



Toxicological profile for Calcium carbonate

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

Calcium;carbonate (PubChem)

1.2. Synonyms

471-34-1: Aeromatt; Akadama; Albacar; Albacar 5970; Albafil; Albaglos; Albaglos SF; Allied Whiting; Antacid; Atomit; Atomite; AX 363; BF 200; Brilliant (15, 1500 or BR 15); Britomya (M or S); BRT (1500 or 30); BS 32; C 50 (carbonate); Cal-Light SA; Cal-Sup; Calcene (CO, NC or TM); Calcoll; Calcidar 40; Calcilit (100 or 8); Calcium carbonate; Calcium carbonate (1:1); Calcium carbonate, precipitated; Calcium monocarbonate; Calibrite; Calmos; Calmote; Calofil (A 4, B 1 or E 2); Calofor U 50; Calofort (S, T or U); Calopake (F, FS, H, high opacity or PC); Calseeds; Caltec; Caltrate; Calwhite; Camel-carb; Camel-tex; Camel-wite; Carbital 90; Carbium; Carbium MM; Carbonic acid calcium salt (1:1); Carbonic acid, calcium salt (1:1); Carborex 2; Carusis P; Caswell No. 139; CCC (G-white or No.AA oolitic); CCRIS 1333; CCW; Chemcarb; Children's Mylanta Upset Stomach Relief; Chooz; CI 77220; C.I. 77220; C.I. Pigment White 18; Clefnon; Crystic prefil S; Dacote; DOMAR; Duramite; Durcal (10, 40, 2NH or C 640305); EINECS 207-439-9; EGRI M 5; EPA Pesticide Chemical Code 073502; Eskalon (100, 1500, 200, 400 or 800); Filtex White Base; Finncarb 6002; Garolite SA; Gilder's whiting; Hakuenka (CC, CCR, DD, O, PX, PZ, R 06 or T-DD); Homocal D; HSDB 927; Hydrocarb; Hydrocarb (60 or 65); K 250; Kotamite; Kredafil (150 Extra or RM 5); KS (1300, 1500, 1800, 2100 or 500); KULU 40; Levigated chalk; Marble white; Marblewhite 325; Marfil; MC-T; Microcarb; Micromic CR 16; Micromya; Microwhite 25; Monocalcium carbonate; MSK (C, G, K, P, PO or V); Multifex MM; Multiflex (MM or SC); Mylanta Soothing Lozenges; N (34 or 43); NCC (45 or P); Neoantacid; Neolite (F, SP or TPS); Non-Fer-Al; NS (100, 200, 2500 or 400); Oyster shell; P-Lite (500 or 700); Pigment white 18; Precipitated chalk; R Jutan; Slaker rejects; T 130-2500; Tums; UNII-H0G9379FGK (ChemIDplus).

1317-65-3: Agricultural limestone; Agstone; Bell mine pulverized limestone; Calcium carbonate; Chalk; Domolite; Franklin; Ground limestone; Limestone; Lithographic stone; Marble; Marble dust; Natural calcium carbonate; Portland stone; Sohnhofen stone; EINECS 215-279-6 (ChemIDplus).

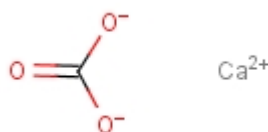
1.3. Molecular formula

471-34-1: Ca.CO₃; C-H2-O3.Ca (ChemIDplus)

1317-65-3: Unspecified (ChemIDplus); CaCO₃ (ChemSpider)

1.4. Structural Formula

471-34-1:



1.5. Molecular weight (g/mol)

471-34-1: 100.09

1.6. CAS registration number

471-34-1, 1317-65-3

1.7. Properties

1.7.1. Melting point

(°C): Aragonite: 825, Calcite: 1339 (CIR, 2017); 800 (CAS RN 471-34-1) (EPISuite, 2017); 800 (decomposes), 825 (decomposes), 899 (decomposes) or 1339 (CAS RN 1317-65-3) (ChemSpider); 825 (CAS RN 471-34-1) (BPDB, 2022); 825-1339 (decomposes) (CAS RNs 471-34-1, 1317-65-3) (PubChem); “does not melt” (CAS RN 1317-65-3) (PPDB, 2021)

1.7.2. Boiling point

(°C): Not applicable; 375.17 (estimated) (CAS RN 471-34-1) (EPISuite, 2017); decomposes before boiling (CAS RNs 471-34-1, 1317-65-3) (BPDB, 2022; PPDB, 2021)

1.7.3. Solubility

Practically insoluble (EFSA, 2011). The amorphous form of calcium carbonate, with particles predominately in the nanoscale range, is reported to be approximately 10 times more soluble than crystalline calcium carbonate (Meiron et al., 2011)” (EFSA, 2011); 1e+006 mg/L at 25°C (estimated) (CAS RN 471-34-1) (EPISuite, 2017); 0–0.002% (CAS RN 1317-65-3) (ChemSpider); 6.6X10⁻⁴ g/100 g water at 20°C (CAS RN 471-34-1, for “white hexagonal crystals or powder” and “white orthorhombic crystals or powder”), 1.1X10⁻³ g/100 g water at 20°C (CAS RN 471-34-1 for “colorless hexagonal crystals”) (HSDB, 2014); 14 mg/L at 20°C (CAS RN 471-34-1) (BPDB, 2022); 0.001% or 14 mg/L at 25°C (CAS RNs 471-34-1, 1317-65-3) (PubChem).

1.7.4. pKa

”Not applicable, no dissociation at 25 °C” (CAS RN 1317-65-3) (PPDB, 2021)

1.7.5. Flashpoint

(°C): Not expected to autoignite; not highly flammable (CAS RN 471-34-1; 1317-65-3) (BPDB, 2022; PPDB, 2021).

1.7.6. Flammability limits (vol/vol%)

Noncombustible solid (CAS RNs 471-34-1, 1317-65-3) (PubChem); “Calcium carbonate is not flammable” (European Commission, 2019a)

1.7.7. (Auto)ignition temperature

(°C): “Calcium carbonate is....not auto-flammable” (European Commission, 2019a)

1.7.8. Decomposition temperature

(°C): 800 (CAS RN 471-34-1) (EPISuite, 2017); 700-900 (CAS RN 471-34-1) (HSDB, 2014); 825 (CAS RNs 471-34-1, 1317-65-3) (PubChem); ≥825 (CAS RN 471-34-1) (GESTIS)

1.7.9. Stability

Stable at normal temperatures and pressure. Unstable in acidic foods (EFSA, 2011); Indefinite shelf-life, stable in air, no rapid reaction with air or water, incompatible with acids, alum, ammonium

salts, fluorine, magnesium (CAS RNs 471-34-1, 1317-65-3) (PubChem); Can react dangerously with heat and carbon dioxide (CAS RN 471-34-1) (GESTIS); "Limestone is known to be stable at room temperature for prolonged periods of time (at least one day)", "Limestone is thermally stable" (European Commission, 2020)

1.7.10. Vapor pressure

Not applicable; 2.73E-015 mm Hg at 25°C (estimated) (CAS RN 471-34-1) (EPISuite, 2017); 0 mm Hg (approximate) (CAS RN 1317-65-3) (ChemSpider); 0 mm Hg (approximate) (CAS RNs 471-34-1, 1317-65-3) (PubChem)

1.7.11. log Kow

-2.12 (estimated) (CAS RN 471-34-1) (EPISuite, 2017); "Not applicable" (CAS RN 471-34-1) (BPDB, 2022); "Calcium carbonate is insoluble in water and n-octanol, hence not applicable" (European Commission, 2019a).

2. General information

2.1. Exposure

EFSA (2011) has estimated the exposure levels of calcium from the use of calcium carbonate in foods. For adults (weighing 60 kg) these are:

- 200-320 mg/day from use as a food additive
- 440-740 mg/day from use as a food additive and from fortified food
- 1240-1540 mg/day from use as a food additive, from fortified food and from supplements.

Concentrations used for these exposure estimates from its use as a food additive and added nutrient source (fortification) are given in the following table (EFSA, 2011):

Food category	Calcium carbonate as a food additive (mg/kg)	Calcium carbonate fortification of food (mg/kg)
Grain milling products	6000	3650
Bread and rolls	3000	9800
Breakfast cereals	2250	13500
Fine bakery products	1970	1825
Cocoa beans and cocoa products	1250	11250
Fish products	2250	0
Chocolate products	1250	11250
Soft drinks	500	0
Baking ingredients	12000	0

Ready to eat soups	500	0
Salt	20000	0
Food supplements	0	8000 mg calcium/day

More detailed information on exposure to calcium carbonate (CAS RN 471-34-1) is provided in the Appendixes to EFSA (2011). EFSA Journal 9(7):2318. Available at <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

The (ex) EU Scientific Committee for Food derived an upper limit of 2500 mg/day for calcium from all sources (SCF, 2003).

Calcium carbonate (CAS RN 471-34-1) is used as an abrasive, buffering, bulking, opacifying and oral care agent and CI 77220 is used as a colorant agent in cosmetics in the EU.

As taken from CosIng, available at <https://ec.europa.eu/growth/tools-databases/cosing/>

In non-medicinal natural health products, limestone (CAS RN 1317-65-3) is used as an anticaking agent and stabilizing agent and calcium carbonate (CAS RN 471-34-1) as an abrasive, binder, buffering agent, bulking agent, colour additive, diluent, dissolution enhancing agent, emulsifying agent, gelling agent, opacifying agent, oral care agent, pH adjuster, stabilizing agent and thickening agent (Health Canada, 2022).

Calcium carbonate (CAS RN 471-34-1) is listed as an ingredient (at given concentrations, where specified) in hobby/craft (1-100%), commercial/institutional (5-60%), home maintenance (1-45%), home office (10-70%), landscaping/yard (0-100%, includes "old" products), pesticide (5->90%, includes "old" products) and pet care (5-40%) products (1-5%), and calcium carbonate (limestone) (CAS RN 1317-65-3) is also listed as an ingredient in hobby/craft (1-60%, includes "old" products), auto (0.5-60%, includes "old" products), commercial/institutional (0-100%), home maintenance (0-100%, includes "old" products), inside the home (5.0-100%), landscaping/yard (0-100%), pesticide (1-70%, includes "old" products), pet care (0.1-70%) and "old" personal care products (1-5%), by the CPID.

Calcium carbonate (CAS RN 471-34-1) is ranked #1 in the US EPA's list of top 20 chemicals used in children's products and #13 in the EPA's list of chemical used in commercial products. As taken from US EPA, 2014.

Permissible exposure limits (PELs) for limestone (CAS RN 1317-65-3) and calcium carbonate (CAS RN 471-34-1):

Particulates not otherwise regulated:

Total dust - 10 mg/m³

Respirable fraction - 5 mg/m³

As taken from Cal/OSHA, available at https://www.dir.ca.gov/title8/5155table_ac1.html#_blank
"Exposure Routes:

Public – Exposure not considered significant – low risk

Occupational – Exposure not considered significant – low risk"

As taken from BPDB, 2022.

"Calcium carbonate is used therapeutically as a phosphate buffer in hemodialysis, as an antacid in gastric hyperacidity for temporary relief of indigestion and heartburn, and as a calcium supplement for preventing and treating osteoporosis."

As taken from PubChem.

AOEL - Acceptable Operator Exposure Level – Systemic – 10 mg/m³ (inhalation) (CAS RN 1317-65-3) (PPDB, 2021).

The Food Safety Commission of Japan has set 2000 mg/day as the upper limit of extra-dietary intake of calcium carbonate (FSCJ, 2020).

“Based on the representative use of the product (as a paste), and the low toxicity profile of the active substance, toxicological reference values (acceptable daily intake (ADI), acute reference dose (ARfD), acceptable operator exposure level (AOEL) and acute acceptable operator exposure level (AAOEL) values are considered not needed for limestone powder (calcium carbonate)).”

“The dermal absorption value for the representative formulation is 25% (default value). In the absence of toxicological reference values, non-dietary exposure estimates are not necessary. It is noted that for the representative use and representative formulation (paste), exposure by inhalation is not expected to be significant. For other potential uses triggering inhalation exposure, e.g. spray, should they be considered for national authorisations, an acceptable operator exposure concentration (AOEC) could be based on the NOAEC of 0.212 mg/L (=212 mg/m³) identified for treatment-related changes in the lower airways (increased lung weight accompanied by slight increases in bronchoalveolar lavage-derived inflammation and cytotoxicity biomarkers) in a 90-day inhalation toxicity study with a nanoform of calcium carbonate. It should however be noted that, concerning inhalation exposure, particular attention should be given to the possible presence of toxicologically relevant crystalline silica in the technical material (see Section 1).”

As taken from EFSA, 2022.

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Baker R and Bishop L. (2005). The pyrolysis of non-volatile tobacco ingredients using a system that stimulates cigarette combustion conditions. J. Anal. Appl. Pyrolysis 74, 145–170.

Ingredient Name & CAS Number	Max. cig. appln. level (ppm)	Purity of Sample (%)	Composition of pyrolysate (Compound, %)	Max. level in smoke (µg)
Calcium carbonate 471-34-1 CaCO ₃	9000	na	No products detected	-

2.3. Ingredient(s) from which it originates

“According to Directive 2008/128/EC calcium carbonate is the product obtained from ground limestone or by the precipitation of calcium ions with carbonate ions” (EFSA, 2011. EFSA Journal 9(7):2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

Calcium carbonate is found naturally in pearls (ranging from ca. 81–95%, depending on source species, natural or cultured).

As taken from Khan IA and Abourashed EA, 2010.

“Calcium Carbonate, as used for industrial purposes, is extracted by mining or quarrying. Pure Calcium Carbonate can be produced from marble, or it can be prepared by passing carbon dioxide into a solution of calcium hydroxide. In the latter case, Calcium Carbonate is derived from the mixture, forming a grade of product called “precipitated calcium carbonate” or PCC. PCC has a

very fine and controlled particle size, diameter of 2 μ , and is particularly useful in the production of paper. The other primary type of industrial product is "ground calcium carbonate" or GCC. The production of GCC involves crushing and processing limestone to create a powdery form graded by size and other properties for many different industrial and pharmaceutical applications."

As taken from CIR, 2017.

"Ground calcium carbonate (CAS: 1317-65-3) results directly from the mining of limestone. The extraction process keeps the carbonate very close to its original state of purity and delivers a finely ground product either in dry or slurry form. Precipitated calcium carbonate (CAS: 471-34-1) is produced industrially by the decomposition of limestone to calcium oxide followed by subsequent recarbonization or as a by-product of the Solvay process (which is used to make sodium carbonate)."

As taken from PubChem.

3. Status in legislation and other official guidance

FIFRA Requirements:

Residues of calcium carbonate are exempted from the requirement of a tolerance when used as a solid diluent, carrier in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest. [40 CFR 180.910; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Residues of calcium carbonate are exempted from the requirement of a tolerance when used as a solid diluent, carrier in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to animals. [40 CFR 180.930; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

FDA Requirements:

Certification of this color additive when used as a color additive in drugs is not necessary for the protection of the public health, and therefore batches thereof are exempt from the certification pursuant to section 721(c) of the act. [21 CFR 73.1070; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

An ingredient whose use in food or food packaging is subject to a prior sanction or approval within the meaning of section 201(s)(4) of the Act is exempt from classification as a food additive. ... Substances classified as stabilizers, when migrating from food-packaging material shall include: ... calcium carbonate. [21 CFR 181.29; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Substance added directly to human food affirmed as generally recognized as safe (GRAS). [21 CFR 184.1191; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. A number of active ingredients have been present in OTC drug products for various uses, as described below. However, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses: calcium carbonate is included in antidiarrheal drug products. [21 CFR 310.545(a)(3); U.S.

National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. A number of active ingredients have been present in OTC drug products for various uses, as described below. However, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses: calcium carbonate is included in digestive aid drug products. [21 CFR 310.545(a)(8); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. A number of active ingredients have been present in OTC drug products for various uses, as described below. However, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses: calcium carbonate is included in weight control drug products. [21 CFR 310.545(a)(20); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Calcium carbonate used as a general purpose food additive in animal drugs, feeds, and related products is generally recognized as safe when used in accordance with good manufacturing or feeding practice. [21 CFR 582.1191; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Calcium carbonate used as a nutrient and/or dietary supplement in animal drugs, feeds, and related products is generally recognized as safe when used in accordance with good manufacturing or feeding practice. [21 CFR 582.5191; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Allowable Tolerances:

Residues of calcium carbonate are exempted from the requirement of a tolerance when used as a solid diluent, carrier in accordance with good agricultural practices as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest. [40 CFR 180.910 U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Residues of calcium carbonate are exempted from the requirement of a tolerance when used as a solid diluent, carrier in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to animals. [40 CFR 180.930 U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

OSHA Standards:

Permissible Exposure Limit: Table Z-1 8-Hr Time Weighted Avg: 15 mg/cu m, total dust. /Calcium carbonate, RN 1317-65-3/ [29 CFR 1910.1000; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Permissible Exposure Limit: Table Z-1 8-Hr Time Weighted Avg: 5 mg/cu m, respirable fraction. /Calcium carbonate, RN 1317-65-3/ [29 CFR 1910.1000; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 21, 2013: <http://www.gpoaccess.gov/ecfr>] **PEER REVIEWED**

Threshold Limit Values:

Substances whose adopted documentation and TLV's were withdrawn. Substance: Calcium carbonate (471-34-1); Year Withdrawn: 2007; Reason: Insufficient data. [American Conference of Governmental Industrial Hygienists TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati, OH, 2013, p. 92] **PEER REVIEWED**

NIOSH Recommendations:

Recommended Exposure Limit: 10 Hr Time-Weighted Avg: 10 mg/cu m (total); 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. 2010-168 (2010)] **PEER REVIEWED**

As taken from HSDB, 2014.

The UK Health and Safety Executive has set workplace exposure limits for CAS RN 1317-65-3 of 10 mg/m³ (inhalable dust) and 4 mg/m³ (respirable fraction) for an 8-h TWA reference period (HSE, 2020). Available at <https://www.hse.gov.uk/pubns/priced/eh40.pdf>

Occupational exposure limits for CAS RN 471-34-1:

OEL-BELGIUM: TWA 0.01 mg(Ag)/m³, MAR2002

OEL-EC: TWA 0.01 mg(Ag)/m³, FEB2006 OEL-FRANCE: VME 10 mg/m³, FEB2006 OEL-JAPAN: OEL 0.01 mg(Ag)/m³, MAY2012 OEL-THE NETHERLANDS: MAC-TGG 10 mg/m³, 2003 OEL-PERU: TWA 10 mg/m³, JUL2005 OEL-UNITED KINGDOM: TWA 0.01 mg(Ag)/m³, OCT2007

As taken from RTECS, 2019.

Occupational exposure limits for CAS RN 1317-65-3:

OEL-BELGIUM: TWA 10 mg/m³, MAR2002 OEL-HUNGARY: TWA 10 mg/m³, SEP2000 OEL-JAPAN: OEL 2 mg/m³ (resp. dust), 84 mg/m³ (total dust), MAY2012 OEL-KOREA: TWA 10 mg/m³, 2006 OEL-MEXICO: TWA 10 mg/m³; STEL 20 mg/m³ (inhalable), 2004 OEL-THE NETHERLANDS: MAC-TGG 10 mg/m³, 2003 OEL-NEW ZEALAND: TWA 10 mg/m³ (inspirable dust), JAN2002 OEL-POLAND: MAC(TWA) dust 10 mg/m³, JAN1999 OEL-RUSSIA: STEL 6 mg/m³, JUN2003 OEL-SWITZERLAND: MAK-W 3 mg/m³, resp, JAN2011 OEL-UNITED KINGDOM: TWA 10 mg/m³ (inhal. dust), OCT2007 OEL-UNITED KINGDOM: TWA 4 mg/m³ (resp. dust), OCT2007 OEL IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN check ACGIH TLV; OEL IN SINGAPORE, VIETNAM check ACGIH TLV

As taken from RTECS, 2013.

US Army Military exposure guidelines (MEGs) for Short-Term exposures to chemicals in ambient air (Record for CAS RN 1317-65-3):

Calcium carbonate (CAS RN 1317-65-3; limestone, marble):

ACGIH TLV withdrawn due to insufficient data.

OSHA PELs: TWA 15 mg/m³ (total dust); 5 mg/m³ (respirable fraction).

NIOSH RELs: TWA (includes CAS RN 471-34-1) 10 mg/m³ (total dust); 5 mg/m³ (respirable fraction).

As taken from ACGIH, 2021.

21 CFR 184.1409 – Ground limestone:

Code of Federal Regulations Title 21, Part 184: DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE, Subpart B--Listing of Specific Substances Affirmed as GRAS.

As taken from FDA, 2022a.

Calcium carbonate (CAS RN 471-34-1) is included on the US FDA's inventory of "Substances Added to Food (formerly EAFUS)" as an anticaking agent or free-flow agent, color or coloring agent, drying agent, formulation aid, humectant, leavening agent, lubricant or release agent, masticatory substance, nutrient supplement, pH control agent and processing aid. It is generally recognised as safe under 21 CFR 184.1191 and is also covered under sections 21 CFR 73.70 (Color additives exempt from certification), 73.1070 (Color additives exempt from certification), 137.105 (Requirements for Specific Standardized Cereal Flours and Related Products. Flour), 137.185 (Requirements for Specific Standardized Cereal Flours and Related Products. Enriched self-rising flour), 137.350 (Requirements for Specific Standardized Cereal Flours and Related Products. Enriched rice), 175.300 (Resinous and polymeric coatings), 176.170 (Components of paper and paperboard in contact with aqueous and fatty foods), 177.1600 (Polyethylene resins, carboxyl modified), 177.1680 (Polyurethane resins), 177.2260 (Filters, resin-bonded), 178.3297 (Colorants for polymers) and 181.29 (Stabilisers).

As taken from FDA, 2022a,b.

"Authorised use and use levels have been defined in Directive 94/36/EC on colours for use in foodstuffs and in Directive 95/2/EC on food additives other than colours and sweeteners, as amended. The following functionalities have been reported: food colour, anti-caking agent, acidity regulator, gelling agent, thickener, bulking agent, hardening agent, glazing agent and release agent. In accordance with Directive 94/36/EC, calcium carbonate is permitted quantum satis in all foodstuffs except for some foodstuffs in which the use of colours (in general) is specifically prohibited or restricted to food colours other than calcium carbonate. In accordance with Directive 95/2/EC, calcium carbonate is generally permitted quantum satis in foodstuffs not referred to in Article 2 (3) of Directive 95/2, unless specifically provided for" (EFSA, 2011. EFSA Journal 9(7):2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

"Calcium carbonate is authorised as a food colour in the EU under Directive 94/36/EC, and is also authorised as an additive generally permitted in foodstuffs under Directive 95/2/EC. It is also included in Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutritional uses, in Directive 2002/46/EC relating to food supplements, and it can be used in fortified foods according to Regulation 1925/2006 on the addition of vitamins and minerals and of certain other substances in food. Calcium carbonate has been evaluated by JECFA in 1965 (JECFA, 1965, 1966), when the Committee established an Acceptable Daily Intake (ADI) not limited. The SCF evaluated calcium carbonate as part of a group of carbonates, and assigned a group ADI not specified (SCF, 1991). Calcium carbonate together with other carbonates has also been reviewed by TemaNord, who concluded that there was no need for a re-evaluation (TemaNord, 2002). The SCF allocated a Tolerable Upper Intake Level (UL) for calcium of 2500 mg/person/day as a nutrient, and also established a Population Reference Intake (PRI) of 700 mg calcium/day (range 400-1200 mg/day depending on age and physiological status) (SCF, 2003). Calcium carbonate is included in Commission Decision 2006/25727, establishing an inventory of ingredients in cosmetic products. In pharmaceuticals calcium carbonate is used as an excipient and as an active ingredient of antacids (Ph. Eur. Comment., 2009). A monograph on calcium carbonate is part of the European Pharmacopoeia (Ph. Eur., 2010). It is also included in Directive 91/414/28 concerning the placing of plant products on the market. Calcium carbonate has been registered under the REACH Regulation 1907/2006/29 (ECHA, 2011). EFSA have previously provided opinions on the calcium salt calcium citrate malate as a source for calcium for use in foods for particular nutritional uses and in foods for the general

population (including food supplements) (EFSA, 2007) and on calcium sulphate as a source of calcium in food supplements (EFSA, 2008b). Calcium carbonate is included in the Food and Drug Administration (FDA) list of food additives that are GRAS for use in nutrient and dietary supplements, and is also certified by the FDA for use in amounts consistent with good manufacturing practice to colour drugs generally (FDA, 2006)” (EFSA, 2011. EFSA Journal 9(7):2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

“The [EFSA] Panel therefore agreed with the group ADI “not specified” established by the SCF in 1991 for a group of carbonates including calcium carbonate and considers that the JECFA definition of “ADI not specified” is applicable to calcium carbonate when used as a food additive” (EFSA, 2011. EFSA Journal 9(7):2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

There is a REACH dossier on calcium carbonate (CAS RN 471-34-1) (ECHA, 2022a), and limestone (CAS RN 1317-65-3) is pre-registered under REACH (“envisaged registration deadline 30 November 2010”) (ECHA).

Calcium carbonate (CAS RN 471-34-1) and limestone (CAS RN 1317-65-3) are included on the Safer Chemical Ingredients List (US EPA, 2022).

Calcium carbonate (CAS RN 471-34-1) and limestone (CAS RN 1317-65-3) are listed in the US EPA InertFinder Database (2022) as cleared for food and non-food use pesticide products. For food use they are covered under 40 CFR sections 180.910 (Inert ingredients used pre- and post-harvest; exemptions from the requirement of a tolerance) and 180.930 (Inert ingredients applied to animals; exemptions from the requirement of a tolerance) (US EPA, 2022).

Calcium carbonate (CAS RN 471-34-1) and limestone (CAS RN 1317-65-3) are both listed on the US EPA Toxic Substances Control Act (TSCA) inventory and also on the US EPA CDR 2020 list (Chemical Data Reporting Rule). Limestone also appears on the CDR 2020 partial exempt list. 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.

The TSCA inventory, 2020 CDR and 2020 CDR partial exempt lists are available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do

Substance	Calcium carbonate			
CAS No.	471-34-1			
	Limit value - Eight hours		Limit value - Short term	
	ppm	mg/m ³	ppm	mg/m ³
Australia		10 (1)		
Canada - Québec		10 (1)		
France		10 inhalable aerosol		
Hungary		10 inhalable aerosol		
Ireland		10 (1)		
		4 (2)		

Latvia		6		
New Zealand		10 (1)		
Poland		10		
Singapore		10 (limestone, marble)		
Switzerland		3 respirable aerosol		
USA - OSHA		15 total dust		
		5 respirable dust		
United Kingdom		10 inhalable aerosol		
		4 respirable aerosol		
	Remarks			
Australia	(1) This value is for inhalable dust containing no asbestos and <1 % crystalline silica.			
Canada - Québec	(1) Total dust			
Ireland	(1) Inhalable fraction (2) Respirable fraction			
New Zealand	(1) The value for inhalable dust containing no asbestos and less than 1% free silica.			
Substance	Marble			
CAS No.	1317-65-3			
	Limit value - Eight hours		Limit value - Short term	
	ppm	mg/m ³	ppm	mg/m ³
Belgium		10		
Japan - JSOH		1 (1)		
		4 (2)		
People's Republic of China		8 (1)		
		4 (2)		
South Korea		10		
Switzerland		3 respirable aerosol		
USA - NIOSH		10 total dust		

		5 respirable fraction		
USA - OSHA		15 inhalable aerosol		
		5 respirable aerosol		
United Kingdom		10 total dust		
		4 respirable dust		
	Remarks			
Japan - JSOH	(1) Respirable dust (2) Total dust: Total dust comprises particles with a flow speed of 50 to 80 cm/sec at the entry of a particle sampler.			
People's Republic of China	(1) Inhalable fraction (2) Respirable fraction			
Substance	Limestone			
CAS No.	1317-65-3			
	Limit value - Eight hours		Limit value - Short term	
	ppm	mg/m ³	ppm	mg/m ³
Belgium		10		
Canada - Québec		10 (1)(2)		
Hungary		10 inhalable aerosol		
Japan - JSOH		2 (1)		
		8 (2)		
People's Republic of China		8 (1)		
		4 (2)		
South Korea		10		
Spain		10 inhalable aerosol		
Switzerland		3 respirable aerosol		
USA - NIOSH		10 (1)(3)		
		5 (2)(3)		
USA - OSHA		15 total dust		

		5 respirable dust		
United Kingdom		10 inhalable aerosol		
		4 respirable aerosol		
	Remarks			
Canada - Québec	(1) Total dust (2) The standard corresponds to dust containing no asbestos and the percentage in crystalline silica is less than 1%			
Japan - JSOH	(1) Respirable dust (2) Total dust: Total dust comprises particles with a flow speed of 50 to 80 cm/sec at the entry of a particle sampler.			
People's Republic of China	(1) Inhalable fraction (2) Respirable fraction			
USA - NIOSH	(1) Total dust (2) Respirable aerosol (3) Calcium carbonate			

As taken from GESTIS, 2021.

Limestone (CAS RN 1317-65-3) and calcium carbonate (CAS RN 471-34-1) are included on the New Zealand Inventory of Chemicals Both of them do not have an individual approval but may be used under an appropriate group standard.,

Calcium carbonate (CAS RN 471-34-1) is classified according to the Environmental Protection Authority of New Zealand (NZ EPA CCID).

Calcium carbonate (CAS RN 471-34-1) and limestone (CAS RN 1317-65-3) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2022b).

Calcium carbonate (E170) is authorised for use as a food additive under EU regulation nos 1129/2011 and 2016/0691 (European Commission, 2018). As a Group I additive it is authorized for use as a food additive under EU regulation nos 1129/2011, 438/2013, 2015/0647, 2015/1832 and 2018/1497. As a Group II additive it is authorized for use as a food additive under EU regulation nos 1129/2011, 380/2012, 438/2013, 0509/2013, 2018/1497 and 1819/2020. (European Commission, 2019b, 2022).

The CIR Expert Panel concluded that calcium carbonate is safe in the present practices of use and concentration, as described in their safety assessment, when formulated to be non-irritating.

As taken from CIR, 2017

Substance	Calcium carbonate
CAS #	471-34-1
EC #	207-439-9 / 215-279-6
Colour index Number / Name of Common Ingredients Glossary	CI 77220
INN/ISO/AN	

Regulation	(EC) 2009/1223
Regulated By	88/667/EEC
Other Directives/Regulations	
Annex/Ref #	IV/124
Colour	White
Product Type, body parts	
Maximum concentration in ready for use preparation	
Other	Purity criteria as set out in Commission Directive 95/45/EC (E 170)
Wording of conditions of use and warnings	
SCCS opinions	
Chemical/IUPAC Name	Calcium carbonate
Identified INGREDIENTS or substances e.g.	CALCIUM CARBONATE CI 77220
Note	
Current Version	v.1

As taken from CosIng

Carbonic acid, calcium salt (1:1) (CAS RN 471-34-1) and limestone (CAS RN 1317-65-3) have been “identified as low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach” and “pose no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” (AICIS, 2012).

Calcium carbonate (CAS RN 471-34-1) 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 exposures:

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)
CALCIUM CARBONATE	AURICULAR (OTIC)	SOLUTION	471341	H0G9379FGK	0.38%w/w	
CALCIUM CARBONATE	BUCCAL	GUM, CHEWING	471341	H0G9379FGK		3492mg
CALCIUM CARBONATE	DENTAL	POWDER, DENTIFRICE	471341	H0G9379FGK	0.5mg/mg	

CALCIUM CARBONATE	INTRAVENOUS	SOLUTION	471341	H0G9379FGK	4.02%w/v	
CALCIUM CARBONATE	ORAL	CAPSULE	471341	H0G9379FGK	349.9mg	
CALCIUM CARBONATE	ORAL	CAPSULE, EXTENDED RELEASE	471341	H0G9379FGK	4mg	
CALCIUM CARBONATE	ORAL	FILM, SOLUBLE	471341	H0G9379FGK		14mg
CALCIUM CARBONATE	ORAL	TABLET	471341	H0G9379FGK		793mg
CALCIUM CARBONATE	ORAL	TABLET, CHEWABLE	471341	H0G9379FGK		1650mg
CALCIUM CARBONATE	ORAL	TABLET, COATED	471341	H0G9379FGK	64.8mg	
CALCIUM CARBONATE	ORAL	TABLET, DELAYED RELEASE	471341	H0G9379FGK	8.4mg	
CALCIUM CARBONATE	ORAL	TABLET, EXTENDED RELEASE	471341	H0G9379FGK	229.7mg	
CALCIUM CARBONATE	ORAL	TABLET, FILM COATED	471341	H0G9379FGK		300mg
CALCIUM CARBONATE	RESPIRATORY (INHALATION)	SOLUTION	471341	H0G9379FGK	4.02%w/v	

As taken from FDA, 2022c.

Calcium carbonate (CAS RN 471-34-1) is listed by the US EPA Office of Pesticide Programs (2021) and was first registered as an antimicrobial pesticide on 22 March 2004. It is noted as being under registration review.

The Food Safety Commission of Japan concluded that calcium carbonate is of no safety concern when used appropriately as a food additive (FSCJ, 2020).

Calcium carbonate (CAS RN 471-34-1) is classified as an NHP [Natural Health Product] in medicinal products, under Schedule 1, item 7 (a mineral) of the Natural Health Products Regulations (Health Canada, 2022).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

“After intestinal absorption, calcium and carbonate/bicarbonate ions enter normal metabolic pathways and body pools. The majority of absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and also faeces and skin) and excess carbonate is

excreted as carbon dioxide via respiration” (EFSA, 2011. EFSA Journal 9(7):2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

After ingestion /of CaCO₃ tablets/, it is converted to sol calcium salts in stomach, and calcium is thereby made available for absorption. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 1528] **PEER REVIEWED**

Calcium excretion varies directly with creatinine clearance. Amount excreted falls far short of amount absorbed, even after weeks of treatment. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 992] **PEER REVIEWED**

As taken from HSDB, 2014

“Calcium ions are inorganic and stable to reduction or oxidation in biological systems. Calcium carbonate plays a wide variety of roles in biological processes, for example acting as a catalytic site for reactions and or transferring atoms or groups to catalytic sites. Calcium is also complexed to important biological molecules such as calmodulin and calbindin (<https://echa.europa.eu/es/registration-dossier/-/registered-dossier/16050/7/2/1>).”

As taken from European Commission, 2019a.

A comparative in vitro metabolism study was not considered necessary given (i) that calcium carbonate is essential to all life forms, (ii) its long history of safe use as a food additive and as a gastric antiacid and (iii) the available toxicokinetic (ADME) information not suggesting any significant metabolic difference between humans and other species.

As taken from EFSA, 2022.

4.2. Absorption, distribution and excretion

“Calcium carbonate dissociates into its constituent ions in the acid milieu of the stomach. Some of the calcium is absorbed, via active transport or passive diffusion, but a large proportion (up to 80 %) is reconverted to calcium carbonate and other insoluble calcium salts and excreted in the faeces. Average absorption of calcium from calcium carbonate over a range of studies has been shown to be in the range of 20-40%. The Panel noted however that recent toxicokinetic data in mice indicate that calcium from nanoparticulate calcium carbonate is more readily absorbed than the microparticulate form, as evidenced by significantly higher serum calcium concentrations in the nano calcium carbonate group than in the micro calcium carbonate group. A slight increase in calcium bioavailability (by 38%) from nanonised pearl powder form by comparison with the corresponding micronised form of calcium carbonate has also been observed in humans. In view of the small increases observed in these studies, the Panel considered that the absorption of calcium from calcium carbonate in nanoparticles will not be markedly different than that from calcium carbonate microparticles” (EFSA, 2011. EFSA Journal 9(7):2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

“As reported by SCF (2003), after intestinal absorption, calcium and carbonate/bicarbonate ions can enter normal metabolic pathways and body pools. The majority of absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and also via faeces and skin) and excess carbonate is excreted as carbon dioxide via respiration” (EFSA, 2011. EFSA Journal 9(7):2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

Amount of calcium absorbed from calcium carbonate is usually stated to be 10%, but ? depends upon amount of gastric acid; in 1 study, 0-2% of single 2 g dose was ... absorbed in achlorhydric persons, 9-16% in normal subjects, and 11-37% in patients with peptic ulcer ... [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of

Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 992] **PEER REVIEWED**

Fraction absorbed seems to be nearly the same when CaCO_3 is given chronically in daily doses of 20 g /as when it is given in single 2 g dose/. ... Amount absorbed probably reaches a plateau at a dose of about 20 g. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 992] **PEER REVIEWED**

... Increased calcium excretion almost always follows admin of antacid doses of calcium carbonate ... [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 992] **PEER REVIEWED**

When calcium carbonate is admin orally, a limited amount of calcium and intestinal bicarbonate are absorbed ... calcium is excreted by kidneys ... [American Hospital Formulary Service. Volumes I and II. Washington, DC: American Society of Hospital Pharmacists, to 1984., p. 56:04] **PEER REVIEWED**

Calcium carbonate is slowly solubilized in stomach and reacts with hydrochloric acid to form calcium chloride, carbon dioxide, and water. About 90% of calcium chloride formed is converted to insol calcium salts (mainly calcium carbonate and to lesser extent calcium phosphate) and calcium soaps in small intestine and is not absorbed. [American Hospital Formulary Service. Volumes I and II. Washington, DC: American Society of Hospital Pharmacists, to 1984., p. 56:04] **PEER REVIEWED**

Normal persons excrete average of 7% of daily intake of 50 mg/kg (approx equiv to 8 g of CaCO_3). Some individuals appear to be hyperexcretors of calcium and are prone to nephrolithiasis. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 992] **PEER REVIEWED**

Calcium carbonate is non-absorbable antacid. It combines with hydrochloric acid to form sol calcium chloride. In small intestine most of calcium chloride is reconverted to insol calcium carbonate or calcium phosphate. [Miller, R. R., and D. J. Greenblatt. Handbook of Drug Therapy. New York: Elsevier North Holland, 1979., p. 1044] **PEER REVIEWED**

Approximately one-fifth to one-third of orally administered calcium is absorbed in the small intestine, depending on presence of vitamin D metabolites, pH in lumen, and on dietary factors, such as calcium binding to fiber or phytates. Calcium absorption is increased when a calcium deficiency is present or when a patient is on a low-calcium diet. In patients with achlorhydria or hypochlorhydria, calcium absorption, especially with the carbonate salt, may be reduced. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 742] **PEER REVIEWED**

Elimination: Renal (20%) - The amount excreted in the urine varies with degree of calcium absorption and whether there is excessive bone loss or failure of renal conservation. Fecal (80%) - Consists mainly of nonabsorbed calcium, with only a small amount of endogenous fecal calcium excreted. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 742] **PEER REVIEWED**

Antacids are cleared from the empty stomach in about 30 min and vary in the extent to which they are absorbed. Antacids that contain aluminum, calcium, or magnesium are less completely absorbed than are those that contain NaHCO_3 ... About 15% of orally admin Ca^{+2} is absorbed, causing a transient hypercalcemia. /Antacids/ [Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1013] **PEER REVIEWED**

After single 4-g dose in normal subjects there is rise in plasma calcium concentration that lasts less than 3 hours; after 8-g dose, hypercalcemia is more persistent. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 992] **PEER REVIEWED****

As taken from HSDB, 2014

“OBJECTIVE: To investigate the characteristics of calcium metabolism in relation to different levels of dietary calcium intakes in premenarche Chinese girls.

METHODS: Forty-nine healthy premenarche girls of Han ethnic (9 - 11.5 years) were recruited, and divided into four groups respectively, receiving four different doses of calcium intakes for 6 days, 600 mg (usual diet), 900 mg (containing 250 ml of milk), 1200 mg (containing 250ml of milk and 750 mg of calcium carbonate) and 1500mg calcium (250 ml of milk and 1500 mg of calcium carbonate) per day. A 3-day urine and stool, and a 3-d duplicated food samples were collected to assess the calcium excretion in urine and feces and input of the dietary calcium during the treatment period.

RESULTS: There were no significant differences in appearance calcium absorption among the four groups (55%, 53%, 52% and 52%). Urine calcium is correlated negatively with dietary protein and dietary phosphorus. And no correlation was found between dietary protein and calcium absorption.

CONCLUSION: The appearance calcium absorption was (53 +/- 0.12) % in Chinese premenarche girls with dietary calcium intakes ranged between 800 to 1600 mg/d, high dietary protein intakes increase urine calcium secrete.” As taken from Luo XL et al. Wei Sheng Yan Jiu. 2005 Jul;34(4):445-8. [Article in Chinese] available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&listuids=16229272&query_hl=18&itool=pubmed_DocSum

“BACKGROUND: Orally administered particles rapidly interact with biological fluids containing proteins, enzymes, electrolytes, and other biomolecules to eventually form particles covered by a corona, and this corona potentially affects particle uptake, fate, absorption, distribution, and elimination in vivo. This study explored relationships between the biological interactions of calcium carbonate particles and their biokinetics. **METHODS:** We examined the effects of food grade calcium carbonates of different particle size (nano [N-Cal] and bulk [B-Cal]: specific surface areas of 15.8 and 0.83 m²/g, respectively) on biological interactions in in vitro simulated physiological fluids, ex vivo biofluids, and in vivo in gastrointestinal fluid. Moreover, absorption and tissue distribution of calcium carbonates were evaluated following a single dose oral administration to rats. **RESULTS:** N-Cal interacted more with biomatrices than bulk materials in vitro and ex vivo, as evidenced by high fluorescence quenching ratios, but it did not interact more actively with biomatrices in vivo. Analysis of coronas revealed that immunoglobulin, apolipoprotein, thrombin, and fibrinogen, were the major corona proteins, regardless of particle size. A biokinetic study revealed that orally delivered N-Cal was more rapidly absorbed into the blood stream than B-Cal, but no significant differences were observed between the two in terms of absorption efficiencies or tissue distributions. Both calcium carbonates were primarily present as particulate forms in gastrointestinal fluids but enter the circulatory system in dissolved Ca(2+), although both types showed partial phase transformation to dicalcium phosphate dihydrate. Relatively low dissolution (about 4%), no remarkable protein-particle interaction, and the major particulate fate of calcium carbonate in vivo gastrointestinal fluids can explain its low oral absorption (about 4%) regardless of particle size. **CONCLUSION:** We conclude that calcium carbonate nanoparticles can act more actively with biological matrices in vitro and ex vivo, but that in vivo, their biological interactions and biokinetics are not affected by particle size.” As taken from Lee JA et al. 2015. Int. J. Nanomedicine 10, 2273-93. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25848250>

“BACKGROUND: Prenatal calcium and iron supplements are recommended in settings of low dietary calcium intake and high prevalence of anemia. However, calcium administration may inhibit iron absorption. To overcome calcium-iron interactions, we developed a multi-micronutrient powder

containing iron (60 mg), folic acid (400 µg), and calcium carbonate granules microencapsulated with a pH-sensitive enteric coating to delay intestinal release. OBJECTIVES: We aimed to establish in vivo evidence that enteric-coated (EC) calcium is bioavailable in pregnant women and to explore the dose-responsiveness of fractional calcium absorption (FCA) in pregnancy. DESIGN: This was a randomized crossover trial in pregnant women (26-28 wk of gestation) in Dhaka, Bangladesh. Participants were allocated to 1 of 3 dose groups (500, 1000, or 1500 mg elemental Ca). FCA was estimated in random order for EC and non-EC (control) granules by a dual-stable-isotope method ((⁴⁴)Ca-labeled granules and intravenous (⁴²)Ca) on the basis of the relative recovery of (⁴⁴)Ca compared with (⁴²)Ca in urine over 48 h. RESULTS: Forty-nine participants with FCA for both EC and non-EC granules were included in the primary analyses. FCA geometric means were as follows: 21.8% (500 mg), 9.2% (1000 mg), and 11.7% (1500 mg) for non-EC granules compared with 3.3% (500 mg), 1.2% (1000 mg), and 2.1% for EC granules. Cumulative 48-h FCA of EC calcium was 85% lower (P<0.001) than that of non-EC calcium, after adjustment for dose. In comparison to 500 mg, the FCA for the 1000-mg dose was 61% lower (P<0.001) and was 42% lower (P = 0.002) for the 1500-mg dose, after adjustment for formulation. CONCLUSIONS: A pH-sensitive enteric coating substantially reduced calcium absorption from a prenatal multi-micronutrient powder. In its current formulation, this novel supplement is not suitable for clinical use. FCA was highly dose-dependent, such that doses of 1000 and 1500 mg delivered only negligibly more bioavailable calcium than the 500-mg dose. This trial was registered at clinicaltrials.gov as NCT01678079.” As taken from Roth DE et al. 2014. Am. J. Clin. Nutr. 100, 1587-95. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25411294>

“Calcium Carbonate (0.40 mCi of calcium ¹⁴C-carbonate pellet) was implanted intraperitoneally into a male rat.²⁶ Approximately 72% of the radiolabeled carbonate was excreted as respiratory carbon dioxide between 2 h and 142 h after implantation (most after 69 h). Approximately 30% of the dose was recovered in unabsorbed pellet. Urinary radioactivity accounted for 0.27% of the dose and fecal radioactivity accounted for approximately 0.07% of the dose; 1% of the absorbed dose was retained by the tissues.”

“The absorbability of calcium from Calcium Carbonate and calcium citrate salts was evaluated in a study in which 37 men and women ingested 300 mg (low load) and 1000 mg (high load) calcium loads as part of a light breakfast meal.²⁸ The subjects were randomly assigned to a sequence of either Calcium Carbonate first and then calcium citrate, or vice versa. Each absorption measurement was conducted over 2 study days. On the first day, the subjects received no supplement; on the second day, the subjects received a 1000 mg calcium load. The test with the 300 mg load was performed similarly. For the high load, absorption was measured by tracer appearance in the serum and by absorptive increment in urinary calcium. For the low load, absorption was measured using the tracer method only. Test materials (in gelatin capsules) were labelled with ⁴⁵Ca. Individual tracer doses were in the range of 5 to 9 µCi. Relative absorption was estimated from the difference in urinary calcium following breakfast on the 2 days and true fractional absorption from the appearance of ⁴⁵Ca in the serum. Mean tracer absorption for both salts combined was 36% at the 300 mg load and 28.4% at the 1000 mg load. In both experiments, the observed mean difference in absorption between the salts was very small. By the tracer method, the within-subject difference (Calcium Carbonate less citrate) was +3.3% ± 1.2% of the ingested dose at the high load and 3.6% ± 2.7% at the low load.

As taken from CIR, 2017.

“Upon oral ingestion, calcium carbonate dissolves slowly in the stomach. It reacts with gastric hydrochloric acid to produce calcium chloride, carbon dioxide and water (Committee on Updating of Occupational Exposure Limits, 2003). Some of the calcium is absorbed, via active transport or passive diffusion, but a large proportion (up to 80%) is reconverted to calcium carbonate and other insoluble calcium salts and excreted in the faeces. Average absorption of calcium from calcium carbonate over a range of studies has been shown to be in the range of 20- 40% (EFSA, 2011c). The large intestine has the ability to absorb large amounts of Ca from CaCO₃, which is very

sparingly soluble in water. FOS-feeding increased the absorption of Ca in rats after CaCO₃ infusion into the stomach (> 40%), but not in rats with CaCO₃ infusion into the caecum (< 40%) The serum concentration of Ca in the FOS fed groups was higher when Ca was infused into the stomach than when Ca was infused into the caecum. Ca content of the femur did not differ among all of the groups, irrespective of FOS feeding (Ohta et al, 1997)."

"After intestinal absorption, calcium and carbonate/bicarbonate ions enter normal metabolic pathways and body pools. The majority of absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and also faeces and skin) and excess carbonate is excreted as carbon dioxide via respiration (EFSA, 2011c).

The calcium ions are distributed in the serum and then throughout the body. The majority of calcium is stored in the skeleton. Calcium, as a bulk metal is found in a variety of proteins and enzymes and is important in the transmission of signals in nerves. At the cellular level, metal ions, such as calcium are used in biology in communication roles to trigger cellular responses. At the macroscopic level, solid calcium compounds also play a structural role as a major component of bones, teeth and shells. As calcium ions are indispensable to life their distribution is tightly regulated systemically as well as intra-cellular. Calcium levels in the body are regulated by homeostatic processes, the same amount is excreted as taken up. These homeostatic processes are able to deal with moderate increases in calcium intake: either by storage in bone or by excretion via urine, faeces or sweat (<https://echa.europa.eu/es/registration-dossier/-/registered-dossier/16050/7/2/1>).

The distribution of C14 in the body and excreta of a mature rat was investigated following implantation of a pellet of CaC14O₃ in each of the lower quadrants of the peritoneal cavity (Armstrong et al, 1948). About 30% of the administered CaC14O₃ remained unabsorbed and about 0.75% of the administered dose (≈1.0% of the absorbed dose) was retained by tissues. A significant incorporation of C14 was found in the inorganic carbonate fraction of bone and in bone protein, the dentin and enamel of teeth, fatty acids, glycerol, haemin, red cell protein, plasma proteins, central nervous system protein, liver and muscle glycogen, muscle protein and in the proteins of the testes and of the thoracic and abdominal viscera. C14 was excreted in expired air (72%), urine (0.27%) and faeces (0.07%)."

"Calcium carbonate has the potential to be inhaled due to its particle size distribution. Its nature as a physiological substance will probably lead to some absorption via the respiratory tract. Non-resorbed particles in the oral cavity, the thorax and the lungs will be transferred to the gastrointestinal tract with the mucus and absorbed there. Therefore, absorption from the gastrointestinal tract will contribute to the total systemic burden of the substance that is inhaled (<https://echa.europa.eu/es/registration-dossier/-/registered-dossier/16050/7/2/1>).

Absorption via the dermal route is expected to be very low based on the inorganic nature of calcium carbonate (<https://echa.europa.eu/es/registration-dossier/-/registered-dossier/16050/7/2/1>)."

As taken from European Commission, 2019a.

Dermal penetration – 15% (CAS RN 1317-65-3) (PPDB, 2021).

4.3. Interactions

... A mixture of 2 parts magnesium oxide and 1 part calcium carbonate produces relatively normal stool for many patients. [American Medical Association, AMA Department of Drugs. AMA Drug Evaluations. 4th ed. Chicago: American Medical Association, 1980., p. 954] **PEER REVIEWED**

Milk-alkali syndrome may occur after prolonged admin of calcium carbonate with concomitant use of sodium bicarbonate and/or homogenized milk containing Vit D. [American Medical Association, AMA Department of Drugs. AMA Drug Evaluations. 4th ed. Chicago: American Medical Association, 1980., p. 955] **PEER REVIEWED**

Certain anions in antacids (carbonate and hydroxide) ... are thought to form insoluble complexes when combined with iron. A study evaluating the effect of antacids and iron absorption used patients with mild iron deficiency and found that when Mylanta II (5 ml) was given with 10 mg of iron, the increase in plasma iron seen two hours after the dose was not significantly different from the iron level seen two hours after a control dose. ... calcium carbonate 500 mg also decreased the two-hour plasma levels, with serum iron levels attaining only one-third of the control dose. [Young, L.Y., M.A. Koda-Kimble (eds.). Applied Therapeutics. The Clinical Use of Drugs. 6th ed. Vancouver, WA., Applied Therapeutics, Inc. 1995., p. 88-7] **PEER REVIEWED**

Concurrent use of excessive amounts of /alcohol, caffeine (usually more than 8 cups of coffee a day), or tobacco/ has been reported to decrease calcium absorption. [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use /of estrogens/ with calcium supplements may increase calcium absorption, which is used to therapeutic advantage when estrogens are prescribed for the treatment of postmenopausal osteoporosis. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use with calcium supplements may decrease effectiveness of cellulose sodium phosphate in preventing hypercalciuria. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use /of other calcium-containing medications or oral magnesium-containing medications/ with calcium supplements may increase serum calcium or magnesium concentration in susceptible patients, mainly patients with impaired renal function, leading to hypercalcemia or hypermagnesemia, respectively. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use of large doses of vitamin D with calcium supplements may excessively increase intestinal absorption of calcium, increasing risk of chronic hypercalcemia in susceptible patients; however, it may be therapeutically advantageous in elderly and high-risk groups when it is necessary to prescribe vitamin D or its derivatives with calcium; careful monitoring of serum calcium concentration is essential during long-term therapy. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Excessive intake, more than 5,000 IU per day, of vitamin A may stimulate bone loss and counteract the effects of calcium supplementation and may cause hypercalcemia. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use /of oral tetracyclines/ with calcium supplements may decrease absorption of tetracyclines because of possible formation of nonabsorbable complexes and increase in intragastric pH; patients should be advised not to take calcium supplements within 1 to 3 hours of taking tetracyclines. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use /of potassium and sodium phosphates/ with calcium supplements may increase potential for deposition of calcium in soft tissues if serum ionized calcium is high. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use decreases the bioavailability of both phenytoin and calcium because of possible formation of a nonabsorbable complex; patients should be advised not to take calcium supplements within 1 to 3 hours of taking phenytoin; also, enteral feeding solutions containing calcium should not

be administered through a nasogastric feeding tube together with phenytoin oral suspension; a 2 hour interval should elapse between the administration of the feeding solution and of the phenytoin. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use /of neuromuscular blocking agents (except succinylcholine)/ with parenteral calcium salts usually reverses the effect of nondepolarizing neuromuscular blocking agents. Also, concurrent use with calcium salts has been reported to enhance or prolong the neuromuscular blocking action of tubocurarine. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent and prolonged use /of milk or milk products/ with calcium supplements may result in the milk-alkali syndrome. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use with calcium supplements may antagonize the effect of gallium nitrate. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use of large amount of fiber or phytates, especially in patients being treated for hypocalcemia, with calcium supplements may reduce calcium absorption by formation of nonabsorbable complexes. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use with calcium supplements may prevent absorption of etidronate; patients should be advised not to take etidronate within 2 hours of calcium supplements. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use /of thiazide diuretics/ with large doses of calcium supplements may result in hypercalcemia because of reduced calcium excretion. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use of parenteral calcium salts with digitalis glycosides may increase the risk of cardiac arrhythmias; therefore, when the parenteral administration of calcium salts to digitalized patients is deemed necessary, caution and close electrocardiographic (ECG) monitoring are recommended. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Concurrent use of /calcium-channel block agents/ with calcium supplements in quantities sufficient to raise serum calcium concentrations above normal may reduce the response to verapamil and probably other calcium-channel blockers. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 743] **PEER REVIEWED**

Itraconazole concn decreased /by/ simultaneous antacids /admin which/ ... decrease gastric acidity. /From table/ [Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1303] **PEER REVIEWED**

Calcium and vitamin D are chemopreventive agents for colorectal neoplasia ... In the current study, adenomatous polyps were transected, approximately 50% were removed for histologic examination, and the remnants tattooed before the administration of either calcium carbonate (1500 mg 3 times daily) plus vitamin D(3) 400 IU or a placebo for 6 months. At study end, polyp remnants were resected completely and were used for histologic examination. Immunohistochemical staining was performed in both flat mucosa and in polyp tissue. Proliferation

was assessed by MIB-1 staining; apoptosis was assessed by terminal deoxyuridine triphosphate-biotin nick-end labeling, BAK, and Bcl-2 staining; and cytokeratin AE1, vitamin D receptor, MUC5AC mucin, and galectin-3 were assessed by immunohistochemistry ... Nineteen patients, including 11 patients in the treatment group and 8 patients in the control group, completed the study. Proliferative indices fell both in flat mucosa and in polyps in the treatment group, and there were no significant changes in the control group. Apoptosis and Bcl-2 immunostaining were unchanged in both groups, but the frequency of BAK-immunostained cells in the interior of polyps rose significantly. Vitamin D receptor staining increased slightly and significantly in flat rectal tissue in the treatment group. There were no significant changes in galectin-3 staining, but a striking reduction in MUC5AC mucin staining in polyps was observed after treatment with calcium plus vitamin D ... The administration of a calcium plus vitamin D chemopreventive regimen resulted in several changes in adenomatous tissue that /the authors believed/ may have contributed to reduced polyp formation. [Holt PR et al; Cancer 106(2):287-96 (2006)] **PEER REVIEWED**

... Studies have shown that the bioavailability of gatifloxacin is decreased with concurrent administration of antacids ... [Mallet L, Huang A; Ann Pharmacother 39(1):150-2 (2005)] **PEER REVIEWED**

For subjects with meal-induced heartburn, treatment with low-dose ranitidine plus antacid is particularly effective in decreasing gastric and oesophageal acidity as well as heartburn severity. [Robinson M et al; Aliment Pharmacol Ther 15(9):1365-74 (2001)] **PEER REVIEWED**

In accordance with the study protocol, calcium carbonate (... 1500 mg = 600 mg as calcium ion) was administered as such (reference) and associated with cholecalciferol (400 IU) (test) for four days (2 doses/day) to 18 healthy male volunteers in a sequential pattern, namely reference followed by test. Urinary excretion of total calcium, and serum concentration of free and total calcium, 25-OH-vitamin D3 and parathyroid hormone were carefully analysed the day before (baseline) and on the 4th day of dosing, with validated methods. The effect of cholecalciferol in promoting calcium absorption was clearly observed from urinary excretion of total calcium, which with the test treatment showed a 16.6% increase in excretion ($p = 0.025$) compared with the reference treatment. The mean excretion values on the 4th day, expressed in mg, were 238.85 and 204.83 with test and reference respectively. Moreover, the results demonstrated an increased serum concentration of both free and total calcium after dosing with test and reference by comparison with the baseline situation. The area under the serum concentration-time curve of total calcium increased from day -1 to day 4 from 550.98 to 575.90 mg.hr/L with test and from 543.03 to 568.16 mg.hr/L with reference. Similarly, ionised calcium increased on day 4 with both the treatments. Parathyroid hormone showed the expected typical decreasing behaviour after dosing with the test and reference drugs. The results of this study suggest that calcium carbonate is absorbed through the intestine when administered either alone or in association with cholecalciferol. [Scotti A et al; Arzneimittelforschung 51(6):493-500 (2001)] **PEER REVIEWED**

A 62-yr-old woman with thyroid carcinoma metastatic to bone, and a history of subclinical hypoparathyroidism was admitted to the hospital in hypocalcemic crisis 5 wk after receiving iv pamidronate. The patient had tetany and laryngospasm. An electrocardiogram showed junctional rhythm with QT segment prolongation. The patient had previously maintained a low-normal serum calcium on 500 to 750 mg of calcium carbonate and 600 IU of vitamin D daily. One week after pamidronate administration the patient's calcium and vitamin D supplementation were inadvertently discontinued. She continued to take daily intranasal calcitonin. At the time of her hospitalization for hypocalcemia, the patient's serum calcium was 4.3 mg/dL. The patient received aggressive calcium and vitamin D supplementation. However, her serum calcium remained below 6 mg/dL for a 2-wk period, and took another week to return to the normal range ... Pamidronate and other bisphosphonates are becoming an integral part of the management of normocalcemic patients with malignant bone disease. Therefore, awareness of risk factors for hypocalcemia and familiarity with avenues available for protection from potentially catastrophic hypocalcemia are both crucial. [Mishra A et al; Endocrine 14(2):159-64 (2001)] **PEER REVIEWED**

... 1,25-Dihydroxyvitamin D₃ and calcium carbonate did not significantly improve bone loss in long-term renal transplant recipients. However, significant osteoclast suppression and a trend to maintain trabecular bone volume and wall thickness as well as improve the axial bone mineral density were observed in the treatment group /of 16 patients compared to 14 controls/. [Cueto-Manzano AM et al; Am J Kidney Dis 35(2):227-36 (2000)] **PEER REVIEWED**

To determine whether calcium supplementation with or without vitamin D is superior to vitamin D alone for the treatment of rickets, 123 Nigerian children (median age, 46 months) with rickets were randomized to treatment with vitamin D (600,000 U intramuscularly at enrollment and at 12 wk), calcium carbonate (1000 mg daily), or a combination of vitamin D and calcium in a double blind, controlled trial for 24 wk. Results showed that the daily dietary calcium intake was low in the children with rickets and the control children (median, 203 mg and 196 mg, respectively). Treatment produced a smaller increase in the mean serum calcium concentration in the vitamin D group (from 7.8 mg/dL at baseline to 8.3 mg/dL) at 24 wk than in the calcium group (from 7.5 mg/dl to 9.0 mg/dL) or the combination-therapy group (from 7.7 to 9.1 mg/dL). A greater proportion of children in the calcium and combination-therapy groups than in the vitamin D group reached the combined end point of a serum alkaline phosphatase concentration of 350 U/L or less and radiographic evidence of nearly complete healing of rickets (61%, 58%, and 19%, respectively). [Thacher TD et al; N Engl J Med 341:563-568 (1999)] **PEER REVIEWED**

In order to assess the effect of calcium on the toxic effects of fluoride, adult female Wistar rats were treated with sodium fluoride (NaF, 500 ppm in drinking water) alone or in combination with calcium carbonate (CaCO₃, 50 mg/ kg by oral intubation) daily for 60 days ... NaF treatment decreased food and water intake, reduced body-weight gain and impaired exploratory motor activity and rota-rod performance. Dental lesions, inhibition of the activities of AchE and N⁺ K⁺ ATPase and a decrease in the concentration of protein, and serum calcium were also observed in these animals. These effects were accompanied by a marked elevation of fluoride concentration in the serum. CaCO₃ decreased the concentration of fluoride in the serum of NaF-treated animals. A decrease in serum fluoride concentration was found also after NaF withdrawal. A prevention of locomotor behavioral, biochemical and dental toxicities of fluoride was observed both in these groups. [Ekambaram P, Paul V; Pharmacol Toxicol 90(2):53-8 (2002)] **PEER REVIEWED**

Recent studies showed that postmenopausal women lost less bone mass when supplemented with calcium or estrogen therapy. ... /The authors concluded that calcium/ supplement with a meal or combined calcium supplement and estrogen therapy is not associated with a significant increased risk of calcium oxalate stone formation in the majority of postmenopausal osteoporotic patients. Determination of urinary saturation for calcium oxalate after calcium and estrogen supplements, especially at the initial phase of treatment, may be helpful in the avoidance of nephrolithiasis. [Domrongkitthaiporn S et al; Maturitas 26;41(2):149-56 (2002)] **PEER REVIEWED**

... This study ... examined the effect of different calcium salts, Ca gluconate (CaG), Ca lactate (CaL), Ca carbonate (CaC) and Ca phosphate (CaP), on the clearance of radiostrontium (*Sr) administered either intraperitoneally (ip) (*Sr-ip group) or orally (*Sr-oral group) in rats. The influence of these Ca salts was examined in a group of animals administered *Sr ip, while the effect of three Ca salts (CaG, CaL and CaP) was studied in another group of rats given *Sr orally and compared with that of Ca alginate (CaA), normally advised for *Sr decorporation. Rats from both groups were subdivided into control and four experimental subgroups and were housed individually. The experimental subgroups were given the respective Ca salts (elemental Ca = 9 mg/rat/day) 2 hr post 85Sr, and thereafter once daily. In the *Sr-ip group, CaG was administered ip while the other Ca salts were given orally. In the *Sr-oral group all Ca salts were administered orally. In addition, the diet of all the experimental subgroups was supplemented with the respective Ca salts to 2% elemental Ca. The whole-body retention (WBR) of *Sr in animals treated with Ca salts was found to be significantly reduced from 50 to 60% at 24 hr to 20 to 30% at the end of 15 days compared with 70 to 80% at 24 hr to 50 to 60% at the end of 15 days in the untreated control animals. The results strongly suggest that CaA could be replaced by any of the commonly used Ca salts for curtailing

the WBR of ^{90}Sr . CaG which was administered ip, in the ^{90}Sr -ip group, was found to be more effective in reducing the WBR of ^{90}Sr . [Jagtap VS et al; J Radiol Prot 23(3):317-26 (2003)] **PEER REVIEWED**

Many trials based on the basic phenomena of isotopic dilution, adsorption, ion exchange, chelation, etc., have been attempted for the decorporation of radiostrontium, particularly Sr, after its entry in the in vivo system. We have recently demonstrated a non-isotopic carrier effect of some common calcium salts (calcium = 9 mg/mL) to reduce the whole body retention of radiostrontium, if administered within 2 hr after radiostrontium exposure and furthermore once daily, in rats, supplemented with calcium fortified diet. However, 25 to 30% of radiostrontium (compared to 50 to 60% in untreated animals) was still found to be retained in the animal even after 2 wk of treatment. Trial of some simple interventional measures, which would not adversely affect the animal metabolism, like pyrophosphate and magnesium sulfate, sodium citrate, chitin (a bio-absorbent), crown ether (a metal-chelator), and ammonium chloride, was therefore attempted to dislodge this remaining radiostrontium by switching over these animals to normal diet and subjecting them to different lines of treatment with these simple interventions through diet and drinking water separately for a further 4 wk. However, this remaining portion of radiostrontium is fixed in the bone and is difficult to dislodge. [Sonawane VR et al; Health Phys 87(1):46-50 (2004)] **PEER REVIEWED**

Pregnancy and lactation are times of physiological stress during which bone turnover is accelerated ... Studies have demonstrated that there is increased mobilization of lead from the maternal skeleton at this time and that calcium supplementation may have a protective effect. Ten immigrants to Australia were provided with either calcium carbonate or a complex calcium supplement (~ 1 g/day) during pregnancy and for 6 months postpartum. Sampling involved monthly venous blood samples throughout pregnancy and every two months postpartum, and quarterly environmental samples and 6-day duplicate diets. The geometric mean blood lead at the time of first sampling was 2.4 ug/dL (range 1.4-6.5). Increases in blood lead during the 3rd trimester, compared with the minimum value observed, varied from about 10 to 50% with a geometric mean of 25%. The increases generally occurred at 6 to 8 months gestation in contrast to our earlier cohort, characterized by very low calcium intakes, where the increases occurred at 3 to 6 months. Large increases in blood lead concentration were found during the postpartum period compared with those during pregnancy; blood lead concentrations increased by between about 30 and 95% (geometric mean 65%; n = 8) from the minimum value observed during late pregnancy. From late pregnancy through postpartum, there were significant increases in the lead isotopic ratios from the minimum value observed during late pregnancy for 3 of 8 subjects ($P < 0.01$). The observed changes are considered to reflect increases in mobilization of lead from the skeleton in spite of calcium supplementation. The identical isotopic ratios in maternal and cord blood provide further confirmation of placental transfer of lead. The extra flux released from bone during late pregnancy and postpartum varies from 50 to 380 ug Pb (geometric mean 145 ug Pb) compared with 330 ug Pb in the earlier cohort. For subjects replete in calcium, the delay in increase in blood lead and halving of the extra flux released from bone during late pregnancy and postpartum may provide less lead exposure to the developing fetus and newly-born infant. [Gulson B et al; Toxicol Appl Pharmacol 197(3):270-1 (2004)] **PEER REVIEWED**

Is an ... antacid and good antidote for oxalic acid /preventing absorption of ingested oxalic acid/. [Gosselin, R.E., H.C. Hodge, R.P. Smith, and M.N. Gleason. Clinical Toxicology of Commercial Products. 4th ed. Baltimore: Williams and Wilkins, 1976., p. II-88] **PEER REVIEWED**

Concomitant administration of levothyroxine and calcium carbonate often results in levothyroxine malabsorption. While in most patients the clinical consequences of this interaction, even with prolonged exposure, are relatively small, overt hypothyroidism may develop in patients with preexisting malabsorption disorders. However ... the clinical manifestations of the initial levothyroxine deficit may not always be apparent and, of all usual laboratory thyroid function tests, only thyrotropin measurement will reliably uncover the exaggerated levothyroxine malabsorption ...

Decreased absorption of levothyroxine when given with calcium carbonate may be particularly pronounced in patients with preexisting malabsorption disorders. Once recognized, a change in drug administration schedule usually minimizes or eliminates this interaction. [Csako G et al; Ann Pharmacother 35(12):1578-83 (2001)] **PEER REVIEWED**

The efficacy of calcium with vitamin D supplementation for preventing hip and other fractures in healthy postmenopausal women remains equivocal ... 36,282 postmenopausal women, 50 to 79 years of age, who were already enrolled in a Women's Health Initiative (WHI) clinical trial /were recruited/ ... Participants /were randomly assigned/ to receive 1000 mg of elemental [corrected] calcium as calcium carbonate with 400 IU of vitamin D3 daily or placebo. Fractures were ascertained for an average follow-up period of 7.0 years. Bone density was measured at three WHI centers ... Hip bone density was 1.06 percent higher in the calcium plus vitamin D group than in the placebo group ($P < 0.01$). Intention-to-treat analysis indicated that participants receiving calcium plus vitamin D supplementation had a hazard ratio of 0.88 for hip fracture (95 percent confidence interval, 0.72 to 1.08), 0.90 for clinical spine fracture (0.74 to 1.10), and 0.96 for total fractures (0.91 to 1.02). The risk of renal calculi increased with calcium plus vitamin D (hazard ratio, 1.17; 95 percent confidence interval, 1.02 to 1.34). Censoring data from women when they ceased to adhere to the study medication reduced the hazard ratio for hip fracture to 0.71 (95 percent confidence interval, 0.52 to 0.97). Effects did not vary significantly according to prerandomization serum vitamin D levels ... Among healthy postmenopausal women, calcium with vitamin D supplementation resulted in a small but significant improvement in hip bone density, did not significantly reduce hip fracture, and increased the risk of kidney stones. [Jackson RD et al; N Engl J Med 354(7):669-83 (2006)] **PEER REVIEWED**

After ingestion /of CaCO_3 tablets/, it is converted to sol calcium salts in bowel, and calcium is thereby made available for absorption. Patients with achlorhydria may not solubilize calcium from ... preparation. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 1528] **PEER REVIEWED**

As taken from HSDB, 2014

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT: Gastric upset is a common side effect of nilotinib therapy, and calcium carbonate is frequently used concomitantly, either as antacid or as calcium supplementation. With the increasing number of oral agents in cancer therapy, oral drug-drug interactions are becoming more relevant. Nilotinib has already been shown to be absorbed to a much lesser extent when co-administered with proton pump inhibitors. Because exposure to sub-therapeutic concentrations of anticancer drugs such as nilotinib may result in selection of resistant clones and ultimately relapse, we studied the effect of a calcium carbonate supplement (Tums Ultra 1000®) on nilotinib pharmacokinetics. WHAT THIS STUDY ADDS: Calcium carbonate may be co-administered with nilotinib without significantly affecting the pharmacokinetics of nilotinib and potentially impacting efficacy. PURPOSE: Nilotinib is a second-generation oral tyrosine kinase inhibitor with superior efficacy compared with imatinib mesylate in the treatment for chronic phase chronic myelogenous leukemia. Calcium carbonate is commonly used as a source of calcium supplementation or as antacid to ameliorate the gastrointestinal side effects associated with nilotinib, which could have unknown effects on nilotinib absorption. The purpose of this study was to provide information on the effect of calcium carbonate on the PK of nilotinib in healthy volunteers. METHODS: Healthy subjects were enrolled in a two-period, open-label, single-institution, randomized, cross-over, fixed-schedule study. In one period, each subject received 400 mg of nilotinib p.o. In the other period, 4,000 mg of calcium carbonate (4 X Tums Ultra 1000®) was administered p.o. 15 min prior to the nilotinib dose. Plasma samples were collected at specified timepoints, concentrations of nilotinib were quantitated by LC-MS, and data were analyzed non-compartmentally. RESULTS: Eleven subjects were evaluable. Calcium supplementation did not significantly affect nilotinib pharmacokinetic parameters including area under the plasma

concentration versus time curve (18.4 µg/mL h alone vs. 16.9 µg/mL h with calcium carbonate, $p = 0.83$; 80 % power); maximum plasma concentration (C(max)) (0.670 µg/mL alone vs. 6.18 µg/mL with calcium carbonate, $p = 0.97$); or half-life (18.9 h alone vs. 17.2 h with calcium carbonate, $p = 0.18$). CONCLUSIONS: Our results indicate that the use of calcium carbonate does not significantly affect nilotinib pharmacokinetics." As taken from Tawbi HA et al. 2013. Cancer Chemother. Pharmacol. 72(5), 1143-7. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24036846>

"PURPOSE: Imatinib mesylate (Gleevec®/Glivec®) has revolutionized the treatment of chronic myeloid leukemias and gastrointestinal stromal tumors, and there is evidence for an exposure response relationship. Calcium carbonate is increasingly used as a calcium supplement and in the setting of gastric upset associated with imatinib therapy. Calcium carbonate could conceivably elevate gastric pH and complex imatinib, thereby influencing imatinib absorption and exposure. We aimed to evaluate whether use of calcium carbonate has a significant effect on imatinib pharmacokinetics. METHODS: Eleven healthy subjects were enrolled in a 2-period, open-label, single-institution, randomized crossover, fixed-schedule study. In one period, each subject received 400 mg of imatinib p.o. In the other period, 4,000 mg calcium carbonate (Tums Ultra®) was administered p.o. 15 min before 400 mg of imatinib. Plasma concentrations of imatinib and its active N-desmethyl metabolite CGP74588 were assayed by LC-MS; data were analyzed non-compartmentally and compared after log transformation. RESULTS: Calcium carbonate administration did not significantly affect the imatinib area under the plasma concentration versus time curve (AUC) (41.2 µg/mL h alone vs. 40.8 µg/mL h with calcium carbonate, $P = 0.99$), maximum plasma concentration (C max) (2.35 µg/mL alone vs. 2.39 µg/mL with calcium carbonate, $P = 0.89$). CONCLUSIONS: Our results indicate that the use of calcium carbonate does not significantly affect imatinib pharmacokinetics." As taken from Tawbi H et al. 2014. Cancer Chemother. Pharmacol. 73(1), 207-11. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24170263>

"OBJECTIVE: This study aims to test the added value of calcium and vitamin D (CaD) in fracture prevention among women taking postmenopausal hormone therapy (HT). METHODS: This is a prospective, partial-factorial, randomized, controlled, double-blind trial among Women's Health Initiative postmenopausal participants aged 50 to 79 years at 40 centers in the United States with a mean follow-up of 7.2 years. A total of 27,347 women were randomized to HT (0.625 mg of conjugated estrogens alone, or 0.625 mg of conjugated equine estrogens plus 2.5 mg of medroxyprogesterone acetate daily), and 36,282 women were randomized to 1,000 mg of elemental calcium (carbonate) plus 400 IU of vitamin D3 daily, each compared with placebo. A total of 16,089 women participated in both arms. The predefined outcomes were adjudicated hip fractures and measured bone mineral density. RESULTS: Interaction between HT and CaD on hip fracture (P interaction = 0.01) was shown. The effect of CaD was stronger among women assigned to HT (hazard ratio [HR], 0.59; 95% CI, 0.38-0.93) than among women assigned to placebo (HR, 1.20; 95% CI, 0.85-1.69). The effect of HT on hip fracture was stronger among women assigned to active CaD (HR, 0.43; 95% CI, 0.28-0.66) than among women assigned to placebo (HR, 0.87; 95% CI, 0.60-1.26). CaD supplementation enhanced the antifracture effect of HT at all levels of personal calcium intake. There was no interaction between HT and CaD on change in hip or spine bone mineral density. CONCLUSIONS: Postmenopausal women at normal risk for hip fracture who are on CaD supplementation experience significantly reduced incident hip fractures beyond HT alone at all levels of personal baseline total calcium intake." As taken from Robbins JA et al. 2014. Menopause 21(2), 116-23. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23799356>

SUMMARY: The Women's Health Initiative (WHI) double-blind, placebo-controlled clinical trial randomly assigned 36,282 postmenopausal women in the U.S. to 1,000 mg elemental calcium carbonate plus 400 IU of vitamin D(3) daily or placebo, with average intervention period of 7.0

years. The trial was designed to test whether calcium plus vitamin D supplementation in a population in which the use of these supplements was widespread would reduce hip fracture, and secondarily, total fracture and colorectal cancer. INTRODUCTION: This study further examines the health benefits and risks of calcium and vitamin D supplementation using WHI data, with emphasis on fractures, cardiovascular disease, cancer, and total mortality. METHODS: WHI calcium and vitamin D randomized clinical trial (CT) data through the end of the intervention period were further analyzed with emphasis on treatment effects in relation to duration of supplementation, and these data were contrasted and combined with corresponding data from the WHI prospective observational study (OS). RESULTS: Among women not taking personal calcium or vitamin D supplements at baseline, the hazard ratio [HR] for hip fracture occurrence in the CT following 5 or more years of calcium and vitamin D supplementation versus placebo was 0.62 (95 % confidence interval (CI), 0.38-1.00). In combined analyses of CT and OS data, the corresponding HR was 0.65 (95 % CI, 0.44-0.98). Supplementation effects were not apparent on the risks of myocardial infarction, coronary heart disease, total heart disease, stroke, overall cardiovascular disease, colorectal cancer, or total mortality, while evidence for a reduction in breast cancer risk and total invasive cancer risk among calcium plus vitamin D users was only suggestive. CONCLUSION: Though based primarily on a subset analysis, long-term use of calcium and vitamin D appears to confer a reduction that may be substantial in the risk of hip fracture among postmenopausal women. Other health benefits and risks of supplementation at doses considered, including an elevation in urinary tract stone formation, appear to be modest and approximately balanced. As taken from Prentice RL et al. 2013. Osteoporos. Int. 24(2), 567-80. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23208074>

“BACKGROUND: The present study was designed to examine the effects of vitamin D plus calcium administration on metabolic profiles and pregnancy outcomes among women at risk for pre-eclampsia. METHODS: In a prospective, double-blind, placebo-controlled trial, 60 women at risk for pre-eclampsia were randomised to take either 50 000 IU vitamin D3 every 2 weeks plus 1000 mg day⁻¹ calcium supplements (as calcium carbonate) (n = 30) or to receive placebos at the same times (n = 30) from 20 to 32 weeks of gestation. Fasting blood samples were taken at baseline and 12 weeks after intervention to determine related variables. Newborn anthropometric measurements were determined. RESULTS: Taking combined cholecalciferol and calcium supplements, compared to placebo, led to significant reductions in fasting plasma glucose (FPG) [mean (SD)] [-5.7 (5.5) versus -0.6 (12.6) mg dL⁻¹ , P = 0.04], serum insulin concentrations [-2.8 (6.0) versus +7.7 (9.8) µIU mL⁻¹ , P<0.001], homeostasis model of assessment-insulin resistance [-0.8 (1.3) versus +1.6 (2.2), P<0.001], homeostatic model assessment-beta cell function [-8.2 (25.8) versus +32.6 (41.3), P<0.001] and a significant rise in quantitative insulin sensitivity check index score [+0.02 (0.02) versus -0.02 (0.02), P<0.001]. Additionally, pregnant women who received cholecalciferol plus calcium supplements had increased serum high-density lipoprotein (HDL)-cholesterol [+4.6 (8.3) versus -2.9 (7.7) mg dL⁻¹ , P = 0.001] and plasma total glutathione (GSH) concentrations [+23.4 (124.0) versus -94.8 (130.2) µm, P = 0.001] compared to placebo. However, after adjustment for the baseline levels, maternal age and baseline body mass index, the effects on FPG levels (P = 0.13) and systolic blood pressure (P = 0.13) disappeared. CONCLUSIONS: Vitamin D plus calcium administration for 12 weeks had beneficial effects on glycaemic status, HDL-cholesterol, GSH and blood pressure among women at risk for pre-eclampsia.” As taken from Samimi M et al. 2016. J. Hum. Nutr. Diet. 29(4), 505-15. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/26467311>

“The present study was carried out to investigate the effects of fluoride toxicity on some biochemical, hormonal, and histological parameters of female rats and the protective role of calcium against such effects. Adult female albino rats were divided into five groups; control group received distilled water for 60 days, calcium group received calcium carbonate with dose of 50 mg/kg three times per week for 60 days, fluoride group received sodium fluoride with dose of 20 mg/kg three times per week for 60 days, calcium + fluoride group received calcium carbonate (50 mg/kg) then after 2 h received sodium fluoride (20 mg/kg) three times per week for 60 days, and

fluoride + calcium group received sodium fluoride (20 mg/kg) three times per week for 30 days then received calcium carbonate (50 mg/kg) three times per week for another 30 days. The results showed that the levels of thiobarbituric acid reactive substances, urea, creatinine, alkaline phosphatase, triiodothyronine, thyroxine, parathormone, phosphorous, magnesium, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and gamma glutamyl transferase were significantly increased in rats treated with fluoride while serum estradiol, calcium, and organ glutathione were significantly decreased. The histological examination of the femur bone revealed that fluoride treatment induced thinning of bone trabeculae with wilding of marrow space, demineralization, and loss of trabeculae interconnections. Also, the histological examination of hepatic and renal tissues of fluoride-treated rats showed some damages in these tissues while administration of calcium carbonate for 30 or 60 days during fluoride treatment minimized such damages. It could be concluded that administration of calcium to female rats can ameliorate the hazardous effects of fluoride observed in the biochemical, hormonal, and histological parameters.” As taken from Mohamed NE. 2016. Biol. Trace Elem. Res. 170(1), 128-44. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/26198134>

“BACKGROUND: Antiretroviral therapy initiation for HIV-1 infection is associated with 2% to 6% loss of bone mineral density (BMD). OBJECTIVE: To evaluate the effect of vitamin D3 plus calcium supplementation on bone loss associated with antiretroviral therapy initiation. DESIGN: 48-week prospective, randomized, double-blind, placebo-controlled study. (ClinicalTrials.gov data-NCT01403051). SETTING: 39 AIDS Clinical Trials Group units. PATIENTS: Adults with antiretroviral therapy-naïve HIV. MEASUREMENTS: BMD by dual-energy x-ray absorptiometry, 25-hydroxyvitamin D levels, and other laboratory assessments. RESULTS: 165 eligible patients were randomly assigned (79 received vitamin D3 plus calcium and 86 received placebo). The study groups were well-balanced at baseline: 90% were men, 33% were non-Hispanic black, and the median CD4 count was 0.341×10^9 cells/L. At 48 weeks, the percentage of decline in total hip BMD was smaller in the vitamin D3 plus calcium group than in the placebo group: Medians were -1.36% (interquartile range [IQR], -3.43% to 0.50%) and -3.22% (IQR, -5.56% to -0.88%), respectively (P = 0.004). Similar results were seen at the lumbar spine. At 48 weeks, 90% of patients achieved HIV-1 RNA levels less than 50 copies/mL. Levels of 25-hydroxyvitamin D3 increased with vitamin D3 plus calcium but not with placebo: Median change was 61.2 nmol/L (IQR, 36.4 to 94.3) versus 1.7 nmol/L (IQR, -13.2 to 10.7) (P<0.001). Overall, 103 patients (62%) reported 1 or more adverse event, with similar distribution between groups; no cases of hypercalcemia and 1 case of nephrolithiasis were reported in the placebo group. LIMITATION: No international sites were included, and follow-up was only 48 weeks. CONCLUSION: Vitamin D3 plus calcium supplementation mitigates the BMD loss seen with initiation of efavirenz/emtricitabine/tenofovir disoproxil fumarate.” As taken from Overton ET et al. 2015. Ann. Intern. Med. 162, 815-24. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26075752>

“PURPOSE: To determine the efficacy in reducing dentin hypersensitivity (DHS) of a combined in-office and home-use desensitizing system, each product containing 8% arginine and calcium carbonate (Test), following a dental scaling procedure, compared to the combination of a conventional prophylactic paste and a potassium nitrate dentifrice (Control), in a group of patients with known dentin hypersensitivity. METHODS: An 8-week clinical study, with 50 subjects, was conducted in Piracicaba, São Paulo, Brazil, using a double-blind/two treatment design. Air blast sensitivity assessments were used to compare the efficacy of the two approaches using both the Schiff scale as well as a Visual Analogue Scale (VAS). RESULTS: Immediately after prophylaxis, the Test treatment provided significant reduction in DHS when compared to baseline values (VAS = 26.2% and Schiff = 29.1%), while for Control treatment this difference was not statistically significant (VAS = 8.1% and Schiff = 6.6%). The comparison between groups after prophylaxis showed a greater DHS reduction for the Test treatment (P<0.05). The reductions in DHS after 2, 4 and 8 weeks were significant for both groups, however, when considering Schiff scale, the Test treatment provided greater DHS reduction after 2 weeks (44.5% for Test versus 27.7% for Control)

and 4 weeks (55.2% for Test and 40.5% for Control), while after 8 weeks there was no significant difference between groups (71.1% for Test versus 61.1% for Control).” As taken from França IL et al. 2015. Am. J. Dent. 28, 45-50. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25864242>

“AIM: The aim of this single-centre, two-cell, double-blind, randomized controlled clinical study was to evaluate the effectiveness of an in-office desensitizing paste containing 8% arginine and calcium carbonate in providing relief on dentine hypersensitivity immediately after scaling and root planing and its sustained relief over a 6-week period. MATERIALS AND METHODS: Fifty periodontitis subjects presenting hypersensitivity were subjected to scaling and root planing and in-office application of either 8% arginine and calcium carbonate desensitizing paste (25 subjects, test group) or fluoride-free prophylaxis paste (25 subjects, control group). Air-blast hypersensitivity was assessed using Schiff and Visual Analogue (VAS) scales at baseline, post-scaling, post-application, 2, 4 and 6 weeks. RESULTS: At all evaluation times, the test group presented significant % reduction in hypersensitivity relative to post-scaling (t-test, $P < 0.05$) (Schiff - Test: 57, 58.6, 60.2, 68; CONTROL: 28.6, 22.2, 23, 23) (VAS - Test: 60, 55.6, 60.1, 68.4; CONTROL: 25.9, 18.2, 20.6, 22.7) and significant % hypersensitivity difference relative to control (ancova, $P < 0.05$) (Schiff: 38.9, 45.9, 47.4, 57.7; VAS: 49.1, 48.9, 52.6, 61). CONCLUSION: The single in-office application of the 8% arginine-calcium carbonate desensitizing paste after scaling and root planing provided significant immediate reduction in dentine hypersensitivity, which sustained over a 6-week period.” As taken from Pepelassi E et al. 2015. J. Clin. Periodontol. 42, 37-45. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25269991>

“This short-term study assessed the efficacy and safety of calcium carbonate combined with calcitonin in the treatment of hypercalcemia in hemodialysis patients. Patients ($n=64$) on hemodialysis for chronic kidney disease for more than 6 months were included based on total serum calcium more than 10.5 mg/dL. All patients were randomized (1:1) to receive calcium carbonate combined with calcitonin (Group I) or lanthanum carbonate (Group II) for 12 weeks. Blood levels of calcium, phosphorus and intact parathyroid hormone (iPTH) were measured every month, bone mass density (BMD) and coronary artery calcium scores (CACS) were measured at 3 months. During the study period, serum calcium decreased from 10.72 ± 0.39 to 10.09 ± 0.28 mg/dL ($P < 0.05$), serum phosphorus decreased from 6.79 ± 1.05 to 5.46 ± 1.18 mg/dL ($P < 0.05$), and serum iPTH levels in the Group I and Group II were not significantly different from the baseline. There were no significant differences in CACS in either group. There were no significant differences in the BMD values between Group I and baseline. In Group II, the BMD values at the lumbar spine and femoral neck were significantly lower than those before the trial and significantly lower than the corresponding values of Group I ($P < 0.05$). Calcium carbonate combined with calcitonin and lanthanum carbonate were equally effective in the suppression of hypercalcemia in hemodialysis patients. There were no serious treatment-related adverse events in treatment with calcium carbonate combined with calcitonin.” As taken from Wei Y et al. 2014. Ther. Apher. Dial. 18, 618-22. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/24674384>

“OBJECTIVE: To analyze the treatment effect of calcium+vitamin D supplementation, hormone therapy, both, and neither on cardiovascular disease risk factors. METHODS: We conducted a prospective, randomized, double-blind, placebo-controlled trial among Women's Health Initiative (WHI) participants. The predefined primary outcome was low-density lipoprotein cholesterol (LDL-C). RESULTS: Between September 1993 and October 1998, a total of 68,132 women aged 50-79 years were recruited and randomized to the WHI-Dietary Modification ($n=48,835$) and WHI-Hormone Therapy trials ($n=27,347$). Subsequently, 36,282 women from WHI-Hormone Therapy (16,089) and WHI-Dietary Modification ($n=25,210$) trials were randomized in the WHI-Calcium+Vitamin D trial to 1,000 mg elemental calcium carbonate plus 400 international units vitamin D3 daily or placebo. Our study group included 1,521 women who participated in both the hormone therapy and calcium+vitamin D trials and were in the 6% subsample of trial participants with blood sample collections at baseline and years 1, 3, and 6. The average treatment effect with 95% confidence interval, for LDL-C, compared with placebo, was -1.6, (95% confidence interval

[CI] -5.5 to 2.2) mg/dL for calcium+vitamin D alone, -9.0 (95% CI -13.0 to -5.1) mg/dL for hormone therapy alone, and -13.8 (95% CI -17.8 to -9.8) mg/dL for the combination. There was no evidence of a synergistic effect of calcium+vitamin D+hormone therapy on LDL-C (P value for interaction=.26) except in those with low total intakes of vitamin D, for whom there was a significant synergistic effect on LDL (P value for interaction=.03). CONCLUSION: Reductions in LDL-C were greater among women randomized to both calcium+vitamin D and hormone therapy than for those randomized to either intervention alone or to placebo. The treatment effect observed in the calcium+vitamin D+hormone therapy combination group may be additive rather than synergistic. For clinicians and patients deciding to begin calcium+vitamin D supplementation, current use of hormone therapy should not influence that decision.” As taken from Schnatz PF et al. 2017. Obstet. Gynecol. 129(1), 121-129. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27926633>

“BACKGROUND: The purpose of the present study was to determine the effect of additional calcium carbonate treatment on fibroblast growth factor 23 (FGF23) levels in patients treated with calcitriol. METHODS: In this randomized, open-labeled, and parallel-group study, a total of 30 patients with early chronic kidney disease (CKD) and vitamin D deficiency were randomly assigned to two groups and received interventions for 8 weeks: 1) a combination of calcium carbonate and calcitriol group; and 2) calcitriol only group. The primary outcome was the difference in percentage change of serum FGF23 levels from baseline between the two groups. Secondary end points included the changes in serum levels of calcium, phosphate, parathyroid hormone (PTH), and 25-hydroxyvitamin D3 (25(OH)D) from baseline. RESULTS: Serum FGF23 levels were more elevated in the combination group than in the calcitriol-alone group. However, both mean change and percentage change in the serum FGF23 levels during the 8-week period were not significantly different between the two groups. Serum calcium level was increased significantly only in the combination treatment group. There was no significant difference in percentage change of serum calcium levels between the two groups. In addition, changes in serum levels of phosphate, 25(OH)D, or PTH were not significantly different between the two groups. In correlation analysis, changes in serum FGF23 levels were positively correlated with changes in serum calcium and phosphate levels, but not with changes in 25(OH)D or PTH levels. No serious adverse events were observed, however, there was one case of mild gastrointestinal discomfort. CONCLUSION: This study revealed that additional calcium carbonate treatment significantly increased serum FGF23 levels in patients treated with calcitriol, with their synergistic effect in promoting intestinal calcium absorption. This suggests that serum FGF23 levels should be monitored regularly, especially in those who use combination of vitamin D and calcium carbonate from the early stages of CKD.” As taken from Han N et al. 2017. Ther. Clin. Risk Manag. 13, 999-1007. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28860784>

“By altering gastric and urinary pH, antacids may alter rates of dissolution and absorption, the bioavailability, and renal elimination of a number of drugs.”

As taken from European Commission, 2020

5. Toxicity

5.1. Single dose toxicity

Record for CAS RN 471-34-1:

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
rat	LD50	oral	6450mg/kg		"Sbornik Vysledku Toxikologickeho Vysetreni Latek A Pripravku," Marhold, J.V., Institut Pro Vychovu

			(6450mg/kg)		Vedoucín Pracovníku Chemického Průmyslu Praha, Czechoslovakia, 1972Vol. -, Pg. 267, 1972.

As taken from ChemIDplus, available at <https://chem.nlm.nih.gov/chemidplus/>

"AFTER SINGLE 4-G DOSE IN NORMAL SUBJECTS THERE IS RISE IN PLASMA CALCIUM CONCENTRATION THAT LASTS LESS THAN 3 HOURS; AFTER 8-G DOSE, HYPERCALCEMIA IS MORE PERSISTENT."

"LARGE DOSES OF CALCIUM CARBONATE (ABOVE 2 G) INCREASE GASTRIC SECRETION FOR A PERIOD OF TIME THAT CONSIDERABLY OUTLASTS ELEVATION OF PH. ... WITH SINGLE DOSES BELOW 2 G, THIS EFFECT IS NEGLIGIBLE."

"NORMAL INDIVIDUAL CAN INGEST 20 G/DAY WITHOUT DEVELOPING CHRONIC HYPERCALCEMIA, BUT CLINICALLY DANGEROUS HYPERCALCEMIA MAY FOLLOW ADMIN OF AS LITTLE AS 3.4 G/DAY TO PATIENTS WITH UREMIA."

Oral rat LD₅₀: 6450 mg/kg bw.

As taken from HSDB, 2014

Oral rat TDLo: 60,000 mg/kg bw.

As taken from IUCLID Dataset (2000), Calcium carbonate (471-34-1).

/LABORATORY ANIMALS: Acute Exposure/ Calcium carbonate ? in form of powder ... found ... /to have/ no toxic effect when applied to surface of rabbit eyes. ... Injecting 8 mg of calcium carbonate powder ... into anterior chamber of rabbit caused moderate rise of intraocular pressure lasting less than 24 hr ... [Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 166-7] **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ Eye, nose, throat, and respiratory irritation /From table/ [Sullivan, J.B. Jr., G.R. Krieger (eds.). Hazardous Materials Toxicology-Clinical Principles of Environmental Health. Baltimore, MD: Williams and Wilkins, 1992., p. 575] **PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ Marginal increase of phospholipids in lung lavage /was found in male rats exposed to 0.0812 mg/L for 90 minutes/ after 21 hr. No effect on lung weight, macrophage concentration, or histopathology. [European Chemicals Bureau; IUCLID Dataset, Calcium carbonate (471-34-1) (2000 CD-ROM edition). Available from, as of July 14, 2006: <http://ecb.jrc.it/esis/esis.php>] **PEER REVIEWED**

LD50 Mouse oral 6450 mg/kg bw [European Chemicals Bureau; IUCLID Dataset, Calcium carbonate (471-34-1) (2000 CD-ROM edition). Available from, as of July 14, 2006: <http://ecb.jrc.it/esis/esis.php>] **PEER REVIEWED**

LD50 Rat oral 6450 mg/kg [Lewis, R.J. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 635] **PEER REVIEWED**

As taken from HSDB, 2014

"In a recent acute toxicity study, no treatment-related effects were seen in female Sprague-Dawley rats (n=5) administered a single oral dose of 2000 mg calcium carbonate/kg bw by gavage. The substance was described as calcium carbonate, with no further details on the form that was tested. The LD50 of calcium carbonate was estimated to be > 2000 mg/kg bw" (EFSA, 2011. EFSA Journal 9(7):2318). <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

Record for CAS RN 471-34-1:

Type of	Route of	Species	Dose	Toxic Effects	Reference

Test	Exposure	Observed	Data		
TDLo - Lowest published toxic dose	Oral	Rodent - rat	60 gm/kg	Gastrointestinal - hypermotility, diarrhea Gastrointestinal - other changes	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: #86940001000,1994
TDLo- Lowest published toxic dose	Oral	Rodent - rat	10 mg/kg	Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	INJPD2 Indian Journal of Pharmacology. (Dept. of Pharmacology, Baranas Hindu Univ., Varanasi 221 005, India) V.1-1968(?)- Volume(issue)/page/year: 38,397,2006

As taken from RTECS, 2019.

Record for CAS RN 1317-65-3:

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TDLo- Lowest published toxic dose	Intravenous	Rodent - rat	30 mg/kg	Vascular - BP lowering not characterized in autonomic section Lungs, Thorax, or Respiration - changes in lung weight Blood - other changes	TOXID9 Toxicologist. (Soc. of Toxicology, Inc., 475 Wolf Ledge Parkway, Akron, OH 44311) V.1-1981- Volume(issue)/page/year: - ,189,2009

As taken from RTECS, 2013.

“Calcium Carbonate (in arachis oil) was administered to 5 female Sprague-Dawley rats at a single oral dose of 2000 mg/kg body weight.²⁸ The dose volume was 10 ml/kg. Dosing was followed by a 14-day observation period and necropsy. None of the animals died and there were no clinical signs of systemic toxicity. Additionally, there were no adverse changes in body weight, and adverse effects were not observed at necropsy. The reported LD50 was > 2000 mg/kg body weight.”.

“The acute inhalation toxicity of Calcium Carbonate was evaluated according to OECD Guideline 403 using 10 (5 males, 5 females) Wistar rats.²⁸ The animals were exposed for 4 h to aerosolized Calcium Carbonate using a nose-only exposure system. Exposure was interrupted for a total of 8 minutes. The flow of air in each tube was 0.97 l/minute, and the mean chemical aerosol concentration was 3 mg/l of air. Mass mean aerodynamic diameters (MMAD) were between 2.28 µm and 2.89 µm, with a geometric standard deviation between 1.5 and 3. Taking these values into consideration, it was assumed that the deposition of particles would occur in the upper and lower respiratory tract. Exposure was followed by a 14-day observation period. All animals were killed and subjected to gross necropsy. Ruffled fur was the only clinical sign that was observed. Slight body weight loss, followed by recovery, was observed. There were no macroscopic findings at necropsy. The reported LC₅₀ was > 3 mg/l of air.”

As taken CIR, 2017.

Record for CAS RN 471-34-1:

Acute oral LD50 rat >2000 mg/kg bw

Dermal LD50 rat 2000 mg/kg bw

Inhalation LC50 rat 4 hr >3.0 mg/l

As taken from BPDB, 2022.

Rat inhalation LC50 >0.08 mg/L (90 min, nose only) (CAS RN 1317-65-3) (PPDB, 2021).

“

Calcium carbonate is considered of low acute toxicity by the oral, dermal and inhalation routes, based on the use as food additive, literature data, history of safe uses, and support by summary information from REACH registration dossiers (Spain, 2020).”

As taken from EFSA, 2021

5.2. Repeated dose toxicity

“WHEN PHOSPHATE INTAKE IS LOW /DURING CHRONIC CALCIUM CARBONATE ADMIN/, HYPOPHOSPHATEMIA MAY OCCUR. HYPERCALCIURIA AND ALKALURIA PREDISPOSE TO NEPHROLITHIASIS.”

“INFREQUENT INSTANCES OF HYPERCALCEMIA WITH ALKALOSIS, CALCINOSIS (INCL NEPHROCALCINOSIS), & AZOTEMIA THAT OCCUR DURING CHRONIC USAGE OF CALCIUM CARBONATE, ESP IN CONJUNCTION WITH MILK & CREAM...RENAL DYSFUNCTION, GI HEMORRHAGE & VOMITING OR ASPIRATION THROUGH NASOGASTRIC TUBE SEEM TO PREDISPOSE TO THE DISORDER.”

“DOSE RELATED CONSTIPATION IS COMMON WHEN 20 TO 40 G IS TAKEN DAILY; HEMORRHOIDS, PAINFUL, BLEEDING ANAL FISSURES...MAY OCCUR. ACUTE APPENDICITIS HAS BEEN PRODUCED BY IMPACTED CALCIUM CARBONATE FECOLITHS.”

“A 40 yr old woman with surgical hyperparathyroidism ingested up to 15 g of calcium daily (calcium carbonate). Her serum calcium level was 5.35 mmol/l (normal 2.20-2.60 mmol/l). She experienced fatigue, anorexia, nausea and vomiting, an elevated blood pressure, hemoconcentration, leukocytosis, metabolic alkalosis, elevated body weight, and hypokalemia.”

