14.4 Packaging group

ADR/RID: -

IMDG: -

IATA: -

14.5 Environmental hazards

ADR/RID: no

IMDG Marine pollutant: no

IATA: no

14.6 Special precautions for user

No data available

Further information

Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out



SECTION 16: Other information

Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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