“HYPERCALCEMIA HAS BEEN OBSERVED IN PATIENTS WHO INGESTED LARGE QUANTITIES OF MILK AND ABSORBABLE ALKALI FOR SEVERAL YEARS FOR TREATMENT OF PEPTIC ULCERS WITHOUT DEMONSTRATING INCREASED LEVELS IN URINE CALCIUM.”

As taken from HSDB, 2014

“Acute hypercalcemia and recurrent nephrolithiasis were observed in 3 subjects regularly ingesting large quantities of calcium carbonate–sodium bicarbonate powders over 20 years.”

As taken from IUCLID Dataset (2000), Calcium carbonate (471-34-1).

LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Twenty one day old male C57B1/6 mice were fed either control AIN-76 diet (0.5% calcium wt/wt; n = 10) or the same supplemented with calcium carbonate (1.0% calcium; n = 10) for 12 weeks. Apoptotic cells in proximal and distal segments were counted and expressed as an apoptotic index (AI: frequency of apoptosis/100 longitudinal crypts). The bromodeoxyuridine (BrdU) labelling index was also determined. Differences were analysed by the student's t test ... In control animals, the AI was significantly higher in the caecum/proximal colon (mean, 28.6; SEM, 2.0) compared with the distal colon (mean, 19.9; SEM, 1.8; p = 0.004). In the calcium treated group, the AI in the caecum/proximal colon (mean, 30.6; SEM, 1.7) was similar to controls (p = 0.71) but the AI in the distal colon was significantly greater (mean, 32.6; SEM, 1.8; p = 0.001) than in control mice and was raised to

values similar to those in the proximal colon. Calcium was also associated with reduced crypt cellularity and, in the proximal colon, a downward shift in the crypt position at which apoptosis occurred. There were no significant differences in the BrdU labelling index between groups or between proximal and distal colonic segments in each group ... /The authors concluded that/ Increased dietary calcium is associated with the induction of apoptosis in normal mouse distal colonic epithelium without affecting cell proliferation. This might contribute to its putative chemopreventive role in colorectal carcinogenesis. [Penman ID et al; J Clin Pathol 53(4):302-7 (2000)] **PEER REVIEWED**

"In a study designed to induce chronic hypercalcaemia, groups of ten male Sprague Dawley rats were given increased amounts of calcium carbonate in their diet (levels of 4% or 8% elemental calcium w/w, equivalent to approximately 5000 or 10 000 mg/kg bw/day calcium carbonate, compared with the normal dietary content of 0.71%) (Puerro Vicente et al., 1993). No details on the form of calcium carbonate used were indicated by the authors. Other groups of rats were given calcium chloride in drinking water (1.5% or 2%) or were given a single intramuscular injection of vitamin D1 on the day on which calcium treatment commenced. One group received 4% elemental calcium in the diet together with 1.5% calcium chloride in drinking water. Groups of 10 rats were killed after 2 and 14 days of treatment respectively, and blood samples taken for determination of total calcium. Other than determination of total calcium in blood, the only parameters investigated in the study were food consumption and body weight gain. Increases in blood calcium were seen after 2 days of treatment with either 4% or 8% elemental calcium (from approximately 100 mg/l to approximately 130 mg/l for either treatment), showing that calcium from calcium carbonate and/or calcium chloride was bioavailable; levels dropped slightly after 14 days of treatment. A reduction in food consumption in the calcium-treated animals resulted in a reduction in body weight gain, manifest throughout the experimental period. No other treatment-related changes were reported by the authors" (EFSA, 2011. EFSA Journal 9(7):2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

"The effects of a high calcium carbonate diet or a high calcium citrate diet in comparison to a control diet on mineral concentrations in several tissues of rats were examined (Takasugi et al., 2005). Male Wistar rats (n= 5 per group) aged 5 weeks were fed one of the experimental diets for 4 weeks. The control diet contained 0.5% calcium as calcium carbonate. The authors did not provide information on the form of calcium carbonate used. High calcium diets contained 2.5% calcium as calcium carbonate or calcium citrate (1250 mg calcium/kg bw/day, equivalent to 3125 mg/kg bw/day calcium carbonate). Feed intake was measured daily and body weight was measured weekly. Blood samples were collected from the abdominal aorta, and the testis, kidney, liver and femur were taken for analysis of calcium content. Calcium, phosphorus, magnesium, iron, copper and zinc concentrations in the tissues and plasma were determined using inductively coupled plasma emission spectrometry. Neither of the high calcium diets affected feed intake but body weight gain was significantly lower in both of the high calcium diet groups (approximately 15% lower, $P < 0.05$) than in the control group. Feed efficiency was also significantly lower in the high calcium groups than in the control group. The high calcium diets did not affect the calcium concentration in the plasma and tissues. Both of the high calcium diets produced a decrease in plasma phosphorus and also in iron levels in the testis, liver and femur. Hepatic copper increased in rats fed the high calcium diets whereas renal copper decreased. The high calcium carbonate diet increased femoral zinc and decreased femoral magnesium but in contrast the high calcium citrate diet did not affect these minerals in the femur. The authors considered that these effects might be due to different effects of the two calcium salts on intestinal pH, with calcium citrate causing a decrease in intestinal pH and thus enhancing the uptake of iron and other minerals. In contrast, as argued by the authors, calcium carbonate may increase the intestinal pH in rats (Koba et al., 2001), thereby reducing the availability of iron (Prather and Miller, 1992). Takasugi and co-workers, based on their results, suggest overall that the effect of excess calcium on minerals partly depends on its form (Takasugi et al., 2005)" (EFSA, 2011. EFSA Journal 9(7):2318). <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

“According to a high anti-osteoporotic efficacy of Sigma Anti-bonding Molecule Calcium Carbonate (SAC), repeated-dose toxicities of SAC were investigated to assess its feasibility as drug or functional food ingredient. Male ICR mice were given drinking water containing 0.006, 0.02 or 0.06% SAC for 4 weeks. SAC feeding decreased the body weights and feed and water consumptions of mice in a dose-dependent manner, especially, leading to severe emaciation and 70% death in 3 weeks in the high-dose (0.06%) group. Not only kidney and heart weights, but also the levels of blood urea nitrogen, creatinine, aspartate transaminase, and creatine phosphokinase significantly increased after SAC administration, indicative of nephrotoxicity and cardiotoxicity. Such renal and cardiac toxicities were also confirmed by microscopic findings, exhibiting renal crystals and cardiac fibrosis, which may be due to the insoluble crystal formation and calcium overload, respectively. In conclusion, it is suggested that no observed adverse effect level of SAC is lower than 0.006% in mice, and that a long-term intake may cause serious adverse effects on renal and cardiac functions.” As taken from Jang JY et al. 2013. Lab. Anim. Res. 29(1), 7-11. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23573102>

Record for CAS RN 471-34-1:

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Human - woman	4.08 gm/kg/30D (intermittent)	Vascular - BP elevation not characterized in autonomic section Gastrointestinal - changes in structure or function of endocrine pancreas Biochemical-Metabolism (Intermediary)- effect on inflammation or mediation of inflammation	MJAUAJ Medical Journal of Australia. (Australasian Medical Pub. Co. Ltd., 71-79 Arundel St., Glebe, N.S.W., Australia) V.1- 1914- Volume(issue)/page/year: 182,350,2005

As taken from RTECS, 2019.

Record for CAS RN 1317-65-3:

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TCLo - Lowest published toxic concentration	Inhalation	Rodent - rat	84 mg/m3/4H/40 W (intermittent)	Lungs, Thorax, or Respiration - fibrosis (interstitial) Liver - other changes Kidney/Ureter/Bladder - other changes	VCVN1* "Vrednie chemicheskije veshestva. Neorganicheskie soedinenija elementov I-IV groopp" (Hazardous substances. Inorganic substances containing I-IV group elements), Filov V.A., Chimia, 1988. Volume(issue)/page/year : -,114,1988
TCLo - Lowest	Inhalation	Rodent -	250 mg/m3/2H/24	Lungs, Thorax, or Respiration - fibrosis,	VCVN1* "Vrednie chemicheskije veshestva.

published toxic concentration		rat	W (intermittent)	focal (pneumoconiosis)	Neorganicheskie soedineniya elementov I- IV grupp" (Hazardous substances. Inorganic substances containing I- IV group elements), Filov V.A., Chimia, 1988. Volume(issue)/page/year : -,115,1988

As taken from RTECS, 2013.

"OBJECTIVE: To determine whether children with calcium-deficiency rickets respond better to treatment with calcium as limestone or as ground fish. STUDY DESIGN: Nigerian children with active rickets (n = 96) were randomized to receive calcium as powdered limestone (920 mg of elemental calcium) or ground fish (952 mg of elemental calcium) daily for 24 weeks. Radiographic healing was defined as achieving a score of 1.5 or less on a 10-point scale. RESULTS: The median (range) age of enrolled children was 35 (6-151) months. Of the 88 children who completed the study, 29 (66%) in the ground fish group and 24 (55%) in the limestone group achieved the primary outcome of a radiographic score of 1.5 or less within 6 months (P = .39). The mean radiographic score improved from 6.2 ± 2.4 to 1.8 ± 2.2 in the ground fish group and from 6.3 ± 2.2 to 2.1 ± 2.4 in the limestone group (P = .68 for group comparison). In an intention to treat analysis adjusted for baseline radiographic score, age, milk calcium intake, and serum 25-hydroxyvitamin D concentration, the response to treatment did not differ between the 2 groups (P = .39). Younger age was associated with more complete radiographic healing in the adjusted model (aOR 0.74 [95% CI 0.57-0.92]). After 24 weeks of treatment, serum alkaline phosphatase had decreased, calcium and 25-hydroxyvitamin D increased, and bone mineral density increased in both groups, without significant differences between treatment groups. CONCLUSION: In children with calcium-deficiency rickets, treatment with calcium as either ground fish or limestone for 6 months healed rickets in the majority of children." As taken from Thacher TD et al. 2015. J. Pediatr. 167, 148-54. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25799193>

"Five rats were fed ^{45}Ca -labeled Calcium Carbonate (0.3 g/kg body weight) in feed for 3 days.²⁶ The strain of rats tested was not identified. All of the animals remained healthy."

As taken from CIR, 2017.

"Six repeated exposure toxicity studies were considered for the evaluation: a 7 and 28-day oral study, a 48-day oral study, a 90-day oral study, a 14 and 28-day subcutaneous exposure study, a 90-day inhalation study and a 1-week inhalation study. All of these studies used rats as test species except for the first one (AS B.6.3.1.2-01) which was conducted using mice. Besides, a 1-year peritoneal tissue response evaluating repeated dose toxicity study with calcium carbonate (AS 6.8.2.3-01) is included in section 2.6.5. None of the three oral toxicity studies revealed relevant signs of toxicity after repeated administration of calcium carbonate to rats or mice. NOAEL in the study in mice was considered to be greater than 1300 mg/kg bw/d after 7 and 28 days of exposure while NOAEL in the 90 day and 48 day studies in rats was > 1000 mg/kg bw/d.

The subchronic inhalation toxicity study (90 days) in rats revealed local effects that could indicate potential irritation of respiratory tract.....NOAEC for systemic toxicity was set > 0.399 mg/L considering the absence of effects at this dose level.....NOAEC in the 1-week inhalation study was > 0.771 mg/L."

"Regarding the available 14 and 28 day subcutaneous toxicity test in rat, in the high dose group of the 14-day toxicity trial (29500 mg/m²) and in the high dose group of the 28-day toxicity trial (5900 mg/m²) severe clinical signs, significant variations in biochemistry and haematology and gross and

histopathological lesions were observed. The medium- (590 mg/m²) and low (59 mg/m²)-dose groups of the 28-day toxicity trial showed moderate to mild lesions."

As taken from European Commission, 2019a.

"[N]o concern was identified by the experts for the long-term toxicity and carcinogenicity of the compound [calcium carbonate]."

As taken from EFSA, 2021

5.3. Reproduction toxicity

"Calcium is among the nutrients of concern during pregnancy and lactation. Few observational studies have suggested an association between calcium intakes during pregnancy with breast milk calcium concentration. The aim of this double blind placebo controlled clinical trial was to determine the effect of calcium supplementation during the third trimester of pregnancy on the breast milk calcium concentration. Methods: Sixty-eight healthy pregnant women were assigned randomly to "Calcium Supplement" (1 g daily, as two calcium carbonate capsules; n= 33) or "Placebo" (n= 35) groups from 28th-30th week of gestation through delivery. Inclusion criteria were: parity <3, singleton pregnancy, intention to breastfeed infant, and intake of no dietary supplements except iron and folic acid. Anthropometric measurements were done according to standard protocols. Breast milk samples were taken on 45th day after delivery. Calcium concentration was measured using atomic absorption spectrometry. Data analyses were done by SPSS (Version 9). Results: There were no significant differences between the two groups with respect to demographic characteristics, anthropometric measures, and dietary energy and calcium intakes. Compliance was similar between the two groups (>80 %). Mean±SD of calcium concentrations in mature breast milk were 228±38 and 235±42 mg/L in "Calcium Supplement" and "Placebo" groups, respectively (p=0.49). Birth weight in the supplemented group (3241±486 g) was significantly higher than the "Placebo" group (3011±434 g). Conclusion: Our study did not support the hypothesis stating that calcium supplementation during the third trimester of pregnancy affects calcium concentration in mature breast milk." As taken from J Pediatr Gastroenterol Nutr 2004 Jun;39(Suppl 1):S472.

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ Rats /fed/ up to 1.25% dietary calcium carbonate for 6 weeks prior to mating and during gestation and found no adverse effects. [Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. 2:229] **PEER REVIEWED

/CASE REPORTS/ ... A case of a 37-year-old multiparous woman who was delivered of 32-week twins via cesarean section for non-reassuring FHR pattern under spinal anesthesia /is reported/. The twins were admitted to the NICU where laboratory investigation revealed that both babies were hypercalcemic (12 and 13 mg/dL) and in acute renal failure (creatinine 2.5 mg/dL). Hence, blood from the mother was sent for analysis and she was also found to be hypercalcemic (12.6 mg/dL) and in ARF (creatinine 2.5 mg/dL). Serum calcium showed minimal change during forced saline and diuretic therapy. However the patient developed chest pain, bradycardia and pulmonary edema with elevation in serum troponin levels (1.8 ng/mL). The cardiologist excluded the diagnosis of myocardial infarction. Subsequently, when a detailed history was obtained, the patient admitted that during the last 3-4 months of pregnancy she was taking 10-15 tablets of Tums (500 mg calcium carbonate tablets) for dyspepsia along with the prescribed multivitamins that contain Vitamin D. The hypercalcemia and renal failure resolved 72 and 96 hours after discontinuing her multivitamin and Tums. Subsequently, it was reported that the serum PTH (1 pcg/mL) and 1,25 dihydroxy-Vitamin D3 (11 pcg/mL) levels were low and 25 hydroxy-Vitamin D3 level (22 ng/mL) was within normal limits. These findings are consistent with a diagnosis of absorptive hypercalcemia secondary to excessive calcium ingestion in the presence of Vitamin D. Although this patient did fine under anesthesia, acute hypercalcemia has been known to cause increased morbidity by involving cardiovascular, cerebral, neurologic, gastrointestinal and renal systems. A detailed history of drug

use, especially non-prescription medications should be mandatory. [Gupta N; Anesthesiology 100(5 Suppl 1):72 (2004)] **PEER REVIEWED**

FDA Pregnancy Risk Category: C /RISK CANNOT BE RULED OUT. Adequate, well controlled human studies are lacking, and animal studies have shown risk to the fetus or are lacking as well. There is a chance of fetal harm if the drug is given during pregnancy; but the potential benefits may outweigh the potential risk./ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 742] **PEER REVIEWED**

As taken from HSDB, 2014

“As reported in section 3.2.2, a combined repeat dose oral toxicity/reproduction/developmental toxicity screening study was carried out in Wistar rats (n= 10/sex/group), in accordance with OECD Guideline 422 (Harlan Laboratories, 2010a). Animals were administered nanoform calcium carbonate (characteristics as already described in Section 3.2.2) by gavage at dose levels of 100, 300 and 1000 mg/kg bw/day for up to 48 days, including a two week maturation phase, pairing, gestation and early lactation for females. Pregnant females were allowed to give birth and maintain their offspring until termination on day 5 post partum. Evaluations of each litter size, litter weight, mean offspring weight by sex, clinical observations and landmark developmental signs were also performed during the post partum period. At termination, all offspring, including those dying during the study, were subjected to a full external and internal examination and any macroscopic abnormalities were recorded. No evidence of treatment-related effects was seen in the parental animals during the study, other than the minor haematological and biochemical changes in males receiving 1000 mg/kg bw/day, as already reported in section 3.2.2. No treatment-related effects were detected on the fertility or mating performance of animals receiving calcium carbonate when compared to controls. All treated animals mated within the first four days of pairing. No treatment-related effects were detected in the length of gestation for treated females when compared to controls. All animals showed gestation lengths between 22 to 23.5 days. All treated females gave birth to a live litter and successfully reared young to day 5 of age. Corpora lutea, implantation counts, litter sizes and pup viability was comparable in all treated groups and controls. There were no differences in litter weights or mean pup body weights between control and treated animals, and no clinical signs of toxicity were detected in pups from treated females when compared to controls. No treatment-related effects were detected for surface righting reflex for pups from treated animals when compared to controls. The incidence, type or distribution of macroscopic findings in pups dying in the post partum period or in pups killed at scheduled termination (day 5 of age) did not indicate any adverse effect of maternal treatment with calcium carbonate. The authors considered that no treatment-related effects were observed on reproduction in this study, and that the NOAEL for reproductive toxicity was 1000 mg/kg bw/day, the highest dose tested. The Panel agreed with this NOAEL” (EFSA, 2011. EFSA Journal 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

“Fairney and Weir maintained female rats throughout pregnancy on a high calcium diet (3% calcium as calcium carbonate in the chow (equivalent to 1500 mg calcium/kg bw/day and 3750 mg calcium carbonate/kg bw/day) and 4% calcium lactate added to the drinking water (equivalent to 882 mg calcium/kg bw/day and 4800 mg calcium lactate/kg bw/day). The number of dams was not given, but the growth rate of 41 offspring from the resulting litters from hypercalcaemic dams was compared with that from 38 offspring from dams maintained on a normal calcium content diet. The mean plasma calcium level in the high calcium group was $12.7 \pm 1.41/100$ ml in the 24 hours after parturition, compared with $10.1 \pm 0.59/100$ ml in the control group. The resulting litters were stated by the authors to be smaller in number (data not provided) and the offspring had lower body weights compared with controls. On macroscopic examination the offspring from hypercalcaemic dams showed alopecia, and the kidneys, liver and heart were paler in appearance compared with control offspring. On histopathological examination the kidneys from the former group showed pyelonephritic scarring, but no soft tissue calcification or nephrocalcinosis was seen on radiological

examination (Fairney and Weir, 1970)” (EFSA, 2011. EFSA Journal 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

“In a study designed to evaluate the developmental effects of increased dietary calcium in rats fed otherwise nutritionally adequate diets, female Charles River CD/VAF Plus were given dietary levels of 0.5% (control level), 0.75%, 1% or 1.25% calcium as calcium carbonate for 6 weeks before mating, during mating and up to Gestation Day (GD) 20 (Shackelford et al. 1993). These levels were equivalent to 375, 500 or 625 mg/kg bw/day calcium and 937.5, 1250 or 1562.5 mg/kg bw/day calcium carbonate. No dose related changes were observed in maternal clinical findings, average numbers of implantations, fetal resorptions and viable fetuses, nor on fetal length and weight. The authors concluded that dietary calcium (given as calcium carbonate) was not fetotoxic or teratogenic at the concentrations used in the study. The Panel agreed with this conclusion and considered that 1562.5 mg/kg bw/day calcium carbonate, the highest dose tested, was a NOAEL for reproductive effects in this study. Dose-related increases of the femoral calcium content were however measured in both pregnant and non-pregnant rats receiving increased dietary calcium, and there were also increases in the phosphorus, magnesium and zinc content of the liver in non-pregnant control rats on the same diets (Shackelford et al., 1994). There were dose-related decreases of the iron and copper contents in kidneys of non-pregnant animals and of iron in the liver of pregnant rats. Fetuses showed dose-related decreases in the whole body content of phosphorus, magnesium, iron and copper. Changes in mineral levels in rats receiving increased dietary calcium were seen in all dose groups compared with controls, although not all minerals in the organs examined were equally affected (Shackelford et al., 1994)” (EFSA, 2011. EFSA Journal 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

“Richards and Greig evaluated the effect of dietary calcium carbonate (0.3%, 0.5%, 0.7% and 1.1%, equivalent to approximately 450, 750, 1050 or 1650 mg calcium carbonate/kg bw/day) on reproductive performance in Swiss mice and on survival and organ pathology of the litters (Richards and Greig, 1952). Mice were given the experimental diets one week before mating and up to GD 21, when the offspring were killed. All diets with a calcium carbonate content of 1.1% resulted in a decreased number of litters and decreased total litter weight. Litters at this dietary level showed hypertrophy of the heart and atrophy of the thymus when killed at age 21 days. Increased heartweights were negatively correlated with haemoglobin levels. Addition of iron to high-calcium diets reduced the degree of cardiac hypertrophy, and the authors concluded that the effects seen were due to a calcium:iron interaction, with consequential reductions in maternal iron levels and anaemia. However, the authors did not provide any information on maternal toxicity in the study, other than an observation of increased heart weight in the mothers and maternal anaemia” (EFSA, 2011. EFSA Journal 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

“The effect of diet-induced maternal hypercalcaemia on fetal development was investigated in the CD-1 mouse (Liebgott et al., 1989). The diet for the control group contained 1.2 % calcium, while that for the experimental group contained 3% calcium as calcium carbonate in the chow (1500 mg calcium/kg bw/day, equivalent to 3750 mg/kg bw/day calcium carbonate) and 4% calcium lactate added to the drinking water. The experimental group was maintained on the high calcium diet for at least 10 days before mating, and during pregnancy until GD-18. The number of litters in both the control and experimental group was identical (n=13). However, fetuses from the treated litters showed significantly lower birth weights and higher frequencies of missing calcification centres in the developing skeletons and dentitions. The authors concluded that high maternal calcium levels can result in fetotoxic effects (Liebgott et al., 1989)” (EFSA, 2011. EFSA Journal 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

“Hypocalcemia is a common, treatable cause of neonatal seizures. A term girl neonate with no apparent risk factors developed seizures on day 5 of life, consisting of rhythmic twitching of all

extremities in a migrating pattern. Physical examination was normal except for jitteriness. Laboratory evaluation was unremarkable except for decreased total and ionized serum calcium levels and an elevated serum phosphorus level. The mother had ingested 3-6 g of calcium carbonate daily during the final 4 months of pregnancy to control morning sickness. The baby's electroencephalogram showed multifocal interictal sharp waves and intermittent electrographic seizures consisting of focal spikes in the left hemisphere accompanied by rhythmic jerking of the right arm and leg. Treatment with intravenous calcium gluconate over several days resulted in cessation of seizures and normalization of serum calcium. The child has remained seizure free and is normal developmentally at 9 years of age. Hypocalcemic seizures in this newborn were likely secondary to excessive maternal calcium ingestion, which led to transient neonatal hypoparathyroidism and hypocalcemia. Inquiry about perinatal maternal medication use should include a search for over-the-counter agents that might not be thought of as "drugs," as in this case, antacids." As taken from Borkenhagen JF et al. 2013. *Pediatr. Neurol.* 48(6), 469-71. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23668874?dopt=AbstractPlus>

"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, 17-22. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26715904>

"Female Swiss mice were bred after being fed (number of animals/feeding duration not stated) a diet supplemented with 0.5%, 1%, or 2% Calcium Carbonate.²⁶ First and second litters were studied. Calcium Carbonate (1% and 2% in diet) yielded an intake of approximately 3000 mg/kg body weight. When compared to the control diet, feeding with the supplemented diet significantly decreased the number and total weight of the weanling mice, and increased the proportion of deaths. Calcium Carbonate (2%) in the diet also caused hypertrophy of the heart and a tendency toward decreased thymus weight in weanling mice."

"In a 62-day developmental toxicity study involving 4 groups of female Charles River CD/1AF Plus rats (mated with male rats), Calcium Carbonate (in the diet) was fed at concentrations of 0.05% (control), 0.75%, 1%, and 1.25% for 6 weeks prior to mating, during mating, and for 20 days of gestation.²⁸ For each dose group, the number of females dosed prior to mating was 69 and the number of pregnant rats dosed (through mating to gestation day 20) ranged from 45 to 48. The male to female ratio per cage was 1:2. Male rats were dosed only during the mating period. On gestation day 20, the animals were killed and Cesarean sections were performed. There was no evidence of test substance-related maternal toxicity or embryotoxic/teratogenic effects. There were no dose-related changes in the average number of implantations, resorptions and viable fetuses, or fetal length or weight. When compared to the control group, there were no statistically significant increases in the litter incidence regarding specific external, visceral, or skeletal variations of the fetuses. The NOAEC for teratogenicity was > 1.25% Calcium Carbonate in the diet, and corresponded to a NOAEL between 1963 and 2188 mg/kg body weight per day."

"A female patient (history of gastritis) at 36 weeks of gestation had ingested 5 glasses of milk and approximately 30 tablets of an antacid containing 500 mg of Calcium Carbonate daily for 2 weeks.²⁸ A Calcium Carbonate intake of > 5.5 g/day for 14 days during pregnancy (later stages) did not cause any observable teratogenic effects."

As taken from CIR, 2017.

"A reproductive toxicity in rats study performed according to OECD guideline 422 was considered acceptable to evaluate the reprotoxicity of calcium carbonate. No effects on parental animals and reproductive performance were observed at all tested dose levels. A reproductive NOAEL > 1000 mg/kg bw/day was set. Besides, in a developmental study available in Table 2.6.6.2, treatment with calcium carbonate began 6 weeks before mating but effects on reproductive toxicity were not observed at all tested dose levels. A reproductive NOAEL in this study > 1420 mg/kg bw/day can be set."

[An] excessive intake [of] calcium carbonate in pregnant women is associated with lower systolic blood pressure in the offspring, particularly among overweight children (AS B.6.9.4.3-03), and can lead to neonatal hypocalcemia (AS B.6.9.3.3-01) but without observable teratogenic effects (AS B.6.9.3.3-02)."

"[In a study] on development performed in rats.....No adverse effects were seen in parental animals. The effects on development observed at the highest tested dose level of 1420 mg/kg bw/d were considered not treatment related. NOAEL for maternal and development was > 1420 mg/kg bw/d. The developmental NOAEL is in line with the conclusions established on this study in the Re-evaluation of calcium carbonate (E 170) as a food additive (EFSA Journal 2011;9(7):2318)."

As taken from European Commission, 2019a.

"Regarding the reproductive toxicity, no adverse effects were observed on reproductive parameters in a combined repeated dose toxicity study with the reproductive and developmental toxicity screening test in rats. In a developmental rat toxicity study, a developmental NOAEL of 1,250 mg/kg bw per day was identified on the basis of increased incidence of external and skeletal foetal findings at the high dose tested (maternal NOAEL). Calcium carbonate is not considered to be teratogenic."

As taken from EFSA, 2021

POD Method	POD Value	POD Owner
HNEL	Not Calculated	PAFA

Lowest-observed effect

Owner	Type	Value	Sites	Effects
PAFA	LOAEL	Not established	NO EFFECTS	NO EFFECTS

No-observed effect

PAFA: HNEL: 1015.0 mg/kg bw/day

Adjustment factors: >Critical study: Reproductive/Developmental Toxicity > Developmental Toxicity (Rat, Oral exposure)

As taken from the COSMOS database available at <https://ng.cosmosdb.eu>

"Management of the pregnant patient with neurologic disease is challenging. Ideally, preconception planning can optimize the woman's neurologic condition before pregnancy. More than half of pregnancies are unplanned which makes careful consideration of medications vitally important. This article focuses on potential toxic risk to the fetus of medications deemed necessary to manage several common maternal neurologic issues: multiple sclerosis, epilepsy, and headache during pregnancy and postpartum. It is important for the practitioner to have an understanding beyond the

category system to understand the potential toxic risks to the infant.” As taken from Hessler A. 2020. Neurol. Clin. 38(4), 897-912. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33040868/>

5.4. Mutagenicity

“Calcium carbonate (with a particle size of 60 - 100 nm when examined by SEM) has given negative results in a range of in vitro genotoxicity assays” (EFSA, 2011. EFSA Journal 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

The mutagenicity of calcium carbonate was assessed in Ames tests in Salmonella typhimurium strains (TA97 and TA102) with doses up to 1 mg/plate in presence or absence of metabolic activation. The results showed that CaCO₃ was not mutagenic.

Mutagenicity Studies:

Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results:	NEGATIVE
Reference:	[FUJITA,H AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. II.; KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 38:423-430, 1987]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results:	NEGATIVE
Reference:	[FUJITA,H AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. II.; KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 38:423-430, 1987]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain	TA97

Indicator:	
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results:	NEGATIVE
Reference:	[FUJITA,H AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. II.; KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 38:423-430, 1987]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results:	NEGATIVE
Reference:	[FUJITA,H AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. II.; KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 38:423-430, 1987]

As taken from CCRIS, 2008

“Relating to genotoxicity / germ cell mutagenicity, five in vitro studies were considered for the evaluation: one bacterial reverse mutation assay, one mammalian cell gene mutation assay and one mammalian chromosome aberration test (those three studies from an ECHA Registration Dossier) and two DNA damage (BrdU) studies (from open literature publications). All five studies reported negative results.”

As taken from European Commission, 2019a.

“Calcium carbonate is unlikely to be genotoxic”.

As taken from EFSA, 2021

“Limestone powder (calcium carbonate) is unlikely to be mutagenic or clastogenic. Aneugenic potential was not investigated but this is not considered needed considering the nature and use of the substance.”

As taken from EFSA, 2022.

“Workers in marble processing plants are at high risk of exposure to high levels of marble dust containing silica, but there are limited studies evaluating the genotoxicity and oxidative stress parameters of workers occupationally exposed to marble dust. In this study, we aimed to clarify how marble dust affects genotoxicity and immunotoxicity mechanisms alongside oxidative stress in the

workers in the marble processing plants of Iscehisar, Turkey. The oxidative stress and immune system parameters were determined spectrophotometrically using commercial kits. Genotoxicity was evaluated by Comet and micronucleus (MN) assays in the lymphocytes and buccal cells, respectively. The enzyme activities of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and the levels of glutathione, and Clara cell secretory protein CC16 in workers (n = 48) were significantly lower than in controls (n = 41), whereas the levels of malondialdehyde, 8-oxo-7,8-dihydro-2'-deoxyguanosine, tumor necrosis factor- α , interleukin-1 β were significantly higher in workers. DNA damage in workers were significantly higher than in controls and there was a clear correlation between the increase in DNA damage and the duration of exposure. Marble workers had significantly higher MN frequencies when compared to controls. The results indicate the possibility of immunotoxic and genotoxic risks to workers in marble industry." As taken from Beci TM et al. 2021. Environ. Res. 197, 111209. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33895109/>

5.5. Cytotoxicity

Exposure to cement dust, a specifically alkaline and irritant dust, is one of the most common occupational dust exposures worldwide. Although several adverse respiratory health effects have been associated with cement dust exposure, the evidence is not conclusive. In the current study, cytotoxic and pro-inflammatory effects as well as oxidative stress elicited by a number of cement dusts, including a limestone and cement clinker sample, were tested using the NR8383 rat alveolar macrophage cell line and primary rat alveolar macrophages. DQ12 quartz and TiO₂ were included as positive and negative controls, respectively. Cytotoxicity was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide assay and the lactate dehydrogenase assay, oxidative stress was determined by measurement of the depletion of total cellular glutathione, and electron spin resonance was applied to determine reactive oxygen species (ROS) generation. The release of the cytokines tumor necrosis factor- α (TNF α), interleukin-1 β (IL-1 β), and macrophage inflammatory protein-2 (MIP-2) was determined by enzyme-linked immunosorbent assay. None of the dust samples were found to cause toxicity to the macrophages or notable glutathione depletion when compared to DQ12. The cement samples also failed to activate macrophages for the generation of ROS and the production of inflammatory cytokines IL-1 β and MIP-2. In contrast, however, most of the cement dusts were found to activate macrophage TNF α production, and this was significantly associated with their content of CaO. Further research is needed to determine the relevance of these in vitro observations for occupational cement dust exposure settings.

As taken from van Berlo et al., Investigation of the Cytotoxic and Proinflammatory Effects of Cement Dusts in Rat Alveolar Macrophages, Chem. Res. Toxicol., 2009, 22 (9), pp 1548–1558, available at <http://pubs.acs.org/doi/abs/10.1021/tx900046x>

"Calcium carbonate rock dust (RD) is used in mining to reduce the explosivity of aerosolized coal. During the dusting procedures, potential for human exposure occurs, raising health concerns. To improve RD aerosolization, several types of anti-caking surface treatments exist. The aim of the study was to evaluate cytotoxicity of four respirable RD samples: untreated/treated limestone (UL/TL), untreated/treated marble (UM/TM), and crystalline silica (SiO₂) as a positive control in A549 and THP-1 transformed human cell lines. Respirable fractions were generated and collected using FSP10 high flow-rate cyclone samplers. THP-1 cells were differentiated with phorbol-12-myristate-13-acetate (20 ng/ml, 48 h). Cells were exposed to seven different concentrations of RD and SiO₂ (0-0.2 mg/ml). RD caused a slight decrease in viability at 24 or 72 h post-exposure and were able to induce inflammatory cytokine production in A549 cells, however, with considerably less potency than SiO₂. In THP-1 cells at 24 h, there was significant dose-dependent lactate dehydrogenase, inflammatory cytokine and chemokine release. Caspase-1 activity was increased in SiO₂- and, on a lesser scale, in TM- exposed cells. To test if the increased toxicity of TM was uptake-related, THP-1 cells were pretreated with Cytochalasin D (CytD) or Bafilomycin A (BafA), followed by exposure to RD or SiO₂ for 6 h. CytD blocked the uptake and significantly decreased

cytotoxicity of all particles, while BafA prevented caspase-1 activation but not cytotoxic effects of TM. Only TM was able to induce an inflammatory response in THP-1 cells, however it was much less pronounced compared to silica.” As taken from Khaliullin TO et al. 2019. *Toxicol. Appl. Pharmacol.* 362, 67-76. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30393145>

“Furthermore, there are two studies about the effects of increased dietary calcium carbonate. In one of them, this is associated with the induction of apoptosis in normal mouse distal colonic epithelium without affecting cell proliferation which might contribute to a chemopreventive role of calcium carbonate in colorectal carcinogenesis (AS B.6.8.2.3-01).”

As taken from European Commission, 2019a.

5.6. Carcinogenicity

“No data are available on the chronic toxicity or carcinogenicity of calcium carbonate. The Panel considered however that it is unlikely that calcium carbonate has carcinogenic potential, given that both calcium and carbonate are natural constituents of the body and normal metabolites of man, animals and plants, and have a long history of safe use as a source of calcium supplementation for humans” (EFSA, 2011. *EFSA Journal* 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

“Food supplements are known to affect the development of gastric adenocarcinoma. In this study, an animal model of gastric resection was used to investigate the effects of calcium carbonate on spontaneous development of gastric adenocarcinoma. Ninety-two Wistar rats with gastric resections (performed to induce spontaneous gastric cancer) and 60 without resections (controls) were used to analyse the carcinogenic potential of different ion supplements in food. Among the resected rats, cancer developed in 3 out of 18 (17%, NS) given NaCl but in 11 out of 18 (61%, $P < 0.01$) exposed to calcium carbonate. No tumours were found in the unresected (unoperated) animals. These findings were further analysed by separately investigating the effects of calcium and carbonate ions on tumorigenesis in the gastric stump model. Cancer developed in one of 26 (4%) resected animals given a diet supplemented with CaHPO_4 , which was lower than the rate observed in the resected control group fed a normal diet, although this difference was not statistically significant. However, tumour development increased significantly in the resected animals given a diet supplemented with NaHCO_3 (tumours in 13 out of 24 rats, 54%; $P < 0.01$). The present results reveal a significant role for carbonate in the induction of gastric carcinoma in the rat. The relevance of this finding is underlined by the fact that carbonate is a major constituent of intestinal reflux into the stomach, and that such reflux is considered to be one of the major causes of gastric cancer.” As taken from Ehrnström RA et al. *Scand J Gastroenterol.* 2006 Jan; 41(1):12-20 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&listuids=16373271&query=hl=1&itool=pubmed_docsum

“In some studies, high calcium intake has been associated with an increased risk of prostate cancer, but no randomized studies have investigated this issue. We randomly assigned 672 men to receive either 3 g of calcium carbonate (1,200 mg of calcium), or placebo, daily for 4 years in a colorectal adenoma chemoprevention trial. Participants were followed for up to 12 years and asked periodically to report new cancer diagnoses. Subject reports were verified by medical record review. Serum samples, collected at randomization and after 4 years, were analyzed for 1,25-(OH) $_2$ vitamin D, 25-(OH) vitamin D, and prostate-specific antigen (PSA). We used life table and Cox proportional hazard models to compute rate ratios for prostate cancer incidence and generalized linear models to assess the relative risk of increases in PSA levels. After a mean follow-up of 10.3 years, there were 33 prostate cancer cases in the calcium-treated group and 37 in the placebo-treated group [unadjusted rate ratio, 0.83; 95% confidence interval (95% CI), 0.52-1.32]. Most cases were not advanced; the mean Gleason's score was 6.2. During the first 6 years (until 2 years

post-treatment), there were significantly fewer cases in the calcium group (unadjusted rate ratio, 0.52; 95% CI, 0.28-0.98). The calcium risk ratio for conversion to PSA >4.0 ng/mL was 0.63 (95% CI, 0.33-1.21). Baseline dietary calcium intake, plasma 1,25-(OH)₂ vitamin D and 25-(OH) vitamin D levels were not materially associated with risk. In this randomized controlled clinical trial, there was no increase in prostate cancer risk associated with calcium supplementation and some suggestion of a protective effect.” As taken from Baron JA et al. *Cancer Epidemiol Biomarkers Prev.* 2005 Mar; 14(3):586-9 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15767334&query hl=14&itool=pubmed_docsum

“Accumulating evidence from observational studies indicates that a high calcium intake may reduce body weight and body fat. However, few randomized trials have been conducted. We examined whether calcium supplementation affects body weight and body fat in young girls and whether a relation exists between habitual calcium intake and body weight and body fat. A randomized, double-blind, placebo-controlled intervention study was conducted in 110 young girls. The subjects were randomly assigned to receive 500 mg Ca/d as calcium carbonate or placebo for 1 y. Two groups of girls were selected according to habitual calcium intake from a large group; one group consumed 1000-1304 mg/d (40th-60th percentile; n = 60) and the other group consumed <713 mg/d (<20th percentile; n = 50). Height, body weight, body fat, and calcium intake were measured at baseline and after 1 y. At baseline a significant negative correlation was observed between habitual dietary calcium intake and percentage of body fat (r = -0.242, P = 0.011). However, calcium supplementation had no effect on height, body weight, or percentage body fat. Habitual dietary calcium intake was inversely associated with body fat, but a low-dose calcium supplement had no effect on body weight, height, or body fat over 1 y in young girls. It is possible that the effect of calcium on body weight is only exerted if it is ingested as part of a meal, or the effect may be due to other ingredients in dairy products, and calcium may simply be a marker for a high dairy intake.” As taken from Lorenzen JK et al. *Am J Clin Nutr.* 2006 Jan; 83(1):18-23 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16400044&query hl=18&itool=pubmed_DocSum

“In earlier observational work, the dietary calcium:protein ratio was directly related to bone accrual in healthy postadolescent women. In this study, we sought to test the hypothesis that augmented calcium intake would increase postadolescent skeletal consolidation, using a double-blind, randomized, placebo-controlled design. We recruited 152 healthy young women (age 23.1 +/- 2.7 y, BMI 22.5 +/- 3.0 kg/m²); their usual diets, as assessed by 7-d food diaries, were low in calcium (605 +/- 181 mg/d; 15.1 +/- 4.5 mmol/d) and in the calcium:protein ratio (10.1 +/- 2.0 mg/g). The subjects were randomly assigned to supplemental calcium [500 mg calcium (12.5 mmol) as the carbonate, 3 times/d, with meals] or placebo capsules identical in appearance; all participants also took a daily multivitamin, and they were followed for up to 36 mo with bone densitometry (dual energy X-ray absorptiometry; DXA) at 6-mo intervals. A total of 121 subjects remained in the study for at least 12 mo (median time in the study, 35 mo), with a mean compliance level (observed/expected tablet consumption) of 87.7%. DXA data for these 121 subjects indicated modest but significant mean rates of increase (i.e., 0.24 to 1.10%/y) in bone mineral content (BMC; total body, total hip, and lumbar spine) and in lumbar spine bone mineral density (BMD) but no change in total hip BMD. None of these rates of change differed by group, i.e., calcium supplementation did not have any measurable effect on bone mass accrual. By midstudy, the calcium content of the subjects' usual diets for both groups had risen by approximately 15%. The combined effect of improved intakes of dietary calcium and the small amount of calcium added by the multivitamin tablets resulted in a mean calcium intake for the control group > 800 mg (20 mmol)/d, possibly at or near the threshold beyond which additional calcium has no further effect on bone accrual.” As taken from Barger-Lux MJ et al. *J Nutr.* 2005 Oct; 135(10):2362-6 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16177196&query hl=18&itool=pubmed_DocSum

LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ This study was done to determine whether a high dietary calcium carbonate concentration could protect against colon tumors in rats. Female Wistar rats were randomly assigned to one of four groups and maintained on an 8% lipid diet for an adaptation period of four weeks. All groups were then fed a 24% lipid diet (sunflower oil), with (Groups 2 and 4) or without (Groups 1 and 3) a 1.5% calcium carbonate supplement. They were intrarectally instilled with saline (Groups 1 and 2) or nitrosomethylurea (NMU) (Groups 3 and 4). Fecal sterol output and pH were analyzed for one week each month. Histological analysis was done at the end of the 32-week experiment. No tumors were found in the non-NMU-treated animals. The NMU-treated rats had tumors: 31% in Group 3 and 30% in Group 4. The calcium carbonate supplement had no effect on this incidence. The lipid and cholesterol excretions of the calcium carbonate-supplemented rats were significantly enhanced. The coprostanol output was not altered, although its fecal concentration of the calcium-supplemented rats was decreased. Although neither lipid overload nor NMU treatment altered the fecal pH, it was significantly increased in both calcium carbonate-supplemented groups. These findings suggest that additional calcium as carbonate has no effect on colon tumor incidence, although the fecal composition is altered. The increased pH of the feces due to the carbonate could have the opposite effect to calcium. [Quilliot D et al; Nutr Cancer 34(2):213-9 (1999)] **PEER REVIEWED**

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ Ninety-two Wistar rats with gastric resections (performed to induce spontaneous gastric cancer) and 60 without resections (controls) were used to analyse the carcinogenic potential of different ion supplements in food ... Among the resected rats, cancer developed in 3 out of 18 (17%, NS) given NaCl but in 11 out of 18 (61%, P<0.01) exposed to calcium carbonate. No tumors were found in the unresected (unoperated) animals. These findings were further analysed by separately investigating the effects of calcium and carbonate ions on tumorigenesis in the gastric stump model. Cancer developed in one of 26 (4%) resected animals given a diet supplemented with CaHPO₄, which was lower than the rate observed in the resected control group fed a normal diet, although this difference was not statistically significant. However, tumor development increased significantly in the resected animals given a diet supplemented with NaHCO₃ (tumors in 13 out of 24 rats, 54%; P<0.01) ... The present results reveal a significant role for carbonate in the induction of gastric carcinoma in the rat. The relevance of this finding is underlined by the fact that carbonate is a major constituent of intestinal reflux into the stomach, and that such reflux is considered to be one of the major causes of gastric cancer. [Ehrnstrom RA et al; Scand J Gastroenterol 41(1):12-20 (2006)] **PEER REVIEWED**

As taken from HSDB, 2014

Tumor Inhibition Studies:

Species:	RAT
Number of Animals Tested:	(21,20)/(20,19)
Strain/Sex:	WISTAR/MALE
Dose (Inhibitor):	0; 7.5% IN DIET FOR FIRST 20 WK THEN BASAL DIET FOR DURATION OF STUDY (STUDY DURATION: 40 WK)
Route (Inhibitor):	ORAL
Carcinogen:	N-METHYL-N'-NITRO-N-NITROSOGUANIDINE ; 70-25-7
Route (Carcinogen):	ORAL

Dose (Carcinogen):	100 MG/L IN DRINKING WATER FOR FIRST 20 WK
Promoter:	NONE USED
Target Tissue: Type of Lesion:	GLANDULAR STOMACH: ADENOCARCINOMA
Endpoint (Incidence):	3/20 (15%), 3/19 (16%), -7%, NOT SIGNIFICANT
Reference:	[KOMATSU,S, MASUDA,T AND HISAMICHI,S; EFFECT OF CALCIUM ON RAT GASTRIC CARCINOGENESIS INDUCED BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE; TOHOKU J. EXP. MED. 165(4):291-297, 1991]
Species:	RAT
Number of Animals Tested:	(21,20)/(20,19)
Strain/Sex:	WISTAR/MALE
Dose (Inhibitor):	0; 7.5% IN DIET FOR FIRST 20 WK THEN BASAL DIET FOR DURATION OF STUDY (STUDY DURATION: 40 WK)
Route (Inhibitor):	ORAL
Carcinogen:	N-METHYL-N'-NITRO-N-NITROSOGUANIDINE ; 70-25-7
Route (Carcinogen):	ORAL
Dose (Carcinogen):	100 MG/L IN DRINKING WATER FOR FIRST 20 WK
Promoter:	NONE USED
Target Tissue: Type of Lesion:	GLANDULAR STOMACH: ADENOCARCINOMA
Endpoint (Incidence):	3/20 (15%), 3/19 (16%), -7%, NOT SIGNIFICANT
Reference:	[KOMATSU,S, MASUDA,T AND HISAMICHI,S; EFFECT OF CALCIUM ON RAT GASTRIC CARCINOGENESIS INDUCED BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE; TOHOKU J. EXP. MED. 165(4):291-297, 1991]
Species:	RAT
Number of Animals Tested:	(60/60)(30/30)
Strain/Sex:	F344/MALE

Dose (Inhibitor):	5% DIET DAILY FOR 34 WK STARTING AFTER CARCINOGEN TREATMENT (STUDY DURATION: 38 WK)
Route (Inhibitor):	ORAL
Carcinogen:	N-BUTYL-N-(4-HYDROXYBUTYL)NITROSAMINE ; 3817-11-6
Route (Carcinogen):	ORAL
Dose (Carcinogen):	0.01% IN DRINKING WATER FOR 4 WK
Promoter:	NONE USED
Target Tissue: Type of Lesion:	URINARY BLADDER: PAPILLOMA
Endpoint (Incidence):	33%, 37%, -12%, NOT SIGNIFICANT
Comments:	PAPILLOMA/10 CM BASEMENT MEMBRANE WAS 0.8 AND 0.4 FOR AGENT AND CONTROL GROUPS RESPECTIVELY (NOT SIGNIFICANT).
Reference:	[FUKUSHIMA,S, HAGIWARA,A, OGISO,T, SHIBATA,M AND ITO,N; PROMOTING EFFECTS OF VARIOUS CHEMICALS IN RAT URINARY BLADDER CARCINOGENESIS INITIATED BY N-NITROSO-N-BUTYL-(4-HYDROXYBUTYL)AMINE; FOOD CHEM. TOXICOL. 21(1):59-68, 1983]
Species:	RAT
Number of Animals Tested:	(?,?)/(?,?)
Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 2% IN DIET FOR 34 WK BEGINNING 1 WK POST LAST CARCINOGEN DOSE (STUDY DURATION: 49 WK)
Route (Inhibitor):	ORAL
Carcinogen:	AZOXYMETHANE ; 25843-45-2
Route (Carcinogen):	SUBCUTANEOUS
Dose (Carcinogen):	12 MG/KG BW IN 0.9% SALINE TWICE
Promoter:	CHOLIC ACID ; 81-25-4
Route (Promoter):	ORAL

Dose (Promoter):	0.5% IN DIET FOR DURATION OF STUDY BEGINNING 1 WK POST LAST CARCINOGEN DOSE
Target Tissue: Type of Lesion:	COLON: ADENOCARCINOMA
Endpoint (Incidence):	26%, 18%, 31%, NOT SIGNIFICANT
Endpoint (Multiplicity):	0.3, 0.2, 33%, NOT SIGNIFICANT
Comments:	BASAL DIET CONTAINED 0.5% CALCIUM.
Reference:	[PENCE,BC, DUNN,DM, ZHAO,C, LANDERS,M AND WARGOVICH,MJ; CHEMOPREVENTIVE EFFECTS OF CALCIUM BUT NOT ASPIRIN SUPPLEMENTATION IN CHOLIC ACID-PROMOTED COLON CARCINOGENESIS: CORRELATION WITH INTERMEDIATE ENDPOINTS; CARCINOGENESIS 16(4):757-765, 1995]
Species:	RAT
Number of Animals Tested:	(?,?)/(?,?)
Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 2% IN DIET FOR 34 WK BEGINNING 1 WK POST LAST CARCINOGEN DOSE (STUDY DURATION: 49 WK)
Route (Inhibitor):	ORAL
Carcinogen:	AZOXYMETHANE ; 25843-45-2
Route (Carcinogen):	SUBCUTANEOUS
Dose (Carcinogen):	12 MG/KG BW IN 0.9% SALINE TWICE
Promoter:	CHOLIC ACID ; 81-25-4
Route (Promoter):	ORAL
Dose (Promoter):	0.5% IN DIET FOR DURATION OF STUDY BEGINNING 1 WK POST LAST CARCINOGEN DOSE
Target Tissue: Type of Lesion:	COLON: ADENOMA
Endpoint (Incidence):	81%, 59%, 27%, P<0.05

Endpoint (Multiplicity):	1.5, 0.9, 40%, NOT SIGNIFICANT
Comments:	BASAL DIET CONTAINED 0.5% CALCIUM.
Reference:	[PENCE,BC, DUNN,DM, ZHAO,C, LANDERS,M AND WARGOVICH,MJ; CHEMOPREVENTIVE EFFECTS OF CALCIUM BUT NOT ASPIRIN SUPPLEMENTATION IN CHOLIC ACID-PROMOTED COLON CARCINOGENESIS: CORRELATION WITH INTERMEDIATE ENDPOINTS; CARCINOGENESIS 16(4):757-765, 1995]
Species:	RAT
Number of Animals Tested:	(?,?)/(?,?)
Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 2% IN DIET FOR 34 WK BEGINNING 1 WK POST LAST CARCINOGEN DOSE (STUDY DURATION: 49 WK)
Route (Inhibitor):	ORAL
Carcinogen:	AZOXYMETHANE ; 25843-45-2
Route (Carcinogen):	SUBCUTANEOUS
Dose (Carcinogen):	12 MG/KG BW IN 0.9% SALINE TWICE
Promoter:	CHOLIC ACID ; 81-25-4
Route (Promoter):	ORAL
Dose (Promoter):	0.5% IN DIET FOR DURATION OF STUDY BEGINNING 1 WK POST LAST CARCINOGEN DOSE
Target Tissue: Type of Lesion:	SMALL INTESTINE: ADENOCARCINOMA
Endpoint (Incidence):	23%, 16%, 30%, NOT SIGNIFICANT
Endpoint (Multiplicity):	0.3, 0.2, 33%, NOT SIGNIFICANT
Comments:	BASAL DIET CONTAINED 0.5% CALCIUM.
Reference:	[PENCE,BC, DUNN,DM, ZHAO,C, LANDERS,M AND WARGOVICH,MJ; CHEMOPREVENTIVE EFFECTS OF CALCIUM BUT NOT ASPIRIN SUPPLEMENTATION IN CHOLIC ACID-PROMOTED COLON CARCINOGENESIS: CORRELATION WITH INTERMEDIATE ENDPOINTS; CARCINOGENESIS 16(4):757-

	765, 1995]
Species:	RAT
Number of Animals Tested:	(?,?)/(?,?)
Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 2% IN DIET FOR 34 WK BEGINNING 1 WK POST LAST CARCINOGEN DOSE (STUDY DURATION: 49 WK)
Route (Inhibitor):	ORAL
Carcinogen:	AZOXYMETHANE ; 25843-45-2
Route (Carcinogen):	SUBCUTANEOUS
Dose (Carcinogen):	12 MG/KG BW IN 0.9% SALINE TWICE
Promoter:	CHOLIC ACID ; 81-25-4
Route (Promoter):	ORAL
Dose (Promoter):	0.5% IN DIET FOR DURATION OF STUDY BEGINNING 1 WK POST LAST CARCINOGEN DOSE
Target Tissue: Type of Lesion:	SMALL INTESTINE: ADENOMA
Endpoint (Incidence):	17%, 7%, 59%, NOT SIGNIFICANT
Endpoint (Multiplicity):	0.2, 0.1, 50%, NOT SIGNIFICANT
Comments:	BASAL DIET CONTAINED 0.5% CALCIUM.
Reference:	[PENCE,BC, DUNN,DM, ZHAO,C, LANDERS,M AND WARGOVICH,MJ; CHEMOPREVENTIVE EFFECTS OF CALCIUM BUT NOT ASPIRIN SUPPLEMENTATION IN CHOLIC ACID-PROMOTED COLON CARCINOGENESIS: CORRELATION WITH INTERMEDIATE ENDPOINTS; CARCINOGENESIS 16(4):757-765, 1995]
Species:	RAT
Number of Animals Tested:	(?,?)/(?,?)
Strain/Sex:	F344/MALE

Dose (Inhibitor):	0; 2% IN DIET FOR 13 WK BEGINNING 36 WK POST LAST CARCINOGEN DOSE (STUDY DURATION: 49 WK)
Route (Inhibitor):	ORAL
Carcinogen:	AZOXYMETHANE ; 25843-45-2
Route (Carcinogen):	SUBCUTANEOUS
Dose (Carcinogen):	12 MG/KG BW IN 0.9% SALINE TWICE
Promoter:	CHOLIC ACID ; 81-25-4
Route (Promoter):	ORAL
Dose (Promoter):	0.5% IN DIET FOR DURATION OF STUDY BEGINNING 1 WK POST LAST CARCINOGEN DOSE
Target Tissue: Type of Lesion:	COLON: ADENOCARCINOMA
Endpoint (Incidence):	26%, 31%, -19%, NOT SIGNIFICANT
Endpoint (Multiplicity):	0.3, 0.3, 0%, NOT SIGNIFICANT
Comments:	BASAL DIET CONTAINED 0.5% CALCIUM.
Reference:	[PENCE,BC, DUNN,DM, ZHAO,C, LANDERS,M AND WARGOVICH,MJ; CHEMOPREVENTIVE EFFECTS OF CALCIUM BUT NOT ASPIRIN SUPPLEMENTATION IN CHOLIC ACID-PROMOTED COLON CARCINOGENESIS: CORRELATION WITH INTERMEDIATE ENDPOINTS; CARCINOGENESIS 16(4):757-765, 1995]
Species:	RAT
Number of Animals Tested:	(?,?)/(?,?)
Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 2% IN DIET FOR 13 WK BEGINNING 36 WK POST LAST CARCINOGEN DOSE (STUDY DURATION: 49 WK)
Route (Inhibitor):	ORAL
Carcinogen:	AZOXYMETHANE ; 25843-45-2
Route	SUBCUTANEOUS

(Carcinogen):	
Dose (Carcinogen):	12 MG/KG BW IN 0.9% SALINE TWICE
Promoter:	CHOLIC ACID ; 81-25-4
Route (Promoter):	ORAL
Dose (Promoter):	0.5% IN DIET FOR DURATION OF STUDY BEGINNING 1 WK POST LAST CARCINOGEN DOSE
Target Tissue: Type of Lesion:	COLON: ADENOMA
Endpoint (Incidence):	81%, 89%, -10%, NOT SIGNIFICANT
Endpoint (Multiplicity):	1.5, 2.6, -73%, P<0.05
Comments:	BASAL DIET CONTAINED 0.5% CALCIUM.
Reference:	[PENCE,BC, DUNN,DM, ZHAO,C, LANDERS,M AND WARGOVICH,MJ; CHEMOPREVENTIVE EFFECTS OF CALCIUM BUT NOT ASPIRIN SUPPLEMENTATION IN CHOLIC ACID-PROMOTED COLON CARCINOGENESIS: CORRELATION WITH INTERMEDIATE ENDPOINTS; CARCINOGENESIS 16(4):757-765, 1995]
Species:	RAT
Number of Animals Tested:	(?,?)/(?,?)
Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 2% IN DIET FOR 13 WK BEGINNING 36 WK POST LAST CARCINOGEN DOSE (STUDY DURATION: 49 WK)
Route (Inhibitor):	ORAL
Carcinogen:	AZOXYMETHANE ; 25843-45-2
Route (Carcinogen):	SUBCUTANEOUS
Dose (Carcinogen):	12 MG/KG BW IN 0.9% SALINE TWICE
Promoter:	CHOLIC ACID ; 81-25-4
Route (Promoter):	ORAL

Dose (Promoter):	0.5% IN DIET FOR DURATION OF STUDY BEGINNING 1 WK POST LAST CARCINOGEN DOSE
Target Tissue: Type of Lesion:	SMALL INTESTINE: ADENOCARCINOMA
Endpoint (Incidence):	23%, 14%, 39%, NOT SIGNIFICANT
Endpoint (Multiplicity):	0.3, 0.2, 33%, NOT SIGNIFICANT
Comments:	BASAL DIET CONTAINED 0.5% CALCIUM.
Reference:	[PENCE,BC, DUNN,DM, ZHAO,C, LANDERS,M AND WARGOVICH,MJ; CHEMOPREVENTIVE EFFECTS OF CALCIUM BUT NOT ASPIRIN SUPPLEMENTATION IN CHOLIC ACID-PROMOTED COLON CARCINOGENESIS: CORRELATION WITH INTERMEDIATE ENDPOINTS; CARCINOGENESIS 16(4):757-765, 1995]
Species:	RAT
Number of Animals Tested:	(?,?)/(?,?)
Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 2% IN DIET FOR 13 WK BEGINNING 36 WK POST LAST CARCINOGEN DOSE (STUDY DURATION: 49 WK)
Route (Inhibitor):	ORAL
Carcinogen:	AZOXYMETHANE ; 25843-45-2
Route (Carcinogen):	SUBCUTANEOUS
Dose (Carcinogen):	12 MG/KG BW IN 0.9% SALINE TWICE
Promoter:	CHOLIC ACID ; 81-25-4
Route (Promoter):	ORAL
Dose (Promoter):	0.5% IN DIET FOR DURATION OF STUDY BEGINNING 1 WK POST LAST CARCINOGEN DOSE
Target Tissue: Type of Lesion:	SMALL INTESTINE: ADENOMA
Endpoint (Incidence):	17%, 14%, 18%, NOT SIGNIFICANT

Endpoint (Multiplicity):	0.2, 0.1, 50%, NOT SIGNIFICANT
Comments:	BASAL DIET CONTAINED 0.5% CALCIUM.
Reference:	[PENCE,BC, DUNN,DM, ZHAO,C, LANDERS,M AND WARGOVICH,MJ; CHEMOPREVENTIVE EFFECTS OF CALCIUM BUT NOT ASPIRIN SUPPLEMENTATION IN CHOLIC ACID-PROMOTED COLON CARCINOGENESIS: CORRELATION WITH INTERMEDIATE ENDPOINTS; CARCINOGENESIS 16(4):757-765, 1995]
Species:	RAT
Number of Animals Tested:	(15,15)/(15,15)
Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 20000 PPM IN DIET FOR DURATION OF STUDY BEGINNING 1 WK POST CARCINOGEN TREATMENT (STUDY DURATION: 25 WK)
Route (Inhibitor):	ORAL
Carcinogen:	N-NITROSOMETHYLBENZYLAMINE ; 937-40-6
Route (Carcinogen):	SUBCUTANEOUS
Dose (Carcinogen):	1 MG/KG 3/WK FOR 5 WK
Promoter:	NONE USED
Target Tissue: Type of Lesion:	ESOPHAGUS: TUMOR
Endpoint (Incidence):	15/15 (100%), 14/15 (93%), 7%, NOT SIGNIFICANT
Endpoint (Multiplicity):	2.9, 3.1, -7%, NOT SIGNIFICANT
Comments:	THE CONTROL (BASAL) DIET CONTAINED 0.5% CALCIUM.
Reference:	[SIGLIN,JC, BARCH,DH AND STONER,GD; EFFECTS OF DIETARY PHENETHYL ISOTHIOCYANATE, ELLAGIC ACID, SULDINAC AND CALCIUM ON THE INDUCTION AND PROGRESSION OF N-NITROSOMETHYLBENZYLAMINE-INDUCED ESOPHAGEAL CARCINOGENESIS IN RATS; CARCINOGENESIS 16(5):1101-1106, 1995]
Species:	RAT
Number of Animals Tested:	(15,15)/(15,15)

Strain/Sex:	F344/MALE
Dose (Inhibitor):	0; 20000 PPM IN DIET FOR DURATION OF STUDY BEGINNING 2 WK PRIOR TO CARCINOGEN TREATMENT (STUDY DURATION: 25 WK)
Route (Inhibitor):	ORAL
Carcinogen:	N-NITROSOMETHYLBENZYLAMINE ; 937-40-6
Route (Carcinogen):	SUBCUTANEOUS
Dose (Carcinogen):	1 MG/KG 3/WK FOR 5 WK
Promoter:	NONE USED
Target Tissue: Type of Lesion:	ESOPHAGUS: TUMOR
Endpoint (Incidence):	15/15 (100%), 13/15 (87%), 13%, NOT SIGNIFICANT
Endpoint (Multiplicity):	2.9, 2.6, 10%, NOT SIGNIFICANT
Comments:	THE MEAN BODY WEIGHTS OF AGENT-TREATED GROUP WERE SLIGHTLY BUT SIGNIFICANTLY LOWER ($P<0.05$) THAN NMBA CONTROLS FROM THE ONSET OF AGENT EXPOSURE THROUGH WK 17.
Reference:	[SIGLIN,JC, BARCH,DH AND STONER,GD; EFFECTS OF DIETARY PHENETHYL ISOTHIOCYANATE, ELLAGIC ACID, SULDINAC AND CALCIUM ON THE INDUCTION AND PROGRESSION OF N-NITROSOMETHYLBENZYLAMINE-INDUCED ESOPHAGEAL CARCINOGENESIS IN RATS; CARCINOGENESIS 16(5):1101-1106, 1995]

As taken from CCRIS, 2008

“BACKGROUND: Processed meat intake has been associated with increased colorectal cancer risk. We have shown that cured meat promotes carcinogen-induced preneoplastic lesions and increases specific biomarkers in the colon of rats. OBJECTIVES: We investigated whether cured meat modulates biomarkers of cancer risk in human volunteers and whether specific agents can suppress cured meat-induced preneoplastic lesions in rats and associated biomarkers in rats and humans. DESIGN: Six additives (calcium carbonate, inulin, rutin, carnosol, α -tocopherol, and trisodium pyrophosphate) were added to cured meat given to groups of rats for 14 d, and fecal biomarkers were measured. On the basis of these results, calcium and tocopherol were kept for the following additional experiments: cured meat, with or without calcium or tocopherol, was given to dimethylhydrazine-initiated rats (47% meat diet for 100 d) and to human volunteers in a crossover study (180 g/d for 4 d). Rat colons were scored for mucin-depleted foci, putative precancer lesions. Biomarkers of nitrosation, lipoperoxidation, and cytotoxicity were measured in the urine and feces of rats and volunteers. RESULTS: Cured meat increased nitroso compounds and lipoperoxidation in human stools (both $P<0.05$). Calcium normalized both biomarkers in rats and human feces, whereas tocopherol only decreased nitro compounds in rats and lipoperoxidation in feces of volunteers (all $P<0.05$). Last, calcium and tocopherol reduced the number of mucin-depleted foci per

colon in rats compared with nonsupplemented cured meat ($P = 0.01$). CONCLUSION: Data suggest that the addition of calcium carbonate to the diet or α -tocopherol to cured meat may reduce colorectal cancer risk associated with cured-meat intake." As taken from Pierre FH et al. 2013. Am. J. Clin. Nutr. 98(5), 1255-62. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24025632>

"Epidemiology suggests that processed meat is associated with colorectal cancer risk, but few experimental studies support this association. We have shown that a model of cured meat made in a pilot workshop promotes preneoplastic lesions, mucin-depleted foci (MDF) in the colon of rats. This study had two aims: to check if real store-bought processed meats also promote MDF, and to test if calcium carbonate, which suppresses heme-induced promotion, can suppress promotion by processed meat. A 14-day study was done to test the effect of nine purchased cured meats on fecal and urinary biomarkers associated with heme-induced carcinogenesis promotion. Fecal water from rats given hot dog or fermented raw dry sausage was particularly cytotoxic. These two cured meats were thus given to rats pretreated with 1,2-dimethylhydrazine, to evaluate their effect on colorectal carcinogenesis. After a 100-days feeding period, fecal apparent total N-nitroso compounds (ATNC) were assayed and colons were scored for MDF. Hot dog diet increased fecal ATNC and the number of MDF per colon compared with the no-meat control diet (3.0 ± 1.7 vs. 1.2 ± 1.4 , $P < 0.05$). In a third study, addition of calcium carbonate ($150 \mu\text{mol/g}$) to the hot dog diet decreased the number of MDF/colon and fecal ATNC compared with the hot dog diet without calcium carbonate (1.2 ± 1.1 vs. 2.3 ± 1.4 , respectively, $P < 0.05$). This is the first experimental evidence that a widely consumed processed meat promotes colon carcinogenesis in rats. It also shows that dietary prevention of this detrimental effect is possible." As taken from Santarelli RL et al. 2013. Int. J. Cancer 133(11), 2533-41. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23712585>

"BACKGROUND & AIMS: Chemopreventive strategies might be used to reduce the recurrence of colorectal adenomas and the incidence of colorectal cancer. We performed a randomized, double-blind, placebo-controlled trial to determine whether a combination of acetylsalicylic acid (aspirin), calcitriol, and calcium carbonate could prevent colorectal adenoma recurrence. METHODS: We included 1107 patients with 1 or more sporadic adenoma(s) removed from the colon or rectum at centers in Europe, Russia, or the United States, from 2004 through 2010. Inclusion criteria were 1 adenoma greater than 1 cm in diameter, more than 1 adenoma of any size, or an adenoma of any size and first-degree relatives with colorectal cancer. Subjects were assigned randomly to groups given $0.5 \mu\text{g}$ calcitriol, 75 mg acetylsalicylic acid, and 1250 mg calcium carbonate ($n = 209$), or placebo ($n = 218$), each day for 3 years. The primary outcome was adenoma recurrence assessed by colonoscopy after 3 years. Secondary outcomes were the proportion of patients with advanced adenomas, the total number of colorectal adenomas, and adenoma size and features. RESULTS: The trial was stopped in October 2010 because of futility. In this analysis, we found no differences between groups in the rate of recurrence (odds ratio [OR], 0.95; 95% confidence interval [CI], 0.61-1.48), adverse effects, or secondary outcomes. Subgroup analyses indicated that the treatment effects may be influenced by smoking status (nonsmokers OR, 0.65; 95% CI, 0.26-1.22 vs current smokers OR, 1.70; 95% CI, 0.70-4.09; P value interaction $< .05$). However, the overall interaction was not significant. CONCLUSIONS: In a prospective study, the combination of calcitriol, aspirin, and calcium carbonate did not prevent recurrence of colorectal adenomas over a 3-year period. The negative results might be owing to the effects of smoking or low doses of the tested agents. Clinicaltrials.gov number: NCT00486512." As taken from Pommergaard HC et al. 2016. Gastroenterology 150, 114-122. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26404953>

"We reviewed patient records with a first-listed diagnosis of urolithiasis-also known as urinary tract or kidney stone disease, nephrolithiasis-upon discharge from Guam's sole civilian hospital during 2006 to 2010 and urinary cancer mortality records from the Guam Cancer Registry for 1970 to 2009 to determine the source of municipal water supplied to the patients' residence. The objective was to investigate a possible relationship between the sources of municipal water supplied to

Guam villages and the incidence of urolithiasis and urinary cancer. We analyzed hospital discharge diagnoses of urolithiasis or renal calculi by calculating the incidence of first-mentioned discharge for urolithiasis or renal calculi and comparing rates across demographic or geographic categories while adjusting by age, sex, and ethnicity/race. We reviewed cancer registry records of urinary cancer deaths by patient residence. The annual incidence of hospitalization for urolithiasis was 5.22 per 10,000. Rates adjusted for sex or age exhibited almost no change. The rate of 9.83 per 10,000 among Chamorros was significantly higher ($P < 0.05$) than the rates among any other ethnic group or race. When villages were grouped by water source, rates of patients discharged with a first-listed diagnosis of urolithiasis, adjusted for ethnicity/race, were similar for villages using either well water (5.44 per 10,000) or mixed source water (5.39 per 10,000), and significantly greater than the rate for villages using exclusively reservoir water (1.35 per 10,000). No statistically significant differences were found between the water source or village of residence and urinary cancer mortality. Some Guam residents living in villages served completely or partly by deep well water high in calcium carbonate may be at increased risk for urolithiasis compared with residents living in villages served by surface waters. Although the risk appears to be highest in villagers of Chamorro ethnicity, residents should be aware of other contributing risk factors and steps to take to avoid developing this health problem.” As taken from Haddock RL et al. 2016. *Int. J. Environ. Res. Public Health* 13(6), E523. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27231922>

5.7. Irritation/immunotoxicity

“CALCIUM CARBONATE...IN FORM OF POWDER...FOUND.../TO HAVE/ NO TOXIC EFFECT WHEN APPLIED TO SURFACE OF RABBIT EYES. ...INJECTING 8 MG OF CALCIUM CARBONATE POWDER...INTO ANTERIOR CHAMBER OF RABBIT CAUSED MODERATE RISE OF INTRAOCULAR PRESSURE LASTING LESS THAN 24 HR.”

As taken from HSDB, 2014

“A 500mg/24h exposure of CaCO_3 on rabbit skin produced a moderately irritating effect. It is believed that CaCO_3 as a pure solid does not entail the irritating effect. Impurities or degradation products may lead to irritant effects on the sweating skin due alkalinity.”

“Calcium carbonate was irritating for humans, causing burns of the skin. The test substance was highly irritating for rabbits eyes and slightly irritating for humans eyes. CaCO_3 has been shown not to be a sensitizer.”

As taken from IUCLID Dataset (2000), Calcium carbonate (471-34-1).

“No data are available indicating that calcium carbonate has allergenic properties or can invoke sensitivity or intolerance reactions in exposed individuals” (EFSA, 2011. *EFSA Journal* 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

Eye Irritation

Species	rabbit
Result	highly irritating
Method	other: Draize test
Remark	0.750 mg/24 h. Test conditions not available but it is expected that CaCO_3 does not lead to eye irritation provided impurities or decomposition products are not present
Source	Solvay S.A. Bruxelles

Species	rabbit
Result	not irritating
Remark	Test conditions not available
Source	Solvay S.A. Bruxelles
Species	rabbit
Result	highly irritating
EC classificat	risk of serious damage to eyes
Source	Guaber SPA Funo di Argelato (BO)

As taken from IUCLID Dataset (2000), Calcium carbonate (471-34-1).

Record for CAS RN 1317-65-3:

Type of Test	Route of Exposure	Species Observed	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration into the eye	Rodent - rabbit	750 ug/24H	Severe	28ZPAK "Sbornik Vysledku Toxilogickeho Vysetreni Latek A Pripravku," Marhold, J.V., Institut Pro Vychovu Vedoucicn Pracovniku Chemickeho Prumyclu Praha, Czechoslovakia, 1972 Volume(issue)/page/year: -,267,1972

As taken from RTECS, 2019.

“BACKGROUND: Exposure to hard water has been suggested as a risk factor for eczema in childhood, based on limited evidence from two ecologic and two cross-sectional studies. OBJECTIVES: We evaluate this hypothesis for the first time in early infancy using prospective data from a mother-child cohort study. METHODS: We used data from the INMA cohorts in Gipuzkoa, Sabadell and Valencia, Spain (N=1638). Current and ever eczema, bathing frequency and duration and covariables were collected by questionnaires at 14 months (14 m) and 4 years (4 y). Calcium carbonate (CaCO₃) level in municipal water was assigned to home addresses at birth, 14 m and 4 y. We calculated Odds Ratio (OR) of eczema related to CaCO₃ at home, bath exposure and a combination of both. RESULTS: Prevalence of eczema ever was 18.4% at 14 m and 33.4% at 4 y. Mean CaCO₃ ranged from 51.6 to 272.8 mg/L among areas. No association was detected between water hardness at home and current or ever eczema. Adjusted OR was 0.79 (95%CI=0.45, 1.39) at 14 m and 0.93 (0.56, 1.52) at 4 y among children in the highest vs. lowest tertiles of CaCO₃. Bath exposure alone or in combination with water hardness did not increase the OR of eczema at 14 m or 4 y either. CONCLUSIONS: We did not find an association between eczema and water hardness at home or bathing exposure during the first four years of life. This first cohort study in a critical age period with improved exposure assessment does not confirm the association suggested among children by previous studies.” As taken from Font-Ribera L et al. 2015. Environ. Res. 142, 579-85. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26298601>

“PURPOSE: To determine the efficacy in reducing dentin hypersensitivity (DHS) of a combined in-office and home-use desensitizing system, each product containing 8% arginine and calcium carbonate (Test), following a dental scaling procedure, compared to the combination of a

conventional prophylactic paste and a potassium nitrate dentifrice (Control), in a group of patients with known dentin hypersensitivity. METHODS: An 8-week clinical study, with 50 subjects, was conducted in Piracicaba, São Paulo, Brazil, using a double-blind/two treatment design. Air blast sensitivity assessments were used to compare the efficacy of the two approaches using both the Schiff scale as well as a Visual Analogue Scale (VAS). RESULTS: Immediately after prophylaxis, the Test treatment provided significant reduction in DHS when compared to baseline values (VAS = 26.2% and Schiff = 29.1%), while for Control treatment this difference was not statistically significant (VAS = 8.1% and Schiff = 6.6%). The comparison between groups after prophylaxis showed a greater DHS reduction for the Test treatment ($P < 0.05$). The reductions in DHS after 2, 4 and 8 weeks were significant for both groups, however, when considering Schiff scale, the Test treatment provided greater DHS reduction after 2 weeks (44.5% for Test versus 27.7% for Control) and 4 weeks (55.2% for Test and 40.5% for Control), while after 8 weeks there was no significant difference between groups (71.1% for Test versus 61.1% for Control).” As taken from França IL et al. 2015. Am. J. Dent. 28, 45-50. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25864242>

“AIM: The aim of this single-centre, two-cell, double-blind, randomized controlled clinical study was to evaluate the effectiveness of an in-office desensitizing paste containing 8% arginine and calcium carbonate in providing relief on dentine hypersensitivity immediately after scaling and root planing and its sustained relief over a 6-week period. MATERIALS AND METHODS: Fifty periodontitis subjects presenting hypersensitivity were subjected to scaling and root planing and in-office application of either 8% arginine and calcium carbonate desensitizing paste (25 subjects, test group) or fluoride-free prophylaxis paste (25 subjects, control group). Air-blast hypersensitivity was assessed using Schiff and Visual Analogue (VAS) scales at baseline, post-scaling, post-application, 2, 4 and 6 weeks. RESULTS: At all evaluation times, the test group presented significant % reduction in hypersensitivity relative to post-scaling (t-test, $P < 0.05$) (Schiff - Test: 57, 58.6, 60.2, 68; CONTROL: 28.6, 22.2, 23, 23) (VAS - Test: 60, 55.6, 60.1, 68.4; CONTROL: 25.9, 18.2, 20.6, 22.7) and significant % hypersensitivity difference relative to control (ancova, $P < 0.05$) (Schiff: 38.9, 45.9, 47.4, 57.7; VAS: 49.1, 48.9, 52.6, 61). CONCLUSION: The single in-office application of the 8% arginine-calcium carbonate desensitizing paste after scaling and root planing provided significant immediate reduction in dentine hypersensitivity, which sustained over a 6-week period.” As taken from Pepelassi E et al. 2015. J. Clin. Periodontol. 42, 37-45. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25269991>

“BACKGROUND: Domestic water hardness and chlorine have been suggested as important risk factors for atopic dermatitis (AD). OBJECTIVE: We sought to examine the link between domestic water calcium carbonate (CaCO_3) and chlorine concentrations, skin barrier dysfunction (increased transepidermal water loss), and AD in infancy. METHODS: We recruited 1303 three-month-old infants from the general population and gathered data on domestic water CaCO_3 (in milligrams per liter) and chlorine (Cl_2 ; in milligrams per liter) concentrations from local water suppliers. At enrollment, infants were examined for AD and screened for filaggrin (FLG) skin barrier gene mutation status. Transepidermal water loss was measured on unaffected forearm skin. RESULTS: CaCO_3 and chlorine levels were strongly correlated. A hybrid variable of greater than and less than median levels of CaCO_3 and total chlorine was constructed: a baseline group of low CaCO_3 /low total chlorine (CaL/CIL), high CaCO_3 /low total chlorine (CaH/CIL), low CaCO_3 /high total chlorine (CaL/CIH) and high CaCO_3 /high total chlorine (CaH/CIH). Visible AD was more common in all 3 groups versus the baseline group: adjusted odds ratio (AOR) of 1.87 (95% CI, 1.25-2.80; $P = .002$) for the CaH/CIL group, AOR of 1.46 (95% CI, 0.97-2.21; $P = .07$) for the CaL/CIH, and AOR of 1.61 (95% CI, 1.09-2.38; $P = .02$) for the CaH/CIH group. The effect estimates were greater in children carrying FLG mutations, but formal interaction testing between water quality groups and filaggrin status was not statistically significant. CONCLUSIONS: High domestic water CaCO_3 levels are associated with an increased risk of AD in infancy. The influence of increased total chlorine levels remains uncertain. An intervention trial is required to see whether installation of a domestic device to decrease CaCO_3 levels around the time of birth can reduce this risk.” As taken from Perkin MR

et al. 2016. J. Allergy Clin. Immunol. 138(2), 509-16. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27241890>

"The ocular irritation potential of Calcium Carbonate was studied using 3 male New Zealand white rabbits.²⁸ The test substance (0.1 ml) was instilled into the right eye, and the observation period was up to 72 h. Left eyes served as controls. Ocular damage/irritation was evaluated according to the Draize scale. Corneal effects were not observed during the study. Iridial inflammation and minimal conjunctival irritation were observed in all treated eyes at 1 h post-instillation. Conjunctival irritation was also observed at 24 h and 48 h post-instillation. All reactions had cleared by 72 h post-instillation. Calcium Carbonate was classified as non-irritating to the eyes of rabbits."

"Also, using an in vitro test for assessing dermal corrosivity potential (Corrositex®), undiluted Calcium Carbonate was not found to be a corrosive agent."

As taken from CIR, 2017.

"Phagocytosis is a physiological process used by immune cells such as macrophages to actively ingest and destroy foreign pathogens and particles. It is the cellular process that leads to the failure of drug delivery carriers because the drug carriers are cleared by immune cells before reaching their target. Therefore, clarifying the mechanism of particle phagocytosis would have a significant implication for both fundamental understanding and biomedical engineering. As far as we know, the effect of particle shape on biological response has not been fully investigated. In the present study, we investigated the particle shape-dependent cellular uptake and biological response of differentiated THP-1 macrophages by using calcium carbonate (CaCO₃)-based particles as a model. Transmission electron microscopy analysis revealed that the high uptake of needle-shaped CaCO₃ particles by THP-1 macrophages because of their high phagocytic activity. In addition, the THP-1 macrophages exposed to needle-shaped CaCO₃ accumulated a large amount of calcium in the intracellular matrix. The enhanced release of interleukin-8 (IL-8) and tumor necrosis factor- α (TNF- α) by the THP-1 macrophages suggested that the needle-shaped CaCO₃ particles trigger a pro-inflammatory response. In contrast, no pro-inflammatory response was induced in undifferentiated THP-1 monocytes exposed to either needle- or cuboidal-shaped CaCO₃ particles, probably because of their low phagocytic activity. We also found that phosphate-coated particles efficiently repressed cellular uptake and the resulting pro-inflammatory response in both THP-1 macrophages and primary peritoneal macrophages. Our results indicate that the pro-inflammatory response of macrophages upon exposure to CaCO₃ particles is shape- and surface property-dependent, and is mediated by the intracellular accumulation of calcium ions released from phagocytosed CaCO₃ particles." As taken from Tabei Y et al. 2017. Biochem. Biophys. Res. Commun. 490(2), 499-505. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28624457>

INTRODUCTION Massive consumption of dietary supplements, including vitamins and minerals, has recently become a serious health issue in Europe. Their use may negatively affect the pharmacological activity of various medications, including antiallergic drugs. Calcium preparations are commonly used in some European countries as a popular remedy for allergy-related skin reactions, such as itching, erythema, and wheals, as well as insect bites. However, so far there have been no reliable studies to prove their action. **OBJECTIVES** The aim of this randomized, double-blind, placebo-controlled study was to investigate the efficacy of calcium salts in allergic reactions, using an allergen-induced skin prick test (SPT). **PATIENTS AND METHODS** Forty adult volunteers with allergic rhinoconjunctivitis or asthma (or both) were recruited to receive oral calcium carbonate (1000 mg) or placebo 3 times a day for 3 days. SPTs were performed with 11 aeroallergen extracts at baseline and at 4 and 72 hours after drug administration. The wheal diameter was measured. We also used the visual analog scale to evaluate the intensity of pruritus. **RESULTS** There was no difference in the wheal size or pruritus between patients receiving calcium or placebo at any of the time points ($P > 0.05$). Calcium preparations were well tolerated. **CONCLUSIONS** We found no evidence to support the efficacy of calcium preparations in allergy-

related skin reactions associated with itching and wheals. Calcium preparations do not suppress SPT responses; moreover, their use in allergic patients should be discouraged due to their possible interference with the absorption of antiallergic drugs." As taken from Matysiak K et al. 2017. Pol. Arch. Intern. Med. 127(9), 582-588. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28724883>

"A respiratory tract irritant" (CAS RN 471-34-1) (BPDB, 2022).

"Irritation eyes, skin, mucous membrane, upper respiratory system; cough, sneezing, rhinorrhea (discharge of thin nasal mucus); lacrimation (discharge of tears)".

As taken from PubChem.

"The subchronic inhalation toxicity study (90 days) in rats revealed local effects that could indicate potential irritation of respiratory tract (slight increases in BAL derived inflammation and cytotoxicity biomarkers, which were largely, but not fully reversible within a 4 week recovery period). This effect was observed after 6h of exposure/5 days during 90 days at 0.399 mg/L, unlike the next lower dose assayed 0.212 mg/L. NOAEC for local effects was established at 0.212 mg/L.....Potential respiratory tract irritation was not seen in the 1-week inhalation toxicity in rats and in the acute inhalation toxicity either."

As taken from European Commission, 2019a.

"[T]he compound [calcium carbonate] was neither skin irritant, eye irritant nor skin sensitiser."

As taken from EFSA, 2021

5.8. All other relevant types of toxicity

HUMAN EXPOSURE STUDIES/ Thirty-two healthy volunteers were exposed to 0 (control), 500, 1000, and 5000 ug/cu m calcium carbonate dust for 3 hours and nasal saccharin transport time (STT), rhinomanometry, and visual analog scales (VAS) on nasal discomfort were obtained. A dose dependent decrease of STT ($P = 0.02$) and nasal patency ($P = 0.04$), and increased sensation of nasal obstruction ($P = 0.002$) and dryness ($P = 0.03$) was observed. The results indicate that acute exposure to low-toxicity particulate matter in concentrations frequently encountered in western agglomeration areas may affect nasal functions and cause nasal symptoms. [Riechelmann H et al; J Occup Environ Med 45(1):54-60 (2003)] **PEER REVIEWED**

/SIGNS AND SYMPTOMS/ Admin of calcium carbonate promotes positive phosphate balance; plasma phosphate concn may or may not be incr, but hyperphosphatemia is usual finding in patients with milk-alkali syndrome. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 992] **PEER REVIEWED**

/SIGNS AND SYMPTOMS/ ... Enough is absorbed to cause systemic and renal effects ... a slight-to-moderate metabolic alkalosis occurs during treatment with CaCO_3 , but it is slow to develop. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 992] **PEER REVIEWED**

/SIGNS AND SYMPTOMS/ When phosphate intake is low /during chronic calcium carbonate admin/, hypophosphatemia may occur. Hypercalciuria and alkaluria predispose to nephrolithiasis. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 993] **PEER REVIEWED**

/SIGNS AND SYMPTOMS/ ... Infrequent instances of hypercalcemia with alkalosis, calcinosis (incl nephrocalcinosis), and azotemia that occur during chronic usage of calcium carbonate, especially in conjunction with milk and cream ... renal dysfunction, GI hemorrhage and vomiting or aspiration

through nasogastric tube seem to predispose to the disorder. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 993] **PEER REVIEWED**

/SIGNS AND SYMPTOMS/ Dose related constipation is common when 20 to 40 g is taken daily; hemorrhoids, painful, bleeding anal fissures ... may occur. Acute appendicitis has been produced by impacted calcium carbonate fecoliths. [American Medical Association, AMA Department of Drugs. AMA Drug Evaluations. 4th ed. Chicago: American Medical Association, 1980., p. 954] **PEER REVIEWED**

/SIGNS AND SYMPTOMS/ Side/Adverse Effects: Those indicating need for medical attention: Incidence more frequent: With parenteral dosage forms only: Hypotension (dizziness); flushing and/or sensation of warmth or heat; irregular heartbeat; nausea or vomiting; skin redness, rash, pain, or burning or injection site; sweating; tingling sensation. Note: Tingling sensation may result when intravenous injection is too rapid; skin redness, rash, pain, or burning may indicate extravasation and can precede sloughing or necrosis of skin. Incidence rare: Hypercalcemic syndrome, acute (drowsiness, continuing nausea and vomiting, weakness); renal calculi, calcific (difficult or painful urination) - with oral dosage forms. Early symptoms of hypercalcemia: Constipation, severe; dryness of mouth; headache, continuing; increased thirst; irritability; loss of appetite; mental depression; metallic taste; unusual tiredness or weakness. Late symptoms of hypercalcemia: Confusion; drowsiness; high blood pressure; increased sensitivity of eyes or skin to light, especially in hemodialysis patients; irregular, fast, or slow heartbeat; nausea and vomiting; unusually large amount of urine or increased frequency of urination. Note: In severe hypercalcemia, ECG changes consisting of shortened Q-T intervals are also seen. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 744] **PEER REVIEWED**

/SIGNS AND SYMPTOMS/ ... /The authors/ examined the relationship between coronary risk factors; metabolic factors, including serum osteoprotegerin (OPG) concentration; and progression of vascular calcification in 26 dialysis patients ... Mean patient age was 52.6 + or - 8.7 (SD) years, and mean duration of dialysis therapy was 7.7 + or - 5.8 years. ACI was measured twice in each patient, and the mean interscan period was 4.9 + or - 0.3 years. Mean ACI changed from 22.2 + or - 24.2 to 33.9 + or - 28.8 overall, and mean change in ACI (DeltaACI) was 12.0 + or - 9.9. Patients were divided into 2 groups: slow progressors, with DeltaACI of 4.1 + or - 3.2 (n = 13), and rapid progressors, with DeltaACI of 19.8 + or - 7.9 (n = 13). Serum fasting glucose and CRP levels of rapid progressors were high, and their serum albumin and intact parathyroid hormone levels were low. Multiple regression analyses showed that serum OPG levels were independently associated with vascular calcification in the hemodialysis patients studied ... Rapid progression of vascular calcification was associated with dose of calcium carbonate prescribed and serum OPG concentration. The clinical significance of these observations remains to be determined. [Nitta K et al; Am J Kidney Dis 42(2):303-9 (2003)] **PEER REVIEWED**

/SIGNS AND SYMPTOMS/ ... When large doses of NaHCO₃ and/or CaCO₃ were ... admin with milk or cream for the management of peptic ulcer, the milk-alkali syndrome occurred frequently. This syndrome results from large quantities of Ca⁺² and absorbable alkali; effects consist of hypercalcemia, reduced secretion of parathyroid hormone, retention of phosphate, precipitation of Ca⁺² salts in the kidney, and renal insufficiency. Therapeutic regimens emphasizing the use of dairy products seldom are employed in current practice. /Antacids/ [Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1013] **PEER REVIEWED**

/CASE REPORTS/ A 40 yr old woman with surgical hyperparathyroidism ingested up to 15 g of calcium daily (calcium carbonate). Her serum calcium level was 5.35 mmol/L (normal 2.20-2.60 mmol/L). She experienced fatigue, anorexia, nausea and vomiting, an elevated blood pressure, hemoconcentration, leukocytosis, metabolic alkalosis, elevated body weight, and hypokalemia. [Ellenhorn, M.J., S. Schonwald, G. Ordog, J. Wasserberger. Ellenhorn's Medical Toxicology:

Diagnosis and Treatment of Human Poisoning. 2nd ed. Baltimore, MD: Williams and Wilkins, 1997., p. 759] **PEER REVIEWED**

/CASE REPORTS/ Severe hypercalcemia, a potentially life-threatening medical emergency, is rare in pregnancy ... A 32-year-old woman /presented/ early in the second trimester with severe hypercalcemia (total calcium 22 mg/dL), alkalosis, and acute renal insufficiency resulting from excessive ingestion of calcium carbonate-containing antacid for gastroesophageal reflux. The patient was treated with aggressive hydration and furosemide, and received 1 dose of intravenous etidronate, leading to short-term symptomatic hypocalcemia. To /the authors'/ knowledge, this is the third reported case of milk-alkali syndrome in pregnancy ... [Picolos MK et al; Obstet Gynecol 104(5 Pt 2):1201-4 (2004)] **PEER REVIEWED**

/CASE REPORTS/ ... The clinical and laboratory data in 3 patients who presented with severe hypercalcemia (corrected serum calcium ≥ 14 mg/dL) /are reported/ and ... the pertinent literature on milk-alkali syndrome /are reviewed/ ... The 3 patients had acute renal insufficiency, relative metabolic alkalosis, and low parathyroid hormone (PTH), PTH-related peptide, and 1,25-dihydroxyvitamin D concentrations. No malignant lesion was found. Treatment included aggressive hydration and varied amounts of furosemide. The 2 patients with the higher serum calcium concentrations received pamidronate intravenously (60 and 30 mg, respectively), which caused severe hypocalcemia. Of the 3 patients, 2 were ingesting acceptable doses of elemental calcium (1 g and 2 g daily, respectively) in the form of calcium carbonate. In addition to /the/ highlighted cases, /the authors/ review the history, classification, pathophysiologic features, and treatment of milk-alkali syndrome and summarize the cases reported from early 1995 to November 2003. [Picolos MK, Orlander PR; Endocr Pract 11(4):272-80 (2005)] **PEER REVIEWED**

/CASE REPORTS/ Development of vascular and systemic calcification is one of the more serious and often occurring complications in hemodialyzed patients. The cardiac valves calcifications are of the essential importance. They are result of the very complicated calcification process connected with hyperphosphatemia, hyperparathyroidism, calcium carbonate administering and active vitamin D3 metabolites as well as using dialysis fluids with high concentration of calcium. Elevated P and Ca x P product are both significant predictors of cardiovascular mortality in hemodialyzed patients. /The authors/ describe two patients with cardiac failure because of cardiac valves calcification. Both patients underwent artificial valves implantation. The patients showed a marked improvement of the quality of life and haemodynamic status. [Dabrowski J et al; Kardiol Pol 61(8):151-5 (2004)] **PEER REVIEWED**

/CASE REPORTS/ Eight continuous ambulatory peritoneal dialysis (CAPD) patients with calciphylaxis were identified in a 3-year period /and/ ... up to five controls for dialysis modality and length of time on dialysis /were matched/ with each case ... All the patients were female, versus only 38% (14/37) of controls ($P < 0.0001$). While not statistically significant, a majority of the patients were diabetic (62.5% (5/8) vs 32% (12/37)). Peak and average levels of serum calcium, phosphate, calcium x phosphate product, parathyroid hormone (PTH), albumin, iron, total iron-binding capacity (TIBC), and ferritin were not significantly different in cases compared with controls. The use of calcitriol alone or with calcium carbonate was not found to be a significant risk factor for the development of calciphylaxis. In a multivariate analysis, iron intake seemed to be protective, contrary to previous reports, while the use of calcium carbonate was associated with a strong trend to increased risk of calciphylaxis development (odds ratio = 1.029/g and 1.011/g calcium ingested per month, at 1 and 2 to 3 months prior to calciphylaxis development; $p = 0.0556$ and 0.0565 , respectively) ... These data, although limited by the small numbers of index cases, suggest that calcium ingestion is a risk factor for calciphylaxis. [Zacharias JM et al; Perit Dial Int 19(3):248-52 (1999)] **PEER REVIEWED

/CASE REPORTS/ Lactation results in dramatic bone resorption and decreased urinary calcium excretion due to rapidly falling estrogen levels and probably increased levels of parathyroid hormone-related protein ... A healthy, 36-year-old woman developed severe hypercalcemia several days after beginning breast-feeding her second child. During and after this pregnancy, she

supplemented a high calcium diet with moderate amounts of calcium carbonate in an attempt to avoid an osteoporotic fracture that occurred while she was breast-feeding her first child ... Because the metabolic changes that occur during lactation predispose a woman to hypercalcemia, the daily recommended daily allowances for calcium should not be exceeded during breast-feeding. [Caplan RH et al; J Reprod Med 49(3):214-7 (2004)] **PEER REVIEWED**

/EPIDEMIOLOGY STUDIES/ To identify potential associations between workplace exposures and cancer mortality risks, job titles collected from 1965 to 1971 for 58,678 men (a subset of a large representative sample of the Canadian workforce) were transformed into probable chemical exposures using a job-exposure matrix developed in Montreal. Mortality follow-up was determined through computerized record linkage with the National Mortality Database in Canada for 1965-1991. Cancer mortality risk was evaluated at two levels of exposure, any and substantial, using Poisson regression controlling for age, calendar period, and social class. Among the 58,678 men, 3,160 died of cancer. Using a liberal reporting criterion, relative risk (RR) >1.0, five or more exposed cancer deaths, $p < \text{or} = 0.100$, several potential associations were identified, including: lung cancer and any exposure to abrasives dust (RR = 2.84), prostate cancer and any exposure to calcium carbonate (RR = 2.46), and prostate cancer and substantial exposure to metallic dust (RR = 2.13). [Weston TL et al; Int J Occup Environ Health 6(3):194-202 (2000)] **PEER REVIEWED**

/OTHER TOXICITY INFORMATION/ About 15% of orally admin Ca^{+2} is absorbed, causing a transient hypercalcemia. Although not a problem in normal patients, the hypercalcemia from as little as 3 to 4 g per day can be problematic in patients with uremia ... /Antacids/ [Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1013] **PEER REVIEWED**

/OTHER TOXICITY INFORMATION/ / CaCO_3 has/ ... capacity to form insoluble cmpd ... The release of CO_2 from bicarbonate and carbonate-containing antacids can cause belching, occasional nausea, abdominal distention, and flatulence. Belching may cause exacerbation of gastroesophageal reflux. /Antacids/ [Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1013] **PEER REVIEWED**

Drug Warnings:

Large doses of calcium carbonate (above 2 g) increase gastric secretion for a period of time that considerably outlasts elevation of pH. ... With single doses below 2 g, this effect is negligible. [Osol, A. and J.E. Hoover, et al. (eds.). Remington's Pharmaceutical Sciences. 15th ed. Easton, Pennsylvania: Mack Publishing Co., 1975., p. 733] **PEER REVIEWED**

After ingestion /of CaCO_3 tablets/, it is converted to sol calcium salts in bowel, and calcium is thereby made available for absorption. Patients with achlorhydria may not solubilize calcium from ... preparation. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 1528] **PEER REVIEWED**

Gastric hypersecretory action is counter productive and may possibly account for various reports that calcium carbonate is less efficacious than other antacids. Calcium carbonate has been known to cause fecal concretions. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 993] **PEER REVIEWED**

Constipating effects and chalky taste of calcium carbonate are clinically disadvantageous. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 993] **PEER REVIEWED**

Admin of calcium carbonate promotes positive phosphate balance; plasma phosphate concn may or may not be incr, but hyperphosphatemia is usual finding in patients with milk-alkali syndrome.

[Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 992] **PEER REVIEWED**

When phosphate intake is low, hypophosphatemia may occur. Hypercalciuria and alkaluria predispose to nephrolithiasis. If no more than 160 mEq (8 g) a day is admin, nephrolithiasis and milk-alkali syndrome can be avoided. [Gilman, A. G., L. S. Goodman, and A. Gilman. (eds.). Goodman and Gilman's The Pharmacological Basis of Therapeutics. 6th ed. New York: Macmillan Publishing Co., Inc. 1980., p. 993] **PEER REVIEWED**

Hypercalcemia has been observed in patients who ingested large quantities of milk and absorbable alkali for several years for treatment of peptic ulcers without demonstrating increased levels in urine calcium. [Evaluations of Drug Interactions. 1st ed. and supplements. Washington, DC: American Pharmaceutical Assn., 1973, 1974., p. 37] **PEER REVIEWED**

Many physicians recommend that pregnant women receive multivitamin and mineral supplements, especially those pregnant women who do not consume an adequate diet and those in high-risk categories (ie, women carrying more than one fetus, heavy cigarette smokers, and alcohol and drug abusers). Taking excessive amounts of a multivitamin and mineral supplement may be harmful to the mother and/or fetus and should be avoided. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 742] **PEER REVIEWED**

Side/adverse effects may be more likely to occur if oral calcium supplements are taken in much larger doses than recommended (greater than 2000 to 2500 mg a day), if they are taken for a longer period of time, or if they are taken by patients with renal function impairment or milk-alkali syndrome. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 744] **PEER REVIEWED**

Side/Adverse Effects: Those indicating need for medical attention: Incidence more frequent: With parenteral dosage forms only: Hypotension (dizziness); flushing and/or sensation of warmth or heat; irregular heartbeat; nausea or vomiting; skin redness, rash, pain, or burning or injection site; sweating; tingling sensation. Note: Tingling sensation may result when intravenous injection is too rapid; skin redness, rash, pain, or burning may indicate extravasation and can precede sloughing or necrosis of skin. Incidence rare: Hypercalcemic syndrome, acute (drowsiness, continuing nausea and vomiting, weakness); renal calculi, calcific (difficult or painful urination) - with oral dosage forms. Early symptoms of hypercalcemia: Constipation, severe; dryness of mouth; headache, continuing; increased thirst; irritability; loss of appetite; mental depression; metallic taste; unusual tiredness or weakness. Late symptoms of hypercalcemia: Confusion; drowsiness; high blood pressure; increased sensitivity of eyes or skin to light, especially in hemodialysis patients; irregular, fast, or slow heartbeat; nausea and vomiting; unusually large amount of urine or increased frequency of urination. Note: In severe hypercalcemia, ECG changes consisting of shortened Q-T intervals are also seen. /Calcium supplements/ [Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006., p. 744] **PEER REVIEWED**

As taken from HSDB, 2014

"Patients with chronic kidney disease (CKD) are given calcium carbonate to bind dietary phosphorus, reduce phosphorus retention, and prevent negative calcium balance; however, data are limited on calcium and phosphorus balance during CKD to support this. Here, we studied eight patients with stage 3 or 4 CKD (mean estimated glomerular filtration rate 36 ml/min) who received a controlled diet with or without a calcium carbonate supplement (1500 mg/day calcium) during two 3-week balance periods in a randomized placebo-controlled cross-over design. All feces and urine were collected during weeks 2 and 3 of each balance period and fasting blood, and urine was collected at baseline and at the end of each week. Calcium kinetics were determined using oral and intravenous (45)calcium. Patients were found to be in neutral calcium and phosphorus balance

while on the placebo. Calcium carbonate supplementation produced positive calcium balance, did not affect phosphorus balance, and produced only a modest reduction in urine phosphorus excretion compared with placebo. Calcium kinetics demonstrated positive net bone balance but less than overall calcium balance, suggesting soft-tissue deposition. Fasting blood and urine biochemistries of calcium and phosphate homeostasis were unaffected by calcium carbonate. Thus, the positive calcium balance produced by calcium carbonate treatment within 3 weeks cautions against its use as a phosphate binder in patients with stage 3 or 4 CKD, if these findings can be extrapolated to long-term therapy.” As taken from Hill KM et al. 2013. Kidney Int. 83(5), 959-66. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23254903?dopt=AbstractPlus>

“Since the active substance is a commonly occurring natural substance no specific effects on endocrine system are expected. The active compound does not fulfil any of the criteria set in the chapter 3 of Guidance for the identification of endocrine disruptors of Regulations (EU) No 528/2012 and (EC) No 1107/2009. There are no toxic effects reported for the compound and the mechanism of action of the repellent is of a physical nature. No specific interaction with endocrine receptors and thus endocrine regulation can be anticipated as the active compound is a mineral that easily dissolves in water into ions. Furthermore, the compound is a common substance found in various matrices in the environment and is essential for many organisms. Similarly, in the literature search there were not found any adverse effects with regard of a risk assessment or potential endocrine activity of the active compound.

Similarly, in the literature search there were not found any adverse effects with regard of a risk assessment or potential endocrine activity of the active compound.”

As taken from European Commission, 2020

6. Functional effects on

6.1. Broncho/pulmonary system

“Some studies have used calcium carbonate to study physiological changes in dust retention, deposition... in the respiratory tract

“There are no reported health effects associated with repeated or prolonged exposure to pure calcium carbonate. If there is more than 1 % crystalline silica (quartz) present in the calcium carbonate, exposure to airborne concentrations may increase the risk of developing a disabling lung disease called silicosis”

As taken from IUCLID Dataset (2000), Calcium carbonate (471-34-1).

“The calcium carbonate dust inhalation (low toxicity particulate matter) can produce sensation of nasal obstruction and nasal dryness (AS B.6.9.4.3-04).”

As taken from European Commission, 2019a.

6.2. Cardiovascular system

“A SLIGHT-TO-MODERATE METABOLIC ALKALOSIS OCCURS DURING TREATMENT WITH CaCO_3 , BUT IT IS SLOW TO DEVELOP.”

“ADMIN OF CALCIUM CARBONATE PROMOTES POSITIVE PHOSPHATE BALANCE; PLASMA PHOSPHATE CONCEN MAY OR MAY NOT BE INCR, BUT HYPERPHOSPHATEMIA IS USUAL FINDING IN PATIENTS WITH MILK-ALKALI SYNDROME.”

As taken from HSDB, 2014

"Milk-alkali syndrome may be a common cause of unexplained hypercalcemia and can be precipitated by small amounts of orally ingested calcium carbonate in susceptible persons. Treatment with hydration, furosemide, and discontinuation of the calcium and vitamin D source is adequate. Pamidronate treatment is associated with considerable risk for hypocalcemia, even in cases of initially severe hypercalcemia." As taken from Picolos MK and Orlander PR Endocr Pract. 2005 Jul-Aug; 11(4):272-80 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16006300&query_hl=12&itool=pubmed_docsum

"This 2 year study shows that calcium carbonate use is continuously associated with progressive arterial calcification in haemodialysis patients. In addition, it suggests that it is also associated with decreased trabecular bone density. However, this latter finding requires confirmation by a study specifically devoted to this issue." As taken from Asmus HG et al. Nephrol Dial Transplant. 2005 Aug;20(8):1653-61 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15930018&query_hl=18&itool=pubmed_DocSum

/SIGNS AND SYMPTOMS/ ... /The authors/ examined the relationship between coronary risk factors; metabolic factors, including serum osteoprotegerin (OPG) concentration; and progression of vascular calcification in 26 dialysis patients ... Mean patient age was 52.6 + or - 8.7 (SD) years, and mean duration of dialysis therapy was 7.7 + or - 5.8 years. ACI was measured twice in each patient, and the mean interscan period was 4.9 + or - 0.3 years. Mean ACI changed from 22.2 + or - 24.2 to 33.9 + or - 28.8 overall, and mean change in ACI (DeltaACI) was 12.0 + or - 9.9. Patients were divided into 2 groups: slow progressors, with DeltaACI of 4.1 + or - 3.2 (n = 13), and rapid progressors, with DeltaACI of 19.8 + or - 7.9 (n = 13). Serum fasting glucose and CRP levels of rapid progressors were high, and their serum albumin and intact parathyroid hormone levels were low. Multiple regression analyses showed that serum OPG levels were independently associated with vascular calcification in the hemodialysis patients studied ... Rapid progression of vascular calcification was associated with dose of calcium carbonate prescribed and serum OPG concentration. The clinical significance of these observations remains to be determined. [Nitta K et al; Am J Kidney Dis 42(2):303-9 (2003)] **PEER REVIEWED**

"Bolland and co-workers have assessed the risk of myocardial infarction and cardiovascular events in individuals given regular calcium supplementation (without co-administered vitamin D) in the management of osteoporosis in a recent study, using a meta-analysis of 15 randomised clinical trials (Bolland et al., 2010). Only 15 of 190 trials originally identified met the pre-specified inclusion (randomised, double blind, placebo controlled trials; elemental calcium was administered at a dose of ≥ 500 mg/day; the participants' mean age at baseline was more than 40 years old; 100 or more participants were randomised; participants of either sex were studied; and the trial duration was more than one year) and exclusion (calcium and vitamin D given together with a placebo comparator; trials in which calcium was administered in the form of dietary modification or a complex nutritional supplement; and trials in which most participants had a major systemic disease other than osteoporosis) criteria. The calcium dose was generally greater than 1000 mg/person (2 studies used doses of 500 and 750 mg/person respectively and one had two dose levels of 600 and 1200 mg/person) whilst dietary calcium ranged from 406 to 1240 mg/day (generally below 1000 mg/day). Five of these trials used patient level data from 8151 participants and eleven used trial level data, involving 11 921 participants with a mean duration of 4 years. In the five studies contributing patient level data, 143 people given calcium supplementation had a myocardial infarction compared with 111 given placebo. These data provided a hazard ratio (HR) of 1.31 (95% confidence interval 1.02 to 1.67, $P=0.035$). Non-significant increases occurred in the incidence of stroke (HR 1.20, 0.96 to 1.50, $P=0.11$), the composite end point of myocardial infarction, stroke, or sudden death (HR 1.18, 1.00 to 1.39, $P=0.057$), and death (HR 1.09, 0.96 to 1.23, $P=0.18$). The meta-analysis of trial level data showed similar results: 296 people had a myocardial infarction (166 in individuals provided with calcium supplementation compared with 130 in individuals given placebo), with an increased incidence of myocardial infarction in the former group (pooled relative

risk 1.27, 95% confidence interval 1.01 to 1.59, $P=0.038$). Pre-specified subgroup analyses showed a significant interaction between treatment allocation and dietary calcium intake for myocardial infarction. There was an increased risk of myocardial infarction in people with dietary calcium intake above the median of 805 mg/day (hazard ratio 1.85, 95% confidence interval 1.28 to 2.67) but no increased risk in those with dietary calcium intake below the median (0.98, 0.69 to 1.38, P for interaction 0.01). The increased risk of myocardial infarction reported by Bolland and co-workers occurred early after calcium supplementation (median follow-up of 3.6 years). The authors concluded that calcium supplementation (without co-administered vitamin D) is associated with an increased risk of myocardial infarction, and noted that given the wide use of calcium supplements by the population, the small increases in risk of cardiovascular disease could translate into a large burden of disease in the population (Bolland et al., 2010)."

"The authors noted that, in contrast to their findings on supplemental calcium, observational studies do not show increased cardiovascular risks with higher dietary calcium intake (Bolland et al., 2010). Although not part of the meta-analysis, the authors have previously reviewed the mechanisms by which calcium supplements might increase the risk of myocardial infarction (Reid et al., 2010). As argued by the authors, calcium supplements acutely increase serum calcium levels to a modest degree, and serum calcium levels have been positively associated with an increased incidence of myocardial infarction in large observational studies. Primary hyperparathyroidism, a condition in which serum calcium levels are raised, has also been associated with an increased risk of cardiovascular events and death. Ingestion of equivalent doses of calcium from dairy products has a much smaller effect than calcium supplements on serum calcium levels, which might account for the absence of a detrimental vascular effect of dietary calcium intake in the observational studies reviewed."

"In a recent update of this study Bolland et al. reanalysed the data used in their earlier studies and unpublished data from two other studies, with the objective of investigating the effect of personal calcium supplement use on cardiovascular risk (Bolland et al., 2011). The authors had hypothesized that the personal use of calcium supplements by women in the trials evaluated in their earlier study might have obscured an adverse effect of calcium and vitamin D on cardiovascular risk. They concluded from this most recent metanalysis that women who were given regular calcium supplementation (with or without co-administered vitamin D) but who were not taking personal calcium supplements were still at increased risk of cardiovascular event, confirming their basic premise that calcium supplements used with or without vitamin D modestly increase cardiovascular event, confirming their basic premise that calcium supplements used with or without vitamin D modestly increase cardiovascular risk (Bolland et al., 2011)" (EFSA, 2011. EFSA Journal 9(7), 2318). <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

"A 5-year, randomised double-blinded placebo controlled trial (Calcium Intake Fracture Outcome Study, CAIFOS) showed no risk of atherosclerotic vascular disease (Lewis et al., 2010). The participants were 1460 women aged 75.1 +/- 2.7 years at baseline (1998), recruited from the general population and randomised to receive 1200 mg of calcium carbonate daily or an identical placebo. All hospital admission and deaths during the 5-year study and the 4.5-year follow-up were derived from the Western Australian Data Linkage Service (WADLS). Hazard ratios for the combined endpoint of atherosclerotic vascular mortality or first-hospitalisation were calculated using pre-specified intention-to-treat and per-protocol models. The intervention group that received calcium supplementation did not have a higher risk of death or first-time hospitalisation from atherosclerotic vascular disease in either the 5-year study (multivariate-adjusted HR 0.938 95% CI 0.690-1.275) or during the total 9.5 years of observational study (multivariate-adjusted HR 0.919 95% CI 0.737-1.146). Further analysis suggested calcium supplementation may reduce the risk of hospitalisation and mortality in patients with pre-existing atherosclerotic cardiovascular disease. The authors concluded that their study provided strong evidence that calcium supplementation of 1200 mg daily does not significantly increase the risk of atherosclerotic vascular disease in elderly women" (EFSA, 2011. EFSA Journal 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

"The Panel noted that although the meta-analysis carried out by Bolland et al. (2010) was a large and robust study with specified inclusion and exclusion criteria, the trends reported were very modest following supplementation with calcium in addition to dietary exposure, and are not supported by the findings of Lewis et al. (2010). The Panel also noted that the trends noted in cardiovascular risks following calcium supplementation contrasted with those found with dietary calcium, and that the authors considered that a plausible mode of action could be described to explain these differences, based on serum levels of calcium following dietary intake and supplementation (EFSA, 2011. EFSA Journal 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

"PURPOSE: To estimate the effects of calcium or vitamin D supplementation or a combination of both on blood pressure and serum lipid and carotenoid levels. METHODS: Ninety-two colorectal adenoma patients were randomized in a pilot, double-blind, placebo-controlled clinical trial of supplemental vitamin D3 800 IU and elemental calcium 2.0 g (as calcium carbonate) alone or in combination in divided doses twice daily with meals over 6 months. RESULTS: Relative to placebo, mean serum triglycerides decreased 30% ($P = .10$) and 32% ($P = .10$) in the calcium and calcium plus vitamin D3 treatment groups, respectively. When the two calcium intervention groups were pooled and compared with the pooled noncalcium groups, the estimated supplemental calcium treatment effects were statistically significant for triglycerides ($P = .04$). Similar but nonstatistically significant decreases (5%-7%) were observed for serum total cholesterol levels. Mean systolic blood pressure increased 6% ($P = .08$) in the calcium group; otherwise, there were no appreciable changes in systolic or diastolic blood pressures in any active treatment group. Mean serum total carotenoid levels decreased 14% ($P = .07$) in the calcium and 9% ($P = .10$) in the calcium plus vitamin D3 groups. CONCLUSIONS: Our results suggest that supplemental calcium alone or combined with vitamin D3 but not vitamin D3 alone may reduce serum lipids and lipophilic micronutrients." As taken from Chai W et al. 2013. Ann. Epidemiol. 23(9), 564-70. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23958407>

"SUMMARY: Calcium supplements have been associated with increased cardiovascular risk, but the mechanism is unknown. We investigated the effects of calcium supplements on the propensity of serum to calcify, based on the transition time of primary to secondary calciprotein particles (T50). Changes in serum calcium were related to changes in T50. INTRODUCTION: Calcium supplements have been associated with increased cardiovascular risk; however, it is unknown whether this is related to an increase in vascular calcification. METHODS: We investigated the acute and 3-month effects of calcium supplements on the propensity of serum to calcify, based on the transition time of primary to secondary calciprotein particles (T50), and on three possible regulators of calcification: fetuin-A, pyrophosphate and fibroblast growth factor-23 (FGF23). We randomized 41 postmenopausal women to 1 g/day of calcium as carbonate, or to a placebo containing no calcium. Measurements were performed at baseline and then 4 and 8 h after their first dose, and after 3 months of supplementation. Fetuin-A, pyrophosphate and FGF23 were measured in the first 10 participants allocated to calcium carbonate and placebo who completed the study. RESULTS: T50 declined in both groups, the changes tending to be greater in the calcium group. Pyrophosphate declined from baseline in the placebo group at 4 h and was different from the calcium group at this time point ($p = 0.04$). There were no other significant between-groups differences. The changes in serum total calcium from baseline were significantly related to changes in T50 at 4 h ($r = -0.32$, $p = 0.05$) and 8 h ($r = -0.39$, $p = 0.01$), to fetuin-A at 3 months ($r = 0.57$, $p = 0.01$) and to pyrophosphate at 4 h ($r = 0.61$, $p = 0.02$). CONCLUSIONS: These correlative findings suggest that serum calcium concentrations modulate the propensity of serum to calcify (T50), and possibly produce counter-regulatory changes in pyrophosphate and fetuin-A. This provides a possible mechanism by which calcium supplements might influence vascular calcification." As taken from Bristow SM et al. 2016. Osteoporos. Int. 27(3), 1209-16. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/26493812>

“INTRODUCTION: Cardiovascular disease is the major cause of mortality in developed countries and has an increasing trend in developing countries. There are some evidences that calcium supplementation may decrease blood pressure and consequently cardiovascular disease, but they are not conclusive and there is no agreement in this respect. The aim of the present study was to assess the effect of supplementary calcium on systolic and diastolic blood pressure in healthy adult women aged 18-30 years. **MATERIALS AND METHODS:** Seventy-five normotensive volunteers were randomly divided into two groups, the treatment group received 1000 mg/day calcium (four doses of 625 mg calcium carbonate) for 1 month and the control group received placebo (dextrose). Systolic and diastolic blood pressure was determined before and after intervention in supine position after 10 min of rest. **RESULTS:** The mean daily calcium intake from food was 773.9 mg in treatment and 721 mg in control group (no significant difference) but in both the groups dietary calcium intake was less than the recommended dietary allowance: After calcium supplementation, the mean change of systolic blood pressure was not significant in the two groups, but diastolic blood pressure reduced in treatment group and increased in control group (-4.9 vs 2.6 mmHg) ($P<0.05$). **CONCLUSIONS:** These results suggest that, calcium supplementation does not have any effect on systolic blood pressure of our volunteers but can decrease diastolic blood pressure significantly and therefore it seems that calcium supplementation may be useful for people with increased diastolic blood pressure, especially for those who receive less calcium than recommended dietary allowance.” As taken from Entezari MH. 2015. J. Educ. Health Promot. 4, 67. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26430694>

“BACKGROUND: Vascular calcification contributes to cardiovascular disease in hemodialysis (HD) patients with diabetes. The randomized controlled trial reported here compared the effects of lanthanum carbonate (LC) and calcium carbonate (CC) on vascular stiffness assessed using brachial-ankle pulse wave velocity (ba-PWV), intima-media thickness (IMT), bone mineral density (BMD), and serum markers of chronic kidney disease - mineral and bone disorder in such patients. **METHODS:** Ba-PWV, IMT, BMD, and the biomarkers osteocalcin (OC) and bone alkaline phosphatase (BAP) were examined in 43 type 2 diabetes HD patients treated with LC ($n=21$) or CC ($n=22$) for 2 years. **RESULTS:** Forty-one patients completed the study (19, LC; 22, CC). The mean ba-PWV significantly increased only in the CC group (median: 2,280.5 to 2,402.5 cm/s, $P<0.05$), after 24-month treatment; it remained unchanged in the LC group (median: 1,830.5 to 2,018.3 cm/s). However, the difference between the groups did not reach statistical significance. Changes in IMT and BMD were not different between the two groups. Changes in serum phosphorus, corrected calcium, and intact parathyroid hormone levels were similar between the groups. The incidence of fracture was 0% (0/19) in the LC group, and 13.6% (3/22) in the CC group ($P=0.2478$). The OC/BAP ratio increased significantly in the LC group (median: 0.83 to 2.47), compared with in the CC group (median: 0.77 to 1.40) ($P=0.036$). **CONCLUSION:** From this study, in Japanese type 2 diabetes HD patients, we conclude that 2-year treatment with LC might have slowed the progression of ba-PWV; however, it did not cause a difference in ba-PWV, IMT, BMD, or fracture, compared with CC. Further, LC increased the OC/BAP ratio to a greater extent than CC.” As taken from Wada K et al. 2015. Int. J. Nephrol. Renovasc. Dis. 8, 111-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26346335>

“BACKGROUND: The present study was designed to examine the effects of vitamin D plus calcium administration on metabolic profiles and pregnancy outcomes among women at risk for pre-eclampsia. **METHODS:** In a prospective, double-blind, placebo-controlled trial, 60 women at risk for pre-eclampsia were randomised to take either 50 000 IU vitamin D3 every 2 weeks plus 1000 mg day(-1) calcium supplements (as calcium carbonate) ($n = 30$) or to receive placebos at the same times ($n = 30$) from 20 to 32 weeks of gestation. Fasting blood samples were taken at baseline and 12 weeks after intervention to determine related variables. Newborn anthropometric measurements were determined. **RESULTS:** Taking combined cholecalciferol and calcium supplements, compared to placebo, led to significant reductions in fasting plasma glucose (FPG) [mean (SD)] [-5.7 (5.5) versus -0.6 (12.6) mg dL(-1) , $P = 0.04$], serum insulin concentrations [-2.8 (6.0) versus +7.7 (9.8) μ IU mL(-1) , $P<0.001$], homeostasis model of assessment-insulin resistance [-0.8 (1.3) versus +1.6

(2.2), $P < 0.001$], homeostatic model assessment-beta cell function $[-8.2 (25.8)$ versus $+32.6 (41.3)$, $P < 0.001$] and a significant rise in quantitative insulin sensitivity check index score $[+0.02 (0.02)$ versus $-0.02 (0.02)$, $P < 0.001$]. Additionally, pregnant women who received cholecalciferol plus calcium supplements had increased serum high-density lipoprotein (HDL)-cholesterol $[+4.6 (8.3)$ versus $-2.9 (7.7)$ mg dL⁻¹], $P = 0.001$] and plasma total glutathione (GSH) concentrations $[+23.4 (124.0)$ versus $-94.8 (130.2)$ μ m, $P = 0.001$] compared to placebo. However, after adjustment for the baseline levels, maternal age and baseline body mass index, the effects on FPG levels ($P = 0.13$) and systolic blood pressure ($P = 0.13$) disappeared. **CONCLUSIONS:** Vitamin D plus calcium administration for 12 weeks had beneficial effects on glycaemic status, HDL-cholesterol, GSH and blood pressure among women at risk for pre-eclampsia." As taken from Samimi M et al. 2016. J. Hum. Nutr. Diet. 29(4), 505-15. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/26467311>

"BACKGROUND: High levels of serum phosphorus both at baseline and during follow-up are associated with increased mortality in dialysis patients, and administration of phosphate binders was independently associated with improved survival among hemodialysis population. Calcium-based phosphate binders are the most commonly used phosphate binders in developing countries for their relatively low costs. **OBJECTIVES:** To compare the efficacy and safety between calcium carbonate and calcium acetate in the treatment of hyperphosphatemia in hemodialysis patients. **METHODS:** PubMed, EMBASE, Cochrane Library, Google scholar and Chinese databases (Wanfang, Weipu, National Knowledge Infrastructure of China) were searched for relevant studies published before March 2014. Reference lists of nephrology textbooks and review articles were checked. A meta-analysis of randomized controlled trials (RCTs) and quasi-RCTs that assessed the effects and adverse events of calcium acetate and calcium carbonate in adult patients with MHD was performed using Review Manager 5.0. **RESULTS:** A total of ten studies (625 participants) were included in this meta-analysis. There was insufficient data in all-cause mortality and cardiovascular events for meta-analysis. Compared with calcium carbonate group, the serum phosphorus was significantly lower in calcium acetate group after 4 weeks' administration (MD -0.15 mmol/L, 95% CI -0.28 to -0.01) and after 8 weeks' administration (MD -0.25 mmol/L, 95% CI -0.40 to -0.11). There was no difference in serum calcium levels or the incidence of hypercalcemia between two groups at 4 weeks and 8 weeks. No statistical difference was found in parathyroid hormone (PTH) levels or serum calcium by phosphorus (Ca x P) product. There was significantly higher risk of intolerance with calcium acetate treatment (RR 3.46, 95% CI 1.48 to 8.26). **CONCLUSIONS:** For hyperphosphatemia treatment, calcium acetate showed better efficacy and with a higher incidence of intolerance compared with calcium carbonate. There are insufficient data to establish the comparative superiority of the two calcium-based phosphate binders on all-cause mortality and cardiovascular end-points in hemodialysis patients." As taken from Wang Y et al. 2015. PLoS One 10(3), e0121376. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25799184>

"OBJECTIVE: To analyze the treatment effect of calcium+vitamin D supplementation, hormone therapy, both, and neither on cardiovascular disease risk factors. **METHODS:** We conducted a prospective, randomized, double-blind, placebo-controlled trial among Women's Health Initiative (WHI) participants. The predefined primary outcome was low-density lipoprotein cholesterol (LDL-C). **RESULTS:** Between September 1993 and October 1998, a total of 68,132 women aged 50-79 years were recruited and randomized to the WHI-Dietary Modification ($n=48,835$) and WHI-Hormone Therapy trials ($n=27,347$). Subsequently, 36,282 women from WHI-Hormone Therapy (16,089) and WHI-Dietary Modification ($n=25,210$) trials were randomized in the WHI-Calcium+Vitamin D trial to 1,000 mg elemental calcium carbonate plus 400 international units vitamin D3 daily or placebo. Our study group included 1,521 women who participated in both the hormone therapy and calcium+vitamin D trials and were in the 6% subsample of trial participants with blood sample collections at baseline and years 1, 3, and 6. The average treatment effect with 95% confidence interval, for LDL-C, compared with placebo, was -1.6 , (95% confidence interval [CI] -5.5 to 2.2) mg/dL for calcium+vitamin D alone, -9.0 (95% CI -13.0 to -5.1) mg/dL for hormone

therapy alone, and -13.8 (95% CI -17.8 to -9.8) mg/dL for the combination. There was no evidence of a synergistic effect of calcium+vitamin D+hormone therapy on LDL-C (P value for interaction=.26) except in those with low total intakes of vitamin D, for whom there was a significant synergistic effect on LDL (P value for interaction=.03). CONCLUSION: Reductions in LDL-C were greater among women randomized to both calcium+vitamin D and hormone therapy than for those randomized to either intervention alone or to placebo. The treatment effect observed in the calcium+vitamin D+hormone therapy combination group may be additive rather than synergistic. For clinicians and patients deciding to begin calcium+vitamin D supplementation, current use of hormone therapy should not influence that decision.” As taken from Schnatz PF et al. 2017. Obstet. Gynecol. 129(1), 121-129. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27926633>

“Recent studies suggest that supplemental Ca (SC) increases the risk of cardiovascular events, whereas dietary Ca (DC) decreases the risk of cardiovascular events. Although frequently consumed with meals, it remains unclear whether Ca can mitigate or aggravate the deleterious effects of a high-fat meal on cardiovascular risk factors. This study aimed to evaluate the effects of SC or DC on blood pressure (BP) and microvascular function (MVF) in the postprandial period in obese women challenged with a high-fat meal. In this cross-over controlled trial, sixteen obese women aged 20-50 years were randomly assigned to receive three test meals (2908 kJ (695 kcal); 48 % fat): high DC (HDCM; 547 mg DC), high SC (HSCM; 500 mg SC-calcium carbonate) and low Ca (LCM; 42 mg DC). BP was continuously evaluated from 15 min before to 120 min after meals by digital photoplethysmography. Before and 120 min after meals, participants underwent evaluation of serum Ca and microvascular flow after postocclusive reactive hyperaemia (PORH) by laser speckle contrast imaging. Ionised serum Ca rose significantly only after HSCM. Systolic BP increased after the three meals, whereas diastolic BP increased after LCM and HDCM. Hyperaemia peak, hyperaemia amplitude and AUC evaluated after PORH decreased with LCM. After HDCM, there was a reduction in hyperaemia peak and hyperaemia amplitude, whereas HSCM decreased only hyperaemia peak. However, comparative analyses of the effects of three test meals on serum Ca, BP and MVF revealed no significant meal×time interaction. This study suggests that in obese women SC and DC do not interfere with the effects of a high-fat meal on BP and MVF.” As taken from Ferreira TD et al. 2016. Br. J. Nutr. 116(9), 1564-1572. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27774917>

“May cause hypotension.”

As taken from BPDB, 2022.

“Although there are data that suggest calcium carbonate supplementation may increase the vascular calcification, these findings have not been confirmed (AS B.6.9.4.2-02).”

“In addition, the calcium carbonate consumption can reduce the overall blood pressure in normotensive men (AS B.6.9.4.3-02)”.

As taken from European Commission, 2019a.

6.3. *Nervous system*

NEUROLOGIC

ACUTE EXPOSURE

Chronic ingestion of calcium carbonate may cause irritability, lethargy, stupor, and coma, depending on the amount and duration of ingestion.

As taken from HSDB, 2014

"In the absence of indications of potential neurotoxic effects in the available studies and considering the history of safe use and the chemical structure of the substance, it has been agreed that calcium carbonate has no potential for neurotoxicity."

As taken from EFSA, 2021

"Several different types of exposure have the potential to produce olfactory and gustatory deficits related to neurotoxicity. Although the literature contains relatively few studies of such chemoreceptive dysfunction in the context of toxic exposure, this review explores the strength of such published associations. Several studies collectively demonstrated moderately strong evidence for an association between manganese dust exposure and olfactory deficits. Evidence of associations between individual chemicals, therapeutics, and composites, such as World Trade Center debris, and olfactory and gustatory deficits remains limited or mixed. Further need for controlled studies for clinical management, exposure limits, and policy development is identified."

As taken from Aulisio MC et al. 2020. *Neurol. Clin.* 38(4), 965-981. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33040872/>

"The cerebellum plays an important role in motor and nonmotor systems, with damage resulting in clinical manifestations presenting as weakness, ataxia, dysarthria, and nystagmus. There are numerous environmental and industrial agents as well as medications that, through either accidental or intentional use, can result in a range of neurologic presentations. The variability in the presentation is important to recognize promptly so that early cessation in exposure, use, or abuse can be initiated to reduce the severity of symptoms. Recognition of an agent causing the particular pathology is important so that the route of exposure, and subsequent treatment options can be identified." As taken from Dolbec K et al. 2020. *Neurol. Clin.* 38(4), 843-852. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33040864/>

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

"The only notable treatment-related effect seen in a 91-day feeding study designed to compare the effects of calcium carbonate to those of calcium citrate malate in rats at dietary calcium levels equivalent to 250 or 500 mg calcium/kg bw/day was nephrocalcinosis, seen in both males and (more marked) in females (EFSA, 2007). These dietary doses correspond to approximately 625 or 1250 mg calcium carbonate/kg bw/day. The Panel considered however that the nephrocalcinosis finding in rats is not relevant for human safety assessment because the rat is a species known to be particularly sensitive to mineralisation of the renal tubule epithelium due to dietary alteration of the calcium and phosphorus homeostasis (Ritskes-Hoitinga et al., 1989, 1991, 1992). Results from a 91-day toxicity study in Beagle dogs designed to compare the effects of calcium carbonate to those of calcium citrate malate at equivalent calcium levels in the diet as in the rat study reported above (providing intakes of approximately 250 and 500 mg calcium/kg bw/day) did not report any signs of nephrocalcinosis or other evidence of toxicity (EFSA, 2007)" (EFSA, 2011. *EFSA Journal* 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

A) Chronic administration (4 to 60 g/day calcium carbonate for 2 to 60 days) has produced the "milk-alkali" syndrome. Chronic renal failure and hemodialysis may predispose a patient.

B) Normal total serum calcium levels are 9 to 10.4 mg/dL (4.5 to 5.2 mEq/L). Symptoms may appear when plasma calcium reaches 6.6 mEq/L.

C) An estimated dose of 11 mEq of elemental calcium intravenously or 1.486 to 6.11 grams of calcium carbonate (21 to 87 mg/kg) orally might be expected to produce a peak serum calcium level of 2 mEq/L above baseline in an adult.

[Rumack BH POISINDEX(R) Information System Micromedex, Inc., Englewood, CO, 2009; CCIS Volume 142, edition expires Nov, 2009. Hall AH & Rumack BH (Eds): TOMES(R) Information

“DISTURBANCES RESULTING FROM LIBERATION OF CO₂ ARE NOT SERIOUS PROBLEM, ALTHOUGH BELCHING OCCURS IN SOME INDIVIDUALS. NAUSEA IS ALSO OCCASIONAL COMPLAINT. ...POSSIBLE CONSTIPATING EFFECT.”

“GASTRIC HYPERSECRETORY ACTION IS COUNTER PRODUCTIVE & MAY POSSIBLY ACCOUNT FOR VARIOUS REPORTS THAT CALCIUM CARBONATE IS LESS EFFICACIOUS THAN OTHER ANTACIDS. CALCIUM CARBONATE HAS BEEN KNOWN TO CAUSE FECAL CONCRETIONS.”

“CONSTIPATING EFFECTS AND CHALKY TASTE OF CALCIUM CARBONATE ARE CLINICALLY DISADVANTAGEOUS.”

As taken from HSDB, 2014

“The Women's Health Initiative (WHI) randomized clinical trial (RCT) of calcium plus vitamin D (CaD) supplements found a 17% excess in urinary tract stone incidence in the supplemented group. This study evaluated whether this risk is modified by participant characteristics. We examined the correlates of urinary tract stone occurrence in the CaD arm of the WHI trial. We analyzed an RCT involving 36,282 postmenopausal women aged 50-79 y from 40 WHI centers: 18,176 women received 500 mg calcium carbonate plus 200 IU vitamin D(3) twice daily (1000 mg and 400 IU daily, respectively), and 18,106 women received a matching placebo for an average of 7.0 y. The incidence of urinary tract stones was determined. The incidence of self-reported clinically diagnosed urinary tract stones was more common in the active CaD medication group than in the placebo group (hazard ratio: 1.17; 95% CI: 1.02, 1.34): 449 women in the CaD group and 381 women in the placebo group reported a stone during the trial. The rates of self-reported stones did not differ between various demographic, anthropomorphic, dietary, and other hypothesized risk factors according to randomization assignment. Neither the total calcium intake nor the use of calcium supplements at baseline was associated with the risk of stones. In sensitivity analyses that censored participants who were below 80% adherence, the findings were similar. Daily supplementation with CaD for 7 y was associated with an increase in the number of self-reported urinary tract stones. These findings have implications for CaD supplement use” (Wallace et al., 2011. American Journal of Clinical Nutrition 94, 270-271). As taken from <http://www.ncbi.nlm.nih.gov/pubmed/21525191>

“The Panel noted that hypercalcaemia and alkalosis can occur in humans taking calcium carbonate with large amounts of milk or cream for the treatment of peptic ulcer (milk-alkali syndrome), often associated with renal dysfunction, metastatic calcification and other symptoms. Similar changes have been reported in individuals taking large amounts of calcium carbonate and other calcium-containing antacids or large amounts of calcium food supplements. The SCF (2003) reported that one third of the cases had consumed both alkali and calcium (between 2.0 and 16.5 g/day of supplementary calcium), while one third developed symptoms as a result of high calcium carbonate intakes alone (between 2 to 10.8 g additional calcium per day from several months to 30 years)” (EFSA, 2011. EFSA Journal 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

“Milk-alkali syndrome is a rare cause of hypercalcemia characterized by the triad of hypercalcemia, renal insufficiency, and metabolic alkalosis that results from the overconsumption of calcium containing products. In the setting of pregnancy where there is a physiologic increase in calcium absorption, milk-alkali syndrome can be potentially life threatening. We report a case of a 26-year-old woman in her second trimester of pregnancy who presented with 2 weeks of flank pain, nausea, vomiting, anorexia, headache, and lightheadedness. The history revealed consumption of a large quantity of milk, calcium carbonate antacid, and calcium-containing prenatal vitamins. Her symptoms and hypercalcemia resolved with intravenous fluids and a loop diuretic. With the

increased use of calcium carbonate for peptic ulcer disease, gastroesophageal reflux disease, and osteoporosis, milk-alkali syndrome has experienced a resurgence and must be considered in the differential diagnosis of hypercalcemia. In this clinical vignette we review the literature on milk-alkali syndrome in pregnancy and discuss important diagnostic and therapeutic considerations when managing the pregnant patient with hypercalcemia" (Kolnick et al. 2011. Journal of General Internal Medicine 26, 939-942). As taken from <http://www.ncbi.nlm.nih.gov/pubmed/21347876>

"A 31 year old man presented with a four day history of anxiety, insomnia, and confusion. Over the past few months he had intermittently noticed red eyes and a "tummy ache." On examination, he was agitated, tachycardic (heart rate 110 beats/min), and he had bilateral conjunctivitis. Initial investigations showed a calcium concentration of 3.54 mmol/L (reference range 2.15-2.6) and metabolic alkalosis. He was treated for acute hypercalcaemia with fluids and discharged when levels normalised." [H]e was readmitted two weeks later with confusion and a witnessed seizure. Investigations again showed a raised calcium (3.80 mmol/L), metabolic alkalosis, and new onset acute renal failure. Plasma glucose was normal. He was once again treated for acute hypercalcaemia and stabilised. On day three of admission, calcium carbonate tablets were discovered on his bedside table. He reported regularly ingesting up to three packets a day to treat symptoms of indigestion." (Ali et al. 2012. British Medical Journal 345:e4443). As taken from <http://dx.doi.org/10.1136/bmj.e4443>

Calcium-alkali-syndrome "has been increasingly observed with high intakes of supplemental calcium carbonate. On the basis of 14 cases (2004-2010) reporting elevated serum calcium (2.64-6.43 mmol/L) and creatinine concentrations in males and females (35-81 years) consuming calcium carbonate at doses >1,000-44,000 mg/day, the IoM [Institute of Medicine] (2011) concluded that supplemental intakes of calcium carbonate $\geq 3,000$ mg/day were hazardous. Duration of supplementation was only reported in seven cases (from weeks to 19 years)" (EFSA, 2012. EFSA Journal 2012;10(7):2814. As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2814/epdf>).

"To summarise clinical support for the anti-caries efficacy of fluoride toothpastes containing sodium monofluorophosphate (SMFP) and to discuss the possible means by which the abrasive particles in calcium carbonate-based SMFP toothpastes might complement and/or enhance fluoride efficacy. The anti-caries efficacy of fluoride has been proven beyond any reasonable doubt, and the efficacy of SMFP, when incorporated into a variety of compatible toothpaste formulations, has been established in numerous clinical trials. Calcium carbonate-based toothpastes may also influence caries by effecting an increase in plaque calcium levels; an inverse relationship between plaque calcium and caries is well-established. It has also been reported that plaque fluoride levels are dependent on plaque calcium levels. Hence elevated plaque calcium resulting from the use of calcium carbonate-based toothpastes has the potential to elevate plaque fluoride, itself linked to reduced caries experience. It has been shown that calcium carbonate particles are retained by plaque and this may also influence caries by neutralising harmful plaque acids and concurrently liberating calcium. Fluoride delivered from calcium carbonate-based SMFP toothpastes is an effective means of reducing caries. Further, calcium carbonate may confer additional benefits through elevation of oral calcium levels and neutralisation of plaque-acids." As taken from Lynch RJ et al. Int Dent J. 2005; 55(3 Suppl 1):175-8 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&listuids=16004249&query=hl=3&itool=pubmed_docsum

"Renal osteodystrophy is a common complication of end-stage renal disease (ESRD) and is a major cause of morbidity in patients with ESRD. High serum levels of phosphorus, calcium and parathyroid hormone are associated with the development of this disease. The effects on bone of treatment with lanthanum carbonate, a new phosphate binder, and calcium carbonate were assessed in patients with ESRD. This was an open-label, multicenter, parallel-group study. Patients were recruited within 12 weeks of commencing dialysis. Following screening, phosphate binder

administration was stopped, tetracycline labeling administered and a transiliac bone biopsy taken. After randomization to lanthanum carbonate or calcium carbonate, patients were titrated to an optimum dose for 8 weeks and maintained at this dose for 44 weeks. The bone was then labeled and a second biopsy taken. Biopsy samples were analyzed histomorphometrically. Paired bone biopsies from 33 lanthanum carbonate- and 30 calcium carbonate-treated patients were suitable for analysis. None of the patients on either treatment developed osteomalacia. Assessment of activation frequency changes showed that 41% of biopsies from lanthanum carbonate-treated patients moved towards normal (observed values at the follow-up biopsy were closer to expected values than were the baseline values, so patients were considered to be improved) compared with 23% of calcium carbonate-treated patients ($p = 0.15$). This study indicates that there was no evidence of aluminum-like toxicity with lanthanum carbonate after 1 year of treatment in ESRD patients commencing dialysis, and there appeared to be a beneficial effect on bone-cell function and activity compared with calcium carbonate." As taken from Freemont AJ et al. Clin Nephrol. 2005 Dec; 64(6):428-37 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&listuids=16370155&query_hl=5&itool=pubmed_docsum

"The studies described in this paper aimed to assess the stain removal efficacy, fluoride efficacy and abrasivity to enamel and dentine of a new whitening toothpaste containing calcium carbonate and perlite, using appropriate in vitro models. Stain removal efficacy was assessed using the pellicle cleaning ratio (PCR) method. Fluoride efficacy was assessed using remineralisation, demineralisation and fluoride-uptake methods. Abrasivity was assessed using an enamel and dentine wear method. The results showed that the new whitening toothpaste was able to remove extrinsic tooth stain more effectively than three commercially available toothpaste formulations. The fluoride efficacy was superior to a non-fluoridated control and was not significantly different to a clinically tested fluoride-containing toothpaste. The abrasivity data showed that the calcium carbonate/perlite toothpaste is no more abrasive to enamel or dentine than two other commercially available whitening toothpastes. The studies show that the new whitening toothpaste is effective in extrinsic stain removal, has an efficacious fluoride source and does not have an undue degree of abrasivity to enamel or dentine compared to other relevant commercially available products." As taken from Pickles MJ et al. Int Dent J. 2005; 55(3 Suppl 1):197-202 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&listuids=16004254&query_hl=16&itool=pubmed_docsum

"Awad and co-workers examined biochemical changes in the apical membranes of the colon as a result of feeding excess calcium to rats maintained on a saturated fatty acid-rich diet. Weanling rats ($n=12$ per group) were fed a semi-synthetic diet (high fat content plus basal calcium content of 0.5%) and excess calcium (1% calcium carbonate, equivalent to 1000 mg/kg bw/day) for 4 weeks. Animals fed the high calcium diet weighed significantly more than the controls after 4 weeks of feeding. By the end of the 4th week on the diet, significant increases in serum calcium were observed, but there were no clinical signs of toxicity. Excess dietary calcium intake resulted in a 54% reduction in faecal water bile acids and a 44% reduction in faecal water free fatty acids. The calcium-supplemented diet had no effect on either the lipid content or the fatty acid composition of the colonic apical membranes, but protein composition was altered. The authors suggested that the observed alterations in protein patterns of these membranes may be due to either the reduction of faecal water bile acids and free fatty acids or may be a direct effect of dietary calcium on membrane protein (Awad et al., 1990)" (EFSA, 2011. EFSA Journal 9(7), 2318). As taken from <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2318/epdf>

"BACKGROUND AND AIMS: *Helicobacter pylori* (Hp) infection causes gastritis and is considered a gastric cancer risk factor. We have previously reported that codfish meal markedly enhanced Hp-induced gastritis in Mongolian gerbils. In the present study, we sought the responsible components in codfish meal. MATERIALS AND METHODS: Codfish were divided into three parts (meat, viscera and 'other parts', including bone), and administered to Hp-infected gerbils. Subsequently, cod bone,

sardine bone and prawn shell were tested, along with major calcium components, hydroxyapatite and calcium carbonate, in bone and shell, respectively. RESULTS: 'Other parts' and cod bone enhanced Hp-induced gastritis, as was observed for whole codfish. Similarly, sardine bone and prawn shell, as well as 0.22-0.88% hydroxyapatite and calcium carbonate, enhanced gastritis. In contrast, administration of a higher dose of the calcium compounds exerted protective effects. CONCLUSION: Intake of calcium compounds may contribute to enhancement of Hp-induced gastritis." As taken from Iimuro M et al. 2013. *Anticancer Res.* 33(9), 3667-74. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24023294>

"AIM: To determine the efficacy of a desensitizing regimen compared to a control in preventing the occurrence and/or alleviating dentin/root sensitivity (DRS) following non-surgical (NSPT) and surgical periodontal treatment (SPT). METHODS: Seventy-four chronic-periodontitis patients (CPP) were randomized into a test group (n = 38) using an in-office prophylaxis paste and a toothpaste at home both containing 8% arginine and calcium carbonate (Pro-ArginTM) Technology) or into a control group (n = 36) receiving a fluoride-free prophylaxis paste and a fluoride toothpaste. The examiner applied the assigned paste onto selected teeth for 3 s following NSPT and for 60 s before flap closure. Patients brushed with the assigned toothpaste twice daily throughout the study. DRS to air stimulus was assessed by the Schiff scale (0-3) and the Visual Analog Scale (VAS: 0-100 mm) six times over 17 weeks. RESULTS: In the test group, VAS scores significantly decreased at 8, 11 and 17 weeks from baseline ($p \leq 0.003$) and Schiff scores at 8 and 11 weeks from baseline ($p \leq 0.014$). The control group exhibited significant increases in VAS and Schiff during the study period ($p \leq 0.006$). Marked inter-group differences were noted at all time points ($P < 0.001$). CONCLUSIONS: The combined use of desensitizing products (8% arginine and calcium carbonate) in-office and at-home prevented DRS development and maintained this effect for 17 weeks following NSPT and SPT." As taken from Giassin PN et al. 2016. *J. Clin. Periodontol.* 43(2), 138-46. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/26660591>

"OBJECTIVE: To report a case of acute pancreatitis associated with the administration of high doses of over-the-counter (OTC) antacids containing calcium carbonate. SETTING: An acute care 276-bed community hospital. CASE SUMMARY: In this case report, a 68-year-old male was admitted to the hospital for nausea, vomiting, and stomach pain. Patient reported taking 15-20 OTC calcium carbonate (4.5-6.0 g elemental calcium) tablets for four to five days prior to admission. Patient's calcium level upon admission was 18.5 mg/dL. Laboratory values of amylase and lipase peaked at 2,768 U/L and 7,091 U/L, respectively. An abdominal computed tomography confirmed acute pancreatitis. For a period of 45 days, treatment in the intensive care unit for acute pancreatitis and latter for necrotizing pancreatitis was conducted. CONCLUSION: This case study supports an association between hypercalcemia and pancreatitis in patients who take greater than recommended dosages of calcium-containing antacids. OTC antacids are popular, but can cause serious adverse events when not used as directed." As taken from Nykamp D & Kraus EJ. 2013. *Consult. Pharm.* 28(4), 247-51. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/23552706?dopt=AbstractPlus>

"OBJECTIVE: To evaluate the efficacy and safety of calcitriol in the prevention and treatment of glucocorticoid-induced osteoporosis. METHODS: 66 patients treated with glucocorticoids (GC) for primary nephrotic syndrome (NS) were randomly assigned to 3 groups. Groups were designated as follows: calcitriol alone (n = 22), calcitriol plus calcium carbonate (n = 23), or calcium carbonate alone (n = 21). Serum markers of bone metabolism and bone mineral density (BMD) were tested at 3 different time points: the initiation of GC treatment (baseline), 12 weeks, and 24 weeks after the initiation of treatment. RESULTS: Levels of serum 25-hydroxy vitamin D, serum osteocalcin, and total serum collagen type N-terminal extension of the peptide were significantly decreased following GC therapy ($P < 0.05$). β -collagen serum-specific sequences were significantly increased following GC therapy. The above-mentioned changes were less dramatic in patients treated with calcitriol, although the differences were significant ($P < 0.05$). Changes in serum levels of calcium,

phosphorus, alkaline phosphatase, and parathyroid hormone (PTH) were not significant. 24 weeks after the initiation of treatment, BMD of the lumbar spine and femoral bone significantly decreased in all of 3 groups. However, patients who received calcitriol had significantly higher BMD of the lumbar spine than patients who received calcium carbonate alone (calcitriol plus calcium carbonate vs. calcium carbonate alone: 0.82 ± 0.19 g/cm² vs. 0.62 ± 0.23 g/cm² $P < 0.05$; calcitriol vs. calcium carbonate alone 0.805 ± 0.203 g/cm² vs. 0.615 ± 0.225 g/cm² $P < 0.05$), respectively. No serious adverse events were observed. CONCLUSION: Calcitriol may be more effective than calcium carbonate in preventing and treating GC-induced osteoporosis in patients with NS." As taken from Chen Y et al. 2015. Clin. Nephrol. 84, 262-9. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26396095>

"BACKGROUND: Vascular calcification contributes to cardiovascular disease in hemodialysis (HD) patients with diabetes. The randomized controlled trial reported here compared the effects of lanthanum carbonate (LC) and calcium carbonate (CC) on vascular stiffness assessed using brachial-ankle pulse wave velocity (ba-PWV), intima-media thickness (IMT), bone mineral density (BMD), and serum markers of chronic kidney disease - mineral and bone disorder in such patients. METHODS: Ba-PWV, IMT, BMD, and the biomarkers osteocalcin (OC) and bone alkaline phosphatase (BAP) were examined in 43 type 2 diabetes HD patients treated with LC (n=21) or CC (n=22) for 2 years. RESULTS: Forty-one patients completed the study (19, LC; 22, CC). The mean ba-PWV significantly increased only in the CC group (median: 2,280.5 to 2,402.5 cm/s, $P < 0.05$), after 24-month treatment; it remained unchanged in the LC group (median: 1,830.5 to 2,018.3 cm/s). However, the difference between the groups did not reach statistical significance. Changes in IMT and BMD were not different between the two groups. Changes in serum phosphorus, corrected calcium, and intact parathyroid hormone levels were similar between the groups. The incidence of fracture was 0% (0/19) in the LC group, and 13.6% (3/22) in the CC group ($P = 0.2478$). The OC/BAP ratio increased significantly in the LC group (median: 0.83 to 2.47), compared with in the CC group (median: 0.77 to 1.40) ($P = 0.036$). CONCLUSION: From this study, in Japanese type 2 diabetes HD patients, we conclude that 2-year treatment with LC might have slowed the progression of ba-PWV; however, it did not cause a difference in ba-PWV, IMT, BMD, or fracture, compared with CC. Further, LC increased the OC/BAP ratio to a greater extent than CC." As taken from Wada K et al. 2015. Int. J. Nephrol. Renovasc. Dis. 8, 111-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26346335>

"OBJECTIVE: To determine whether children with calcium-deficiency rickets respond better to treatment with calcium as limestone or as ground fish. STUDY DESIGN: Nigerian children with active rickets (n = 96) were randomized to receive calcium as powdered limestone (920 mg of elemental calcium) or ground fish (952 mg of elemental calcium) daily for 24 weeks. Radiographic healing was defined as achieving a score of 1.5 or less on a 10-point scale. RESULTS: The median (range) age of enrolled children was 35 (6-151) months. Of the 88 children who completed the study, 29 (66%) in the ground fish group and 24 (55%) in the limestone group achieved the primary outcome of a radiographic score of 1.5 or less within 6 months ($P = .39$). The mean radiographic score improved from 6.2 ± 2.4 to 1.8 ± 2.2 in the ground fish group and from 6.3 ± 2.2 to 2.1 ± 2.4 in the limestone group ($P = .68$ for group comparison). In an intention to treat analysis adjusted for baseline radiographic score, age, milk calcium intake, and serum 25-hydroxyvitamin D concentration, the response to treatment did not differ between the 2 groups ($P = .39$). Younger age was associated with more complete radiographic healing in the adjusted model (aOR 0.74 [95% CI 0.57-0.92]). After 24 weeks of treatment, serum alkaline phosphatase had decreased, calcium and 25-hydroxyvitamin D increased, and bone mineral density increased in both groups, without significant differences between treatment groups. CONCLUSION: In children with calcium-deficiency rickets, treatment with calcium as either ground fish or limestone for 6 months healed rickets in the majority of children." As taken from Thacher TD et al. 2015. J. Pediatr. 167, 148-54. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25799193>

"BACKGROUND: High levels of serum phosphorus both at baseline and during follow-up are associated with increased mortality in dialysis patients, and administration of phosphate binders

was independently associated with improved survival among hemodialysis population. Calcium-based phosphate binders are the most commonly used phosphate binders in developing countries for their relatively low costs. OBJECTIVES: To compare the efficacy and safety between calcium carbonate and calcium acetate in the treatment of hyperphosphatemia in hemodialysis patients. METHODS: PubMed, EMBASE, Cochrane Library, Google scholar and Chinese databases (Wanfang, Weipu, National Knowledge Infrastructure of China) were searched for relevant studies published before March 2014. Reference lists of nephrology textbooks and review articles were checked. A meta-analysis of randomized controlled trials (RCTs) and quasi-RCTs that assessed the effects and adverse events of calcium acetate and calcium carbonate in adult patients with MHD was performed using Review Manager 5.0. RESULTS: A total of ten studies (625 participants) were included in this meta-analysis. There was insufficient data in all-cause mortality and cardiovascular events for meta-analysis. Compared with calcium carbonate group, the serum phosphorus was significantly lower in calcium acetate group after 4 weeks' administration (MD -0.15 mmol/L, 95% CI -0.28 to -0.01) and after 8 weeks' administration (MD -0.25 mmol/L, 95% CI -0.40 to -0.11). There was no difference in serum calcium levels or the incidence of hypercalcemia between two groups at 4 weeks and 8 weeks. No statistical difference was found in parathyroid hormone (PTH) levels or serum calcium by phosphorus (Ca x P) product. There was significantly higher risk of intolerance with calcium acetate treatment (RR 3.46, 95% CI 1.48 to 8.26). CONCLUSIONS: For hyperphosphatemia treatment, calcium acetate showed better efficacy and with a higher incidence of intolerance compared with calcium carbonate. There are insufficient data to establish the comparative superiority of the two calcium-based phosphate binders on all-cause mortality and cardiovascular end-points in hemodialysis patients." As taken from Wang Y et al. 2015. PLoS One 10(3), e0121376. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25799184>

"PURPOSE: We conducted this review to assess the relative efficacy and safety of lanthanum carbonate versus calcium-based phosphate binders in chronic kidney disease. METHODS: We systematically searched PubMed, EMBASE, the Cochrane Controlled Trial Register of Controlled Trials and Chinese Biological Medical Database for randomized controlled trials comparing lanthanum carbonate with calcium-based phosphate binders in adult patients with chronic kidney disease. Study quality was assessed using the criteria outlined in the Cochrane Handbook for Systematic Reviews of intervention. Meta-analysis was conducted by reviewer manager software, version 5.3. RESULTS: Eleven trials with 1,501 participants were included. Lanthanum carbonate appeared to be associated with a significant reduction in progression of vascular calcification and a beneficial effect on bone outcomes without aluminum-like toxicity. Lanthanum carbonate achieved similar proportions of phosphate-controlled patients (RR 0.63, 95% CI 0.27-1.44) with lower incidence of hypercalcemia (RR 0.13, 95% CI 0.05-0.35) in comparison with calcium-based phosphate binders. Lanthanum carbonate was associated with significantly lower serum calcium, similar serum Ca x P product and higher serum iPTH compared with calcium salts in patients with chronic kidney disease. CONCLUSION: Lanthanum carbonate could delay the progression of vascular calcification and benefit chronic kidney disease patients on bone outcomes. Lanthanum carbonate could achieve similar proportion of phosphate-controlled patients as calcium-based phosphate binders with lower incidence of hypercalcemia." As taken from Zhai CJ et al. 2015. Int. Urol. Nephrol. 47, 527-35. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25399356>

"BACKGROUND: Bariatric surgery, especially Roux-en-Y gastric bypass, can cause serious nutritional complications arising from poor absorption of essential nutrients. Secondary hyperparathyroidism is one such complications that leads to increased parathyroid hormone levels due to a decrease in calcium and vitamin D, which may compromise bone health. AIM: To compare calcium carbonate and calcium citrate in the treatment of secondary hyperparathyroidism. METHOD: Patients were selected on the basis of their abnormal biochemical test and treatment was randomly done with citrate or calcium carbonate. RESULTS: After 60 days of supplementation, biochemical tests were repeated, showing improvement in both groups. CONCLUSION: Supplementation with calcium (citrate or carbonate) and vitamin D is recommended after surgery

for prevention of secondary hyperparathyroidism.” As taken from Baretta GA et al. 2015. Arq. Bras. Cir. Dig. 28(Suppl. 1), 43-5. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26537273>

“BACKGROUND: The present study was designed to examine the effects of vitamin D plus calcium administration on metabolic profiles and pregnancy outcomes among women at risk for pre-eclampsia. METHODS: In a prospective, double-blind, placebo-controlled trial, 60 women at risk for pre-eclampsia were randomised to take either 50 000 IU vitamin D3 every 2 weeks plus 1000 mg day(-1) calcium supplements (as calcium carbonate) (n = 30) or to receive placebos at the same times (n = 30) from 20 to 32 weeks of gestation. Fasting blood samples were taken at baseline and 12 weeks after intervention to determine related variables. Newborn anthropometric measurements were determined. RESULTS: Taking combined cholecalciferol and calcium supplements, compared to placebo, led to significant reductions in fasting plasma glucose (FPG) [mean (SD)] [-5.7 (5.5) versus -0.6 (12.6) mg dL(-1) , P = 0.04], serum insulin concentrations [-2.8 (6.0) versus +7.7 (9.8) µIU mL(-1) , P<0.001], homeostasis model of assessment-insulin resistance [-0.8 (1.3) versus +1.6 (2.2), P<0.001], homeostatic model assessment-beta cell function [-8.2 (25.8) versus +32.6 (41.3), P<0.001] and a significant rise in quantitative insulin sensitivity check index score [+0.02 (0.02) versus -0.02 (0.02), P<0.001]. Additionally, pregnant women who received cholecalciferol plus calcium supplements had increased serum high-density lipoprotein (HDL)-cholesterol [+4.6 (8.3) versus -2.9 (7.7) mg dL(-1) , P = 0.001] and plasma total glutathione (GSH) concentrations [+23.4 (124.0) versus -94.8 (130.2) µm, P = 0.001] compared to placebo. However, after adjustment for the baseline levels, maternal age and baseline body mass index, the effects on FPG levels (P = 0.13) and systolic blood pressure (P = 0.13) disappeared. CONCLUSIONS: Vitamin D plus calcium administration for 12 weeks had beneficial effects on glycaemic status, HDL-cholesterol, GSH and blood pressure among women at risk for pre-eclampsia.” As taken from Samimi M et al. 2016. J. Hum. Nutr. Diet. 29(4), 505-15. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/26467311>

“The present study was carried out to investigate the effects of fluoride toxicity on some biochemical, hormonal, and histological parameters of female rats and the protective role of calcium against such effects. Adult female albino rats were divided into five groups; control group received distilled water for 60 days, calcium group received calcium carbonate with dose of 50 mg/kg three times per week for 60 days, fluoride group received sodium fluoride with dose of 20 mg/kg three times per week for 60 days, calcium + fluoride group received calcium carbonate (50 mg/kg) then after 2 h received sodium fluoride (20 mg/kg) three times per week for 60 days, and fluoride + calcium group received sodium fluoride (20 mg/kg) three times per week for 30 days then received calcium carbonate (50 mg/kg) three times per week for another 30 days. The results showed that the levels of thiobarbituric acid reactive substances, urea, creatinine, alkaline phosphatase, triiodothyronine, thyroxine, parathormone, phosphorous, magnesium, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and gamma glutamyl transferase were significantly increased in rats treated with fluoride while serum estradiol, calcium, and organ glutathione were significantly decreased. The histological examination of the femur bone revealed that fluoride treatment induced thinning of bone trabeculae with wilding of marrow space, demineralization, and loss of trabeculae interconnections. Also, the histological examination of hepatic and renal tissues of fluoride-treated rats showed some damages in these tissues while administration of calcium carbonate for 30 or 60 days during fluoride treatment minimized such damages. It could be concluded that administration of calcium to female rats can ameliorate the hazardous effects of fluoride observed in the biochemical, hormonal, and histological parameters.” As taken from Mohamed NE. 2016. Biol. Trace Elem. Res. 170(1), 128-44. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/26198134>

“This short-term study assessed the efficacy and safety of calcium carbonate combined with calcitonin in the treatment of hypercalcemia in hemodialysis patients. Patients (n=64) on hemodialysis for chronic kidney disease for more than 6 months were included based on total serum calcium more than 10.5 mg/dL. All patients were randomized (1:1) to receive calcium

carbonate combined with calcitonin (Group I) or lanthanum carbonate (Group II) for 12 weeks. Blood levels of calcium, phosphorus and intact parathyroid hormone (iPTH) were measured every month, bone mass density (BMD) and coronary artery calcium scores (CACS) were measured at 3 months. During the study period, serum calcium decreased from 10.72 ± 0.39 to 10.09 ± 0.28 mg/dL ($P < 0.05$), serum phosphorus decreased from 6.79 ± 1.05 to 5.46 ± 1.18 mg/dL ($P < 0.05$), and serum iPTH levels in the Group I and Group II were not significantly different from the baseline. There were no significant differences in CACS in either group. There were no significant differences in the BMD values between Group I and baseline. In Group II, the BMD values at the lumbar spine and femoral neck were significantly lower than those before the trial and significantly lower than the corresponding values of Group I ($P < 0.05$). Calcium carbonate combined with calcitonin and lanthanum carbonate were equally effective in the suppression of hypercalcemia in hemodialysis patients. There were no serious treatment-related adverse events in treatment with calcium carbonate combined with calcitonin." As taken from Wei Y et al. 2014. Ther. Apher. Dial. 18, 618-22. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/24674384>

"BACKGROUND: Phosphate binders have an impact on fibroblast growth factor 23 (FGF23); however, the effect of phosphate binders on serum hepcidin has not been explored. We conducted a 24-week multicenter randomized controlled trial to investigate the effects of lanthanum carbonate or calcium carbonate monotherapy on serum phosphate, FGF23, and hepcidin levels in chronic hemodialysis patients. METHODS: Forty-six patients were recruited, and daily dietary phosphorus was controlled between 600-800 mg. Serum calcium, phosphate, albumin, alkaline phosphatase (ALP), FGF23, intact parathyroid hormone (iPTH), hepcidin, high-sensitivity CRP (hsCRP), 25(OH)D, 1,25(OH)2D, fetuin-A, and osteopontin were checked as scheduled. RESULTS: Twenty-five patients completed the study. Mean serum FGF23 level was significantly decreased after a 24-week treatment with lanthanum (8677.5 ± 7490.0 vs. 4692.8 ± 5348.3 pg/mL, $p = 0.013$, $n = 13$), but not with calcium ($n = 12$). The reduction of serum hepcidin in lanthanum group was positively correlated with the decrement of serum phosphate ($r = 0.631$, $p = 0.021$) and serum hsCRP ($r = 0.670$, $p = 0.012$) levels, respectively. Serum ALP, iPTH, vitamin D, fetuin-A, and osteopontin revealed no significant inter- or intragroup differences. CONCLUSIONS: In summary, a decrease in serum FGF23 levels and a trend of decline in hepcidin levels were observed only in lanthanum group." As taken from Chang YM et al. 2016. Clin. Exp. Nephrol. 21(5), 908-916. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/27928636>

"Two experiments were conducted to determine the requirement for standardized total tract digestible (STTD) Ca by 11- to 25-kg pigs based on growth performance, bone ash, or Ca and P retention and to determine the effect of dietary Ca on expression of genes related to Ca transport in the jejunum and kidneys. Six diets were formulated to contain 0.36% STTD P and 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca by including increasing quantities of calcium carbonate in the diets at the expense of cornstarch. Two additional diets contained 0.72% STTD Ca and 0.33% or 0.40% STTD P to determine if 0.36% STTD P had negative effects on the Ca requirement. The same batch of all diets was used in both experiments. In Exp. 1, 256 pigs (11.39 ± 1.21 kg initial BW) were randomly allotted to the 8 diets with 4 pigs per pen and 8 replicate pens per diet in a randomized complete block design. On the last day of the experiment, 1 pig from each pen was euthanized and the right femur and intestine and kidney samples were collected. Results indicated that ADG and G:F started to decline (linear and quadratic, < 0.05) at 0.54 and 0.50% STTD Ca, respectively. In contrast, bone ash increased (quadratic, < 0.05) as dietary Ca increased and reached a plateau indicating that the requirement for STTD Ca to maximize bone ash was 0.48%. Bone ash, but not ADG or G:F, increased (linear, < 0.01) as STTD increased in the diets. The mRNA expression of genes related to transcellular Ca transport decreased (linear, < 0.01) in the jejunum and in kidneys (linear and quadratic, < 0.01) as dietary Ca increased. In Exp. 2, 80 pigs (13.12 ± 1.79 kg initial BW) were placed in metabolism crates and randomly allotted to the 8 diets with 10 replicate pigs per diet in a randomized complete block design. Fecal and urine samples were collected using the marker-to-marker approach. Results indicated that the requirement for STTD Ca to maximize Ca and P retention (g/d) was 0.60 and 0.49%, respectively. In conclusion,

the STTD Ca requirement by 11- to 25-kg pigs to maximize bone ash was 0.48%; however, ADG and G:F declined if more than 0.54 or 0.50% STTD Ca, respectively, was fed, and the minimum concentration of Ca needed to maximize ADG and G:F could not be determined under the conditions of this experiment. Increasing dietary Ca decreased the mRNA expression of several genes related to transcellular Ca transport in the jejunum and the kidneys” As taken from González-Vega JC et al. 2016. J. Anim. Sci. 94(8), 3321-3334. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27695786>

“We reviewed patient records with a first-listed diagnosis of urolithiasis-also known as urinary tract or kidney stone disease, nephrolithiasis-upon discharge from Guam's sole civilian hospital during 2006 to 2010 and urinary cancer mortality records from the Guam Cancer Registry for 1970 to 2009 to determine the source of municipal water supplied to the patients' residence. The objective was to investigate a possible relationship between the sources of municipal water supplied to Guam villages and the incidence of urolithiasis and urinary cancer. We analyzed hospital discharge diagnoses of urolithiasis or renal calculi by calculating the incidence of first-mentioned discharge for urolithiasis or renal calculi and comparing rates across demographic or geographic categories while adjusting by age, sex, and ethnicity/race. We reviewed cancer registry records of urinary cancer deaths by patient residence. The annual incidence of hospitalization for urolithiasis was 5.22 per 10,000. Rates adjusted for sex or age exhibited almost no change. The rate of 9.83 per 10,000 among Chamorros was significantly higher ($P<0.05$) than the rates among any other ethnic group or race. When villages were grouped by water source, rates of patients discharged with a first-listed diagnosis of urolithiasis, adjusted for ethnicity/race, were similar for villages using either well water (5.44 per 10,000) or mixed source water (5.39 per 10,000), and significantly greater than the rate for villages using exclusively reservoir water (1.35 per 10,000). No statistically significant differences were found between the water source or village of residence and urinary cancer mortality. Some Guam residents living in villages served completely or partly by deep well water high in calcium carbonate may be at increased risk for urolithiasis compared with residents living in villages served by surface waters. Although the risk appears to be highest in villagers of Chamorro ethnicity, residents should be aware of other contributing risk factors and steps to take to avoid developing this health problem.” As taken from Haddock RL et al. 2016. Int. J. Environ. Res. Public Health 13(6), E523. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27231922>

“We discuss a case of an 81-year-old man who presented to hospital with a case of severe hypercalcaemia (5.07 mmol/L). The two most common causes of hypercalcaemia, making up around 90% of the cases, are malignancy and primary hyperparathyroidism. Initial investigations are guided by this knowledge. In this case, no underlying malignancy or evidence of a parathyroid adenoma causing primary hyperparathyroidism was found. Instead, on further history, it was found that this patient had troublesome dyspepsia symptoms for which he had ingested 10-12 calcium carbonate tablets a day for the preceding 2 years. This amounts to 6.6-7.9 g of calcium carbonate per day compared with the guideline daily intake of calcium of 1 g. His presentation of severe hypercalcaemia was therefore diagnosed as milk-alkali syndrome secondary to calcium carbonate tablet abuse.” As taken from Stoney B and Bagchi G. 2017. BMJ Case Rep. bcr-2017-219611. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28473363>

“During a diagnostic esophagogastroduodenoscopy (EGD) and colonoscopy, a foreign material was found coating a patient's stomach and proximal colon. Polypectomy with a hot snare and cold forceps proved unsuccessful, as the endoscope channels clogged. Thereafter, the patient confessed to taking one bottle of Tums (GlaxoSmithKline, St. Louis, Missouri, US) daily for an unknown duration. The medication was discontinued and a repeat colonoscopy showed complete resolution. The costs of repeat procedures, reduced efficacy, as well as equipment damage or refurbishment are substantial, and so providers should note that this may be the result of excessive calcium carbonate (similarly to barium) and instruct the patients to adjust intake accordingly.” As taken from Anderson J et al. 2018. Cureus 10(7), e3052. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30271697>

“In the other study, the excess dietary calcium carbonate intake in rats resulted in a reduction in faecal water bile acids and in faecal water free fatty acids and it was not toxic to rats (AS B.6.8.2.3-02).”

“In addition, the calcium carbonate consumption....can stimulate gastric acid secretion in men with and without duodenal ulcer (AS B.6.9.4.3-01).”

As taken from European Commission, 2019a.

“High doses or prolonged use [of calcium carbonate] may lead to gastric hypersecretion and acid rebound.”

“Because of its capacity to enhance secretion and to form insoluble compounds, CaCO_3 has unpredictable effects on gastrointestinal motility.”

As taken from European Commission, 2020.

“Gut dysbiosis in patients with chronic kidney disease (CKD) may induce chronic inflammation and increase morbidity. Phosphate-binding agents, generally used in patients with CKD, may potentially change the composition of the gut microbiota. This study aimed to compare the microbiota composition in hemodialysis patients treated with ferric citrate or calcium carbonate. The stool microbiota was investigated in hemodialysis patients treated with ferric citrate ($n = 8$) and calcium carbonate ($n = 46$) using 16S rRNA gene amplicon sequencing profiling using linear discriminant analysis of effect size. Further predictive functional profiling of microbial communities was obtained with Tax4Fun in R. Hemodialysis patients treated with calcium carbonate had a significantly reduced microbial species diversity (Shannon index and Simpson index) and an increased microbial alteration ratio compared with patients treated with ferric citrate. A distinct microbial community structure was found in patients treated with ferric citrate, with an increased abundance of the Bacteroidetes phylum and a decreased abundance of the phylum Firmicutes. Members of the order Lactobacillales were enriched in patients treated with calcium carbonate, whereas taxa of the genera Ruminococcaceae UCG-004, Flavonifractor, and Cronobacter were enriched in patients treated with ferric citrate phosphate binder. In conclusion, Ferric citrate therapy results in a more diverse microbiome community compared to calcium carbonate therapy in hemodialysis patients with phosphate binder treatment. The gut microbiome reflects the phosphate binder choice in hemodialysis patients, further affecting the physiological environment in the gastrointestinal tract.”

As taken from Wu PH et al. 2020. Microorganisms 8(12), 2040. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33419265/>

“Milk-alkali syndrome is characterized by a triad of elevated levels of calcium, metabolic alkalosis, and acute kidney injury that commonly occurs as a result of the combined intake of large amounts of calcium and absorbable alkali. The syndrome can have an acute onset with the rapid development of hypercalcemia and, if left untreated, may progress to the development of acute renal failure and metastatic calcification.

The syndrome was first recognized in the early 20th century when Bertram Sippy introduced a treatment regimen for peptic ulcer disease. The ‘Sippy regimen’ consisted of multiple daily doses of milk and cream combined with an absorbable alkali such as magnesium oxide, sodium bicarbonate, or bismuth subcarbonate to protect the gastric ulcer from further erosion by gastric acid. The results were highly favorable, and it soon became a popular therapy. Soon after, various toxic effects, including hypercalcemia and metabolic alkalosis, were reported. Some cases with acute kidney injury were also reported. With the advent of newer drugs for the treatment of peptic ulcer disease, such as histamine type-2 receptor blockers in the 1980s, the syndrome virtually vanished from the world.

Recently, however, an increased number of cases of the milk-alkali syndrome have been reported. This is likely due to the common use of over-the-counter preparations of calcium for the prevention and treatment of osteoporosis in post-menopausal women. Calcium carbonate is also frequently prescribed to patients with chronic kidney disease for the prevention of secondary

hyperparathyroidism. Various scholars have also suggested changing the name of the syndrome to calcium-alkali syndrome due to the changing etiopathology. Milk-alkali syndrome now accounts for more than 10% of the cases of hypercalcemia and is the third most common cause of hypercalcemia in hospitalized patients.”

As taken from Ali R et al. 2022

7. Addiction

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

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998).

Tobacco smoke condensates from cigarettes containing calcium carbonate and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of calcium carbonate. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	9,000 (471-34-1) -	Baker et al., 2004a JTI NTM Study Report(s)
In vitro genotoxicity	9,000 (471-34-1) -	Baker et al., 2004c JTI NTM Study Report(s)
In vitro cytotoxicity	9,000 (471-34-1) -	Baker et al., 2004c JTI NTM Study Report(s)
Inhalation study	9,000 (471-34-1)	Baker et al., 2004c

In comparison with a CSC of a reference cigarette with sideseam adhesives/cigarette paper corresponding to representative specifications for the majority of commercial cigarettes no differences were observed either in the bacterial mutagenicity, cytotoxicity or mammalian cell genotoxicity of the smoke condensate prepared from cigarettes with sideseam adhesives/cigarette paper containing Calcium Carbonate at 15.0156 mg/cig. The smoke chemistry data between test and reference cigarette revealed small changes towards both higher and lower yields per cigarette over all analytical groups. These differences were well within the variability of the analytical methods (JTI NTM Study Report(s)).

Calcium carbonate was used as a component of reconstituted tobacco sheet which when incorporated into cigarettes gave a 75% reduction in tars, nicotine, PAH'S and carbonyl compounds (Briskin 1979).

Calcium carbonate was used as a component of cigarette and cigarillo wrapper. When tested the products had reduced nicotine and tar deliveries (Detert & Ruchholz 1974).

Six products containing 25% sheet (incorporating up to 50% calcium carbonate) were tested for effects on mainstream smoke delivery of condensate,nicotine and CO. Condensate and nicotine levels were reduced in all products. CO was reduced in all but one product where a 12% increase was determined (Eicher et al 1985).

Calcium carbonate was used as a component of reconstituted cigar tobacco sheet the use of which gave a reduction in CO and wet tar levels (Fiore et al 1975).

9. Heated/vapor emissions toxicity

Aerosol from heated tobacco stick(s) containing Calcium carbonate 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	8.84	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	8.84	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	8.84	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

10. Ecotoxicity

10.1. Environmental fate

EPISuite provides the following information for CAS RN 471-34-1:

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method :	Incomplete
Group Method:	Incomplete
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 3.595E-022 atm-m3/mole (3.643E-017 Pa-m3/mole) VP: 2.73E-015 mm Hg (source: MPBPVP) WS: 1E+006 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:

Can Not Estimate (can not calculate HenryLC)

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	0.6999 0.8303 2.9780 (weeks) 3.7033 (days-weeks) 0.4144 0.4101 0.8361
Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:

Vapor pressure (liquid/subcooled):	0.00441 Pa (3.31E-005 mm Hg)

Log Koa ():	not available
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	0.00068 not available

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	0.024
Mackay model:	0.0516
Octanol/air (Koa) model:	not available

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	0.0000 E-12 cm3/molecule-sec
Half-Life =	-----
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 0.0378(Junge-Pankow, Mackay avg) Not available (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	1.75 L/kg (MCI method)
Log Koc:	0.243 (MCI method)
Koc :	0.3336 L/kg (Kow method)
Log Koc:	-0.477 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:

Rate constants can NOT be estimated for this structure!

Volatilization from Water:

Henry LC: 3.6E-022 atm-m3/mole (calculated from VP/WS)

Half-Life from Model River:	1.629E+018 hours (6.788E+016 days)
Half-Life from Model Lake:	1.777E+019 hours (7.405E+017 days)

Removal In Wastewater Treatment:

Total removal:	1.85 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.75 percent

Total to Air:	0.00 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	2.96e-007	1e+005	1000
Water	36.9	360	1000
Soil	63	720	1000
Sediment	0.0702	3.24e+003	0

Persistence Time: 593 hr

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that carbonic acid calcium salt (1:1) (CAS RN 471-34-1) is persistent in the environment.

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

Natural Pollution Sources:

EGG SHELLS ARE 94% CALCIUM CARBONATE. LIMESTONE...IS NATURAL SOURCE OF TECHNICAL OR AGRICULTURAL GRADES.

[Rossoff, I.S. Handbook of Veterinary Drugs. New York: Springer Publishing Company, 1974., p. 65]
 PEER REVIEWED

...OYSTER SHELLS ARE COMPOSED LARGELY OF CALCIUM CARBONATE. ... IT IS ALSO A PRINCIPAL COMPONENT OF DOLOMITE...

[Farm Chemicals Handbook 1980. Willoughby, Ohio: Meister, 1980., p. B-25] **PEER REVIEWED**

Exists in nature as minerals aragonite, calcite and vaterite. Two crystal forms are of commercial importance: aragonite, orthorhombic...calcite hexagonal-rhombohedral...

[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 279] **PEER REVIEWED**

As taken from HSDB, 2014

10.2. Aquatic toxicity

/AQUATIC SPECIES/ Catostomus commersoni ...(Suckers, 200-600 g) were surgically fitted with caudal artery of vein cannulae for repetitive blood sampling and exposed to sublethal levels of acidity (pH4-4.5 H₂SO₄) in both hard ([Ca⁺⁺] = 1.5-2.5 meq/L) and soft water ([Ca⁺⁺] = 0.1-.0.3 meq/L). ...During 4 days of acid exposure, similar disturbances in blood acid-base status and plasma ion levels occurred in both experimental media, but with more marked changes in soft water. A mixed respiratory and metabolic blood acidosis developed, manifested by significant depressions of plasma pH and bicarbonate, and simultaneous elevation of carbon dioxide tension. Plasma Na⁺ and Cl⁻ loss was reflected in a significantly reduced osmolality. Plasma K⁺ increased while levels of divalent cations (Ca⁺⁺, Mg⁺⁺) were unaltered. An increase of diffusive permeability of the gill epithelium and net branchial acid entry may explain the greater ion loss and blood

acidosis seen in soft water acid exposed suckers. In contrast, previous investigations on trout (*Oncorhynchus mykiss*) report fundamental differences in the responses to acidified hard and soft water. This suggests that the mechanism(s) of action of low pH may be species dependent. [European Chemicals Bureau; IUCLID Dataset, Calcium carbonate (471-34-1) (2000 CD-ROM edition). Available from, as of July 14, 2006: <http://ecb.jrc.it/esis/esis.php>] **PEER REVIEWED**

/AQUATIC SPECIES/ The physiological responses of 1 to 2 year old *Oncorhynchus mykiss* (rainbow trout) to low pH are dependant on the environmental Ca^{++} concentration. Trout were maintained for 5 days in acidified water. ...In moderately hard water ($[\text{Ca}^{++}] = 1.6\text{-}2.7 \text{ meq/L}$) at a mean pH of 4.3, trout developed a major blood acidosis but exhibited only a minor depression in plasma ion levels. In acidified soft water ($[\text{Ca}^{++}] = 0.3 \text{ mg/L}$), only a minor acidosis occurred, but plasma ion levels fell and there were substantially greater mortalities. Lethal bioassays performed on fingerling trout over a range of pH levels (3.0-4.8) revealed an important influence of external $[\text{Ca}^{++}]$ on resistance to acid exposure. Terminal physiological measurements on adult fish succumbing to low pH in soft water indicate the singular importance of iono-regulatory failure as the toxic mechanism of action under these circumstances. [European Chemicals Bureau; IUCLID Dataset, Calcium carbonate (471-34-1) (2000 CD-ROM edition). Available from, as of July 14, 2006: <http://ecb.jrc.it/esis/esis.php>] **PEER REVIEWED**

/AQUATIC SPECIES/ The effects of diet and water hardness, alone and in combination, on life history characteristics of *Daphnia magna* were determined in 2 laboratory tests. Water hardness: 50 to 350 mg/L (as CaCO_3). ...Number of young on the 1st day of reproduction, total young, and the number of generations were greater with increasing hardness. At the max test hardness, about 65% more young were produced than at the lowest hardness. Furthermore, time to sexual maturity was about 1 day shorter in the harder culture water. Daphnids fed the combination of green alga, trout chow, and dehydrated alfalfa were >3-fold more productive than daphnids fed only the alga or only trout chow and alfalfa. The combination of the algae-reinforced diet with hard culture water provided for optimal productivity. [European Chemicals Bureau; IUCLID Dataset, Calcium carbonate (471-34-1) (2000 CD-ROM edition). Available from, as of July 14, 2006: <http://ecb.jrc.it/esis/esis.php>] **PEER REVIEWED**

/AQUATIC SPECIES/ A significant number of moribund and dead lobsters *Homarus americanus* were reported to New York state authorities by lobster fishers in Long Island Sound (LIS) during the summer of 2002. Morbid lobsters were characterised by an orange discolouration of the abdomen, lethargy, an excess of epibionts and poor post-capture survival. On necropsy, severe extensive multifocal or diffuse mineralised granulomatous inflammation of the gills and antennal glands was the most striking pathology. In the gills, granulomas often occluded the lumen of filaments, resulting in congestion, ischemia and coagulative necrosis of gill tissues. In the antennal glands, granulomas were concentrated along the border between the coelomosac and labyrinth. No significant pathogens were recovered from diseased individuals. In prechronic individuals, however, it was evident that granulomas were focused around calcium carbonate (aragonite) crystals. This disease may result from anomalously high sea-bottom temperatures in LIS (approximately 23 deg C) during the summer of 2002 and associated disruptions of the calcium chemistry of lobsters in favour of deposition of minerals in soft tissues. The ultimate cause of death of affected lobsters is probably respiratory failure due to reduced effective surface area of the gills, exacerbated by hypermetabolic temperatures and an abundance of epibionts. [Dove AD et al; Dis Aquat Organ 58(2-3):215-21 (2004)] **PEER REVIEWED**

As taken from HSDB, 2014

Acute/Prolonged Toxicity to Fish

Type:	field observation
Species:	Gambusia affinis (Fish, fresh water)

Exposure period:	96 hour(s)
Unit:	mg/l Analytical monitoring: no data
TLm :	> 56000
Remark:	At the dosage of 56000 mg/l of CaCO ₃ , the turbidity was reduced from an initial value of 260 mg/l to 35 mg/l.
Source:	Solvay S.A. Bruxelles (26)
Test condition:	The authors studied the effect of adding calcium carbonate to highly turbid waters containing the fishes at 19–21 degree C.
Species:	Poecilia latipinna (Fish, estuary)
Exposure period:	96 hour(s)
Unit:	mg/l
NOEC:	> 200
Source:	Solvay S.A. Bruxelles(27)
Exposure period:	96 hour(s)
Unit:	mg/kg soil dw
TLm/96 :	> 1000
Source:	Guaber SPA Funo di Argelato (BO)

Chronic Toxicity to Fish

Species:	Catostomus commersoni (Fish, fresh water)
Endpoint:	other: blood disturbances
Remark:	During 4 days of acid exposure, similar disturbances in blood acid–base status and plasma ion levels occurred in both experimental media, but with more marked changes in soft water. A mixed respiratory and metabolic blood acidosis developed, manifested by significant depressions of plasma pH and bicarbonate, and simultaneous elevation of carbon dioxide tension. Plasma Na ⁺ and Cl [–] loss was reflected in a significantly reduced osmolality. Plasma K ⁺ increased while levels of divalent cations (Ca ⁺⁺ , Mg ⁺⁺) were unaltered. An increase of diffusive permeability of the gill epithelium and net branchial acid entry may explain the greater ion loss and blood acidosis seen in soft water acid exposed suckers. In contrast, previous investigations on trout (<i>Salmo gairdneri</i>) report fundamental differences in the responses to acidified hard and soft water. This suggests that the mechanism(s) of action of low pH may be species dependent.
Source:	Solvay S.A. Bruxelles (28) (29)
Test condition:	Suckers (200–600 g) were surgically fitted with caudal artery of vein cannulae for repetitive blood sampling and exposed to sublethal levels of acidity (pH 4–4.5 H ₂ SO ₄) in both hard

	([Ca ⁺⁺] = 1.5–2.5 meq/l) and soft water ([Ca ⁺⁺] = 0.1–0.3 meq/l).	
Species:	Salmo gairdneri (Fish, estuary, fresh water)	
Endpoint:	other: physiological response	
Exposure period:	5 day	
Remark:	In moderately hard water ([Ca ⁺⁺] = 1.6–2.7 meq/l) at a mean pH of 4.3, trout developed a major blood acidosis but exhibited only a minor depression in plasma ion levels. In acidified soft water ([Ca ⁺⁺] = 0.3 meq/l), only a minor acidosis occurred, but plasma ion levels fell and there were substantially greater mortalities. Lethal bioassays performed on fingerling trout over a range of pH levels (3.0–4.8) revealed an important influence of external [Ca ⁺⁺] on resistance to acid exposure. Terminal physiological measurements on adult fish succumbing to low pH in soft water indicate the singular importance of iono-regulatory failure as the toxic mechanism of action under these circumstances.	
Source:	Solvay S.A. Bruxelles (30) (31)	
Test condition:	The physiological responses of 1 to 2 year old rainbow trout to low pH are dependant on the environmental Ca ⁺⁺ concentration. Trouts were maintained for 5 days in acidified water.	

Chronic Toxicity to Aquatic Invertebrates

Species:	Daphnia magna (Crustacea)
Endpoint:	reproduction rate
Result:	No. of young on the 1st day of reproduction, total young, and the no. of generations were greater with increasing hardness. At the max. test hardness, ~ 65% more young were produced than at the lowest hardness. Furthermore, time to sexual maturity was ~ 1 day shorter in the harder culture water. Daphnids fed the combination of green alga, trout chow, and dehydrated alfalfa were > 3-fold more productive than daphnids fed only the alga or only trout chow and alfalfa. The combination of the algae-reinforced diet with hard culture water provided for optimal productivity.
Source:	Solvay S.A. Bruxelles
Test condition:	The effects of diet and water hardness, alone and in combination, on life history characteristics of D. Magna were determined in 2 laboratory tests. Water hardness : 50 to 350 mg/l (as CaCO ₃).

As taken from IUCLID Dataset (2000), Calcium carbonate (471-34-1).

Record for carbonic acid calcium salt (1:1) (CAS RN 471-34-1):

Spec. Sci. Name	Exp. Type	Media Type	Resp. Site	Endpoint	Trend	Effect	Conc (Standardized)	Stat. Signif.
Spec. Common Name	Chem. Anal.	Loc	Obs. Dur. (Days)	BCF	Eff %	Effect Meas.	Appl. Rate	Sig. Level

Americam ysis bahia Opossum Shrimp	R M	SW LAB	7			GRO GGRO/	T (28000- 212000) ug/L	
Americam ysis bahia Opossum Shrimp	R M	SW LAB	7			REP GREP/	T (28000- 212000) ug/L	
Americam ysis bahia Opossum Shrimp	R M	SW LAB	(3 - 6)			GRO GGRO/	T (30000- 198000) ug/L	
Oreochro mis mossambi cus Mozambiq ue Tilapia	AQU A - NR U	FW LAB	LI 1	LOEC	DEC 62.90/	BCM MLDH	T 50000 ug/L	ASIG <0.01
Oreochro mis mossambi cus Mozambiq ue Tilapia	AQU A - NR U	FW LAB	LI 2	LOEC	DEC 53.59/	BCM MLDH	T 50000 ug/L	ASIG <0.01
Oreochro mis mossambi cus Mozambiq ue Tilapia	AQU A - NR U	FW LAB	LI 3	NOEC	DEC 11.06/	BCM MLDH	T 50000 ug/L	ANOSI G <0.05
Oreochro mis mossambi cus Mozambiq ue Tilapia	AQU A - NR U	FW LAB	LI 4	NOEC	DEC 35.48/	BCM MLDH	T 50000 ug/L	ANOSI G <0.05
Oreochro mis mossambi cus Mozambiq	AQU A - NR U	FW LAB	LI 7	NOEC	INC 14.84/	BCM MLDH	T 50000 ug/L	ANOSI G <0.05

ue Tilapia								
Acipenser sinensis Chinese Sturgeon	R U	FW LAB	6	NR-ZERO	NEF 0	MOR MORT	T 320000 ug/L	
Poecilia sp. Mollies	U	LAB	4			MOR/ MORT	T 200000* ug/L	
Ictalurus punctatus Channel Catfish	E U	FW FIEL DA	103	NOEC	DEC 82.1	MOR SURV	T 8 kg/ha	ANOSI G >0.05
Ictalurus punctatus Channel Catfish	E U	FW FIEL DA	103	NOEC	INC	PHY FDCV	T 8 kg/ha	ANOSI G >0.05
Ictalurus punctatus Channel Catfish	E U	FW FIEL DA	WO 103	NOEC	DEC	POP BMAS	T 8 kg/ha	ANOSI G >0.05
Cyprinus carpio Common Carp	FD U	FW LAB	MUL/ 7			PHY CAAS/	T 0.38 %	
Oncorhync hus mykiss Rainbow Trout	F U	FW LAB	PL 0.042	LOEC	DEC	BCM CLCO	T (4000- 320000) ug/L	ASIG <=0.05
Oncorhync hus mykiss Rainbow Trout	F U	FW LAB	PL 0.042	LOEC	DEC	BCM NACO	T (4000- 320000) ug/L	ASIG <=0.05
Oncorhync hus mykiss Rainbow Trout	F U	FW LAB	PL 0.042	LOEC	DEC	PHY CO2T	T (4000- 320000) ug/L	ASIG <=0.05

Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.042	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.042	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.042	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.042	LOEC	INC	BCM PHPH	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.083	LOEC	DEC	BCM CLCO	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.083	LOEC	DEC	BCM NACO	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.083	LOEC	DEC	PHY CO2T	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.083	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.083	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05

hus mykiss Rainbow Trout	U	LAB	0.083			AMMO/	320000) ug/L	<=0.05
Oncorhync hus mykiss Rainbow Trout	F U	FW LAB	PL 0.083	LOEC	INC	BCM AMMO/	T (4000- 320000) ug/L	ASIG =<0.05
Oncorhync hus mykiss Rainbow Trout	F U	FW LAB	PL 0.083	LOEC	INC	BCM PHPH	T (4000- 320000) ug/L	ASIG =<0.05
Oncorhync hus mykiss Rainbow Trout	F U	FW LAB	PL 0.125	LOEC	DEC	BCM CLCO	T (4000- 320000) ug/L	ASIG =<0.05
Oncorhync hus mykiss Rainbow Trout	F U	FW LAB	PL 0.125	LOEC	DEC	BCM NACO	T (4000- 320000) ug/L	ASIG =<0.05
Oncorhync hus mykiss Rainbow Trout	F U	FW LAB	PL 0.125	LOEC	DEC	PHY CO2T	T (4000- 320000) ug/L	ASIG =<0.05
Oncorhync hus mykiss Rainbow Trout	F U	FW LAB	PL 0.125	LOEC	INC	BCM AMMO/	T (4000- 320000) ug/L	ASIG =<0.05
Oncorhync hus mykiss Rainbow Trout	F U	FW LAB	PL 0.125	LOEC	INC	BCM AMMO/	T (4000- 320000) ug/L	ASIG =<0.05
Oncorhync hus	F	FW	PL	LOEC	INC	BCM	T (4000- 320000) ug/L	ASIG

mykiss Rainbow Trout	U	LAB	0.125			AMMO/		<=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.125	LOEC	INC	BCM HCO3	T (4000-320000) ug/L	ASIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.125	LOEC	INC	BCM PHPH	T (4000-320000) ug/L	ASIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.167	LOEC	DEC	BCM CLCO	T (4000-320000) ug/L	ASIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.167	LOEC	DEC	BCM NACO	T (4000-320000) ug/L	ASIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.167	LOEC	DEC	PHY CO2T	T (4000-320000) ug/L	ASIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.167	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.167	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG <=0.05
Oncorhynchus mykiss	F U	FW LAB	PL 0.167	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG <=0.05

Rainbow Trout								
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.167	LOEC	INC	BCM HCO3	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.167	LOEC	INC	BCM PHPH	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.208	LOEC	DEC	BCM CLCO	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.208	LOEC	DEC	BCM NACO	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.208	LOEC	DEC	PHY CO2T	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.208	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.208	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss	F U	FW LAB	PL 0.208	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05

Rainbow Trout								
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.208	LOEC	INC	BCM HCO3	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.208	LOEC	INC	BCM PHPH	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.25	LOEC	DEC	PHY CO2T	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.25	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.25	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.25	LOEC	INC	BCM AMMO/	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.25	LOEC	INC	BCM HCO3	T (4000-320000) ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow	F U	FW LAB	PL 0.25	LOEC	INC	BCM PHPH	T (4000-320000) ug/L	ASIG ≤0.05

Trout								
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 1	LOEC	DEC	PHY CO2T	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 1	LOEC	INC	BCM AMMO/	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 1	LOEC	INC	BCM AMMO/	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 1	LOEC	INC	BCM AMMO/	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 1	LOEC	INC	BCM PHPH	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 2	LOEC	DEC	PHY CO2T	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 2	LOEC	INC	BCM AMMO/	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 2	LOEC	INC	BCM AMMO/	T 4000 ug/L	ASIG ≤0.05

Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 2	LOEC	INC	BCM AMMO/	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 2	LOEC	INC	BCM PHPH	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 4	LOEC	DEC	BCM HCO3	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 4	LOEC	DEC	BCM NACO	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 4	LOEC	DEC	PHY CO2T	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 4	LOEC	INC	BCM AMMO/	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 4	LOEC	INC	BCM AMMO/	T 4000 ug/L	ASIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 4	LOEC	INC	BCM AMMO/	T 4000 ug/L	ASIG ≤0.05

hus mykiss Rainbow Trout	U	LAB	4			PHPH		<=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 8	LOEC	DEC	BCM CLCO/	T 32000 0 ug/L	SIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 8	LOEC	DEC	BCM HCO3/	T 32000 0 ug/L	SIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 8	LOEC	DEC	BCM NACO/	T 32000 0 ug/L	SIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 8	LOEC	DEC	PHY CO2T/	T 32000 0 ug/L	SIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 8	LOEC	INC	BCM AMMO/	T 32000 0 ug/L	SIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 8	LOEC	INC	BCM AMMO/	T 32000 0 ug/L	SIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 8	LOEC	INC	BCM AMMO/	T 32000 0 ug/L	SIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 8	LOEC	INC	BCM PHPH/	T 32000 0 ug/L	SIG <=0.05
Oncorhynchus mykiss	FD M	FW LAB	BLC ~ 7	LOEC	DEC	PHY CAUP	T 64.71 mg/g	SIG <0.05

Rainbow Trout							fd	
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI ~ 7	LOEC	DEC	PHY CAUP	T 64.71 mg/g fd	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI 28	LOEC	INC	BCM CDCO	T 65.8 mg/g fd	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI ~ 15	LOEC	DEC	PHY CAUP	T (51.27- 55.72) mg/g fd	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI ~ 30	LOEC	INC	PHY CAUP	T (51.27- 55.72) mg/g fd	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI 21	LOEC	INC	BCM CDCO	T 65.8 mg/g fd	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	42	LOEC	DEC	PHY FDCV/	T 61000 ppm	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL ~ 7	LOEC	DEC	PHY CAUP	T 64.71 mg/g fd	SIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 28	LOEC	DEC	ACC RSDE	T 65.8 mg/g fd	ASIG <0.05

Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 28	LOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR ~ 15	LOEC	DEC	PHY CAUP	T (51.27- 55.72) mg/g fd	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR ~ 30	LOEC	INC	PHY CAUP	T (51.27- 55.72) mg/g fd	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	SH 42	LOEC	INC	BCM PBCO/	T 61000 ppm	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO ~ 30	LOEC	INC	PHY FLUX/	T (51.27- 55.72) mg/g fd	ASIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO ~ 7	LOEC	DEC	PHY CAUP	T 64.71 mg/g fd	SIG <0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	BL 1	NOEC	NEF	BCM UREN	T 32000 0 ug/L	ANOSIG ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	BL 2	NOEC	INC	BCM UREN	T 32000 0 ug/L	ANOSIG ≤0.05
Oncorhynchus mykiss	F	FW	BL	NOEC	INC	BCM	T 32000	ANOSIG

Rainbow Trout	U	LAB	4			UREN	0 ug/L	<=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	BL 8	NOEC	DEC	BCM UREN/	T 32000 0 ug/L	NOSIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.042	NOEC	INC	BCM HCO3	T (4000-32000 0) ug/L	ANOSIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.083	NOEC	INC	BCM HCO3	T (4000-32000 0) ug/L	ANOSIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.25	NOEC	DEC	BCM CLCO	T (4000-32000 0) ug/L	ANOSIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 0.25	NOEC	DEC	BCM NACO	T (4000-32000 0) ug/L	ANOSIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 1	NOEC	DEC	BCM HCO3	T 32000 0 ug/L	ANOSIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 1	NOEC	INC	HRM CRTS	T 32000 0 ug/L	ANOSIG <=0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 2	NOEC	DEC	HRM CRTS	T 32000 0 ug/L	ANOSIG <=0.05
Oncorhynchus	F	FW	PL	NOEC	DEC	HRM	T	NOSIG

mykiss Rainbow Trout	U	LAB	8			CRTS/	32000 0 ug/L	<=0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BL 42	NOEC	DEC	BCM HMGL/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BL 42	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BL 42	NOEC	DEC	BCM PRCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BL 42	NOEC	DEC	ENZ ALAD/	T 61000 ppm	SIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BL 42	NOEC	INC	BCM ZNCO/	T 61000 ppm	SIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BLC ~ 7	NOEC	DEC	ACC RSDE/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BLC ~ 7	NOEC	DEC	BCM NACO/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BLC ~ 7	NOEC	DEC	PHY NAUP	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus	FD	FW	BLC	NOEC	INC	BCM	T	ANOSIG

mykiss Rainbow Trout	M	LAB	~ 7			CLCO/	64.71 mg/g fd	<0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BLC ~ 7	NOEC	INC	PHY CAUP	T 34.00 mg/g fd	NOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BR 14	NOEC	NEF	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BR 28	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	BR 42	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	ER 21	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	ER 35	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	ER 7	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI ~ 15	NOEC	DEC	PHY CAUP	T (51.27- 55.72) mg/g fd	ANOSIG <0.05

Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI ~ 30	NOEC	INC	PHY CAUP	T (51.27-55.72) mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI ~ 7	NOEC	DEC	ACC RSDE/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI ~ 7	NOEC	DEC	BCM CLCO/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI ~ 7	NOEC	DEC	BCM NACO/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI ~ 7	NOEC	DEC	PHY NAUP	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI 14	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI 14	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	GI 21	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss	FD	FW	GI	NOEC	DEC	BCM	T 61000 ppm	ANOSIG

Rainbow Trout	M	LAB	28			PBCO/		<0.05
Oncorhynchus mykiss	FD	FW	GI	NOEC	INC	ENZ	T 61000	ANOSIG
Rainbow Trout	M	LAB	35			NKAT/	ppm	<0.05
Oncorhynchus mykiss	FD	FW	GI	NOEC	INC	BCM	T 61000	ANOSIG
Rainbow Trout	M	LAB	42			PBCO/	ppm	<0.05
Oncorhynchus mykiss	FD	FW	GI	NOEC	INC	BCM	T 65.8	ANOSIG
Rainbow Trout	M	LAB	7			CDCO	mg/g fd	<0.05
Oncorhynchus mykiss	FD	FW	GT/	NOEC	INC	BCM	T 61000	ANOSIG
Rainbow Trout	M	LAB	14			PBCO/	ppm	<0.05
Oncorhynchus mykiss	FD	FW	GT/	NOEC	INC	BCM	T 61000	ANOSIG
Rainbow Trout	M	LAB	28			PBCO/	ppm	<0.05
Oncorhynchus mykiss	FD	FW	GT/	NOEC	INC	BCM	T 61000	ANOSIG
Rainbow Trout	M	LAB	42			PBCO/	ppm	<0.05
Oncorhynchus mykiss	FD	FW	IN/	NOEC	INC	BCM	T 61000	ANOSIG
Rainbow Trout	M	LAB	14			PBCO/	ppm	<0.05
Oncorhynchus mykiss	FD	FW	IN/	NOEC	INC	BCM	T 61000	ANOSIG
Rainbow Trout	M	LAB	14			PBCO/	ppm	<0.05
Oncorhynchus mykiss	FD	FW	IN/	NOEC	DEC	BCM	T 61000	ANOSIG
Rainbow Trout	M	LAB	28			PBCO/	ppm	<0.05

Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	IN/ 28	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	IN/ 35	NOEC	DEC	ENZ NKAT/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	IN/ 35	NOEC	INC	ENZ NKAT/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	IN/ 35	NOEC	INC	ENZ NKAT/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	IN/ 42	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	IN/ 42	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI ~ 30	NOEC	INC	PHY CAUP	T (51.27- 55.72) mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI ~ 7	NOEC	DEC	ACC RSDE/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI ~ 7	NOEC	DEC	BCM CLCO/	T 64.71 mg/g fd	ANOSIG <0.05

Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI ~ 7	NOEC	DEC	BCM NACO/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI ~ 7	NOEC	DEC	PHY CAUP	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI ~ 7	NOEC	INC	PHY NAUP	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI 14	NOEC	INC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI 21	NOEC	INC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI 28	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI 7	NOEC	INC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI/ 14	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI/ 14	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05

Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI/ 28	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI/ 28	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	KI/ 42	NOEC	DEC	BCM PBCO/	T 61000 ppm	SIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI ~ 15	NOEC	DEC	PHY CAUP	T (51.27- 55.72) mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI ~ 7	NOEC	DEC	ACC RSDE/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI ~ 7	NOEC	DEC	PHY CAUP	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI ~ 7	NOEC	INC	BCM CLCO/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI ~ 7	NOEC	INC	BCM NACO/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus	FD	FW	LI	NOEC	INC	PHY	T	ANOSIG

mykiss Rainbow Trout	M	LAB	~ 7			NAUP	64.71 mg/g fd	<0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI 14	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI 14	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI 28	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI 28	NOEC	INC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI 42	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI 42	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	LI 7	NOEC	INC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	MU 14	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss	FD M	FW LAB	MU 28	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05

Rainbow Trout								
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	MU 42	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	14	NOEC	DEC 2.54	MOR MORT	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	30	NOEC	2-5	MOR MORT	T (51.27- 55.72) mg/g fd	ANOSIG >0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	42	NOEC	DEC	GRO COND/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL ~ 15	NOEC	DEC	PHY CAUP	T (51.27- 55.72) mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL ~ 30	NOEC	INC	PHY CAUP	T (51.27- 55.72) mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL ~ 7	NOEC	DEC	ACC RSDE/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL ~ 7	NOEC	DEC	BCM CLCO/	T 64.71 mg/g fd	ANOSIG <0.05

Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL ~ 7	NOEC	DEC	PHY CAUP	T 34.00 mg/g fd	NOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL ~ 7	NOEC	DEC	PHY NAUP	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL ~ 7	NOEC	INC	BCM NACO/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 21	NOEC	DEC	ACC RSDE/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 21	NOEC	DEC	BCM MGCO/	T 61000 ppm	SIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 21	NOEC	DEC	BCM NACO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 21	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 35	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss	FD M	FW LAB	PL 35	NOEC	INC	ACC RSDE/	T 61000 ppm	SIG <0.05

Rainbow Trout								
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 35	NOEC	INC	BCM NACO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 7	NOEC	DEC	ACC RSDE/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 7	NOEC	DEC	BCM NACO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 7	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	PL 7	NOEC	NEF	BCM MGCO/	T 61000 ppm	SIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR ~ 7	NOEC	DEC	BCM CLCO/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR ~ 7	NOEC	DEC	PHY CAUP	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR ~ 7	NOEC	INC	BCM NACO/	T 64.71 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss	FD M	FW LAB	RR ~ 7	NOEC	INC	PHY NAUP	T 64.71 mg/g	ANOSIG <0.05

Rainbow Trout							fd	
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR 14	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR 14	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR 21	NOEC	INC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR 28	NOEC	NEF	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR 28	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR 42	NOEC	NEF	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	RR 7	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	SH 14	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	SH 14	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05

Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	SH 21	NOEC	INC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	SH 28	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	SH 28	NOEC	INC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	SH 7	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	SP 14	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	SP 28	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	SP 42	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	VE 14	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	VE 28	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOSIG <0.05
Oncorhynchus mykiss	FD	FW	VE	NOEC	DEC	BCM	T 61000 ppm	ANOSIG

Rainbow Trout	M	LAB	42			PBCO/		<0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO ~ 15	NOEC	DEC	PHY FLUX/	T (51.27-55.72) mg/g fd	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO ~ 7	NOEC	INC	PHY CAUP	T 34.00 mg/g fd	NOSI G <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 14	NOEC	DEC	BCM PBCO/	T 61000 ppm	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 14	NOEC	DEC	GRO GAIN	T 64.71 mg/g fd	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 14	NOEC	INC	BCM CDCO	T 65.8 mg/g fd	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 21	NOEC	INC	BCM CDCO	T 65.8 mg/g fd	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 28	NOEC	INC	BCM PBCO/	T 61000 ppm	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 28	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 30	NOEC		GRO SPGR	T (51.27-55.72) mg/g fd	ANOS IG >0.05

Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 42	NOEC	DEC	GRO SPGR/	T 61000 ppm	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 42	NOEC	DEC	GRO WGHT/	T 61000 ppm	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 42	NOEC	NEF	BCM PBCO/	T 61000 ppm	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO 7	NOEC	DEC	BCM CDCO	T 65.8 mg/g fd	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO (0 - 7)	NOEC	DEC	GRO SPGR	T 65.8 mg/g fd	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO (14 - 21)	NOEC	DEC	GRO SPGR	T 65.8 mg/g fd	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO (14 - 21)	NOEC	INC	GRO SPGR	T 65.8 mg/g fd	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO (7 - 14)	NOEC	INC	GRO SPGR	T 65.8 mg/g fd	ANOS IG <0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	42	NR-ZERO	NEF 0	MOR MORT/	T 61000 ppm	
Oncorhynchus	F	FW	BL		CHG	PHY	T	

s mykiss Rainbow Trout	U	LAB	(0.042 - 0.25)			FLUX/	(4000-320000) ug/L	
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	(1 - 8)		CHG	MOR MORT	T (4000-320000) ug/L	
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 1		DEC	BCM CLCO	T (4000-320000) ug/L	MULT ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 1		DEC	BCM NACO	T (4000-320000) ug/L	MULT ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 2		DEC	BCM CLCO	T (4000-320000) ug/L	MULT ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 2		DEC	BCM HCO3	T (4000-320000) ug/L	MULT ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 2		DEC	BCM NACO	T (4000-320000) ug/L	MULT ≤0.05
Oncorhynchus mykiss Rainbow Trout	F U	FW LAB	PL 4		CHG	HRM CRTS	T (4000-320000) ug/L	MULT ≤0.05
Oncorhynchus mykiss	F	FW	PL		DEC	BCM	T (4000-	MULT

Rainbow Trout	U	LAB	4			CLCO	320000) ug/L	<=0.05
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	MUL/ ~ 7		CHG	PHY CDUP	T (34.00-64.71) mg/g fd	
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	28		NEF	MOR MORT	T 65.8 mg/g fd	
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	VE (~15 - ~30)		CHG	PHY CAUP	T (51.27-55.72) mg/g fd	
Oncorhynchus mykiss Rainbow Trout	FD M	FW LAB	WO ~ 7		CHG	PHY NAFX	T (34.00-64.71) mg/g fd	
Gambusia affinis Western Mosquitofish	S U	FW LAB	1	LC50/	INC	MOR MORT	T > 56000000 ug/L	
Gambusia affinis Western Mosquitofish	S U	FW LAB	2	LC50/	INC	MOR MORT	T > 56000000 ug/L	
Gambusia affinis Western Mosquitofish	S U	FW LAB	4	LC50/	INC	MOR MORT	T > 56000000 ug/L	
Echinochloa frumentacea Billion Dollar Grass	S U	FW LAB	RO 7	LOEC	DEC 0.0/	GRO LGTH	F 1300 umol/L	SIG <0.05
Echinochloa	S	FW	SO	LOEC	DEC	GRO	F 1300	SIG

frumentacea Billion Dollar Grass	U	LAB	7		0.0/	LGTH	umol/L	<0.05
Echinochloa frumentacea Billion Dollar Grass	S U	FW LAB	RO 7	NOEC	DEC 44.4/	GRO LGTH	F 250 umol/L	NOSI G <0.05
Echinochloa frumentacea Billion Dollar Grass	S U	FW LAB	SO 7	NOEC	DEC 98.6/	GRO LGTH	F 250 umol/L	NOSI G <0.05
Culex quinquefasci atus Southern House Mosquito	AQU A - NR U	FW LAB	1		INC 89-92	MOR MORT	T (50000- 500000) ug/L	
Aquatic community Aquatic Community	E U	FW FIELD A	72	LOEC	DEC	PHY PSYN/	T 8 kg/ha	ASIG >0.05
Aquatic community Aquatic Community	E U	FW FIELD A	72	LOEC	DEC	PRS PPRO/	T 8 kg/ha	ASIG >0.05
Aquatic community Aquatic Community	E U	FW FIELD A	WO 72	NOEC	INC	BCM CHLA	T 8 kg/ha	ANOS IG >0.05
Plankton sp. Plankton	E U	FW FIELD N	91.32		DEC	POP ABND	T ug/L 250 kg	
Plankton sp. Plankton	E U	FW FIELD N	91.32		DEC	POP BMAS	T ug/L 250 kg	
Plankton sp. Plankton	S U	FW LAB	0.167			PRS PPRO/	T (5000- 600000) ug/L	

Elimia flava Freshwater Snail	S U	FW LAB	HL 3	LOEC	DEC	BCM CO2C	T 125000 ug/L	SIG 0.05
Elimia flava Freshwater Snail	S U	FW LAB	HL 3	LOEC	INC	BCM NACO	T 12500 ug/L	ASIG 0.05
Elimia flava Freshwater Snail	S U	FW LAB	HL 3	LOEC	INC	BCM PHPH	T 12500 ug/L	ASIG 0.05
Elimia flava Freshwater Snail	S U	FW LAB	HL 3	NOEC	DEC	BCM CO2C	T 12500 ug/L	NOSI G 0.05
Elimia flava Freshwater Snail	S U	FW LAB	HL 3	NOEC		BCM CACO	T 125000 ug/L	ANOS IG 0.05
Elimia flava Freshwater Snail	S U	FW LAB	HL 3	NOEC		BCM HCO3	T 125000 ug/L	ANOS IG 0.05
Elimia flava Freshwater Snail	S U	FW LAB	HL 3	NOEC		BCM KCON	T 125000 ug/L	ANOS IG 0.05
Elimia flava Freshwater Snail	S U	FW LAB	HL 3	NOEC		PHY OSMO	T 125000 ug/L	ANOS IG 0.05

As taken from the EPA ECOTOX database.

Record for limestone (CAS RN 1317-65-3):

Spec. Sci. Name Spec. Common Name	Exp. Type Chem. Anal.	Media Type Loc	Resp. Site Obs. Dur. (Days)	Endpoint BCF	Trend Eff %	Effect Effect Meas.	Conc (Standardized) Appl. Rate	Stat. Signif. Sig. Level
Salmo salar Atlantic Salmon	E U	FW FIELDA	3	NR-ZERO	NEF 0	MOR MORT	F 45000 ug/L	
Salmo salar Atlantic Salmon	E U	FW FIELDA	3	NR-ZERO	NEF 0	MOR MORT	F 5300 ug/L	
Salmo salar Atlantic Salmon	E U	FW FIELDA	(0 - ~ 68)		CHG	MOR MORT	F (1800-5300) ug/L	
Salmo salar Atlantic Salmon	E U	FW FIELDA	(0 - ~ 68)		CHG	MOR MORT	F (3000-45000) ug/L	

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that there is no inherent toxicity to aquatic organisms from carbonic acid calcium salt (1:1) (CAS RN 471-34-1).

Data accessed February 2017 on the OECD website:

<http://webnet.oecd.org/CCRWeb/Search.aspx>

Record for CAS RN 471-34-1:

Fish (Oncorhynchus mykiss) Acute 96 hour LC50 >100 mg/l

Fish(Oncorhynchus mykiss) Chronic 21 day NOEC >200mg/l

Aquatic invertebrates (Daphnia magna) Acute 48 hour EC50 >100 mg/l

Algae Acute 72 hour EC50, growth >42.0mg/l

As taken from BPDB, 2016.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

Arbuscular mycorrhizal (AM) development in different soil types, and the influence of AM fungal hyphae on their original soil were investigated. *Plantago lanceolata*, which can grow in soils of a very wide pH range, was grown in two closely related limestone soils and an acid soil from rock habitats. Plants were colonised by the indigenous AM fungal community. The use of compartmented systems allowed us to compare soil with and without mycorrhizal hyphae. Root colonisation of *P. lanceolata* was markedly higher in the limestone soils (30–60%) than in the acid soil (5–20%), both in the original habitat and in the experimental study. Growth of extraradical AM fungal hyphae was detected in the limestone soils, but not in the acid soil, using the signature fatty acid 16:1 ω 5 as biomass indicator. Analysis of signature fatty acids demonstrated an increased microbial biomass in the presence of AM fungal hyphae as judged for example from an increased amount of NLFA 16:0 with 30 nmol g⁻¹ in one of the limestone soils. Bacterial activity, but not soil phosphatase activity, was increased by around 25% in the presence of mycorrhizal hyphae in the first harvest of limestone soils. AM fungal hyphae can thus stimulate microorganisms. However, no effect of AM hyphae was observed on the soil pH or organic matter content in the limestone soils and the available P was not depleted.

As taken from van Aarle et al., Growth and interactions of arbuscular mycorrhizal fungi in soils from limestone and acid rock habitats, *Soil Biology and Biochemistry*, Volume 35, Issue 12, December 2003, Pages 1557-1564. Science Direct, 2010 available at www.sciencedirect.com

A greenhouse study involving four levels each of Mo and limestone indicated that without application of both Mo and lime, the cauliflower (*Brassica oleracea*, var. *botrytis* L.) crop produced very poor yields on the three soils investigated. Alfalfa (*Medicago sativa* L., var. 'Narragansett') was also a failure without added lime. At soil pH values of 5.5 and above, Mo applications increased the yield of alfalfa on Culloden sandy loam and O'Leary sandy clay loam soils, but gave no response on Acadia silty clay loam soil. Bromegrass (*Bromus inermis* Leyss., var. 'Saratoga') showed a marked response to application of lime, but very little response to application of Mo. Cauliflower yield showed a highly significant Mo $\times$ lime interaction on Culloden and O'Leary soils, where without added Mo, lime treatment up to pH 6.5 failed to show any increase in yield. Addition of different levels of lime did not change the exchangeable Mo content of any of the three soils, after growing crops.

The application of Mo or lime increased the Mo content of all crop tissues up to the highest level of applied Mo or lime. Application of lime substantially increased the Mo content of cauliflower and alfalfa while bromegrass showed less increase. There was a marked Mo $\times$ lime interaction on the Mo content of all crops, with much higher Mo contents in the plant when lime and Mo were applied together than when either treatment was applied alone. Liming to pH 6.5 without added Mo did not increase the Mo content of crops over 1.5 ppm.

In the absence of added Mo, cupping and drying of the edges of true leaves of cauliflower appeared at the early stage of growth whether or not lime had been applied to the soils. During the later stages of growth, these symptoms in most cases were associated with yellowing between the veins of leaves.

As taken from Umesh C. Gupta; Effect and Interaction of Molybdenum and Limestone on Growth and Molybdenum Content of Cauliflower, Alfalfa, and Bromegrass on Acid Soils; *American Society of Agronomy*, Vol. 33 No. 6, p. 929-932, 1969, available at <https://www.agronomy.org/publications/sssaj/abstracts/33/6/SS0330060929>

Record for carbonic acid calcium salt (1:1) (CAS RN 471-34-1):

Spec. Sci Name Spec. Common Name	Exp. Type Chem Anal.	Media Type Loc	Resp. Site Obs. Dur. (Days)	Endpoint BCF	Trend Eff %	Effect Effect Meas.	Conc (Standardized) Appl. Rate	Stat. Signif. Sig. Level

Agelaius phoeniceus Red-Winged Blackbird	19	NAT FIELDA	FD U	2	NOEL	AVO FOOD	ANOSIG <0.05	T 25 % w/w
Citrus hybrid Citrus Hybrid	RO 220	NAT LAB	DA M	4 OR		BCM CUCO		NC (386-1090) mg/kg
Citrus jambhiri Rough Lemon	RO 220	NAT LAB	DA M	4 OR		BCM CUCO		NC (386-1090) mg/kg
Citrus jambhiri Rough Lemon	RO 220	NAT LAB	DA M	4 OR		BCM CUCO		NC (380-867) mg/kg
Citrus hybrid Citrus Hybrid	MUL/ 220	NAT LAB	DA M	4 OR		POP BMAS		NC (386-1090) mg/kg
Citrus jambhiri Rough Lemon	MUL/ 220	NAT LAB	DA M	4 OR		POP BMAS		NC (386-1090) mg/kg
Citrus jambhiri Rough Lemon	MUL/ 220	NAT LAB	DA M	4 OR		POP BMAS		NC (380-867) mg/kg
Anagasta kuehniella Mediterranean Flour Moth	47.1	NONE LAB	FD U	4	LOEL	DVP DVLP	ASIG 0.05	T 1.0 %w/w
Anagasta kuehniella	(55.5 -	NONE	FD	4	LOEL	DVP	SIG	T 2.0 %w/w

Mediterranean Flour Moth	60.4)	LAB	U			DVLP	0.05	
Anagasta kuehniella	>= 47.1	NONE	FD	4	LOEL	REP	ASIG	T 1.0 %w/w
Mediterranean Flour Moth		LAB	U			FCND	0.05	
Anagasta kuehniella	>= 55.5	NONE	FD	4	LOEL	REP	ASIG	T 1.0 %w/w
Mediterranean Flour Moth		LAB	U			FCND	0.05	
Anagasta kuehniella	(55.5 - 60.4)	NONE	FD	4	NOEL	DVP	NOSIG	T 1.0 %w/w
Mediterranean Flour Moth		LAB	U			DVLP	0.05	
Apis mellifera	0.042	NONE	HS	2	NOEL	AVO	ANOSIG	T 10 g/L
Honey Bee		FIELDN	U			CHEM		
Apis mellifera	0.167	NONE	HS	2	NOEL	AVO	ANOSIG	T 10 g/L
Honey Bee		FIELDN	U			CHEM		

As taken from the EPA ECOTOX database.

10.5. All other relevant types of ecotoxicity

EPISuite provides the following information for CAS RN 471-34-1:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-2.4456 days (HL = 0.003584 days)
Log BCF Arnot-Gobas method (upper trophic):	-0.049 (BCF = 0.8931)

Log BAF Arnot-Gobas method (upper trophic):	-0.049 (BAF = 0.8931)
log Kow used:	-2.12 (estimated)

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that the bioaccumulative potential of carbonic acid calcium salt (1:1) (CAS RN 471-34-1) in the environment has not been determined.

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

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13. Last audited

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**SPECIFICATIONS FOR THE IDENTITY AND
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TOXICOLOGICAL EVALUATION:
SOME ANTIMICROBIALS, ANTIOXIDANTS,
EMULSIFIERS, STABILIZERS,
FLOUR-TREATMENT AGENTS,
ACIDS, AND BASES**

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JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Rome, 13-20 December 1965

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Monographs containing specifications for identity and purity, biological data and toxicological evaluation will be issued later by FAO and WHO in separate documents entitled :

Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Antimicrobials and Antioxidants.

FAO Nutrition Meetings Report Series No. 40A

WHO/Food Add./66.29 (in preparation).

Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Flour-Treatment Agents.

FAO Nutrition Meetings Report Series No. 40B

WHO/Food Add./66.30 (in preparation).

Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Acids and Bases.

FAO Nutrition Meetings Report Series No. 40C

WHO/Food Add./66.31 (in preparation).

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EMULSIFIERS, STABILIZERS, FLOUR-TREATMENT
AGENTS, ACIDS AND BASES**

**Ninth Report of the Joint FAO/WHO Expert Committee
on Food Additives**

INTRODUCTION

A Joint FAO/WHO Expert Committee on Food Additives met in Rome from 13 to 20 December 1965. The meeting was opened by Dr M. Autret, Director, Nutrition Division, FAO. Dr O. E. Fischnich, Assistant Director-General, Technical Department, FAO, addressed the Committee on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and of the World Health Organization. Professor R. Truhaut and Professor J. F. Reith were unanimously elected Chairman and Vice-Chairman respectively. Professor A. C. Frazer agreed to serve as Rapporteur.

As a result of the recommendations of the Joint FAO/WHO Conference on Food Additives held in September 1955,¹ eight Joint FAO/WHO Expert Committees on Food Additives have met and issued the following reports : " General Principles Governing the Use of Food Additives : First Report ",² " Procedures for the Testing of Intentional Food Additives to Establish their Safety for Use : Second Report ",³ " Specifications for Identity and Purity of Food Additives (Antimicrobial Preservatives and Antioxidants) : Third Report ",⁴ " Specifications for Identity and Purity

¹ *FAO Nutrition Meetings Report Series*, 1956, No. 11 ; *Wld Hlth Org. techn. Rep. Ser.*, 1956, 107.

² *FAO Nutrition Meetings Report Series*, 1957, No. 15 ; *Wld Hlth Org. techn. Rep. Ser.*, 1957, 129.

³ *FAO Nutrition Meetings Report Series*, 1958, No. 17 ; *Wld Hlth Org. techn. Rep. Ser.*, 1958, 144.

⁴ These specifications were subsequently revised and published as *Specifications for identity and purity of food additives. Vol. I. Antimicrobial preservatives and antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962.

of Food Additives (Food Colours) : Fourth Report ”,¹ “ Evaluation of the Carcinogenic Hazards of Food Additives : Fifth Report ”,² “ Evaluation of the Toxicity of a Number of Antimicrobials and Antioxidants : Sixth Report ”,³ “ Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Emulsifiers, Stabilizers, Bleaching and Maturing Agents : Seventh Report ”,⁴ “ Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Food Colours and Some Antimicrobials and Antioxidants : Eighth Report ”.⁵

This meeting was convened on recommendations made in the previous reports of the Joint FAO/WHO Expert Committee on Food Additives. Its terms of reference were to draw up specifications for and to make a toxicological evaluation of some food additives belonging to the classes of antimicrobials, antioxidants, emulsifiers, stabilizers and flour-treatment agents not considered at earlier meetings. The Committee was also asked to re-evaluate some food additives already considered and to review their specifications or toxicological evaluation in the light of new biological and chemical data.

Some acids and bases were also considered at this meeting at the request of the Second Joint FAO/WHO Conference on Food Additives.⁶

Relationship to the Codex Alimentarius Commission

The relationship between the Joint FAO/WHO Expert Committee on Food Additives and the Codex Alimentarius Commission will in future be of paramount importance for the functioning of the Expert Committee. The links between these bodies are described in Annex 4, and a flowsheet (Annex 5) is provided that depicts the stages involved in the submission of a food additive for assessment and its subsequent consideration and evaluation.

¹ These specifications were subsequently revised and published as *Specifications for identity and purity of food additives. Vol. II. Food Colours*, Rome, Food and Agriculture Organization of the United Nations, 1963.

² *FAO Nutrition Meetings Report Series*, 1961, No. 29 ; *Wld Hlth Org. techn. Rep. Ser.*, 1961, 220.

³ *FAO Nutrition Meetings Report Series*, 1962, No. 31 ; *Wld Hlth Org. techn. Rep. Ser.*, 1962, 228.

⁴ *FAO Nutrition Meetings Report Series*, 1964, No. 35 ; *Wld Hlth Org. techn. Rep. Ser.*, 1964, 281.

⁵ *FAO Nutrition Meetings Report Series*, 1965, No. 38 ; *Wld Hlth Org. techn. Rep. Ser.*, 1965, 309.

⁶ *FAO Nutrition Meetings Report Series*, 1963, No. 34 ; *Wld Hlth Org. techn. Rep. Ser.*, 1963, 264.

1. GENERAL CONSIDERATIONS

1.1 Principles

The Committee agreed to base its considerations on the general principles set out in the first, second, fifth and sixth reports.

1.2 Publication of experimental results

The Committee wished to re-emphasise the great value of publishing experimental results. Work that is published in the scientific literature is subject to scientific examination, criticism, refutation or confirmation. Unpublished reports, on the other hand, are not necessarily submitted to this scrutiny. For this reason, when considering scientific information, much greater weight will usually be given to published than to unpublished work. It is an accepted principle that the Joint FAO/WHO Expert Committee prefers published information as the basis for its decisions; only under exceptional circumstances will the Committee use unpublished information; confidential reports will not be considered. In the last year or two, a considerable amount of information on the biological effects of food additives has been published, and much of this work is referred to in this report.

1.3 Supply of information to FAO and WHO

The policy with regard to the supply of information to FAO and WHO is as follows.

Preference will always be given to the consideration of published work, copies of which should be submitted. If work can be submitted only in unpublished form, this must be submitted as an official document sponsored by a scientist of standing; these documents will be placed on file at WHO (toxicological data) or FAO (data on specifications) and will be available for consultation by any scientist who is entitled to use the facilities of FAO or WHO. Photostats or other copies of appropriate sections of these documents will be supplied to scientists on request, without further reference to the investigators or to those responsible for the original submission. Under no circumstances will confidential documents be accepted or consulted.

2. SPECIFICATIONS

2.1 Removal of substances from the agenda

Certain substances that had been included on the agenda did not appear to the Committee to be used to a significant extent as food additives and were not considered further. These were : the calcium and sodium salts of methyl, ethyl, propyl and butyl *p*-hydroxybenzoate, calcium benzoate, sodium sorbate, sodium dithionite, nitrofurazone, 2,4,5-trihydroxybutyrophenone, ethylcellulose and gluconic acid. In other cases, adequate chemical information on the substance was not available. Specifications were not prepared for these substances : namely, stearyl citrate, diacetyl-tartaric acid esters of monoglycerides and diglycerides, hydroxypropyl-methylcellulose, propylene glycol alginate, propylene glycol monostearate and tannic acids. Details are mentioned under the appropriate section, and consideration of these substances, as well as of brominated vegetable oils, was postponed.

2.2 Limitation of selenium content in sulfur dioxide and related substances

The Committee was asked to reduce the limits of selenium in sulfur dioxide and related compounds from 30 ppm to 10 ppm. Since no toxicological problems were involved, and since it was not known whether all manufacturers could achieve the revised limit or whether the available test would be effective in controlling the selenium content at the lower level, the Committee decided that the maximum permitted level of selenium in sulfur dioxide and related substances should remain at 30 ppm. However, it was recommended that enquiries should be instituted by FAO to find out whether a lower level might be adopted at a later date.

2.3 Formulation of food additives

A food additive may be marketed as a formulated preparation consisting of a mixture of the main ingredient with a vehicle and possibly other substances. The specifications elaborated by the Committee refer only to the main ingredient as a pure substance, with the exception of acetone peroxide, which is diluted with starch to control its explosive property. Formulation does not affect the toxicological evaluation, provided that the substances added are known to be acceptable and that they do not alter the absorption or metabolism of the food additive in such a way that the biological data are thereby invalidated.

3. BIOLOGICAL DATA

The Committee agreed that the monographs dealing with the evaluation of the biological data should be drafted along the lines adopted in the previous reports.

3.1 Biochemical and metabolic studies

The Committee emphasised the importance of metabolic and biochemical studies in the investigation of the biological effects of a food additive. It may be possible to dispense with elaborate long-term studies if it can be convincingly shown that the substance is not absorbed or is broken down completely before absorption into well-known substances that are generally recognized as having no deleterious action. In any case, a proper understanding of the changes that the food additive may undergo in the food, in the gastro-intestinal tract or in the body is necessary for the full interpretation of the biological and toxicological data.

3.2 Zones of acceptability

The Committee decided to retain the method of expressing the daily acceptable intake level that had been adopted in earlier reports and, in appropriate cases, to continue to divide the overall zone of acceptability into two parts: unconditional and conditional. It was felt that any failure to understand this system was not due to inadequate explanation in earlier reports. The method was discussed in detail in the seventh report.¹ The Committee did agree, however, that footnotes or other devices should be used to ensure that the meaning of unconditional and conditional zones of acceptability is made clear in any table summarizing the values agreed by the Committee, without the necessity for referring to the text.

In previous reports, acceptable daily intake has usually been expressed as milligrams of the substance in question per kilogram of body weight. There are, however, certain food additives that are more appropriately limited in terms of levels of treatment; for example, flour-treatment agents. There are also certain additives that are common components of food or normal body constituents; in such cases it is unreasonable to set specific limits. The appropriate method of expressing acceptable levels of use must be decided in each case.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1964, **281**, 10.

3.3 Grouping of related food additives

As a number of food additives are closely related chemically and toxicologically, the Committee adopted a system of grouping of food additives. The acceptable daily intake level is expected to cover all the specified members of the group that may be included in the diet. In some cases, a given food additive may be related to two groups, in which case the level in the diet must not exceed the maximum acceptable level for either group. The problem is not as complicated as it may appear at first sight, since many of the substances in a group of additives are likely to be used as alternatives to each other.

3.4 Request for further work

Previous monographs have often listed further work that seemed to be desirable. However, it is only in relatively few cases that the studies requested have been done, although in some cases the work may be urgently needed in the interests of the consumer. The Committee therefore agreed to request, in the monographs, only work that is urgently needed in the interests of safety. All other suggestions for further work that may seem desirable, but not so urgently needed, will be included under the heading "Comments on experimental studies reported". The Committee draws the attention of manufacturers and users of food additives to the fact that, in future, if a statement about further work being required is included in a monograph, this matter should be given urgent attention. If the additive in question is one that is already in use, its continued use will be supported by the Committee only if the further work required has been carried out and the results justify continuance of the use of the additive. If no action is taken to provide the further evidence that is required, it will be assumed that neither the manufacturers nor the users are interested in continuing the use of the additive. In this case, the Committee may well decide to recommend the prohibition of its use. If the food additive is a new substance not yet in use and if further work has been required, a conditional intake level may be established for a limited period to allow the results of this additional work to be submitted and studied.

3.5 References

Authors who have submitted papers relevant to a given monograph but who are not mentioned in it may rest assured that their work has been consulted. In the interest of brevity, however, the bibliographies of some monographs may be restricted to the more recent or more extensive investigations in the field in question. The pioneering achievements of the earlier workers in these fields are fully appreciated.

4. COMMENTS ON SUBSTANCES ON THE AGENDA

4.1 Antimicrobials and antioxidants

The monographs on these food additives will appear in a publication to be entitled "Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Antimicrobials and Antioxidants"¹ and the recommended acceptable daily intakes are summarized in Annex 1.

The biological data on butyl *p*-hydroxybenzoate were insufficient to allow an evaluation to be made. Specifications for this substance were drawn up.

It was not possible to establish satisfactory specifications for the calcium and sodium salts of the methyl, ethyl, propyl and butyl esters of *p*-hydroxybenzoic acid.

No information was available on the use of calcium benzoate and sodium dithionite in food technology. Sodium sorbate was considered to be too unstable for satisfactory use in food. For these various reasons further study of these substances was postponed.

Because nitrofurazone is used in human therapeutics and for toxicological reasons the Committee considered that its use as a direct additive to food is highly undesirable and recommended that it should not be used for this purpose. It was understood that its use as a food additive was likely to be terminated by the end of 1965. No further consideration was therefore given to this substance.

There was no objection to the use of the calcium disodium salt of ethylenediaminetetra-acetic acid (EDTA), but the Committee considered that the use of the disodium salt, which will chelate calcium, requires careful control. There is no objection to its use in food provided that the amount added is accurately assessed and corresponds to the amount of calcium to be chelated. The end-product in the food will then be calcium disodium EDTA; an excessive amount of disodium EDTA in food would be highly undesirable.

The Committee agreed to the use of hydrogen peroxide in milk only as an emergency measure in those places where other methods of microbiological control, such as pasteurization, are not available; the treatment of milk with hydrogen peroxide may be justified if the alternatives are no milk or infected milk. The Committee strongly recommended that every effort should be made to find other methods of milk treatment to deal with problems of this nature. The Committee also understood that hydrogen

¹ *FAO Nutrition Meetings Report Series*, No. 40A; WHO/Food Add./66.29 (in preparation).

peroxide is used as a food additive for other special purposes in some countries. Provided that these special uses do not contravene the general principle that a food additive must not be used to mislead the consumer as to the nature and quality of food,¹ it is suggested that they receive consideration by a future meeting of the Committee.

A specification was elaborated for potassium metabisulfite. This substance contributes to the total dietary intake of sulfur dioxide, which should not exceed that recommended in the sixth report.²

Specifications, toxicological evaluation and acceptable daily intake levels for potassium benzoate, propionic acid and calcium and potassium sorbate will appear in the monographs.

Diethyl pyrocarbonate has been studied in detail from the biochemical and metabolic aspects, and the end-products of its breakdown, as well as products of interaction with constituents of beverages, are substances that are acceptable at the concentrations present and in the quantities likely to be consumed. It was considered advisable to recommend a maximum level of treatment of beverages rather than an acceptable daily intake.

Sufficient information was not available to allow for the establishment of a specification for stearyl citrate, which is an antioxidant synergist. Although the toxicological work on this substance was done some years ago, it was done thoroughly. The Committee did not suggest that the use of stearyl citrate should be discontinued, but did require that a satisfactory specification for its use should be sent to FAO as soon as possible and preferably before the next meeting (October 1966). Long-term studies using modern techniques were considered desirable, but the Committee did not insist on these studies as an urgent requirement.

The Committee understood that 2,4,5-trihydroxybutyrophenone is no longer marketed as a food additive and it was therefore given no further consideration.

4.2 Emulsifiers and stabilizers

Diacetyltartaric acid esters of mono- and di-glycerides had been thoroughly studied from a biochemical and metabolic standpoint. It was clear that complete hydrolysis of these substances occurred, giving rise to acetic acid, tartaric acid, and mono- and di-glycerides. All these substances occur in the diet in amounts in excess of those that might arise from the use of the mixed esters as a food additive. The Committee agreed that the use of this additive could be accepted without limitation. Unfortunately, no

¹ *FAO Nutrition Meetings Report Series*, 1957, No. 15 ; *Wld Hlth Org. techn. Rep. Ser.*, 1957, **129**, 5.

² *FAO Nutrition Meetings Report Series*, 1962, No. 31 ; *Wld Hlth Org. techn. Rep. Ser.*, 1962, **228**, 96.

specification could be elaborated because of lack of an assay method. The Committee stressed the importance of supplying to FAO all the information required for the elaboration of specifications at the same time that the biological data are sent to WHO (see flowsheet, Annex 5). It is hoped that this substance can be further considered at the next meeting of the committee (in October 1966).

The use of brominated vegetable oils presents a problem. These brominated oils are used to adjust the density of essential oil contained in certain beverages and to produce a clouding effect. The quantity of brominated oils used is likely to be small. Nevertheless, large quantities of beverages may be consumed over long periods of time, and no evidence was available on the possible accumulation of the brominated fatty acids in the body lipids, with subsequent release of bromine. The Committee was unable to evaluate brominated oils, since evidence from appropriate long-term studies, with special reference to possible cumulative effects, was not available. The results of such studies should be submitted to WHO if the use of brominated oils as food additives is to be continued.

Ethylcellulose is a packaging material and not a food additive. It was therefore not considered.

Hydroxypropylmethylcellulose has been investigated extensively. However, there appears to be some doubt as to whether the various substances that have been studied toxicologically were identical. The Committee recommended that the specifications for the different forms should be effectively correlated; no evaluation can be made until this has been done.

Sufficient information was not available to allow a specification for the propylene glycol esters of alginic acid to be elaborated.

No satisfactory information, either chemical or toxicological, was available on tannic acids. Further study of these substances was therefore postponed. The Committee appreciated the complexity of the problem but considered that the information required on tannic acids should be provided as soon as possible.

The additive "propylene glycol monostearate and palmitate" was considered, but no specifications were prepared because of lack of appropriate chemical information.

4.3 Flour-treatment agents

The monographs on these substances will appear in a publication entitled "Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Flour-Treatment Agents";¹ some information is given in summary form in Annex 2.

¹ *FAO Nutrition Meetings Report Series*, No. 40B; WHO/Food Add./66.30 (in preparation).

Differences in rate of oxidation may have considerable importance in bread technology and may even have some relevance to labour utilization. It is for this reason that some new agents are being brought forward for consideration that are much more rapid in action than those at present available. Such a rapidly acting agent is of particular value in the modern methods of bread-making that do not involve bulk fermentation. Since the main toxicological and nutritional considerations are concerned with treated flour rather than with the food additive itself, the Committee followed the procedure adopted in the seventh report for benzyl peroxide, chlorine dioxide, and potassium bromate¹ and recommended levels of flour treatment for this group of additives instead of setting an acceptable level of daily intake.

Specifications are essential before any evaluation of the biological data is possible. In the case of chlorine and nitrogen oxides, satisfactory specifications were not available to the Committee. However, both these additives have been used for the treatment of flour for many years. The Committee understood that the use of nitrogen oxides has greatly declined in recent times. Lack of adequate information prevented the Committee from advising the prohibition of the use of nitrogen oxides in flour treatment at this time. If their use is to be continued, however, further information would be required on the possible formation of nitrosamines and the effect of long-term ingestion of bread made with flour treated at various levels with nitrogen oxides. The Committee recommended that urgent action be taken to obtain information about possible nitrosamine formation so that this substance could be reconsidered at the next meeting (October 1966). The Committee also stated that the results of long-term studies as indicated should be made available to it by the end of 1969.

The Committee understood that chlorine is used for the treatment of flour for special purposes, such as cake and biscuit manufacture. Since the amount of chlorine-treated flour involved is probably not more than a small proportion of total flour consumption, the Committee did not recommend the prohibition of the use of chlorine for flour treatment at this time. Nevertheless, if chlorine treatment of flour is to continue, long-term studies using appropriate products made from flour treated with chlorine at various levels will be needed. The results of these studies should be submitted to WHO as soon as possible and, in any case, not later than the end of 1969. When these biological studies are done, it will, of course, be necessary to have adequate specifications for the gases used in flour treatment.

A specification was prepared for acetone peroxides, but no adequate biological information was available. Since the residues from acetone

¹ *FAO Nutrition Meetings Report Series*, 1964, No. 35; *Wld Hlth Org. techn. Rep. Ser.*, 1964, 281, 155, 159, 164.

peroxides are likely to be different from those from other peroxides, it was not possible to predict what biological effects they might produce. The biological evaluation of this substance was therefore postponed.

Biological information on calcium peroxides and calcium stearyl-2-lactylate was also inadequate, and further study of these additives was postponed.

Consideration was given to the use of potassium or calcium iodate as a flour-treatment agent. At the level of treatment proposed, it seemed likely that a considerable increase in the daily iodine intake would result. Although 10 ppm of iodide or iodate is the level that is commonly used for iodized table salt, there is a great difference between the daily intake of table salt and the daily intake of bread. While there may be some differences of opinion about the possible hazards of increasing the daily iodine intake to perhaps five or ten times the level usually recommended (100-200 $\mu\text{g/day}$), the Committee considered that the use as a food additive for the treatment of a staple, such as flour, of a substance having such physiological significance and potency as iodate is highly undesirable. The Committee therefore recommended that iodates should not be used for flour treatment.

Although ammonium and potassium persulfates have a long history of use, there are insufficient toxicological data for an acceptable treatment level for flour to be estimated.

Specifications, toxicological evaluation and acceptable levels of flour treatment for azodicarbonamide and stearyl tartrate can be found in the monographs.

4.4 Acids and bases

The monographs on these substances will appear in a publication entitled "Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Acids and Bases";¹ a summary of the information is given in Annex 3.

A group of acids that are well-known substances involved in intermediary metabolism was discussed. Included were acetic, lactic and malic acids. Propionic acid (see section 4.1) might also have been conveniently added to this list. It was agreed that no limit need be prescribed for any of these acids when used as food additives, on the assumption that only reasonable quantities of them are likely to be used. The following points were, however, considered to be relevant to the food-additive use of these acids.

¹ *FAO Nutrition Meetings Report Series*, No. 40C; WHO/Food Add./66.31 (in preparation).

As far as acetic acid is concerned, its acid taste is likely to be a safeguard against its ingestion in excessive quantities.

Fumaric acid has been shown to cause testicular damage in animals when administered in high dosage, especially if given parenterally. However, this observation was not regarded as pertinent when fumaric acid is used only in small quantities as a flavouring agent.

Both lactic and malic acids may occur in the D(−) form as well as in the L(+) form. In small infants, it has been shown that the enzyme responsible for converting the D(−) to the L(+) form is relatively deficient; consequently D(−) lactic acid may be toxic in early infancy. The Committee recommended that D(−) lactic acid should not be added to the diet of very young infants, and that, for adults, the use of the D(−) forms of lactic and malic acids should be restricted, as indicated in Annex 3 of the present report and in the monographs. In the case of malic acid, there is also the danger of contamination with maleic acid, which is a toxic substance. The Committee recommends that the maleic acid content of malic acid should not exceed 0.05%.

No details were available on the use of gluconic acid as a food additive, and it was not further considered.

Hydrochloric acid is not regarded as a toxic substance in the concentrations that are used in food technology. A specification for hydrochloric acid is included in the monograph as a safeguard against toxic impurities.

The specification, toxicological evaluation and acceptable daily intake of adipic acid are given in the monograph on this substance.

Monocalcium phosphate is used in flour. The Committee had no objection to its use, provided that this is taken into account in assessing the overall dietary load of calcium and phosphate; a limit for the latter has been recommended.

The bases used in food technology are required for pH adjustment. The amounts and concentrations used are not likely to have any toxicological significance. The Committee placed no restriction on the food-additive use of the bases listed, provided that the contribution made to the dietary load of sodium, potassium, calcium and magnesium is assessed and considered to be acceptable.

4.5 Re-evaluation

The Committee was asked to re-evaluate a number of substances. The decisions are given in Annex 1. Details on these substances will appear in a publication to be entitled "Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some Antimicrobials

and Antioxidants".¹ Successive Joint FAO/WHO Expert Committees on Food Additives have considered and reconsidered many additives belonging to these two categories. A table will be included in the above-mentioned publication indicating in which document the specification for and toxicological evaluation or re-evaluation of each substance can be found.

In the case of butylated hydroxytoluene (BHT), there was an excellent response to the request, in the sixth report,² for additional information, and a large amount of further published evidence became available to the Committee. After consideration of these data, the Committee recommended that BHT should be treated similarly to butylated hydroxyanisole (BHA), and that the acceptable daily intake already approved for BHA should now apply to both BHA and BHT, which should be considered together as a food-additive group.

A considerable amount of further work is being done on hexamethylenetetramine, and it is understood that much of this will be completed by the end of 1966 or thereabouts. The Committee recommended that no further action should be taken with regard to the food-additive use of hexamethylenetetramine until this further information is available. The Committee will evaluate this substance as soon as possible.

It was suggested that sorbitol might be accepted without an indication of an acceptable daily intake. The Committee agreed with this suggestion. The earlier decision recorded in the seventh report³ is revised accordingly.

Further evidence was presented on sulfur dioxide and sulfites and the Committee was asked to re-examine the limits proposed in the sixth report.⁴ The Committee studied this further information and recommended that the limits proposed in the sixth report should remain unchanged. The details of this new information are given in the monograph.

In accordance with the principle of grouping chemically and toxicologically related food additives (p. 10), the following substances were considered and grouped with related substances :

- (a) methyl, ethyl and propyl *p*-hydroxybenzoates
- (b) the calcium, potassium and sodium salts of propionic acid
- (c) sorbic acid
- (d) sulfur dioxide, sodium sulfite, sodium metabisulfite and sodium hydrogen sulfite.

¹ *FAO Nutrition Meetings Report Series*, No. 40A ; WHO/Food Add./66.29 (in preparation).

² *Wld Hlth Org. techn. Rep. Ser.*, 1962, **228**, 45.

³ *Wld Hlth Org. techn. Rep. Ser.*, 1964, **281**, 101.

⁴ *Wld Hlth Org. techn. Rep., Ser.* 1962, **228**, 96.

5. RECOMMENDATIONS TO FAO AND WHO

The second Joint FAO/WHO Conference on Food Additives recommended that the Joint FAO/WHO Expert Committee on Food Additives should serve as the advisory body to the Codex Alimentarius Commission on matters relating to specifications and toxicological evaluation of food additives. It is essential that the Committee meets annually in order that it may keep up with the progress made, through the said Commission, in the Food Standards Program. So that the criteria used for setting acceptable daily intakes may be reviewed in the light of new scientific knowledge at such meetings, WHO should further consider convening, as soon as possible, an additional meeting to discuss procedures for investigating food contaminants and intentional food additives in order to establish their safety to the consumer.

6. RECOMMENDATIONS TO THE CODEX COMMITTEE ON FOOD ADDITIVES

The Joint FAO/WHO Expert Committee on Food Additives would like the Codex Committee on Food Additives to consider, when establishing lists of food additives for consideration by future Joint Committees, whether they could recommend substances belonging to the same group(s) of food additives rather than substances belonging to a number of different groups. The group(s) recommended for consideration should, if possible, contain all the substances belonging to it (or them). This would greatly facilitate the work of the Joint FAO/WHO Expert Committee on Food Additives.

Furthermore, each additive recommended by the Codex Committee for consideration should be accompanied by data on the technological justification and the levels of use in the foods to which it is added. If an additive is recommended, it is assumed that biological data have already been sent to WHO and information from which a chemical specification can be elaborated has been sent to FAO.

Annex 1

**ACCEPTABLE DAILY INTAKES FOR MAN
OF SOME ANTIMICROBIALS AND ANTIOXIDANTS**

Compounds considered	Specifications available	Over-all daily intake zone ^a (mg/kg body-weight)	
		Unconditional	Conditional
Benzoic acid	Yes	0-5 ^b	5-10 ^b
Sodium benzoate	Yes		
Potassium benzoate	Yes		
Methyl <i>p</i> -hydroxybenzoate	Yes	0-2 ^c	2-7 ^c
Ethyl <i>p</i> -hydroxybenzoate	Yes		
Propyl <i>p</i> -hydroxybenzoate	Yes		
Butyl <i>p</i> -hydroxybenzoate	Yes	Decision postponed (p. 11)	
Butylated hydroxyanisole	Yes	0-0.5 ^d	0.5-2.0 ^d
Butylated hydroxytoluene	Yes		
Disodium EDTA	Yes	0-1.25 ^e	1.25-2.5 ^e
Calcium disodium EDTA	Yes		
Hexamethylenetetramine	No	Decision postponed (p. 17)	
Hydrogen peroxide	Yes	Decision postponed (p. 11)	
Calcium propionate	Yes	0-10 ^f	10-20 ^f
Potassium propionate	No		
Sodium propionate	Yes		
Propionic acid	Yes		
Calcium sorbate	Yes	0-12.5 ^g	12.5-25 ^g
Potassium sorbate	Yes		
Sorbic acid	Yes		
Sorbitol	Yes	Not limited	
Sulfur dioxide	Yes	0-0.35 ^h	0.35-1.5 ^h
Sodium sulfite	Yes		
Sodium metabisulfite	Yes		
Potassium metabisulfite	Yes		
Sodium hydrogen sulfite	Yes		
Antimicrobial for certain beverages			
Diethyl pyrocarbonate	Yes	0-300 (p. 12)	

^a The first part of the acceptable daily intake zone has been termed the unconditional zone of acceptability, and this represents levels of use that are effective technologically, at least for some purposes, and can be safely employed without further expert advice. The second part consists of a conditional zone of acceptability and represents levels of use that can be employed safely but at which it is thought desirable that some degree of expert supervision and advice should be readily available.

^b As sum of benzoic acid and sodium and potassium benzoate (calculated as benzoic acid).

^c As sum of methyl, ethyl and propyl esters of *p*-hydroxybenzoic acid.

^d As sum of butylated hydroxytoluene and butylated hydroxyanisole.

^e As calcium disodium EDTA. The use of disodium EDTA is recommended only for accurately chelating calcium.

^f As sum of propionic acid and calcium, sodium and potassium propionate (calculated as propionic acid).

^g As sum of sorbic acid and calcium and potassium sorbate (calculated as sorbic acid).

^h As sum of sulfur dioxide, sodium sulfite, sodium and potassium metabisulfite and sodium hydrogen sulfite (calculated as SO₂).

Annex 2

ACCEPTABLE TREATMENT LEVELS FOR SOME
FLOUR-TREATMENT AGENTS

Compounds considered	Specifications available	Acceptable levels of treatment (in parts per million)
Chlorine	No	No level set (p. 14)
Nitrogen oxides	No	No level set (p. 14)
Acetone peroxides	Yes	No level set (p. 14)
Calcium peroxides	Yes	No level set (p. 15)
Calcium iodate	Yes ^a	Use not recommended (p. 15)
Potassium iodate	Yes ^a	Use not recommended (p. 15)
Ammonium persulfate	Yes	No level set (p. 15)
Potassium persulfate	Yes	No level set (p. 15)
Azodicarbonamide	Yes	0-45
Stearyl tartrate	Yes	0-500
Calcium stearyl-2-lactylate	Yes	No level set (p. 15)

^a Identification tests only.

Annex 3

ACCEPTABLE DAILY INTAKES FOR MAN
OF SOME ACIDS AND BASES

Compounds considered	Specification available	Over-all daily intake zone ^a (mg/kg body-weight)	
		Unconditional	Conditional
Acids			
Acetic acid	Yes	Not limited	
Adipic acid	Yes		0-5
Calcium phosphate, monobasic	Yes	Not limited ^b	
Fumaric acid	Yes	0-6	6-10
Hydrochloric acid	Yes	Not limited	
DL-Lactic acid	Yes		0-100 ^c
DL-Malic acid	Yes		0-100 ^d
Bases			
Carbonates of ammonium, calcium, magnesium, potassium and sodium	Yes	Not limited ^e	(p. 16)
Hydrogen carbonates of ammonium, potassium and sodium	Yes		
Hydroxides of ammonium, calcium, magnesium, potassium and sodium	Yes		
Oxides of calcium and magnesium	Yes		

^a The first part of the acceptable daily intake zone has been termed the unconditional zone of acceptability and this represents levels of use that are effective technologically, at least for some purposes, and can be safely employed without further expert advice. The second part consists of a conditional zone of acceptability and represents levels of use that can be employed safely, but at which it is thought desirable that some degree of expert supervision and advice should be readily available.

^b Subject to limits of phosphorus load given in the seventh report (*Wld Hlth Org. techn. Rep. Ser.*, 1964, 281, 31).

^c Refers to content of D(—)-lactic acid.

^d Refers to content of D(—)-malic acid. (See also p. 16.)

^e Provided that use is in accordance with good manufacturing practice.

Annex 4**RELATIONSHIP BETWEEN THE JOINT FAO/WHO EXPERT
COMMITTEE ON FOOD ADDITIVES AND THE CODEX
ALIMENTARIUS COMMISSION**

The Joint FAO/WHO Expert Committee on Food Additives consists of scientists who are chosen because of their knowledge and experience in the field under discussion ; they act as independent experts, and their decisions are based on scientific considerations alone. The FAO/WHO Codex Alimentarius Commission¹ consists of representatives of the governments of member countries ; they have a direct link with governments and with governmental agencies. The ultimate objective of the FAO/WHO Food Standards Program is to obtain agreement by governments, through the Codex Alimentarius Commission, on the acceptability and usage of food and food additives in order to ensure the safety of the consumer and to facilitate international trade. The FAO/WHO Expert Committee on Food Additives has been working for more than ten years on the elaboration of specifications for important food additives and on the evaluation of biological information on the effects of these food additives, leading to recommendations about safe usage. The Codex Alimentarius Commission and its subsidiary bodies, which have been in existence since 1963, have now become regular parts of FAO and WHO. This development is welcome, since it should greatly assist in the implementation of the scientific advice that has been put forward in the reports of the FAO/WHO Expert Committee. The Codex Alimentarius Commission has established a Codex Committee on Food Additives, which is the responsibility of the Netherlands Government and is under the Chairmanship of Professor M. J. L. Dols, who is also the current Chairman of the Codex Alimentarius Commission. This Codex Committee on Food Additives will apply the recommendations of the FAO/WHO Expert Committee to the practical problems of food and nutrition in the various member countries. Thus, the Codex Committee will examine such problems as the effects of using the appropriate food additives in different food commodities, technological problems concerned with the production of food or the use of food additives in accordance with the recommendations of the FAO/WHO Expert Committee, the total load of individual food additives or food-additive groups and the problems that may arise nationally

¹ Codex Alimentarius Commission (1965) *Report of the Third Session ...*, Rome (Alinorm 65/30).

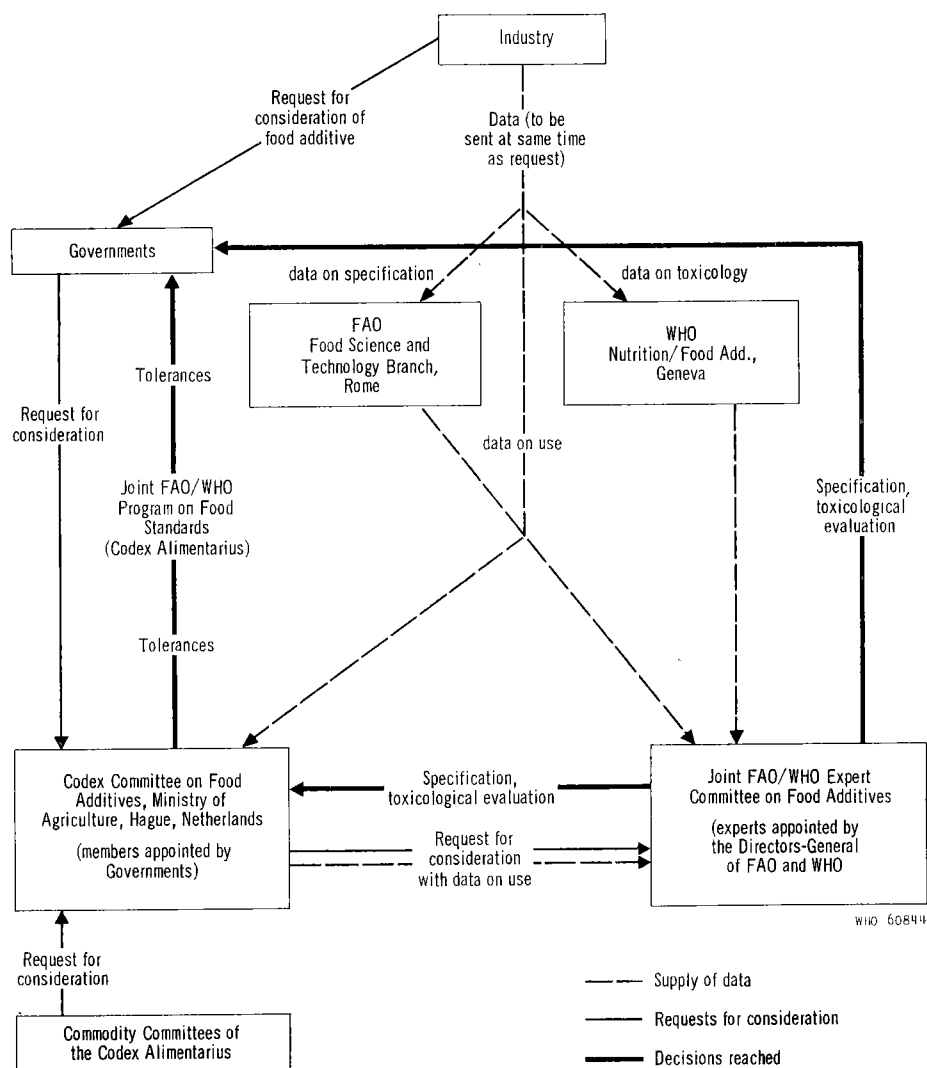
as a result of disagreement of a dietary or legal nature. When the Codex Committee on Food Additives has reached decisions with regard to the application of this information to food production and to nutrition, these decisions regarding specific tolerances for particular foods will be communicated to the appropriate Codex Commodity Committee, and in due time the information will be included in the appropriate food standards published by the Codex Alimentarius Commission. The Codex Alimentarius is also expected to contain the main details about each food additive, and these will be based on the information supplied by the FAO/WHO Expert Committee. This procedure for the handling of these matters has been approved by both FAO and WHO and it has already come into operation.

The FAO/WHO Expert Committee will continue to elaborate specifications, to evaluate biological data and to make recommendations about the acceptable daily intakes for man. This information will be published in reports as previously, but it will also be transmitted to the Codex Committee on Food Additives for their attention.

In the past, it has proved difficult to select the food additives most deserving of consideration by the Expert Committee. Sometimes substances have been included on the agenda for which there appeared to be little demand or for which chemical and biological information was inadequate; at other times, important new additives that had been extensively studied scientifically did not appear on the agenda. The introduction of the Codex Committee on Food Additives, with its direct association with governments and governmental agencies in each of the member countries and through them, presumably, with the chemical and food industries in these countries, should make it easier to determine which food additives require consideration by the FAO/WHO Expert Committee. It will, however, be necessary to ensure that there is adequate publicity about the activities of FAO and WHO in the field of food standards and the safe use of food additives, that sufficient contact with the chemical and food industries in member countries is achieved and maintained, and that adequate notice is given to those concerned with the introduction of a new additive to ensure that full information is made available at the appropriate time, preferably in the form of published papers. The FAO/WHO Expert Committee has already reported on a considerable number of the more important food additives. This is fortunate, as it makes it possible for the Codex Committee on Food Additives to proceed with the application of the recommendations contained in the FAO/WHO Expert Committee's reports. However, there are still many food additives that require consideration by the FAO/WHO Expert Committee, further new food additives will be introduced, and new uses will be found for existing additives; for these and other reasons re-evaluation is likely to be requested quite frequently.

Thus, the FAO/WHO Expert Committee still has a great deal of work to do, and this situation is likely to continue for many years. In all its future deliberations, it will be of great importance that the closest possible liaison be maintained between the FAO/WHO Expert Committee and the Codex Committee on Food Additives ; frequent exchange of information in both directions is essential for the effective working of the procedure adopted.

FLOW DIAGRAM FOR INTERNATIONAL ACCEPTANCE OF FOOD ADDITIVES



I U C L I D

D a t a s e t

Existing Chemical	Substance ID: 471-34-1
CAS No.	471-34-1
EINECS Name	calcium carbonate
EINECS No.	207-439-9
Molecular Formula	CH2O3.Ca

Dataset created by: EUROPEAN COMMISSION - European Chemicals Bureau

This dossier is a compilation based on data reported by the European Chemicals Industry following 'Council Regulation (EEC) No. 793/93 on the Evaluation and Control of the Risks of Existing Substances'. All (non-confidential) information from the single datasets, submitted in the IUCLID/HEDSET format by individual companies, was integrated to create this document.

The data have not undergone any evaluation by the European Commission.

Creation date: 18-FEB-2000

Number of Pages: 58

Chapters: all

Edition: Year 2000 CD-ROM edition

Flags: non-confidential

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European Chemicals Bureau

1.0.1 OECD and Company Information

Name: 3M United Kingdom PLC
Town: Bracknell
Country: United Kingdom

Name: Amway Europe
Street: Ikaroslaan, 2B
Town: 1930 Zaventem
Country: Belgium
Phone: 027209953
Telefax: 027253298

Name: Bakelite Italia S.p.A.
Street: Via Mazzini, 792-4
Town: I-21058 Solbiate Olona (VA)
Country: Italy
Phone: +39/(0)331/355-225
Telefax: +39/(0)331/376-390

Name: BASF AG
Street: Karl-Bosch-Str
Town: 67056 Ludwigshafen
Country: Germany

Name: BASF Antwerpen N. V.
Town: 2040 Antwerpen 4
Country: Belgium

Name: Chemische Fabrik Kalk GmbH
Town: 51103 Koeln
Country: Germany

Name: Du Pont de Nemours (Luxembourg) S.A.
Street: Contern
Town: L-2984 Luxembourg
Country: Luxembourg
Phone: 3666-1
Telefax: 3666-5006

Name: Faxe Kalk sarl
Street: 2, rue de Messine
Town: 75008 Paris
Country: France
Phone: 1 42 56 92 92
Telefax: 1 42 56 92 93

1. General Information

date: 18-FEB-2000
Substance ID: 471-34-1

Name: Guaber SPA
Street: Via Gobetti, 4
Town: 40050 Funo di Argelato (BO)
Country: Italy
Phone: +390516649111
Telefax: +390516649251

Name: Helm AG
Street: Nordkanalstrasse 28
Town: 20097 Hamburg
Country: Germany
Phone: +49402375-0
Telefax: +49402375-90
Telex: 2170150

Name: Honeywill and Stein Ltd
Street: 1 Finsbury Circus
Town: EC2M 7BA London
Country: United Kingdom
Phone: +44(0)171 496 4000
Telefax: +44(0)171 496 4516

Name: Hydro Agri AB
Street: P.O. Box 516
Town: 26124 Landskrona
Country: Sweden
Phone: 46 418 76 100
Telefax: 46 418 17 225
Telex: 72588 supra s

Name: INDÚSTRIAS LEVER PORTUGUESA, LDA.
Street: Rua Cidade de Goa, 22-24
Town: 2685 SACAV M Sacavém
Country: Portugal
Phone: 9413161
Telefax: 9413390

Name: Keyser & Mackay
Street: Leidsegracht 21
Town: 1017NA amsterdam
Country: Netherlands
Phone: 020-6263323
Telefax: 020-6254559

Name: LEVER BROTHERS LTD
Street: LEVER HOUSE, 3 St JAMES ROAD
Town: KT1 2BA KINGSTON UPON THAMES
Country: United Kingdom
Phone: 0044 51 641 4505
Telefax: 0044 51 641 4308

1. General Information

date: 18-FEB-2000
Substance ID: 471-34-1

Name: Lever GmbH
Street: P.O. Box 104180
Town: 20028 Hamburg
Country: Germany

Name: Lever Italy
Street: Via Nino Bonnet, 6/A
Town: 20154 Milan
Country: Italy

Name: Lever Nederland B.V.
Street: Molenbaan 2
Town: 2908 LM Chapelle a/d Ijssel
Country: Netherlands

Name: Lubrizol Great Britain Limited
Street: P.O.Box 88
Town: DE56 1QN Belper, Derby
Country: United Kingdom
Phone: 332-842211
Telefax: 332-842303
Telex: 37445

Name: Metallgesellschaft AG
Street: Reuterweg 14
Town: 60323 Frankfurt a.M
Country: Germany
Phone: +49.69.159.0
Telefax: +49.69.159.2125

Name: Mobil Oil Italiana
Street: Via Vitaliano Brancati, 60
Town: 00144 Rome
Country: Italy
Phone: 39-6-50280261
Telefax: 39-6-50280290

Name: NEUBER GES.M.B.H.
Street: BRÜCKENGASSE 1
Town: 1060 WIEN
Country: Austria
Phone: 0222/599950
Telefax: 0222/5970200

Name: Novartis Agro S.A. (formerly Ciba-Geigy Agro S.A.) France
Street: 14, boulevard Richelieu
Town: 92845 Rueil-Malmaison Cedex
Country: France
Phone: 33 1 55 47 82 00
Telefax: 33 1 55 47 82 20

1. General Information

date: 18-FEB-2000
Substance ID: 471-34-1

Name: Rhone-Poulenc Chemicals Limited
Town: WD1 1QH Watford, Hertforshire
Country: United Kingdom

Name: Riedel-de Haën AG
Street: Wunstorfer Str.40
Town: 30926 Seelze
Country: Germany
Phone: 05137 999 0
Telefax: 05137 999 123
Telex: 9 21 295 rdhs d

Name: S.A. CHAUX ET DOLOMIES DU BOULONNAIS
Street: Rue de Rivoli 122
Town: F-755038-62132 PARIS
Country: France
Phone: +33-1 40 41 72 72
Telefax: +33-1 42 21 48 40

Name: Schaefer Kalk
Street: Postfach 1361
Town: 65572 Diez
Country: Germany
Phone: 06432/503-0
Telefax: 06432/503-269

Name: SCORA S.A.
Street: Rue de Rivoli 122
Town: F-75038 PARIS
Country: France
Phone: +33-1 40 41 72 72
Telefax: +33-1 42 21 48 40

Name: Siemens Matsushita Components OHG
Street: Siemensstraße 43
Town: 8530 Deutschlandsberg
Country: Austria

Name: SKW Trostberg
Street: Postfach 1262
Town: 83303 Trostberg
Country: Germany
Phone: 08621/86-0

Name: SMTPI
Street: SAINT FERREOL
Town: 12490 SAINT ROME DE TARN
Country: France
Phone: 65 62 54 62
Telefax: 65 62 59 16

1. General Information

date: 18-FEB-2000
Substance ID: 471-34-1

Name: Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Street: Via Varesina 1
Town: 21021 Angera VA
Country: Italy

Name: Solvay Alkali GmbH
Street: Postfach 110270
Town: 42662 Solingen
Country: Germany

Name: Solvay Oesterreich AG
Street: Parkring 12
Town: 1010 Wien
Country: Austria
Phone: +43 1 515880
Telefax: +43 1 5158860

Name: Solvay S.A.
Street: Rue du Prince Albert 33
Town: 1050 Bruxelles
Country: Belgium

Name: TFL ITALIA Spa
Street: L. Magnolini 18
Town: 25135 BRESCIA
Country: Italy
Phone: 030 3691.1
Telefax: 030 3691.210

Name: Tioxide Europe Limited
Town: W14 ORL London
Country: United Kingdom

Name: ZENECA Specialties
Street: PO Box 42
Town: M9 3DA Manchester
Country: United Kingdom

1.0.2 Location of Production Site

-

1.0.3 Identity of Recipients

-

1.1 General Substance Information

Substance type: inorganic
Physical status: liquid

Substance type: inorganic
Physical status: solid

Substance type: organic
Physical status: solid

Substance type: organic
Physical status:

Substance type:
Physical status: solid

1.1.1 Spectra

-

1.2 Synonyms

a: Calciumcarbonat; b: Kalk

Source: Metallgesellschaft AG Frankfurt a.M

CaCO₃p

Remark: Carbonate de calcium précipité

Source: S.A. CHAUX ET DOLOMIES DU BOULONNAIS PARIS

Calcit, Aragonit, Vaterit, gefälltes Calciumcarbonat, Schaefer CCP, Schaefer PCC, Schaefer Precarb

Source: Schaefer Kalk Diez

CALCITE

Source: Lever Nederland B.V. Chapelle a/d Ijssel
Lever Italy Milan
LEVER BROTHERS LTD KINGSTON UPON THAMES
Lever GmbH Hamburg

Calcite

Source: INDÚSTRIAS LEVER PORTUGUESA, LDA. Sacavém

Calcium carbonate

Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

Calcium carbonate (1:1)

Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

Calcium carbonate (CaCO₃) (6CI)

Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

Calcium monocarbonate

Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

Calcium(2+) carbonate

Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

Calciumcarbonat

Source: SKW Trostberg Trostberg
Riedel-de Haën AG Seelze

Carbonate de calcium

Source: Solvay S.A. Bruxelles
SCORA S.A. PARIS
Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA
Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

Carbonate de calcium précipité

Remark: formule : CaCO₃p
Source: S.A. CHAUX ET DOLOMIES DU BOULONNAIS PARIS

Carbonate de calcium précipité

Remark: abréviation : CCP
Source: SCORA S.A. PARIS

Carbonato di calcio

Source: Bakelite Italia S.p.A. Solbiate Olona (VA)

CARBONIC ACID CALCIUM SALT

Source: Lever Nederland B.V. Chapelle a/d Ijssel
Lever Italy Milan
LEVER BROTHERS LTD KINGSTON UPON THAMES
Lever GmbH Hamburg

Carbonic acid calcium salt

Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

carbonic acid calcium salt

Source: Amway Europe Zaventem

Carbonic acid calcium salt (1:1) (8CI, 9CI)

Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

Carbonic acid, calcium salt

Source: Honeywill and Stein Ltd London

Carbonic acid, calcium salt (1:1)

Remark: CAS name.

Source: Solvay S.A. Bruxelles
Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA
Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

Carbonic acid, calcium salt (1:1)

Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

Carbonic acid, calcium salt (1:1)

Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

Carbonic acid, calcium salt (1:1)

Source: BASF AG Ludwigshafen

Carbonic acid, calcium salt (1:1)

Source: BASF AG Ludwigshafen

Carbonic acid, calcium salt (1:1)

Source: BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

Carbonic acid, calcium salt (1:1)

Source: BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

CCP

Remark: Usual abbreviation for "Carbonate de Calcium Precipite".

Source: Solvay S.A. Bruxelles
Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA
Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

CCP

Remark: Abréviation usuelle du "carbonate de calcium précipité"**Source:** S.A. CHAUX ET DOLOMIES DU BOULONNAIS PARIS

COATED PRECIPITATED CALCIUM CARBONATE

Source: ZENECA Specialties Manchester

CPCC

Source: ZENECA Specialties Manchester

Kalkstein

Source: Du Pont de Nemours (Luxembourg) S.A. Luxembourg

Lime

Source: Hydro Agri AB Landskrona

Limestone

Source: Du Pont de Nemours (Luxembourg) S.A. Luxembourg

Marmorpulver, Kalkstein, Calcit

Source: NEUBER GES.M.B.H. WIEN

Monocalcium carbonate

Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

PCC

Remark: zabréviation du " Precipitated calcium carbonate "**Source:** S.A. CHAUX ET DOLOMIES DU BOULONNAIS PARIS

PCC

Source: Rhone-Poulenc Chemicals Limited Watford, Hertforshire

Precipitated calcium carbonate

Source: Solvay S.A. Bruxelles
Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA
Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien
Rhone-Poulenc Chemicals Limited Watford, Hertforshire
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

Præcipiteret calciumcarbonat, fældet kalk

Source: Faxe Kalk sarl Paris

SOCAL

Remark: Solvay Registered Trade Mark.**Source:** Solvay S.A. Bruxelles
Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA
Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien

Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

Spezialkalk

Source: SKW Trostberg Trostberg

SYNTHETIC ARAGONITE

Source: Lever Nederland B.V. Chapelle a/d Ijssel
Lever Italy Milan
LEVER BROTHERS LTD KINGSTON UPON THAMES
Lever GmbH Hamburg

Synthetic aragonite

Source: Solvay S.A. Bruxelles
Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA
Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

SYNTHETIC CALCITE

Source: Lever Nederland B.V. Chapelle a/d Ijssel
Lever Italy Milan
LEVER BROTHERS LTD KINGSTON UPON THAMES
Lever GmbH Hamburg

Synthetic calcite

Source: Solvay S.A. Bruxelles
Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA
Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

Remark: abréviation du " Precipitated calcium carbonate "

Source: SCORA S.A. PARIS

Remark: CALCIUM CARBONATE AS REPORTED HERE IS IMPORTED IN THE FORM OF AN OIL SOLUBLE MIXTURE. IT IS NOT MANUFACTURED SEPARATE FROM THE OTHER INGREDIENTS WHICH AID IN KEEPING THE CALCIUM CARBONATE IN SOLUTION. WHEN FOUND IN NATURE CALCIUM CARBONATE IS KNOWN AS LIMESTONE. IT IS NOT POSSIBLE TO DISSOLVE POWDERED LIMESTONE IN OIL. PROPRIETARY ART IS USED TO MANUFACTURE THIS FORM OF CALCIUM CARBONATE. THERE IS NO REASON TO BELIEVE THAT IF ISOLATED THIS FORM OF CALCIUM CARBONATE SHOULD HAVE PROPERTIES DIFFERENT FROM LIMESTONE.

Source: Lubrizol Great Britain Limited Belper, Derby

1.3 Impurities

-

1.4 Additives

-

1.5 Quantity**Quantity** more than 1 000 000 tonnes**1.6.1 Labelling**

-

1.6.2 Classification

-

1.7 Use Pattern

Type:	type
Category:	Non dispersive use
Type:	type
Category:	Use in closed system
Type:	type
Category:	Use resulting in inclusion into or onto matrix
Type:	type
Category:	Wide dispersive use
Type:	industrial
Category:	Agricultural industry
Type:	industrial
Category:	Basic industry: basic chemicals
Type:	industrial
Category:	Chemical industry: used in synthesis
Type:	industrial
Category:	Electrical/electronic engineering industry
Type:	industrial
Category:	Leather processing industry
Type:	industrial
Category:	Paints, lacquers and varnishes industry
Type:	industrial
Category:	Paper, pulp and board industry
Type:	industrial
Category:	Personal and domestic use

Type:	industrial
Category:	Polymers industry
Type:	industrial
Category:	Public domain
Type:	industrial
Category:	other: Kalkkomponente fuer Zementherstellung, Emissionsreduzierung in Ziegeleien
Type:	industrial
Category:	other: used in product of detergents
Type:	industrial
Category:	other
Type:	use
Category:	Absorbents and adsorbents
Type:	use
Category:	Adhesive, binding agents
Type:	use
Category:	Anti-set-off and anti-adhesive agents
Type:	use
Category:	Cleaning/washing agents and disinfectants
Type:	use
Category:	Cosmetics
Type:	use
Category:	Dustbinding agents
Type:	use
Category:	Fertilizers
Type:	use
Category:	Fillers
Type:	use
Category:	Food/foodstuff additives
Type:	use
Category:	Intermediates
Type:	use
Category:	Lubricants and additives
Type:	use
Category:	pH-regulating agents
Type:	use
Category:	Pesticides

Type: use
Category: Pharmaceuticals

Type: use
Category: Reprographic agents

Type: use
Category: Stabilizers

Type: use
Category: Viscosity adjustors

Type: use
Category: other: Fillers, food/food stuff additives, pharmaceuticals.

Type: use
Category: other: alimentation animale

Type: use
Category: other: pH-regulating agents, viscosity adjusters.

Type: use
Category: other: stabilisation des sols

Type: use
Category: other

1.7.1 Technology Production/Use

-

1.8 Occupational Exposure Limit Values

Type of limit: MAC (NL)
Limit value: 10 mg/m³
Remark: Total dust
Source: Keyser & Mackay amsterdam

Type of limit: MAC (NL)
Limit value: 5 mg/m³
Remark: inhalable
Source: Keyser & Mackay amsterdam

Type of limit: MAK (DE)
Limit value:
Remark: Kein MAK-Wert festgelegt.
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

(1)

Type of limit: OES (UK)
Limit value: 10 mg/m³
Source: Rhone-Poulenc Chemicals Limited Watford, Hertfordshire

(2)

Type of limit: TLV (US)
Limit value: 10 mg/m³
Remark: ACGIH (8h -TWA) : total dust
Source: Lever Nederland B.V. Chapelle a/d Ijssel
Lever Italy Milan
LEVER BROTHERS LTD KINGSTON UPON THAMES
Lever GmbH Hamburg

Type of limit: TLV (US)
Limit value: 10 mg/m³
Remark: ACGIH (8h-TWA) : total dust.
Source: Solvay S.A. Bruxelles

Type of limit: TLV (US)
Limit value: 10 mg/m³
Remark: bezieht sich auf 'Calcium carbonate' CAS-Nr. 1317-65-3.
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(3)

Type of limit: TLV (US)
Limit value: 10 mg/m³
Remark: bezieht sich auf 'Calcium carbonate' CAS-Nr. 1317-65-3.
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4

(3)

Type of limit: TLV (US)
Limit value: 10 mg/m³
Source: INDÚSTRIAS LEVER PORTUGUESA, LDA. Sacavém

Type of limit: TLV (US)
Limit value: 10 mg/m³
Remark: ACGIH (8h-TWA) : total dust.
Source: Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA

Type of limit: TLV (US)
Limit value: 10 mg/g
Source: Mobil Oil Italiana Rome

1. General Information

date: 18-FEB-2000
Substance ID: 471-34-1

Type of limit: TLV (US)
Limit value: 10 mg/m3
Remark: ACGIH (8h-TWA) : total dust.
Source: Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien

Type of limit: TLV (US)
Limit value: 10 mg/m3
Remark: bezieht sich auf 'Calcium carbonate' CAS-Nr. 1317-65-3.
Source: BASF AG Ludwigshafen

(3)

Type of limit: TLV (US)
Limit value: 10 mg/m3
Remark: bezieht sich auf 'Calcium carbonate' CAS-Nr. 1317-65-3.
Source: BASF AG Ludwigshafen

(3)

Type of limit: TLV (US)
Limit value: 10 mg/m3
Remark: bezieht sich auf 'Calcium carbonate' CAS-Nr. 1317-65-3.
Source: BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

(3)

Type of limit: TLV (US)
Limit value: 10 mg/m3
Remark: bezieht sich auf 'Calcium carbonate' CAS-Nr. 1317-65-3.
Source: BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

(3)

Type of limit: TLV (US)
Limit value: 10 mg/m3
Remark: ACGIH (8h-TWA) : total dust.
Source: Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

Type of limit: other
Limit value:
Remark: No data
Source: Bakelite Italia S.p.A. Solbiate Olona (VA)

Type of limit: other
Limit value:
Remark: No exposure limit was found for the material having this CAS number.
Source: Lubrizol Great Britain Limited Belper, Derby

1. General Information

date: 18-FEB-2000
Substance ID: 471-34-1

Type of limit: other: GOST (SU)
Limit value: 6 mg/m3
Remark: Limestone
Source: Lever Nederland B.V. Chapelle a/d Ijssel
Lever Italy Milan
LEVER BROTHERS LTD KINGSTON UPON THAMES
Lever GmbH Hamburg

Type of limit: other: GOST (SU)
Limit value: 6 mg/m3
Remark: Limestone.
Source: Solvay S.A. Bruxelles

Type of limit: other: GOST (SU)
Limit value: 6 mg/m3
Remark: Limestone.
Source: Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA

Type of limit: other: GOST (SU)
Limit value: 6 mg/m3
Remark: Limestone.
Source: Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien

Type of limit: other: GOST (SU)
Limit value: 6 mg/m3
Remark: Limestone.
Source: Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

Type of limit: other: OSHA-PEL
Limit value: 10 mg/m3
Remark: total dust
Source: Lever Nederland B.V. Chapelle a/d Ijssel
Lever Italy Milan
LEVER BROTHERS LTD KINGSTON UPON THAMES
Lever GmbH Hamburg

Type of limit: other: OSHA-PEL (US)
Limit value: 5 mg/m3
Remark: Respirable fraction
Source: Lever Nederland B.V. Chapelle a/d Ijssel
Lever Italy Milan
LEVER BROTHERS LTD KINGSTON UPON THAMES
Lever GmbH Hamburg

Type of limit: other: OSHA-PEL (US)
Limit value: 10 mg/m³
Remark: Total dust.
Source: Solvay S.A. Bruxelles
Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA
Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

Type of limit: other: OSHA-PEL (US)
Limit value: 5 mg/m³
Remark: Respirable fraction.
Source: Solvay S.A. Bruxelles

Type of limit: other: OSHA-PEL (US)
Limit value: 5 mg/m³
Remark: Respirable fraction.
Source: Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesia S.P.A. - SGIM
Angera VA

Type of limit: other: OSHA-PEL (US)
Limit value: 5 mg/m³
Remark: Respirable fraction.
Source: Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien

Type of limit: other: OSHA-PEL (US)
Limit value: 5 mg/m³
Remark: Respirable fraction.
Source: Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

Type of limit: other: TLV (F)
Limit value: 10 mg/m³
Source: SKW Trostberg Trostberg

Type of limit: other: TLV (NL)
Limit value: 10 mg/m³
Remark: aerosol
Source: SKW Trostberg Trostberg

Type of limit:**Limit value:****Remark:** néant**Source:** S.A. CHAUX ET DOLOMIES DU BOULONNAIS PARIS
SCORA S.A. PARIS**Type of limit:****Limit value:** 10 mg/m³**Remark:** Grænseværdi for ufarligt (tørt) støv er i Danmark 10 mg/m³.
Produktet fremstilles og sælges som en vandig suspension med
20 % tørstofindhold, og giver ingen anledning til støvning.**Source:** Faxe Kalk sarl Paris**1.9 Source of Exposure****Remark:** Consumer and professional exposures related to the use
pattern are described in section 1.7.

Possibility of a natural background exposure.

Calcium carbonate is one of the most common forms of calcium
in the sedimentary rocks; CaCO₃ is present in sandstone and
detrital rock as a cement between particles or a partial
filling of interstices.Weathering of rocks involves the dissolution of CaCO₃ with
removal of calcium as ion-form Ca²⁺.

Countries where Solvay production plants are located in EU:

- France
- Germany
- Italy

Source: Solvay S.A. Bruxelles
Solvay S.A. Bruxelles
Societa Generale per L Industria della Magnesita S.P.A. - SGIM
Angera VA
Solvay S.A. Bruxelles
Solvay Alkali GmbH Solingen

(4)

Remark: néant**Source:** S.A. CHAUX ET DOLOMIES DU BOULONNAIS PARIS**Remark:** RISQUE A LA PLUIE**Source:** SMTPI SAINT ROME DE TARN**Remark:** Os operadores estão em contacto com a substância em
condições controladas. Os consumidores estão em contacto nas
condições de utilização.**Source:** INDÚSTRIAS LEVER PORTUGUESA, LDA. Sacavém**Remark:** No data**Source:** Bakelite Italia S.p.A. Solbiate Olona (VA)

Remark: Consumer and professional exposures related to the use pattern are described in section 1.7.

Possibility of a natural background exposure.
Calcium carbonate is one of the most common forms of calcium in the sedimentary rocks; CaCO_3 is present in sandstone and detrital rock as a cement between particles or a partial filling of interstices.
Weathering of rocks involves the dissolution of CaCO_3 with removal of calcium as ion-form Ca^{2+} .

Countries where Solvay production plants are located in EU:
- France
- Germany
- Italy
- Declared in 1995: Austria

Source: Solvay S.A. Bruxelles
Solvay Oesterreich AG Wien

(4)

Remark: Product is locked into polymers. Emission of product during course of end product life is of negligible impact. At completion of end-product, products disposed of by landfill will not give rise to any environmental exposure; products disposed of by incineration will lead to the formation of calcium salts.

Source: ZENECA Specialties Manchester

Remark: The industrial production process consists of burning of natural limestone, hydration to calcium hydroxide (Ca(OH)_2) and then recarbonation to calcium carbonate (CaCO_3).

Source: Exposure through normal handling by user.
Rhone-Poulenc Chemicals Limited Watford, Hertfordshire

Remark: EXPOSURE FROM WORKER HANDLING IS EXPECTED TO BE LOW SINCE THE SUBSTANCE AS REPORTED HERE WOULD BE HANDLED AS A LIQUID IN SEMI-CLOSED SYSTEMS. THERE WOULD BE NO EXPOSURE TO DUST AS THERE MAY BE WITH SOLID CALCIUM CARBONATE.

EXPOSURE TO RELATIVELY LOW CONCENTRATIONS MAY OCCUR DUE TO THE PUBLIC CHANGING CRANKCASE LUBRICANTS IN AUTOMOBILES.

IN THE RARE EVENT OF A SPILL, MATERIAL COULD ENTER WATERWAYS. SUBMITTER KNOWS OF NO OCCURENCES OF A LARGE SPILL OF FINISHED LUBRICATING OIL.

Source: Lubrizol Great Britain Limited Belper, Derby

Remark: Im industriellen Produktionsprozess wird natürlicher Kalkstein zu CaO gebrannt, dieses wird mit Wasser zu Ca(OH)_2 gelöscht und anschließend mit CO_2 zu CaCO_3 recarbonatisiert. Normale Handhabung durch den Benutzer.

Source: Schaefer Kalk Diez

1.10.1 Recommendations/Precautionary Measures

-

1.10.2 Emergency Measures

-

1.11 Packaging

-

1.12 Possib. of Rendering Subst. Harmless

-

1.13 Statements Concerning Waste

-

1.14.1 Water Pollution

Classified by: KBWS (DE)
Labelled by: KBWS (DE)
Class of danger: 0 (generally not water polluting)
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
SKW Trostberg Trostberg
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

1.14.2 Major Accident Hazards

Legislation: Stoeerfallverordnung (DE)
Substance listed: no
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

(5)

1.14.3 Air Pollution

Classified by: TA-Luft (DE)
Labelled by: TA-Luft (DE)
Number: 3.1.3 (total dust)
Class of danger:
Source: BASF AG Ludwigshafen
BASF Antwerpen N. V. Antwerpen 4
BASF AG Ludwigshafen
Chemische Fabrik Kalk GmbH Koeln

Classified by: other: keine Stoffklasse bzw. Emissionsbegrenzung nach TA Luft. Der Stand der Technik ist einzuhalten.

Labelled by:

Number:

Class of danger:

Source: SKW Trostberg Trostberg

1.15 Additional Remarks

Remark: néant

Source: S.A. CHAUX ET DOLOMIES DU BOULONNAIS PARIS

Source: SCORA S.A. PARIS

Remark: No data

Source: Bakelite Italia S.p.A. Solbiate Olona (VA)

Country: ANNO 1991 : PAESE ESPORTATORE AUSTRIA

Source: TFL ITALIA Spa BRESCIA

Country: ANNO 1992: PAESE ESPORTATORE AUSTRIA

Source: TFL ITALIA Spa BRESCIA

Country: ANNO 1993: PAESE ESPORTATORE AUSTRIA

Source: TFL ITALIA Spa BRESCIA

Country: ANNO 1994 . PAESE ESPORTATORE AUSTRIA

Source: TFL ITALIA Spa BRESCIA

Remark: Product can be disposed of safely to landfill.
Product is transported in 25kg paper sacks, in intermediate bulk containers of up to 1 ton capacity and road tankers of capacity up to 25 tonnes.

Source: ZENECA Specialties Manchester

Remark: The Company Honeywill and Stein was part of BP Chemicals Ltd until sold on 31st July 1998 to Internatio-Muller of Rotterdam.

Source: Honeywill and Stein Ltd London

1.16 Last Literature Search

-

1.17 Reviews

-

1.18 Listings e.g. Chemical Inventories

-

2.1 Melting Point

Value: = 825 degree C
Decomposition: yes
Sublimation: no
Source: Guaber SPA Funo di Argelato (B0)

Value: 899 degree C
Decomposition: yes
Method: other
Remark: THE "HANDBOOK OF CHEMISTRY AND PHYSICS, 66TH EDITION" GIVES A DECOMPOSITON TEMPERATURE OF 898 DEGREES CENTIGRADE.
Source: Lubrizol Great Britain Limited Belper, Derby

Value:
Decomposition: yes
Remark: Decomposition temperature : 700-900 degree C (825 degree C for aragonite, and 899 degree C for calcite).
Source: Solvay S.A. Bruxelles

(6)

Value:
Remark: CaCO₃ zersetzt sich, abhängig von seiner kristallinen Struktur, oberhalb von 800 Grad C.
Source: Schaefer Kalk Diez

2.2 Boiling Point

Value:
Decomposition: yes
Source: Solvay S.A. Bruxelles

(6)

Value:
Decomposition: yes
Source: Lubrizol Great Britain Limited Belper, Derby

2.3 Density

Type: density
Value: = 2.71 at 18 degree C
Remark: THE "HANDBOOK OF CHEMISTRY AND PHYSICS, 66TH EDITION" GIVES THE VALUE FOR THE DENSITY REPORTED HERE.
Source: Lubrizol Great Britain Limited Belper, Derby

Type: bulk density
Value: .2 - .6 g/cm³ at 20 degree C
Source: Schaefer Kalk Diez

Type: density
Value: 2.7 - 2.9 g/cm³ at 20 degree C
Source: Schaefer Kalk Diez

Type: relative density
Value: = 2.93 at 20 degree C
Source: Solvay S.A. Bruxelles
Test substance: Aragonite.

(6)

Type: relative density
Value: ca. 27 g/cm³ at 20 degree C
Source: Guaber SPA Funo di Argelato (B0)

Type: relative density
Value: = 2.71
Source: Solvay S.A. Bruxelles
Test substance: Calcite.

(6)

Type: bulk density
Value: 700 - 1400 kg/m³
Method: other
Source: SMTPI SAINT ROME DE TARN

Type: bulk density
Value: ca. 780 kg/m³
Source: Solvay S.A. Bruxelles
BASF AG Ludwigshafen

(7)

Type: bulk density
Value: ca. 1100 kg/m³
Source: INDÚSTRIAS LEVER PORTUGUESA, LDA. Sacavém

2.3.1 Granulometry

-

2.4 Vapour Pressure

Value:
Method: other (calculated): not applicable
Source: Solvay S.A. Bruxelles

Value:
Remark: Zersetzt sich (s. 2.1 Bemerkungen).
Source: Schaefer Kalk Diez

2.5 Partition Coefficient

log Pow:
Method: other (calculated): not applicable for an inorganic compound which dissociates
Year:
Source: Solvay S.A. Bruxelles

2.6.1 Water Solubility

Value: >= 0 - .13 mg/l at 10.5 degree C

pH: >= 9 - 12

Source: SMTPI SAINT ROME DE TARN

Value: = .0014 at 25 degree C

Remark: SOURCE OF VALUE IS "HANDBOOK OF CHEMISTRY AN PHYSICS, 66TH EDITION. THE VALUE GIVEN IS FOR THE CALCITE FORM.

Source: Lubrizol Great Britain Limited Belper, Derby

Value: ca. .02 g/l at 25 degree C

pH: ca. 9.5 at 50 g/l and 20 degree C

Source: Solvay S.A. Bruxelles

BASF AG Ludwigshafen

(7)

Value: = 14 mg/l at 25 degree C

Source: Solvay S.A. Bruxelles

Test substance: Calcite.

(6)

Value: = 15.3 mg/l at 25 degree C

Source: Solvay S.A. Bruxelles

Test substance: Aragonite.

(6)

pH: 8.5 - 9.5

Source: INDÚSTRIAS LEVER PORTUGUESA, LDA. Sacavém

2.6.2 Surface Tension

-

2.7 Flash Point

Value:

Type:

Method: other: not applicable

Year:

Source: Solvay S.A. Bruxelles

(6)

Value:

Type:

Method:

Year:

Remark: nicht entflammbar

Source: Schaefer Kalk Diez

2.8 Auto Flammability

Value:
Method: other: not applicable
Source: Solvay S.A. Bruxelles (6)

Value:
Remark: nicht selbstentzündlich
Source: Schaefer Kalk Diez

2.9 Flammability

Result: non flammable
Source: SMTPI SAINT ROME DE TARN

Result:
Method: other: not applicable
Source: Solvay S.A. Bruxelles (6)

Result:
Remark: nicht entzündlich
Source: Schaefer Kalk Diez

2.10 Explosive Properties

Result: not explosive
Source: Solvay S.A. Bruxelles (6)

Result:
Remark: nicht explosiv
Source: Schaefer Kalk Diez

2.11 Oxidizing Properties

Result:
Remark: keine brandfördernden Eigenschaften
Source: Schaefer Kalk Diez

2.12 Additional Remarks

Remark: pH may vary from 6 to 10 depending on the CO₂ pressure (from 15-25 degree C)
Source: Solvay S.A. Bruxelles (8)

3.1.1 Photodegradation

Type: other: no data available
Method:
Year: **GLP:**
Test substance:
Source: Solvay S.A. Bruxelles

3.1.2 Stability in Water

Type: abiotic
Method: other: CALCIUM complexation/precipitation reaction
Year: **GLP:**
Test substance:

Remark: In general, calcium salts are not as strong electrolytes as those of sodium or potassium (they are less completely dissociated at comparable concentrations), and some calcium complexes are stable in aqueous systems.

Result: Predominant calcium complexes in freshwater and seawater:

Complex	Freshwater (ca. 28 mg/l Ca)	Seawater (ca. 412 mg/l Ca)
Ca ²⁺	95.0 %	91.0 %
CaHCO ₃ ⁺	1.9 %	1.0 %
CaCO ₃	0.9 %	0.2 %
CaSO ₄	2.3 %	8.0 %

- In both freshwater and seawater systems, the calcium ion complexes are present at low concentrations compared with that of the uncomplexed aqueous ion.
- In freshwater, the concentrations of both the calcium sulfate (CaSO₄) and calcium bicarbonate (CaHCO₃⁺) complexes are significant, representing 2.3 % and 1.9 % of the total calcium content.
- In seawater, the concentration of CaSO₄ represents 8 % of the total calcium content.

Carbonate and hydroxide pairs of calcium have been reported, but their concentrations are generally significant only in strongly alkaline waters.

Source: Solvay S.A. Bruxelles

(9) (10)

Type: abiotic**Method:** other: CALCIUM complexation reaction with organic ligands**Year:** **GLP:****Test substance:**

Remark: Calcium forms strong complexes with organic ligands; however, the influence of these ligands on the concentration of calcium in natural waters is not likely to be significant in most natural water systems, as the concentration of Ca^{2+} is considerably higher than that of the complexing species.

Result: Complexation equilibrium constants (K) for calcium complexes with organic ligands:

Complexes	log K (25 degC)
(Ca ²⁺)(HIDA)	4.77
(Ca ²⁺)(EDTA)	10.61
(Ca ²⁺)(TPP)	5.20
(Ca ²⁺)(CIT)	3.55 (20 degC)

HIDA: Ethanolaminediacetic acid

EDTA: Ethylenediaminetetraacetic acid

TPP: Tripolyphosphoric acid

CIT: Citric acid

The pH of a solution markedly affects the extent of complexation of an organic species with a metal since H^+ competes for the basic coordination site.

Source: Solvay S.A. Bruxelles

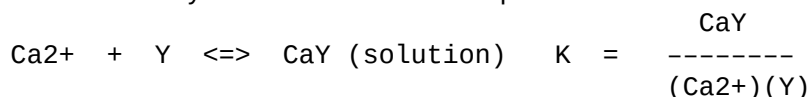
(4) (11)

Type: abiotic**Method:** other: CALCIUM complexation/precipitation reaction**Year:** **GLP:****Test substance:**

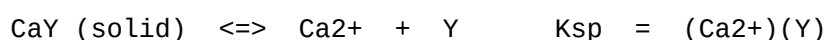
Remark: Calcium complexes/precipitates are in equilibrium with free ions; this equilibrium depends on environmental conditions, such as temperature, pH,...

The global equilibrium between complex, precipitate and free ions can be described by two constants:

1/ Complex stability constant of the equilibrium:



2/ Solubility product constant of the equilibrium:



=> Solubility of soluble complex (CaY) is constant:



Result: Complex stability constants and solubility constants for various calcium-complexes (25 degC):

	Complex / Precipitate	Complex stability constant log K	Solubility product constant log Ksp	Solubility of soluble complex form
	CaCO ₃	3.15	- 8.35	10 exp-5.20
	CaSO ₄	2.31	- 5.04	10 exp-2.73
	Ca(OH) ₂	3.56	- 5.19	10 exp-1.63
Source:	Solvay S.A. Bruxelles			(12) (13)
Type:	abiotic			
Method:	other: CARBONATE hydrolysis as a function of pH			
Year:	GLP:			
Test substance:				
Remark:	The carbonate system in water is important because of the ubiquity of carbon dioxide and carbonate-bearing-minerals in the environment: - Carbon dioxide gas, after dissolving in water, is hydrolyzed to form carbonic acid: $\text{CO}_2 (\text{gas}) \rightleftharpoons \text{CO}_2 (\text{solution})$ $\text{CO}_2 (\text{solution}) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$ - Carbonate minerals (such as calcite CaCO₃) dissolve in water with formation of carbonate and a cation: $\text{CaCO}_3 (\text{solid}) \rightleftharpoons (\text{Ca})^{2+} + (\text{CO}_3)^{2-}$ Initial species of carbonates in aqueous environment are H₂CO₃ and (CO₃)²⁻ which are in equilibrium with other forms depending on environmental conditions: closed or open system, pH, temperature,...: 1/ Closed system, such groundwater: ----- CO₂ exchange with the surrounding environment is minimal or very slow (thus, total carbonate concentration including different species is constant); equilibrium exists between H₂CO₃/HCO₃⁻/(CO₃)²⁻ as a function of pH: - pH > 10: (CO₃)²⁻ is predominant - 6 < pH < 10: HCO₃⁻ is predominant - pH < 6: H₂CO₃ is predominant 2/ Open system, such river: ----- Carbonate solution is exposed to the atmosphere and the total carbonate concentration varies with the amount of atmospheric CO₂ that dissolves in the solution; since the partial pressure of CO₂ is constant under normal atmospheric conditions, the concentration of aqueous CO₂ is also constant once equilibrium is attained and can be determined in accordance with the Henry's Law: $\text{H}_2\text{CO}_3 (\text{solution}) = \text{CO}_2 (\text{solution}) = H \cdot \text{PCO}_2 (\text{gas})$			

- H: Henry's Law constant
- PCO2: partial pressure of CO2

In consequence, total carbonate concentration increases at pH > 6 as H2CO3/CO2 (solution) hydrolyses in HCO3-.

Source: Solvay S.A. Bruxelles

(14)

Type: abiotic

Method: other: CARBONATE complexation/precipitation reaction

Year:

GLP:

Test substance:

Remark: COMPLEXATION

Aqueous carbonate complex readily with metal ions; sodium carbonate complex is formed in both fresh and salt water:

- in freshwater, only small concentration of carbonate complex is formed; 99 % of the total carbonate carbon exists as free ions in freshwater;
- in seawater, much larger proportions of (CO3)2- exist as complexes; in various models of seawater, 8-10 % of (CO3)2- exist as free ions, 16-19 % as NaCO3-, 44-67 % as MgCO3 (soluble form) and 7-21 % as CaCO3 (soluble form).

PRECIPITATION

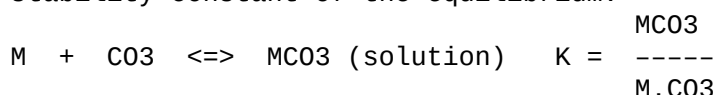
-
- The solubilities of carbonate minerals are intermediate between those of highly soluble chlorides and sulfates (e.g. NaCl, MgSO4) and highly insoluble phosphates (e.g. Ca3(PO4)2);
 - As the concentrations of HCO3- and (CO3)2- in natural waters depend on the pCO2 to which the solutions are exposed, bicarbonate and carbonate solubilities vary with pCO2.

Result: Complex stability constants and solubility constants for various metal-carbonate complexes (25 degC) :

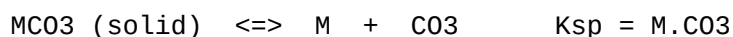
Complex / Precipitate	Complex stability constant log K	Solubility product constant log Ksp	Solubility of soluble complex form

BaCO3	2.78	- 8.30	10 exp-5.52
CaCO3	3.15	- 8.35	10 exp-5.20
CdCO3	4.40	-13.74	10 exp-9.34
MgCO3	2.92	- 7.46	10 exp-4.54
ZnCO3	4.80	-10.00	10 exp-5.20

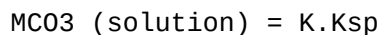
Complex stability constant of the equilibrium:



Solubility product constant of the equilibrium:



Solubility of soluble complex (MC03) is constant:



In conclusion, as free carbonate concentration increases, free metal concentration decreases.

Source: Solvay S.A. Bruxelles

(15) (11) (16) (10)

Type: abiotic

t1/2 pH 12 : >= year

Method:

Year:

GLP:

Test substance:

Source: SMTPI SAINT ROME DE TARN

Type: abiotic

Method:

Year:

GLP:

Test substance:

Remark: Das Produkt ist wegen der sehr geringen Löslichkeit in Wasser sehr stabil (s. 2.6). Bei einem pH-Wert von < 7 zersetzt sich das Produkt abhängig von der Art der Säure, der Temperatur und anderen Bedingungen.

Source: Schaefer Kalk Diez

3.1.3 Stability in Soil

Type: other: CARBONATE acid-base reaction

Radiolabel:

Concentration:

Cation exch.

capac.

Microbial

biomass:

Method:

Year:

GLP:

Test substance:

Remark:

- The content of CO₂ plays an important role in soil chemistry.
- The amount of CO₂ in the soil solution is a function of microbial and root activity and the rate of upward diffusion of CO₂.
- The CO₂ concentration and the soil solution pH determine the speciation of inorganic carbon in the soil:
 - 1/ at low pH, concentration of the carbonate ion in solution is small, being converted to HCO₃⁻ and H₂CO₃;
 - 2/ in alkaline soils, carbonate minerals become important and may determine the limits of the solubilities of many metal ions.

- The partial pressure of CO₂ in well-drained agricultural soils (0.01 atm and lesser value for drier and warmer soils) ranges from 10 to 100 times the partial pressure of CO₂ in the atmosphere (0.0003 atm); because the rate of diffusion in aqueous solutions is several orders of magnitude less than in air, flooded soils in which respiration (biological oxidation of organic matter) is taking place may develop pCO₂ concentrations close to the partial pressure of O₂ in the atmosphere (0.209 atm) as O₂ is converted in CO₂.

Such a high CO₂ concentration can lower the pH substantially; the change in CO₂ concentration (and hence of pH and speciation) can occur within a few hours, leading to large differences between field and laboratory measurements.

Source: Solvay S.A. Bruxelles

(17) (14)

Type:
Concentration:
Cation exch.
capac.
Microbial
biomass:

Radiolabel:

Method:
Year:
Test substance:
Remark:

GLP:

Das Produkt ist unter natürlichen Bedingungen im Boden sehr stabil, unter sauren Bedingungen löst es sich auf.

Source: Schaefer Kalk Diez

Type:
Concentration:
Cation exch.
capac.
Microbial
biomass:

Radiolabel:

Method:
Year:
Test substance:
Remark:

other: CALCIUM complexation reaction

GLP:

Environmental calcium complexes are in equilibrium with soil Ca or calcite as a function of gaseous CO₂, inorganic ligand concentrations and pH.

Result:

- The chloride complexes of calcium contribute very little to the total soluble calcium.
- The CaHCO₃⁺ ion is insignificant in acid soils but becomes more significant in submerged soils, where the pH approaches neutrality and the partial pressure of CO₂ (g) increases.
- Calcite supports the neutral ion pair, CaCO₃, at a concentration of 10 exp-5.27 M independant of CO₂ (g) and pH; however, this ion pair is not important in acidic soil.
- CaSO₄ is significant at sulfate concentrations greater than 10 exp-4 M.
- None of the phosphate complexes (CaH₂PO₄⁺, CaHPO₄, CaPO₄⁻)

are significant in acid soils; however in neutral and calcareous soils, CaHPO_4 and CaPO_4^- are significant.
- The hydrolysis species (CaOH^+ and Ca(OH)_2) and nitrate complexes (CaNO_3^+ and $\text{Ca(NO}_3)_2$) are not important in the pH range of most soils.

Source: Solvay S.A. Bruxelles

Test condition: Representative soil pore-water concentrations:

Free complexing ion	Concentration
Ca ²⁺	10 exp-2.5 M
Cl ⁻	10 exp-4 to 10 exp-2 M
(SO ₄) ²⁻	10 exp-4 to 10 exp-2 M
H ₂ PO ₄ ⁻	10 exp-6 to 10 exp-4 M
NO ₃ ⁻	10 exp-4 to 10 exp-2 M
CO ₂ (g)	10 exp-3.5 to 10 exp-2.5 M

(18)

3.2 Monitoring Data (Environment)

Type of measurement: background concentration

Medium: surface water

Result: CALCIUM concentration (mg/l): mean value

Jambes : 65.7
Nameche : 75.3
Liege : 74.1
Grobbendonk : 71.6
Lier : 70.6
Broechem : 69.6

Source: Solvay S.A. Bruxelles

Test condition: Belgium, Meuse, Canal Albert, Kempische kanalen, various cities, 1993.

(19)

Type of measurement: background concentration

Medium: surface water

Result: Measured CALCIUM concentrations in natural waters: (information of the rock type being drained by the freshwater system is given):

Aqueous System (type of rock drained)	Calcium Concentration (mg/l)
- Seawater	412
- Groundwater (limestone)	80
- Stream (granite)	4
- River/unpolluted	13.4
- River/including contri-	14.7

bution of human activities
 - Public water Supply/100 U.S. cities 26 (median)
 0-145
Source: Solvay S.A. Bruxelles (20) (4) (21) (10) (22)

Type of measurement:

Medium:

Remark: Nicht anwendbar, da CaCO₃/Kalkstein allgegenwärtig ist.

Source: Schaefer Kalk Diez

Type of measurement: other

Medium:

Remark: - No monitoring data available as CARBONATE system (H₂CO₃/CO₂/HCO₃⁻/(CO₃)₂⁻) is ubiquitous.

- Determination of CARBONATE concentration (as CaCO₃) is used as an indicator of alkalinity and hardness in most natural waters; typical value of alkalinity for a river is about 100 mg/l as CaCO₃.

Source: Solvay S.A. Bruxelles

(23)

3.3.1 Transport between Environmental Compartments

Type: adsorption

Media: water - soil

Method: other: CALCIUM adsorption

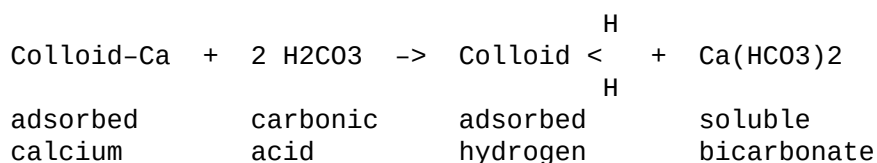
Year:

Remark:

- Ion exchange of calcium with a variety of surface sites in soils governs the concentration of calcium ions in soil water and the soil itself.
- Colloid fractions of clays and hydrous oxides especially contribute to these surface sites.
- Much of the calcium present in soils is held onto the colloidal surface as adsorbed cations.
- However, calcium ions may be released to the soil solution by exchange with other positively charged ions and subsequently readsorbed.

Result:

- During liming, when soluble calcium ions are added to the soil, the colloidal surface can adsorb large quantities of calcium ions.
- The following reaction, which involves the exchange of protons (from carbonic acid) for adsorbed calcium ions, shows the dynamic cation exchange process:



Although all cations may be adsorbed by soil colloids,

certain cations, such as calcium, are favored under natural conditions.
In humid regions, the order of adsorption of cations is H^+ , Al^{3+} and Ca^{2+} first, Mg^{2+} second, then K^+ and Na^+ third.
In well-drained arid and semiarid regions, the order is usually Ca^{2+} and Mg^{2+} first, Na^+ and K^+ second, and H^+ last.
Source: Solvay S.A. Bruxelles (24)

Type: other: weathering process

Media: other: soil - water

Method: other: not mentioned

Year:

Remark:

- Natural water systems acquire their chemical characteristics by dissolution and by chemical reactions with solids, liquids and gases with which they come in contact during the hydrological cycle.
- Weathering of rocks (dissolution) and formation of soils/sediments (precipitation) are the chemical processes that regulate the composition of natural waters.
- Weathering reactions, which involve the interaction of water and the atmosphere with the earth's crust and subsequent removal of the major cations, occur because the minerals contained in the uppermost layer of the lithosphere are thermodynamically unstable in the presence of water and the acidity of the atmosphere.
- The solubilities of the various calcium minerals govern the levels of calcium in solution.

Result: Reactions and equilibrium constants for weathering reactions of selected calcium minerals:

Reaction	log K (25 degC)
-----	-----
$CaCO_3 + 2 H^+ \rightleftharpoons Ca^{2+} + CO_2 + H_2O$ (calcite) (g)	9.74
$CaCO_3 + 2 H^+ \rightleftharpoons Ca^{2+} + CO_2 + H_2O$ (aragonite) (g)	9.97
$CaO + 2 H^+ \rightleftharpoons Ca^{2+} + H_2O$ (lime)	32.95
$Ca(OH)_2 + 2 H^+ \rightleftharpoons Ca^{2+} + 2 H_2O$ (portlandite)	22.80

Source: Solvay S.A. Bruxelles (18)

Type:

Media:

Method:

Year:

Remark: nicht anwendbar

Source: Schaefer Kalk Diez

3.3.2 Distribution

Media: other

Method:

Year:

Result:

- There is no mathematical model giving the distribution of calcium and chloride ions between the different compartments of the environment.
- Natural occurrence:

CALCIUM:

- 1/ Calcium, a metallic element, is fifth in abundance in the earth's crust, of which it forms approximately 3.6 %.
- 2/ Concentrations in igneous and sedimentary rocks are 3.5 % and 3.3 %, respectively.
- 3/ The average calcium concentration in soils is reported to be approximately 1.4 %.

CARBONATE:

- Carbonate minerals, primarily calcite (CaCO_3) and dolomite ($\text{CaMg}(\text{CO}_3)_2$), have been estimated to contain more than 83 % of the carbon on the earth.
- In soil systems, inorganic carbon exists mostly as CaCO_3 .
- Carbonate species (e.g. H_2CO_3 , HCO_3^- , $(\text{CO}_3)^{2-}$) naturally present in rain tend to accumulate in soils of arid areas. Where evapotranspiration exceeds precipitation, the flux of water into the soil is not sufficient to remove sparingly soluble salts like CaCO_3 from the soil.
- Another source of calcium carbonate in soils is groundwater: as groundwater nears the surface, evapotranspiration and CO_2 loss to the atmosphere (which decreases CaCO_3 solubility) lay cause CaCO_3 to precipitate.

Source: Solvay S.A. Bruxelles

(17) (4) (25) (18) (21) (10)

Media:

Method:

Year:

Remark: nicht anwendbar

Source: Schaefer Kalk Diez

3.4 Mode of Degradation in Actual Use

- Remark:**
- Ca^{2+} and carbonate are used physiologically by living organisms.
 - Ca^{2+} and carbonate are persistent indefinitely in the environment as equilibrium between various ionic forms, complexes and precipitates.

- Stability of that equilibrium depends on environmental conditions such as pH, temperature, concentration of complexing ions (major or trace elements).

Source: Solvay S.A. Bruxelles

Remark: THE SUBSTANCE AS REPORTED HERE IS A COMPONENT OF AUTOMOTIVE ENGINE CRANKCASE LUBRICANTS. IT REACTS IN THE LUBRICANT WITH ACIDIC MATERIALS RESULTING FROM OXIDATION OR THE INTRODUCTION OF ACIDIC GASES FROM THE COMBUSTION CHAMBER PASSING BY THE OIL AND COMPRESSION RINGS OF THE ENGINE CYLINDERS. THE PRODUCT FROM THE NEUTRALIZATION IS A MIXTURE OF CALCIUM SALTS WHICH REMAINS SOLUBLE IN THE LUBRICANT.

Source: Lubrizol Great Britain Limited Belper, Derby

Remark: nicht anwendbar

Source: Schaefer Kalk Diez

3.5 Biodegradation

Type:

Inoculum:

Result: other: not applicable for an inorganic compound

Method:

Year:

GLP:

Test substance:

Source: Solvay S.A. Bruxelles

Type:

Inoculum:

Method:

Year:

GLP:

Test substance:

Remark: Natürliches CaCO₃/Kalkstein wird als Düngemittel verwendet.

Source: Schaefer Kalk Diez

3.6 BOD₅, COD or BOD₅/COD Ratio

Method: other

Remark: Not applicable for an inorganic compound.

Source: Solvay S.A. Bruxelles

Remark: nicht anwendbar

Source: Schaefer Kalk Diez

3.7 Bioaccumulation**Species:****Exposure period:****Concentration:****BCF:****Elimination:****Method:** other**Year:** GLP:**Test substance:****Remark:** Not bioaccumulable.
log P o/w is not applicable for an inorganic compound which dissociates.**Source:** Solvay S.A. Bruxelles**Species:****Exposure period:****Concentration:****BCF:****Elimination:****Method:****Year:** GLP:**Test substance:****Remark:** Wird CaCO₃ zusammen mit der Nahrung aufgenommen, so werden ca. 10% vom Organismus absorbiert, 90% werden ausgeschieden. Es gibt keine Anreicherung der Substanz im Gewebe.**Source:** Schaefer Kalk Diez**3.8 Additional Remarks****Remark:** Calcium carbonate is a poorly soluble complex; as the environmental fate and pathways, presented in chapter 3, are related to the free ionized forms Ca²⁺ and (CO₃)²⁻, they are only applicable to the soluble complex form of CaCO₃, with a solubility of 10 exp-5.20 M (see section 3.1.2). CaCO₃ exists mainly as precipitated form.**Source:** Solvay S.A. Bruxelles

AQUATIC ORGANISMS**4.1 Acute/Prolonged Toxicity to Fish**

Type: field observation
Species: Gambusia affinis (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
TLm : > 56000
Method:
Year: **GLP:**
Test substance: no data
Remark: At the dosage of 56000 mg/l of CaCO₃, the turbidity was reduced from an initial value of 260 mg/l to 35 mg/l.
Source: Solvay S.A. Bruxelles
Test condition: The authors studied the effect of adding calcium carbonate to highly turbid waters containing the fishes at 19-21 degree C.

(26)

Type:
Species: Poecilia latipinna (Fish, estuary)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
NOEC: > 200
Method:
Year: **GLP:**
Test substance:
Source: Solvay S.A. Bruxelles

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Type:
Species:
Exposure period: 96 hour(s)
Unit: mg/kg soil dw **Analytical monitoring:**
TLm/96 : > 1000
Method:
Year: **GLP:**
Test substance:
Source: Guaber SPA Funo di Argelato (B0)

Type:
Species:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: nicht ökotoxisch
Source: Schaefer Kalk Diez

4.2 Acute Toxicity to Aquatic Invertebrates

Species:

Exposure period:

Unit:

Analytical monitoring:

Method:

Year:

GLP:

Test substance:

Remark: nicht ökotoxisch

Source: Schaefer Kalk Diez

4.3 Toxicity to Aquatic Plants e.g. Algae

Species:

Endpoint:

Exposure period:

Unit:

Analytical monitoring:

Method:

Year:

GLP:

Test substance:

Remark: nicht ökotoxisch

Source: Schaefer Kalk Diez

4.4 Toxicity to Microorganisms e.g. Bacteria

Type:

Species:

Exposure period:

Unit:

Analytical monitoring:

Method:

Year:

GLP:

Test substance:

Remark: nicht ökotoxisch

Source: Schaefer Kalk Diez

4.5 Chronic Toxicity to Aquatic Organisms**4.5.1 Chronic Toxicity to Fish****Species:** Catostomus commersoni (Fish, fresh water)**Endpoint:** other: blood disturbances**Exposure period:****Unit:****Analytical monitoring:** no data**Method:****Year:****GLP:****Test substance:** no data**Remark:** During 4 days of acid exposure, similar disturbances in blood acid-base status and plasma ion levels occurred in both experimental media, but with more marked changes in soft water.

A mixed respiratory and metabolic blood acidosis developed, manifested by significant depressions of plasma pH and bicarbonate, and simultaneous elevation of carbon dioxide tension.

Plasma Na⁺ and Cl⁻ loss was reflected in a significantly reduced osmolality. Plasma K⁺ increased while levels of divalent cations (Ca⁺⁺, Mg⁺⁺) were unaltered.

An increase of diffusive permeability of the gill epithelium and net branchial acid entry may explain the greater ion loss and blood acidosis seen in soft water acid exposed suckers.

In contrast, previous investigations on trout (Salmo gairdneri) report fundamental differences in the responses to acidified hard and soft water. This suggests that the mechanism(s) of action of low pH may be species dependent.

Source: Solvay S.A. Bruxelles**Test condition:** Suckers (200-600 g) were surgically fitted with caudal artery of vein cannulae for repetitive blood sampling and exposed to sublethal levels of acidity (pH 4-4.5 H₂SO₄) in both hard ([Ca⁺⁺] = 1.5-2.5 meq/l) and soft water ([Ca⁺⁺] = 0.1-0.3 meq/l).

(28) (29)

Species: Salmo gairdneri (Fish, estuary, fresh water)
Endpoint: other: physiological response
Exposure period: 5 day
Unit: **Analytical monitoring:** no data
Method: **GLP:**
Year:
Test substance: no data
Remark: In moderately hard water ($[Ca^{++}] = 1.6-2.7$ meq/l) at a mean pH of 4.3, trout developed a major blood acidosis but exhibited only a minor depression in plasma ion levels.
In acidified soft water ($[Ca^{++}] = 0.3$ meq/l), only a minor acidosis occurred, but plasma ion levels fell and there were substantially greater mortalities.
Lethal bioassays performed on fingerling trout over a range of pH levels (3.0-4.8) revealed an important influence of external $[Ca^{++}]$ on resistance to acid exposure. Terminal physiological measurements on adult fish succumbing to low pH in soft water indicate the singular importance of iono-regulatory failure as the toxic mechanism of action under these circumstances.
Source: Solvay S.A. Bruxelles
Test condition: The physiological responses of 1 to 2 year old rainbow trout to low pH are dependant on the environmental Ca^{++} concentration.
Trouts were maintained for 5 days in acidified water.
(30) (31)

Species:
Endpoint:
Exposure period:
Unit: **Analytical monitoring:**
Method: **GLP:**
Year:
Test substance:
Remark: nicht ökotoxisch
Source: Schaefer Kalk Diez

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Endpoint: reproduction rate
Exposure period:
Unit: **Analytical monitoring:** no data
Method:
Year: **GLP:**
Test substance: no data
Result: No. of young on the 1st day of reproduction, total young, and the no. of generations were greater with increasing hardness. At the max. test hardness, ~ 65% more young were produced than at the lowest hardness.
Furthermore, time to sexual maturity was ~ 1 day shorter in the harder culture water.
Daphnids fed the combination of green alga, trout chow, and dehydrated alfalfa were > 3-fold more productive than daphnids fed only the alga or only trout chow and alfalfa. The combination of the algae-reinforced diet with hard culture water provided for optimal productivity.
Source: Solvay S.A. Bruxelles
Test condition: The effects of diet and water hardness, alone and in combination, on life history characteristics of D. magna were determined in 2 laboratory tests.
Water hardness : 50 to 350 mg/l (as CaCO₃).

(32)

Species:
Endpoint:
Exposure period:
Unit: **Analytical monitoring:**
Method:
Year: **GLP:**
Test substance:
Remark: nicht ökotoxisch
Source: Schaefer Kalk Diez

TERRESTRIAL ORGANISMS**4.6.1 Toxicity to Soil Dwelling Organisms**

Type:

Species:

Endpoint:

Exposure period:

Unit:

Method:

Year:

GLP:

Test substance:

Remark: nicht ökotoxisch

Source: Schaefer Kalk Diez

4.6.2 Toxicity to Terrestrial Plants

Species:

Endpoint:

Expos. period:

Unit:

Method:

Year:

GLP:

Test substance:

Remark: Natürliches CaCO₃/Kalkstein wird als Düngemittel verwendet.

Source: Schaefer Kalk Diez

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

Species:

Endpoint:

Expos. period:

Unit:

Method:

Year:

GLP:

Test substance:

Remark: Gefälltes Calciumcarbonat wird als Futter- und Nahrungsmittelzusatz verwendet.

Source: Schaefer Kalk Diez

4.7 Biological Effects Monitoring

Remark: nicht anwendbar

Source: Schaefer Kalk Diez

4.8 Biotransformation and Kinetics

Type:

Remark: nicht anwendbar

Source: Schaefer Kalk Diez

4.9 Additional Remarks

Remark: Gefälltes Calciumcarbonat ist ein synthetisches Produkt, das die gleichen chemischen Eigenschaften, wie das natürliche Calciumcarbonat/Kalkstein aufweist. Dieses ist am Aufbau der Lithospähre beteiligt.

Source: Schaefer Kalk Diez

5.1 Acute Toxicity**5.1.1 Acute Oral Toxicity**

Type: LD50
Species: rat
Sex:
Number of
Animals:
Vehicle:
Value: 6450 mg/kg bw
Method:
Year: GLP:
Test substance:
Source: Solvay S.A. Bruxelles

(33)

Type: LD50
Species: mouse
Sex:
Number of
Animals:
Vehicle:
Value: = 6450 mg/kg bw
Method:
Year: GLP:
Test substance:
Source: Guaber SPA Funo di Argelato (B0)

Type:
Species:
Sex:
Number of
Animals:
Vehicle:
Value:
Method:
Year: GLP:
Test substance:
Remark: nicht toxisch (s. 4.6.3)
Source: Schaefer Kalk Diez

5.1.2 Acute Inhalation Toxicity**Type:** LC0**Species:** rat**Sex:****Number of
Animals:****Vehicle:****Exposure time:** 90 minute(s)**Value:** .0812 mg/l**Method:****Year:****GLP:****Test substance:****Remark:** (male rat). Marginal increase of phospholipids in lung lavage after 21 h. No effect on lung weight, macrophage concentration, or histopatology.**Source:** Solvay S.A. Bruxelles

(34) (35)

Type:**Species:****Sex:****Number of
Animals:****Vehicle:****Exposure time:****Value:****Method:****Year:****GLP:****Test substance:****Remark:** Nicht toxisch.
Jeder Staub vermag, abhängig von der Dauer der Exposition, eine leichte Reizung der oberen Atemwege zu verursachen.**Source:** Schaefer Kalk Diez**5.1.3 Acute Dermal Toxicity****Type:** other**Species:** rabbit**Sex:****Number of
Animals:****Vehicle:****Value:** = 500 mg/kg bw**Method:****Year:****GLP:****Test substance:****Source:** Guaber SPA Funo di Argelato (B0)

5. Toxicity

date: 18-FEB-2000
Substance ID: 471-34-1

Type: other
Species: human
Sex:
Number of
Animals:
Vehicle:
Value:
Method:
Year: GLP:
Test substance:
Source: SMTPI SAINT ROME DE TARN

Type:
Species:
Sex:
Number of
Animals:
Vehicle:
Value:
Method:
Year: GLP:
Test substance:
Remark: nicht anwendbar
Source: Schaefer Kalk Diez

5.1.4 Acute Toxicity, other Routes

Type:
Species:
Sex:
Number of
Animals:
Vehicle:
Route of admin.:
Value:
Method:
Year: GLP:
Test substance:
Remark: nicht anwendbar
Source: Schaefer Kalk Diez

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation**Species:** rabbit**Concentration:****Exposure:****Exposure Time:****Number of****Animals:****PDII:****Result:** moderately irritating**EC classificat.:****Method:** other: Draize test**Year:****GLP:****Test substance:****Remark:** 500 mg/24 h.

test conditions are not available but it is believed that CaCO₃ as a pure solid does not entail the irritating effect. Impurities or degradation products may lead to irritant effects on the sweating skin due alkalinity.

Source: Solvay S.A. Bruxelles

(33)

Species: human**Concentration:****Exposure:****Exposure Time:****Number of****Animals:****PDII:****Result:** irritating**EC classificat.:** corrosive (causes burns)**Method:****Year:****GLP:****Test substance:****Source:** SMTPI SAINT ROME DE TARN**Species:****Concentration:****Exposure:****Exposure Time:****Number of****Animals:****PDII:****Result:****EC classificat.:****Method:****Year:****GLP:****Test substance:****Remark:** Die Substanz ist nicht hautreizend.**Source:** Schaefer Kalk Diez

5.2.2 Eye Irritation

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: highly irritating
EC classificat.:
Method: other: Draize test
Year: **GLP:**
Test substance:
Remark: 0.750 mg/24 h.
Test conditions not available but it is expected that CaCO₃ does not lead to eye irritation provided impurities or decomposition products are not present.
Source: Solvay S.A. Bruxelles

(33)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: not irritating
EC classificat.:
Method:
Year: **GLP:**
Test substance:
Remark: Test conditions not available.
Source: Solvay S.A. Bruxelles

(36)

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: highly irritating
EC classificat.: risk of serious damage to eyes
Method:
Year: **GLP:**
Test substance:
Source: Guaber SPA Funo di Argelato (B0)

5. Toxicity

date: 18-FEB-2000
Substance ID: 471-34-1

Species: human
Concentration:
Dose:
Exposure Time:
Comment:
Number of
Animals:
Result: slightly irritating
EC classificat.: irritating
Method:
Year: GLP:
Test substance:
Source: SMTPI SAINT ROME DE TARN

Species:
Concentration:
Dose:
Exposure Time:
Comment:
Number of
Animals:
Result:
EC classificat.:
Method:
Year: GLP:
Test substance:
Remark: Jeder Staub kann Beschwerden verursachen.
Source: Schaefer Kalk Diez

5.3 Sensitization

Type:
Species:
Number of
Animals:
Vehicle:
Result:
Classification:
Method:
Year: GLP:
Test substance:
Remark: nicht sensibilisierend
Source: Schaefer Kalk Diez

5.4 Repeated Dose Toxicity

Species: Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of
treatment:
Post. obs.
period:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Remark: nicht kanzerogen
Source: Schaefer Kalk Diez

5.5 Genetic Toxicity 'in Vitro'

-

5.6 Genetic Toxicity 'in Vivo'

-

5.7 Carcinogenicity

-

5.8 Toxicity to Reproduction

Type:
Species: Sex:
Strain:
Route of admin.:
Exposure Period:
Frequency of
treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Remark: nicht reproduktionstoxisch
Source: Schaefer Kalk Diez

5.9 Developmental Toxicity/Teratogenicity

Species: Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Remark: nicht anwendbar
Source: Schaefer Kalk Diez

5.10 Other Relevant Information

Type: adsorption
Remark: Calcium carbonate is converted to calcium chloride by gastric acid. Some of the calcium is absorbed from the intestines but 80 % is reconverted to insoluble calcium salts such as the carbonate and stearate, and excreted.
Source: Solvay S.A. Bruxelles (37)

Type: other
Remark: 2 ml einer 10 % Calciumcarbonat-Suspension wurden 6 Meerschweinchen i.p. appliziert. Je 2 Tiere wurden nach 30, 60 und 90 Tagen getoetet und histologisch untersucht. Die Substanz wurde komplett absorbiert, es konnten keine Hinweise einer Antikoerperreaktion festgestellt werden.
Source: BASF AG Ludwigshafen (38)

5.11 Experience with Human Exposure

Remark: Acute hypercalcemia and recurrent nephrolithiasis were observed in 3 subjects regularly ingesting large quantities of calcium carbonate-sodium bicarbonate powders over 20 years.
Source: Solvay S.A. Bruxelles (39)

Remark: some studies have used calcium carbonate to study physiological changes in dust retention, deposition... in the respiratory tract.
Source: Solvay S.A. Bruxelles (40) (41) (42) (43) (44)

- Remark:** Because no adverse health effects have been reported in the literature among workers using calcium carbonate, it being a nuisance dust, a TLV of 10 mg/m³ total dust is recommended.
- Source:** Solvay S.A. Bruxelles (45)
- Remark:** calcium carbonate like other calcium salt may cause constipation. Hypercalcemia can occur as can alkalosis following the regular use of large doses. Similar effects with renal dysfunction, metastatic calcification, nausea and absence of hypercalciuria have developed in some patients taking calcium carbonate with large quantities of milk or cream in the treatment of peptic ulcer.
- Source:** Solvay S.A. Bruxelles (37)
- Remark:** There are no reported health effects associated with repeated or prolonged exposure to pure calcium carbonate. If there is more than 1 % crystalline silica (quartz) present in the calcium carbonate, exposure to airborne concentrations may increase the risk of developing a disabling lung disease called silicosis
- Source:** Solvay S.A. Bruxelles (46)
- Remark:** Starke Konjunktivitis bds. nach lokaler akzidentieller Einwirkung bei einer Person beobachtet, die zur weiteren Behandlung in die Klinik eingewiesen wurde.
- Source:** BASF AG Ludwigshafen (47)

- (1) TRGS (1993)
- (2) H&SE EH40 "Occupational Exposure Limits";
- (3) ACGIH (1991-1992)
- (4) Hem J.D. (1970), Study and interpretation of chemical characteristics of natural waters, Water supply paper N.1473, 2nd ed., U.S. Geological Survey, Washington, D.C.
- (5) Stoerfall-Verordnung vom 20.09.1991
- (6) Solvay S.A. (1993), "Socal", Material Safety Data Sheet 147.
- (7) BASF AG, Sicherheitsdatenblatt Calciumcarbonat GG (16.12.1993)
- (8) Gmelins Handbuch der Anorganischen Chemie : CALCIUM Teil B - Lieferung 3, System - Nummer 28 (1961) Verlag Chemie GMBH.
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- (11) Martell A.E., Smith R.M. (1976 and 1982), Critical stability constants, vol. 4, Inorganic Complexes and vol. 5, First supplement, Plenum Press, New York.
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7.1 Risk Assessment

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SCIENTIFIC OPINION

Scientific Opinion on the Tolerable Upper Intake Level of calcium¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to re-evaluate the safety in use of calcium. The Panel was requested to consider if the Tolerable Upper Intake Level (UL) for calcium established by the SCF in 2003 (2,500 mg/day for adults, including pregnant and lactating women), which was based on different intervention studies of long duration in which total daily calcium intakes of 2,500 mg from both diet and supplements were tolerated without adverse effects, needed to be changed on the basis of new available evidence. A number of placebo controlled human intervention studies in adults published since then also showed that total daily calcium intakes of 2,500 mg from both diet and supplements are tolerated without adverse effects. The Panel considers that no relationship has been established between long-term calcium intakes from diet and supplements and increased risk of nephrolithiasis, cardiovascular disease or prostate cancer. No new data have become available which would require a revision of the UL for calcium for adults, including pregnant and lactating women, of 2,500 mg. No new data have become available which would allow the setting of a UL for infants, children or adolescents. Data from European populations indicate that intakes of calcium in high consumers among adult males can be close to the UL. Although available data do not allow the setting of a UL for infants, children or adolescents, no risk has been identified with highest current levels of calcium intake in these age groups. © European Food Safety Authority, 2012

KEY WORDS

Calcium, supplements, hypercalcaemia, hypercalciuria, UL, safety.

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SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to re-evaluate the safety in use of calcium and to provide, if necessary, revised Tolerable Upper Intake Levels (ULs) of calcium for all relevant population groups.

Calcium is important for the maintenance of healthy teeth and bones, cell signalling, coagulation, muscle contraction, neural transmission and many other functions. Calcium is the fifth most abundant element in the human body. Some 99 % of the total calcium of the body is located in bones and teeth, mostly as calcium hydroxyapatite. Bone mineral provides structure and strength to the body, and a reservoir of calcium that helps to maintain a constant concentration of calcium in the blood.

Foods vary widely in calcium content. The best sources are milk and milk products, which provide about 45 to 70 % of the dietary calcium in European diets. Some plants, drinking and mineral water, and food supplements are also good sources of well absorbable calcium.

A number of potential adverse effects of excessive calcium intakes have been proposed. These include hypercalciuria, deterioration of kidney function, kidney stone formation, the milk-alkali syndrome (MAS), vascular calcification, increased risk of cardiovascular disease and increased risk of prostate cancer.

The SCF (2003) based the derivation of a UL for calcium on the evidence of different intervention studies of long duration, some of which were placebo controlled, in which total daily calcium intakes of 2,500 mg from both diet and supplements were tolerated without adverse effects. Because of the abundance of data, the application of an uncertainty factor was considered unnecessary. A UL of 2,500 mg of calcium per day from all sources was proposed for adults, and for pregnant and lactating women.

A number of placebo controlled human intervention studies in adults published since then also show that total daily calcium intakes of 2,500 mg from both diet and supplements are tolerated without adverse effects.

The Panel notes that new case reports have become available on consumption of calcium supplements and the CAS/MAS syndrome. However, the Panel considers that no dose-response relationships can be derived from these.

The Panel considers that no relationship has been established between long-term calcium intakes from diet and supplements and increased risk of nephrolithiasis, cardiovascular disease or prostate cancer.

The Panel concludes that no new data have become available which would require a revision of the UL for calcium for adults, including pregnant and lactating women, of 2,500 mg, and that no new data have become available which would allow the setting of a UL for infants, children or adolescents.

Data from European populations indicate that intakes of calcium in high consumers among adult males can be close to the UL. Although available data do not allow the setting of a UL for infants, children or adolescents, no risk has been identified with highest current levels of calcium intake in these age groups.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Calcium has been assessed in the past by the Scientific Committee on Food. In the Opinion on the Tolerable Upper Intake Level (UL) of calcium of 4 April 2003 an UL was established for adults of 2500mg of calcium per day for calcium intake from all sources. The Committee could not derive an UL for children and adolescents due to insufficient data.

On 30 November 2010, the American Institute of Medicine (IoM) published a report on “*Dietary Reference Intakes for Calcium and Vitamin D*”. In this report, the IoM proposes new reference values and UL values for calcium which, as stated in the report “*are based on much more information and higher-quality study results than were previously available*”.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to:

- re-evaluate the safety in use of calcium,
- if necessary, provide revised tolerable upper intake levels, that are unlikely to pose a risk of adverse health effects, for calcium for all relevant population groups.

ASSESSMENT

1. Introduction

Calcium is important for the maintenance of healthy teeth and bones, cell signalling, coagulation, muscle contraction, neural transmission and many other functions. Calcium is the fifth most abundant element in the human body. Some 99 % of the total calcium of the body is located in bones and teeth, mostly as calcium hydroxyapatite. Bone mineral provides structure and strength to the body, and a reservoir of calcium that helps to maintain a constant concentration of calcium in the blood.

In 2003, the Scientific Committee on Food (SCF) established a Tolerable Upper Intake Level (UL) of calcium for adults, including pregnant and lactating women, of 2,500 mg/day. This UL was based on different long-term intervention studies in which a total daily intake of 2,500 mg of calcium/day from all sources (diet and supplements) was tolerated without adverse effects. The Committee was unable to derive a UL for children and adolescents from the very few data available from intervention studies, and it was considered inappropriate to derive such age-specific ULs via extrapolation from data collected in older age groups.

The UL for calcium of the SCF (2003) was the same as that from the US Institutes of Medicine (IoM, 1997), which was based on a LOAEL of 4,000-5,000 mg/day identified by using the milk alkali syndrome as the critical endpoint and applying an uncertainty factor of 2 because of observed associations between calcium intake and the potential hazard of kidney stones, hypercalciuria, and interference of dietary calcium with the bioavailability of some minerals in vulnerable populations. The IoM also defined the same UL of 2,500 mg of calcium/day for adults >70 years of age, for children and adolescents 1-18 years of age, and for pregnant and lactating women, but could not establish a UL for infants.

In 2011, the IoM published its re-assessment of the UL for calcium, which for adults ≥ 51 years old was based on a LOAEL of 2,000 mg of calcium/day with nephrolithiasis as the critical endpoint (Jackson et al., 2006). No uncertainty factor was applied. For adults 19 to 50 years of age, the UL was the same as in 1997 and was derived by interpolation between the UL for older adults and the new UL of 3,000 mg for children aged 9-13 years and adolescents aged 14-18 years. This latter value was set taking into account a presumably higher need of, and consequently a higher tolerance for, calcium in the age group 9-18 years in connection with the pubertal growth spurt. A UL of 1,500 mg of calcium/day was derived from a NOAEL of 1,750 mg/day observed in one intervention study on infants aged 2.5 to 5 months at baseline and 9 months at the end of the intervention (Dalton et al., 1997; Sargent et al., 1999). Uncertainty factors of 2 and 1.2 were applied to adjust for the weight increment during the intervention and the results were rounded up to ULs of 1,000 mg/day for infants aged 0-6 months and of 1,500 mg/day for infants aged 6-12 months, respectively. No new data were available for the age group 1-8 years and the UL of 2,500 mg/day was kept. For pregnant and lactating women, the age-specific UL for adolescents aged 14-18 years of 3,000 mg/day and the UL of 2,500 mg/day for women >19 years of age were considered adequate.

The Panel was requested to consider whether the UL for calcium established by the SCF in 2003 needed to be changed on the basis of new available evidence.

2. Dietary Intake

2.1. Food sources including dietary supplements

Foods vary widely in calcium content. The best sources are milk (120 mg/100 g) and milk products (up to about 1,100 mg/100 g), from which about 32 % of calcium is absorbable (Weaver, 2001). In

European diets, about 45 to 70 % of the dietary calcium intake is provided by dairy products (Guéguen and Pointillart, 2000; IUNA (Irish Universities Nutrition Alliance), 2001). Some plants are good sources of well absorbable calcium, for example brassica, almonds and dried apricots. However, some vegetables (rhubarb, spinach) contain considerable amounts of calcium which is poorly absorbed because of a high content in oxalate, which forms sparingly soluble calcium oxalate. Drinking water and mineral waters (>150 mg calcium/L) can also be good sources of absorbable calcium.

In the European Union, the following forms of calcium are authorised for addition to foods and for use in food supplements (Annex II of the Regulation (EC) No 1925/2006⁴ and Annex II of Directive 2002/46/EC⁵): carbonate, chloride, citrate malate, gluconate, glycerophosphate, lactate, hydroxide, oxide, acetate, L-ascorbate, bisglycinate, salts of citric acid, pyruvate, salts of orthophosphoric acid, succinate, L-lysinate, malate, L-pidolate, L-threonate, sulphate.

2.2. Dietary intakes

Mean intakes of calcium in European countries vary according to sex, age, and supplementation habits (Appendices A and B). There is a large diversity in the methodology used to assess the individual intakes of children, adolescents and adults. These differences in dietary assessment methods make direct comparisons difficult. Data from Poland based on a single 24-h recall have been listed in Appendices A and B for completeness, but have not been considered in the text. Age classifications may not be uniform and comparability is also hindered by differences in food composition tables used for the conversion of food consumption data to nutrient intake data (Deharveng et al., 1999). Although these differences have an impact on the accuracy of between-country comparisons, the data presented give a rough overview of average calcium intakes and intakes in high consumers in a number of European countries.

2.2.1. Adults

Mean calcium intakes from food only were between 623 mg/day (Belgium, ≥75 years) and 1,374 mg/day (Denmark, men, 18-24 years), and median intakes were within this range. Available values for high percentiles of intake were between 1,045 mg/day (Spain, P95, women, 18-64 years) and 2,422 mg/day (Germany, P95, men, 19-24 years). Mean calcium intakes in the surveys considering food and food supplements combined were within these ranges, as well as values for the highest percentiles of calcium intake.

2.2.2. Infants (≤1 year)

Mean calcium intakes from food were available from one country only (The Netherlands) and varied between 730 mg/day (0.75 years) and 824 mg/day (1 year). The value for the high percentile was 1,085 mg/day (P90, 1 year). Mean calcium intakes for the entire sample and for the highest percentiles of consumption in the only study (Finland) which considered food and food supplements together were within these ranges.

2.2.3. Children (generally between 1 and 14 years)

For young children (0-3 years), mean calcium intakes from food were between 664 mg/day (Italy, 0-<3 years) and 1,024 mg/day (Greece, 1-5 years). Values for the high percentiles of calcium intake

⁴ Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, p. 26-38.

⁵ Directive 2002/46/EC of the European Parliament and of 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. 51-57.

were between 1,070 mg/day (Italy, P95, 0-3 years, including fortified food) and 1,392 mg/day (Greece, P90, 1-5 years). Mean calcium intakes for the entire sample and for the highest percentiles of consumption in the surveys considering food and supplements together were within these ranges.

For older children (about 3-14 years) mean calcium intakes from food were between 804 mg/day (Ireland, girls, 5-12 years; United Kingdom, 4-10 years) and 1,072 mg/day (Denmark, aged 4-14 years), and median intakes were within that range. Values for the high percentiles of intake were between 1,123 mg/day (Spain, P95, 4-10 years) and 1,776 mg/day (Denmark, P95, 4-14 years). Calcium intakes were slightly higher in food surveys considering food and food supplements together. In these surveys, mean calcium intakes were between 724 mg/day (The Netherlands, 4-6 years) and 1,103 mg/day (Finland, boys, 6 years), and median intakes were within that range. Values for the high percentiles of intake varied between 1,151 mg/day (Spain, P95, 4-10 years, including fortified food) and 1,623 mg/day (The Netherlands, P95, boys, 9-13 years).

2.2.4. Adolescents (generally between 10 and 18 years)

Mean calcium intakes from food varied from 734 mg/day (Ireland, girls, 13-17 years) to 1,337 mg/day (Germany, 12-17 years). The two available median values were within that range. Values for the high percentiles of intake varied between 1,231 mg/day (Spain, P95, 11-17 years) and 2,400 mg/day (Germany, P95, 12-17 years).

Mean calcium intakes from food and supplements combined were between 786 mg/day (United Kingdom, 11-18 years) and 1,396 mg/day (Germany, 12-17 years, including fortified food). Available median intakes were within that range. The values for the high percentiles varied between 1,267 mg/day (Spain, P95, 11-17 years, including fortified food) and 2,515 mg/day (P95, Germany, 12-17 years, including fortified food).

3. Hazard identification

3.1. Calcium physiology and homeostasis

Calcium homeostasis is under endocrine and genetic control. Systemic and local factors regulate intestinal absorption, influx and efflux from bone, and calcium excretion and re-absorption by the kidney. Because of this regulatory network, there is no biomarker for calcium status.

3.1.1. Serum calcium and its regulation

Calcium is present in the blood in three different forms: as free Ca^{2+} ions, bound to protein (about 45 %), and complexed to citrate, phosphate, sulphate and carbonate (about 10 %). Calcium in the blood (and in extracellular fluid) is kept constant at 2.5 mmol/L (range 2.25-2.6 mmol/L), but it is ionised calcium (between 1.1-1.4 mmol/L) which is controlled by the interrelated action of three hormones, namely parathyroid hormone (PTH), 1,25-dihydroxycholecalciferol ($1,25\text{-(OH)}_2\text{-D}$) and calcitonin. The first two determine how much Ca^{2+} moves out of or into the body, whilst PTH determines how Ca^{2+} moves between the extracellular fluid and bone.

A decrease in serum concentrations of Ca^{2+} induces the release of PTH via the calcium-sensing receptor (CaSR) which is located on the cell surface of the parathyroid glands. The PTH stimulates $1,25\text{-(OH)}_2\text{-D}$ synthesis in the kidney, bone resorption, and renal re-absorption of calcium (Perez et al., 2008). Synthesis of $1,25\text{-(OH)}_2\text{-D}$ is also stimulated by low serum phosphorus concentrations and decreases with high phosphorus concentrations. An increase in serum concentrations of Ca^{2+} inhibits PTH secretion via the CaSR and $1,25\text{-(OH)}_2\text{-D}$ synthesis, and stimulates calcitonin secretion by the parafollicular C cells of the thyroid gland. Other locations of the CaSR include the intestine, kidney,

thyroid gland, lung, brain, skin, bone marrow, and osteoblasts. According to population-based genome-wide association studies, individual serum calcium concentrations within the normal range are influenced by some single-nucleotide polymorphisms of the CaSR gene (O'Seaghdha et al., 2010; Riccardi and Brown, 2010).

Several genetic disorders have been described where this homeostatic regulation is disturbed and hypercalcaemia results. The latter is considered to be a consequence of either due to loss-of-function mutations of CYP24A1 which encodes 25-hydroxyvitamin D 24-hydroxylase leading to idiopathic infantile hypercalcaemia (OMIM 143880), or inactivating mutations of the CaSR gene (Schlingmann et al., 2011). Such mutations can result in either neonatal severe primary hyperparathyroidism (OMIM 239200) or familial hypocalciuric hypercalcaemia (OMIM 145980). Rarely, inactivating auto-antibodies against CaSR cause hypocalciuric hypercalcaemia (Riccardi and Brown, 2010). Activating mutations of the CaSR gene can be the cause of autosomal dominant hypoparathyroidism (OMIM 601298) with hypocalcaemia and hypercalciuria, or of the Bartter syndrome type V.

Hypercalcaemia is defined by serum calcium concentrations >2.75 mmol/L (11 mg/dL). Values used for the diagnosis of hypercalcaemia may change across laboratories and will be defined in this Opinion according to the cut-off selected by the authors in each individual study. The most common causes of hypercalcaemia include malignant tumours, hyperparathyroidism of different aetiology, and less frequently excessive calcium and/or vitamin D intakes. Clinical symptoms of persistent hypercalcaemia are fatigue, muscular weakness, anorexia, nausea, vomiting, constipation, tachycardic arrhythmia, soft tissue calcification, failure to thrive and weight loss. Hypercalcaemia can lead to hypercalciuria when the renal capacity of calcium re-absorption is exceeded, and to renal concentration defects resulting in polyuria through activation of the renal CaSR.

Consequences of severe chronic hypercalcaemia are nephrolithiasis and impairment of kidney function, resulting in loss of the concentrating ability of the kidney (i.e., a decrease in salt and water reabsorption), and in volume and salt depletion. Chronic hypercalcaemia may also lead to calcification of soft tissues (e.g., nephrocalcinosis and vascular calcification), particularly when phosphorus concentrations in the blood are also high, as in renal insufficiency. The age-related decrease in urinary calcium excretion and renal function increases the sensitivity of older people to excess calcium intakes.

3.1.2. Calcium absorption in the intestine

Calcium must be in a soluble form or bound to soluble organic molecules to be absorbable. Depending on solubility, chemical form and other factors of the food, the fractional absorption of dietary calcium in adults is around 25 % (range 10 to 40 %). Fractional absorption decreases with high calcium content of diets and increases with low-calcium diets. This adaptation is modulated by PTH and $1,25-(\text{OH})_2\text{-D}$ in response to lower or higher serum calcium concentrations. Fractional calcium absorption is highest (up to 60%) in infancy (Abrams et al., 1997), declines thereafter to rise again before puberty, and declines after puberty to the adult value of 25 %. During pregnancy calcium absorption is twice as high (Moser-Veillon et al., 2001). Dietary factors also modulate calcium absorption. Most calcium salts used in fortified foods or dietary supplements are absorbed to a similar extent as calcium from dairy foods. A difference in the kinetics of calcium taken as a supplement and calcium taken with food is manifested in earlier and higher rises of serum calcium with supplemental calcium than with calcium ingested with food, whilst the total amount of calcium absorbed may be enhanced with food (Heaney et al., 1989). Significant differences in the time course of rises in serum calcium and reactive decreases in serum PTH concentrations following the consumption of different calcium salts (formate $\gg$ citrate $>$ carbonate) in the fasting state have been observed (Hanzlik et al., 2005). The clinical significance with respect to calcium uptake into bone and excretion of excess calcium into urine is not clear. Ionised calcium in serum following the ingestion of 1,000 mg of calcium as a supplement just before a meal by healthy subjects rose in 20 of 40 trials above the upper

limit of normal (specified as 1.28 mmol/L) within two to four hours, and was accompanied by a significant increase in calcium excretion in the urine and by an increase of the calcium/creatinine ratio by up to 0.13 (Reid et al., 1986). This finding indicates that rapidly absorbed calcium can transitorily lead to increased concentrations of serum and urinary calcium. The bulk of unabsorbed calcium forms complexes with bile acids, free fatty acids, oxalic acid and phytic acid, and excreted in the faeces (Heaney, 2002).

There are two main mechanisms for calcium transport in the intestine: an active transcellular process and a passive paracellular process. Both are regulated by hormones, nutrients and other factors.

Active transcellular transport in the small intestine is saturable and regulated by dietary intake and the needs of the body. The efficiency of this transport improves with diets low in calcium and in situations of increased need for calcium (e.g. growth, pregnancy and lactation), and it decreases with age. Regulation of the uptake of calcium ions (Ca^{2+}) at the apical side of the epithelium, and the release at the basolateral membrane, involves epithelial Ca^{2+} channels TRPV5 and TRPV6 (which are also expressed in the kidney and other organs), calbindins, the plasma-membrane Ca-ATPase (PMCA), and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX1), whose activation and/or expression is under the control of $1,25\text{-(OH)}_2\text{-D}$ by binding to the vitamin D receptor (VDR).

Passive para-cellular diffusion through the tight junctions of the intestinal epithelium, particularly of the jejunum and ileum, follows down an electrochemical gradient together with water, sodium and glucose, which is determined by the concentration of soluble calcium in the gut lumen. Passive para-cellular diffusion becomes important when dietary calcium is high and the trans-cellular pathway is down-regulated. This process is independent of age (Bronner, 1992).

3.1.3. Renal excretion and re-absorption of calcium

The majority of calcium absorbed is stored in the skeleton. Excess of absorbed calcium is excreted in urine, faeces and sweat. Calcium balance is positive in healthy children, adolescents and young adults before bone growth and bone modelling cease. Renal calcium excretion is the net result of glomerular filtration and tubular passive (collecting duct) or active (proximal tubule, loop of Henle, distal tubule) reabsorption (normal over 98 % of the filtered load). Active transport is under the control of PTH, calcitonin and $1,25\text{-(OH)}_2\text{-D}$, the levels of which are set via the CaSR by the calcium concentrations in extracellular fluid (Hoenderop et al., 2002). Average 24-h excretion of calcium is 40 mg in young children, 80 mg in prepubertal children and reaches about 150-200 mg in healthy adults. Small changes in the levels of Ca^{2+} which is filtrated into the urine can lead to substantial changes in urinary calcium. Below a serum calcium concentrations of 2 mmol/L, little (if any) calcium is excreted in the urine (<30 mg/24 h). The maximum amount of calcium which can be excreted by healthy humans is about 1,000 mg/24 h. Higher amounts are associated with the risk of calcium deposition in the renal tissue or with the formation of kidney stones because urine is supersaturated with calcium and phosphate. Stone formation and renal calcification are enhanced by an alkaline environment.

Urinary calcium excretion is increased by dietary sodium intake (30 to 40 mg of calcium excreted per every two grams of dietary sodium) (Matkovic et al., 1995) and in chronic metabolic acidosis (Bushinsky, 2001). Calcium excretion rises with excess dietary protein intake (by 0.5 mg for each gram of dietary protein, when intake is >47 g/day) (Walker and Linkswiler, 1972; Whiting et al., 1998).

Hypercalciuria is defined by a calcium excretion >0.3 mg/mg creatinine in 24-hour urine of adults, by a calcium excretion >250 mg/day in women and >275-300 mg/day in men, or by a calcium excretion >4 mg/kg bw/day for both men and women. Calcium/creatinine ratios in urine are higher in infancy and decrease with age. In randomly collected urine samples, calcium/creatinine ratios of 0.9, 0.6, 0.42 and 0.22 have been reported for ages <7 months, 7-18 months, 19 months to 6 years, and adults,

respectively (Sargent et al., 1993). Calcium/creatinine ratios may differ somewhat in different populations because of differences in dietary habits, sunshine exposure, drinking water composition and ethnicity. An increase in the calcium/creatinine ratio in a random urine sample can be an incidental occurrence only.

Chronic hypercalciuria may be the consequence of increased intestinal absorption of calcium (absorptive) or of decreased renal calcium reabsorption (resorptive). Causes of chronic hypercalciuria include excessive calcium intake (without accompanying hypercalcaemia), excessive vitamin D intake, hyperparathyroidism, hyperthyroidism, renal tubular acidosis, sarcoidosis or malignant tumors. Idiopathic (primary) hypercalciuria is characterised by the lack of concomitant hypercalcaemia. Pregnant women are often hypercalciuric from the 12th gestational week onwards even with “normal” calcium intakes. The hypercalciuria results from the doubling of intestinal calcium absorption in early pregnancy, which leads to positive calcium balance (Heaney and Skillman, 1971).

Persistent hypercalciuria is associated with decreased bone mineral density and higher risk of kidney stone formation, particularly in subjects with idiopathic hypercalciuria (Audran et al., 1991; Bataille et al., 1991). Kidney stones affect more than 5 % of the population in Europe, and up to 20-40 % of patients with calcium containing stones (about 80 % of kidney stones) have idiopathic hypercalciuria. As stones form in urine that is supersaturated, high concentrations of calcium (and oxalate) in urine, rather than the total amount of calcium excreted, increase the risk of nephrolithiasis (kidney stones). Other dietary risk factors for nephrolithiasis include low fluid intakes and high intakes of protein, salt, sucrose, alcohol and oxalate. Pregnancy is associated with an increased risk of kidney stone formation.

3.2. Adverse effects of excess calcium intake

A number of potential adverse effects of excessive calcium intakes have been proposed. These include hypercalciuria, deterioration of kidney function, kidney stone formation, the milk-alkali syndrome (MAS), vascular calcification, increased risk of cardiovascular disease and increased risk of prostate cancer (IoM, 2011).

3.2.1. Milk-alkali syndrome/calcium-alkali-syndrome

The adverse effects of combined therapy for peptic ulcers with milk and absorbable antacids (mostly sodium bicarbonate or calcium carbonate) were named collectively MAS (Burnett et al., 1949; Cope, 1936). Milk-alkali syndrome is characterised by metabolic alkalosis and hypercalcaemia with dehydration, renal failure, nephrocalcinosis and nephrolithiasis in variable combinations and severity. The SCF (2003) compiled case reports from 82 patients (24-95 years) with MAS, in which total calcium intakes were generally inadequately documented but reported to range between 400 and 23,000 mg/day. Milk-alkali syndrome was not observed in the course of a number of intervention studies which involved between 11 and 2,295 individuals (children, pregnant and pre- or perimenopausal women, elderly subjects), tested the effects of calcium supplements (500 to 2,000 mg/day) on different outcomes, and lasted between 12 weeks to four years (SCF, 2003).

Since then the therapy including milk for peptic ulcers has become obsolete but the syndrome has been increasingly observed with high intakes of supplemental calcium carbonate and MAS has been re-named calcium-alkali-syndrome (CAS) (Patel and Goldfarb, 2010). On the basis of 14 cases (2004-2010) reporting elevated serum calcium (2.64-6.43 mmol/L) and creatinine concentrations in males and females (35-81 years) consuming calcium carbonate at doses >1,000-44,000 mg/day, the IoM (2011) concluded that supplemental intakes of calcium carbonate $\geq 3,000$ mg/day were hazardous. Duration of supplementation was only reported in seven cases (from weeks to 19 years).

Extensive calcification of renal convoluted tubular cells and the tubular lamina have been described in kidney biopsies of patients with MAS or CAS, and which in severe cases were associated with interstitial fibrosis, areas of inflammatory changes and peri-glomerular fibrosis. The extent of calcium deposition was proportional to renal dysfunction. Calcium deposits in the cornea and conjunctiva, and less commonly in peri-articular tissue, subcutaneous tissue, the central nervous system, liver, adrenal glands, bone and lungs, have also been observed in chronic cases of MAS (Medarov, 2009).

The Panel notes that supplemental alkaline calcium intakes at doses >1,000 mg/day may increase the risk of CAS, but these case reports do not allow an estimation the amount of calcium or the duration of the treatment at which the risk increases.

A decrease in calcium excretion or in kidney function (e.g. in old age), an increase in calcium re-absorption (e.g. thiazide diuretics), or conditions leading to metabolic alkalosis (e.g. hyperemesis in pregnancy or bulimia) are risk factors for developing CAS when individuals are taking alkaline calcium supplements (Medarov, 2009).

3.2.2. Hypercalciuria, kidney function and kidney stones

3.2.2.1. Adults

The SCF (2003) reported on a higher frequency (compared to placebo) of incidental hypercalciuria (>350 mg/day) in post-menopausal women consuming calcium supplements (1,600 mg/day), in addition to dietary calcium intakes of about 800 mg/day, for four years. No subject receiving calcium supplements developed kidney stones, nephrocalcinosis or a reduction in glomerular filtration rate (Riggs et al., 1998). A trend for an increase in serum creatinine (by 1.2 µmol/L) with total calcium intakes of 2-3 g/day had been observed in a study involving 130 peri-menopausal women (Elders et al., 1994), whereas no effect was reported in 46 women aged 50-70 years at comparable calcium intakes (Schaafsma et al., 2002). It was unclear whether compromised glomerular function as indicated by increases in serum creatinine could have been attributed to high calcium intakes in the first study.

The effect of different doses of calcium on hypercalciuria and/or kidney function has not been systematically investigated, and no new data have become available since 2003.

The SCF (2003) also reported no increased risk of kidney stones in approximately 5,000 subjects (from different intervention studies) who received between 500 and 2,000 mg calcium/day as a supplement in addition to 300 to 1,800 mg of calcium/day from the diet (total intakes between 1,300 and 3,000 mg calcium/day) during three months to four years. None of these studies were designed to investigate the effects of calcium supplements on kidney stone formation. In two prospective cohort studies with 45,619 men aged between 40 and 75 years followed over four years (the Health Professionals Follow-up Study, Curhan et al., 1993) and 91,731 women aged between 34 and 59 years followed over 12 years (the Nurses' Health Study I, Curhan et al., 1997), without kidney stones at the beginning of the observation period, total calcium intakes >1,050 mg/day in men and >1,100 mg/day in women decreased the risk of kidney stone formation by approximately 35 % compared to lower intakes. In both studies, an inverse association was reported between dietary calcium and risk of kidney stones after adjustment for confounders, whereas calcium supplements in daily amounts below 100 mg increased the risk for stone formation by 20 % in women, with no further increase in the relative risk at higher intakes. In a population-based study which involved 1,309 women aged 20 to 92 years, women with kidney stones (n=44) ingested on average 250 mg less calcium per day than women without stones (840 vs 1,070 mg/day). Calcium supplements were not associated with the risk of kidney stone formation (Sowers et al., 1998).

The SCF (2003) concluded that both observational studies on the relationship between total calcium intake and kidney stone incidence and intervention studies with calcium supplements did not allow an estimation of the calcium intake on a population basis which would promote kidney stone formation.

A longer follow-up of the Health Professionals Study (Taylor et al., 2004) and results from a new Nurses' Health Study II (Curhan et al., 2004) have been published since then. In addition, the IoM (2011) established the UL for adults aged >51 years (LOAEL of 2,000 mg of calcium/day with nephrolithiasis as the critical endpoint) on the basis of a secondary analysis of the Women's Health Initiative (WHI) intervention trial (Jackson et al., 2006), for which a subsequent publication has become available (Wallace et al., 2011).

In the 14-year follow-up of the Health Professionals Study (Taylor et al., 2004), self-administered food frequency questionnaires (FFQs) were used to assess dietary (and supplemental) intakes at baseline and every four years in 45,619 men aged 40 to 75 years without history of nephrolithiasis. A total of 1,473 incident symptomatic kidney stones were documented. After adjusting for relevant risk factors, a higher dietary calcium intake was associated with a reduced risk of kidney stones (RR 0.69, 95 % CI 0.56-0.87, for men in the highest vs. the lowest quintile of calcium intake) in men aged <60 years. Median calcium intakes from the diet were 503 mg/day in the lowest and 1,194 mg/day in the highest quintiles, respectively. There was no association between dietary calcium and stone formation in men aged ≥60 years. Supplemental calcium intake was not associated with risk of developing kidney stones.

Similarly, in the eight-year follow-up of the Nurse's Health Study II (Curhan et al., 2004), self-administered food frequency questionnaires were used to assess dietary (and supplemental) intakes at baseline and at four years of follow-up in 96,245 female participants aged 27 to 44 years with no history of kidney stones. A total of 1,223 incident symptomatic kidney stones were documented. After adjusting for relevant risk factors, a higher dietary calcium intake was associated with a reduced risk of kidney stones (RR 0.73, 95 % CI 0.59-0.90, for women in the highest vs. the lowest quintile of calcium intake). Calcium intakes from diet were <626 mg/day in the lowest and >1,129 mg/day in the highest quintiles, respectively. Supplemental calcium intake was not associated with risk of kidney stone formation.

The Panel notes that dietary calcium intakes in the range of the most recent dietary recommendations do not promote kidney stone formation on a population basis. The Panel also notes that calcium supplements have not been associated with an increased risk of kidney stones in large prospective cohort studies.

In the Women's Health Initiative trial (Jackson et al., 2006), 36,282 post-menopausal women aged 50 to 79 years (mean age 62 years) were randomly assigned to consume with meals either a placebo (n=18,106) or 1,000 mg of elemental calcium (calcium carbonate) with 10 µg (400 IU) of vitamin D₃ per day (n=18,176) for an average period of seven years. Hypercalcaemia and kidney stones were among the exclusion criteria. The primary outcomes were bone fractures and measures of bone density. Subjects were allowed to continue the use of personal supplements up to 1,000 mg of calcium and up to 12.5 µg of vitamin D per day. Mean baseline intake (diet and non-trial supplements) of calcium was approximately 1,150 mg/day, and mean baseline intake of vitamin D was about 9 µg/day. About 40 % of the subjects consumed >1,200 mg calcium/day at baseline and 29 % consumed personal supplements with >500 mg calcium daily. Compliance (≥80 % of the assigned study supplements) ranged from 60 % to 63 % during the first three years of follow-up, with an additional 13 % to 21 % of the participants taking at least half of their study pills. At the end of the trial, 76 % of the women were still taking the study supplements, of which 59 % were compliant. Dietary calcium intake during the trial remained stable, but intake from supplements increased by 100 mg/day in both groups. Supplementation with calcium and vitamin D resulted in an increased risk (17 %) of self-reported clinically diagnosed kidney stones. Kidney stones were reported by 449 women in the supplemented group, as compared to 381 women in the placebo group (hazard ratio, 1.17; 95 %

CI 1.02-1.34). The Panel notes that although excluded by protocol design, 161 participants in the active arm and 172 in the placebo arm had a self-reported history of urinary tract stones and were included in the intention-to-treat analysis. It is unclear whether (and the extent to which) women with history of kidney stone formation contributed to the cases in each group. In a further analysis of the same study (Wallace et al., 2011), neither total calcium intake nor the use of calcium supplements at baseline were associated with the risk of kidney stones. The risk of kidney stones was not significantly different between the treatment and placebo groups when only subjects compliant with the study treatments were considered (hazard ratio: 1.21; 95 % CI 0.98-1.34). Confirmation of diagnosis upon hospital admission occurred in a small proportion of women (35 cases in each group) and was not significantly different between groups. The Panel notes that this study did not assess the relationship between dietary, supplemental (sum of both personal and trial supplements) or total calcium intakes in relation to the risk of kidney stone formation, but rather the effect of an additional amount of calcium (and vitamin D) over widely variable baseline calcium intakes from food and personal supplements (from <400 mg/day to >1,490 mg/day), and this additional amount of calcium was unrelated to the risk of developing kidney stones. The Panel also notes that additional calcium (and vitamin D) supplementation did not increase the risk of self-reported kidney stones in subjects who complied with the study treatment, or the proportion of women with objective diagnosis of kidney stones requiring hospital admission. The Panel considers that this study does not provide evidence for an increased risk of kidney stones which could be attributed to high calcium intakes.

The Panel notes that calcium intakes up to about 2,400 mg/day have not been associated with an increased risk of chronic hypercalciuria or impaired kidney function, and that calcium intakes up to 3,000 mg/day have not been associated with an increased risk of nephrolithiasis in the general adult population.

3.2.2.2. Infants

One intervention study assessed calcium excretion in urine and renal function in relation to calcium intakes in infants. The formation of kidney stones in infants is rare.

A double-blind RCT (Dalton et al., 1997; Sargent et al., 1999) was performed to investigate the efficacy of an iron-fortified (12.8 mg/L) cow's milk-based formula with added calcium glycerophosphate (1,800 mg calcium/L, 1,390 mg phosphorus/L) vs. the same (control) formula without added calcium and phosphate (calcium 465 mg/L and phosphorus 317 mg/L) on the prevention of lead absorption in infants. The effects of the study formulas on calcium and iron metabolism were also assessed among other indicators of safety. Eligibility was tested pre-randomisation in 314 formula-fed healthy infants who consumed the test formula during a one-month run-in phase. Three infants developed hypercalciuria (calcium/creatinine >0.8 mg/mg) and were excluded from randomisation for this reason. A total of 103 eligible infants aged 3.5-6.0 months were randomised to consume either formula for nine months. Blood samples were obtained at baseline and at four and nine months, and urine samples at baseline, one, two, four, six and nine months for calcium and creatinine analysis. At the end of the intervention the mean age of the children was 14 months. Eleven children dropped out in each of the study groups for various reasons. Calcium intake from formula was five times higher in the intervention group (n=41) than in controls (n=40) at baseline and at four and nine months (1,740±450, 1,710±460 and 1,560±700 mg/day vs 480±90, 400±130 and 340±173 mg/day, respectively). Serum calcium concentrations did not differ between groups. One child in the control formula group had a serum calcium concentration >2.72 mmol/L after four months which returned to normal after nine months. There were no significant differences in urinary calcium/creatinine ratios between groups at baseline, or at four or nine months. Incidental hypercalciuria was observed in five infants in each group, which only persisted in a repeat sample in one subject of each group. Haematuria was not observed in any subject.

The Panel notes that in one small study, dietary calcium intakes up to about 1,750 mg/day for nine months did not increase the risk of hypercalcaemia, clinically significant hypercalciuria, or symptoms of kidney stones in infants.

3.2.2.3. Children and adolescents

In 2003, the SCF (2003) noted that no adverse effects of calcium citrate-malate supplements (500 to 1,000 mg calcium over 1.5 to 3 years) or of extra dairy foods or foods fortified with milk extracts (700 to 820 mg/day extra calcium over one year) were reported in 217 children (from five intervention studies) between 6 and 14 years and between 6.6 and 11 years, respectively, in comparison to non-supplemented controls. Mean calcium intakes from diet in groups receiving calcium supplements were about 900-1,000 mg/day. The SCF considered these data as insufficient to derive a UL for children or adolescents.

In a double-blind RCT aimed at investigating the role of calcium supplementation on lead poisoning, 88 “moderately poisoned” children aged 1-6 years were randomised to consume calcium (dose adjusted to reach 1,800 mg/day from food and supplements) or placebo for three months. Data from 67 children were available for analysis (35 in the calcium group). Calcium intake at enrolment was $1,108 \pm 465$ mg and 973 ± 409 mg for the placebo controls and supplemented groups, respectively. Urinary calcium/creatinine ratios did not differ between children in the intervention (total calcium intake of $1,701 \pm 121$ mg /day) and control ($1,012 \pm 454$ mg Ca/day) groups, and were 0.1 ± 0.1 throughout the study. Haematuria was not observed (Markowitz et al., 2004).

The Panel notes that, in one small study of short duration, calcium intakes of about 1,800 mg/day for three months did not affect renal function in children aged 1-6 years. No new data are available for adolescents.

3.2.3. Risk of cardiovascular disease

Chronic hypercalcaemia can lead to calcification of soft tissues, particularly if phosphorus levels are also high, but no relationship between calcium intake and the occurrence of nephrocalcinosis or calcification of vascular tissue has been established in humans except in the presence of decreased kidney function. Coronary artery calcification has been described in patients with renal insufficiency on dialysis and treated with calcium-containing phosphate binders (Asmus et al., 2005; Block et al., 2005; Goodman et al., 2000; Russo et al., 2007), but not in healthy subjects.

It has been hypothesised that excess calcium intakes leading to chronic hypercalcaemia could increase the risk of cardiovascular events through arterial calcification. Arterial calcification has been associated with an increased risk of cardiovascular morbidity and mortality in men and women in a number of prospective cohort studies (Jacobs et al., 2010; Levitzky et al., 2008; Wilson et al., 2001; Wong et al., 2009).

Few studies have investigated the relationship between calcium intake and vascular calcification in healthy subjects and none of these was designed for that purpose.

In a RCT trial of two years duration in 163 healthy men (age 57 ± 10 years), no difference in calcification of the coronary arteries or the abdominal aorta assessed by computed tomography (CT) was observed between subjects on calcium supplements (1,200 mg/day) and subjects on placebo (Van Pelt et al., 2009). Calcium intakes from diet were not reported.

In contrast, in a retrospective analysis of a two-year RCT of similar size which investigated the effect of supplemental calcium (1,000 mg/day) and vitamin D₃ (20 µg/day) given as fortified milk (vs. no supplemental milk) on bone mass in 167 men >50 years of age (Daly et al., 2006), men with

calcification of the abdominal aorta measured by CT at baseline and receiving the fortified milk showed a significantly greater progression of aortic calcification than controls (Daly et al., 2006). Baseline mean dietary calcium intake was about 900 mg/day and serum 25(OH)D was 75 nmol/L.

In the Epidemiology of Coronary Artery Calcification study conducted between 1991 and 2008 (Bhakta et al., 2009), 1,376 randomly selected asymptomatic adults of all ages were repeatedly examined by electron beam computed tomography for coronary artery calcification (CAC) or aortic valve calcification (AVC). A retrospective analysis was performed on the subgroup of 257 participants (144 female) >60 years who had CAC or AVC at baseline and at least one follow-up measurement. None of the males and only 25 of the 144 female subjects reported taking calcium supplements (500-2,000 mg/day). The CAC and AVC scores at baseline or their progression rates over time (mean follow-up 3.7±0.9 years) were not significantly different between calcium supplement female users and non-users. The Panel notes that this study may have been underpowered for the CAC outcome.

The Panel notes that the data available on the relationship between calcium intake and risk of vascular calcification in humans are inconsistent.

A number of large prospective cohort studies have found a positive association between serum calcium concentrations and increased risk of myocardial infarction, stroke or death in the general adult population and in patients with coronary artery disease (Foley et al., 2008; Grandi et al., 2012; Jorde et al., 1999; Lind et al., 1997). However, these studies do not allow conclusions to be drawn on the relationship between dietary calcium intakes and cardiovascular disease (CVD) risk because calcium intakes were not reported.

A systematic review (Chung et al., 2009) which investigated the relationship between calcium intakes (food and supplements) and incidence of cardiovascular (CV) disease (i.e., total, fatal and non-fatal CV events; total, fatal and non-fatal myocardial infarction and stroke) identified ten longitudinal cohort studies (Al-Delaimy et al., 2003; Ascherio et al., 1998; Bostick et al., 1999; Iso et al., 1999; Larsson et al., 2008; Marniemi et al., 2005; Umesawa et al., 2006; 2008; Van der Vijver et al., 1992; Weng et al., 2008), one nested case-control study (Ross et al., 1997), and no RCTs which had evaluated such a relationship. Except for two studies with a sample size of 755 (Marniemi et al., 2005) and 1,340 (Van der Vijver et al., 1992) subjects, the cohorts included >17,700 and up to about 44,000 subjects. Baseline calcium intakes were assessed by FFQs in all studies and were analysed as predictors of long-term cardiovascular outcomes. Calcium intake within the studied populations varied widely across studies. Mean intakes in the lowest and highest quintiles ranged from about 250 and 667 mg/day in the Japan Collaborative Cohort (JACC) Study (Umesawa et al., 2006) to 876 and 1,916 mg/day in a Finnish male cohort (Larsson et al., 2008). Calcium intakes in studies on Asian populations were generally low (Umesawa et al., 2006; 2008; Weng et al., 2008). Two studies addressed CV death in men and women separately with follow-ups of 9 and 28 years (Umesawa et al., 2006; Van der Vijver et al., 1992); three studies assessed cardiac events (fatal and non fatal combined) either in both sexes together or in men with follow-ups of 10-13 years (Al-Delaimy et al., 2003; Marniemi et al., 2005; Umesawa et al., 2008); cardiac death was analysed in four studies, separately in men and women, at 8, 9, 12 or 28 years of follow-up (Al-Delaimy et al., 2003; Bostick et al., 1999; Umesawa et al., 2008; Van der Vijver et al., 1992), and non-fatal cardiac events in one study in men with 12 years of follow-up (Al-Delaimy et al., 2003); six studies assessed total incidence of stroke in men, women, or both sexes combined with follow-ups of 8-14 years (Ascherio et al., 1998; Iso et al., 1999; Larsson et al., 2008; Marniemi et al., 2005; Umesawa et al., 2008; Weng et al., 2008); and two studies addressed fatal stroke in men and women separately with follow-ups of 10 and 13 years (Ross et al., 1997; Umesawa et al., 2006). One study (the Iowa Women's Health Study, Bostick et al., 1999) found a significant association between calcium intake <696 mg/day and higher risk of ischemic heart disease death compared to an intake of >1,425 mg calcium/day in white women aged 55 to 69 years. A Japanese (Umesawa et al., 2008) and a Taiwanese (Weng et al., 2008) study in men and women (40-79 years and ≥40 years, respectively) found progressively lower risks for stroke

in subjects in higher quintiles of calcium intake in populations with relatively low calcium intakes, whereas one study in women (32-57 years) found significantly higher stroke risk in subjects with calcium intake <500 mg/day compared with women in the next two higher quintiles (Iso et al., 1999). No other associations between calcium intake (total, from food or from supplements) and risk of CVD (any outcome, men, women, or both sexes combined) were reported.

Three additional prospective cohort studies on the relationship between calcium intakes and risk of CVD have been published.

In the Kuopio Osteoporosis Risk Factor and Prevention Study (Pentti et al., 2009), 10,555 females aged 52-62 years without coronary heart disease (CHD) in 1994 were followed-up for seven years. Information about health events and supplemental calcium intake was obtained at baseline via questionnaires, whereas dietary calcium intakes were calculated from liquid milk products and cheese. Dietary calcium intakes in the 2,723 (25.8 %) users and the 7,832 non-users of calcium or calcium plus vitamin D supplements according to the 1994 questionnaire were 773 ± 351 mg/day and 818 ± 404 mg/day, respectively. In age-adjusted analysis of the entire cohort, the hazard ratio (HR) for CHD was 1.14 (95 % CI 0.94–1.39) in calcium supplement users compared with non-users. The multivariate adjusted HR for supplement users compared to non users with respect to CHD was 1.24 (95 % CI 1.02-1.52) for the total sample and 1.26 (95 % CI 1.01-1.57) for postmenopausal women. The Panel notes that calcium intakes from supplements were not reported.

In the European Prospective Investigation into Cancer and Nutrition (EPIC) study (Li et al., 2012), 23,980 participants aged 35-64 years with no history of major CV events were followed up for about 11 years. Dietary calcium was assessed at baseline using a FFQ, whereas use of dietary supplements (including calcium) was checked by questionnaires at baseline and regularly during follow-up. Outcome measures were incidence of myocardial infarction, incidence of stroke, and CVD mortality. A total of 354 cases of myocardial infarction, 260 cases of stroke and 267 cases of CV death were documented during follow-up. Between 3 and 4 % of participants reported use of calcium supplements (either alone or in combination with other vitamins and minerals), but doses of supplemental calcium were not reported. Mean dietary calcium intake at baseline was 766 mg/day in supplement non-users and about 820 mg/day in supplement users. After adjustment for potential confounders, none of the linear trend tests were statistically significant. A statistically significant inverse association was only found between total dietary calcium intake and risk of myocardial infarction for the third (820 mg/day) quartile compared with the lowest (513 mg/day) quartile (HR 0.69; 95 % CI 0.50-0.94) in both sexes combined. The association was also significant for women (HR 0.43; 95 % CI 0.22-0.82), but not for men. Users of calcium supplements had a statistically significantly increased risk of myocardial infarction in comparison with non-users of any supplements (HR 1.86; 95 % CI 1.17-2.96). This association was more pronounced for calcium-only supplement users (HR 2.39; 95 % CI 1.12-5.12), and related to most recent but not to cumulative calcium supplementation. No significant association was found between calcium supplementation and risk of either stroke or overall CVD mortality.

In a subsequent publication (Mursu et al., 2011) of the Iowa Women's Health Study (Bostick et al., 1999), 38,772 women aged 55-69 years were followed-up for 19 years. Diet was assessed using a FFQ in 1986 and 2004, and the use of supplemental calcium by a specific questionnaire in 1986, 1987, 1989, 1992, 1997 and 2004. After adjustment for confounders, use of calcium-containing supplements was associated with a significantly lower rate of total mortality (HR 0.91; 95 % CI 0.88-0.94) and cardiovascular mortality (HR 0.87; 95 % CI 0.82-0.92). The inverse association between supplemental calcium and total mortality was not significant at the highest doses of supplemental calcium (>1,300 mg calcium/day). The Panel notes that dietary (and thus total) calcium intakes were not reported in this follow-up analysis.

The Panel notes that calcium intakes up to about 2,000 mg/day from food and supplements have not generally been associated with an increased risk of CV events in a high number of large prospective cohort studies.

No RCTs specifically designed to investigate the effects of supplemental calcium on CVD risk are available.

In a systematic review and meta-analysis (Wang et al., 2010), four RCTs which reported on the occurrence of CV events with calcium supplementation versus placebo were identified (Baron et al., 1999; Bolland et al., 2008; Prince et al., 2006; Reid et al., 2008). The study by Baron et al., (1999) reported similar proportions of subjects hospitalised for cardiac disease (10 % vs. 11 %) or stroke (2 % vs. 3 %) among those who received 1,200 mg/day of supplemental calcium (n=464) and those who received placebo (n=466) for four years (mean calcium intakes from diet 880 mg/day). Prince et al., (2006) also found similar rates of CHD in women receiving supplemental calcium (n=730) or placebo (n=730) for five years (mean calcium intakes from diet 915 mg/day). Consumption of calcium supplements (1,000 mg/day; n=732) for five years did not increase the risk of myocardial infarction, stroke or composite CVD endpoint (myocardial infarction, stroke, sudden death) in post-menopausal women (mean calcium intakes from diet 860 mg/day) when unreported events obtained from a national database were included in the analysis (Bolland et al., 2008). One trial in men of two years duration found significantly more self-reported composite CV events in the calcium supplemented group (600 mg or 1,200 mg/day; n=216) than in the placebo group (n=107) (Reid et al., 2008). The Panel notes the low number of cases reported in each group (three in the calcium and one in the placebo groups). The pooled relative risk of CVD for all trials combined was 1.14 (95 % CI 0.92-1.41). The Panel considers that this meta-analysis does not show an increased risk of CV events with calcium supplements of 600 to 1,200 mg/day consumed for two to five years in addition to dietary calcium intakes of about 900 mg/day.

A systematic review and meta-analysis (Bolland et al., 2010) considered 11 double-blind RCTs (Baron et al., 1999; Bolland et al., 2008; Bonithon-Kopp et al., 2000; Bonnicksen et al., 2007; Dawson-Hughes et al., 1990; Grant et al., 2005; Lappe et al., 2007; Lappe and Heaney, 2008; Prince et al., 2006; Reid et al., 1993, 1995; Reid et al., 2006; Reid et al., 2008; Riggs et al., 1998) which used calcium supplements at doses ≥ 500 mg/day for more than one year in subjects aged ≥ 40 years at baseline for the prevention or treatment of osteoporosis or colorectal adenomas. Trials were excluded when calcium plus vitamin D were compared to placebo. The studies included collectively about 12,000 subjects (6,116 in the calcium supplemented groups; 5,805 in the placebo groups) and the outcome measures of interest were myocardial infarction, stroke, sudden death, and death from all causes. Only the four RCTs included in the meta-analysis by Wang et al. (2010) had reported on these outcomes. Baseline age of participants ranged from 56 to 77 years and trial duration from two to five years. Doses of supplemental calcium ranged from 500 to 2,000 mg/day in addition to average dietary calcium intakes of 400-1,240 mg/day. Calcium supplementation did not increase the risk for any outcome in any of the individual studies, or the risk of myocardial infarction, stroke, and sudden death combined, the risk of death, or the risk of stroke in the meta-analyses performed. Allocation to calcium supplementation was associated with an increased risk of myocardial infarction (RR 1.27, 95 % CI 1.01-1.59). One trial (Grant et al., 2005) accounted for 66 % of the subjects and for >50 % of the CV events in the meta-analysis. The compliance rate with the study pills in that study was only 54.5 % (about 45 % in the calcium arms), and the clinical characteristics of participants who did and did not return the completed health questionnaires used for the allocation of events were unknown, which may have introduced bias in between-group comparisons. The Panel notes that cardiovascular events, including myocardial infarction, were not end points in a number of trials but the events were rather attributed on the basis of self-reported health questionnaires and were not verified. The Panel also notes that event frequency and differences in the number of events between the calcium and placebo groups were small, and that the use of multiple end points without adjustment of the level of significance for multiple testing increases the likelihood of chance findings. The Panel considers that

this meta-analysis does not show an increased risk of CV events (myocardial infarction or stroke) with calcium supplements of 500 to 2,000 mg/day consumed for two to five years in addition to dietary calcium intakes of 400-1,240 mg/day.

The same authors (Bolland et al., 2011) updated the above mentioned meta-analysis by including data from two study arms comparing calcium and vitamin D supplements against placebo (Grant et al., 2005; Lappe et al., 2007), and from a sub-sample of subjects participating in the WHI intervention trial (supplementation with calcium and vitamin D versus placebo) described in Section 3.2.2.1. In that study, no effect of calcium (and vitamin D) supplements on confirmed cases of myocardial infarction, stroke, or coronary heart disease death, which were pre-specified secondary efficacy outcomes of the study, had been reported by the authors (Hsia et al., 2007). In subgroup analyses, women with higher total calcium intake (diet plus supplements) at baseline were not at higher risk for coronary events or stroke if assigned to the active calcium/vitamin D group. In the meta-analysis by Bolland et al. (2011), only data from subjects not taking personal supplements at baseline were considered. The Panel notes that the reasons for the selection are unclear, and that subjects were not randomised on the basis of taking personal supplements. The Panel also notes that the additional data considered (supplementation with calcium plus vitamin D against placebo) does not provide information about the risk associated with supplemental calcium intakes *per se*. The Panel considers that this meta-analysis suffers from the same limitations as the previous meta-analysis by the same authors (Bolland et al., 2010), and that it does not provide additional information about the relationship between calcium intakes and risk of CVD.

The Panel considers that long-term calcium intakes from diet and supplements up to 2,500-3,000 mg/day are not associated with an increased risk of cardiovascular disease in adults.

3.2.4. Prostate cancer

The majority of epidemiological studies and the few RCTs which have reported cancer as an outcome found an inverse or no association between calcium intakes from food and/or supplements and the risk of cancer (incidence and mortality), with the exception of prostate cancer (Chung et al., 2009; IoM, 2011; WCRF/AICR, 2007).

The report from the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR, 2007), reviewed nine prospective cohort studies (Baron et al., 2005; Berndt et al., 2002; Chan et al., 2000; Chan et al., 2001; Giovannucci et al., 1998; Laaksonen et al., 2004; Platz et al., 2004; Rodriguez et al., 2003; Schuurman et al., 1999; Tseng et al., 2005), 12 case-control studies (Chan et al., 1998; Du et al., 1997; Hayes et al., 1999; Hodge et al., 2004; Kaul et al., 1987; Key et al., 1997; Ohno et al., 1988; Oishi et al., 1988; Ramon et al., 2000; Tavani et al., 2001; Tavani et al., 2005; Tzonou et al., 1999; Vlainjac et al., 2001; Vlainjac et al., 1997; Walker et al., 2005) and two ecological studies (Boing et al., 1985; Liaw et al., 2003) which investigated the association between calcium intake and risk of prostate cancer. A positive association between calcium intake and risk of prostate cancer was reported in three cohort studies (Chan et al., 2001; Giovannucci et al., 1998; Tseng et al., 2005), one case-control study (Vlainjac et al., 2001) and one ecological study (Boing et al., 1985), whereas no significant association was observed in the remaining studies. An increased risk of advanced/aggressive prostate cancer was also associated with increased intakes of milk and dairy products in one (Giovannucci et al., 1998; 2006) out of the four cohort studies which reported on this outcome. The WCRF/AICR (2007) concluded that there was a “probable” association between diets high in calcium and prostate cancer risk.

Chung et al. (2009) reviewed 12 cohort studies that reported on the association between calcium intake and the risk of prostate cancer (Baron et al., 2005; Chan et al., 2001; Giovannucci et al., 2006; Koh et al., 2006; Kurahashi et al., 2008; Mitrou et al., 2007; Park et al., 2007a; 2007b; Rodriguez et al., 2003; Rohrmann et al., 2007; Schuurman et al., 1999; Tseng et al., 2005). The incidence of prostate cancer in these studies ranged from 0.008 to 0.10. Total calcium intake ranged from

<500 mg/day to $\geq 2,000$ mg/day and the time between dietary assessment and the diagnosis of prostate cancer varied from 1 to 17 years. Seven studies did not find an association between calcium intake and the risk of prostate cancer (Baron et al., 2005; Koh et al., 2006; Kurahashi et al., 2008; Park et al., 2007a; 2007b; Rohrmann et al., 2007; Schuurman et al., 1999), whereas the remaining five studies reported a higher risk associated with higher (vs. lower) calcium intakes. The higher and lower calcium intakes ranged from 921 to >2,000 mg and from 455 to 1,000 mg/day, respectively.

One multicentre RCT (Baron et al., 2005) which investigated the efficacy of calcium supplementation in the prevention of colorectal carcinomas also assessed the risk of prostate cancer as a secondary outcome. Men (mean age 61.8 years) received either 1,200 mg of calcium (n=327) or placebo (n=327) daily for four years and were followed for up to 12 years for prostate cancer diagnosis. Dietary calcium intake at baseline was comparable in the two groups (about 800 mg/day). Over the entire study period, the risk for prostate cancer was not significantly different in the calcium group compared to the placebo (rate ratio, 0.83; 95 % CI 0.52–1.32). Total calcium intakes were not associated with prostate cancer risk.

The Panel notes that a number of case-control (one out of 12) and prospective cohort studies (five out of 12, three of which were of good quality) have reported an increased risk of prostate cancer with total calcium intakes of 1,000 to >2,000 mg/day compared to intakes ranging from 500 to <1,000 mg/day. However, the Panel also notes that these studies were uncontrolled for factors other than calcium and which may have been responsible for the effect, and that the only RCT which reported on this outcome showed no effect of calcium supplementation at doses of 1,200 mg/day (up to about 2,000 mg/day of total calcium) on the risk of prostate cancer.

The Panel considers that long-term calcium intakes from diet and supplements above 2,000 mg/day are not associated with an increased risk of prostate cancer.

3.2.5. Interactions between calcium and dietary minerals

Calcium has been shown to interfere with both iron and zinc absorption in short term trials or single-dose experiments. This effect has not been observed in long-term observational and intervention studies at dietary calcium intakes in the range of current recommendations or at supplemental calcium intakes up to 2,000 mg/day in adults (SCF, 2003).

The Panel notes that no new data have become available since 2003 on this outcome.

4. Dose response assessment and derivation of a Tolerable Upper Intake Level

4.1. Adults

The SCF (2003) based the derivation of a UL for calcium on the evidence of different intervention studies of long duration, some of which were placebo controlled, in which total daily calcium intakes of 2,500 mg from both diet and supplements were tolerated without adverse effects. Because of the abundance of data, the application of an uncertainty factor was considered unnecessary. A UL of 2,500 mg of calcium per day from all sources was proposed for adults, and for pregnant and lactating women.

A number of placebo controlled human intervention studies in adults published since then also show that total daily calcium intakes of 2,500 mg from both diet and supplements are tolerated without adverse effects.

The Panel notes that new case reports have become available on consumption of calcium supplements and the CAS/MAS syndrome. However, the Panel considers that no dose-response relationships can be derived from these.

The Panel considers that no relationship has been established between long-term calcium intakes from diet and supplements and increased risk of nephrolithiasis, cardiovascular disease or prostate cancer.

The Panel proposes a UL of 2,500 mg/day of calcium from all sources.

4.2. Pregnant and lactating women

The UL for pregnant and lactating women established by the SCF (2003) was the same as for adults.

The Panel notes that no new data have become available since 2003 for this population subgroup.

The Panel proposes a UL of 2,500 mg/day of calcium from all sources.

4.3. Infants

In 2003, the SCF concluded that the data available were insufficient to establish a UL for infants.

The Panel notes that no new data have become available for this population subgroup since 2003.

The Panel concludes that the data are insufficient to establish a UL for infants.

4.4. Children and adolescents

In 2003, the SCF concluded that the data available were insufficient to establish a UL for children and adolescents.

The Panel notes that calcium intakes of about 1,800 mg/day did not affect renal function in children aged 1-6 years in one small, short-term (three month) RCT (Markowitz et al., 2004). However, the Panel considers that these data are not sufficient for establishing a UL for children or adolescents.

The Panel concludes that the data are insufficient to establish a UL for children or adolescents.

5. Characterisation of the risk

Data from European populations indicate that intakes of calcium in high consumers among adult males can be close to the UL.

Although available data do not allow the setting of a UL for infants, children or adolescents, no risk has been identified with highest current levels of calcium intake in these age groups.

CONCLUSIONS

The Panel proposes a UL for calcium of 2,500 mg for adults, and for pregnant and lactating women. The Panel considers that the available data are insufficient to set a UL for infants, children or adolescents.

Data from European populations indicate that intakes of calcium in high consumers among adult males can be close to the UL. Although available data do not allow the setting of a UL for infants,

children or adolescents, no risk has been identified with highest current levels of calcium intake in these age groups.

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APPENDICES

A. INTAKE OF CALCIUM AMONG ADULTS IN EUROPEAN COUNTRIES

Nutrient source	Sex	Country	References	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95	P97.5
Food	Women	Austria	(Elmadfa et al., 2009)	24-hour recall	426	< 25	> 35	pregnant 2nd trimester	919.0				
					76	< 25	25	pregnant 2nd trimester	798.0				
					288	25	35	pregnant 2nd trimester	919.0				
					62	35	> 35	pregnant 2nd trimester	1,078.0				
		Denmark	(Pedersen et al., 2010)	7-day record (data collected in 2003-2008)	150	18	24		1,040.0			1,619.0	
					340	25	34		1,106.0			1,843.0	
					412	35	44		1,058.0			1,787.0	
					359	45	54		961.0			1,585.0	
					326	55	64		926.0			1,559.0	
					198	65	75		880.0			1,582.0	
			(Tetens et al., 2011)	7-day record and interview (data collected in 2000-2004)	671	18	49	Non supplement users	939.0			1,675.0	
					825	18	49	Supplements users	1,014.0			1,712.0	
					280	50	75	Non supplement users	834.0			1,524.0	
					599	50	75	Supplements users	864.0			1,589.0	
		Finland	(Paturi et al., 2008)	48-hour recall and two 3-day records for the usual intakes over the past year (assessment of underreporters)	641	25	74	Usual intakes. Underreporters excluded	1,000.0			1,700.0	
		Germany	(MRI, 2008)	24-hour recall + Dietary History	510	19	24		1,039.0			1,756.0	
					972	25	34		1,061.0			1,804.0	
					2,694	35	50		1,067.0			1,815.0	
					1,840	51	64		1,011.0			1,700.0	
					1,562	65	80		918.0			1,553.0	
		Ireland	(IUNA (Irish Universities Nutrition Alliance), 2011)	4-day record	255	18	35		772.0			1,388.0	1,590.0
					232	36	50		775.0			1,289.0	1,436.0
					153	51	64		782.0			1,226.0	1,397.0

Nutrient source	Sex	Country	References	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95	P97.5
Food	Women	Italy	(Sette et al., 2010)	Consecutive 3-day food record	1,245	18	< 65	Including fortified food	730.0			1,233.0	
					316	65	99	Including fortified food	754.0			1,285.0	
		Poland	(Flynn et al., 2009)	24-hour recall	1,656	18	96		516.0			1,113.0	
		Spain	(Flynn et al., 2009)	Two non-consecutive 24-hour recalls (one for all, a second for 62 % of the sample)	895	18	64	Data collected in Catalonia	767.0			1,045.0	
		Sweden	(Becker and Pearson, 2002)	7-day record	67	17	24		937.0			1,436.0	
					128	25	34		973.0			1,421.0	
					143	35	44		888.0			1,362.0	
					118	45	54		901.0			1,451.0	
					68	55	64		927.0			1,607.0	
					65	65	74		937.0			1,504.0	
		The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	347	19	30			910.0		1,482.0	
					351	31	50			953.0		1,540.0	
					353	51	69			985.0		1,583.0	
Food	Men	Denmark	(Pedersen et al., 2010)	7-day record (data collected in 2003-2008)	105	18	24		1,374.0			2,182.0	
					234	25	34		1,235.0			2,071.0	
					318	35	44		1,182.0			1,967.0	
					336	45	54		1,073.0			1,898.0	
					336	55	64		955.0			1,742.0	
					240	65	75		952.0			1,669.0	
					663	18	49	non supplement users	1,043.0			1,937.0	
					587	18	49	supplements users	1,125.0			1,931.0	
		Finland	(Paturi et al., 2008)	48-hour recall and two 3-day records for the usual intakes over the past year (assessment of underreporters)	363	50	75	non supplement users	829.0			1,492.0	
					491	50	75	supplements users	859.0			1,639.0	
					637	25	74	Usual intakes. Underreporters excluded	1,286.0			2,000.0	

Nutrient source	Sex	Country	References	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95	P97.5
Food	Men	Germany	(MRI, 2008)	24-hour recall + Dietary History	510	19	24		1,281.0			2,422.0	
					690	25	34		1,252.0			2,226.0	
					2,079	35	50		1,167.0			2,109.0	
					1,633	51	64		1,071.0			1,908.0	
					1,469	65	80		970.0			1,669.0	
		Ireland	(IUNA (Irish Universities Nutrition Alliance), 2011)	4-day record	276	18	35		1,105.0			1,856.0	2,091.0
		Ireland	(IUNA (Irish Universities Nutrition Alliance), 2011)	4-day record	205	36	50		1,023.0			1,836.0	2,103.0
					153	51	64		957.0			1,589.0	1,812.0
		Italy	(Sette et al., 2010)	Consecutive 3-day food record	1,068	18	< 65	Including fortified food	799.0			1,433.0	
					202	65	99	Including fortified food	825.0			1,403.0	
		Poland	(Flynn et al., 2009)	24-hour recall	1,324	18	96		658.0			1,428.0	
		Spain	(Flynn et al., 2009)	Two non-consecutive 24-hour recalls (one for all, a second for 62 % of the sample)	718	18	64	Data collected in Catalonia.	821.0			1,203.0	
		Sweden	(Becker and Pearson, 2002)	7-day record	67	17	24		1,201.0			2,073.0	
					128	25	34		1,090.0			1,719.0	
					143	35	44		1,029.0			1,638.0	
					118	45	54		1,041.0			1,657.0	
					68	55	64		1,035.0			1,699.0	
					65	65	74		1,064.0			1,868.0	
		The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	356	19	30			1,080.0		1,777.0	
					348	31	50			1,136.0		1,852.0	
					351	51	69			1,099.0		1,803.0	

Nutrient source	Sex	Country	References	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95	P97.5
Food	Men and women	Belgium	(De Vriese et al., 2006)	Two 24-hour recall	873	19	59		813.3		978.0		
					822	60	74		695.4		847.0		
					744	75	> 75		623.7		755.0		
		France	(Dufour et al., 2010) (IUNA (Irish Universities Nutrition Alliance), 2011)	7-day record	1,918	18	79	Non under-reporters. Without fortified food.	913.1			1,487.5	1,652.0
		Ireland	(Lopes et al., 2006)	4-day record	226	65	> 65		840.0			1,518.0	1,654.0
		Portugal	(Lopes et al., 2006)	Food frequency questionnaire	478	18	39	Data collected in Porto	1,027.3			1,714.3	
					537	40	49		915.1			1,600.7	
					789	50	64		922.6			1,629.3	
					585	65	> 65		882.8			1,514.7	
		United Kingdom	(Bates et al., 2011)	4-day record	807	19	64		830.0				1,542.0
					224	65	> 65		871.0				1,630.0
Supplements	Women	Austria	(Elmadfa et al., 2009)	Quantitative consumption frequency questionnaire	28	18	65	Assessment of the consumption of supplements among 282 adults in all Austria (77 supplement users)		200.0	353.0		
								Users of calcium supplements					
		Germany	(MRI, 2008)	24-hour recall + Dietary History	40	19	24	Users of calcium supplements	288.0			500.0	
					84	25	34	Users of calcium supplements	198.0			500.0	
					285	35	50	Users of calcium supplements	272.0			800.0	
					324	51	64	Users of calcium supplements	353.0			1,000.0	
					338	65	80	Users of calcium supplements	409.0			1,200.0	

Nutrient source	Sex	Country	References	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95	P97.5
Supplements	Men	Austria	(Elmadfa et al., 2009)	Quantitative consumption frequency questionnaire	21	18	65	Assessment of the consumption of supplements among 282 adults in all Austria (77 supplement users)		162.0	341.0		
		Germany	(MRI, 2008)	24-hour recall + Dietary History	38	19	24	Users of calcium supplements	188.0			500.0	
					53	25	34	Users of calcium supplements	203.0			620.0	
					152	35	50	Users of calcium supplements	236.0			600.0	
					146	51	64	Users of calcium supplements	245.0			620.0	
					182	65	80	Users of calcium supplements	319.0			1,200.0	
Food and supplements	Women	Denmark	(Tetens et al., 2011)	7-day record and interview (data collected in 2000-2004)	825	18	49	Supplements users	1,130.0			1,881.0	
					599	50	75	Supplements users	1,018.0			1,899.0	
		Finland	(Paturi et al., 2008) (IUNA (Irish Universities Nutrition Alliance), 2011)	48-hour recall and two 3-day records for the usual intakes over the past year (assessment of underreporters)	641	25	74	Usual intakes. Underreporters excluded	1,190.0			1,830.0	
		Ireland		4-day record	255	18	35		794.0			1,394.0	1,655.0
					232	36	50		824.0			1,394.0	1,702.0
					153	51	64		874.0			1,630.0	1,842.0
		Poland	(Flynn et al., 2009)	24-hour recall	1,656	18	96		523.0			1,121.0	
		Spain	(Flynn et al., 2009)	Two non-consecutive 24-hour recalls (one for all, a second for 62 % of the sample)	895	18	64	Including fortified food. Data collected in Catalonia.	779.0			1,069.0	
		The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	347	19	30			918.0		1,501.0	
					351	31	50			983.0		1,602.0	
					353	51	69			1,026.0		1,686.0	

Nutrient source	Sex	Country	References	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P95	P97.5
Food and supplements	Men	Denmark	(Tetens et al., 2011)	7-day record and interview	587	18	49	Supplements users	1,228.0			2,029.0	
					491	50	75	Supplements users	970.0			1,785.0	
		Finland	(Paturi et al., 2008) (IUNA (Irish Universities Nutrition Alliance), 2011)	48-hour recall and two 3-day records for the usual intakes over the past year (assessment of underreporters)	637	25	74	Usual intakes. Underreporters excluded	1,300.0			2,100.0	
		Ireland	(Flynn et al., 2009)	4-day record	276	18	35		1,122.0			1,877.0	2,091.0
					205	36	50		1,036.0			1,836.0	2,103.0
					153	51	64		981.0			1,600.0	1,830.0
		Poland	(Flynn et al., 2009)	24-hour recall	1,324	18	96		662.0			1,428.0	
		Spain	(Flynn et al., 2009)	Two non-consecutive 24-hour recalls (one for all, a second for 62 % of the sample)	718	18	64	Including fortified food. Data collected in Catalonia	831.0			1,217.0	
		The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	356	19	30			1,091.0		1,791.0	
					348	31	50			1,154.0		1,869.0	
					351	51	69			1,109.0		1,818.0	
Food and supplements	Men and women	Ireland	(IUNA (Irish Universities Nutrition Alliance), 2011)	4-day record	226	65	> 65		954.0			2,106.0	2,337.0
		United Kingdom	(Bates et al., 2011)	4-day record	807	19	64		850.0				1,663.0
					224	65	> 65		902.0				1,677.0

NB: white: less than ~50 years. grey: over ~50 years

B. INTAKE OF CALCIUM AMONG CHILDREN IN EUROPEAN COUNTRIES

Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P90	P95	P97.5
Food	Boys and girls	The Netherlands	(de Boer et al., 2006)	2-day record (independent days)	333	0.75	0.75	Including fortified food	730.0		812.0	915.0		
					306	1	1	Including fortified food	824.0		938.0	1,085.0		
Food and supplements	Girls	Finland	(Kyttälä et al., 2008; 2010)	3-day record	198	1	1	non breast-fed infants genetically at-risk of type 1 diabetes	696.0		885.0			
Food and supplements	Boys	Finland	(Kyttälä et al., 2008; 2010)	3-day record	257	1	1	non breast-fed infants genetically at-risk of type 1 diabetes	670.0		886.0			
Food	Girls	Denmark	(Andersen et al., 1996)	7-day record	149	1	3		996.0				1,325.0	
		The Netherlands	(Ocke et al., 2008)	2-day record (independent days)	313	2	3		734.0				1,089.0	
Food	Boys	Denmark	(Andersen et al., 1996)	7-day record	129	1	3		910.0				1,359.0	
		The Netherlands	(Ocke et al., 2008)	2-day record (independent days)	327	2	3		788.0				1,180.0	
Food	Boys and girls	Greece	(Manios et al., 2008)	3-day record (weighed food records and 24 h recall or food diaries)	2,317	1	5	Non under-reporters. Possible inclusion of supplements not mentioned	1,024.0			1,392.0		
		Italy	(Sette et al., 2010)	Consecutive 3-day food records	52	0	< 3	Including fortified food	664.0				1,070.0	
		The Netherlands	(de Boer et al., 2006)	2-day record (independent days)	302	1.5	1.5	Including fortified food	866.0			1,082.0		
		United Kingdom	(Bates et al., 2011)	4-day food diary	219	1.5	3		773.0					1,365.0
Food and supplements	Girls	Finland	(Kyttälä et al., 2008; 2010)	3-day record	118	2	2	Children genetically at-risk for type 1 diabetes	870.0		1,019.0			
					235	3	3		881.0		1,062.0			
		The Netherlands	(Ocke et al., 2008)	2-day record (independent days)	313	2	3		740.0				1,099.0	

Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P90	P95	P97.5
Food and supplements	Boys	Finland	(Kyttälä et al., 2008; 2010)	3-day record	112	2	2	Children genetically at-risk for type 1 diabetes	844.0		1,037.0			
					236	3	3		925.0		1,109.0			
		The Netherlands	(Ocke et al., 2008)	2-day record (independent days)	327	2	3		795.0				1,184.0	
Food and supplements	Boys and girls	United Kingdom	(Bates et al., 2011)	4-day food diary	219	1.5	3		775.0					1,365.0
Food	Girls	Ireland	(IUNA (Irish Universities Nutrition Alliance), a)	7-day record	301	5	12		804.0				1,274.0	1,434.0
					151	7	8			817.0			1,353.0	
		The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	352	9	13			846.0			1,393.0	
Food	Boys	Ireland	(IUNA (Irish Universities Nutrition Alliance), a)	7-day record	293	5	12		907.0				1,488.0	1,578.0
					153	7	8			878.0			1,495.0	
		The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	351	9	13			943.0			1,587.0	
Food	Boys and girls	Denmark	(Pedersen et al., 2010)	7-day record	903	4	14		1,072.0				1,776.0	
		Italy	(Sette et al., 2010)	Consecutive 3-day food records	52	3	< 10	Including fortified food	749.0				1,197.0	
		Germany	(Flynn et al., 2009)	3-day record	1,234	6	11		870.0				1,352.0	
		Poland	(Flynn et al., 2009)	24-hour recall	455	4	10		595.0				1,134.0	

Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P90	P95	P97.5
Food	Boys and girls	Spain	(Flynn et al., 2009)	24-hour recall and food frequency questionnaire, a second 24-hour recall in 25-30 % of the sample	723	4	10		867.0				1,123.0	
		The Netherlands	(Flynn et al., 2009)	2-day record (independent days)	639	4	6	Including fortified food	716.0				1,155.0	
		United Kingdom	(Bates et al., 2011)	4-day food diary	423	4	10		804.0					1,339.0
Food and supplements	Girls	Finland	(Kyttälä et al., 2008; 2010)	3-day record	247	4	4	Children genetically at-risk for type 1 diabetes	930.0		1,089.0			
					349	6	6		991.0		1,172.0			
		The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	151	7	8			852.0			1,408.0	
					352	9	13			856.0			1,418.0	
Food and supplements	Boys	Finland	(Kyttälä et al., 2008; 2010)	3-day record	307	4	4	Children genetically at-risk for type 1 diabetes	983.0					
					364	6	6		1,103.0					
		The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	153	7	8			892.0			1,505.0	
					351	9	13			964.0			1,623.0	
Food and supplements	Boys and girls	Germany	(Flynn et al., 2009)	3-day record	1,234	6	11		874.0				1,352.0	
		Ireland	(IUNA (Irish Universities Nutrition Alliance), a) (Flynn et al., 2009)	7-day record	594	5	12		862.0				1,433.0	
		Poland	(Flynn et al., 2009)	24-hour recall	455	4	10		598.0				1,134.0	

Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P90	P95	P97.5
Food and supplements	Boys and girls	Spain	(Flynn et al., 2009) (Enghardt-Barbieri et al., 2006)	24-hour recall and food frequency questionnaire, a second 24-hour recall in 25-30 % of the sample	723	4	10	Including fortified food	898.0				1,151.0	
				4-day record	590	4	4		855.0				1,330.0	
		Sweden			889	8	9		959.0				1,537.0	
					1,016	11	12		878.0				1,519.0	
		The Netherlands	(Flynn et al., 2009)	2-day record (independent days)	639	4	6		724.0				1,165.0	
					639	4	6	Including fortified food	811.0				1,247.0	
		United Kingdom	(Bates et al., 2011)	4-day food diary	423	4	10		804.0					1,339.0
Food	Girls	Denmark	(Pedersen et al., 2010)	7-day record	134	14	17		966.0				1,699.0	
		Ireland	(IUNA (Irish Universities Nutrition Alliance), b)	7-day record	217	13	17		734.0				1,376.0	1,554.0
		Italy	(Sette et al., 2010)	Consecutive 3-day food records	139	10	< 18	Including fortified food	770.0				1,306.0	
		The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	354	14	18			876.0			1,435.0	

Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P90	P95	P97.5
Food	Boys	Denmark	(Pedersen et al., 2010)	7-day record	101	14	17		1,288.0				2,245.0	
		Ireland	(IUNA (Irish Universities Nutrition Alliance), b)	7-day record	224	13	17		1,063.0				1,905.0	2,047.0
		Italy	(Sette et al., 2010)	Consecutive 3-day food records	108	10	< 18	Including fortified food	892.0				1,435.0	
		The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	352	14	18			1,010.0			1,679.0	
Food	Boys and girls	Belgium	(De Vriese et al., 2006)	Two 24-hour recall	806	15	18		787.9		966.0			
		Germany	(Flynn et al., 2009)	3-day record	1,272	12	17		1,337.0				2,400.0	
		Spain	(Flynn et al., 2009)	24-hour recall and food frequency questionnaire, a second 24-hour recall in 25-30 % of the sample	1,137	11	17		878.0				1,231.0	
		United Kingdom	(Bates et al., 2011)	4-day food diary	453	11	18		785.0					1,522.0
Supplements	Girls	Germany	(MRI, 2008)	24-hour recall + Dietary History	34	14	18		189.0				1,000.0	
Supplements	Boys	Germany	(MRI, 2008)	24-hour recall + Dietary History	43	14	18		189.0				500.0	
Food and supplements	Girls	The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	354	14	18			872.0			1,433.0	
Food and supplements	Boys	The Netherlands	(van Rossum et al., 2011)	Two non-consecutive 24-hour dietary recalls	352	14	18			1,010.0			1,692.0	

Nutrient source	Sex	Country	Reference	Dietary method	n	Age min	Age max	Population / Fortified foods included or excluded	mean	median	P75	P90	P95	P97.5
Food and supplements	Boys and girls	Germany	(Flynn et al., 2009)	3-day record	1,272	12	17		1,350.0				2,422.0	
					1,272	12	17	Including fortified food	1,396.0				2,515.0	
		Ireland	(IUNA (Irish Universities Nutrition Alliance), b) (Flynn et al., 2009)	7-day record	441	13	17		906.0				1,657.0	
					581	11	17		1,398.0				2,975.0	
		Spain	(Flynn et al., 2009)	24-hour recall and food frequency questionnaire, a second 24-hour recall in 25-30 % of the sample	1,137	11	17	Including fortified food	900.0				1,267.0	
					453	11	18		786.0					1,522.0
		United Kingdom	(Bates et al., 2011)	4-day food diary										

NB: First white section: infants. First grey section: ~1-3 years. Second white section: older children. Second grey section: ~teenagers

GLOSSARY AND ABBREVIATIONS

1,25-(OH) ₂ -D	1,25-dihydroxycholecalciferol
25(OH)D	25-Hydroxy-vitamin D
AVC	Aortic valve calcification
BMI	Body Mass Index
CAC	Coronary artery calcification
CAS	Calcium-alkali-syndrome
CaSR	Calcium-sensing receptor
CHD	Coronary heart disease
CI	Confidence interval
CT	Computed tomography
CV	Cardiovascular
CVD	Cardiovascular disease
CYP	Cytochrome
PEPIC	European Prospective Investigation into Cancer and Nutrition
FFQ	Food frequency questionnaire
HR	Hazard ratio
IoM	Institute of Medicine
IU	International Unit
JACC	Japan Collaborative Cohort
LOAEL	Lowest-observed-adverse-effect-level
MAS	Milk-alkali syndrome
NCX1	Na/Ca Exchanger 1
NOAEL	No observable adverse effect level
OMIM	Online Mendelian Inheritance in Man
PMCA	Plasma-membrane Ca-ATPase
PTH	Parathyroid hormone

RCT	Randomised controlled trial
RR	Relative risk
SCF	Scientific Committee on Food
TRPV	Transient Receptor Potential Vanilloid
UL	Tolerable Upper Intake Level
VDR	Vitamin D receptor
WCRF/AICR	World Cancer Research Fund/American Institute for Cancer Research
WHI	Women's Health Initiative

CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance limestone¹

European Food Safety Authority²

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Limestone is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004³, as amended by Commission Regulation (EC) No 1095/2007⁴.

Limestone was included in Annex I to Directive 91/414/EEC on 1 September 2009 pursuant to Article 24b of the Regulation (EC) No 2229/2004 (hereinafter referred to as ‘the Regulation’), and has subsequently been deemed to be approved under Regulation (EC) No 1107/2009⁵, in accordance with Commission Implementing Regulation (EU) No 540/2011⁶, as amended by Commission Implementing Regulation (EU) No 541/2011⁷. In accordance with Article 25a of the Regulation, as amended by Commission Regulation (EU) No 114/2010⁸, the European Food Safety Authority (EFSA) is required to deliver by 31 December 2012 its view on the draft review report submitted by the European Commission in accordance with Article 25(1) of the Regulation. This review report was established as a result of the initial evaluation provided by the designated rapporteur Member State in the Draft Assessment Report (DAR). The EFSA therefore organised a peer review of the DAR. The conclusions of the peer review are set out in this report.

Austria being the designated rapporteur Member State submitted the DAR on limestone in accordance with the provisions of Article 22(1) of the Regulation, which was received by the EFSA on 28 November 2007. The peer review was initiated on 25 June 2008 by dispatching the DAR to the notifier Agro Radomyšl a.s. and on 20 October 2010 to the Member States for consultation and comments. Following consideration of the comments received on the DAR, it was concluded that EFSA should conduct a focused peer review in the area of mammalian toxicology, and deliver its conclusions on limestone.

The conclusions laid down in this report were reached on the basis of the evaluation of the representative uses of limestone as a repellent for use in forestry, as proposed by the notifier. Full details of the representative uses can be found in Appendix A to this report.

¹ On request from the European Commission, Question No EFSA-Q-2009-00261, issued on 30 June 2011.

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³ OJ L 379, 24.12.2004, p.13

⁴ OJ L 246, 21.9.2007, p.19

⁵ OJ L 309, 24.11.2009, p.1

⁶ OJ L 153, 11.6.2011, p.1

⁷ OJ L 153, 11.6.2011, p.187

⁸ OJ L 37, 10.2.2010, p.12

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It should be emphasized that the substance notified as limestone is mainly composed of calcium carbonate.

A data gaps was identified in the section on physical and chemical properties.

No data gaps or critical areas of concern were identified in the mammalian toxicology section.

No significant residues in plant or animal matrices are expected based on the representative uses. Therefore a consumer risk assessment was not required.

Limestone is a naturally occurring sedimentary rock composed largely of the mineral calcite (calcium carbonate: CaCO_3). Considering the nature of the substance and the limited usage leading to negligible levels of environmental exposure, a definition of residue in the environment for monitoring is deemed to be unnecessary for limestone (CaCO_3).

Due to negligible levels of environmental exposure arising from the representative uses, the risk can be considered low for non-target organisms. No data gaps or critical areas of concern were identified in the ecotoxicology section.

KEY WORDS

Limestone, calcium carbonate, peer review, risk assessment, pesticide, repellent

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BACKGROUND

Limestone is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004⁹, as amended by Commission Regulation (EC) No 1095/2007¹⁰.

Limestone was included in Annex I to Directive 91/414/EEC on 1 September 2009 pursuant to Article 24b of the Regulation (EC) No 2229/2004 (hereinafter referred to as 'the Regulation'), and has subsequently been deemed to be approved under Regulation (EC) No 1107/2009¹¹, in accordance with Commission Implementing Regulation (EU) No 540/2011¹², as amended by Commission Implementing Regulation (EU) No 541/2011¹³. In accordance with Article 25a of the Regulation, as amended by Commission Regulation (EU) No 114/2010¹⁴ the European Food Safety Authority (EFSA) is required to deliver by 31 December 2012 its view on the draft review report submitted by the European Commission in accordance with Article 25(1) of the Regulation (European Commission, 2008). This review report was established as a result of the initial evaluation provided by the designated rapporteur Member State in the Draft Assessment Report (DAR). The EFSA therefore organised a peer review of the DAR. The conclusions of the peer review are set out in this report.

Austria being the designated rapporteur Member State submitted the DAR on limestone in accordance with the provisions of Article 22(1) of the Regulation, which was received by the EFSA on 28 November 2007 (Austria, 2007). The peer review was initiated on 25 June 2008 by dispatching the DAR to the notifier Agro Radomyšl a.s. and on 20 October 2010 to the Member States for consultation and comments. In addition, the EFSA conducted a public consultation on the DAR. The comments received were collated by the EFSA and forwarded to the RMS for compilation and evaluation in the format of a Reporting Table. The notifier was invited to respond to the comments in column 3 of the Reporting Table. The comments and the notifier's response were evaluated by the RMS in column 3 of the Reporting Table.

The scope of the peer review was considered in a telephone conference between the EFSA, the RMS, and the European Commission on 15 February 2011. On the basis of the comments received and the RMS' evaluation thereof it was concluded that the EFSA should organise a consultation with Member State experts in the area of mammalian toxicology.

The outcome of the telephone conference, together with EFSA's further consideration of the comments is reflected in the conclusions set out in column 4 of the Reporting Table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, including those issues to be considered in consultation with Member State experts, and additional information to be submitted by the notifier, were compiled by the EFSA in the format of an Evaluation Table.

The conclusions arising from the consideration by the EFSA, and as appropriate by the RMS, of the points identified in the Evaluation Table, together with the outcome of the expert discussions where these took place, were reported in the final column of the Evaluation Table.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in May – June 2011.

This conclusion report summarises the outcome of the peer review of the risk assessment on the active substance and the representative formulation evaluated on the basis of the representative uses as a repellent for use in forestry, as proposed by the notifier. A list of the relevant end points for the active

⁹ OJ L 379, 24.12.2004, p.13

¹⁰ OJ L 246, 21.9.2007, p.19

¹¹ OJ L 309, 24.11.2009, p.1

¹² OJ L 153, 11.6.2011, p.1

¹³ OJ L 153, 11.6.2011, p.187

¹⁴ OJ L 37, 10.2.2010, p.12

substance as well as the formulation is provided in Appendix A. In addition, a key supporting document to this conclusion is the Peer Review Report, which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The Peer Review Report (EFSA, 2011) comprises the following documents, in which all views expressed during the course of the peer review, including minority views, can be found:

- the comments received on the DAR,
- the Reporting Table (15 February 2011),
- the Evaluation Table (27 June 2011),
- the report(s) of the scientific consultation with Member State experts (where relevant),
- the comments received on the draft EFSA conclusion.

Given the importance of the DAR including its addendum (compiled version of May 2011 containing all individually submitted addenda (Austria, 2011)) and the Peer Review Report, both documents are considered respectively as background documents A and B to this conclusion.

THE ACTIVE SUBSTANCE AND THE FORMULATED PRODUCT

Limestone is considered by the International Organization for Standardization not to require a common name. The active substance notified as limestone is formed of calcium carbonate (IUPAC).

The representative formulated product for the evaluation was 'Neoponit L', a paste, water-based, film-forming composition (PA) containing 750 g/kg limestone.

The representative uses evaluated comprise manual applications by gloves or brush on forest plantations as a repellent to prevent browsing damage. The formulation is used only as a protective coat on the outside of the trees. Full details of the representative uses can be found in the list of end points in Appendix A.

CONCLUSIONS OF THE EVALUATION

1. Identity, physical/chemical/technical properties and methods of analysis

The following guidance document was followed in the production of this conclusion: SANCO/3030/99 rev.4 (European Commission, 2000).

The minimum purity of limestone is 980 g/kg expressed as calcium carbonate. No FAO specification exists.

The assessment of the data package revealed no issues that need to be included as critical areas of concern with respect to the identity, physical, chemical and technical properties of limestone or the representative formulation. It should be noted that the active substance is composed of calcium carbonate. A data gap was identified for the parameters cited from the open literature on the physical/chemical properties of limestone. The main data regarding the identity of limestone and its physical and chemical properties are given in Appendix A.

Adequate analytical methods are available for the determination of calcium carbonate or impurities in the technical material and for the determination of calcium carbonate in the representative formulation. The need for methods of analysis for monitoring this compound in food of plant and animal origin and in the environment has been waived due to the nature of the compound. A method for body fluids and tissues is not required as the active substance is not classified as toxic or very toxic.

2. Mammalian toxicity

Limestone was discussed at the PRAPeR TC 55 Experts' Teleconference on mammalian toxicology.

Overall, it was noted that the database is quite limited and mainly based on summaries of assessments performed for purposes other than the pesticide use.

The limited data available indicate that limestone is not acutely toxic by the oral route, and it is irritating to skin and eyes, and to the respiratory system, however it was not possible to draw a firm conclusion. No relevant data were submitted for short- and long-term toxicity, genotoxicity, carcinogenicity and reproductive toxicity, however, as the paintbrush and gloves applications of a paste were not considered to be a source of significant exposure based on the unlikely dermal absorption of limestone and the negligible inhalation exposure, it was agreed that there is no need to set an Acceptable Operator Exposure Level (AOEL), or an Acceptable Daily Intake (ADI) or an Acute Reference Dose (ARfD), based on the representative uses.

3. Residues

Metabolism and residue studies were not considered relevant for evaluation due to the nature of the active substance and the representative uses. Limestone, which is composed of different crystal forms of calcium carbonate, will be used only as protection coating for trees in forestry. Crops destined for

human and animal consumption are not treated, and therefore no residues on food and feed may occur. A consumer risk assessment was therefore not required.

4. Environmental fate and behaviour

Limestone is a naturally occurring sedimentary rock composed largely of the mineral calcite (calcium carbonate: CaCO_3). The active substance is pulverized limestone.

After application (by brush or gloves) the formulation dries and forms a protective coating. The dried formulation is not water soluble. Because of the method of application leading to negligible levels of environmental exposure and the natural presence of calcium carbonate in soils and aquatic sediments, further consideration of its fate and behaviour in the environment was concluded to be unnecessary.

5. Ecotoxicology

It is noted that the available database in this section is limited, a few studies with aquatic organisms were submitted. Based on the available data, no classification is necessary for limestone with regard to aquatic organisms.

Because of the method of application leading to negligible levels of environmental exposure, the risk can be considered low for birds and mammals, aquatic organisms, bees, non-target arthropods, earthworms, soil macro- and micro-organisms, terrestrial non-target plants and biological methods for sewage treatment plants.

6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments

6.1. Soil

Compound (name and/or code)	Persistence	Ecotoxicology
Not applicable Considering the nature of the substance and the limited exposure from the representative uses a definition of residue in the environment for risk assessment by other disciplines is deemed to be unnecessary for limestone (CaCO ₃).	Not applicable	-

6.2. Ground water

Compound (name and/or code)	Mobility in soil	>0.1 µg/L 1m depth for the representative uses (at least one FOCUS scenario or relevant lysimeter)	Pesticidal activity	Toxicological relevance	Ecotoxicological activity
Not applicable Considering the nature of the substance and the limited exposure from the representative uses a definition of residue in the environment for groundwater exposure assessment is deemed to be unnecessary for limestone (CaCO ₃).	Not applicable	Not applicable	-	-	-

6.3. Surface water and sediment

Compound (name and/or code)	Ecotoxicology
Not applicable Considering the nature of the substance and the limited exposure from the representative uses a definition of residue in the environment for risk assessment by other disciplines is deemed to be unnecessary for limestone (CaCO ₃).	-

6.4. Air

Compound (name and/or code)	Toxicology
Not applicable Considering the nature of the substance and the limited exposure from the representative uses a definition of residue in the environment for risk assessment by other disciplines is deemed to be unnecessary for limestone (CaCO ₃).	-

LIST OF STUDIES TO BE GENERATED, STILL ONGOING OR AVAILABLE BUT NOT PEER REVIEWED

This is a complete list of the data gaps identified during the peer review process, including those areas where a study may have been made available during the peer review process but not considered for procedural reasons (without prejudice to the provisions of Article 7 of Directive 91/414/EEC concerning information on potentially harmful effects).

- Studies for the parameters cited from the open literature on the physical/chemical properties of limestone (relevant for all representative uses evaluated; submission date proposed by the notifier: unknown; see section 1)

PARTICULAR CONDITIONS PROPOSED TO BE TAKEN INTO ACCOUNT TO MANAGE THE RISK(S) IDENTIFIED

- None.

ISSUES THAT COULD NOT BE FINALISED

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles of Annex VI to Directive 91/414/EEC and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

- None.

CRITICAL AREAS OF CONCERN

An issue is listed as a critical area of concern where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles of Annex VI to Directive 91/414/EEC, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

- None.

REFERENCES

- Austria, 2007. Draft Assessment Report (DAR) on the active substance limestone prepared by the rapporteur Member State Austria in the framework of Directive 91/414/EEC, November 2007.
- Austria, 2011. Final Addendum to Draft Assessment Report on limestone, compiled by EFSA, May 2011.
- EFSA (European Food Safety Authority), 2011. Peer Review Report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance limestone.
- European Commission, 2008. Review Report for the active substance limestone finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 28 October 2008 in view of the inclusion of limestone in Annex I of Directive 91/414/EEC. SANCO/2618/08 - rev.2, 23 October 2008.
- European Commission, 2000. Technical Material and Preparations: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414. SANCO/3030/99 rev.4, 11 July 2000.

APPENDICES

APPENDIX A – LIST OF END POINTS FOR THE ACTIVE SUBSTANCE AND THE REPRESENTATIVE FORMULATION

Identity, Physical and Chemical Properties, Details of Uses, Further Information

Active substance (ISO Common Name) ‡	Limestone (pulverized) - no ISO common name required
Function (e.g. fungicide)	Repellent
Rapporteur Member State	Austria

Identity (Annex IIA, point 1)

Chemical name (IUPAC) ‡	Calcium carbonate
Chemical name (CA) ‡	Calcium carbonate
CIPAC No ‡	852
CAS No ‡	1317-65-3 for limestone (471-34-1 for CaCO ₃)
EC No (EINECS or ELINCS) ‡	215-279-6 (EINECS) for limestone
FAO Specification (including year of publication) ‡	No FAO specification
Minimum purity of the active substance as manufactured ‡	980 g/kg (expressed as CaCO ₃)
Identity of relevant impurities (of toxicological, ecotoxicological and/or environmental concern) in the active substance as manufactured	None
Molecular formula ‡	CaCO ₃ as the main component of limestone
Molecular mass ‡	100.09 g/mol
Structural formula ‡	$\text{Ca}^{+2} \left[\begin{array}{c} \text{O} \\ \diagdown \\ \text{C} = \text{O} \\ \diagup \\ \text{O} \end{array} \right]^{-2}$

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Physical and chemical properties (Annex IIA, point 2)

Melting point (state purity) ‡	Limestone does not melt
Boiling point (state purity) ‡	Not applicable
Temperature of decomposition (state purity)	Data gap
Appearance (state purity) ‡	White colour when high purity (literature) Various shades of yellow, cream, red grey and dark hues possible
Vapour pressure (state temperature, state purity) ‡	Not applicable
Henry's law constant ‡	Not applicable
Solubility in water (state temperature, state purity and pH) ‡	Data gap
Solubility in organic solvents ‡ (state temperature, state purity)	Not applicable
Surface tension ‡ (state concentration and temperature, state purity)	Not applicable
Partition co-efficient ‡ (state temperature, pH and purity)	Not applicable
Dissociation constant (state purity) ‡	Not applicable
UV/VIS absorption (max.) incl. ϵ ‡ (state purity, pH)	Not applicable
Flammability ‡ (state purity)	Not highly flammable (statement)
Explosive properties ‡ (state purity)	Not explosive (statement)
Oxidising properties ‡ (state purity)	Not oxidizing (statement)

Summary of representative uses evaluated (*Limestone pulverized*)

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc. of a.s. (i)	method kind (f-h)	growth stage & season (j)	number min/max (k)	interval between applications (min)	g a.s./hL min – max	water L/ha min – max	g a.s./ha min – max		
Forest plantation Forest tree nursery	EU (Czech Republic)	Neoponit L	F	Red deer (<i>Cervus elaphus</i> L.) Roe deer (<i>Capreolus capreaolus</i> L.) Brown hare (<i>Lepus europaeus</i> L.)	PA	750 g/kg	Manually using gloves or special forestry brush	September - November (when shoots are sufficiently mature)	1	1 year			45 - 90	none	60 kg Neoponit L/ha for 5000 pcs/ha 120 kg Neoponit L/ha for 10000 pcs/ha

(a) For crops, the Codex and EU (or other) classifications should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)

(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)

(c) e.g. biting or suckling insects, soil borne insects, foliar fungi, weeds

(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) GCFP Codes – GIFAP Technical monograph No 2, 1989

(f) All abbreviations used must be explained

(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drenching

(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated

(i) g/kg or g/l

(j) Growth stage at last treatment (BBCH Monograph, Growth Stage of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(k) Indicate the minimum and maximum number of application possible under practical conditions of use

(l) PHI – minimum pre-harvest interval

(m) Remarks may include: Extent of use/economic importance/restrictions

Methods of Analysis

Analytical methods for the active substance (Annex IIA, point 4.1)

Technical a.s. (analytical technique)	Acid-base titration (CO ₃) ²⁻ Optical emission spectroscopy with inductively coupled plasma (Ca, Mg)
Impurities in technical a.s. (analytical technique)	None
Plant protection product (analytical technique)	Acid-base titration (CO ₃) ²⁻ Optical emission spectroscopy with inductively coupled plasma (Ca, Mg)

Analytical methods for residues (Annex IIA, point 4.2)

Residue definitions for monitoring purposes

Food of plant origin	No residue definition. According to the representative uses limestone is used as a repellent which is applied to the shoots with brushes or gloves (forest plantation, forest tree nursery). Since coating of trees is the only use of limestone no crops are treated directly, and therefore no residues on food and/or feed may occur.
Food of animal origin	No residue definition. See statement above.
Soil	No residue definition. Limestone is a ubiquitous substance. It consists mainly of CaCO ₃ which is a highly abundant element in the earth's crust. It is impossible to differentiate analytically between applied limestone and naturally present limestone.
Water surface	No residue definition. See statement above.
drinking/ground	No residue definition. See statement above.
Air	No residue definition. See statement above.

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	Not relevant. See statement above.
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	Not relevant. See statement above.
Soil (analytical technique and LOQ)	Not relevant. See statement above.
Water (analytical technique and LOQ)	Not relevant. See statement above.

Air (analytical technique and LOQ)

Not relevant. See statement above.

Body fluids and tissues (analytical technique and LOQ)

No method required since the active substance is not classified as toxic or highly toxic.

Classification and proposed labelling with regard to physical and chemical data (Annex IIA, point 10)

Active substance

RMS/peer review proposal

Limestone will not be classified from a physical/chemical viewpoint.

Impact on Human and Animal Health

Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA, point 5.1)

Rate and extent of oral absorption ‡	Data available of limited validity, no further data needed.
Distribution ‡	No data available, not needed.
Potential for accumulation ‡	No data available, not needed.
Rate and extent of excretion ‡	Data available of limited validity, no further data needed.
Metabolism in animals ‡	Data available of limited validity, no further data needed.
Toxicologically relevant compounds ‡ (animals and plants)	No data available, not needed.
Toxicologically relevant compounds ‡ (environment)	No data available, not needed.

Acute toxicity (Annex IIA, point 5.2)

Rat LD ₅₀ oral ‡	Data available of limited validity, no further data needed.	-
Rat LD ₅₀ dermal ‡	No data available, not needed.	-
Rat LC ₅₀ inhalation ‡	Data available of limited validity, no further data needed.	
Skin irritation ‡	Data available of limited validity, no further data needed.	
Eye irritation ‡	Data available of limited validity, no further data needed.	
Skin sensitisation ‡	No data available, not needed.	-

Short term toxicity (Annex IIA, point 5.3)

Target / critical effect ‡	No data available, not needed.	
Relevant oral NOAEL ‡	No data available, not needed.	-
Relevant dermal NOAEL ‡	No data available, not needed.	-
Relevant inhalation NOAEL ‡	No data available, not needed.	-

Genotoxicity ‡ (Annex IIA, point 5.4)

No data available, not needed.	-

Long term toxicity and carcinogenicity (Annex IIA, point 5.5)

Target/critical effect ‡	No data available, not needed.	
Relevant NOAEL ‡	No data available, not needed.	
Carcinogenicity ‡	No data available, not needed.	-

Reproductive toxicity (Annex IIA, point 5.6)

Reproduction toxicity

Reproduction target / critical effect ‡	No data available, not needed.	-
Relevant parental NOAEL ‡	No data available, not needed.	-
Relevant reproductive NOAEL ‡	No data available, not needed.	-
Relevant offspring NOAEL ‡	No data available, not needed.	-

Developmental toxicity

Developmental target / critical effect ‡	No data available, not needed.	-
Relevant maternal NOAEL ‡	No data available, not needed.	-
Relevant developmental NOAEL ‡	No data available, not needed.	-

Neurotoxicity (Annex IIA, point 5.7)

Acute neurotoxicity ‡	No data available, not needed.	-
Repeated neurotoxicity ‡	No data available, not needed.	-
Delayed neurotoxicity ‡	No data available, not needed.	-

Other toxicological studies (Annex IIA, point 5.8)

Mechanism studies ‡	No data available, not needed.
Studies performed on metabolites or impurities ‡	No data available, not needed.

Medical data ‡ (Annex IIA, point 5.9)

Overdose: Hypercalcaemia, alkalosis, abdominal distention, nausea, flatulence, eructation and belching with acid reflux, reduced secretion of parathyroid hormone, retention of phosphate, precipitation of Ca^{2+} salts in the kidney and renal insufficiency.

Therapeutic doses of calcium carbonate used as an antacide range up to 1 g, and in case of treatment of hypocalcaemic tetany up to 4 g. Hypercalcaemia from as little as 3 to 4 g a day can be problematic in uremia patients. Clinical cases of poisoning incidents are described after 20-30 years of regular consumption of about 7-15 g calcium carbonate.

Summary (Annex IIA, point 5.10)

	Value	Study	Safety factor
ADI ‡	No data available, not required.	-	-
AOEL ‡	No data available, not required.	-	-
ARfD ‡	No data available, not required.	-	-

Dermal absorption ‡ (Annex IIIA, point 7.3)

Formulation (Neoponit L 75 % PA)	Negligible.

Exposure scenarios (Annex IIIA, point 7.2)

Operator	Paintbrush and gloves applications of a paste were not considered to be a source of significant exposure.
Workers	Paintbrush and gloves applications of a paste were not considered to be a source of significant exposure.
Bystanders	Paintbrush and gloves applications of a paste were not considered to be a source of significant exposure.

Classification and proposed labelling with regard to toxicological data (Annex IIA, point 10)

	RMS/peer review proposal
Limestone	Data available of limited validity to conclude, no further data needed.

Residues

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plant groups covered

Rotational crops

Metabolism in rotational crops similar to metabolism in primary crops?

Processed commodities

Residue pattern in processed commodities similar to residue pattern in raw commodities?

Plant residue definition for monitoring

Plant residue definition for risk assessment

Conversion factor (monitoring to risk assessment)

Not required. No residue definition.

According to the representative uses limestone is used as a repellent, applied to the shoots with brushes or gloves (forest plantation, forest tree nursery).

Since coating of trees is the only use of limestone no crops are treated directly, and therefore no residues on food and/or feed may occur.

Metabolism in livestock (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

Animals covered

Time needed to reach a plateau concentration in milk and eggs

Animal residue definition for monitoring

Animal residue definition for risk assessment

Conversion factor (monitoring to risk assessment)

Metabolism in rat and ruminant similar (yes/no)

Fat soluble residue: (yes/no)

Not required. No residue definition.

See general statement under metabolism in plants.

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

Not required. See general statement under metabolism in plants.

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 Introduction)

Not required. See general statement under metabolism in plants.

Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

Expected intakes by livestock ≥ 0.1 mg/kg diet (dry weight basis) (yes/no - If yes, specify the level)

Potential for accumulation (yes/no):

Metabolism studies indicate potential level of residues ≥ 0.01 mg/kg in edible tissues (yes/no)

Ruminant:	Poultry:	Pig:
Conditions of requirement of feeding studies		
Not required. See general statement under metabolism in plants.		

Muscle

Liver

Kidney

Fat

Milk

Eggs

Feeding studies (Specify the feeding rate in cattle and poultry studies considered as relevant)

Residue levels in matrices : Mean (max) mg/kg

Not required. See general statement under metabolism in plants.

Summary of residues data according to the representative uses on raw agricultural commodities and feedingstuffs (Annex IIA, point 6.3, Annex IIIA, point 8.2)

Crop	Northern or Mediterranean Region, field or glasshouse, and any other useful information	Trials results relevant to the representative uses (a)	Recommendation/comments	MRL estimated from trials according to the representative use	HR (c)	STMR (b)
Not required. See general statement under metabolism in plants.						

(a) Numbers of trials in which particular residue levels were reported *e.g.* 3 x <0.01, 1 x 0.01, 6 x 0.02, 1 x 0.04, 1 x 0.08, 2 x 0.1, 2 x 0.15, 1 x 0.17

(b) Supervised Trials Median Residue *i.e.* the median residue level estimated on the basis of supervised trials relating to the representative use

(c) Highest residue

Consumer risk assessment (Annex IIA, point 6.9, Annex IIIA, point 8.8)

ADI	Not relevant, see section on mammalian toxicology
TMDI (% ADI) according to WHO European diet	
TMDI (% ADI) according to national (to be specified) diets	
IEDI (WHO European Diet) (% ADI)	
NEDI (specify diet) (% ADI)	
Factors included in IEDI and NEDI	
ARfD	Not relevant, see section on mammalian toxicology
IENTI (% ARfD)	
NESTI (% ARfD) according to national (to be specified) large portion consumption data	
Factors included in IENTI and NESTI	

Processing factors (Annex IIA, point 6.5, Annex IIIA, point 8.4)

Crop/ process/ processed product	Number of studies	Processing factors		Amount transferred (%) (Optional)
		Transfer factor	Yield factor	
Not required. See general statement under metabolism in plants.				

Proposed MRLs (Annex IIA, point 6.7, Annex IIIA, point 8.6)

Not required. See general statement under metabolism in plants.

Fate and behaviour in the environment**Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1)**

Not relevant. Due to the natural occurrence of calcium and carbonate in the environment, conducting specific environmental fate studies is not required.

Route of degradation in soil - Supplemental studies (Annex IIA, point 7.1.1.2)

No data available. Not required.

Rate of degradation in soil (Annex IIA, point 7.1.1.2, Annex IIIA, point 9.1.1)

No data available. Not required.

Soil adsorption/desorption (Annex IIA, point 7.1.2)

Due to the natural occurrence of calcium and carbonate in the environment, conducting specific environmental fate studies is not required.

Much of the calcium present in soils is held onto the colloidal surface as adsorbed cations. However, calcium ions may be released to the soil solution by exchange with other positively charged ions, and subsequently re-adsorbed. Although all cations may be adsorbed by soil colloids, certain cations, such as calcium, are favoured under natural conditions. In humid regions, the ranking of adsorption of cations is H^+ , Al^{3+} and Ca^{2+} first, Mg^{2+} second, then K^+ and Na^+ third. In well-drained arid and semiarid regions the ranking is usually Ca^{2+} and Mg^{2+} first, Na^+ and K^+ second, and H^+ last.

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Due to the natural occurrence of calcium and carbonate in the environment, conducting specific environmental fate studies is not required.

PEC (soil) (Annex IIIA, point 9.1.3)

Not relevant for environmental exposure assessment. Owing to the manual application by coating forest trees with gloves or brush no entry of the active substance into soil is expected. Therefore the calculation of PEC_{soil} is not considered necessary.

Route and rate of degradation in water (Annex IIA, point 7.2.1)

Due to the natural occurrence of calcium and carbonate in the environment, conducting specific environmental fate studies is not required.

PEC (surface water) and PEC sediment (Annex IIIA, point 9.2.3)

Not relevant for environmental exposure assessment. Owing to the manual application by coating forest trees with gloves or brush no entry of the active substance into soil and surface water is expected. Therefore the calculation of PEC_{SW} or EC_{SED} is not considered necessary.

PEC (ground water) (Annex IIIA, point 9.2.1)

Not relevant for environmental exposure assessment. Owing to the manual application by coating forest trees with gloves or brush no entry of the active substance into soil and water is expected. Therefore the calculation of PEC_{GW} is not considered necessary.

Fate and behaviour in air (Annex IIA, point 7.2.2, Annex III, point 9.3)

Not relevant. Due to the natural occurrence of calcium and carbonate in the environment, conducting specific environmental fate studies is not required.

PEC (air)

Carbon dioxide is the main component that would be released to the atmosphere. CO₂ is naturally occurring in the environment. Therefore it is not reasonable to perform a calculation of PEC_A.

Residues requiring further assessment

Environmental occurring residues requiring further assessment by other disciplines (toxicology and ecotoxicology), and or for which consideration for groundwater exposure is necessary.

Not necessary. Calcium and carbonate ions are naturally occurring in the environment.

Monitoring data, if available (Annex IIA, point 7.4)

Soil (indicate location and type of study)	Not applicable. Calcium and carbonate ions are naturally occurring in the environment.
Surface water (indicate location and type of study)	Not applicable. Calcium and carbonate ions are naturally occurring in the environment.
Ground water (indicate location and type of study)	Not applicable. Calcium and carbonate ions are naturally occurring in the environment.
Air (indicate location and type of study)	Not applicable. Calcium is not volatile and CO ₂ is naturally occurring in the environment.

Points pertinent to the classification and proposed labelling with regard to fate and behaviour data

Biodegradability assessment not applicable for a mineral.
No classification proposed.

Ecotoxicology

Effects on terrestrial vertebrates (Annex IIA, point 8.1, Annex IIIA, points 10.1 and 10.3)

Species	Test substance	Time scale	End point (mg/kg bw/(day))	End point (mg/kg feed)
Birds ‡				
No data, not required.				
Mammals ‡				
Rat	a.s.	Acute	Data available of limited validity, no further data needed.	
Rat	Preparation	Acute		
Additional higher tier studies ‡ No data				

Toxicity/exposure ratios for terrestrial vertebrates (Annex IIIA, points 10.1 and 10.3)

Indicator species	Time scale	ETE	TER	Annex VI Trigger
Birds	As the treated plant material does not constitute an attractive food item for birds and as it is likely that the product also has a slight repellent effect against birds, the risk for birds is considered to be low.			
Mammals	Mammals that could be at risk by feeding on the tree shoot, such as deer and hare, belong to the target-organisms and are repelled by the mechanical effect of the product. Therefore the risk for mammals is considered to be low.			

Toxicity data for aquatic species (most sensitive species of each group) (Annex IIA, point 8.2, Annex IIIA, point 10.2)

Group	Test substance	Time-scale (Test type)	End point	Toxicity ¹ (mg a.s./L)
Laboratory tests ‡				
Fish				
Mosquitofish (<i>Gambusia affinis</i>)	a.s. (calcium carbonate)	96 hr (static)	mortality, EC ₅₀	>56000 a.s. (nom)
Guppy (<i>Poecilia reticulata</i>)	Vinylacrylat, main co- formulant of the lead formulation	96 hr (semi static)	Mortality, EC ₅₀	> 100 (nom)
Aquatic invertebrate				
Waterflea (<i>Daphnia magna</i>)	a.s. (calcium carbonate)	125 d (semi static)	no adverse effects at 350 mg/L, therefore an EC ₅₀ >350 mg/L was estimated	>350 (im)

Group	Test substance	Time-scale (Test type)	End point	Toxicity ¹ (mg a.s./L)
Waterflea (<i>Daphnia magna</i>)	Vinylacrylat, main co- formulant of the lead formulation	48 h (test type not reported)	Mortality, EC ₅₀ NOEC	87.9 _(nom) 80 _(nom)
Algae				
Green alga (<i>Scenedesmus subspicatus</i>)	Vinylacrylat main co- formulant of the lead formulation	72 h (test type not reported)	Biomass: E _b C ₅₀	> 100 _(nom)

¹ based on nominal (_{nom}) or mean initial concentrations (_{im}).

Toxicity/exposure ratios for the most sensitive aquatic organisms (Annex IIIA, point 10.2)

Due to the type of application as coating on current year shoot of forest trees exposure of aquatic organisms is considered to be low. Therefore a calculation of TER values for aquatic organisms is not considered necessary.

Bioconcentration

No data, not required.

As limestone is a naturally occurring compound and exposure is unlikely, no adverse effects on aquatic organisms from bioaccumulation of limestone are expected to occur. Moreover, limestone is biologically almost inert.

Effects on honeybees (Annex IIA, point 8.3.1, Annex IIIA, point 10.4)

Test substance	Acute oral toxicity (LD ₅₀ µg/bee)	Acute contact toxicity (LD ₅₀ µg/bee)
No data, not required.	The representative formulation is used as a coating on current year shoot of forest trees (manually applied) and hence exposure of bees is considered to be low. No studies are required according to Directive 91/414/EEC in cases where preparations containing the active substance are for exclusive use in situations where bees are not likely to be exposed.	

Hazard quotients for honey bees (Annex IIIA, point 10.4)

Test substance	Route	Hazard quotient	Annex VI Trigger
No data, not relevant			

Effects on other arthropod species (Annex IIA, point 8.3.2, Annex IIIA, point 10.5)

Laboratory tests with standard sensitive species

Species	Test Substance	End point	Effect (LR ₅₀ g/ha)
No data, not relevant			

Further laboratory and extended laboratory studies ‡

Species	Life stage	Test substance, substrate and duration	Dose (g/ha)	End point	% effect	Trigger value
No data, not relevant						

Effects on earthworms, other soil macro-organisms and soil micro-organisms (Annex IIA points 8.4 and 8.5, Annex IIIA, points, 10.6 and 10.7)

Test organism	Test substance	Time scale	End point
Earthworms			
No data, not relevant			
Other soil macro-organisms			
Soil mite			
No data, not relevant			
Collembola			
No data, not relevant.			
Soil micro-organisms			
No data, not relevant			

Toxicity/exposure ratios for soil organisms

Due to the representative uses and the special type of application technique exposure of earthworms and soil organisms is considered to be low.

Moreover, calcium and carbonate are typical compounds of all natural systems and environmental compartments including water, soil, plant and animal tissues. For this reason no studies to assess effects on earthworms and soil organisms are required.

Effects on non target plants (Annex IIA, point 8.6, Annex IIIA, point 10.8)

Preliminary screening data

No data, not relevant.

Effects on biological methods for sewage treatment (Annex IIA 8.7)

No data, not required.	No inhibitory effects on aerobic waste water microorganisms are expected if the representative formulation is used according to the GAP as a coating on current year shoot of forest trees (manually applied). The active substance, pulverized limestone, contains mainly CaCO_3 in concentrations of min. 98 %. Calcium and carbonate are typical compounds of all natural materials that are present in soils, sediments and surface water.

Ecotoxicologically relevant compounds (consider parent and all relevant metabolites requiring further assessment from the fate section)

Compartment	
soil	None
water	None
sediment	None
groundwater	None

Classification and proposed labelling with regard to ecotoxicological data (Annex IIA, point 10 and Annex IIIA, point 12.3)

Limestone	RMS/peer review proposal
	No classification proposed
'Neoponit L'	RMS/peer review proposal
	No classification proposed

ABBREVIATIONS

1/n	slope of Freundlich isotherm
λ	wavelength
ε	decadic molar extinction coefficient
°C	degree Celsius (centigrade)
μg	microgram
μm	micrometer (micron)
a.s.	active substance
AChE	acetylcholinesterase
ADE	actual dermal exposure
ADI	acceptable daily intake
AF	assessment factor
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
AR	applied radioactivity
ARfD	acute reference dose
AST	aspartate aminotransferase (SGOT)
AV	avoidance factor
BCF	bioconcentration factor
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CIPAC	Collaborative International Pesticides Analytical Council Limited
CL	confidence limits
cm	centimetre
d	day
DAA	days after application
DAR	draft assessment report
DAT	days after treatment
DM	dry matter
DT ₅₀	period required for 50 percent disappearance (define method of estimation)
DT ₉₀	period required for 90 percent disappearance (define method of estimation)
dw	dry weight
EbC ₅₀	effective concentration (biomass)
EC ₅₀	effective concentration
ECHA	European Chemical Agency
EEC	European Economic Community
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMDI	estimated maximum daily intake
ER ₅₀	emergence rate/effective rate, median
ErC ₅₀	effective concentration (growth rate)
EU	European Union
EUROPOEM	European Predictive Operator Exposure Model
f(twa)	time weighted average factor
FAO	Food and Agriculture Organisation of the United Nations
FIR	Food intake rate
FOB	functional observation battery
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
g	gram
GAP	good agricultural practice

GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GGT	gamma glutamyl transferase
GM	geometric mean
GS	growth stage
GSH	glutathion
h	hour(s)
ha	hectare
Hb	haemoglobin
Hct	haematocrit
hL	hectolitre
HQ	hazard quotient
ICP-OES	inductively coupled plasma optical emission spectroscopy
IEDI	international estimated daily intake
IENTI	international estimated short-term intake
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint Meeting on the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
K _{doc}	organic carbon linear adsorption coefficient
kg	kilogram
K _{Foc}	Freundlich organic carbon adsorption coefficient
L	litre
LC ₅₀	lethal concentration, median
LD ₅₀	lethal dose, median; dosis letalis media
LDH	lactate dehydrogenase
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOQ	limit of quantification (determination)
m	metre
M/L	mixing and loading
MAF	multiple application factor
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
mg	milligram
mL	millilitre
mm	millimetre
MRL	maximum residue limit or level
MSDS	material safety data sheet
MTD	maximum tolerated dose
MWHC	maximum water holding capacity
NESTI	national estimated short-term intake
ng	nanogram
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
OM	organic matter content
PA	paste
Pa	pascal
pcs	pieces
PD	proportion of different food types
PEC	predicted environmental concentration
PEC _{air}	predicted environmental concentration in air

PEC _{gw}	predicted environmental concentration in ground water
PEC _{sed}	predicted environmental concentration in sediment
PEC _{soil}	predicted environmental concentration in soil
PEC _{sw}	predicted environmental concentration in surface water
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIE	potential inhalation exposure
pK _a	negative logarithm (to the base 10) of the dissociation constant
P _{ow}	partition coefficient between <i>n</i> -octanol and water
PPE	personal protective equipment
ppm	parts per million (10 ⁻⁶)
ppp	plant protection product
PT	proportion of diet obtained in the treated area
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r ²	coefficient of determination
RPE	respiratory protective equipment
RUD	residue per unit dose
SC	suspension concentrate
SD	standard deviation
SFO	single first-order
SSD	species sensitivity distribution
STMR	supervised trials median residue
t _{1/2}	half-life (define method of estimation)
TER	toxicity exposure ratio
TER _A	toxicity exposure ratio for acute exposure
TER _{LT}	toxicity exposure ratio following chronic exposure
TER _{ST}	toxicity exposure ratio following repeated exposure
TK	technical concentrate
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TRR	total radioactive residue
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UV	ultraviolet
W/S	water/sediment
w/v	weight per volume
w/w	weight per weight
WBC	white blood cell
WHO	World Health Organisation
wk	week
yr	year

SCIENTIFIC OPINION

Scientific Opinion on re-evaluation of calcium carbonate (E 170) as a food additive¹

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of calcium carbonate (E 170). Calcium carbonate is an inorganic salt authorised as a food additive in the EU, and is also included in the list of substances that may be added for specific nutritional purposes in foods for particular nutritional uses and in Directive 2002/46/EC relating to food supplements. Calcium carbonate was previously evaluated by JECFA in 1965, when the Committee established an ADI not limited. The SCF evaluated calcium carbonate in 1990 as part of a group of carbonates, when the Committee assigned a group ADI not specified. The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that the available toxicological database on calcium carbonate is limited, but does not give rise to concern. The few effects seen in studies in humans and animals are associated with high calcium carbonate intakes, and are also seen with other calcium salts. The Panel agrees with the group ADI “not specified” assigned by the SCF to a group of carbonates including calcium carbonate, when considering the use of calcium carbonate as a food additive. The Panel notes that the estimated exposures to calcium from all sources, including the use of calcium carbonate as a food additive, taken together with intakes of calcium from supplements and from food fortification are below the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003. The Panel concludes that trace levels of adventitious nanoscale material within macroscale calcium carbonate are not of toxicological concern.

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KEY WORDS

Calcium carbonate, E 170, CAS Registry Number 471-34-1, calcite, chalk, carbonic acid calcium salt, CI Pigment White 18, food additive, food colouring substance, food supplement, food fortification.

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of calcium carbonate (E 170) when used as a food additive⁴.

Calcium carbonate is a food additive generally authorised in foodstuffs under Directive 95/2/EC⁵ on food additives and is a permitted food colouring substance under Directive 94/36/EC⁶ on colours for use in foodstuffs. It is also included in Directive 2001/15/EC⁷ on substances that may be added for specific nutritional purposes in foods for particular nutritional uses, in Directive 2002/46/EC⁸ relating to food supplements and it can be used in fortified foods according to Regulation 1925/2006⁹ on the addition of vitamins and minerals and of certain other substances in food.

Calcium carbonate was previously evaluated by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA) in 1965, when the Committee established an Acceptable Daily Intake (ADI) not limited. The EU Scientific Committee for Food (SCF) evaluated calcium carbonate in 1990 as part of a group of carbonates, when the Committee assigned a group ADI not specified.

Specifications for calcium carbonate have been defined in Commission Directive 2008/128/EC¹⁰ and by JECFA. The purity is specified as not less than 98% calcium carbonate. The Panel noted that calcium carbonate can have an amorphous or microcrystalline structure, and that particle size varies according to the form and manufacturing conditions. Particles of amorphous calcium carbonate are reported to be characteristically 40 to 120 nm diameter spherules, in contrast to the 1 to 10 µm diameter crystals typical of the crystalline forms of calcium carbonate. Nanoform calcium carbonate (as used in some of the toxicological studies reported in this opinion) was reported to have a particle size of 60 - 100 nm when examined by Scanning Electron Microscopy (SEM). The typical average particle size (d50) of food grade calcium carbonate is stated by industry to be about 5 µm, with an upper range (d98%) of 65 µm and less than 1% of particles having a diameter below 100 nm; the presence of unintentional nanoscale particles at trace levels in the product cannot be excluded.

Calcium carbonate dissociates into its constituent ions in the acid milieu of the stomach. Some of the calcium is absorbed, via active transport or passive diffusion, but a large proportion (up to 80 %) is reconverted to calcium carbonate and other insoluble calcium salts and excreted in the faeces. Average absorption of calcium from calcium carbonate over a range of studies has been shown to be in the range of 20-40%. The Panel noted however that recent toxicokinetic data in mice indicate that calcium from nanoparticulate calcium carbonate is more readily absorbed than the microparticulate form, as evidenced by significantly higher serum calcium concentrations in the nano calcium carbonate group than in the micro calcium carbonate group. A slight increase in calcium bioavailability (by 38%) from nanonised pearl powder form by comparison with the corresponding

⁴ The following functionalities have been reported: food colour, anti-caking agent, acidity regulator, gelling agent, thickener, bulking agent, hardening agent, glazing agent, release agent.

⁵ European Parliament and Council Directive 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1–63.

⁶ European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 237, 10.9.94, p. 13-29.

⁷ Commission Directive 2001/15/EC of February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 52, 2.2.2001, p 19.

⁸ Commission Directive 2002/46/EC of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183 of 12.7.2002 p.51.

⁹ Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ L 404, 30.12.2006, p. 26-38.

¹⁰ Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1.2009, p. 20-63.

micronised form of calcium carbonate has also been observed in humans. In view of the small increases observed in these studies, the Panel considered that the absorption of calcium from calcium carbonate in nanoparticles will not be markedly different than that from calcium carbonate microparticles.

After intestinal absorption, calcium and carbonate/bicarbonate ions enter normal metabolic pathways and body pools. The majority of absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and also faeces and skin) and excess carbonate is excreted as carbon dioxide via respiration.

Limited toxicological data are available on calcium carbonate. A number of short term studies (generally less than 90 days) have been carried out with calcium carbonate in rats, mice and cats, to investigate the effects of high calcium diets on various physiological and biochemical parameters. These studies have also assessed the toxicity of high levels of calcium carbonate in the diet, as manifest by effects on growth and food consumption, and have overall not demonstrated any evidence of toxicity attributable to calcium carbonate.

The only notable treatment-related effect seen in a 91-day feeding study with calcium carbonate in rats at dietary calcium levels equivalent to 250 or 500 mg/kg bw/day was nephrocalcinosis, seen in both males and (more marked) in females. The Panel considered however that the nephrocalcinosis finding in rats is not relevant for human safety assessment because the rat is a species known to be particularly sensitive to mineralisation of the renal tubule epithelium due to dietary alteration of the calcium and phosphorus homeostasis. Results from a 91-day toxicity study in Beagle dogs fed calcium carbonate at doses to achieve equivalent calcium levels in the diet as in the rat study (~ 250 and 500 mg calcium/kg bw/day) did not report any signs of nephrocalcinosis.

Nephrocalcinosis was also not observed in a recent combined repeat dose oral toxicity/reproduction/developmental toxicity screening study with calcium carbonate (having a particle size of 60 - 100 nm when examined by SEM) carried out in Wistar rats at dose levels of up to 1000 mg/kg bw/day for up to 48 days. The only changes seen in this study were slight but statistically significant haematological and biochemical effects in males receiving 1000 mg/kg bw/day, and significant reductions in plasma phosphate levels in all male treated groups. The Panel considered that these changes were non-adverse and agreed with the NOAEL of 1000 mg/kg bw/day identified by the authors of the study, the highest dose tested. No evidence of toxicity was reported in a study in which mice were administered calcium carbonate (described by the authors as nano calcium carbonate) by oral gavage at dose levels up to 1300 mg/kg bw/day for 28 days. The Panel noted however that only limited investigations were carried out in this study.

Calcium carbonate (including calcium carbonate having a particle size of 60 - 100 nm when examined by SEM) has given negative results in a range of *in vitro* genotoxicity assays. No data are available on the chronic toxicity or carcinogenicity of calcium carbonate. The Panel considered however that it is very unlikely that calcium carbonate has carcinogenic potential, given that both calcium and carbonate are natural constituents of the body and normal metabolites of man, animals and plants, and have a long history of safe use as a source of calcium supplementation for humans.

Several reproductive and developmental toxicity studies in rats and mice have been carried out with calcium carbonate. In rats fed diets containing calcium carbonate at levels up to approximately 1500 mg/kg bw/day, no dose-related changes indicative of developmental toxicity were reported. In a recent OECD guideline-compliant combined repeat dose oral toxicity/reproduction study, no effects on reproduction, including developmental toxicity, were reported at dose levels of nanoparticulate calcium carbonate up to 1000 mg/kg bw/day, the highest dose tested. Other studies have shown evidence of fetotoxicity of calcium (as calcium carbonate) when administered during pregnancy at levels in the diet greater than 1500 mg/kg bw/day calcium carbonate. Overall, the Panel noted that in

rodents high doses of calcium carbonate (> 1500 mg/kg bw/day) causing hypercalcaemia during gestation can result in adverse effects on reproduction, fetotoxicity and elemental imbalances in the offspring. However a recent repeat dose oral toxicity/reproduction study provided a NOAEL of 1000 mg/kg bw/day, the highest dose tested, for developmental toxicity and the Panel considered overall that there is no concern for the reproductive effects of calcium carbonate at intakes below 1500 mg/kg bw/day.

No data are available indicating that calcium carbonate has allergenic properties or can invoke sensitivity or intolerance reactions in exposed individuals.

The Panel noted that a number of studies have been carried out with calcium carbonate in humans, showing that hypercalcaemia and alkalosis can occur when calcium carbonate (between 2.0 and 16.5 g/day of supplementary calcium) is taken with large amounts of milk or cream for the treatment of peptic ulcer (milk-alkali syndrome). The hypercalcaemia is often associated with renal dysfunction, metastatic calcification and other symptoms, and similar changes have been reported in individuals taking large amounts of calcium carbonate and other calcium-containing antacids or large amounts of calcium food supplements.

The Panel also noted overall that although a recent meta-analysis reported by Bolland et al. in 2010 and 2011, showing an increased risk of myocardial infarction in individuals given regular calcium supplementation in the management of osteoporosis, was a very large and robust study, the trends reported were very modest, and are not supported by the findings in a similar study carried out by Lewis et al. and reported in 2010. The Panel also noted that the trends noted in cardiovascular risks following calcium supplementation contrasted with those found with dietary calcium, and that a plausible mode of action could be described to explain these differences, based on serum levels of calcium following dietary intake and supplementation.

Overall the Panel considered that the available toxicological database on calcium carbonate is limited, but it does not give rise to concern. The Panel considered that further toxicological studies on calcium carbonate are not necessary for the reasons already stated by the SCF in 1991, namely that calcium carbonate is a natural constituent of man, animals and plants, and therefore occurs naturally in foodstuffs. Calcium and carbonate/bicarbonate also constitute some of the major electrolytes present in all biological materials. The few effects seen in studies in humans and animals are associated with high calcium carbonate intakes, and are also found after large intakes of other calcium salts. The Panel considered that these high-calcium intake effects are not relevant to the evaluation of calcium carbonate as a food additive, and that the SCF established an UL for calcium in 2003, taking these effects into account.

The Panel therefore agreed with the group ADI “not specified” established by the SCF in 1991 for a group of carbonates including calcium carbonate and considers that the JECFA definition of “ADI not specified” is applicable to calcium carbonate when used as a food additive.

The Panel considered however that intakes of calcium resulting from the use of calcium carbonate as a food additive, taken together with intakes of calcium from supplements and from food fortification, should be below the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003.

Based on data made available to the Panel by industry and Member States, the Panel estimated mean calcium intake from use of calcium carbonate as food additive and added nutrient source (fortification) and including all calcium intake from food supplements (scenario 3) as ranging from 1105-1375 mg calcium/day for toddlers, 1270-1780 mg calcium/day for children, 1330-1730 mg calcium/day for adolescents and 1240-1540 mg calcium/day for adults. The ranges of high percentile intakes are estimated at 1270-1705 mg calcium/day for toddlers, 1450-2500 mg calcium/day for children, 1730-2380 mg calcium/day for adolescents and 1420-2380 mg calcium/day for adults.

Estimated exposure to calcium from all sources based on data presented in the literature for six Member States showed that mean calcium intakes (including food supplements) in all population groups were of comparable size, ranging from 664 – 795 mg calcium/day for toddlers, 749 – 869 mg calcium/day for children, 738 – 1070 mg calcium/day for adolescents and 730 – 1164 mg calcium/day for adults. The range of high percentile intakes were 1070 – 1184 mg calcium/day for toddlers, 1124–1453 mg calcium/day for children, 1306 – 1905 mg calcium/day for adolescents and 1233 – 1851 mg calcium/day for adults. Mean intake for the elderly (>64 years old) ranges from 754 – 954 mg calcium/day and 1285 – 2106 mg calcium/day for high percentile consumers.

The Panel noted that both the intake estimates calculated by the Panel, which are considered to be conservative, and the total dietary intake of calcium reported in literature are all below the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003. Therefore, the Panel concluded that, based on the available data described in this opinion, the use of calcium carbonate as a food additive at the current maximum reported use levels is not of safety concern.

The Panel noted that calcium carbonate is currently permitted at *quantum satis* in the vast majority of food categories in which it is allowed (generally permitted in all foods with certain exceptions), however information gathered by the Panel for this re-evaluation shows that calcium carbonate is only used at defined amounts in a number of food categories. The Panel thus recommended that the legislation be updated to reflect actual usage levels evaluated in this opinion.

The Panel also noted that a range of studies have been performed with calcium carbonate described as nanoform (having a particle size of 60 - 100 nm when examined by SEM), however none of these provided comprehensive data on characterisation of the nano material. Nor do these studies provide a full toxicological database in line with the guidance provided in the EFSA Scientific Committee Opinion on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety. According to the information provided by the industry, the presence of unintentional nanoscale particles at trace levels in calcium carbonate when used as a food additive cannot be excluded. Whilst the data are inadequate to reach definitive conclusions on calcium carbonate predominantly in the nanoscale, the Panel considered that the available data are sufficient to conclude that the current levels of adventitious nanoscale material within macroscale calcium carbonate would not be an additional toxicological concern.

The Panel also noted that the JECFA specification for lead in calcium carbonate is ≤ 3 mg/kg whereas the EC specification is ≤ 10 mg/kg.

Additionally the Panel noted that limestone (a source of calcium carbonate) may contain variable amounts of aluminium. The Panel noted that aluminium intake from use of calcium carbonate as a food additive could significantly contribute (50-100%) to the weekly intake of aluminium, for which a tolerable weekly intake (TWI) of 1 mg aluminium/kg bw/week has been established, and therefore specifications for the maximum level of aluminium in calcium carbonate may be required

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by the EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010¹¹. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU¹² of 2001. The report “Food additives in Europe 2000¹³” submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of the adoption of Regulation (EU) 257/2010 the 2003 Terms of Reference are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedure and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

¹¹ OJL 80, 26.03.2010, p19

¹² COM(2001) 542 final.

¹³ Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002:560.

ASSESSMENT

1. INTRODUCTION

The present opinion deals with the re-evaluation of the safety of calcium carbonate (E 170) when used as a food additive. The following functionalities have been reported: food colour, anti-caking agent, acidity regulator, gelling agent, thickener, bulking agent, hardening agent, glazing agent and release agent.

Calcium carbonate is a food additive generally authorised in foodstuffs under Directive 95/2/EC¹⁴ on food additives and is a permitted food colouring substance under Directive 94/36/EC¹⁵ on colours for use in foodstuffs. Calcium carbonate was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1965 (JECFA, 1965, 1966). The EU Scientific Committee for Food (SCF) evaluated calcium carbonate in 1990 as part of a group of carbonates (SCF, 1991), and a review of carbonates as food additives has been carried out by TemaNord (TemaNord, 2002). Calcium carbonate has also been registered under the REACH Regulation 1907/2006¹⁶ (ECHA, 2011).

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data^{17,18}.

2. TECHNICAL DATA

2.1. Identity of the substance

Calcium carbonate is an inorganic salt with CAS Registry Number 471-34-1, Colour Index No. 77220 and EINECS No. 207-439-9. Synonyms are carbonic acid calcium salt, calcite, chalk, CI Pigment White 18, INS No. 170(i). The chemical formula is CaCO_3 and the molecular weight is 100.1 g/mol. Calcium carbonate is an odourless white micro-crystalline or amorphous powder with six known forms (polymorphs). Five of these are crystalline and one is amorphous (hydrated amorphous calcium carbonate (Meiron et al., 2011)). Of the five crystalline forms, three are anhydrous crystalline (i.e., calcite, aragonite, and vaterite) and two are hydrated crystalline (i.e., crystalline monohydrocalcite and ikaite) (Meiron et al., 2011). The most thermodynamically stable of these forms is calcite, whereas the least stable is the amorphous form (Nebel et al., 2008; Meiron et al., 2011).

Particles of amorphous calcium carbonate are reported to be characteristically 40 to 120 nm diameter spherules, in contrast to the 1 to 10 μm diameter crystals typical of the other forms (Meiron et al., 2011). According to the Calcium Carbonate Association (CCA-Europe) engineered nanoscale calcium carbonate is not used as a food additive. According to CCA-Europe, the typical average particle size (d50) of food grade calcium carbonate is stated by CCA-Europe to be about 5 μm , with an upper range (d98%) of 65 μm , but the presence of unintentional nanoscale particles at trace levels in the product cannot be excluded (CCA-Europe, 2011). According to CCA-Europe, in two samples of food grade calcium carbonate the total number of particles with a diameter of less than 100 nm was

¹⁴ European Parliament and Council Directive 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1–63.

¹⁵ European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 237, 10.9.94, p. 13–29.

¹⁶ Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 396, 30.12.2006, p. 1–849.

¹⁷ Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available from: <http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm>

¹⁸ Call for scientific data on calcium carbonate (E 170i). Published: 10 December 2010. Available from: <http://www.efsa.europa.eu/en/dataclosed/call/ans101210.htm>.

estimated to be less than 1% (0.3% and 0.65% respectively, and 0.04% and 0.13% when a correction factor was applied, to allow for the maximum possible dissolved content arising as a result of the determination method). Some recent toxicological studies have been carried out with nanoparticulate calcium carbonate, as reported in Section 3 of this opinion. Information on the particle size of the calcium carbonate used in these studies has been provided in this opinion, when available.

The Panel noted that in characterising the particle size of calcium carbonate as a food additive, the considerations laid out in the EFSA Scientific Committee Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain should be taken into account (EFSA, 2011).

Calcium carbonate is practically insoluble in water and ethanol (FCC 7, 2010), but is soluble with effervescence in hydrochloric and other acids (Commission Directive 2008/128/EC¹⁹). According to the available information in the ECHA database the solubility of calcium carbonate (conventional) in water (OECD Guideline 105) is 16.6 mg/l (20°C, pH 9-9.4), while that of the aragonite and calcite forms is 6.6 mg/l (20°C) and of the valerite form 11 mg/l (20°C) (ECHA, 2011). The amorphous form of calcium carbonate, with particles predominately in the nanoscale range, is reported to be approximately 10 times more soluble than crystalline calcium carbonate (Meiron et al., 2011).

2.2. Specifications

Specifications have been defined in Commission Directive 2008/128/EC and by JECFA (JECFA, 2006) (Table 1).

Table 1: Specifications for calcium carbonate according to Commission Directive 2008/128/EC and JECFA (2006)

	Commission Directive 2008/128/EC	JECFA, 2006
Assay (on anhydrous basis)	≥ 98%	≥ 98%
Loss on drying (200 °C, 4 hours)	≤ 2.0%	≤ 2%
Acid-insoluble substances	≤ 0.2%	≤ 0.2%
Free alkali	-	≤ 0.05%
Magnesium and alkali salts	≤ 1.5%	≤ 1.0%
Fluoride	≤ 50 mg/kg	≤ 50 mg/kg
Antimony (as Sb) Copper (as Cu) Chromium (as Cr) Zinc (as Zn) Barium (as Ba)	≤ 100 mg/kg, singly or in combination	≤ 0.03 %
Arsenic	≤ 3 mg/kg	≤ 3 mg/kg
Lead	≤ 10 mg/kg	≤ 3 mg/kg
Cadmium	≤ 1 mg/kg	-

The Panel noted that the JECFA specification for lead in calcium carbonate is ≤ 3 mg/kg, whereas the EC specification is ≤ 10 mg/kg.

The Panel noted that there is no available information indicating that calcium carbonate is used as an aluminium lake for colouring purposes. The Panel noted that limestone (a source of calcium carbonate) may contain variable amounts of aluminium, one report indicating approximate

¹⁹ Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1.2009, p. 20-63.

concentrations of between 1 and 134 mg/kg (EPA, 1995). Additionally, the results of the analysis of some samples of food grade calcium carbonate were provided to the Panel, showing a range of aluminium concentrations from 10 to 190 mg/kg (Chemische Fabrik Budenheim KG, 2011). The Panel noted that aluminium intake from use of calcium carbonate as a food additive could add to the weekly intake of aluminium, for which a tolerable weekly intake (TWI) of 1 mg aluminium/kg bw/week has been established (EFSA, 2008a), and therefore specifications for the maximum level of aluminium in calcium carbonate may be required.

2.3. Manufacturing process

According to Directive 2008/128/EC calcium carbonate is the product obtained from ground limestone or by the precipitation of calcium ions with carbonate ions. The most common industrial process for obtaining calcium carbonate involves the following steps (Domingo et al., 2004): (a) calcination of limestone (natural calcium carbonate rocks) to produce quicklime (CaO) and carbon dioxide (CO₂); (b) the slaking process, in which the quicklime is transformed to slaked lime slurry (a Ca(OH)₂ suspension) by controlled addition of water; and, finally (c) the carbonation reaction, in which CO₂ is bubbled through an aqueous slurry of slaked lime. The carbonation reaction is the crucial step determining the morphology of the obtained product. Particle size of the product can be varied by manipulation of the production conditions, such as partial pressure of CO₂, temperature, pH and presence of additives such as carboxylic acids (Tai and Chen, 1995; Franke and Mersmann 1995; García-Carmona et al., 2003; Domingo et al., 2004; Reiger et al., 2007; Henderson et al., 2008, Graham et al., 2008).

2.4. Methods of analysis in food

Determination of either calcium or the carbonate anion as result of use as a food additive in foodstuffs is only possible to a limited extent, since both occur endogenously in most foods. The most commonly used analytical techniques for determining the calcium carbonate content in foods rely on the measurement of total calcium by Atomic Absorption Spectroscopy (AAS), Atomic Emission Spectroscopy (AES) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS) or other related techniques following acid digestion of the sample (Scotter, 2011). Carbonate can be determined by measurement of evolved carbon dioxide volumetrically following acidification with hydrochloric acid and capture in a Chittick apparatus or gravimetrically after precipitation with barium hydroxide solution. In beer and wine carbonate can be determined by Ion Chromatography (IC) using conductivity detection (Scotter, 2011).

2.5. Reaction and fate in food

Calcium carbonate can be anticipated to be unstable in acidic foods.

2.6. Case of need and proposed uses

Authorised use and use levels have been defined in Directive 94/36/EC on colours for use in foodstuffs and in Directive 95/2/EC on food additives other than colours and sweeteners, as amended. The following functionalities have been reported: food colour, anti-caking agent, acidity regulator, gelling agent, thickener, bulking agent, hardening agent, glazing agent and release agent.

In accordance with Directive 94/36/EC, calcium carbonate is permitted *quantum satis* in all foodstuffs except for some foodstuffs in which the use of colours (in general) is specifically prohibited or restricted to food colours other than calcium carbonate.

In accordance with Directive 95/2/EC, calcium carbonate is generally permitted *quantum satis* in foodstuffs not referred to in Article 2 (3) of Directive 95/2, unless specifically provided for (see Table 2).

Table 2 summarises those beverages and foodstuffs that are permitted to contain calcium carbonate up to specified Maximum Permitted Levels (MPLs) set by Directive 94/36/EC and Directive 95/2/EC.

Table 2: Maximum Permitted Levels (MPLs) of use of calcium carbonate in beverages and foodstuffs according to European Parliament and Directives 94/36/EC and 95/2/EC and maximum reported use levels of calcium carbonate in beverages and foodstuffs used for the refined exposure assessment

Foodstuff Category	Legislation	Maximum Permitted Levels	Maximum Reported Use Level
Generally permitted in foodstuffs not referred to in Article 2 (3) of Directive 95/2	Directive 95/2/EC	<i>quantum satis</i>	See Annex B
Cocoa and chocolate products as defined in Directive 73/241/EEC ²⁰	Directive 95/2/EC	7 % on dry matter without fat expressed as potassium carbonates	
Grape juice as defined in Directive 93/77/EEC ²¹	Directive 95/2/EC	<i>quantum satis</i>	
Ripened cheese	Directive 95/2/EC	<i>quantum satis</i>	
Sliced and grated ripened cheese	Directive 95/2/EC	<i>quantum satis</i>	
Carriers or carrier solvents for food additives	Directive 95/2/EC		
Processed cereal based foods and baby foods	Directive 95/2/EC	<i>quantum satis</i> (only for pH adjustment)	See Annex B
Foodstuffs mentioned in Annex V Part 2 and in all other foodstuffs other than those listed in Annexes II and III of Directive 96/34/EC	Directive 96/34/EC	<i>quantum satis</i>	

Actual levels of use of Calcium Carbonate

In the framework of Regulation (EC) No 1333/2008²² on food additives and of Commission Regulation (EU) No 257/2010²³ regarding the re-evaluation of approved food additives, EFSA issued a public call for scientific data on calcium carbonate (E 170i)¹⁹ related to:

- The conditions of use (function, uses and use levels) other than as a food colour of calcium carbonate as a miscellaneous food additive.

²⁰ Council Directive 73/241/EEC of 24 July 1973 on the approximation of the laws of the Member States relating to cocoa and chocolate products intended for human consumption. OJ L 228, 16.8.1973, p 23.

²¹ Council Directive 93/77/EEC of 21 September 1993 relating to fruit and certain similar products. OJ L 244, 30.9.1993, p23.

²² Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p16-33.

²³ Commission Regulation (EU) No 257/2010 of 15 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

- The use and use levels of calcium carbonate as a nutrient source added for specific nutritional purposes in foods for particular nutritional uses (PARNUTs) and in food supplements.

Table 7 in Annex B summarises the information on current use levels of calcium carbonate made available to the Panel for several food categories following this call for data¹⁹.

2.7. Information on existing authorisations and evaluations

Calcium carbonate is authorised as a food colour in the EU under Directive 94/36/EC, and is also authorised as an additive generally permitted in foodstuffs under Directive 95/2/EC. It is also included in Directive 2001/15/EC²⁴ on substances that may be added for specific nutritional purposes in foods for particular nutritional uses, in Directive 2002/46/EC²⁵ relating to food supplements, and it can be used in fortified foods according to Regulation 1925/2006²⁶ on the addition of vitamins and minerals and of certain other substances in food. Calcium carbonate has been evaluated by JECFA in 1965 (JECFA, 1965, 1966), when the Committee established an Acceptable Daily Intake (ADI) not limited. The SCF evaluated calcium carbonate as part of a group of carbonates, and assigned a group ADI not specified (SCF, 1991). Calcium carbonate together with other carbonates has also been reviewed by TemaNord, who concluded that there was no need for a re-evaluation (TemaNord, 2002). The SCF allocated a Tolerable Upper Intake Level (UL) for calcium of 2500 mg/person/day as a nutrient, and also established a Population Reference Intake (PRI) of 700 mg calcium/day (range 400-1200 mg/day depending on age and physiological status) (SCF, 2003).

Calcium carbonate is included in Commission Decision 2006/257²⁷, establishing an inventory of ingredients in cosmetic products. In pharmaceuticals calcium carbonate is used as an excipient and as an active ingredient of antacids (Ph. Eur. Comment., 2009). A monograph on calcium carbonate is part of the European Pharmacopoeia (Ph. Eur., 2010). It is also included in Directive 91/414²⁸ concerning the placing of plant products on the market. Calcium carbonate has been registered under the REACH Regulation 1907/2006²⁹ (ECHA, 2011). EFSA have previously provided opinions on the calcium salt calcium citrate malate as a source for calcium for use in foods for particular nutritional uses and in foods for the general population (including food supplements) (EFSA, 2007) and on calcium sulphate as a source of calcium in food supplements (EFSA, 2008b). Calcium carbonate is included in the Food and Drug Administration (FDA) list of food additives that are GRAS for use in nutrient and dietary supplements, and is also certified by the FDA for use in amounts consistent with good manufacturing practice to colour drugs generally (FDA, 2006).

2.8. Exposure Assessment

The Panel agreed to follow the principles of the stepwise approach, which were used in the report of the Scientific Co-operation (SCOOP) Task 4.2, to estimate additives' intakes (EC, 1998). In the tiered approach, Tier 1 is based on theoretical food consumption data and MPLs for food additives as permitted by relevant Community legislation. The Second and Third Tiers refer to assessment at the

²⁴ Commission Directive 2001/15/EC of February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 52, 2.2.2001, p. 19.

²⁵ Commission Directive 2002/46/EC of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183 of 12.7.2002, p. 51.

²⁶ Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ L 404, 30.12.2006, p. 26-38.

²⁷ Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97, 5.4.2006, p. 1-528.

²⁸ Council Directive 91/414/EEC concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1-32.

²⁹ Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 396, 30.12.2006, p. 1-849.

level of individual Member States, combining national data on food consumption with the maximum permitted usage levels for the food additive (Tier 2) and with its actual usage patterns (Tier 3).

2.8.1. Crude estimates (Budget Method)

As calcium carbonate is permitted at *quantum satis* in all foodstuffs, the Panel could not estimate the dietary exposure using the Budget method (Tier 1).

2.8.2. Refined estimates

As with the exception of cocoa and cocoa products all applications are *quantum satis*, no Tier 2 calculations were performed.

Tier 3

Exposure estimates have been performed by the Panel based on the EFSA Comprehensive European Food Consumption Database (EFSA, 2011), which provides summary statistics for a number of European countries. For the purposes of this assessment, exposure estimates have been calculated based on data suitable for long-term (chronic) intake assessment for the population groups mentioned in Table 3:

Table 3. Population groups considered for the exposure estimates of calcium carbonate

Population	Age range	Countries with chronic food consumption statistics
Adults	from 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, UK
Adolescents	from 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Children	from 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Toddlers	from 12 up to and including 35 months of age	Belgium, Bulgaria, Finland, Germany, Italy, Netherlands, Spain

In order to refine the exposure assessment for children and adults to food additives, the Panel has defined some rules to identify maximum reported use levels based on maximum actual usage or maximum analytical data. The rules followed in order to deal with *quantum satis* authorisation (for use as colour), with use level data or observed analytical data, are given in Annex A.

As calcium carbonate is generally permitted at *quantum satis* in all foodstuffs, exposure estimates are based on food-categories for which usage information was received (see Table 7 in Annex B). A number of assumptions had to be made in assigning concentration values to food categories, which are detailed in Annex B.

Usage data in the following food categories (Table 4) were provided and were included in the exposure assessment taking into account *quantum satis* rules for colours (see Annex A) and assumptions detailed in Annex B.

Table 4. Concentration levels of calcium carbonate used for exposure estimates from its use as a food additive and added nutrient source (fortification)

Food category	Calcium carbonate as a food additive (mg/kg)	Calcium carbonate as added nutrient source (fortification) (mg/kg)
Grain milling products	6000 ¹	3650 ¹
Bread and rolls	3000 ¹	9800 ¹
Breakfast cereals	2250 ¹	13 500 ¹
Fine bakery wares	1970 ¹	1825 ¹
Cocoa beans and cocoa products	1250 ²	11 250 ¹
Fish products	2250 ¹	0
Chocolate (cocoa) products	1250 ²	11 250 ¹
Soft drinks	500 ³	0
Baking ingredients	12 000 ¹	0
Cereal-based food for infants and young children	100 ¹	1000 ⁴
Ready-to-eat meal for infants and young children	100 ¹	1000 ⁴
Ready to eat soups	500 ³	0
Salt	20 000 ¹	0
Food Supplements	0	800 mg calcium/day

¹ Based on usage data received from industry or Member States following the call for data (see Annex B for individual data contributions)

² According to Directive 95/2/EC and based on Directive 73/241/EEC (see Annex B for assumptions made)

³ According to *quantus satis* rules for food colours in Directive 96/34/EC

⁴ Use of calcium based on Directive 2006/15 (see Annex B for assumptions made)

Three exposure estimates based on the Comprehensive Food Consumption Database and usage data provided by industry and Member States have been calculated taking into account different sources of exposure. These were:

- Scenario 1 Exposure to calcium carbonate/calcium from the use of calcium carbonate as a food additive
- Scenario 2 Exposure to calcium carbonate/calcium from the use of calcium carbonate as a food additive and added nutrient source (fortification)
- Scenario 3 Exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (fortification) and from consumption of food supplements

Table 5 summarises the anticipated exposure to calcium from the use of calcium carbonate in the three scenarios mentioned above in the different population groups (adults, adolescents, children and toddlers). For full details of the estimates for scenarios 1-3 see Tables 8-10 in Annex C. In order to facilitate comparison with the UL for calcium established by the SCF in 2003, all exposure values calculated for calcium carbonate in scenarios 1-3 have also been converted to calcium.

None of these three scenarios, however, take into account intake of naturally occurring calcium. To provide information on calcium intake from all dietary sources, data from the literature on total

calcium intake available from various Member States and for different population groups has also been provided (see Table 6).

Table 5. Anticipated exposure to calcium from the use of calcium carbonate in the three scenarios in adult, adolescent, children and toddler populations.

	Daily intake of calcium (mg/day)							
	Adults (60 kg)		Adolescents (50 kg)		Children (30 kg)		Toddlers (15 kg)	
	Mean	95 th percentile	Mean	95 th percentile	Mean	95 th percentile	Mean	95 th percentile
Scenario 1	200-320	320-560	230-430	380-680	230-410	350-650	170-260	245-425
Scenario 2	440-740	620-1580	530-930	930-1580	470-980	650-1700	305-575	470-905
Scenario 3	1240-1540	1420-2380	1330-1730	1730-2380	1270-1780	1450-2500	1105-1375	1270-1705

Table 5 provides the ranges for mean and 95th percentile intake estimates for scenarios 1-3, calculated for adults, adolescents, children and toddlers, based on food consumption data contained in the comprehensive food consumption database provided by a number of different Member States (EFSA, 2011).

The estimated mean calcium intake from the use of calcium carbonate as food additive (scenario 1) ranges from 170-260 mg calcium/day for toddlers, 230-410 mg calcium/day for children, 230-430 mg calcium/day for adolescents and 200-320 mg calcium/day for adults. The ranges of high percentile intakes are 245-425 mg calcium/day for toddlers, 350-650 mg calcium/day for children, 380-680 mg calcium/day for adolescents and 320-560 mg calcium/day for adults.

The estimated mean calcium intake from use of calcium carbonate as food additive and added nutrient source (fortification) (scenario 2) ranges from 305-575 mg calcium/day for toddlers, 470-980 mg calcium/day for children, 530-930 mg calcium/day for adolescents and 440-740 mg calcium/day for adults. The ranges of high percentile intakes are 470-905 mg calcium/day for toddlers, 650-1700 mg calcium/day for children, 930-1580 mg calcium/day for adolescents and 620-1580 mg calcium/day for adults.

The estimated mean calcium intake from use of calcium carbonate as food additive and added nutrient source (fortification) and including all calcium intake from food supplements (scenario 3) ranges from 1105-1375 mg calcium/day for toddlers, 1270-1780 mg calcium/day for children, 1330-1730 mg calcium/day for adolescents and 1240-1540 mg calcium/day for adults. The ranges of high percentile intakes are 1270-1705 mg calcium/day for toddlers, 1450-2500 mg calcium/day for children, 1730-2380 mg calcium/day for adolescents and 1420-2380 mg calcium/day for adults.

As can be seen in Table 5, the use of calcium carbonate as added nutrient source (fortification) in addition to its use as food additive, doubles or in some cases triples intake estimates of calcium across all population groups (scenario 2 compared to scenario 1). This is expected, as reported use levels of calcium carbonate as added nutrient source are, for the majority of food categories, much higher than reported use levels for its use as additive (see Table 4). The intake of calcium-containing supplements (estimated at 800 mg calcium/day) contributes between 32-72% to the overall estimated intake of calcium from use as a food additive, nutrient source and intake from food supplements (scenario 3), whilst food additive only application (scenario 1) contributes between 15-30%.

2.8.3. Total estimated intake of calcium from all sources (food additive, fortification, supplement and natural sources) based on published dietary survey data

Further information on total estimated intake of calcium from all sources, including naturally occurring calcium, is available for a number of Member States, based on compositional information of foodstuffs consumed. For all countries, compositional nutrient data used to derive total calcium intake originate from analyses conducted in laboratories, supplemented by other sources. Other data sources include scientific publications, food composition tables, derived nutrients from comparable foods, calculations from recipes and estimations and information from manufacturers. Albeit not all encompassing, the so estimated calcium intake already takes into account use as a food additive and added nutrient source to a large degree. Table 6 provides a summary of these data, presenting the ranges of mean and 95th percentile intakes (for full details see Table 11, Annex D).

Information on major dietary contributors to calcium intake was available for some countries listed in Table 11. For Dutch children (Ocke et al, 2008), dairy products were the main sources of calcium (69%). For UK adults (Henderson et al, 2003), milk and milk products contributed 43% and cereal and cereal products contributed 30%. For Irish adults (IUNA, 2011), milk, yoghurt and cheese contributed 39%, followed by breads and breakfast cereals (25%). In France, (AFSSA, 2009), milk and dairy products contributed 43% to total calcium intake in adults and 49% in children and adolescents (3-17yrs), respectively. These findings suggest that dairy products are the single most important contributor to calcium intake from the diet.

Table 6. Anticipated exposure to calcium from all dietary sources, based on published dietary survey data

Daily intake of calcium (mg/day)							
Adults (60 kg)		Adolescents (50 kg)		Children (30 kg)		Toddlers (15 kg)	
Mean	95 th percentile	Mean	95 th percentile	Mean	95 th percentile	Mean	95 th percentile
730-1164	1233-2106	738-1070	1306-1905	749-869	1124-1453	664-795	1070-1184

Table 6 shows that mean calcium intakes (including from food additives, fortification and food supplements) in all population groups are of comparable size, ranging from 664 – 795 mg calcium/day for toddlers, 749 – 869 mg calcium/day for children, 738 – 1070 mg calcium/day for adolescents and 730 – 1164 mg calcium/day for adults. The range of high percentile intakes are 1070 – 1184 mg calcium/day for toddlers, 1124-1453 mg calcium/day for children, 1306 – 1905 for adolescents and 1233 – 1851 for adults. Mean intake for the elderly (>64 years old) ranges from 754 – 954 mg calcium/day and 1285 – 2106 mg calcium/day for high percentile consumers.

The range of mean calcium intakes from use as a food additive, added nutrient source (fortification) and intake of food supplements calculated for the different age groups in this opinion (Scenario 3) are somewhat higher than the values reported in Table 6. This is expected as exposure estimates calculated in this opinion are rather conservative, as they are based on maximum reported use levels and are assumed to always be present in all foods covered by the assessment, which in practice is unlikely.

2.8.4. Dietary exposure to aluminium

The Panel derived anticipated exposure to aluminium arising from the intake of calcium from all sources, as estimated by the Panel and presented in Table 6. Assuming that calcium carbonate contributes approximately 20% to the total calcium intakes provided in Table 6 (Panel estimate), and assuming a concentration of 200 mg aluminium/kg calcium carbonate, the ranges of the mean and 95th percentile of the aluminium intakes across the population groups are 0.07-0.09 and 0.11-0.16 mg/kg bw/day, respectively, which is equivalent to an aluminium mean intake of 0.49-0.63 mg/kg bw/week

and 95th percentile intake of 0.77-1.12 mg/kg bw/week. Hence, the aluminium intake contributions from the consumption of calcium carbonate to the TWI for aluminium of 1 mg/kg bw/week would be 49-63% at the mean and 77-112% at the 95th percentile.

3. BIOLOGICAL AND TOXICOLOGICAL DATA

No toxicological or biological information was submitted for the re-evaluation of calcium carbonate following a public call for data¹⁷, prior to the start of this re-evaluation. A literature search was conducted on the most commonly available online databases for toxicological and biological information (PubMed, Science Direct, Toxline and Web of Knowledge), to cover recent published literature on calcium carbonate. An additional source of information was the registration dossier provided by industry for calcium carbonate under the REACH Regulation 1907/2006, published by the European Chemicals Agency (ECHA, 2011) and unpublished study reports provided by CCA-Europe (2011).

The present opinion briefly summarises a number of the most relevant toxicological studies on calcium carbonate that were reviewed by the SCF in its 2003 opinion, establishing an UL for calcium (SCF, 2003). It describes in more detail any new toxicological data published since the SCF opinion (2003), or earlier published studies that were not included in that opinion, including new information available in the ECHA registration dossier (ECHA, 2011). It also provides a brief overview of the adverse effects of high calcium intakes in humans as described in the SCF opinion (2003) establishing a UL for calcium (SCF, 2003). Given that the opinion is directed towards an evaluation of the safety of calcium carbonate as a food additive and that the safety of calcium in terms of amounts that may be consumed is outside the remit of this Panel, further details of the human studies on which the UL is based are not included, and the SCF opinion should be consulted for this information. However, details of some new human studies published since the SCF review in 2003 that are considered relevant to the safety assessment of calcium carbonate as a food additive are included.

3.1. Absorption, distribution, metabolism, excretion

Calcium carbonate dissociates into its constituent ions in the acid milieu of the stomach. Some of the calcium is absorbed, via active transport or passive diffusion, the bulk (89-90%) of unabsorbed calcium is complexed to bile acids, free fatty acids and oxalic acid and excreted with the faeces (Heaney, 2002). As reported by EFSA, absorption of calcium from calcium carbonate in rats and humans is comparable to that from calcium citrate malate, average absorption of calcium from calcium carbonate over a range of studies being in the range of 20-40% (EFSA, 2007).

As reported by SCF (2003), after intestinal absorption, calcium and carbonate/bicarbonate ions can enter normal metabolic pathways and body pools. The majority of absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and also via faeces and skin) and excess carbonate is excreted as carbon dioxide via respiration (SCF, 2003).

Animal studies not included in the SCF opinion (2003)

Meiron and co-workers have examined the bioavailability of calcium from a stable amorphous calcium carbonate (ACC) (Meiron et al. 2011). ACC was prepared by a novel synthetic method using phosphoaminoacids to stabilise this form of calcium carbonate, as described by Bentov et al. (2010). The study compared the solubility and fractional absorption of ACC (particle diameter 40 to 120 nm), ACC with chitosan (ACC-C), and crystalline calcium carbonate (CCC) (particle diameter 1 to 10 µm). Solubility was evaluated by dissolving these preparations in dilute phosphoric acid. The results demonstrated that both ACC and ACC-C were more soluble than CCC, ACC being reported to be approximately 10 times more soluble than CCC (see also section 2.1). Fractional absorption was

evaluated by intrinsically labelling calcium carbonate preparations with ^{45}Ca , which were then orally administered (12.5 mg of CCC, 15 mg of ACC, or 18.5 mg of ACC-C) to rats (17 per group) using gelatin capsules. Fractional absorption was determined by evaluating the percentage of the administered radioactive dose per millilitre in the serum, calcium deposition in the femur, and whole-body retention over a 34-hour period. Calcium serum analysis revealed that calcium absorption from ACC and ACC-C preparations was up to 40% higher than from CCC, whereas retention of ACC and ACC-C was up to 26.5% higher than from CCC. Absorbed calcium in the femurs of rats given ACC was 30% higher than in CCC-treated animals, whereas 15% more calcium was absorbed following ACC-C treatment than following CCC treatment. The authors considered that their study demonstrated the enhanced solubility and bioavailability of ACC compared with CCC (Meiron et al. 2011). The Panel noted that the information provided on the particle size of the tested amorphous calcium carbonate indicates that this preparation contained particles with sizes within the nanoscale range.

The absorption of calcium from calcium carbonate in microparticulate form (particle diameter $3.77 \pm 0.76 \mu\text{m}$) or in nanoparticulate form (as described by the authors, particle diameter $0.15 \pm 0.02 \mu\text{m}$, prepared from the microparticulate form with the aid of a pulsed air-flow pulverizer) was compared in ovariectomised ICR mice (Huang et al., 2009). Female mice ($n=6$ per group) were given vitamin D₃ (261 U/kg bw) together with either micro calcium carbonate or nano calcium carbonate daily by gavage for 28 days at a dose of 1300 mg/kg bw. Two additional groups of mice were either ovariectomised or sham-ovariectomised, and received Vitamin D₃ but no calcium salt supplementation. Calcium absorption was measured by determination of serum calcium concentrations at 2, 6, 12 or 24 hours after the last calcium supplementation, and bone mineral density (BMD) of the whole body was determined by dual-energy x-ray absorptiometry (pDEXA). At 6 hours post-administration, increased serum calcium concentrations were observed in the nano calcium carbonate group when compared to the micro calcium carbonate group. The mean increase was small (9.1%), and no significant differences were observed at 2, 12 or 24 hours post-administration. The BMD of the whole body in ovariectomised mice was significantly lower than in sham-ovariectomised mice, and was greater in both the micro- and nano calcium carbonate-treated mice than in the controls. The BMD of the nano calcium carbonate-treated mice was significantly increased (by 7.1%) when compared to the micro calcium carbonate-treated mice (Huang et al., 2009). In view of the small increases in serum calcium observed in this study, the Panel considered that the absorption of calcium from nanoparticulate calcium carbonate would be not markedly different than that of microparticulate calcium carbonate.

Human studies not included in the SCF opinion (2003)

Absorption of calcium from radiolabelled (^{45}Ca) calcium carbonate or calcium citrate was compared in 37 healthy adult men and women given a single dose of either 300 mg (17 women only) or 1000 mg calcium (10 men and 10 women), ingested as part of a light breakfast meal (Heaney et al., 1999). The authors did not provide details on the form of calcium carbonate used. Absorption of calcium was measured following the 1000 mg calcium dose by tracer appearance in serum and by the absorptive increment in urinary calcium and at the 300 mg calcium dose by the tracer method only. Mean tracer absorption (for both salts combined) was 36.0% at the 300 mg calcium load and 28.4% at the 1000 mg calcium load. When the extent of absorption of the two salts of calcium was compared, the mean difference in absorption was small, calcium from calcium carbonate being significantly better absorbed than from calcium citrate at the 1000 mg calcium dose, while at the 300 mg calcium dose the reverse was reported. There was no significant difference between the men and the women tested in the 1000 mg calcium load experiment.

In contrast, in a study in 18 postmenopausal normal women given 500 mg calcium either as calcium carbonate or calcium citrate, the investigators found that calcium from calcium citrate was 2.5 fold more bioavailable than from calcium carbonate, based on measurement of AUC for serum calcium

(Heller et al, 1999). The authors did not provide details on the form of calcium carbonate used, other than it was a commercial calcium carbonate supplement.

In a study designed to evaluate the effect of calcium carbonate and sevelamer hydrochloride (SEV), a calcium-free phosphate binder, on serum calcium levels and urinary calcium excretion, twelve healthy male individuals were given calcium carbonate (500 mg, no details on the form of calcium carbonate used was indicated by the authors) or placebo daily for 6 days with 1-week washout between the treatment periods (Heinrich et al., 2008). SEV (800 mg) was administered in parallel to either calcium carbonate or placebo. During the study, weekly blood samples were taken and 24-hour urine was collected each day for measurement of calcium, magnesium, phosphorous, chloride and intact parathyroid hormone. Intake of calcium carbonate as compared to placebo was associated with an increase in the total amount of calcium absorbed and increased urinary calcium excretion in healthy individuals while serum calcium concentrations remained unaffected.

The calcium bioavailability of orally administered pearl powder (PP, a form of calcium carbonate prepared with a dry grinder) in nanonised form (NPP, pearl powder nanonised, particle diameter 0.04 to 0.4 μm) and micronised form (MPP, pearl powder micronised, particle diameter 4 to 300 μm) was compared in human subjects (Chen et al., 2008). A group of 28 adults (14 females, 14 males) free from hyperthyroidism, hypercalcemia and hypocalcemia were recruited as the subjects for the study. The bioavailability of NPP and MPP was measured by determination of serum calcium concentrations at 2, 4 and 6 hours after administration of either preparation. Serum intact parathyroid hormone (iPTH) was also measured at 0 and 4 hours after administration, and urinary creatinine was measured within 1 hour of administration, for determination of urinary calcium/creatinine ratio. At 2 hours post-administration, increased serum calcium concentrations were observed in the nano calcium carbonate (NPP) group when compared to the micro calcium carbonate (MPP) group. No significant differences were observed at 4 or 6 hours post-administration. The comparative bioavailability, $\text{AUC}_{0-6 \text{ hours}}$ for NPP was 38% greater than that for MPP, indicating better absorption of calcium from NPP than from MPP. According to the authors, the results showed better absorption and retention of calcium from NPP than from MPP, as reflected by a shorter elapsed time before the maximum concentration of calcium appeared in the serum and higher maximum calcium concentration (C_{max} at 2 hours post-administration) in serum after administration. iPTH was reduced after administration of pearl powder, the reduction being greater after administration of NPP than after MPP. However, no significant differences in calcium/creatinine ratio were observed between the NPP and MPP treatments. The authors concluded that nanonisation of pearl powder improves calcium bioavailability from this source in humans. Although a conventional form of calcium carbonate used was not directly tested in this study, the Panel noted that these results would support the data in ovariectomised mice from Huang et al. (2009) described above, which indicated a small increase in absorption of calcium from calcium carbonate in nanoparticles by comparison to the corresponding microparticles. However, the Panel noted that both studies were of short duration.

3.2. Toxicological data

3.2.1. Acute toxicity

The LD_{50} of calcium carbonate has been reported to be 6450 mg/kg bw, indicating that it is of low acute toxicity (Marhold, 1972).

In a study comparing the acute toxicity of nanoparticulate versus microparticulate calcium carbonate, groups of 8 male and 8 female ICR mice were administered a single dose of 1300 mg/kg bw of either the nano or the micro form by gavage, together with 261 U vitamin D3/kg bw, and observed for a 7 day period after dosing (Huang et al., 2009). No mortality or other treatment-related changes were observed in either group.

In a recent acute toxicity study, no treatment-related effects were seen in female Sprague-Dawley rats (n=5) administered a single oral dose of 2000 mg calcium carbonate/kg bw by gavage. The substance was described as calcium carbonate, with no further details on the form that was tested. The LD₅₀ of calcium carbonate was estimated to be > 2000 mg/kg bw (SafePharm Laboratories, 2009).

3.2.2. Short-term and subchronic toxicity

In a study designed to induce chronic hypercalcaemia, groups of ten male Sprague Dawley rats were given increased amounts of calcium carbonate in their diet (levels of 4% or 8% elemental calcium w/w, equivalent to approximately 5000 or 10 000 mg/kg bw/day calcium carbonate, compared with the normal dietary content of 0.71%) (Puerro Vicente et al., 1993). No details on the form of calcium carbonate used were indicated by the authors. Other groups of rats were given calcium chloride in drinking water (1.5% or 2%) or were given a single intramuscular injection of vitamin D₁ on the day on which calcium treatment commenced. One group received 4% elemental calcium in the diet together with 1.5% calcium chloride in drinking water. Groups of 10 rats were killed after 2 and 14 days of treatment respectively, and blood samples taken for determination of total calcium. Other than determination of total calcium in blood, the only parameters investigated in the study were food consumption and body weight gain. Increases in blood calcium were seen after 2 days of treatment with either 4% or 8% elemental calcium (from approximately 100 mg/l to approximately 130 mg/l for either treatment), showing that calcium from calcium carbonate and/or calcium chloride was bioavailable; levels dropped slightly after 14 days of treatment. A reduction in food consumption in the calcium-treated animals resulted in a reduction in body weight gain, manifest throughout the experimental period. No other treatment-related changes were reported by the authors.

The effects of a high calcium carbonate diet or a high calcium citrate diet in comparison to a control diet on mineral concentrations in several tissues of rats were examined (Takasugi et al., 2005). Male Wistar rats (n= 5 per group) aged 5 weeks were fed one of the experimental diets for 4 weeks. The control diet contained 0.5% calcium as calcium carbonate. The authors did not provide information on the form of calcium carbonate used. High calcium diets contained 2.5% calcium as calcium carbonate or calcium citrate (1250 mg calcium/kg bw/day, equivalent to 3125 mg/kg bw/day calcium carbonate). Feed intake was measured daily and body weight was measured weekly. Blood samples were collected from the abdominal aorta, and the testis, kidney, liver and femur were taken for analysis of calcium content. Calcium, phosphorus, magnesium, iron, copper and zinc concentrations in the tissues and plasma were determined using inductively coupled plasma emission spectrometry. Neither of the high calcium diets affected feed intake but body weight gain was significantly lower in both of the high calcium diet groups (approximately 15% lower, $p < 0.05$) than in the control group. Feed efficiency was also significantly lower in the high calcium groups than in the control group. The high calcium diets did not affect the calcium concentration in the plasma and tissues. Both of the high calcium diets produced a decrease in plasma phosphorus and also in iron levels in the testis, liver and femur. Hepatic copper increased in rats fed the high calcium diets whereas renal copper decreased. The high calcium carbonate diet increased femoral zinc and decreased femoral magnesium but in contrast the high calcium citrate diet did not affect these minerals in the femur. The authors considered that these effects might be due to different effects of the two calcium salts on intestinal pH, with calcium citrate causing a decrease in intestinal pH and thus enhancing the uptake of iron and other minerals. In contrast, as argued by the authors, calcium carbonate may increase the intestinal pH in rats (Koba et al., 2001), thereby reducing the availability of iron (Prather and Miller, 1992). Takasugi and co-workers, based on their results, suggest overall that the effect of excess calcium on minerals partly depends on its form (Takasugi et al., 2005).

As part of a series of studies designed to examine the bioavailability, acute and subacute toxicity of nanoparticulate (as described by the authors, 151 ± 19 nm) calcium carbonate and calcium citrate (396 ± 41 nm) ICR male and female mice (n=8 per group) were given 0, 13, 130 or 1300 mg/kg bw/day of nano calcium carbonate or calcium citrate orally by gavage for 28 days. The animals were monitored

for clinical signs of toxicity, body weights were recorded at the commencement of the study and at termination, and a macroscopic pathological examination was carried out. No treatment-related changes in any of these parameters were reported in any group (Huang et al., 2009).

A combined repeat dose oral toxicity/reproduction/developmental toxicity screening study with “nano” calcium carbonate was carried out in Wistar rats (HanTM:HsdRccHanTM:WIST strain, n= 10 animals/sex/group), in accordance with OECD Guideline 422 (Harlan Laboratories, 2010a). According to CCA-Europe the material tested had a particle size of 60 - 100 nm when examined by SEM, although particle size determination using other methods such as Sedigraph and Malvern showed higher apparent particle size due to aggregation (CCA-Europe, 2011). A particle size analysis submitted by CCA-Europe for the nano material using Sedigraph indicated a median particle diameter of $0.58 \pm 0.6 \mu\text{m}$. Animals were administered the nano calcium carbonate by gavage as a suspension in distilled water at dose levels of 100, 300 and 1000 mg/kg bw/day for up to 48 days, including a two week maturation phase, pairing, gestation and early lactation for females. The study was preceded by a 14-day preliminary toxicity study using levels of up to 1000 mg/kg bw/day nano calcium carbonate administered by gavage, in which no toxicologically significant treatment-related changes were seen. Males were killed on day 43 of the study, while females were killed on day 5 post partum. Full clinical, behavioural, biochemical, haematological gross pathological and histopathological examinations were carried out on 5 males and 5 females per group. One male treated with 1000 mg/kg bw/day was killed *in extremis* on day 39, due to misdosing. Otherwise, no treatment-related effects were seen on clinical behaviour and in the functional observation battery. Body weights and body weight gain, food and water consumption and food efficiency were comparable between control and treated groups throughout the study. Males treated with 1000 mg calcium carbonate/kg bw/day showed a statistically significant reduction in mean corpuscular haemoglobin (5.4% decrease) and mean corpuscular volume (5.8% decrease), but individual values were within the normal ranges for rats of the strain and age used and were considered by the study authors not to be of toxicological importance. All other haematological results were comparable in test animals and controls. Males treated with 1000 mg calcium carbonate/kg bw/day also showed a statistically significant reduction in total plasma protein (5.4% decrease) and a statistically significant increase in plasma chloride concentration (1.9% increase), while males from all treatment groups also showed statistically significant reductions in plasma phosphorus levels (from 1.74 mmol/l in controls to 1.28, 1.24 and 1.22 mmol/l respectively in the groups receiving 100, 300 or 1000 mg calcium carbonate/kg bw/day). Individual values for these parameters were however within the normal ranges for rats of the strain and age used, and were considered by the study authors not to be of toxicological importance. Similar changes were not reported in the female rats receiving nano calcium carbonate, other than a slight, not statistically significant decrease in plasma phosphorus levels in females receiving either 300 or 1000 mg calcium carbonate/kg bw/day. While isolated statistically significant differences were seen in organ weights (absolute and relative) in treated groups relative to controls, none of these were dose-related and they were considered by the study authors to be unrelated to treatment. No treatment-related macroscopic or microscopic abnormalities were reported. The authors concluded that the No-Observed-Adverse-Effect-Level (NOAEL) for systemic toxicity was 1000 mg calcium carbonate/kg bw/day, the highest dose tested. The Panel agreed with this NOAEL, considering the minor changes in haematological parameters in males treated with 1000 mg calcium carbonate/kg bw/day to be non-adverse. The results of the reproductive/developmental phase of this study are reported in Section 3.2.5.

As reported by EFSA, a 91-day feeding study (preceded by a 28-day study of similar design) in Sprague-Dawley rats compared the effects of calcium carbonate to those of calcium citrate malate (EFSA, 2007). The authors did not provide any details on the form of calcium carbonate used. One hundred (50 male and 50 female) rats divided into 5 treatment groups were fed a semi-purified diet. Three groups were fed calcium sources to deliver 0.5% calcium in the diet (equivalent to ~250 mg calcium/kg bw/day): a calcium carbonate group (group 1), a calcium carbonate plus citric and malic acids group (group 2) receiving the same levels of the individual acids as group 3, and a calcium

citrate malate group (group 3). Two more groups were fed calcium sources to deliver 1% calcium in the diet (equivalent to ~500 mg calcium/kg bw/day), a calcium carbonate plus calcium citrate malate group (group 4) and a calcium carbonate group (group 5). Parameters evaluated included body weight and food consumption, in-life and gross necropsy observations, clinical chemistry, haematology, and urinalysis, kidney calcium content; spleen and liver iron content, fat-free dry weight, ash, and magnesium and phosphorus content of the left femur; selected organ weights, and histology of kidneys, thyroid glands, parathyroid glands, and left tibia. Daily and weekly observations revealed no treatment-related effects. No statistically significant differences in body weight gain or feed efficiency were reported in rats of either sex supplemented with calcium as calcium carbonate, compared to those receiving calcium citrate malate. Male and female rats in group 4 and female rats in group 5 showed increases in feed consumption compared to group 1 animals (low calcium carbonate). These were attributed to differences in utilisation efficiency of the diets since increased feed consumption was not associated with an increase in body weight. In female rats supplemented with calcium carbonate plus calcium citrate malate phosphorus content of femurs was significantly higher. The histopathological evaluation of kidneys revealed nephrocalcinosis in all groups for both sexes. In males, this condition was less severe in groups 1, 2, and 3 (fed ~250 mg calcium/kg bw/day) compared to groups 4 and 5 (fed ~500 mg calcium/kg bw/day). Nephrocalcinosis was observed in all females except one animal in group 3 and was more severe than that of the males in the corresponding treatment groups (EFSA, 2007).

In a study designed to examine the effects of increasing calcium on apoptosis and cell proliferation in normal murine colonic crypt epithelium, 21-day old male mice (n= 10 per group) were fed either control diet or one supplemented with 1% calcium carbonate (equivalent to 1500 mg/kg bw/day) for 12 weeks (Penman et al., 2000). Initial and final body weights and body weight changes were similar between the two groups, and no signs of toxicity were noted in the calcium-supplemented group. Calcium treatment was associated with an increase in apoptotic index in the distal but not proximal colon. In the proximal colon of the calcium treated group there was also a minor but significant shift towards the crypt base as the mean position at which apoptotic cells were recorded (Penman et al., 2000).

As reported by EFSA (EFSA, 2007), a 91-day feeding study comparing the effects of calcium carbonate to those of calcium citrate malate was conducted in groups of five male and five female Beagle dogs using a similar experimental design as that for the rat study described above. No treatment-related effects of biological significance were observed in any of the groups. Notably, contrary to the findings in the rat studies, it was reported that there were no apparent treatment-related histopathological lesions (e.g. kidney) and that any pathology seen was either of low incidence or present across all groups equally (EFSA, 2007).

Adult cats and kittens were maintained on high calcium (17.7 mmol calcium/MJ) diets or normal calcium diets (9.5 mmol calcium/MJ) for up to 31 weeks, in a study designed to compare the efficacy of calcium carbonate and calcium chloride as sources of calcium (Pastoor et al., 1994). In both cats and kittens fed high calcium diets, urinary concentrations of magnesium and phosphorus and apparent absorption of these minerals were lower than after feeding the normal calcium diets. Urinary pH and phosphorus concentration were lower in cats and kittens fed diets with calcium chloride instead of calcium carbonate. Body weight gain and tibia growth in the kittens tended to be greater after feeding the diets with calcium chloride. Calcium chloride versus calcium carbonate and also supplemental calcium chloride in the high calcium diet significantly stimulated femur density and reduced renal calcium concentrations (Pastoor et al., 1994).

3.2.3. Genotoxicity

Nanoform calcium carbonate was tested in three in vitro assays according to OECD guidelines and in compliance with GLP (CCA-Europe, 2011). According to CCA-Europe the material tested had a

particle size of 60 - 100 nm when examined by SEM, although particle size determination using Sedigraph showed higher apparent particle size due to aggregation (CCA-Europe, 2011). This was the same material as was tested in the combined repeat dose oral toxicity/reproduction/developmental toxicity screening study described in Section 3.2.2. This nanoform calcium carbonate gave negative results in a bacterial reverse mutation assay according to OECD TG 471, in *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *E. coli* WP2 uvr A and using both the direct plate incorporation and pre-incubation methods (Harlan Laboratories, 2010b). In this study nano calcium carbonate was dissolved in dimethyl sulphoxide to give final test concentrations of 50, 150, 500, 1500 or 5000 µg/plate, with and without metabolic activation. No evidence of toxicity was seen at any of the concentrations tested, and there was no precipitation of test material when the direct plate incorporation method was used. However, when the pre-incubation method was used precipitation of test material was evident at both the 1500 and 5000 µg/plate test concentrations, in the absence and presence of S9-mix, respectively. There was no evidence of a treatment-related increase in revertants in any of the treatment groups (Harlan Laboratories, 2010b).

A negative result was also obtained for this nanoform calcium carbonate in a mammalian cell gene mutation assay (OECD TG 476) using mouse lymphoma L5178Y TK+/- cells (Harlan Laboratories, 2010c). In this study nano calcium carbonate was dissolved in dimethyl sulphoxide to give final test concentrations in the range of 7.81 to 250 µg/ml, and exposure periods of 4 hours with and without metabolic activation and 24 hours without metabolic activation were used. In a preliminary toxicity test there was no evidence of marked toxicity of the test substance and maximum test concentrations achievable were limited by precipitation of calcium carbonate, which was observed at and above 62.5 µg/ml at the 4 hour exposure period and above 15.6 µg/ml at the 24 hour exposure period. No evidence of a dose-related increase in mutant frequency was seen at any dose level in any of the 3 exposure groups (Harlan Laboratories, 2010c).

Nanoparticulate calcium carbonate (median particle diameter of 0.58 ± 0.6 µm) was not clastogenic in human lymphocytes in an *in vitro* mammalian chromosome aberration test conducted in accordance with OECD TG 473 (Harlan Laboratories, 2010d). Three treatment conditions were used in the study, namely a 4 hour exposure period in the absence of metabolic activation followed by a 20 hour expression period, a 4 hour exposure period in the presence of metabolic activation followed by a 20 hour expression period, and a 24 hour continuous exposure period in the absence of metabolic activation. Nano calcium carbonate was dissolved in dimethyl sulphoxide to give final test concentrations in the range of 31.25 to 1000 µg/ml for each of the three treatment conditions. Toxicity was not marked up to the highest concentration level of 1000 µg/ml, and there was no marked dose-related inhibition of mitotic index. Although precipitation of calcium carbonate was observed at concentrations of 125 µg/ml which persisted onto the slides at and above 500 µg/ml in the 4- and 20-hour group without S9 and at 1000 µg/ml in the 4- and 20-hour group with S9 and in the 24-hour group without S9, this did not impede the scoring of chromosome aberrations except at the highest test concentration of 1000 µg/ml calcium carbonate. There was no evidence of a dose-related increase in chromosome aberrations up to test concentrations of 500 µg/ml calcium carbonate (Harlan Laboratories, 2010d).

Overall, the Panel considered that based on these data there is no concern regarding the genotoxicity of calcium carbonate

3.2.4. Chronic toxicity and carcinogenicity

No chronic toxicity or carcinogenicity studies on calcium carbonate have been reported in the open literature or were provided to EFSA.

3.2.5. Reproductive and developmental toxicity

As reported in section 3.2.2, a combined repeat dose oral toxicity/reproduction/developmental toxicity screening study was carried out in Wistar rats ($n = 10/\text{sex}/\text{group}$), in accordance with OECD Guideline 422 (Harlan Laboratories, 2010a). Animals were administered nanoform calcium carbonate (characteristics as already described in Section 3.2.2) by gavage at dose levels of 100, 300 and 1000 mg/kg bw/day for up to 48 days, including a two week maturation phase, pairing, gestation and early lactation for females. Pregnant females were allowed to give birth and maintain their offspring until termination on day 5 post partum. Evaluations of each litter size, litter weight, mean offspring weight by sex, clinical observations and landmark developmental signs were also performed during the post partum period. At termination, all offspring, including those dying during the study, were subjected to a full external and internal examination and any macroscopic abnormalities were recorded.

No evidence of treatment-related effects was seen in the parental animals during the study, other than the minor haematological and biochemical changes in males receiving 1000 mg/kg bw/day, as already reported in section 3.2.2. No treatment-related effects were detected on the fertility or mating performance of animals receiving calcium carbonate when compared to controls. All treated animals mated within the first four days of pairing. No treatment-related effects were detected in the length of gestation for treated females when compared to controls. All animals showed gestation lengths between 22 to 23.5 days. All treated females gave birth to a live litter and successfully reared young to day 5 of age. Corpora lutea, implantation counts, litter sizes and pup viability was comparable in all treated groups and controls. There were no differences in litter weights or mean pup body weights between control and treated animals, and no clinical signs of toxicity were detected in pups from treated females when compared to controls. No treatment-related effects were detected for surface righting reflex for pups from treated animals when compared to controls. The incidence, type or distribution of macroscopic findings in pups dying in the post partum period or in pups killed at scheduled termination (day 5 of age) did not indicate any adverse effect of maternal treatment with calcium carbonate. The authors considered that no treatment-related effects were observed on reproduction in this study, and that the NOAEL for reproductive toxicity was 1000 mg/kg bw/day, the highest dose tested. The Panel agreed with this NOAEL.

Fairney and Weir maintained female rats throughout pregnancy on a high calcium diet (3% calcium as calcium carbonate in the chow (equivalent to 1500 mg calcium/kg bw/day and 3750 mg calcium carbonate/kg bw/day) and 4% calcium lactate added to the drinking water (equivalent to 882 mg calcium/kg bw/day and 4800 mg calcium lactate/kg bw/day). The number of dams was not given, but the growth rate of 41 offspring from the resulting litters from hypercalcaemic dams was compared with that from 38 offspring from dams maintained on a normal calcium content diet. The mean plasma calcium level in the high calcium group was $12.7 \pm 1.41/100 \text{ ml}$ in the 24 hours after parturition, compared with $10.1 \pm 0.59/100 \text{ ml}$ in the control group. The resulting litters were stated by the authors to be smaller in number (data not provided) and the offspring had lower body weights compared with controls. On macroscopic examination the offspring from hypercalcaemic dams showed alopecia, and the kidneys, liver and heart were paler in appearance compared with control offspring. On histopathological examination the kidneys from the former group showed pyelonephritic scarring, but no soft tissue calcification or nephrocalcinosis was seen on radiological examination (Fairney and Weir, 1970).

In a study designed to evaluate the developmental effects of increased dietary calcium in rats fed otherwise nutritionally adequate diets, female Charles River CD/VAF Plus were given dietary levels of 0.5% (control level), 0.75%, 1% or 1.25% calcium as calcium carbonate for 6 weeks before mating, during mating and up to Gestation Day (GD) 20 (Shackelford et al. 1993). These levels were equivalent to 375, 500 or 625 mg/kg bw/day calcium and 937.5, 1250 or 1562.5 mg/kg bw/day calcium carbonate. No dose related changes were observed in maternal clinical findings, average numbers of implantations, fetal resorptions and viable fetuses, nor on fetal length and weight. The

authors concluded that dietary calcium (given as calcium carbonate) was not fetotoxic or teratogenic at the concentrations used in the study. The Panel agreed with this conclusion and considered that 1562.5 mg/kg bw/day calcium carbonate, the highest dose tested, was a NOAEL for reproductive effects in this study. Dose-related increases of the femoral calcium content were however measured in both pregnant and non-pregnant rats receiving increased dietary calcium, and there were also increases in the phosphorus, magnesium and zinc content of the liver in non-pregnant control rats on the same diets (Shackelford et al., 1994). There were dose-related decreases of the iron and copper contents in kidneys of non-pregnant animals and of iron in the liver of pregnant rats. Fetuses showed dose-related decreases in the whole body content of phosphorus, magnesium, iron and copper. Changes in mineral levels in rats receiving increased dietary calcium were seen in all dose groups compared with controls, although not all minerals in the organs examined were equally affected (Shackelford et al., 1994).

Richards and Greig evaluated the effect of dietary calcium carbonate (0.3%, 0.5%, 0.7% and 1.1%, equivalent to approximately 450, 750, 1050 or 1650 mg calcium carbonate/kg bw/day) on reproductive performance in Swiss mice and on survival and organ pathology of the litters (Richards and Greig, 1952). Mice were given the experimental diets one week before mating and up to GD 21, when the offspring were killed. All diets with a calcium carbonate content of 1.1% resulted in a decreased number of litters and decreased total litter weight. Litters at this dietary level showed hypertrophy of the heart and atrophy of the thymus when killed at age 21 days. Increased heart weights were negatively correlated with haemoglobin levels. Addition of iron to high-calcium diets reduced the degree of cardiac hypertrophy, and the authors concluded that the effects seen were due to a calcium:iron interaction, with consequential reductions in maternal iron levels and anaemia. However, the authors did not provide any information on maternal toxicity in the study, other than an observation of increased heart weight in the mothers and maternal anaemia.

The effect of diet-induced maternal hypercalcaemia on fetal development was investigated in the CD-1 mouse (Liebrott et al., 1989). The diet for the control group contained 1.2 % calcium, while that for the experimental group contained 3% calcium as calcium carbonate in the chow (1500 mg calcium/kg bw/day, equivalent to 3750 mg/kg bw/day calcium carbonate) and 4% calcium lactate added to the drinking water. The experimental group was maintained on the high calcium diet for at least 10 days before mating, and during pregnancy until GD-18. The number of litters in both the control and experimental group was identical (n=13). However, fetuses from the treated litters showed significantly lower birth weights and higher frequencies of missing calcification centres in the developing skeletons and dentitions. The authors concluded that high maternal calcium levels can result in fetotoxic effects (Liebrott et al., 1989).

3.2.6. Allergenicity, sensitivity and intolerance

No data are available indicating that calcium carbonate has allergenic properties or can invoke sensitivity or intolerance reactions in exposed individuals.

3.2.7. Other studies

3.2.7.1. Effects of calcium carbonate and other sources of calcium in humans

A number of studies on the effects of calcium carbonate in humans have been published, as summarised by the SCF in their opinion establishing an UL for calcium (SCF, 2003). In summary, calcium carbonate like other calcium salts can cause constipation if taken in large amounts as an antacid (Martindale, 2002). Hypercalcaemia and alkalosis can occur in individuals taking calcium carbonate (between 2.0 and 16.5 g/day of supplementary calcium) with large amounts of milk or cream for the treatment of peptic ulcer (milk-alkali syndrome), often associated with renal dysfunction, metastatic calcification and other symptomology in the absence of hypercalciuria (Orwoll, 1982; Abreo et al., 1993, as reported in SCF, 2003). Acute hypercalcaemia and recurrent

nephrolithiasis were reported in three subjects regularly consuming large quantities (7 to 15 g daily) of calcium carbonate-sodium bicarbonate powders over a period of 10 years (Robson and Heading, 1978).

Bolland and co-workers have assessed the risk of myocardial infarction and cardiovascular events in individuals given regular calcium supplementation (without co-administered vitamin D) in the management of osteoporosis in a recent study, using a meta-analysis of 15 randomised clinical trials (Bolland et al., 2010). Only 15 of 190 trials originally identified met the pre-specified inclusion (randomised, double blind, placebo controlled trials; elemental calcium was administered at a dose of ≥ 500 mg/day; the participants' mean age at baseline was more than 40 years old; 100 or more participants were randomised; participants of either sex were studied; and the trial duration was more than one year) and exclusion (calcium and vitamin D given together with a placebo comparator; trials in which calcium was administered in the form of dietary modification or a complex nutritional supplement; and trials in which most participants had a major systemic disease other than osteoporosis) criteria. The calcium dose was generally greater than 1000 mg/person (2 studies used doses of 500 and 750 mg/person respectively and one had two dose levels of 600 and 1200 mg/person) whilst dietary calcium ranged from 406 to 1240 mg/day (generally below 1000 mg/day). Five of these trials used patient level data from 8151 participants and eleven used trial level data, involving 11 921 participants with a mean duration of 4 years. In the five studies contributing patient level data, 143 people given calcium supplementation had a myocardial infarction compared with 111 given placebo. These data provided a hazard ratio (HR) of 1.31 (95% confidence interval 1.02 to 1.67, $P=0.035$). Non-significant increases occurred in the incidence of stroke (HR 1.20, 0.96 to 1.50, $P=0.11$), the composite end point of myocardial infarction, stroke, or sudden death (HR 1.18, 1.00 to 1.39, $P=0.057$), and death (HR 1.09, 0.96 to 1.23, $P=0.18$). The meta-analysis of trial level data showed similar results: 296 people had a myocardial infarction (166 in individuals provided with calcium supplementation compared with 130 in individuals given placebo), with an increased incidence of myocardial infarction in the former group (pooled relative risk 1.27, 95% confidence interval 1.01 to 1.59, $P=0.038$). Pre-specified subgroup analyses showed a significant interaction between treatment allocation and dietary calcium intake for myocardial infarction. There was an increased risk of myocardial infarction in people with dietary calcium intake above the median of 805 mg/day (hazard ratio 1.85, 95% confidence interval 1.28 to 2.67) but no increased risk in those with dietary calcium intake below the median (0.98, 0.69 to 1.38, P for interaction 0.01). The increased risk of myocardial infarction reported by Bolland and co-workers occurred early after calcium supplementation (median follow-up of 3.6 years). The authors concluded that calcium supplementation (without co-administered vitamin D) is associated with an increased risk of myocardial infarction, and noted that given the wide use of calcium supplements by the population, the small increases in risk of cardiovascular disease could translate into a large burden of disease in the population (Bolland et al., 2010).

The authors noted that, in contrast to their findings on supplemental calcium, observational studies do not show increased cardiovascular risks with higher dietary calcium intake (Bolland et al., 2010). Although not part of the meta-analysis, the authors have previously reviewed the mechanisms by which calcium supplements might increase the risk of myocardial infarction (Reid et al., 2010). As argued by the authors, calcium supplements acutely increase serum calcium levels to a modest degree, and serum calcium levels have been positively associated with an increased incidence of myocardial infarction in large observational studies. Primary hyperparathyroidism, a condition in which serum calcium levels are raised, has also been associated with an increased risk of cardiovascular events and death. Ingestion of equivalent doses of calcium from dairy products has a much smaller effect than calcium supplements on serum calcium levels, which might account for the absence of a detrimental vascular effect of dietary calcium intake in the observational studies reviewed.

In a recent update of this study Bolland et al. reanalysed the data used in their earlier studies and unpublished data from two other studies, with the objective of investigating the effect of personal

calcium supplement use on cardiovascular risk (Bolland et al., 2011). The authors had hypothesised that the personal use of calcium supplements by women in the trials evaluated in their earlier study might have obscured an adverse effect of calcium and vitamin D on cardiovascular risk. They concluded from this most recent meta-analysis that women who were given regular calcium supplementation (with or without co-administered vitamin D) but who were not taking personal calcium supplements were still at increased risk of cardiovascular event, confirming their basic premise that calcium supplements used with or without vitamin D modestly increase cardiovascular risk (Bolland et al., 2011).

A 5-year, randomised double-blinded placebo controlled trial (Calcium Intake Fracture Outcome Study, CAIFOS) showed no risk of atherosclerotic vascular disease (Lewis et al., 2010). The participants were 1460 women aged 75.1 +/- 2.7 years at baseline (1998), recruited from the general population and randomised to receive 1200 mg of calcium carbonate daily or an identical placebo. All hospital admission and deaths during the 5-year study and the 4.5-year follow-up were derived from the Western Australian Data Linkage Service (WADLS). Hazard ratios for the combined endpoint of atherosclerotic vascular mortality or first-hospitalisation were calculated using pre-specified intention-to-treat and per-protocol models. The intervention group that received calcium supplementation did not have a higher risk of death or first-time hospitalisation from atherosclerotic vascular disease in either the 5-year study (multivariate-adjusted HR 0.938 95% CI 0.690-1.275) or during the total 9.5 years of observational study (multivariate-adjusted HR 0.919 95% CI 0.737-1.146). Further analysis suggested calcium supplementation may reduce the risk of hospitalisation and mortality in patients with pre-existing atherosclerotic cardiovascular disease. The authors concluded that their study provided strong evidence that calcium supplementation of 1200 mg daily does not significantly increase the risk of atherosclerotic vascular disease in elderly women.

The Panel noted that although the meta-analysis carried out by Bolland et al. (2010) was a large and robust study with specified inclusion and exclusion criteria, the trends reported were very modest following supplementation with calcium in addition to dietary exposure, and are not supported by the findings of Lewis et al. (2010). The Panel also noted that the trends noted in cardiovascular risks following calcium supplementation contrasted with those found with dietary calcium, and that the authors considered that a plausible mode of action could be described to explain these differences, based on serum levels of calcium following dietary intake and supplementation.

3.2.7.2. Biochemical changes in the colon of rats following administration of calcium carbonate

Awad and co-workers examined biochemical changes in the apical membranes of the colon as a result of feeding excess calcium to rats maintained on a saturated fatty acid-rich diet. Weanling rats (n=12 per group) were fed a semi-synthetic diet (high fat content plus basal calcium content of 0.5%) and excess calcium (1% calcium carbonate, equivalent to 1000 mg/kg bw/day) for 4 weeks. Animals fed the high calcium diet weighed significantly more than the controls after 4 weeks of feeding. By the end of the 4th week on the diet, significant increases in serum calcium were observed, but there were no clinical signs of toxicity. Excess dietary calcium intake resulted in a 54% reduction in faecal water bile acids and a 44% reduction in faecal water free fatty acids. The calcium-supplemented diet had no effect on either the lipid content or the fatty acid composition of the colonic apical membranes, but protein composition was altered. The authors suggested that the observed alterations in protein patterns of these membranes may be due to either the reduction of faecal water bile acids and free fatty acids or may be a direct effect of dietary calcium on membrane protein (Awad et al., 1990).

4. DISCUSSION

The Panel was not provided with a newly submitted dossier on calcium carbonate and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Calcium carbonate is a food additive generally permitted in foodstuffs under Directive 95/2/EC on food additives and is an authorised food colouring substance under Directive 94/36/EC on colours for use in foodstuffs. It is also included in Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutritional uses, in Directive 2002/46/EC relating to food supplements and it can be used in fortified foods according to Regulation 1925/2006 on the addition of vitamins and minerals and of certain other substances in food.

Calcium carbonate was previously evaluated by JECFA in 1965 (JECFA, 1965, 1966), when the Committee established an ADI not limited. The SCF evaluated calcium carbonate in 1990 as part of a group of carbonates, when the Committee assigned a group ADI not specified (SCF, 1991). A review of carbonates as food additives has also been carried out by TemaNord (TemaNord, 2002). The SCF concluded that both calcium and carbonate were natural constituents of man, animals and plants, and occur naturally in foodstuffs. The Committee noted that these cations and anions constitute the major electrolytes present in all biological materials and, while noting that exhaustive systematic toxicological studies had not been carried out with the individual ions (or their salts), they concluded that *“no safety problems are likely to arise from their use in food, provided the contributions from food intake do not disturb the homeostatic mechanisms controlling the electrolyte balance of the body.”* (SCF, 1991). The SCF based their ADI not specified for carbonates on these considerations. The SCF have also allocated an UL for calcium, from the diet and from supplements, of 2500 mg/person/day as a nutrient (SCF, 2003).

Specifications for calcium carbonate have been defined in Commission Directive 2008/128/EC and by JECFA (JECFA, 2006). The purity is specified as not less than 98% calcium carbonate. The Panel noted that the JECFA specification for lead in calcium carbonate is ≤ 3 mg/kg whereas the EC specification is ≤ 10 mg/kg. The Panel also noted that calcium carbonate can have an amorphous or microcrystalline structure, and that particle size varies according to the form and manufacturing conditions. Particles of amorphous calcium carbonate are reported to be characteristically 40 to 120 nm diameter spherules, in contrast to the 1 to 10 μm diameter crystals typical of the crystalline forms (Meiron et al., 2011). Nanoform calcium carbonate (as used in some of the toxicological studies reported in this opinion) was reported to have a particle size of 60 - 100 nm when examined by SEM, although particle size determination using Sedigraph showed higher apparent particle size due to aggregation (CCA-Europe, 2011). The typical average particle size (d50%) of food grade calcium carbonate is stated by CCA-Europe to be about 5 μm , with an upper range (d98%) of 65 μm and less than 1% of particles having a diameter below 100 nm; the presence of unintentional nanoscale particles at trace levels in the product cannot be excluded (CCA-Europe, 2011).

Calcium carbonate dissociates into its constituent ions in the acid milieu of the stomach. Some of the calcium is absorbed, via active transport or passive diffusion (SCF, 2003), the bulk of unabsorbed calcium is complexed to bile acids, free fatty acids and oxalic acid and excreted with the faeces (Heaney, 2002). Average absorption of calcium from calcium carbonate over a range of studies has been shown to be in the range of 20-40% (EFSA, 2007). A recent 28-day oral feeding study in mice has shown that calcium from nanoparticulate calcium carbonate is 9.1% more bioavailable (mean of 9.1% higher plasma levels) than from the microparticulate form, as evidenced by significantly higher serum calcium concentrations in the nano calcium carbonate group than in the micro calcium carbonate group. The bone mineral density in the nano calcium carbonate-treated mice was increased by 7.1% when compared to the micro calcium carbonate-treated mice (Huang et al., 2009). A slight increase in calcium bioavailability (by 38%) from nanonised pearl powder form by comparison with the corresponding micronised form of calcium carbonate was also observed in humans (Chen et al., 2008).

In view of the small increases in calcium absorption observed in these studies, the Panel considered that the absorption of calcium from calcium carbonate in nanoparticles would be not markedly different than that from calcium carbonate microparticles.

After intestinal absorption, calcium and carbonate/bicarbonate ions enter normal metabolic pathways and body pools. The majority of absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and also faeces and skin) and excess carbonate is excreted as carbon dioxide via respiration (SCF, 2003).

A number of short term studies (generally less than 90 days) have been carried out with calcium carbonate in rats and mice, to investigate the effects of high calcium diets on calcium levels in the body (Puerro-Vicente et al., 1993) and on homeostasis of other minerals (Takasugi et al., 2005). Effects of high calcium on membrane biochemistry in the colon (Awad et al., 1990) or effects on apoptosis and cell proliferation in the colon (Penman et al., 2000) have also been investigated. These studies have also assessed the toxicity of high levels of calcium carbonate in the diet, as manifest by effects on growth and food consumption, and have overall not demonstrated any evidence of toxicity attributable to calcium carbonate. Similarly, a 31-week study in cats and kittens, comparing the effects of high calcium carbonate and high calcium chloride diets revealed changes in homeostasis of calcium and other minerals in the body, but no overt toxicity (Pastoor et al., 1994). The Panel considered that the investigations carried out in these studies were of limited value in assessing the safety of calcium carbonate.

The only notable treatment-related effect seen in a 91-day feeding study designed to compare the effects of calcium carbonate to those of calcium citrate malate in rats at dietary calcium levels equivalent to 250 or 500 mg calcium/kg bw/day was nephrocalcinosis, seen in both males and (more marked) in females (EFSA, 2007). These dietary doses correspond to approximately 625 or 1250 mg calcium carbonate/kg bw/day. The Panel considered however that the nephrocalcinosis finding in rats is not relevant for human safety assessment because the rat is a species known to be particularly sensitive to mineralisation of the renal tubule epithelium due to dietary alteration of the calcium and phosphorus homeostasis (Ritskes-Hoitinga et al., 1989, 1991, 1992). Results from a 91-day toxicity study in Beagle dogs designed to compare the effects of calcium carbonate to those of calcium citrate malate at equivalent calcium levels in the diet as in the rat study reported above (providing intakes of approximately 250 and 500 mg calcium/kg bw/day) did not report any signs of nephrocalcinosis or other evidence of toxicity (EFSA, 2007).

Nephrocalcinosis was also not observed in a recent combined repeat dose oral toxicity/reproduction/developmental toxicity screening study with nanoform calcium carbonate (having a particle size of 60 - 100 nm when examined by SEM), carried out in Wistar rats at dose levels of up to 1000 mg/kg bw/day (Harlan Laboratories, 2010a). The only changes seen in this study, in which dosing was continued for up to 48 days, were slight but statistically significant haematological and biochemical effects in males receiving 1000 mg/kg bw/day, and significant reductions in plasma phosphate levels in all male treated groups. The Panel considered that these changes were non-adverse and agreed with the NOAEL of 1000 mg/kg bw/day identified by the authors of the study, the highest dose tested.

No evidence of toxicity was reported in a study in which mice were administered calcium carbonate (described by the authors as nano calcium carbonate) by oral gavage at dose levels up to 1300 mg/kg bw/day for 28 days (Huang et al., 2009). The Panel noted however that only limited investigations were carried out in this study.

Calcium carbonate (with a particle size of 60 - 100 nm when examined by SEM) has given negative results in a range of *in vitro* genotoxicity assays. No data are available on the chronic toxicity or carcinogenicity of calcium carbonate. The Panel considered however that it is unlikely that calcium carbonate has carcinogenic potential, given that both calcium and carbonate are natural constituents of the body and normal metabolites of man, animals and plants, and have a long history of safe use as a source of calcium supplementation for humans.

Several reproductive and developmental toxicity studies in rats and mice have been carried out with calcium carbonate. In rats fed diets containing calcium carbonate at levels up to approximately 1500 mg/kg bw/day, no dose-related changes indicative of developmental toxicity were reported (Shackelford et al., 1993). The Panel considered that a NOAEL of 1500 mg/kg bw/day could be identified for reproductive effects of calcium carbonate in this study. However, there were dose-related increases of the femoral calcium content and decreases in tissue phosphorus, magnesium, iron and copper in both dams and fetuses (Shackelford et al., 1994). The Panel also noted the results of a recent OECD guideline-compliant combined repeat dose oral toxicity/reproduction/developmental toxicity screening study carried out with calcium carbonate (having a particle size of 60 - 100 nm when examined by SEM) in Wistar rats at dose levels up to 1000 mg/kg bw/day in which no effects on reproduction, including developmental toxicity, were reported (Harlan Laboratories, 2010a). Other studies have shown evidence of fetotoxicity of calcium (as calcium carbonate) when administered during pregnancy at levels in the diet equivalent to 3750 mg/kg bw/day calcium carbonate, together with additional calcium intake from calcium lactate in drinking water (Fairney and Weir, 1970; Liebgott et al., 1989). In the study of Richards and Greig in mice reported in 1952, diets with a calcium carbonate content of 1.1% (1650 mg calcium carbonate/kg bw/day) resulted in a decreased number and total weight of litters and in cardiac hypertrophy and thymic atrophy in the offspring when killed at age 21 days. Overall, the Panel noted that in rodents high doses of calcium carbonate (> 1500 mg/kg bw/day) causing hypercalcaemia during gestation can result in adverse effects on reproduction, fetotoxicity and elemental imbalances in the offspring. However a recent repeat dose oral toxicity/reproduction study provided a NOAEL of 1000 mg/kg bw/day, the highest dose tested, for developmental toxicity and the Panel considered overall that there is no concern for the reproductive effects of calcium carbonate at intakes below 1500 mg/kg bw/day.

No data are available indicating that calcium carbonate has allergenic properties or can invoke sensitivity or intolerance reactions in exposed individuals.

The Panel noted that hypercalcaemia and alkalosis can occur in humans taking calcium carbonate with large amounts of milk or cream for the treatment of peptic ulcer (milk-alkali syndrome), often associated with renal dysfunction, metastatic calcification and other symptoms. Similar changes have been reported in individuals taking large amounts of calcium carbonate and other calcium-containing antacids or large amounts of calcium food supplements. The SCF (2003) reported that one third of the cases had consumed both alkali and calcium (between 2.0 and 16.5 g/day of supplementary calcium), while one third developed symptoms as a result of high calcium carbonate intakes alone (between 2 to 10.8 g additional calcium per day from several months to 30 years).

The Panel additionally noted that while the meta-analysis carried out by Bolland and co-workers (Bolland et al., 2010, 2011), showing an increased risk of myocardial infarction in individuals given regular calcium supplementation in the management of osteoporosis, was a very large and robust study, the trends reported were very modest, and are not supported by the findings in a similar study carried out by Lewis et al. (2010). The Panel also noted that the trends in cardiovascular risks following calcium supplementation contrasted with those found with dietary calcium, and that the authors considered that a plausible mode of action could be identified to explain these differences, based on serum levels of calcium following dietary intake and supplementation (Bolland et al., 2010, 2011).

Overall the Panel considered that the available toxicological database on calcium carbonate is limited, but it does not give rise to concern. The Panel considered that further toxicological studies on calcium carbonate are not necessary for the reasons already stated by the SCF in 1991, namely that calcium carbonate is a natural constituent of man, animals and plants, and therefore occurs naturally in foodstuffs. Calcium and carbonate/bicarbonate also constitute some of the major electrolytes present in all biological materials. The few effects seen in studies in humans and animals are associated with high calcium carbonate intakes (2 – 16 g/person/day in humans and > 1500 mg/kg

bw/day in animals), and are also found after large intakes of other calcium salts. The Panel considered that these high-calcium intake effects are not relevant to the evaluation of calcium carbonate as a food additive, and that the SCF established an UL for calcium taking these effects into account (SCF, 2003).

The Panel therefore agrees with the group ADI “not specified” established by the SCF for a group of carbonates including calcium carbonate (SCF, 1991), and considers that the definition of ADI “not specified”³⁰ is applicable to calcium carbonate when used as a food additive.

The Panel considered however that intakes of calcium resulting from use of calcium carbonate as a food additive, taken together with intakes of calcium from supplements and food fortification, should be below the UL of 2500 mg/day for calcium from all food sources established by the SCF (SCF, 2003).

In assessing exposure to calcium carbonate as a food additive, since it is permitted at *quantum satis* in all foodstuffs, the Panel could not estimate the dietary exposure using the Budget method (Tier 1). No Tier 2 calculations were performed, as with the exception of cocoa and cocoa products all applications are *quantum satis*. Refined exposure estimates have thus been performed only for Tier 3 using the maximum reported use levels presented in Annex B, for adult adolescent, children and toddler populations.

The Panel has derived estimates for three exposure scenarios taking into account different sources of exposure, namely Scenario 1: exposure to calcium carbonate/calcium from use of calcium carbonate as a food additive, Scenario 2: exposure to calcium carbonate/calcium from use of calcium carbonate as a food additive and added nutrient source (fortification), and Scenario 3: exposure to calcium from use of calcium carbonate as a food additive and added nutrient source (fortification) and from consumption of food supplements. In addition, the Panel has estimated exposure to calcium from all sources based on published data from dietary surveys presented in the literature for six Member States.

Anticipated exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (including food supplements) in adult, adolescent, children and toddler populations were calculated by the Panel (Table 5).

Based on data made available to the Panel by industry and Member States, the Panel estimated mean calcium intake from use of calcium carbonate as food additive and added nutrient source (fortification) and including all calcium intake from food supplements (scenario 3) as ranging from 1105-1375 mg calcium/day for toddlers, 1270-1780 mg calcium/day for children, 1330-1730 mg calcium/day for adolescents and 1240-1540 mg calcium/day for adults. The ranges of high percentile intakes are estimated at 1270-1705 mg calcium/day for toddlers, 1450-2500 mg calcium/day for children, 1730-2380 mg calcium/day for adolescents and 1420-2380 mg calcium/day for adults.

³⁰ JECFA definition of “ADI not specified: “A term applicable to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e., it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance”. Available at <http://www.who.int/foodsafety/chem/jecfa/glossary.pdf>

The Panel noted that the use of calcium carbonate as added nutrient source (fortification) in addition to its use as food additive, doubles or in some cases trebles intake estimates of calcium across all population groups (scenario 2 compared to scenario 1). This is expected, as reported use levels of calcium carbonate as added nutrient source are, for the majority of food categories, much higher than reported use levels for its use as additive (Table 4). The intake of calcium-containing supplements (estimated at 800 mg calcium/day) contributes between 32-72% to the overall estimated intake of calcium from use as a food additive, nutrient source and intake from food supplements (scenario 3), whilst additive only application (scenario 1) contributes between 15-30%.

Estimated exposure to calcium from all sources based on data presented in the literature for six Member States (Table 6) showed that mean calcium intakes (including food supplements) in all population groups were of comparable size, ranging from 664 – 795 mg calcium/day for toddlers, 749 – 869 mg calcium/day for children, 738 – 1070 mg calcium/day for adolescents and 730 – 1164 mg calcium/day for adults. The range of high percentile intakes were 1070 – 1184 mg calcium/day for toddlers, 1124-1453 mg calcium/day for children, 1306 – 1905 mg calcium/day for adolescents and 1233 – 1851 mg calcium/day for adults. Mean intake for the elderly (>64 years old) ranged from 754 – 954 mg calcium/day and 1285 – 2106 mg calcium/day for high percentile consumers.

The range of mean calcium intakes from use as a food additive and added nutrient source (fortification) calculated for the different age groups in this opinion (Scenario 3, Table 5) are somewhat higher than the values provided for the estimated exposure to calcium from all sources based on data presented in the literature for six Member States (Table 6). This is expected as exposure estimates calculated in this opinion are rather conservative, as they are based on maximum reported use levels and are assumed to always be present in all foods covered by the assessment, which in practice is unlikely.

The Panel noted that both the intake estimates calculated by the Panel, which are considered to be conservative, and the total dietary intake of calcium reported in literature are all below the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003. Therefore, the Panel concluded that, based on the available data described in this opinion, the use of calcium carbonate as a food additive at the current maximum reported use levels is not of safety concern.

The Panel noted that a range of studies have been performed with calcium carbonate described as nanoform (having a particle size of 60 - 100 nm when examined by SEM), however none of these provided comprehensive data on characterisation of the nano material. Nor do these studies provide a full toxicological database in line with the guidance provided by EFSA Scientific Committee Opinion on Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain (EFSA, 2011). According to the information provided by the industry the presence of unintentional nanoscale particles at trace levels in calcium carbonate when used as a food additive cannot be excluded (CCA-Europe, 2011). Whilst the data are inadequate to reach definitive conclusions on calcium carbonate predominantly in the nanoscale, the Panel considered that the available data are sufficient to conclude that the current levels of adventitious nanoscale material within microscale calcium carbonate would not be an additional toxicological concern.

The Panel additionally noted that limestone may contain variable amounts of aluminium, reported concentrations ranging between 1 and 134 mg/kg (EPA, 1995), and that analysis of food grade calcium carbonate has shown aluminium concentrations from 10 to 190 mg/kg (Chemische Fabrik Budenheim KG, 2011). Assuming that calcium carbonate contributes approximately 20% to the total calcium intake shown in Table 6 (Panel estimate), and assuming a concentration of 200 mg aluminium/kg calcium carbonate, the Panel estimated a mean intake for aluminium from calcium carbonate of 0.49-0.63 mg/kg bw/week and 95th percentile intake of 0.77-1.12 mg/kg bw/week. Hence, the aluminium intake contributions from the consumption of calcium carbonate to the TWI for aluminium of 1mg/kg bw/week would be 49-63% at the mean and 77-112% at the 95th percentile.

CONCLUSIONS

Calcium carbonate is an inorganic salt authorised as a food additive and food colour in the EU and previously evaluated by JECFA in 1965, when the Committee established an ADI not limited. The SCF evaluated calcium carbonate in 1990 as part of a group of carbonates, when the Committee also assigned a group ADI not specified.

The Panel agrees with the group ADI “not specified” assigned by the SCF when considering the use of calcium carbonate as a food additive and food colour. The Panel concurs with the JECFA definition of “ADI not specified” and considers it applicable to calcium carbonate when used as a food additive.

The Panel therefore concludes that, based on the available data described in this opinion, the use of calcium carbonate as a food additive at the current maximum reported use levels is not of safety concern provided that the total intake of calcium from all sources does not exceed the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003.

The Panel notes that both the estimated intake of calcium calculated by the Panel, considered to be conservative and the total dietary intake of calcium reported in the literature are all below the UL of 2500 mg/day for calcium from all sources established by the SCF in 2003.

The Panel noted that calcium carbonate is currently permitted at *quantum satis* in the vast majority of food categories in which it is allowed (generally permitted in all foods with certain exceptions), however information gathered by the Panel for this re-evaluation shows that calcium carbonate is only used at defined amounts in a number of food categories. The Panel thus recommends that the legislation be updated to reflect actual usage levels evaluated in this opinion.

The Panel notes that the presence of unintentional nanoscale particles at trace levels in food additive grade calcium carbonates cannot be excluded. Whilst the data are inadequate to reach definitive conclusions on calcium carbonate predominantly in the nanoscale, the Panel concluded that the available data are sufficient to conclude that the current levels of adventitious nanoscale material within microscale calcium carbonate would not be an additional toxicological concern.

The Panel also notes that the JECFA specification for lead in calcium carbonate is ≤ 3 mg/kg whereas the EC specification is ≤ 10 mg/kg.

Additionally the Panel notes that limestone (a source of calcium carbonate) may contain variable amounts of aluminium. The Panel noted that aluminium intake from use of calcium carbonate as a food additive could significantly contribute (50-100%) to the weekly intake of aluminium, for which a TWI of 1 mg aluminium/kg bw/week has been established, and therefore specifications for the maximum level of aluminium in calcium carbonate may be required

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ANNEX A

Rules defined by the Panel to deal with *quantum satis* (QS) authorisation, usage data or observed analytical data for for all regulated food additives to be re-evaluated.

Figure 1: Rules defined by the Panel to deal with usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules.



Figure 2: Rules defined by the Panel to deal with *quantum satis* (QS) authorisation.



ANNEX B

Table 7. Overview of information provided on usage of calcium carbonate as a food additive or added nutrient source.

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
Tennant (2008)	Additive		Non-alcoholic beverages		No use	No use		
SYFAB	Additive	Anti-caking agent	Baking powder	g/kg	n.s.	6		6000
Belgium Authority/CIAA	Additive	Anti-caking agent, Acidity regulator	Baking powders for production of fine bakery wares		n.s.	n.s.		
GEMEF	Additive	Anti-caking agent	Baking improvers for bread in countries outside the EU with shipping and storage at high temperature (final product)	%	0.02		200	
CIAA	Additive	n.s.	Baking powders for production of fine bakery wares (rarely used)	mg/kg	1000	900-12 000	1000	12 000
CIAA	Additive	n.s.	Fine bakery ware filled croissants (Limited representation of the European Market)	mg/kg	1970	n.s.	1970	
CIAA	Additive	n.s.	Fine bakery ware stol (limited representation of the Dutch Market)	mg/kg	1000	n.s.	1000	
CIAA	Additive	n.s.	Melkbrood (representative for Dutch market)	mg/kg	100	500	100	500
DTU	Additive	Acidity regulator	Fine bakery ware (nougat and almond cream containing biscuits: Nougat Pie Royal		n.s.	n.s.		

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
			Biscuit, Mazariner Royal Biscuit, Mazariner Royal Biscuit)					
DTU	Additive	Acidity regulator	Fine bakery ware (dessert waffles: Dessertvafler u. sukker (frost))		n.s.	n.s.		
Ireland, INFID	Additive	Anti-caking agent	Hot cross buns		n.s.			
CIAA	Additive	n.s.	Confectionery coating for fine bakery wares (very rarely used)	mg/kg	n.s.	85 000		85 000
CIAA	Additive	n.s.	Rusk (limited representation of the German market)	mg/kg	5000	12 000	5000	12 000
CIAA	Additive	n.s.	Fine bakery ware biscuits (Limited representation of the European Market)	mg/kg	<10 000	n.s.	10 000	
CIAA	Nutrient	Nutrient	Fine bakery ware		n.s.	n.s.		
Hovis	Nutrient	Mandatory Fortification (UK)	White and brown flour (excluding wholemeal)	mg/kg	n.s.	3650		3650
Hovis	Nutrient	Nutrient	Bread	mg/kg	9800	n.s.	9800	
Hovis	Nutrient	Nutrient	Rolls	mg/kg	8900	n.s.	8900	
Ireland, INFID	Nutrient	Nutrient	Bread (McCann's White Sliced Pan, Irish Sliced Stoneground Wheaten Rankin selection)		n.s.	n.s.		
Belgium Authority/CIAA	Additive	Anti-caking agent	Breakfast cereals (Use during the production of some of the extruded products such as rings, crisps, shells, spheres,		n.s.	n.s.		

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
			etc. to improve the flowing properties of the flour. Also e.g. rice crisps to be included in some kinds of muesli.)					
CIAA	Additive	n.s.	Muesli (limited to German market)	mg/kg	600	1500	600	1500
GEMEF	Additive	Acidity regulator	Cereal bars (extruded cereal component)	%	0.2	0.5	2000	5000
CIAA	Nutrient	Fortification	Breakfast Cereals	mg/kg	13 000	15 000	13 000	15 000
Polish Agency	Nutrient	Nutrient	Breakfast Cereals	mg/g (as Ca)	120	264-450	3000	11 250
Ireland, INFID	Nutrient	Nutrient	Breakfast Cereals		n.s.	n.s.		
CIAA	Nutrient	Fortification	Cereal bars	mg/kg	12 000	15 000	12 000	15 000
Ireland, INFID	Nutrient	Nutrient	Cereal Bars		n.s.	n.s.		
Ireland, INFID	Nutrient	Nutrient	Chocolate bar		n.s.	n.s.		
Belgium Authority	Additive	Bulking Agent	Chocolate (to give products less sweet taste (to replace sugar)		n.s.	n.s.		
Belgium Authority	Additive	Bulking Agent	Chocolate/chocolate fillings (E.g. a hazelnut filling containing calcium carbonate (+/- 5%) to give products less sweet taste)	%	5	n.s.	50 000	
CIAA	Additive	n.s.	Chocolate fillings (hazelnut fillings)	mg/kg	50 000	n.s.	50 000	

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
SYFAB	Additive	Release Agent	Confectionery (residual amount)	mg/kg	400	n.s.	400	
Belgium Authority/CIAA	Additive	Colour	Confectionery: Surface of citric jellified confectionery (limited representation of Belgian market)	mg/kg	n.s.	7200		7200
GEMEF	Additive	Acidity regulator	Chocolate confectionery (extruded cereal products)	%	0.2	0.5	2000	5000
GEMEF	Additive	Acidity regulator	Chocolate bars with crispy cereals (extruded cereal products)	%	0.2	0.5	2000	5000
GEMEF	Additive	Acidity regulator	Toppings for ice creams (extruded cereal products)	%	0.2	0.5	2000	5000
GEMEF	Additive	Acidity regulator	Chocolate coatings (extruded cereal products)	%	0.2	0.5	2000	5000
Ireland, INFID	Additive	Glazing agent	Chocolate covered peanuts		n.s.	n.s.		
Ireland, INFID	Additive	Glazing agent	Chocolate covered peanuts		n.s.	n.s.		
Polish Agency	Nutrient	Nutrient	Cocoa	mg/100g as calcium	120	264-450	3000	11 250
CIAA	Additive	Firming agent	Fruit and vegetable preparations excluding compote (limited use on German		n.s.	n.s.		

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
			market). The acid liberates Ca and enables complexation with natural tomato pectin					
DTU	Additive	Acidity Regulator	Frugt muckis kvark dessert (Milk based dessert with fruit)		n.s.	n.s.		
Ireland, INFID	Nutrient	Nutrient	Edible ice		n.s.	n.s.		
Polish Agency	Additive	colour acidity regulator, stabiliser, carrier or anticaking agent	Not specified		n.s.	n.s.		
Belgium Authority	Additive	Hardening Agent	Oils and fats (to improve texture of oils (hardening effect), to allow for the use of less saturated fat. (1 to 5% calcium carbonate).		currently not used	currently not used		
Südsalz	Additive	Anti-caking agent	salt and salt substitutes	g/kg	10	20	10 000	20 000
Belgium Authority	Additive	Colour	Prepared salads (as bread spread) In the surimi ingredient, to make it white when otherwise greyish		n.s.	n.s.		
CIAA	Additive	Colour	Few surimi applications	mg/kg		1500-2000		
DTU	Additive	n.s.	Seafood mix, surimi		n.s.	n.s.		
CIAA	Additive	Colour	Meat curing surface treatment in dry sausages (limited representation of Belgian and German market)	g/kg		7		

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
Heiploeg	Additive	Gelling agent/Thickener/Colour	Surimi	%	n.s.	1.5		15 000
Belgium Authority/CIAA	Nutrient	Nutrient	Water and water based beverages (added mineral salt)		n.s.	n.s.		
GSK Nutritional Healthcare	Nutrient	Nutrient	Malt beverage	g/25g powder	1	n.s.	40	
Ireland, INFID	Nutrient	Nutrient	Drinking chocolate powder		n.s.	n.s.		
Belgium Authority	Additive	Colour	Soft drink		n.s.	n.s.		
CIAA	Additive	Colour	Soft drink (limited representation)	mg/l	100	1000		
Ireland, INFID	Additive	Colour	Cup-a-soup (mushroom)		n.s.	n.s.		
Belgium Authority	Additive	Bulking Agent	Food supplement	%	1	n.s.	10 000	
CIAA	Nutrient	n.s.	Food supplements	mg/kg	10 000	n.s.	10 000	
EHPM	Additive	Bulking Agent	Food supplements (limited use)	g/kg	n.s.	500		500 000
EHPM	Additive	Colour	Food supplements (very limited use)	mg/kg	n.s.	7500		7500
Belgium Authority	Nutrient	Nutrient	Food supplements (effervescent tablets, tablets, chewable tablets, capsules, drinks, gelules, powder)	mg daily intake as calcium	120	1600	300	4000
EHPM	Nutrient	Nutrient	Food supplements (Ca min RDA at 120 mg=300 mg CaCO ₃) max 100% RDA =	mg daily intake	300	2000	300	2000

Data Provider	Category	Principal Function	Food Group	Unit	Reported range of typical use levels	Reported range of maximum use levels	Reported range of typical use levels	Reported range of maximum use levels
				as reported			Calcium carbonate expressed as mg/kg	
			2000 mg CaCO ₃					
CIAA	Additive	Acidity Regulator	Weaning foods for infants and young children in good health (for pH adjustment)	mg/kg	n.s.	100		100
Belgium Authority	Nutrient	Nutrient	Food for particular nutritional uses (e.g. infant formulae, follow up formulae, cereal based foods for infants and young children, baby food, foods for special medical purposes, replacement meals).		n.s.	n.s.		
Belgium Authority	Nutrient	Nutrient	Biscuits nutritional substance in baby biscuits (according to Directive 2006/125 ³¹)		n.s.	n.s.		
CIAA	Nutrient	Nutrient	Biscuits nutritional substance in baby biscuits (according to Directive 2006/125)		n.s.	n.s.		
Belgium Authority	Nutrient	Nutrient	Breakfast cereals, biscuits, soy drinks and other beverages, cereal bars, confectionary, desserts, ...)	mg daily intake as calcium	120	1600		

n.s. = not specified

³¹ Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 5.12.2006, 16-35..

Assumptions made:

Grain milling products

Information received on the use of calcium carbonate	Assumptions made
Use as anti-caking agent in flour at a concentration of 6 g/kg flour.	Anti caking use at 6000 mg/kg flour
Use as added nutrient source, due to mandatory fortification (UK only) in white and brown flour. Typical use of 3650 mg/kg	Mandatory fortification at 3650 mg/kg

The maximum reported use level as a food additive was 6000 mg/kg in bread.

The maximum reported use level as added nutrient source was 3650 mg/kg in bread/rolls.

Bread and rolls

Information received on the use of calcium carbonate	Assumptions made
Use as anti-caking agent in flour at a concentration of 6 g/kg flour.	Bread contains on average 50% flour, resulting in a concentration of 3000 mg/kg bread
Use as anti-caking agent in bread in countries outside the EU with shipping and storage at high temperature. Concentration in the final product 0.02%	200 mg/kg residual amount in bread.
Use as nutrient, due to mandatory fortification (UK only) in white and brown flour. Typical use of 3650 mg/kg	Assuming a 50% content of flour in bread, the level in the final product calculates at 1825 mg/kg bread

Use as nutrient in bread (UK market). Typical use level 9800 mg/kg	Nutrient use at 9800 mg/kg
Use as nutrient in rolls (UK market). Typical use level 8900 mg/kg	Nutrient use at 8900 mg/kg

The maximum reported use level as a food additive was 3000 mg/kg in bread.

The maximum reported use level as added nutrient source was 9800 mg/kg in bread/rolls.

Breakfast cereals (including cereal bars)

Information received on the use of calcium carbonate	Assumptions made
Use as anti-caking agent breakfast cereals (use during production of extruded products such as rings, crisps, shells and spheres). Use in rice crisps to be included in muesli. No usage information reported	No usage level reported. Usage as anti-caking agent in flour reported at 6 g/kg. Assuming a 50% flour content, the usage level is estimated at 3000 mg/kg extruded breakfast cereal
Unspecified use in muesli (limited to German market). Typical use 600 mg/kg, maximum use 1500 mg/kg.	The maximum use of 1500 mg/kg was used for exposure from muesli.
Use as acidity regulator in cereal bars in the extruded cereal component. Typical usage 0.2%, maximum use 0.5%	Based on labelled cereal content of a popular brand of cereal bars, cereal content was estimated at 25%, which calculates at a maximum usage of 1250 mg/kg cereal bar
Use as nutrient in cereal bars. Typical use 12 000 mg/kg, maximum use 15 000 mg/kg	Nutrient use at a maximum of 15 000 mg/kg
Use as nutrient in breakfast cereals. Typical use 13 000 mg/kg, max use 15 000 mg/kg	Nutrient use at a maximum of 15 000 mg/kg
Use as nutrient in breakfast cereals. Typical use 120 mg calcium/100 g food and maximum use of 264-450 mg calcium/100 g food.	Expressed as calcium carbonate per kg food: Typical 3000 mg/kg, maximum 11 250 mg/kg

The maximum reported use level as a food additive was 3000 mg/kg in extruded breakfast cereals.
The maximum reported use level as a food additive was 1500 mg/kg in muesli.
The maximum reported use level as a food additive was 1250 mg/kg in cereal bars.
The maximum reported use level as added nutrient source was 15 000 mg/kg in breakfast cereals and cereal bars.

For the purposes of estimating exposure of calcium carbonate from breakfast cereals including mueslis and cereal bars, the following fractions have been assigned based on the observed range of contributions of the individual foodgroups to the overall breakfast cereal foodgroup in the comprehensive food consumption database:

- Extruded breakfast cereals: 60% at a level of 3000 mg/kg (additive use)
- Mueslis and Cereal Bars: 30% at a level of 1500 mg/kg (additive use)
- Extruded Breakfast Cereals, mueslis and Cereal Bars: 90% at a level of 15 000 mg/kg (use as added nutrient source)

Fine bakery wares

Information received on the use of calcium carbonate	Assumptions made
Unspecified rare use in baking powders for production of fine bakery wares. Typical use 1000 mg/kg. Maximum use 900-12 000 mg/kg	According to the Irish recipes database, on average 1.8% of baking powder is used in fine bakery ware. This calculates at a maximum of 216 mg/kg in the final product
Unspecified use in filled croissants (limited representation of the European Market). Typical use of 1970 mg/kg	Typical use level 1970 mg/kg
Unspecified use in Stol (limited representation of the Dutch market. Typical use 1000 mg/kg	Typical use level 1000 mg/kg
Unspecified use in Melkbrood (representative for Dutch market. Typical use 100 mg/kg. Maximum use 500 mg/kg	Maximum use level 500 mg/kg

Use as acidity regulator in nougat and almond cream containing biscuits and dessert waffles. No usage level reported	-
Use as anti-caking agent in hot cross buns. No usage level specified	-
Unspecified very rare use in confectionery coating for fine bakery ware. Maximum use 85 000 mg/kg	Maximum use level 85 000 mg/kg (coating). As no information on type and amount of quantity of coating is known and use has been indicated to be very rare, this information was not used in the exposure estimate.
Information received	Assumptions made
Unspecified rare use in baking powders for production of fine bakery wares. Typical use 1000 mg/kg. Maximum use 900-12 000 mg/kg	According to the Irish recipes database, on average 1.8% of baking powder is used in fine bakery ware. This calculates at a maximum of 216 mg/kg in the final product
Unspecified use in filled croissants (limited representation of the European Market). Typical use of 1970 mg/kg	Typical use level 1970 mg/kg
Unspecified use in Stol (limited representation of the Dutch market. Typical use 1000 mg/kg	Typical use level 1000 mg/kg
Unspecified use in Melkbrood (representative for Dutch market. Typical use 100 mg/kg. Maximum use 500 mg/kg	Maximum use level 500 mg/kg
Use as acidity regulator in nougat and almond cream containing biscuits and dessert waffles. No usage level reported	-
Use as anti-caking agent in hot cross buns. No usage level specified	-
Unspecified very rare use in confectionery coating for fine bakery ware. Maximum use 85 000 mg/kg	Maximum use level 85 000 mg/kg (coating). As no information on type and amount of quantity of coating is known and use has been indicated to be very rare, this

	information was not used in the exposure estimate.
Information received	Assumptions made
Unspecified rare use in baking powders for production of fine bakery wares. Typical use 1000 mg/kg. Maximum use 900-12 000 mg/kg	According to the Irish recipes database, on average 1.8% of baking powder is used in fine bakery ware. This calculates at a maximum of 216 mg/kg in the final product
Unspecified use in filled croissants (limited representation of the European Market). Typical use of 1970 mg/kg	Typical use level 1970 mg/kg
Unspecified use in Stol (limited representation of the Dutch market. Typical use 1000 mg/kg	Typical use level 1000 mg/kg
Unspecified use in Melkbrood (representative for Dutch market. Typical use 100 mg/kg. Maximum use 500 mg/kg	Maximum use level 500 mg/kg
Use as acidity regulator in nougat and almond cream containing biscuits and dessert waffles. No usage level reported	-
Use as anti-caking agent in hot cross buns. No usage level specified	-
Unspecified very rare use in confectionery coating for fine bakery ware. Maximum use 85 000 mg/kg	Maximum use level 85 000 mg/kg (coating). As no information on type and amount of quantity of coating is known and use has been indicated to be very rare, this information was not used in the exposure estimate.
Unspecified use in rusk (limited representation of the German market). Typical use 5000 mg/kg, maximum use 12 000 mg/kg	Maximum use level of 12 000 mg/kg. As this usage is very specific and not considered representative for fine bakery ware (it is a very specific double baked item commonly available only in Germany) it was not used in the exposure estimate.
Unspecified use in biscuits (limited representation of the European market). Typical	Typical use level <1000 mg/kg

use less than 1000 mg/kg	
Use as nutrient in fine bakery ware. No usage level reported	-
Use as nutrient, due to mandatory fortification (UK only) in white and brown flour. Typical use of 3650 mg/kg	Assuming a 50% content of flour in bakery ware, the level in the final product calculates at 1825 mg/kg

The maximum reported use level as a food additive was 1970 mg/kg in fine bakery ware.

The maximum reported use level as added nutrient source was 1825 mg/kg in fine bakery ware.

Cocoa beans and cocoa products (Cocoa beans, powder, mass and powder for beverage preparation)

Information received on the use of calcium carbonate	Assumptions made
Use as added nutrient source in cocoa. Typical use 120 mg calcium/100 g food, maximum use 264 – 450 mg calcium/100 g food	Expressed as calcium carbonate per kg food: typical 3000 mg/kg food, maximum 11 250 mg/kg food
Use as added nutrient source in drinking chocolate powder. No usage level reported	

No information on the use of calcium carbonate in this food category was provided by industry. Directive 95/2/EC permits a usage of 7% expressed as potassium carbonate based on the dry weight excluding fat. The equivalent amount of calcium carbonate calculates at 5g/kg dry cocoa excluding fat. Based on a minimum content of 2.5% dry non-fat cocoa solids in milk chocolate (as required by legislation, Directive 73/241/EEC), a usage of 1250 mg/kg calcium carbonate in chocolate can be calculated.

The maximum reported use level as added nutrient source in cocoa was 11 250 mg/kg

Fish products

Information received on the use of calcium carbonate	Assumptions made

Use as colour in prepared salads as bread spread in the surimi ingredient. Maximum concentration 1500 mg-2000 mg/kg	
Use as gelling agent, thickener and colour in Surimi. Typical use 1.5%	Calculated at 15 000 mg/kg surimi

The maximum reported use level as a food additive was 15 000 mg/kg surimi.

For the purposes of estimating exposure of calcium carbonate from surimi, a factor of 15% was assigned to the fish product group, as it contains a number of different food groups (such as fish fingers, fish cakes, etc), which do not contain calcium carbonate (E170).

Dried cured sausages covering

Use as a food colour for meat curing surface treatment in dry sausages, maximum use of 7g/kg.	Maximum use level 7 g/kg. As no information on consumption of sausage and the amount that is covering the sausage (probably the skin of the sausage is not eaten) is available, this information was not used in the exposure estimate.

Fermented milk products

Information received on the use of calcium carbonate	Assumptions made
Use as acidity regulator in milked based desserts containing fruit (Frugt Muckis Kvark dessert). No usage level reported	

No usage level was reported and there was insufficient information to approximate likely usage levels. No exposure from this foodgroup could be calculated.

Cheese

This food category is specifically mentioned in Directive 95/2/EC (ripened cheese and sliced and grated ripened cheese, *quantum satis*). Industry did not submit any information on usage of calcium carbonate in cheese and therefore no exposure from this foodgroup was calculated.

Fruit juice

This food category is specifically mentioned in Directive 95/2/EC (grape juice as defined in Directive 93/77/EEC, *quantum satis*). Industry did not submit any information on usage of calcium carbonate in grapefruit juice and therefore no exposure from this foodgroup was calculated.

Chocolate (Cocoa) products (Chocolate, filled chocolate, chocolate bars, etc)

Information received on the use of calcium carbonate	Assumptions made
Use as bulking agent in chocolate (to replace sugar). No usage level reported	-
Use as bulking agent in chocolate/chocolate fillings (e.g. hazelnut filling containing calcium carbonate (+/- 5%) to give products a less sweet taste.	Assuming 50% filling, the concentration in the final product calculates at 25 000 mg/kg hazelnut filled chocolate confectionery
Use as release agent in confectionery with a residual amount of 400 mg/kg	Typical residual amount of 400 mg/kg
Use as colour in confectionery (surface of citric jellified confectionery). Maximum use 7200 mg/kg	Maximum use level 7200 mg/kg. As no information on consumption of citrified jellies with whitened surface powder is available, this information was not used in the exposure estimate
Unspecified use in chocolate fillings (hazelnut fillings). Typical use 50 000 mg/kg	Assuming 50% filling, the concentration in the final product calculates at 25 000 mg/kg
Use as acidity regulator in chocolate confectionary and chocolate bars (in the extruded cereal part). Typical levels 0.2%, maximum levels 0.5%	Based on labelled cereal content of a popular brand of chocolate bars (3.5-11%), cereal content was estimated at 10%, which calculates at a maximum usage of 500

	mg/kg cereal containing chocolate bar
Use as acidity regulator for chocolate coatings (extruded cereal products). Typical use 0.2%, maximum use 0.5%	Maximum use level 5000 mg/kg (coating). As no information on type and amount of quantity of coating is known, this information was not used in the exposure estimate.
Use as glazing agent in chocolate covered peanuts. No usage level reported	Assuming 50% chocolate coating, the use level can be calculated based on 5 g/kg permitted in dry non fat cocoa solids. Assuming a minimum content of 2.5% dry non fat cocoa solids, the estimated usage calculates at 625 mg/kg chocolate covered peanuts.
Use as nutrient in malt beverage. Typical use 1 g/25 g powder (to be reconstituted with 200 ml milk)	Calculates at 40 mg/kg powder or 5 g/l reconstituted beverage

The maximum reported use level as a food additive was 25 000 mg/kg in filled chocolate

The maximum reported use level as a food additive was 500 mg/kg in chocolate bars.

The maximum reported use level as a food additive was 625 mg/kg in chocolate coated peanuts.

Directive 95/2/EC permits a usage level of 7% expressed as potassium carbonate based on the dry weight excluding fat. The equivalent amount of calcium carbonate calculates at 5g/kg dry cocoa excluding fat. Based on a minimum content of 2.5% dry non-fat cocoa solids in milk chocolate (as required by legislation Directive 73/241/EEC), a usage level of 1250 mg/kg calcium carbonate in chocolate can be calculated. This level was applied to the food category chocolate confectionery as the reported usage referred to very specific food items only.

Soft drinks

Information received on the use of calcium carbonate	Assumptions made
Use as food colour in soft drinks-Typical use level 100 mg/l, maximum use level 1000 mg/l	500 mg/kg use (in accordance with <i>quantum satis</i> rules for colours) was assigned.

Use as added nutrient source in water and water based beverages as added mineral salt. No usage data reported.	

No usage data for calcium carbonate used as food colour in soft drink was provided. In accordance with *quantum satis* rules for food colours (see Annex A), an MPL of 500 mg/kg has been assigned for the exposure estimate. The usage data reported by industry only became available at a very late stage and after exposure estimates had already been finalised. The exposure estimates are based on an MPL of 500 mg/kg based on *quantum satis* rules for food colours (see Annex A). In view of the very limited use reported by industry, the Panel was satisfied that the applied level of 500 mg/kg was sufficient to estimate exposure from soft drinks. Since calcium carbonate is a white colour and its use is assumed to be limited (due to limited availability of white soft drinks), a factor of 10% has been assigned to avoid gross overestimation from this food category.

Seasoning or extracts

Information received on the use of calcium carbonate	Assumptions made
Use as anti-caking agent in salt. Typical use level 10 g/kg, maximum use level 20 g/kg	Maximum use level 20 000 mg/kg

The maximum reported use level as a food additive was 20 000 mg/kg.

As the comprehensive food consumption database does not provide detailed information on salt intake, exposure to calcium carbonate from salt consumption has been estimated based on an average daily intake of 10 g of salt per day, which is in line with reported salt intake in the European Union (EC, 2008).

Baking ingredients

Information received on the use of calcium carbonate	Assumptions made
Use as anti-caking agent and as acidity regulator in bread improvement products. No usage level supplied	-

Unspecified rare use in baking powders for production of fine bakery wares. Typical use level 1000 mg/kg. Maximum use 900-12 000 mg/kg	Maximum use level 12 000 mg/kg

The maximum reported use level as a food additive was 12 000 mg/kg.

Ready to eat soups

Information received on the use of calcium carbonate	Assumptions made
Use as food colour in mushroom soup. No usage level reported	500 mg/kg use (in accordance with <i>quantum satis</i> rules for food colours) was assigned.

No usage level for calcium carbonate used as food colour in soups was provided. In accordance with *quantum satis* rules for colours (see Annex A), an MPL of 500 mg/kg has been assigned for the exposure estimate. Since calcium carbonate is a white colour and its use is assumed to be limited, a factor of 10% has been assigned to avoid gross overestimation from this food category.

Vegetable products (pureed, mashed pickled, etc)

Information received on the use of calcium carbonate	Assumptions made
Use as firming agent in fruit and vegetable preparations excluding compote (to enable complexation with natural tomato pectin). Limited use on German market. No usage level reported.	

No usage level was reported and there was insufficient information to approximate likely usage levels. No exposure from this food category could be calculated.

Animal and vegetable fats and oils

Information received on the use of calcium carbonate	Assumptions made
Potential use as hardening agent in oils and fats to improve texture and allow for the use of less saturated fat. Usage 1-5% .Currently not used.	As currently not in use, this has not been included in the exposure estimates.

The maximum reported potential use level as a food additive was 500 mg/kg, however, as this application is currently not in use it has not been included in the exposure estimate.

Dietary supplements

Information received on the use of calcium carbonate	Assumptions made
Use as bulking agent in food supplements at 1%	Calculates at 10 000 mg/kg food supplement
Limited use as bulking agent in food supplements. Maximum use 500 g/kg	Maximum usage 500 000 mg/kg
Very limited use as colour in food supplements. Typical use 7500 mg/kg	Typical use 7500 mg/kg
Use as nutrient in food supplements (effervescent tablets, tablets, chewable tablets, capsules, drinks, gelules, powder, etc). Typical daily intake 120 mg calcium/day maximum intake 1600 mg calcium/day.	Converted to calcium carbonate. Usual daily intake 300 mg. Maximum daily intake 4000 mg.
Use as nutrient in food supplements. Typical daily intake 120 mg calcium/day, maximum intake 800 mg calcium/day	Converted to calcium carbonate. Usual daily intake 300 mg. Maximum daily intake 2000 mg.

The maximum reported use level as a food additive was 500 000 mg/kg.

The maximum reported use level as added nutrient source was expressed as daily intake at 4000 mg/day

The use as bulking agent was not considered representative for estimation of exposure from consumption of food supplements. Estimation of intake from food supplements was based on a daily intake of 4000 mg as reported.

Cereal-based food for infants and young children

Information received on the use of calcium carbonate	Assumptions made
Use as acidity regulator in weaning foods for infants and young children in good health (for pH adjustment). Maximum usage 100 mg/kg	Maximum usage 100 mg/kg
Use as nutrient in food for particular nutritional uses as specified by legislation	
Use as nutrient in baby biscuits according to Directive 2006/125	Use of calcium according to Directive 2006/125: Rusks and biscuits which are to be used either directly or, after pulverisation, with the addition of water, milk or other suitable liquids; (min 50mg/100 kcal (for milk biscuits) max 100 mg/100 kcal)

The maximum reported use level as a food additive was 100 mg/kg

The maximum reported use level as added nutrient source was not specified. According to Directive 2006/125 a maximum of 100 mg calcium per 100 kcal baby biscuit is permitted. Based on labelled ingredient information of a popular brand, 100 kcal equates to 24 g of biscuit, giving a total of 416 mg calcium/kg biscuit. This can be converted to 1040 mg calcium carbonate/kg biscuit.

Ready-to-eat meal for infants and young children

Information received on the use of calcium carbonate	Assumptions made
Use as acidity regulator in weaning foods for infants and young children in good health (for pH adjustment). Maximum usage 100 mg/kg	Maximum usage 100 mg/kg
Use as nutrient in food for particular nutritional uses as specified by legislation	Use of calcium according to Directive 2006/125 (a) ‘processed cereal-based foods’ which are divided into the following four categories: (i) simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids; (maximum 180 mg/100kcal) (ii) cereals with an added high protein food which are or have to be reconstituted with water or other protein-free liquid (minimum 80 mg/100 kcal, maximum 180 mg/100kcal) (iii) pastas which are to be used after cooking in boiling water or other appropriate liquids(maximum 80 mg/100 kcal) (iv) rusks and biscuits which are to be used either directly or, after pulverisation, with the addition of water, milk or other suitable liquids (minimum 50 mg/100 kcal for milk biscuits maximum 100 mg/100 kcal) (b) ‘baby foods’ other than processed cereal-based foods. (max 80 mg/100kcal)

The maximum reported use level as a food additive was 100 mg/kg.

The maximum reported use level as added nutrient source was assumed to be of similar range as calculated for biscuits and a value of 1040 g/kg was assigned.

Ices and Desserts

Use as acidity regulator in toppings for ice creams (extruded cereals), typical use 0.2%, maximum use 0.5%	Maximum use level 5000 mg/kg. As no information on type and amount of quantity of topping is known, this information was not used in the exposure estimate.

ANNEX C

Exposure Scenarios (1 - 3)

Table 8 summarises the anticipated exposure of adults, adolescents, children and toddlers to calcium carbonate from use as a food additive, Table 9 summarises the total anticipated exposure from its use as a food additive and added nutrient source (food fortification) and Table 10 summarises the anticipated exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (including food supplements). For all scenarios, exposure from consumption of salt was calculated separately, as consumption information in the comprehensive database was not sufficiently detailed to allow for incorporation of this food group in the calculation.

Exposure to calcium carbonate from salt consumption has been estimated based on an average daily intake of 10 g of salt per day, which is in line with reported salt intake in the European Union (EC, 2008). However, no specific information on salt intake by children or toddlers was specified in the former mentioned report and the assumed intake of 10 g/day may present an over-estimate in these population groups.

Scenario 1 - Exposure from use of calcium carbonate as a food additive

Table 8. Anticipated exposure of adults, adolescents, children and toddlers to calcium carbonate from its use as a food additive

Daily intake (mg/kg bw) as CaCO ₃ from use as a food additive only								
	Adults		Adolescents		Children		Toddlers	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
BEL	8	14	10	16	23	35	27	43
BUL					28	48	28	43
CZE	11	18	19	28	24	34		
CYP			8	22				
DK	10	15	15	23	26	38		
FIN	11	20			17-23	29-39	29	57
F	7	14	12	18	18	27		
DE	7	13	9	17	18-19	27-28	18-18	27-32
HU	10	17						
IRL	8	14						
GRE					13	23		
ITA	9	15	14	26	21	34	16	41
LV	9	17	12	30	16	40		
NL	9	14			20	31	23	37
ES	6-6	11-11	8-12	14-23	16-16	26-28		
SE	8	11	10	15	15	23		
UK	8	12						
ALL	6-11	11-20	8-19	14-30	13-28	23-48	14-29	27-57
Daily intake (mg) as CaCO ₃								
	Adults (60 kg)		Adolescents (50 kg)		Children (30 kg)		Toddlers (15 kg)	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
ALL	360-660	660-1200	400-950	700-1500	390-840	690-1440	210-435	405-855
ALL including salt	560-860	860-1400	600-1150	900-1700	590-1040	890-1640	410-635	605-1055

Daily intake (mg) converted to Ca								
	Adults (60 kg)		Adolescents (50 kg)		Children (30 kg)		Toddlers (15 kg)	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
ALL	120-240	240-480	150-350	300-600	150-330	270-570	90-180	165-345
ALL including salt	200-320	320-560	230-430	380-680	230-410	350-650	170-260	245-425

Estimates calculated for the adult population give a range of mean dietary exposure to calcium carbonate of 560 - 680 mg/day (200-320 mg calcium /day) and a range of 860 – 1400 mg/day (320 - 560 mg calcium /day) for high level (95th percentile) consumers.

Estimates calculated for the adolescent give a range of mean dietary exposure to calcium carbonate of 600-1150 mg/day (230 - 430 mg calcium /day) and a range of 900 – 1700 mg/day (380 - 680 mg calcium a/day) for high level (95th percentile) consumers.

Estimates calculated for the children give a range of mean dietary exposure to calcium carbonate of 590-1040 mg/day (230-410 mg calcium /day) and a range of 890-1640 mg/day (350-650 mg calcium /day) for high level (95th percentile) consumers.

Estimates calculated for the toddler give a range of mean dietary exposure to calcium carbonate of 410-635 mg/day (170-260 mg calcium /day) and a range of 605-1055 mg/day (245-425 mg calcium /day) for high level (95th percentile) consumers.

For all population groups, potential exposure to calcium carbonate from intake of 10 g salt per day has been calculated at 200 mg/day which can be considered a significant contributor to the overall exposure.

For adults, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (53.3 to 72.6% in 14 countries). Grain milling products accounted for 8.8 to 89.4% in 11 countries, breakfast cereals accounted for 5.1 to 18.3% in 4 countries, fine bakery wares accounted for 9.5 to 21.9% in 12 countries and baking ingredients accounted for 5.3% in 1 country.

For adolescents, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (42.2 to 71.2%). Grain milling products accounted for 6.8 to 34.2% in 7 countries, breakfast cereals accounted for 6.4 to 9.1% in 5 countries and fine bakery wares accounted for 13.6 to 28.6% in 11 countries.

For children, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (34.4 to 67.3% in 16 countries). Grain milling products accounted for 8 to 83.9% in 11 countries, breakfast cereals accounted for 5.7 to 27.5% in 10 countries, fine bakery wares accounted for 10.1 to 38.4% in 15 countries and chocolate (cocoa) products accounted for 5.1% in 1 country.

For toddlers, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (42.4 to 71.6% in 8 countries). Grain milling products accounted for 5.5 to 92.6% in 8 countries, breakfast cereals accounted for 5.7 to 7.1% in 2 countries, fine bakery wares accounted for 8.2 to 22.1% in 8 countries, chocolate (cocoa) products accounted for 5.4% in 1 country and ready-to-eat meals for infants and young children accounted for 5.6% in 1 country.

Scenario 2 - Exposure from use of calcium carbonate as additive and added nutrient source

Table 9. Anticipated exposure of adults, adolescents, children and toddlers to calcium carbonate from its use as food additive and added nutrient (excluding exposure from food supplements)

Daily intake mg/kg bw as CaCO ₃ from use as additive and added nutrient source								
	Adults		Adolescents		Children		Toddlers	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
BEL	23	45	31	51	68	109	84	137
BUL					69	135	67	137
CZE	26	49	43	75	57	136		
CYP			26	45				
DK	27	44	40	66	74	112		
FIN	14	23			33-59	48-131	37	65
F	22	41	31	51	47	75		
DE	22	41	28	53	58-59	86-91	61-62	91-106
HU	26	47						
IRL	26	43						
GRE					34	66		
ITA	21	41	32	60	49	92	38	92
LV	25	52	35	68	48	110		
NL	26	45			62	98	71	117
ES	17-18	32-34	24-36	41-71	51-51	81-91		
SE	25	62	29	48	45	92		
UK	24	38						
ALL	14-27	23-62	24-43	41-75	33-75	48-136	37-84	65-137

Daily intake mg as CaCO ₃								
	Adults (60kg)		Adolescents (50kg)		Children (30kg)		Toddlers (15 kg)	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
All	840-1620	1380-3720	1200-2150	2050-3750	990-2250	1440-4080	555-1260	975-2055
All including salt	1040-1820	1580-3920	1400-2350	2250-3950	1190-2450	1640-4280	755-1460	1175-2255
Daily intake mg converted to Ca								
	Adults (60kg)		Adolescents (50kg)		Children (30kg)		Toddlers (15 kg)	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
All	360-660	540-1500	450-850	850-1500	390-900	570-1620	225-495	390-825
All including salt	440-740	620-1580	530-930	930-1580	470-980	650-1700	305-575	470-905

The use of calcium carbonate as added nutrient source (in fortification) in addition to its use as a food additive results in an up to 3 fold increased intake estimate of calcium carbonate.

Estimates calculated for the adult population give a range of mean dietary exposure to calcium carbonate of 1040 - 1820 mg/day (440 - 740 mg calcium /day) and a range of 1580 - 3920 mg/day (620 - 1580 mg calcium /day) for high level (95th percentile) consumers.

Estimates calculated for the adolescent give a range of mean dietary exposure to calcium carbonate of 1400 - 2350 mg/day (530 - 930 mg calcium/day) and a range of 2250 - 3950 mg/day (930 - 1580 mg calcium /day) for high level (95th percentile) consumers.

Estimates calculated for the children give a range of mean dietary exposure to calcium carbonate of 1190 - 2,450 mg/day (470 - 980 mg calcium /day) and a range of 1640 - 4280 mg/day (650 - 1700 mg calcium /day) for high level (95th percentile) consumers.

Estimates calculated for the toddler give a range of mean dietary exposure to calcium carbonate of 755-1460 mg/day (305 - 575 mg calcium /day) and a range of 1175 - 2255/day (470 - 905 mg calcium /day) for high level (95th percentile) consumers.

For all population groups, potential exposure to calcium carbonate from intake of 10 g salt per day has been calculated at 200 mg/day which can be considered a significant contributor to the overall exposure.

For adults, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (54.6 to 80.3% in 14 countries). Grain milling products accounted for 5.4 to 70.6% in 6 countries, breakfast cereals accounted for 5.4 to 33.3% in 11 countries, fine bakery wares accounted for 5.3 to 8.1% in 10 countries and chocolate (cocoa) products accounted for 5.6 to 9.2% in 8 countries

For adolescents, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (51.6 to 76.1%). Grain milling products accounted for 5.5 to 15.4% in 4 countries, breakfast cereals accounted for 6 to 18.3% in 10 countries, fine bakery wares accounted for 5.8 to 10.8% in 10 countries, cocoa beans and cocoa products accounted for 5.5% in 1 country and chocolate (cocoa) products accounted for 5.4 to 14% in 10 countries.

For children, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (6.7 to 77.8%). Grain milling products accounted for 5.2 to 59.5% in 6 countries, breakfast cereals accounted for 6.6 to 47.9% in 15 countries, fine bakery wares accounted for 5.9 to 14.6% in 12 countries, cocoa beans and cocoa products accounted for 7.2% in 1 country and chocolate (cocoa) products accounted for 5.4 to 15% in 14 countries.

For toddlers, the main contributors to the total anticipated exposure to calcium carbonate (>5% in all countries), excluding contribution from salt were bread and rolls (48.4 to 78.5% in 8 countries). Grain milling products accounted for 7.3 to 73.5% in 4 countries, breakfast cereals accounted for 5.4 to 15% in 7 countries, fine bakery wares accounted for 5.2 to 9.1% in 5 countries, cocoa beans and cocoa products accounted for 12.5% in 1 country, chocolate (cocoa) products accounted for 5.2 to 15.6% in 4 countries, cereal-based food for infants and young children accounted for 6.5% in 1 country and ready-to-eat meals for infants and young children accounted for 6.7 to 16.3% in 4 countries.

Scenario 3 - Exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (fortification) including food supplements

Estimation of exposure to calcium carbonate/calcium from food supplements was hampered by lack of detail in the comprehensive food consumption database and did not permit calculation of reliable intake estimates. It is also not known how much of the calcium contained in food supplements is in the form of calcium carbonate. Information received from the Belgium authorities indicated a daily intake range between 15-200% of the RDA for calcium (120 – 1600 mg/day). Therefore for the purposes of

estimating long-term additional exposure to calcium from consumption of food supplements in this opinion, an intake of 800 mg calcium/day (100% RDA) in all population groups was assumed.

Table 10 summarises the total anticipated exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (from fortification) as calculated in scenario 2 (see Table 9) and from consumption of calcium-containing food supplements.

Table 10. Anticipated exposure to calcium from the use of calcium carbonate as a food additive and added nutrient source (including food supplements) in adult, adolescent, children and toddlers populations.

Daily intake of calcium (mg)							
Adults (60 kg)		Adolescents (50 kg)		Children (30 kg)		Toddlers (15 kg)	
Mean	95 th percentile	Mean	95 th percentile	Mean	95 th percentile	Mean	95 th percentile
1240-1540	1420-2380	1330-1730	1730-2380	1270-1780	1450-2500	1105-1375	1270-1705

Estimates calculated for the adult population give a range of mean dietary exposure to calcium of 1240 - 1540 mg/day and a range of 1420 – 2380 mg/day for high level (95th percentile) consumers.

Estimates calculated for adolescents give a range of mean dietary exposure to calcium of 1330 - 1730 mg/day and a range of 1730 – 2380 mg/day for high level (95th percentile) consumers.

Estimates calculated for children give a range of mean dietary exposure to calcium of 1270 – 1780 mg/day and a range of 1450 - 2500 mg/day for high level (95th percentile) consumers.

Estimates calculated for toddlers give a range of mean dietary exposure to calcium of 1105 – 1375 mg/day and a range of 1270 - 1705 mg/day for high level (95th percentile) consumers.

ANNEX D

Total estimated dietary intake of calcium from all sources based on published dietary survey data

Table 11. Mean and 95th/97.5th percentile calcium intakes (mg/day) from food and supplements (where available)

where available)

Toddlers										
					plus supplements			without supplements		
Country	Year	N	sex	age	mean	P95	P97.5	mean	P95	P97.5
					(mg/kg)					
NL	2005/2006	327	Male	2-3	795	1184		788	1180	
		313	Female	2-3	740	1099		734	1089	
ITA	2005/2006	52	All	0-2.9	664	1070				
UK	2008/2009	120	All	1.5-3				771		1310
Children										
					plus supplements			without supplements		
Country	Year	N	sex	age	mean	P95	P97.5	mean	P95	P97.5
					(mg/kg)					
NL	2005/2006	327	Male	4-6	863	1345		854	1335	
		312	Female	4-6	756	1124		748	1113	
F	2006/2007	570	All	3-10				804	1219	1339
IRL	2003/2004	145	Male	5-8	869	1453	1603	852	1414	1538
		151	Female	5-8	815	1213	1425	810	1213	1338
ITA	2005/2006	193	All	3-9.9	749	1197				
UK	2008/2009	238	All	4-10				787		1318
Adolescents										
					plus supplements			without supplements		
Country	Year	N	sex	age	mean	P95	P97.5	mean	P95	P97.5
F	2006/2007	874	All	11-17				809	1299	1432
IRL	2005/2006	224	Male	13-17	1070	1905				
		217	Female	13-17	738	1383				
	2003/2004	148	Male	9-12	965	1507	1655	961	1507	1655
		151	Female	9-12	800	1393	1487	798	1425	1487
ITA	2005/2006	108	Male	10-17.9	892	1435				
		139	Female	10-17.9	770	1306				
UK	2008/2009	224	All	11-18				813		1522
		548	All	19-64				824		1492
Adults										
					plus supplements			without supplements		
Country	Year	N	sex	age	mean	P95	P97.5	mean	P95	P97.5
UK	2000/2001	833	Male	19-64	1016		1794	1007		1783
		891	Female	19-64	809		1550	777		1372
F	2006/2007	1918	All	18-79				914	1488	1652
IRL	2008-2010	1274	All	18-64	941	1690	1872			
		226	All	>65	954	2106	2337			
		1068	Male	18-64.9	799	1433				
ITA	2005/2006	1245	Female	18-64.9	730	1233				
		202	Male	>65	825	1403				
		316	Female	>65	754	1285				
UK	2008/2009	548	All	19-64				824		1492
NL	2003	398	Female	19-30	1008	1626				
		352	Male	19-30	1164	1851				

N: Number of survey population

P95: 95th percentile

P97.5: 97.5th percentile

Netherlands (NL): Ocké et al., 2008; Hulshof et al., 2004.

United Kingdom (UK): Henderson et al., 2003; FSA, 2010.

France (F): AFFSA, 2009

Ireland (IRL): IUNA, 2006, 2008, 2011

Italy (ITA): Sette et al., 2010.

GLOSSARY AND ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
ACC	Amorphous Calcium Carbonate
ADI	Acceptable Daily Intake
AFSSA	L'Agence Française de Sécurité Sanitaire des Aliments
ANS	Panel on Food Additives and Nutrient Sources added to Food
BMD	Bone Mineral Density
CAIFOS	Calcium Intake Fracture Outcome Study
CAS	Chemical Abstracts Service
CCA-Europe	Calcium Carbonate Association-Europe
CCC	Crystalline Calcium Carbonate
CIAA	Confederation of the Food and Drink Industries of the EU
DTU	Danmarks Tekniske Universitet
EC	European Commission
EFSA	European Food Safety Authority
EHPM	European Federation of Associations of Health Product Manufacturers
EPA	US Environmental Protection Agency
EU	European Union
EXPOCHI	Refers to EFSA Article 36 2008 call for Proposals Focused on Children and Food Consumption
FAO/WHO	Food and Agriculture Organization/World Health Organization
FDA	Food and Drug Administration
GD	Gestation Day
GRAS	Generally recognized as safe
HR	Hazard Ratio
ICP	Inductively Coupled Plasma
IC	Ion Chromatography
INFID	Irish National Food Ingredient Database
iPTH	intact Parathyroid Hormone

IUNA	Irish Universities Nutrition Alliance
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOAEL	Lowest Observed Adverse Effect Level
MJ	MegaJoule
MPL	Maximum Permitted Level
MPP	Micronised Pearl Powder
MS	Mass Spectrometry
NOAEL	No Observed Adverse Effect Level
NPP	Nanosised Pearl Powder
Ph. Eur.	European Pharmacopoeia
PRI	Population Reference Intake
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety
SCF	Scientific Committee for Food
SYFAB	Syndicat national des Fabricants de Produits intermédiaires pour boulangerie, pâtisserie et biscuiterie
UL	Tolerable Upper Intake Level
WADLS	Western Australian Data Linkage Service

Safety Assessment of Carbonate Salts as Used in Cosmetics

Status: Final Report
Release Date: January 11, 2017
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The 2016 Cosmetic Ingredient Review Expert Panel members are: Chair, 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 Lillian J. Gill, D.P.A. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Bart Heldreth, Ph.D., Chemist.

ABSTRACT: The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of 6 carbonate salts which function as absorbents, bulking agents, opacifying agents, pH adjusters, buffering agents, abrasives, and oral care agents in cosmetic products. The Panel reviewed relevant data relating to the safety of these ingredients, and concluded that these carbonate salts are safe in the present practices of use and concentration in cosmetics when formulated to be non-irritating.

INTRODUCTION

The safety of the following 6 carbonate salts as used in cosmetics is reviewed in this safety assessment:

Magnesium Carbonate
Ammonium Bicarbonate
Ammonium Carbonate
Calcium Carbonate
Potassium Bicarbonate
Potassium Carbonate

According to the *International Cosmetic Ingredient Dictionary and Handbook*, the functions of these ingredients in cosmetic products include: absorbents, bulking agents, opacifying agents, pH adjusters, buffering agents, abrasives, and oral care agents (**Table 1**).¹ Ingredient definitions are also included in **Table 1**.

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) has evaluated the safety of Sodium Sesquicarbonate, Sodium Bicarbonate, and Sodium Carbonate in cosmetic products, and concluded that these ingredients are “safe as presently used in cosmetics”.² A CIR final report with this conclusion was published in 1987. Subsequently, during a Panel re-review of the safety of these ingredients in 2004, the conclusion originally determined by the Panel was reaffirmed.³

Data from chemical registration dossiers submitted to the European Chemicals Agency (ECHA) that relate to some of the ingredients that are being reviewed were found on the ECHA website. A chemical registration dossier may contain data on the cosmetic ingredient that is being reviewed or pertinent data on a surrogate chemical. ECHA data, whether on the cosmetic ingredient or on a surrogate chemical, are included and referenced in the report text.

CHEMISTRY

Definition and General Characterization

The carbonate salts are alkaline salts that may be formed by treating carbonic acid with an appropriate base (e.g., adding carbonic acid to sodium hydroxide will produce sodium carbonate (and water); Figure 1).

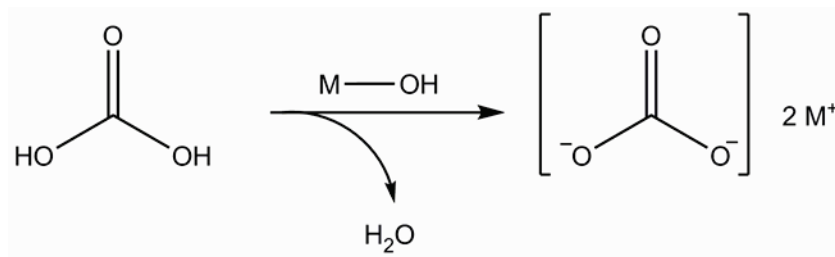


Figure 1. Carbonic Acid and Salts Thereof.

However, most of these salts are also naturally occurring as minerals. All of the ingredients in this report are related as either alkaline earth metal (column I or II) or ammonium salts of carbonic acid. This group comprises carbonate salts with differences in properties that can be attributed to differences in the cation component. Assessing the safety of all of these ingredients in a single report facilitates a coherent analysis, taking into account comparabilities and differences in properties among these ingredients. This enables a more informative and efficient safety assessment of the ingredients than would be likely in separate reports that each assesses a single ingredient. The definitions of the carbonate salts that are included in this safety assessment are presented in **Table 1**. These salts are classified as generally recognized as safe (GRAS) by the U.S Food and Drug Administration (FDA) for use in food. Daily consumption of these GRAS foods would result in much larger

systemic exposures than what is expected from use in cosmetic products, even if there was 100% dermal absorption of the cosmetic product. Thus, the systemic toxicity potential of these carbonate salts via oral exposure is not addressed further in this report. The primary focus of the safety assessment is the review of the safety of topical exposure to these ingredients.

Physical and Chemical Properties

These ingredients are typically colorless or white solids with low formula weights (**Table 2**). While the carbonate salts may be fairly alkaline in concentrated solution, an acceptable pH can be easily obtained in formulation.

Method of Manufacture

Ammonium Carbonate

Ammonium Carbonate may be prepared from gaseous ammonia, carbon dioxide, and steam.⁴

Calcium Carbonate

Calcium Carbonate, as used for industrial purposes, is extracted by mining or quarrying.⁵ Pure Calcium Carbonate can be produced from marble, or it can be prepared by passing carbon dioxide into a solution of calcium hydroxide. In the latter case, Calcium Carbonate is derived from the mixture, forming a grade of product called “precipitated calcium carbonate” or PCC. PCC has a very fine and controlled particle size, diameter of 2 μ , and is particularly useful in the production of paper. The other primary type of industrial product is “ground calcium carbonate” or GCC. The production of GCC involves crushing and processing limestone to create a powdery form graded by size and other properties for many different industrial and pharmaceutical applications.

Composition/Impurities

Magnesium Carbonate

The following specifications for Magnesium Carbonate are referenced in the “Evaluation of the Health Aspects of Magnesium Salts as Food Ingredients” by the Select Committee on GRAS Substances: not less than 40% and not greater than 43.5% magnesium oxide, not greater than 3 ppm arsenic, not greater than 30 ppm heavy metals, not greater than 10 ppm lead, and not greater than 0.6% calcium oxide.⁶

The *Food Chemicals Codex* specification for Magnesium Carbonate impurities states that this chemical should contain no more than 2 mg/kg lead and no more than 0.6% calcium oxide.⁷

Ammonium Bicarbonate

According to the *Food Chemicals Codex*, Ammonium Bicarbonate contains not less than 99% and not more than 100.5% NH_4HCO_3 .⁷ The following specifications for impurities in Ammonium Bicarbonate are stated as follows: chloride ($\leq 0.003\%$), lead (≤ 3 mg/kg), and sulfate ($\leq 0.007\%$).

Ammonium Carbonate

According to the *Food Chemicals Codex*, Ammonium Carbonate consists of ammonium bicarbonate (NH_4HCO_3) and ammonium carbamate ($\text{NH}_2\text{COONH}_2$), and should contain not less than 30% NH_3 and not more than 43% NH_3 . The specifications for Ammonium Carbonate impurities are as follows: chloride ($\leq 0.003\%$), lead (≤ 3 mg/kg), and sulfate ($\leq 0.005\%$).⁷

Calcium Carbonate

According to the *Food Chemicals Codex*, Calcium Carbonate contains not less than 98% and not more than 100.5% CaCO_3 on a dried basis.⁷ The following specifications for impurities in Calcium Carbonate are stated as follows: acid-insoluble substances ($\leq 0.2\%$), arsenic (≤ 3 mg/kg), fluoride ($\leq 0.005\%$), lead (≤ 3 mg/kg), and magnesium and alkali salts ($\leq 1\%$).

The European Commission’s purity criteria for Calcium Carbonate as a color for use in foodstuffs are as follows: loss on drying ($\leq 2\%$), acid-insoluble substances ($\leq 0.2\%$), magnesium and alkali salts ($\leq 1.5\%$), fluoride (≤ 50 mg/kg), antimony (≤ 100 mg/kg, singly or in combination), copper (≤ 100 mg/kg, singly or in combination), chromium (≤ 100

mg/kg, singly or in combination), zinc (≤ 100 mg/kg, singly or in combination), barium (≤ 100 mg/kg, singly or in combination), arsenic (≤ 3 mg/kg), lead (≤ 10 mg/kg), and cadmium (≤ 1 mg/kg).⁸

Potassium Bicarbonate and Potassium Carbonate

According to the *Food Chemicals Codex*, Potassium Bicarbonate is not less than 99% and not more than 101.5% KHCO_3 , calculated on the dried basis, and Potassium Carbonate is not less than 99% and not more than 100.5% K_2CO_3 on the dried basis.⁷ The specification for impurities in Potassium Bicarbonate and Potassium Carbonate states that each chemical should contain no more than 2 mg/kg lead.

USE

Cosmetic

The safety of the carbonate salts included in this assessment is evaluated based on data received from the FDA and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by Industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category. Collectively, the use frequency and use concentration data (**Table 3**) indicate that 5 of the 6 ingredients in this safety assessment are currently being used in cosmetic products; Potassium Bicarbonate is not reported as being used.

According to 2016 VCRP data, the greatest reported use frequency is for Magnesium Carbonate (317 product formulations, mostly leave-on products), followed by Calcium Carbonate (174 product formulations, mostly leave-on products) (**Table 3**).⁹ The results of a concentration of use survey conducted in 2015 indicate that Ammonium Bicarbonate has the highest maximum concentration of use; it is used at concentrations up to 93.4% in rinse-off products (hair bleaches). The maximum concentration of use in leave-on products is being reported for Calcium Carbonate (concentrations up to 35% in eyebrow pencils) (**Table 3**).¹⁰

Cosmetic products containing carbonate salts may be applied to the skin and hair or, incidentally, may come in contact with the eyes (e.g., Calcium Carbonate at maximum use concentrations up to 35% in eye area cosmetics) and mucous membranes (e.g., Calcium Carbonate at maximum use concentrations up to 10% in dentifrices). Additionally, some of these ingredients are being used in products that may result in incidental ingestion. For example, Calcium Carbonate is being used in dentifrices at maximum use concentrations up to 10%, and in lipstick at maximum use concentrations up to 8%. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Magnesium Carbonate is used in aerosol color hair sprays at maximum use concentrations up to 0.18%, and in face powders at maximum use concentrations up to 4%. Calcium Carbonate is used in powders (dusting and talcum, excluding aftershave talc) at maximum use concentrations up to 5%, and in face powders at maximum use concentrations up to 15%. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm , with propellant sprays yielding a greater fraction of droplets/particles below 10 μm , compared with pump sprays.^{11,12,13,14} 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.^{11,12} 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.^{15,16,17}

Calcium Carbonate and Magnesium Carbonate appear on the list of colorants allowed in cosmetic products that are marketed within the European Union.¹⁸ The purity criteria that have been established for Calcium Carbonate are presented in the Composition/Impurities section of this safety assessment.⁸ These criteria must be met for Calcium Carbonate when this ingredient is used as a colorant in cosmetic products.

Noncosmetic

The following carbonate salts are direct food additives that are classified as GRAS in the United States: Magnesium Carbonate, Ammonium Bicarbonate, Ammonium Carbonate, Calcium Carbonate, Potassium Bicarbonate, and Potassium Carbonate.¹⁹

The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives has determined that Magnesium Carbonate, Ammonium Carbonate, Calcium Carbonate, and Potassium Carbonate are not limited in terms of overall daily intake (mg/kg body weight).²⁰ The Committee noted that these bases are required for pH adjustment in food technology, and that the amounts and concentrations used are not likely to have any toxicological significance. Furthermore, the Committee placed no restriction on the food-additive use of these bases, provided that the contribution made to the dietary load of potassium, calcium, and magnesium is assessed and considered to be acceptable.

A grade of Calcium Carbonate that is referred to as “precipitated calcium carbonate” or PCC is particularly useful in the production of paper.⁵ Combinations of viscous xylocaine, aluminum hydroxide-Magnesium Carbonate, and diphenhydramine hydrochloride have been used to treat mucosal toxicity resulting from chemotherapy and radiotherapy in the treatment of esophageal cancer.²¹

Carbonate salts are also used as inactive ingredients in FDA-approved drug products, and these uses are summarized below:

Magnesium Carbonate

Magnesium Carbonate is used as an inactive ingredient (maximum potency of 10 mg to 250 mg) in oral drug products that have been approved by FDA.²²

Calcium Carbonate

Calcium Carbonate is used as an inactive ingredient in drug products that have been approved by FDA for otic application (maximum ingredient potency: 0.38%), buccal application (maximum ingredient potency : 145.7 mg), and inhalation exposure (maximum ingredient potency: 4.02%).²² Calcium Carbonate is also an FDA-approved inactive ingredient in oral drug products (maximum ingredient potency: 4 mg to 550 mg).

Potassium Bicarbonate

Potassium Bicarbonate is used as an inactive ingredient (maximum potency: 1.06 mg to 500 mg) in oral drug products that have been approved by FDA.²² This ingredient is also an FDA-approved inactive ingredient (maximum potency of 8 mg) in drug products administered via the transmucosal route.

Potassium Carbonate

Potassium Carbonate is used as an inactive ingredient in oral drug products (maximum ingredient potency: up to 27.69 mg) and in topical drug products (maximum ingredient potency not stated).²²

TOXICOKINETIC STUDIES

Absorption, Distribution, Metabolism, and Excretion

Animal

Oral

Magnesium Sulfate

An acid solution of magnesium chloride containing 200 μ curies of magnesium (²⁸Mg) was neutralized with 1 N sodium hydroxide and the precipitate was dissolved in 1 N sulfuric acid.²⁵ A slightly acidic solution of magnesium sulfate in distilled water (contained 5 meq of magnesium) was fed (in feed) to 3 groups of 6 rabbits (domestic rabbits of mixed breed). The 3 groups were treated as follows: group 1 (feed and water withheld for 17 h), Group 2 (animals starved for 36 h), and Group 3 (animals starved for 48 h). In the 3 groups, mean urinary excretion of radioactivity in 48 h ranged between 10% and 12.5%. An external survey of Group 2 animals at 24 h revealed a maximal concentration of radioactivity in the mid abdomen. Two of the animals were killed, and the cecum and its contents were found to contain 78% of the ingested dose. The results of this study indicate that poor gastrointestinal absorption of magnesium accounts for its low renal excretion. Additionally, absorption does not appear to occur from the large intestine.

Intraperitoneal

Calcium Carbonate

Calcium Carbonate (0.40 mCi of calcium ¹⁴C-carbonate pellet) was implanted intraperitoneally into a male rat.²⁶ Approximately 72% of the radioactivity was excreted as respiratory carbon dioxide between 2 h and 142 h after implantation (most after 69 h). Approximately 30% of the dose was recovered in unabsorbed pellet. Urinary radioactivity accounted for 0.27% of the dose and fecal radioactivity accounted for approximately 0.07% of the dose; 1% of the absorbed dose was retained by the tissues.

Sodium Bicarbonate

Rats (number, strain and sex not stated) received 5 intraperitoneal (i.p.) injections of ¹¹C-sodium bicarbonate, made at 30-minute intervals.²⁷ The dose/concentration of the test substance was not stated. The animals were killed 30 minutes after the last injection. Approximately 60% of the radioactivity was accounted for. The urine contained 1.3% of the radioactivity, and > 50% of the radioactivity occurred as respiratory ¹¹C-carbon dioxide.

Human

Oral

Calcium Carbonate

The absorbability of calcium from Calcium Carbonate and calcium citrate salts was evaluated in a study in which 37 men and women ingested 300 mg (low load) and 1000 mg (high load) calcium loads as part of a light breakfast meal.²⁸ The subjects were randomly assigned to a sequence of either Calcium Carbonate first and then calcium citrate, or vice versa. Each absorption measurement was conducted over 2 study days. On the first day, the subjects received no supplement; on the second day, the subjects received a 1000 mg calcium load. The test with the 300 mg load was performed similarly. For the high load, absorption was measured by tracer appearance in the serum and by absorptive increment in urinary calcium. For the low load, absorption was measured using the tracer method only. Test materials (in gelatin capsules) were labelled with ⁴⁵Ca. Individual tracer doses were in the range of 5 to 9 µCi. Relative absorption was estimated from the difference in urinary calcium following breakfast on the 2 days and true fractional absorption from the appearance of ⁴⁵Ca in the serum. Mean tracer absorption for both salts combined was 36% at the 300 mg load and 28.4% at the 1000 mg load. In both experiments, the observed mean difference in absorption between the salts was very small. By the tracer method, the within-subject difference (Calcium Carbonate less citrate) was +3.3% ± 1.2% of the ingested dose at the high load and 3.6% ± 2.7% at the low load.

Potassium Bicarbonate

Following ingestion, Potassium Bicarbonate rapidly dissociates in gastric juice to yield carbonate ions (bicarbonate and carbonate) and potassium ions.²⁹ At this stage, the minor alkalinity is neutralized by the stomach acid. The undissociated Potassium Bicarbonate is not expected to be systemically available in the body under normal handling and use conditions.

Potassium Carbonate

Like Potassium Bicarbonate, following ingestion, Potassium Carbonate rapidly dissociates in the gastric juice to yield carbonate ions and potassium ions.³⁰ Similarly, the dissociation into ions (in gastric juice) of the following other carbonate salts reviewed in this safety assessment would be expected: Magnesium Carbonate, Ammonium Bicarbonate, Ammonium Carbonate, and Calcium Carbonate.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Ammonium Carbonate

The acute dermal toxicity of Ammonium Carbonate was evaluated in accordance with OECD Guideline 402 using 10 (5 male, 5 female) rats of the CRL:(WI) strain.³¹ The test substance (200 mg/kg body weight) was applied to 10% of the total body surface for 24 h. The application site was covered with a gauze pad that was secured with a semi-occlusive plastic wrap. Dosing was followed by a 14-day observation period, after which necropsy was performed. None of the animals died and there were no clinical signs of toxicity. Additionally, there were no effects on body weight. It was concluded that the LD₅₀ was > 2000 mg/kg body weight.

Ammonium Carbamate

The acute dermal toxicity of ammonium carbamate (43.6% ammonium and 56.3% carbon dioxide) was studied using 10 (5 male, 5 female) rats of the Crl:CD(SD) strain.³² The test substance (in water; 5000 mg/kg weight) was applied, under a semi-occlusive patch, to the back for 24 h. Dosing was followed by a 14-day observation period and necropsy. None of the animals died and there were no test substance-related clinical findings. There also were no remarkable body weight changes during the study. At necropsy, scabbing at the application site was observed in 3 animals. The reported LD₅₀ was > 5000 mg/kg. Results relating to skin irritation are included in the section on Skin Irritation and Sensitization.

Calcium Carbonate

Calcium Carbonate (nano form) was tested in an acute dermal toxicity study involving 10 (5 male, 5 female) Wistar rats.²⁸ The test substance was applied to the back and flanks (~ 10% of total body surface area; 2000 mg/kg body weight) of each animal, and the application site was covered with a semi-occlusive patch for 24 h. The animals were killed at the end of the study and subjected to gross necropsy. None of the animals died and there were no clinical signs of systemic toxicity or dermal irritation. There was no evidence of abnormalities at necropsy. The reported acute dermal LD₅₀ was > 2000 mg/kg body weight.

Potassium Bicarbonate

The acute dermal toxicity of Potassium Bicarbonate was studied using New Zealand white rabbits (5 males, 5 females).²⁹ Potassium Bicarbonate was applied to the back at a dose level of 2000 mg/kg body weight, and the application site was covered with a 10 cm x 10 cm occlusive patch. The test substance was applied to the application site at a rate of approximately 0.05 g/cm² during a 24-h exposure period. Dosing was followed by a 14-day observation period. All animals were killed and subjected to gross necropsy. None of the animals died and they all appeared clinically normal throughout the study. With the exception of an incidental dermal finding in 1 animal, there were no visible lesions at necropsy. The slight to moderate dermal irritation observed had cleared in all animals by day 10. The dermal LD₅₀ was reported to be > 2000 mg/kg body weight.

Potassium Carbonate

An acute dermal toxicity evaluation using data on Potassium Carbonate containing a pesticide (identity and concentration of pesticide not stated; concentration of Potassium Carbonate not stated) was performed according to the U.S. EPA pesticide assessment guidelines.³⁰ The test substance, moistened with distilled water, was applied to the skin of young adult, New Zealand white rabbits (5 males, 5 females [fasted]) for 24 h. The area of application, dose per cm², and whether or not the site was covered were not stated. Application of the test substance was followed by a 14-day observation period. None of the animals died. Dermal irritation was observed at the test site; however, irritation scores were not provided. There were no gross findings at necropsy, and neither adverse pharmacologic effects nor abnormal behavior were observed. The reported dermal LD₅₀ was > 2000 mg/kg body weight for Potassium Carbonate containing a pesticide.

Ammonium Sulfate

The acute dermal toxicity of ammonium sulfate was evaluated according to OECD Guideline 434 using groups of 6 (3 males, 3 females per group) Wistar rats.²⁷ Ammonium sulfate (in water-acetone solution; single dose of 2000 mg/kg body weight) was applied to a 3 x 4 cm² area of the back. The application site was not covered. Dosing was followed by a 14-day observation period. The reported LD₅₀ was > 2000 mg/kg body weight.

Oral

Ammonium Bicarbonate

An LD₅₀ of 1576 mg/kg was reported for rats (number and strain not stated) in an acute oral toxicity study on Ammonium Bicarbonate.³³ Additional study details were not reported.

Ammonium Carbonate

The acute oral toxicity of Ammonium Carbonate (in 0.5% aqueous carboxymethyl cellulose) was studied using groups of 5 male and 5 female Wistar rats.²⁷ Ammonium Carbonate was administered to 4 groups at the following oral doses, respectively: 215 mg/kg, 681 mg/kg, 1470 mg/kg, and 2150 mg/kg. The maximum dose volume per group was 10 ml. Untreated rats served as controls. Dosing was followed by a 14-day non-treatment period. Five rats dosed with 2150 mg/kg and 3 rats dosed with 1470 mg/kg died. There were no mortalities in the 2 lower dose groups. Except for paresis, observed only in male rats (1470 mg/kg dose group), the following clinical signs were observed in male and female rats (number affected not stated) of the 1470 mg/kg dose group prior to death: dyspnea, apathy, abnormal position, staggering, tonic convulsions, exophthalmos, salivation, and poor general state. Necropsy findings in animals that died included general congestion of the glandular stomach (mucosa slightly red). There were no pathological findings in animals that were killed. An LD₅₀ of 1576 mg/kg was reported for male/female rats.

Ammonium Carbamate

Groups of male and female rats (1 to 5/group) were dosed orally (by gavage) with ammonium carbamate at up to 4000 mg/kg body weight.³² The 800, 1000, and 2000 mg/kg body weight groups consisted of 5 animals each; the remaining groups consisted of 1 animal per group. Deaths occurred within 30 minutes of exposure, and the clinical signs observed included apathy, convulsions, and accelerated respiration. Gross pathology data were not available. An LD₅₀ of 1380 mg/kg body weight was reported.

Calcium Carbonate

Calcium Carbonate (in arachis oil) was administered to 5 female Sprague-Dawley rats at a single oral dose of 2000 mg/kg body weight.²⁸ The dose volume was 10 ml/kg. Dosing was followed by a 14-day observation period and necropsy. None of the animals died and there were no clinical signs of systemic toxicity. Additionally, there were no adverse changes in body weight, and adverse effects were not observed at necropsy. The reported LD₅₀ was > 2000 mg/kg body weight.

Potassium Bicarbonate

A single oral dose of Potassium Bicarbonate (in distilled water; 2000 mg/kg body weight) was administered by gavage to Sprague-Dawley rats (5 males, 5 females).²⁹ Dosing was followed by a 14-day observation period and necropsy. None of the animals died. Except for piloerection observed in all animals during the first 30 minutes post-application, there were no treatment-related clinical signs or changes in body weight. There also were no treatment-related necropsy findings. Potassium Bicarbonate was classified as non-toxic and the reported LD₅₀ was > 2000 mg/kg body weight.

Potassium Carbonate

In an acute oral toxicity study using rats (number and strain not stated), a mean LD₅₀ of 1870 (range: 1340 to 2600) mg/kg was reported after gastric intubation with Potassium Carbonate (0.20 g/ml).³⁴ Additional study details were not presented.

In a study summarized in a REACH dossier on Potassium Carbonate, "potash calc." (composition not stated) was evaluated in an acute oral toxicity study using a procedure that was equivalent to that of the now discontinued OECD Guideline 401.³⁰ Fasted Sprague-Dawley rats (5 males, 5 females) were dosed with the test substance; dosing was followed by a 14-day observation period. None of the animals died, and there were no treatment-related clinical signs, necropsy findings, or changes in body weight. The LD₅₀ was > 2000 mg/kg body weight.

Inhalation

Ammonium Bicarbonate and Ammonia

The acute inhalation toxicity of ammonia was studied using 3 groups of male ICR mice (number per group not stated).³² The 3 groups were exposed for 1 h to ammonia (method not stated) at the following concentrations: 2408 mg/m³ (3440 ppm), 2954 mg/m³ (4220 ppm), and 3402 mg/m³ (4860 ppm). Dosing was followed by a 14-day observation period and necropsy. The lungs of mice that died (number not stated) were diffusely hemorrhagic. Acute vascular congestion and

diffuse intra-alveolar hemorrhage were observed microscopically. Mild to moderate chronic focal pneumonitis was also observed. In animals that survived, focal atelectasis and liver damage were observed in animals that were killed after the observation period. Other findings in surviving animals included: swelling and increased cytoplasmic granularity of hepatocytes (at 3340 ppm), scattered foci of frank cellular necrosis of hepatocytes (at 4220 ppm), increased necrosis of hepatocytes (at 4860 ppm), and follicular hyperplasia in the spleen (test concentrations not stated). This finding for the spleen was not observed in animals that died during exposure. It was noted that the liver lesions may have resulted from the compromised nutritional state of the mice. The LC_{50} for Ammonium Bicarbonate was deduced using the maximum quantity of NH_3 possibly released from ammonium carbamate (i.e., 43.6%). The calculated LC_{50} values for Ammonium Bicarbonate (in mice) were: 6.8 mg/L air (for 1 h exposure) and 1.7 mg/L air (4534 ppm [extrapolated using Haber's law]).

Calcium Carbonate

The acute inhalation toxicity of Calcium Carbonate was evaluated according to OECD Guideline 403 using 10 (5 males, 5 females) Wistar rats.²⁸ The animals were exposed for 4 h to aerosolized Calcium Carbonate using a nose-only exposure system. Exposure was interrupted for a total of 8 minutes. The flow of air in each tube was 0.97 l/minute, and the mean chemical aerosol concentration was 3 mg/l of air. Mass mean aerodynamic diameters (MMAD) were between 2.28 μm and 2.89 μm , with a geometric standard deviation between 1.5 and 3. Taking these values into consideration, it was assumed that the deposition of particles would occur in the upper and lower respiratory tract. Exposure was followed by a 14-day observation period. All animals were killed and subjected to gross necropsy. Ruffled fur was the only clinical sign that was observed. Slight body weight loss, followed by recovery, was observed. There were no macroscopic findings at necropsy. The reported LC_{50} was > 3 mg/l of air.

Potassium Bicarbonate

The acute inhalation toxicity of Potassium Bicarbonate was evaluated using Sprague-Dawley rats (5 males, 5 females).²⁹ The animals were exposed for 45.5 h to aerosolized Potassium Bicarbonate at a mean gravimetric chamber concentration of 4.88 ± 0.6 mg/l. Approximately 1% of the particles were of a size $< 1 \mu m$; 25% were $< 3 \mu m$. The mass mean aerodynamic diameter was $\sim 4.7 \mu m$. The following signs were observed during the first hour of exposure: decreased activity, ocular discharge, and hunched posture. Similar signs as well as facial staining and/or nasal discharge were observed after removal of the animals from the exposure chamber. All of the animals recovered from these signs within 24 h. Gross necropsy findings were unremarkable, and all tissues and organs appeared normal. The LC_{50} was reported to be $> 4.88 \pm 0.60$ mg/l air.

Potassium Carbonate

In a REACH dossier on Potassium Carbonate, an acute inhalation toxicity evaluation using data on Potassium Carbonate containing a pesticide (identity and concentration of pesticide not stated; concentration of Potassium Carbonate not stated) was performed according to the U.S. EPA pesticide assessment guidelines.³⁰ Sprague-Dawley rats (5 males, 5 females) were exposed to the aerosolized test substance (mass mean aerodynamic diameter $\approx 3.6 \mu m$) for 4.5 h. The gravimetric chamber concentration was 4.96 ± 1.14 mg/L, with approximately 3% of the particles below 1 μm , and 38% below 3 μm . Exposure was followed by a 14-day observation period. None of the animals died. Dermal necrosis and corneal opacity were observed in all animals, and damage was most severe around the mouth and on the forelimbs. There were no test substance-related gross necropsy findings. The LC_{50} was $> 4.96 \pm 1.14$ mg/L air.

Sodium Bicarbonate

The acute inhalation toxicity of sodium bicarbonate was evaluated using groups of 10 (5 males, 5 females per group) Sprague-Dawley rats.²⁷ The mass mean aerodynamic diameter of the test substance was $2.9 \pm 1.77 \mu m$. The animals were exposed to aerosolized sodium bicarbonate (4.74 ± 1.03 mg/l) for 4.5 h, followed by a 14-day observation period. None of the animals died, and necropsy was performed at the end of the observation period. Ocular/nasal discharge was observed in 6 of 10 rats. Moderately red lung tissue was reported for 1 male and 1 female. One male had slightly red lung tissue. The necropsy findings were classified as unremarkable. The reported LC_{50} was > 4.74 mg/L air.

Intravenous

Ammonium Bicarbonate

The acute intravenous (i.v.) toxicity of Ammonium Bicarbonate (in 0.03 M sodium hydroxide) was evaluated using groups of 10 young albino mice, and a mean LD_{50} value of 3.10 ± 0.28 mM/kg body weight was reported.³⁵ Additional study details were not included.

Ammonium Bicarbonate and Ammonium Carbamate

The acute i.v. toxicity of an ammonium carbamate/Ammonium Bicarbonate mixture (in 0.03 M sodium hydroxide) was evaluated using groups of 10 young albino mice.³⁵ The test substance was defined as commercial reagent grade Ammonium Carbonate, and was described as a mixture of approximately equal parts Ammonium Bicarbonate and ammonium carbamate. A mean LD₅₀ value of 1.02 ± 0.11 mM/kg body weight was reported. Additional study details were not provided.

Short-Term Toxicity Studies

Oral

Magnesium Chloride Hexahydrate

The repeated dose toxicity of magnesium chloride hexahydrate was studied using groups of Wistar rats.²⁵ The dose groups were as follows: 250 mg/kg body weight/day (10 males, 10 females), 500 mg/kg body weight/day (12 males, 12 females), and 1000 mg/kg body weight/day (15 males, 15 females). The vehicle (water) control group consisted of 12 males and 12 females. The test substance was administered orally (by gavage) to males and females daily during 14 days pre-mating and 14 days of mating. The test substance was also administered to females during gestation and up to postnatal day (PND) 3, and to males for 28 to 29 days. There were no treatment-related mortalities or major toxicological findings. There were no remarkable clinical signs or effects on hematology or clinical chemistry. The same was true for necropsy and histopathological findings relating to non-reproductive organs. It was concluded that the NOAEL for magnesium chloride hexahydrate was 1000 mg/kg body weight/day. Furthermore, it was determined that the equivalent NOAEL for Magnesium Carbonate was 414 mg/kg body weight/day. Results relating to toxic effects of magnesium chloride hexahydrate on reproductive organs are included in the section on Developmental and Reproductive Toxicity.

Calcium Carbonate

Five rats were fed ⁴⁵Ca-labeled Calcium Carbonate (0.3 g/kg body weight) in feed for 3 days.²⁶ The strain of rats tested was not identified. All of the animals remained healthy.

The oral toxicity of Calcium Carbonate (nano form) was evaluated in a 14-day study using groups of 6 (3 male, 3 female) Wistar rats.²⁸ The 4 groups consisted of the control and 250, 500, and 1000 mg/kg bodyweight/day groups. The test substance was administered once daily by gavage at a dosage volume of 5 ml/kg. Control animals were dosed with distilled water. There were no unscheduled deaths or treatment-related toxicologically significant macroscopic abnormalities. There also were no clinical signs of toxicity or adverse effects on body weight development. The NOAEL was 1000 mg/kg body weight/day.

Potassium Bicarbonate

In a 4-week toxicity study, groups of 10 male and 10 female SPF-bred Wistar rats (CpB:WU;Wistar random) were fed unsupplemented rodent diet (control) or a diet containing 2% or 4% Potassium Bicarbonate.³⁶ The animals were killed at the end of the study. None of the animals died during the study, and no treatment-related abnormalities were reported. There were no consistent or treatment-related effects on red blood cell variables, clotting potential or total and differential white blood cell counts in any of the groups. Relative kidney weights (relative to body weight) were increased; however, this finding was not consistent or considered to be dose-related. At necropsy, macroscopic examination did not reveal any significant differences among test and control groups, except for macroscopic lesions in the urinary bladder of some rats. Most of the histopathological changes observed represented background pathology that is normal for SPF-bred Wistar rats (CpB:WU; Wistar random).

Ammonium Chloride

The oral toxicity of ammonium chloride was evaluated in a 28-day study involving groups of 10 male and female Wistar rats.²⁷ Ammonium chloride was fed at a concentration of 2% (2214.5 mg/kg body weight/day) or 4% (4228.5 mg/kg body weight/day) in the diet daily for 28 days. The animals were killed at the end of the study and necropsied. Organs were subjected to microscopic examination. Significant weight loss (18% to 25% below the control value; p < 0.01) was noted for males and females of the higher dose group; food intake was decreased. Growth was also markedly decreased (p < 0.05) in the lower dose group. None of the animals died and there were no treatment-related clinical signs. Urinalyses revealed a dose-dependent increase in net acid excretion. There were no consistent or treatment-related defects on red blood cell

variables, clotting potential or total and differential white blood cell counts. Alkaline phosphatase activity was increased in the higher dose group (27.7% over controls, both sexes) and in low-dose males (27.7% over controls). There were no consistent or treatment-related changes in the following organ weights: liver, spleen, ovaries, pituitary, thyroid, thymus, or heart. Relative kidney weight (relative to body weight) was increased in both dose groups. Relative adrenal weight was increased only in males of the higher dose group. There were no significant differences in necropsy findings between test and control groups. Most of the histopathological changes observed were nearly equally distributed among control and test groups, and represented normal background pathology for rats of this strain and age. It was noted that most of the changes could be regarded as physiological adaptations to the feeding of acid-forming salt.

Ammonium chloride was fed (in the diet) to 10 male Sprague-Dawley rats at 684 mg/kg body weight/day (7 days per week) for 70 days.³² Negative and positive control (tributyl phosphate) groups were included in the study. A 7-day post-exposure period was observed, and gross and microscopic examinations of the bladder were performed. There were no significant differences in food consumption among the 3 groups. The difference also was not significant when animals fed ammonium chloride were compared to the negative control group. Body weight was significantly decreased in the positive control group. Gross and microscopic examination results were negative for animals fed ammonium chloride and for the negative control group. Hyperplasia of the bladder was observed in the positive control group. The NOEL for ammonium chloride was < 684 mg/kg body weight/day.

Inhalation

Potassium Carbonate

The potential for short-term toxicity and neurotoxicity of a Potassium Carbonate-based scrubbing solution (containing 30.8% (w/v) Potassium Carbonate) used in petroleum refineries was evaluated in Sprague-Dawley CrI:CD BR rats.³⁷ Inhalation exposures were to aerosols of a “used” scrubbing solution in a whole-body exposure chamber, 6 h/day for 21 consecutive days at target concentrations of 0 (filtered air – control), 0.1, 0.2, or 0.4 mg/L (30 animals/sex/group). Five rats per sex per group were allowed a 14-day recovery period (satellite recovery group) and killed on study day 35 for either systemic or neurotoxic evaluation. Functional observation battery examinations and locomotor activity tests were conducted. No apparent adverse effects were noted at any exposure level, as determined by clinical observations, food consumption measurements, hematology, serum chemistry, ophthalmologic observations, and gross pathology evaluations. Statistically significant increases in lung weights were noted at all concentrations; all lung weights returned to control values at the end of exposure, except for the 0.4 mg/L group (females). There were no significant changes in other organ weights. Histopathologic findings were restricted to the respiratory tract and were characterized by minimal to moderate epithelial hyperplasia, epithelial necrosis, and cytoplasmic vacuolation at levels I and II of the nasal cavities. The mild cytoplasmic vacuolization of the olfactory epithelium was observed in the 0.2 and 0.4 mg/l exposure groups. Minimal epithelial necrosis at level II of the nasal cavities was observed in the 0.4 mg/l exposure group. Lung bronchiolization and alveolar macrophage infiltration were also observed in the 0.2 and 0.4 mg/l exposure groups. The respiratory tract findings were considered a local response to the high alkalinity of the test material, as substantiated by the return to normal upon cessation of exposure. Exposure to the scrubbing solution had no adverse effect on functional observation battery endpoints or locomotor activity, brain weight and size, and neuropathologic assessments. The authors concluded that inhalation exposure to a Potassium Carbonate-based scrubbing solution aerosol for 21 days did not result in any persistent systemic toxicity, including neurotoxicity, in male or female rats.

Subchronic Toxicity Studies

Oral

Potassium Bicarbonate

In a 13-week toxicity study, groups of 10 male and 10 female SPF-bred Wistar rats (CpB:WU;Wistar random) were fed unsupplemented rodent diet or a diet containing 2% or 4% Potassium Bicarbonate.³⁶ The results reported in this study were identical to those stated in the 4-week study on Potassium Bicarbonate (in the Short-term Oral Toxicity section), except for the following: Zona glomerulosa hypertrophy (classified as a non-neoplastic histopathological change) was observed at a concentration of 4%, and this finding was statistically significant ($p < 0.01$) compared to the control. The finding of oncocytic kidney tubules (also classified as non-neoplastic histopathological change) was statistically significant, compared to the control, at a concentration of 4% ($p < 0.01$).

The effect of Potassium Bicarbonate (2% or 4%) on rat urinary bladder epithelium was studied without prior exposure to a bladder tumor initiator.³⁸ In 4 studies, ranging in duration from 4 to 130 weeks, equimolar amounts of K⁺ were administered in the diet to male and female weanling, SPF-bred Wistar rats (Cpb:WU; Wistar random) (85 rats/sex/group) as

Potassium Bicarbonate. Increased urinary volume and potassium levels were observed, and urinary pH was increased. Results relating to carcinogenicity are included in the Carcinogenicity section

Groups of 10 weanling male SPF-bred albino rats (Cpb: WU; Wistar random) were fed a basal diet or diet supplemented with Potassium Bicarbonate (2.5% in the diet) for up to 13 weeks.³⁹ A group of 10 rats was also fed 6% monosodium glutamate (MSG) in the diet. Feeding with MSG induced slight growth retardation, decreased food intake (mainly with the purified diet), and increased kidney-to-body weight ratios. The addition to stock diet of 2.5% Potassium Bicarbonate, instead of MSG, induced changes in growth rate, food intake, and kidney weight that were similar to those observed with 6% MSG. Results relating to carcinogenicity are included in the Carcinogenicity section.

Another 13-week study was performed using groups of 10 weanling male SPF-bred albino rats (Cpb: WU; Wistar random) to compare the effects of 5% Potassium Bicarbonate in a stock diet and in a purified diet.³⁹ Unsupplemented stock and purified diets served as controls. The rats were gradually accustomed to the high level of Potassium Bicarbonate (5%) by feeding 1% in the diet during week 1, 2% in the diet during week 2, 3% in the diet during week 3, 4% in the diet during weeks 4 and 5, and 5% in the diet from week 6 on. Growth was retarded by 5% Potassium Bicarbonate in the groups on either basal diet; the difference was not statistically significant for the rats fed 5% Potassium Carbonate in the stock diet, but it was statistically significant in the rats fed 5% Potassium Carbonate in the purified diet. None of the rats had any abnormalities in condition or behavior. At microscopic examination, there were no treatment-related changes in the following organs: ureters, kidneys, liver, testes, thyroid with parathyroids, adrenals and. Results relating to hyperplastic changes in the bladder epithelium are included in the Carcinogenicity section.

Chronic Toxicity Studies

Oral

Potassium Bicarbonate

In an 18-month toxicity study, groups of 15 male and 15 female SPF-bred Wistar rats (CpB:WU;Wistar random) were fed unsupplemented rodent diet or a diet containing 2% or 4% Potassium Bicarbonate.³⁶ The animals were killed at the end of the study. There were no consistent or treatment-related effects on red blood cell variables, clotting potential or total and differential white blood cell counts in any of the groups. At necropsy, macroscopic examination did not reveal any significant differences among test and control groups, except for macroscopic lesions in the urinary bladder of some rats. Most histopathological changes observed were considered equally distributed among the treatment groups and the controls, and represented normal background pathology for rats of this strain and age. The following statistically significant changes (compared to the control) were observed: zona glomerulosa hypertrophy (classified as a non-neoplastic histopathological change) at a concentration in feed of 4% ($p < 0.01$); oncocytic kidney tubules (classified as a non-neoplastic histopathological change) at a concentration of 2% ($p < 0.05$) and 4% ($p < 0.01$); simple urothelial hyperplasia of the urinary bladder (classified as a non-neoplastic histopathological change) at concentrations of 2% and 4% ($p < 0.05$); and papillary/nodular hyperplasia of the urinary bladder (classified as a non-neoplastic histopathological change) at a concentration of 2% ($p < 0.01$). The papillary/nodular hyperplasia of the urinary bladder observed in the 4% dietary group was not statistically significant.

In a 30-month carcinogenicity study, groups of 50 SPF-bred weanling Wistar rats (CpB:WU;Wistar random) per sex were fed a natural ingredient diet (controls) or diet supplemented with 2% or 4% Potassium Bicarbonate.³⁶ There were no treatment-related mortalities. At necropsy, macroscopic examination did not reveal any significant differences among test and control groups, except for macroscopic lesions in the urinary bladder of some rats. Most histopathological changes observed were considered equally distributed among the treatment groups and the controls and represented normal background pathology for rats of this strain and age. Results relating to carcinogenicity are included in the Carcinogenicity section.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Magnesium Chloride Hexahydrate

The reproductive and developmental toxicity of magnesium chloride hexahydrate was studied using groups of Wistar rats.²⁵ The dose groups were as follows: 250 mg/kg body weight/day (10 males, 10 females), 500 mg/kg body weight/day (12 males, 12 females), and 1000 mg/kg body weight/day (15 males, 15 females). The vehicle (water) control group consisted of 12 males and 12 females. The test substance was administered orally (by gavage) to males and females daily during 14 days pre-mating and 14 days of mating. The test substance was also administered to females during gestation

and up to PND 3, and to males for 28 to 29 days. No test substance-related histopathological lesions were observed in the reproductive organs of male or female rats dosed with the test substance (all dose groups). There were no treatment-related effects with respect to the following when compared to controls: mean number of corpora lutea, number of implantation sites, total number of pups born, number of males, number of females, sex ratio, live pups, still birth, runt on PND 0, and total number of live pups and sex ratio on PND 4, pre-implantation loss and post-implantation loss. The survival of pups (in all treatment groups) from PND 0 to PND 4 was not affected by treatment. At necropsy, there were no gross external abnormalities in pups from any dose group. It was concluded that the NOAEL for reproductive/developmental toxicity of magnesium chloride hexahydrate was 1000 mg/kg body weight. Furthermore, it was determined that the equivalent NOAEL for Magnesium Carbonate was 414 mg/kg body weight/day. Additional results from this study are included in the Short-Term Oral Toxicity section of this report.

The teratogenicity of magnesium chloride hexahydrate (in distilled water) was evaluated using groups of 22 pregnant female Wistar rats.²⁵ The 3 dose groups received oral doses (by gavage) of 200, 400, and 800 mg/kg/day, respectively, on gestation days 6 to 15 (10 days). A fourth group served as the control. The animals were killed on day 20 of pregnancy. Regarding maternal toxicity, there were no clinical signs or deaths. Dosing (all groups) did not cause increased incidences of the following: number of implantations, number of corpora lutea, % implantation loss, number of offspring alive, sex ratio of offspring, offspring weight or embryo/intrauterine fetal death. Gross malformation was observed in the dose groups, but without any intergroup differences. Bone malformation was observed in one fetus of the 800 mg/kg/day dose group. There were no intergroup differences in bone abnormality, effects on lumbocostale, extra ribs, sacrococcygea, metacarpal bone or its ossification. Visceral malformations were observed in 4 to 6 fetuses from each dose group, but without intergroup differences. It was concluded that magnesium chloride hexahydrate was not teratogenic in rats dosed by gavage. The NOAEL was estimated to be > 800 mg/kg body weight/day for pregnant rats and their fetuses. The equivalent NOAEL for Magnesium Carbonate was determined to be > 331 mg/kg body weight/day.

Calcium Carbonate

Female Swiss mice were bred after being fed (number of animals/feeding duration not stated) a diet supplemented with 0.5%, 1%, or 2% Calcium Carbonate.²⁶ First and second litters were studied. Calcium Carbonate (1% and 2% in diet) yielded an intake of approximately 3000 mg/kg body weight. When compared to the control diet, feeding with the supplemented diet significantly decreased the number and total weight of the weanling mice, and increased the proportion of deaths. Calcium Carbonate (2%) in the diet also caused hypertrophy of the heart and a tendency toward decreased thymus weight in weanling mice.

The reproductive toxicity of Calcium Carbonate (nano form) was studied using groups of 20 (10 male, 10 female) Wistar rats.²⁸ Three groups received oral dosage rates of 100, 300, and 1000 mg/kg body weight/day, respectively. The doses were administered daily for 48 consecutive days (including a 2-week maturation phase, pairing, gestation, and early lactation). Untreated animals served as controls. The dosage volume was 5 ml/kg. Males were killed on day 43 and females were killed on day 5 post-partum. Gross pathology and histopathology were performed. There were no test substance-related mortalities or toxicologically significant macroscopic or microscopic findings in parental animals. All offspring were subjected to a full external and internal examination; any macroscopic abnormalities were recorded. There were no test substance-related effects on reproductive performance and length of gestation. When compared to controls, there were no significant differences with respect to corpora lutea and implantation counts. Litter sizes and viability for treated groups were also comparable to controls. There also were no obvious clinical signs of toxicity in offspring from treated females, or test substance-related gross pathological findings in offspring. Because there were no treatment-related effects on reproduction, the NOEL for reproductive toxicity was considered to be 1000 mg/kg body weight per day.

In a 62-day developmental toxicity study involving 4 groups of female Charles River CD/VAF Plus rats (mated with male rats), Calcium Carbonate (in the diet) was fed at concentrations of 0.05% (control), 0.75%, 1%, and 1.25% for 6 weeks prior to mating, during mating, and for 20 days of gestation.²⁸ For each dose group, the number of females dosed prior to mating was 69 and the number of pregnant rats dosed (through mating to gestation day 20) ranged from 45 to 48. The male to female ratio per cage was 1:2. Male rats were dosed only during the mating period. On gestation day 20, the animals were killed and Cesarean sections were performed. There was no evidence of test substance-related maternal toxicity or embryotoxic/teratogenic effects. There were no dose-related changes in the average number of implantations, resorptions and viable fetuses, or fetal length or weight. When compared to the control group, there were no statistically significant increases in the litter incidence regarding specific external, visceral, or skeletal variations of the fetuses. The NOAEC for teratogenicity was > 1.25% Calcium Carbonate in the diet, and corresponded to a NOAEL between 1963 and 2188 mg/kg body weight per day.

Potassium Carbonate

The teratogenicity of Potassium Carbonate was evaluated using groups of 22 to 25 CD-1 mice, according to a protocol similar to OECD Test Guideline 414.^{30,40} The test substance was administered, by gavage, at doses of 0, 2.9, 13.5, 62.5, or 290 mg/kg body weight/day, on gestation days 6 through 15. On day 17, Caesarean section was performed on all of the dams, and the following information was recorded: sex, numbers of corpora lutea, implantation sites, resorption sites, live and dead fetuses, and body weights of live pups. The urogenital tract of each dam was examined in detail for anatomical normality. All of the fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses in each litter were subjected to detailed visceral examinations, and the remaining two-thirds were examined for skeletal defects. There were no effects on mortality, body weight gain, or the urogenital tracts of dams. The no-observed-effect-level (NOEL) for maternal toxicity was 290 mg/kg body weight/day (i.e., the highest dose tested). There were no effects on any of the following: numbers of corpora lutea, live litters, implantations, resorptions, live and dead fetuses, the sex ratio of the fetuses, or the average fetal weight. The incidence of soft tissue and skeletal abnormalities within groups treated with Potassium Carbonate did not differ from that of sham-treated controls. The NOEL for developmental toxicity/teratogenicity was 290 mg/kg body weight/day.

The teratogenicity of Potassium Carbonate was also evaluated using groups of 22 to 25 albino rats (Wistar-derived stock).⁴⁰ The test substance was administered (by oral intubation), on gestation days 6 through 15, at dose rates of 0, 1.8, 8.4, 38.8, or 180 mg/kg body weight/day according to the procedure in the preceding experiment, except that Caesarean section was performed on day 20. There were no discernible effects on nidation or on maternal or fetal survival. Furthermore, the number of abnormalities observed in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Sodium Bicarbonate

The embryotoxicity/teratogenicity of sodium bicarbonate was evaluated using groups of 25 female Wistar rats.²⁷ After mating, groups of female rats were dosed orally (intubation) with sodium bicarbonate (in water) on gestation days 6 through 15. The following dosages were administered to the 4 groups, respectively: 3.4, 15.8, 73.3, and 340 mg/kg body weight. Two additional groups were sham-exposed and dosed with aspirin (positive control), respectively. The animals were killed on gestation day 17. There was no evidence of embryotoxicity or teratogenicity. The number of abnormalities observed in either soft or skeletal tissues of test animals did not differ from the number occurring spontaneously in sham-treated controls.

The embryotoxicity/teratogenicity of sodium bicarbonate was evaluated using groups of nulliparous female Sprague-Dawley rats of the Crj:CD(SD) strain.³¹ After mating was confirmed (designated as gestation day 0), the animals were dosed orally with 2% sodium bicarbonate (in drinking water) on gestation days 15 through 20. Two groups were dosed orally (by gavage) with 0.5% aqueous methylcellulose on day 16 of gestation, and were given either tap water (control group) or 2% sodium bicarbonate solution in drinking water. An untreated group served as the concurrent control. The animals were killed on gestation day 20 and fetal external examinations performed. Dosing with sodium bicarbonate had no effect on the number of implants, % resorptions, or the number of live fetuses per litter. The average body weights of the live fetuses of pregnant females treated with sodium bicarbonate were comparable to those of the control group. There were no treatment-related abnormalities in groups treated with sodium bicarbonate.

The developmental toxicity of sodium bicarbonate was studied using groups of 50 (25 males, 25 females/group) CD-1 mice.³² Four groups were dosed orally (by gavage) with sodium bicarbonate at 5.8 mg/kg body weight, 27 mg/kg body weight, 125 mg/kg body weight, and 580 mg/kg body weight, respectively, on days 6 through 15 of gestation (17 days). A fifth group served as the sham-treated control. The number of abnormalities observed in soft or skeletal tissues of the treatment groups did not differ from the number occurring spontaneously in the sham-treated controls.

Inhalation

Potassium Carbonate

The developmental toxicity potential of a scrubbing solution containing 30.8% Potassium Carbonate, used extensively in petroleum refineries to remove CO₂ from hydrogen gas streams, was evaluated.⁴¹ Pregnant female CD (Sprague-Dawley) rats (number not stated) were exposed to aerosols of a "used" scrubbing solution at 0.05, 0.1, 0.2, or 0.3 mg/L for 6 h/day on days 6-19 of pregnancy. Control animals were exposed to filtered air under the same exposure conditions. Dams were killed on day 20 of pregnancy and a laparohysterectomy was performed. The mass median aerodynamic diameter of aerosol particles ranged from 1.6 to 2.8 μ , with geometric standard deviations between 2.0 and 2.3 μ . The overall pregnancy rate was high (> 95%) and equivalent across all groups. All pregnant dams had live litters, and 22-24 litters were examined in each group. Treatment-related clinical signs consisted of rales, observed at all exposure levels, and gasping only at the 0.3 mg/L exposure level. The occurrence of rales was presumably a localized effect on the

respiratory tract, and was likely due to the irritating properties of the scrubbing solution. Maternal toxicity was exhibited in the 0.3 mg/L group, including reduced body weight, weight gain, and food consumption, and one possible treatment-related death on gestation day 17. At the scheduled necropsy, there were no treatment-related, gross pathological observations and no statistically significant differences in measurements of reproductive and developmental parameters. The incidences of fetuses with skeletal variations involving the sternum were clustered in two litters at the highest exposure level, with atypically low-term fetal body weights. Under the conditions of this investigation, Potassium Carbonate scrubbing solution was not a developmental toxicant.

GENOTOXICITY STUDIES

In Vitro

Ammonium Carbamate

Ammonium Carbonate is a mixture of Ammonium Bicarbonate and Ammonium Carbamate. The genotoxicity of ammonium carbamate was evaluated in the Ames test at concentrations up to 5000 µg/plate with and without metabolic activation.³² The positive controls were as follows: 4-nitroquinoline-*N*-oxide, 9-aminoacridine, 2-aminoanthracene (2-AA), *N*-methyl-*N*-nitroso-*N*-nitrosoguanidine (MNNG), and 4-nitro-*o*-phenylenediamine. Ammonium carbamate was not genotoxic.

Calcium Carbonate

The genotoxicity of Calcium Carbonate (nano form) was studied using the mammalian chromosome aberration test, performed in accordance with OECD Guideline 473.²⁸ Human lymphocytes were incubated with the test substance at concentrations up to 1000 µg/ml with and without metabolic activation. Cyclophosphamide and mitomycin C served as positive controls. Calcium Carbonate (nano form) did not cause a statistically significant increase in the frequency of cells with chromosome aberrations either with or without metabolic activation. The test substance was classified as non-clastogenic.

Potassium Bicarbonate

The genotoxicity of Potassium Bicarbonate was evaluated in the Ames test (with and without metabolic activation) using *Salmonella typhimurium* (*S. typhimurium*) strains TA1535, TA1537, TA1538, TA98, and TA100, and *Saccharomyces cerevisiae* strain D4.⁴² Potassium Bicarbonate was tested at concentrations up to 0.1580% in bacteria and at concentrations up to 3.3% in yeast. Test results were negative with and without metabolic activation, and Potassium Bicarbonate was classified as non-genotoxic.

Potassium Carbonate

In the Ames test, Potassium Carbonate was not genotoxic in *Saccharomyces cerevisiae* strain D4 (yeast) or in the following bacterial strains with or without metabolic activation: *S. typhimurium* strains: TA1535, TA1537, and TA1538. Details relating to the test procedure were not included.²⁶

Potassium Chloride

The following data on potassium chloride (surrogate compound) are included in a REACH dossier on Potassium Carbonate. The genotoxicity of potassium chloride was evaluated in the L5178Y mouse lymphoma cell mutagenesis assay, at concentrations up to 5000 µg/ml with and without metabolic activation.^{30,43} Ethyl methanesulfonate and 3-methylcholanthrene served as positive controls. Results were negative with and without metabolic activation. The positive controls were genotoxic.

Ammonium Sulfate

The genotoxicity of ammonium sulfate (in acetone) was evaluated in the mammalian cell gene mutation assay using V79 Chinese hamster fibroblasts.²⁷ The test procedure was consistent with OECD Guideline 476. Ammonium sulfate was tested at concentrations up to 1320 µg/ml with and without metabolic activation. Results were negative with and without metabolic activation.

Magnesium Chloride

Magnesium chloride was evaluated in the mammalian chromosome aberration test, without metabolic activation, in accordance with OECD Guideline 473.²⁵ The test substance (in physiological saline) was evaluated at doses up to 2 mg/ml in Chinese hamster lung fibroblast (V79) cultures incubated for up to 48 h. Untreated and vehicle-treated cultures served as controls. The incidence of polyploid cells at 48 h was 1%, and the incidence of cells with structural chromosomal aberrations at 24 h was 2%. It was concluded that magnesium chloride was non-genotoxic in this assay.

In Vivo

Ammonium Chloride

The genotoxicity of ammonium chloride (in saline) was evaluated in the micronucleus test.²⁷ Groups of 6 male mice of the ddY strain received i.p. doses up to 500 mg/kg body weight. Control mice were injected i.p. with mitomycin C. Bone marrow from the femur was analyzed. One thousand polychromatic erythrocytes per mouse were scored and the number of micronucleated erythrocytes was recorded. Ammonium chloride was not genotoxic in this assay.

ANTI-GENOTOXICITY STUDIES

In Vitro

Magnesium Carbonate

The anti-genotoxicity of Magnesium Carbonate in the presence of hydrogen peroxide was evaluated in the Ames test using *S. typhimurium* strain 102.⁴⁴ Magnesium Carbonate was tested at a concentration of 25 mM or 50 mM, and each concentration was tested in the presence of 82 mM or 164 mM hydrogen peroxide. Magnesium Carbonate did not cause a decrease in the number of revertants induced by hydrogen peroxide. The number of revertants induced by hydrogen peroxide (164 mM) alone was 695.50 ± 62.7 . The combination of Magnesium Carbonate (50 mM) + hydrogen peroxide (164 mM) yielded 746 ± 202 revertants. A control value of 334.20 ± 47.98 revertants was reported.

Magnesium Carbonate was also evaluated for anti-genotoxicity at concentrations of 50 mM and 100 mM (in the presence of hydrogen peroxide) in the suspension test using strain D7 of *Saccharomyces cerevisiae*.⁴⁴ Both stationary and logarithmic phase cells were used. The high concentration of Magnesium Carbonate (100 mM) was found to be cytotoxic only in cells in the logarithmic growth phase. Magnesium Carbonate significantly decreased the gene conversion frequency that was induced by 200 mM hydrogen peroxide. Also, the point reverse mutations induced by 200 mM and 400 mM hydrogen peroxide were statistically significantly decreased in the presence of Magnesium Carbonate. In the logarithmic growth phase, Magnesium Carbonate caused a significant decrease in the gene conversion frequency that was induced by 50 mM and 100 mM hydrogen peroxide. The anti-genotoxic effect of Magnesium Carbonate was not found to be dose-dependent.

The effects of Magnesium Carbonate on the genotoxicity induced by nickel subsulfide were examined using Chinese hamster ovary (CHO) cells and BALB/3T3 fibroblast cells.⁴⁴ The cells were incubated, with and without nickel subsulfide (at 1 µg/ml), in the presence of various concentrations of Magnesium Carbonate (0.6, 1.2, 2.4 µg/ml) to give final molar ratios of 0.25, 0.5, and 1.0. The suppression of up to 64% of the proliferation of BALB/3T3 fibroblasts by nickel subsulfide (1 µg/ml) was reversed by Magnesium Carbonate, having recovered slowly in a dose-dependent manner. The nickel compound increased not only the number of micronuclei, but also the amount of DNA-protein cross-links examined with CHO and BALB/3T3 cells, respectively. These genotoxic effects of nickel were again lessened by Magnesium Carbonate. The nickel subsulfide at 1 µg/ml increased the number of micronuclei from 12 to 54 in controls, out of 500 binucleated cells. This number was reduced to 34 upon Magnesium Carbonate co-treatment at 2.4 µg/ml. The DNA-protein cross-links coefficient of 1.63 obtained in the presence of nickel was decreased to 1.39 with Magnesium Carbonate co-treatment at 2.4 µg/ml.

CARCINOGENICITY STUDIES

Oral

Potassium Bicarbonate

The effect of Potassium Bicarbonate (2% or 4%) on rat urinary bladder epithelium was studied without prior exposure to a bladder tumor initiator.³⁸ In 4 studies, ranging in duration from 4 to 130 weeks, equimolar amounts of potassium were administered in the diet to male and female weanling, SPF-bred Wistar rats (Cpb:WU; Wistar random) (85 rats/sex/group) as Potassium Bicarbonate. Control rats were fed a cereal-based open formula diet. The feeding of Potassium Bicarbonate (2% and 4% concentrations) resulted in simple epithelial hyperplasia and, after prolonged administration, in papillary/nodular hyperplasia, papillomas, and transitional cell carcinomas of the urinary bladder. The incidence of hyperplastic and neoplastic bladder lesions tended to be higher in rats fed 4% Potassium Bicarbonate than in those fed 2% Potassium Bicarbonate, suggesting a dose-response relationship. Based on these results, the authors concluded that Potassium Bicarbonate is capable of inducing urinary bladder cancer in rats without prior application of an initiator.

Groups of 10 weanling male SPF-bred albino rats (Cpb: WU; Wistar random) were fed a basal diet or diet supplemented with Potassium Bicarbonate (2.5% in the diet) for up to 13 weeks.³⁹ A group of 10 rats was also fed 6% monosodium glutamate (MSG) in the diet. The rats that received 6% MSG in the diet showed an increased incidence and degree of focal and diffuse hyperplasia of the bladder epithelium. The group that received Potassium Bicarbonate in the diet also had epithelial hyperplasia in the urinary bladder. Hyperplasia of the epithelium lining the renal pelvis or papilla was not observed in these animals.

Another 13-week study was performed using groups of 10 weanling male SPF-bred albino rats (Cpb: WU; Wistar random) to compare the effects of 5% Potassium Bicarbonate in a stock diet and in a purified diet.³⁹ Unsupplemented stock and purified diets served as controls. The rats were gradually accustomed to the high level of Potassium Bicarbonate (5%) by feeding 1% in the diet during week 1, 2% in the diet during week 2, 3% in the diet during week 3, 4% in the diet during weeks 4 and 5, and 5% in the diet from week 6 on. The microscopic examinations of the urinary bladder, ureters, kidneys, liver, testes, thyroid with parathyroids, adrenals and bone revealed changes considered to be related to treatment only in the bladder epithelium. These changes comprised various forms and degrees of epithelial hyperplasia and very small intra-epithelial cysts. An increased incidence and severity of hyperplasia occurred in each of the two groups that received Potassium Bicarbonate. Generally, the hyperplastic changes were diffuse, and their degree varied from minimal to moderate. More severe hyperplasia (papillomatous) was present in one rat fed Potassium Bicarbonate in the stock diet. Both the incidence and the degree of the epithelial changes indicated a more marked effect of Potassium Bicarbonate in the stock diet than in the purified diet.

In a 30-month carcinogenicity study, groups of 50 SPF-bred weanling Wistar rats (CpB:WU;Wistar random) per sex were fed a natural ingredient diet (controls) or diet supplemented with 2% or 4% Potassium Bicarbonate.³⁶ There were no treatment-related mortalities. At necropsy, macroscopic examination did not reveal any significant differences among test and control groups, except for macroscopic lesions in the urinary bladder of some rats. Most histopathological changes observed were considered equally distributed among the treatment groups and the controls and represented normal background pathology for rats of this strain and age.

Dose-related increases in the incidence of zona glomerulosa hypertrophy (classified as a non-neoplastic histopathological change) occurred in all treatment groups (both sexes) and was statistically significant ($p < 0.01$) when compared to the control. At week 13, oncocytic tubules were noted in males and females fed 2 or 4% Potassium Bicarbonate; after 30 months, the incidence of this lesion was much higher in the treated rats when compared to the background incidence in controls. No progression to oncocytomas was noted. The incidences of simple epithelial hyperplasia and of papillary/nodular hyperplasia of the urinary bladder were increased in the 2% and 4% Potassium Bicarbonate groups. Urothelial hyperplasia (classified as a non-neoplastic histopathological change) and papillary/nodular hyperplasia of the urinary bladder were statistically significant ($p < 0.01$), compared to the control. The incidence of (multiple) transitional cell papilloma (benign) in the urinary bladder was 2 (in males) and 6 (in females; $p < 0.05$) for animals dosed with 4% Potassium Bicarbonate. The incidence of transitional cell carcinoma (malignant) in the urinary bladder was 1 (in males) and 3 (in females) dosed with 4% Potassium Bicarbonate. These changes indicate an association between prolonged treatment with Potassium Bicarbonate and urinary bladder cancer. Except for the preneoplastic and neoplastic lesions in the urinary bladder, there were no treatment-related changes in any specific tumor type among the groups. In females, relatively high incidences of adenocarcinomas were found in the uterus with 4% Potassium Bicarbonate, but because these changes were not accompanied by preneoplastic alterations in this 30-month study or in the 18-month chronic oral study (in Chronic Oral Toxicity section) and because their incidences were within the range of historical control data, they were not deemed treatment-related.

Additionally, the total number of rats with tumors and the total incidence of tumors were not affected by treatment. Although the number of Potassium Bicarbonate-fed males with malignant tumors reached the level of statistical significance, the difference compared to the controls was not statistically significant when the number of urinary bladder lesions was excluded from the evaluation. In summary, apart from the effects on the urinary bladder, treatment with Potassium Bicarbonate did not affect the type, incidence, or multiplicity of tumors, or the time of tumor appearance or the ratio of benign-to-malignant tumors.³⁶

Co-Carcinogenicity

Sodium Bicarbonate

The co-carcinogenicity of sodium bicarbonate in the presence of *o*-phenylphenol was evaluated using groups of Fischer 344 male rats.³² The 6 dietary groups (31 rats/group) in this study were: Group 1 (2% sodium *p*-phenylphenol [OPP-Na] in diet), Group 2 (1.25% OPP + 0.64% sodium bicarbonate in diet), Group 3 (1.25% OPP + 0.32% sodium bicarbonate in diet), Group 4 (1.25% OPP + 0.16% sodium bicarbonate in diet), Group 5 (1.25% OPP or 0.64% sodium bicarbonate in diet), and Group 6 (1.25% OPP or 0.64% sodium bicarbonate in diet). The control group (fed plain diet) consisted of 30 rats. The groups were fed continuously for 104 weeks. In week 104, the % survival was 84% (26 of 31 rats) for animals exposed to sodium bicarbonate and 73% (22 of 30 rats) in the control group. When compared to the control, the final body weight was statistically significantly lower in all dietary groups. The relative weight (organ/body weight %) of the kidneys and liver was statistically significantly increased when compared to the control. Also, when compared to the control group, animals fed sodium bicarbonate in the diet did not have a statistically significant increase in the number of tumors. The first bladder tumor was observed in the rat that died in week 49. The number of rats that survived for 104 weeks is unknown. Sodium bicarbonate alone in the diet did not have a carcinogenic effect on the urinary bladder of rats. Papillary or nodular hyperplasia and papilloma incidence did not differ between test animals and the control group.

Tumor Promotion

Sodium Bicarbonate

The following data on sodium bicarbonate (surrogate compound) are included in a REACH dossier on Ammonium Carbonate.³¹ The tumor promotion potential of sodium bicarbonate in male 344 rats dosed with the carcinogen *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) was evaluated in a 32-week study. Ten groups of 20 rats received drinking water with 0.05% BBN and 2 groups of 10 rats (control groups) received drinking water without BBN for the first 4 weeks of the study. At 3 days after the cessation of BBN treatment, 4 groups received a powdered basal diet containing 0.375%, 0.75%, 1.5%, and 3% sodium bicarbonate, respectively; the control group received basal diet only. The remaining 5 groups consisted of 4 groups that received powdered basal diet containing the 4 concentrations of sodium bicarbonate, respectively, + 5% ascorbic acid and the control group that received basal diet + 5% ascorbic acid for 32 weeks. The 2 groups of 10 rats (controls) that received drinking water without BBN were maintained on powdered basal diet containing 3% sodium bicarbonate or 5% ascorbic acid + 3% sodium bicarbonate for 32 weeks. The total observation period was 36 weeks and 3 days. In a second experiment, the rats were randomly divided into 4 groups of 5 rats and 4 groups received powdered basal diet with the following components, respectively: 3% sodium bicarbonate, 5% ascorbic acid, 3% sodium bicarbonate + 5% ascorbic acid. The fifth group (control) received basal diet with no added chemicals.

In 3 of the 10 groups dosed with BBN, the urinary bladder had multiple large tumors. Papillary or nodular hyperplasia and papillomas were observed. Urinary pH and sodium concentrations were increased in rats fed sodium bicarbonate only if they had been pretreated with BBN. Similar results were not reported for animals fed sodium bicarbonate only. The results of this study confirmed that the dose-dependent increase in both urinary pH and sodium concentration and the dose-dependent promotion of urinary bladder carcinogenesis were parallel effects of sodium bicarbonate. Study results also indicated that ascorbic acid administered orally acted as an amplifier (a co-promoter), though this vitamin had no promotion potential.³¹

OTHER RELEVANT STUDIES

Nephrotoxicity

The possible toxic effects of a Potassium Carbonate emulsion (pH not stated) on some biomarkers of tissue damage was investigated using groups of 4 California rabbits.⁴⁵ The rabbits received Potassium Carbonate emulsion orally, via drinking, at doses of 50 mg/L and 100 mg/L for 14 consecutive days. The control group received physiological saline. At

the higher concentration (100 mg/L), the emulsion significantly increased uric acid, creatinine, and urea by 126.3%, 48.6% and 458.8%, respectively, compared to the control ($P < 0.05$). Oral administration of the emulsion at a concentration of 50 mg/L caused a statistically significant increase in urea, creatinine, and uric acid by 253.8%, 38.6% and 88.8%, respectively, compared to the control. Also, Potassium Carbonate emulsion statistically significantly increased serum blood urea nitrogen (BUN) at concentrations of 50 mg/L and 100 mg/L. The results of this study suggested that oral exposure to excessive amounts of Potassium Carbonate emulsion repeatedly over an extended period could precipitate kidney damage.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Skin Irritation and Sensitization

Skin irritation and sensitization studies on carbonate salts are summarized in Table 4.^{25,27,28,29,30,31,32,46} The results for *in vitro* skin irritation studies were negative, however, a surrogate chemical for Potassium Carbonate was classified as a skin irritant in rabbits and ammonium carbamate (component of Ammonium Carbonate) induced skin irritation in an acute dermal toxicity study involving rats. Additionally, negative results were reported in local lymph node assays evaluating sensitization potential, and the same was true for animal sensitization tests (Buehler and maximization tests included) involving mice or guinea pigs.

OCULAR IRRITATION STUDIES

In Vitro

Magnesium Carbonate

The ocular irritation potential of Magnesium Carbonate (concentration not stated) was evaluated in the *in vitro* bovine corneal opacity and permeability test (BCOP).⁴⁷ In the BCOP test method, changes in corneal opacity caused by chemical damage are determined by measuring decreases in light transmission through the cornea. Changes in permeability of the cornea resulting from chemical damage are determined by measuring increases in the quantity of sodium fluorescein dye that passes through all corneal cell layers. Both measurements are used to calculate an *in vitro* irritancy score (IVIS), which is used to predict the *in vivo* ocular irritation/corrosion potential of a test substance. The following scores are considered positive: corneal opacity (CO) or iris (IR) score ≥ 1 or conjunctival chemosis (CC) or conjunctival redness (CR) ≥ 2 . There was no evidence of CC or CR or lesions of the iris. A CO score of 1 was reported, and the reaction cleared by day 3. Therefore, Magnesium Carbonate caused only corneal opacity in this test.

Ammonium Bicarbonate

The Hen's Egg Chorioallantoic Membrane Test (HET-CAM) was used to evaluate the ocular irritation potential of Ammonium Bicarbonate.²⁷ Undiluted Ammonium Bicarbonate (25 μ L) or 10% aqueous Ammonium Bicarbonate (0.3 mL) was applied topically to the chorioallantoic membrane of fertilized and incubated hen eggs (3 eggs per test concentration). There was no evidence of irritation at the 10% concentration. Undiluted Ammonium Bicarbonate caused moderate intravascular coagulation in all eggs within 50 seconds. Hemorrhagia was not noted during the observation period. It was concluded that, under the conditions of this test, Ammonium Bicarbonate did not produce changes that were indicative of serious eye damage.

Animal

Magnesium Carbonate

In a study involving 2 New Zealand white rabbits, the ocular irritation potential of Magnesium Carbonate (10% w/v aqueous, pH 9.9) was evaluated.²⁵ Magnesium Carbonate (10 ml) was instilled into the conjunctival sac of the right eye. The left eye served as the untreated control. Eyes were not rinsed after instillation of the test substance. Moderate conjunctival irritation was observed at 1 h post-instillation, and minimal conjunctival irritation was observed at 24 h and 48 h post-instillation. Reactions had cleared by 72 h post-instillation. Reactions were not observed in the cornea or iris. Magnesium Carbonate was classified as non-irritating to the eyes of rabbits.

Ammonium Carbonate

The ocular irritation potential of Ammonium Carbonate was evaluated using 3 New Zealand white rabbits.³¹ Ammonium Carbonate (0.1 ml; ~ 66 mg) was instilled into one eye. The following reactions were observed: slight corneal opacity, moderate conjunctival redness, slight to marked conjunctival chemosis, and slight to severe discharge. Additionally, discharge of blood, scleral vessels injected in a circumscribed area, or circular and marginal vascularization of the cornea in a circumscribed area were observed. The ocular reactions were reversible in 2 rabbits within 7 days and in 1 rabbit within 14 days after instillation. Ammonium Carbonate was classified as non-irritating.

Calcium Carbonate

The ocular irritation potential of Calcium Carbonate was studied using 3 male New Zealand white rabbits.²⁸ The test substance (0.1 ml) was instilled into the right eye, and the observation period was up to 72 h. Left eyes served as controls. Ocular damage/irritation was evaluated according to the Draize scale. Corneal effects were not observed during the study. Iridial inflammation and minimal conjunctival irritation were observed in all treated eyes at 1 h post-instillation. Conjunctival irritation was also observed at 24 h and 48 h post-instillation. All reactions had cleared by 72 h post-instillation. Calcium Carbonate was classified as non-irritating to the eyes of rabbits.

Potassium Bicarbonate

Undiluted Potassium Bicarbonate (0.1 ml) was instilled into the eyes of 6 male New Zealand white rabbits.²⁹ The eyes were not rinsed and untreated eyes served as controls. Instillation of the test substance was followed by a 7-day observation period. Mild or moderate conjunctival redness and discharge and moderate or severe chemosis were observed in all animals at 1 h post instillation. At day 4, chemosis and discharge were fully reversible in all animals. The test substance did not induce substantial corneal opacity or iritis. The study was terminated before all ocular reactions were completely reversed. However, the authors noted observations relating to the healing process indicated that the complete reversibility of ocular reactions was likely. Therefore, Potassium Bicarbonate was classified as non-irritating to the eyes of rabbits.

Sodium Carbonate Monohydrate

Data on sodium carbonate monohydrate were used to evaluate the ocular irritation potential of Potassium Carbonate in 9 New Zealand white rabbits.³⁰ The test substance (0.1 ml; concentration not stated) was instilled into the conjunctival sac, after which the eye was either rinsed (3 rabbits) or not rinsed (6 rabbits). Untreated eyes served as controls. Reactions were scored according to the Draize scale for up to 14 days post-instillation. Conjunctival redness was observed in all 6 rabbits that were not subjected to ocular rinsing, and in 1 rabbit after ocular rinsing. Conjunctival chemosis was observed in all 6 rabbits (no ocular rinsing) and in 2 rabbits after ocular rinsing. Corneal opacity, ulceration, and pannus were also observed in rinsed eyes. Necrosis/ulceration, alopecia, and bleeding were observed in eyes that were not rinsed. Signs of ocular irritation persisted to the end of the study in rabbits (unrinsed eyes) and in one rabbit (no ocular rinsing). It was concluded that sodium carbonate monohydrate was irritating to the eye.

CLINICAL STUDIES

Case Reports

Calcium Carbonate

A female patient (history of gastritis) at 36 weeks of gestation had ingested 5 glasses of milk and approximately 30 tablets of an antacid containing 500 mg of Calcium Carbonate daily for 2 weeks.²⁸ A Calcium Carbonate intake of > 5.5 g/day for 14 days during pregnancy (later stages) did not cause any observable teratogenic effects.

Potassium Carbonate

A male crystal factory worker with a 1-month history of eczema on the hands, arms, and legs was patch-tested with a 1% aqueous solution of Potassium Carbonate.³⁰ The patch test procedure was not stated. Patch test results were negative for skin sensitization.

SUMMARY

The carbonate salts have the following functions in cosmetic products: absorbents, bulking agents, opacifying agents, pH adjusters, buffering agents, abrasives, and oral care agents.

Collectively, information supplied to FDA by industry as part of the VCRP and a survey of ingredient use concentrations conducted by the Council indicate that the following carbonate salts are being used in cosmetic products: Magnesium Carbonate, Ammonium Bicarbonate, Ammonium Carbonate, Calcium Carbonate, and Potassium Carbonate. The highest use frequency is reported for Magnesium Carbonate (317 uses). The Council survey data also indicate that the carbonate salts are being used in cosmetics at maximum ingredient use concentrations up to 93.4% (i.e., Ammonium Bicarbonate in rinse-off products [hair bleaches]). The highest maximum concentration of use in leave-on products is being reported for Calcium Carbonate (concentrations up to 35% in eyebrow pencils).

Calcium Carbonate (0.40 mCi of calcium ^{14}C -carbonate pellet) was implanted intraperitoneally into a male rat.²⁶ Approximately 72% of the radiolabeled carbonate was excreted as respiratory carbon dioxide between 2 h and 142 h after implantation.

After magnesium sulfate was fed (in the diet) to rabbits, mean urinary excretion of radioactivity ranged between 10% and 12.5% and the cecum and its contents were found to contain 78% of the ingested dose. After ^{11}C -sodium bicarbonate was injected intraperitoneally into rats, the urine contained 1.3% of the radioactivity and >50% of the radioactivity occurred as respiratory ^{11}C -carbon dioxide.

Following ingestion, Potassium Carbonate rapidly dissociates in the gastric juice to yield carbonate ions and potassium ions. Similarly, after ingestion, Potassium Bicarbonate rapidly dissociates in gastric juice to yield carbonate ions (bicarbonate and carbonate) and potassium ions. The absorbability of calcium from Calcium Carbonate and calcium citrate salts was compared in a study in which 37 men and women ingested 300 mg (low load) and 1000 mg (high load) calcium loads as part of a light breakfast meal. Relative absorption was estimated from the difference in urinary calcium following breakfast on the 2 days and true fractional absorption from the appearance of ^{45}Ca in the serum. Mean tracer absorption for both salts combined was 36% at the 300 mg load and 28.4% at the 1000 mg load.

In an acute dermal toxicity study on a tradename material (Potassium Carbonate containing pesticide), the LD_{50} in rabbits was > 2000 mg/kg body weight. The same results (LD_{50} > 2 000 mg/kg) were reported in acute dermal toxicity studies on Ammonium Carbonate, Calcium Carbonate, Potassium Bicarbonate, and Ammonium Sulfate.

An acute oral LD_{50} of ~ 2000 mg/kg was reported for Ammonium Bicarbonate, Ammonium Carbonate and Potassium Carbonate in studies involving rats. The acute oral LD_{50} for Calcium Carbonate and Potassium Bicarbonate was > 2000 mg/kg.

In an acute inhalation toxicity study on ammonia (1-h exposure) involving mice, the lungs of animals that died were diffusely hemorrhagic (mild to moderate pneumonitis also observed) and liver damage was observed at each concentration of exposure (3440 ppm, 4220 ppm, and 4860 ppm). It was noted that the liver lesions may have resulted from the compromised nutritional state of the mice. An acute inhalation LC_{50} value (1-h exposure) of 6.8 mg/L air for Ammonium Bicarbonate was deduced based on the results of this study.

An acute inhalation LC_{50} of > 3 mg/l of air was reported for rats exposed to aerosolized Calcium Carbonate. In acute inhalation toxicity studies on Potassium Bicarbonate and sodium bicarbonate, an LC_{50} value of > 5 mg/l of air was reported. In an acute inhalation toxicity study on a tradename material (Potassium Carbonate containing pesticide), a mean LC_{50} of > 5 mg/L air (rats) was reported.

Mean acute i.v. toxicity values of 3.10 and 1.02 mM/kg were reported for Ammonium Bicarbonate and Ammonium Carbonate, respectively, in studies involving albino rats.

A NOAEL of 1000 mg/kg body weight/day was reported in a study in which male and female rats received oral doses of magnesium chloride hexahydrate during 14 days pre-mating and 14 days of mating. The same results were reported for female rats dosed orally during gestation and up to PND 3 and for male rats dosed orally for 28 to 29 days. Based on the results of the preceding 2 experiments, it was determined that the equivalent NOAEL for Magnesium Carbonate was 4128 mg/kg body weight/day. Rats fed Calcium Carbonate at 300 mg/kg for 3 days had no indication of toxicity, and the same was true for Calcium Carbonate (nano form) at oral doses up to 1000 mg/kg body weight/day for 14 days. There were no test substance-related changes in rats fed doses up to 4228.5 mg/kg body weight/day ammonium chloride for 28 days.

In repeated dose oral toxicity studies (4-week, 13-week, 18-month, and 30-month studies) of Potassium Bicarbonate (2% or 4% in diet), most of the histopathological changes observed were considered equally distributed among treatment

groups, and represented normal background pathology for SPF-bred Wistar rats. Neither gross nor microscopic changes of the urinary bladder were observed in rats fed ammonium chloride (in the diet) in a short-term (70 days) oral feeding study. The NOEL was < 684 mg/kg body weight/day.

Inhalation exposure to an aerosolized Potassium Carbonate-based scrubbing solution for 21 days did not result in any persistent systemic toxicity or neurotoxicity in either male or female rats.

The results of a study in which rabbits were dosed orally (via drinking) with Potassium Carbonate emulsion (50 mg/L and 100 mg/L) for 14 consecutive days suggested that Potassium Carbonate emulsion exposure could precipitate kidney damage.

The reproductive and developmental toxicity of magnesium chloride hexahydrate was evaluated in male and female rats that received oral doses up to 1000 mg/kg body weight/day during 14 days pre-mating and 14 days of mating. Additionally, female rats received the same doses during gestation and up to PND 3 and male rats were dosed similarly for 28 to 29 days. The NOEL for the reproductive/developmental toxicity of magnesium chloride hexahydrate was 1000 mg/kg body weight/day; the equivalent NOEL for Magnesium Carbonate was determined to be 414 mg/kg body weight/day. In another study, pregnant female rats were dosed orally with magnesium chloride hexahydrate at doses (in distilled water) up to 800 mg/kg body weight/day on gestation days 6 to 15. Visceral malformations were observed in 4 to 6 fetuses from each dose group, but without intergroup differences. The NOEL for magnesium chloride hexahydrate was estimated to be > 800 mg/kg body weight/day; the equivalent NOEL for Magnesium Carbonate was determined to be > 331 mg/kg body weight/day.

Female Swiss mice were bred after feeding with a diet supplemented with 0.5%, 1%, or 2% Calcium Carbonate. Calcium Carbonate (2%) in the diet caused hypertrophy of the heart and decreased thymus weight in weanling mice. In a reproductive toxicity study, rats were fed oral doses of Calcium Carbonate (nano form) up to 1000 mg/kg body weight/day for 48 consecutive days. The maximum dose administered was the NOEL in this study. In a 62-day developmental toxicity study, rats were fed Calcium Carbonate at concentrations up to 1.25% in the diet, and the NOEL for teratogenicity was determined to be > 1.25% in the diet.

There was no evidence of embryotoxicity or teratogenicity in the offspring of rats dosed orally with sodium bicarbonate (up to 340 mg/kg body weight) on gestation days 6 through 15. In another study on sodium bicarbonate, there were no test substance-related abnormalities in the offspring of rats that received sodium bicarbonate at concentrations of 0.5% and 2% (in drinking water) on gestation days 15 through 20. The number of abnormalities observed in soft or skeletal tissues in groups of mice that received oral doses of sodium bicarbonate up to 580 mg/kg on gestation days 6 through 15 did not differ from the number occurring spontaneously in the sham-treated controls.

In teratogenicity studies involving rats and mice, NOELs of 290 mg/kg and 180 mg/kg (highest dose in each study), respectively, were reported for Potassium Carbonate. The results of an inhalation developmental toxicity study on a Potassium Carbonate scrubbing solution (up to 3 mg/l) were negative.

A female patient at 36 weeks of gestation ingested 5 glasses of milk and approximately 30 tablets of an antacid containing 500 mg of Calcium Carbonate daily for 2 weeks. Teratogenic effects were not observed.

In the chromosome aberrations assay, Calcium Carbonate was non-clastogenic to human lymphocytes and magnesium chloride and ammonium sulfate were non-genotoxic to Chinese hamster lung fibroblast (V79) cell cultures. Ames test results for ammonium carbamate, Potassium Bicarbonate, and Potassium Carbonate were negative for genotoxicity. Results were negative for potassium chloride in the L5178Y mouse lymphoma cell mutagenesis assay. In the *in vivo* micronucleus test, ammonium chloride was not genotoxic.

Additionally, anti-genotoxic effects of Magnesium Carbonate have been reported in *in vitro* tests involving *Saccharomyces cerevisiae* strain D4, Balb 3T3 fibroblast cells, or Chinese hamster ovary cells, but not in the Ames test using *S. typhimurium* strain 102.

In a co-carcinogenicity study in which rats were fed sodium bicarbonate (up to 0.64% in diet) + OPP (1.25% in the diet) continuously for 104 weeks, there was no statistically significant increase in the number of bladder tumors when compared to the control group. Also, sodium bicarbonate alone did not have a carcinogenic effect on the urinary bladder.

Bladder cancer has been associated with rats fed Potassium Bicarbonate in the diet (2% or 4%) for up to 130 weeks. The results of a 32-week tumor promotion study involving rats dosed with sodium bicarbonate (up to 3% in the diet) and

0.05% BBN confirmed that the dose-dependent increase in both urinary pH and sodium concentration and the dose-dependent promotion of urinary bladder carcinogenesis were parallel effects of sodium bicarbonate.

Undiluted Magnesium Carbonate, Ammonium Bicarbonate, and Ammonium Carbonate were non-irritating/non-corrosive to the reconstituted human epidermis model *in vitro*. Also, using an *in vitro* test for assessing dermal corrosivity potential (Corrositex®), undiluted Calcium Carbonate was not found to be a corrosive agent.

Undiluted Calcium Carbonate was not a corrosive agent when applied to the skin of rabbits. In the local lymph node assay, Calcium Carbonate (solid nanomaterial) was applied to the skin of mice at concentrations up to 25%, and the results of this assay were negative.

Potash hydrate (surrogate chemical for Potassium Carbonate; 500 mg in saline) was moderately irritating to the skin of 6 rabbits and Potassium Bicarbonate (500 mg in saline) was classified as mildly irritating to the skin of 6 rabbits. Reactions were observed at abraded sites, but not intact sites. Potassium Bicarbonate (0.2 g in deionized water) was classified as a non-sensitizer in the Buehler test (10 guinea pigs). Reactions were not observed during induction or the challenge phase. In a repeated insult patch test, a Potassium Carbonate tradename material (tested at 95% w/w) also was not a skin sensitizer in the Buehler test (10 guinea pigs). In the local lymph node assay, ammonium acetate was applied to the skin of mice at concentrations up to 50%, and the results of this assay were negative (non-sensitizer). Ammonium carbamate also produced negative results in the local lymph node assay (non-sensitizer) when applied to the skin of mice at concentrations up to 50%.

Magnesium Carbonate caused only corneal opacity in the *in vitro* bovine corneal opacity and permeability test. In the HET-CAM test, it was concluded that Ammonium Bicarbonate (undiluted or 10% aqueous) did not produce changes that were indicative of serious eye damage. Undiluted Ammonium Bicarbonate, but not the 10% concentration, caused intravascular coagulation in all eggs.

Magnesium Carbonate (10% aqueous) was classified as non-irritating to the eyes of 2 rabbits. However, transient ocular irritation was observed. Undiluted Ammonium Carbonate (3 rabbits tested), Calcium Carbonate (3 rabbits tested), and Potassium Bicarbonate (6 rabbits tested) were classified as non-irritating to the eyes of rabbits. The ocular reactions observed were reversible. In a study involving 9 rabbits, undiluted sodium carbonate monohydrate was classified as an ocular irritant.

DISCUSSION

The Panel noted that this safety assessment contains toxicity data on ammonium carbamate because the cosmetic ingredient Ammonium Carbonate is made up of Ammonium Bicarbonate and ammonium carbamate.

The Panel expressed concern about the potential for skin and ocular irritation from exposures to carbonate salts. For example, animal studies reported that Potassium Carbonate and sodium carbonate monohydrate (a closely related chemical that is not a cosmetic ingredient) were skin and ocular irritants, respectively. Sodium carbonate monohydrate is not used; however, other carbonate salts are being used in eye products, but are not irritants. The Panel noted that the carbonate salts alone would not likely be irritating at concentrations used in cosmetic products, but that these ingredients may contribute to the irritation potential of other ingredients in cosmetic formulations. Thus, the Panel determined that cosmetic products containing carbonate salts should be formulated to be non-irritating.

The Panel noted studies that reported renal toxicity and neoplastic lesions of the urinary bladder in animals fed Potassium Bicarbonate. However, the Panel concluded that the effects reported in these studies are attributable to irritation resulting from the ingredient in the urine after repeated daily exposure to high dietary concentrations of Potassium Bicarbonate over an extended period. The Panel agreed that the dietary exposures tested in these studies do not reflect the much lower exposures that can reasonably be expected from the use of carbonate salts in cosmetic products.

Concern about heavy metals that may be present in carbonate salts was expressed by the Panel. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities in the ingredient before blending into cosmetic formulations.

The Panel discussed the issue of incidental inhalation exposure from propellant hair sprays and face powders. They considered pertinent data indicating that incidental inhalation exposures to these ingredients in such cosmetic products would not cause adverse health effects, specifically, short-term (repeated exposures for 21 days) inhalation toxicity data and

developmental toxicity data on a scrubbing solution containing 30.8% Potassium Carbonate in studies involving rats. The Panel also noted that droplets/particles from spray and loose-powder cosmetic products would not be respirable to any appreciable amount. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at <http://www.cir-safety.org/cir-findings>.

CONCLUSION

The CIR Expert Panel concluded that the following 6 carbonate salts are safe in the present practices of use and concentration, as described in this safety assessment, when formulated to be non-irritating.

Magnesium Carbonate
Ammonium Bicarbonate
Ammonium Carbonate
Calcium Carbonate
Potassium Bicarbonate*
Potassium Carbonate

*Not reported to be in current use. Were the ingredient in this group not in current use to be used in the future, the expectation is that it would be used in product categories and at concentrations comparable to others in this group.

Table 1. Definitions, Structures, and functions of the ingredients in this safety assessment (INCI Dictionary¹; CIR Staff)

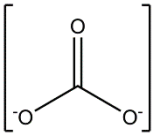
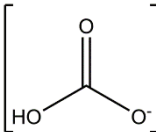
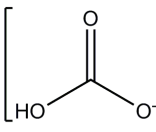
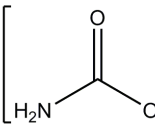
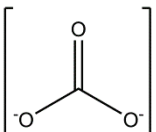
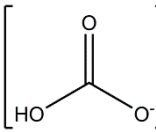
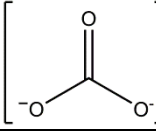
Ingredient/CAS No.	Definition & Structure	Function
Magnesium Carbonate 546-93-0 7757-69-9	Magnesium Carbonate is a basic dehydrated Magnesium Carbonate or a normal hydrated Magnesium Carbonate.  Mg^{2+}	Absorbents; Bulking Agents; Opacifying Agents; pH Adjusters
Ammonium Bicarbonate 1066-33-7	Ammonium Bicarbonate is an inorganic salt that conforms to the formula:  NH_4^+	pH Adjusters
Ammonium Carbonate 10361-29-2 (unspecified) 506-87-6 (diammonium) 8000-73-5 (mixture)	Ammonium Carbonate is a mixture of Ammonium Bicarbonate and ammonium carbamate.  NH_4^+ ammonium bicarbonate  NH_4^+ ammonium carbamate	Buffering Agents; pH Adjusters
Calcium Carbonate 471-34-1	Calcium Carbonate is the inorganic salt that conforms to the formula:  Ca^{2+}	Abrasives; Buffering Agents; Bulking Agents; Opacifying Agents; Oral Care Agents
Potassium Bicarbonate 298-14-6	Potassium Bicarbonate is the inorganic salt that conforms to the formula:  K^+	Buffering Agents; pH Adjusters
Potassium Carbonate 584-08-7	Potassium Carbonate is the inorganic salt that conforms to the formula:  2 K^+	pH Adjusters

Table 2. Properties of Carbonate Salts.⁴

Property	Value	Background Information
Magnesium Carbonate		
Formula Weight (g/mol)	84.31. ⁴⁸	
Density (g/cm³)	3.0. ⁴⁹	
Melting Point (°C)	900. ⁴⁹	
Ammonium Bicarbonate		
Form/Odor	Shiny, hard, colorless or white prisms or crystalline mass. Faint odor of ammonia.	Comparatively stable at room temperature. Volatile with decomposition at ~ 60°. Decomposes in hot water.
Formula Weight (g/mol)	79.06	
Solubility	Soluble in water: 14% (10°C); 17.4% (20°C); 21.3% (30°C). Insoluble in alcohol and acetone.	
Ammonium Carbonate		
Form	Flat, columnar, prismatic crystals or elongated flakes.	Commercial preparations are usually a mixture with ammonium carbamate and Ammonium Bicarbonate.
Formula Weight (g/mol)	79.06-78.07	
Melting Point (°C)	43	
Calcium Carbonate		
Form	Odorless, tasteless powder or crystals.	Two crystal forms are of commercial importance: Aragonite (orthorhombic; melting point: 825°C (decomposes); density: 2.83; formed at temperatures above 30°; Calcite (hexagonal-rhombohedral; melting point 1339°C; density: 25.2; formed at temperatures below 30°.
Formula Weight (g/mol)	100.09	
Solubility	Soluble in 1N acetic acid, 3N hydrochloric acid, 2N nitric acid. Practically insoluble in water. Insoluble in ethanol.	
Potassium Bicarbonate		
Form	Colorless, transparent crystals, white granules or powder.	Contains not less than 99% KHCO ₃ .
Formula Weight (g/mol)	100.11	
Water Solubility (g/L)		
@ 20°C & pH 7	357	
@ 50°C & pH 7	500	
Solubility	Almost insoluble in ethanol.	
Potassium Carbonate		
Form	Hygroscopic, odorless granules or granular powder.	
Formula Weight (g/mol)	138.20	

Table 2. Properties of Carbonate Salts.⁴

Property	Value	Background Information
Potassium Carbonate		
Solubility	Soluble in 1 part cold water and in 0.7 part boiling water. Practically insoluble in alcohol.	
Density (g/ml)	2.29	
Melting Point (°C)	891	

Table 3. Frequency and Concentration of Use According to Duration and Type of Exposure.^{9,10}

	Magnesium Carbonate		Ammonium Bicarbonate		Ammonium Carbonate	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	317	0.1-14.4	70	3-93.4	27	0.6-6.5
Duration of Use						
<i>Leave-On</i>	225	0.1-7	2	NR	3	0.6-3
<i>Rinse off</i>	90	0.12-14.4	68	3-93.4	24	2-6.5
<i>Diluted for (bath) Use</i>	2	NR	NR	NR	NR	NR
Exposure Type						
<i>Eye Area</i>	70	0.2-2	NR	NR	NR	NR
<i>Incidental Ingestion</i>	2	NR	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	9*	0.18	1***	NR	3*	NR
<i>Incidental Inhalation- Powders</i>	109	0.5-4	1***	NR	NR	NR
<i>Dermal Contact</i>	283	0.1-7	1	NR	1	NR
<i>Deodorant (underarm)</i>	NR	NR	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	3	NR	9	3-6.1	2	0.6-3
<i>Hair-Coloring</i>	27	0.12-14.4	60	1.5-93.4	24	2-6.5
<i>Nail</i>	NR	NR	NR	NR	NR	NR
<i>Mucous Membrane</i>	5	NR	NR	NR	NR	NR
<i>Baby Products</i>	3	NR	NR	NR	NR	NR
	Calcium Carbonate		Potassium Carbonate			
	# of Uses	Conc. (%)	# of Uses	Conc. (%)		
Totals/Conc. Range	174	0.0036-35	4	0.0019-2.5		
Duration of Use						
<i>Leave-On</i>	113	0.001-35	1	0.002-0.0068		
<i>Rinse off</i>	59	0.0036-25	3	0.0019-2.5		
<i>Diluted for (bath) Use</i>	2	NR	NR	NR		
Exposure Type						
<i>Eye Area</i>	16	2-35	NR	0.002-0.005		
<i>Incidental Ingestion</i>	41	0.045-10	NR	NR		
<i>Incidental Inhalation- Sprays</i>	17*	NR	1*	NR		
<i>Incidental Inhalation- Powders</i>	42	0.047-15; 25**	NR	0.006-0.0068**		
<i>Dermal Contact</i>	129	0.001-35	4	0.0019-0.0068		
<i>Deodorant (underarm)</i>	NR	NR	NR	NR		
<i>Hair - Non-Coloring</i>	1	0.01-6	NR	NR		
<i>Hair-Coloring</i>	NR	< 0.01-8	NR	2-2.5		
<i>Nail</i>	1	10-15	NR	NR		
<i>Mucous Membrane</i>	54	0.0036-10	NR	NR		
<i>Baby Products</i>	NR	NR	NR	NR		

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for Bath Product Uses.

*It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.

** It is possible that these products may be powders, but it is not specified whether the reported uses are powders.

***Not specified whether a powder or spray, so this information is captured for both categories of incidental inhalation.

Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

Table 4. Skin Irritation/Sensitization Studies

Test Substance	In Vitro Assays/Animals Tested	Test Protocol	Results
<i>Skin Irritation Studies</i>			
<u>In Vitro Studies</u>			
Magnesium Carbonate	Reconstituted human epidermis model.	OECD Guideline 431. Solid material (20 mg) applied topically to model, followed by addition of 0.9% w/v sodium chloride solution for wetting of test material. Duplicate tissues treated for exposure periods of 3, 60, and 240 minutes. Negative control (0.9% w/v sodium chloride solution) and positive control (glacial acetic acid).	Non-corrosive to the skin. ²⁵
Ammonium Bicarbonate	Reconstructed 3-dimensional human epidermis model	OECD Guideline 431. 25 µl (concentration not stated) applied to epidermal tissue samples for up to 1 h. Colorimetric test to determine tissue destruction by measuring metabolic activity of tissue. Reduction of mitochondrial dehydrogenase activity measured by reduced formazan production after incubation with tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)].	Ammonium Bicarbonate unable to reduce MTT directly in this assay. Mean viability of treated tissues was 105% after 3-minute exposure and 36% after 1-h exposure. ²⁷
Ammonium Bicarbonate	Reconstructed 3-dimensional human epidermis model	Skin corrosion test (OECD Guideline 431). Epidermal tissue samples incubated with test substance for 1 h, followed by 42-h post-incubation period.	Mean viability of treated tissues was 71% after 1-h exposure + 42-day post-incubation period. Test substance classified as non-irritating. ²⁷
Ammonium Carbonate	3-dimensional human epidermis model	OECD Guideline 439. Test substance (50 µl, concentration not stated) applied to disks of tissue samples, followed by 15-minute incubation period and 42-h post-incubation period. Viability of each disk assessed by incubating tissues for 3 h with MTT solution.	Mean tissue viability was 115%. Test substance classified as non-irritating. ³¹
Calcium Carbonate	3-dimensional human epidermis model.	In vitro test method for assessing dermal corrosivity potential. Methodology based on ability of corrosive chemical to pass through a biobARRIER and elicit color change in underlying liquid Chemical Detection System (CDS). BiobARRIER composed of a hydrated collagen matrix in supporting filter membrane. CDS composed of water and pH indicator dyes.	Test substance classified as non-corrosive. ⁴⁶
<u>Animal Studies</u>			
Calcium Carbonate	Rabbits (number and strain not stated)	Undiluted test substance applied to intact skin. Evaluation for corrosion within 3 minutes and at 1 h or 4 h post-application.	Test substance classified as non-corrosive. ⁴⁶

Table 4. Skin Irritation/Sensitization Studies

Test Substance	In Vitro Assays/Animals Tested	Test Protocol	Results
Potassium Bicarbonate	6 New Zealand White rabbits	Test substance (500 mg in saline) applied to dorsal area of trunk (abraded or intact skin). Site covered with 2.5 cm ² occlusive patch for 24 h.	At 24 h and 72 h post-application, no signs of erythema or edema at intact skin sites. However, at both times, very slight erythema (score = 1), with slightly white discolorations, at abraded sites of all animals. No evidence of edema. Primary irritation index (abraded and intact skin; maximum possible index = 8) = 0.5. Test substance classified as mild skin irritant. ²⁹
Potash Hydrate (composition not stated; surrogate for Potassium Carbonate)	6 New Zealand White rabbits	Test substance (500 mg moistened with saline) applied, under 2.5 cm x 2.5 cm occlusive dressing, to abraded and intact dorsal skin for 24 h.	Skin irritation not observed at intact sites at 24 h or 72 h post-application. For abraded sites, erythema score of 4 and edema score of 2 in all rabbits at 24 h. At 72 h, erythema score of 4 (abraded skin) in all rabbits. Primary irritation index (abraded and intact skin scores) = 2.5. Test substance classified as moderate skin irritant. ³⁰
Ammonium Carbamate (component of Ammonium Carbonate)	2 Vienna White rabbits	Test substance (40% or 80% aqueous; 1 ml) applied, under occlusive patch, to skin for 1, 5, or 15 minutes. Application followed by 24-h observation period.	Neither local effects nor clinical symptoms observed. Test substance classified as non-irritant at both concentrations. ³²
Ammonium Carbamate (component of Ammonium Carbonate). Contained 43.6% ammonium and 56.3% carbon dioxide.	10 rats (5 males, 5 females) of the CrI:CD(SD) strain	Test substance (in water; dose = 5000 mg/kg body weight) applied, under semi-occlusive patch, to the back for 24 h.	Erythema (very slight to severe), edema (very slight to moderate), eschar, necrosis, exfoliation, scabbing, and desquamation were reported. Number of animals with reactions not stated. ³²
<i>Sensitization Studies</i>			
<u>In Vitro Studies</u>			
Calcium Carbonate (solid nanomaterial, white powder)	Three groups of 4 mice of the CBA/Ca (CBA/caOlaHsd) strain	Local lymph node assay (OECD Guideline 429). Three groups exposed to concentrations of 5%, 10%, and 25% w/w, respectively. Each concentration (in formamide) applied (25 µl) to dorsal surface of ear for 3 consecutive days. Hexyl cinnamic aldehyde served as positive control.	Calcium Carbonate was a non-sensitizer. Positive control was a sensitizer; stimulation index, expressed as the mean radioactive (³ HTdR) incorporation into lymph node cells for the treatment group divided by the mean radioactive incorporation of the control groups, was 5.6. ²⁸
Ammonium Acetate (surrogate for Ammonium Carbonate)	3 groups of 4 CBA female mice	Local lymph node assay (OECD Guideline 429). Same as above procedure except for test concentrations of 10% w/v, 25% w/v, and 50% w/v. Tested as 25 µl in acetone/olive oil [4:1 v/v] mixture.	No signs of irritation at application site in any treatment group. Stimulation index values were 1.6 (for 10%), 1.7 (for 25%) and 1.3 (for 50%). Stimulation index for positive control was 10.7. Non-sensitizer. ³¹

Table 4. Skin Irritation/Sensitization Studies

Test Substance	In Vitro Assays/Animals Tested	Test Protocol	Results
Ammonium Carbamate (component of Ammonium Carbonate)	3 groups of 5 mice of the CBA/J inbred SPF strain	Local lymph node assay (OECD Guideline 429). Same test concentrations as in above procedure. Tested as 25 µl in propylene glycol.	Test substance was a non-sensitizer. Stimulation index values were 1.1 (for 10% ammonium carbamate), 1.2 (for 25% ammonium carbamate), and 0.6 (for 50% ammonium carbamate). ³²
<u>Animal Studies</u>			
Potassium Bicarbonate	Groups of Hartley guinea pigs of the (CrI:HA)BR strain (10 test guinea pigs, 10 negative controls, and 4 positive controls)	Buehler test procedure. Induction: test substance (0.2 g in deionized water) applied, under an occlusive patch (Hill Top Chamber®, 25-mm diameter), for 6 h to the anterior left flank once per week for 3 weeks. Challenge: Test substance (0.2 g in deionized water) applied to anterior right flank. Positive controls treated with 0.3% w/v 2,4-dinitrochlorobenzene.	Test substance was non-sensitizer. ²⁹
Potassium Carbonate	Groups of guinea pigs of unspecified strain (10 test guinea pigs, 10 negative controls, and 5 positive controls)	Buehler test procedure. Test substance (moistened with distilled water) applied for 24 h, under occlusive patch, to the skin at a concentration of 95% w/w during 3-week induction period and challenge phase. Dinitrochlorobenzene served as positive control.	Test substance was non-irritant and non-sensitizer. ³⁰
Ammonium Chloride (surrogate for Ammonium Bicarbonate)	20 Pirbright-Hartley guinea pigs	Maximization test. Induction phase consisted of single intracutaneous induction exposure to 5% ammonium chloride and a single 48-h epicutaneous induction exposure to 25% ammonium chloride. Intradermal injection occurred on day 1 and epicutaneous induction occurred on day 9. For epicutaneous induction, a 2 x 4 cm occlusive patch containing 0.5 ml of the test substance was applied to the area of the intradermal injection site. On day 22, animals received a 24-h challenge application (occlusive patches) of 10% ammonium chloride.	Two guinea pigs had + reactions (barely perceptible erythema) with very slight to slight edema. 10% of the animals tested had a positive reaction after being challenged with ammonium chloride. Results were interpreted as the absence of skin sensitization potential. ²⁷

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CONCLUSION ON PESTICIDES PEER REVIEW

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Peer review of the pesticide risk assessment of the active substance calcium carbonate

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Abstract

The conclusions of EFSA following the peer review of the initial risk assessments carried out by the competent authorities of the rapporteur Member State Spain and co-rapporteur Member State Hungary for the pesticide active substance calcium carbonate are reported. The context of the peer review was that required by Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The conclusions were reached on the basis of the evaluation of the representative uses of calcium carbonate as a repellent on deciduous and coniferous trees in forestry. The reliable end points, appropriate for use in regulatory risk assessment, are presented. Missing information identified as being required by the regulatory framework is listed. No concerns were identified.

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Summary

Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659, lays down the procedure for the renewal of the approval of active substances submitted under Article 14 of Regulation (EC) No 1107/2009. The list of those substances is established in Commission Implementing Regulation (EU) No 686/2012 as amended by Commission Implementing Regulation (EU) No 2016/183. Calcium carbonate is one of the active substances listed in that Regulation.

In accordance with Article 1 of Regulation (EU) No 844/2012, the rapporteur Member State (RMS), Spain, and co-rapporteur Member State (co-RMS), Hungary, received an application from Flügel GmbH for the renewal of approval of the active substance calcium carbonate.

An initial evaluation of the dossier on calcium carbonate was provided by the RMS in the renewal assessment report (RAR) and subsequently, a peer review of the pesticide risk assessment on the RMS evaluation was conducted by the European Food Safety Authority (EFSA) in accordance with Article 13 of Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The following conclusions are derived.

The uses of calcium carbonate according to the representative uses as a repellent, by applications by painting individual plants and by spraying individual plants with a low-pressure handheld sprayer, on deciduous and coniferous trees in forestry as proposed at Central Europe, result in a sufficient repellent efficacy against game browsing and fraying the antlers.

It should be emphasised that the substance applied for as calcium carbonate is mined limestone and therefore calcium carbonate and limestone are considered the same compound in the course of this peer review.

In the area of physical-chemical properties and analytical methods, the assessment did not reveal any issue not finalised or areas of concern.

In the area of mammalian toxicology, the assessment of the data package revealed no issues that could not be finalised for the representative uses as a repellent neither areas of concerns.

In the area of residues, issues not finalised or areas of concerns were not identified. Calcium carbonate should be considered by risk managers for inclusion in Annex IV of Regulation 396/2005 and the maximum residue level (MRL) review according to article 12 becomes obsolete.

Calcium carbonate is the main component of naturally occurring sedimentary rocks composed largely of the mineral calcite (limestone). The information available and its evaluation regarding the environmental fate and behaviour of calcium carbonate were considered sufficient to complete the assessments necessary regarding the environmental exposure assessment at the European Union (EU) level for the representative uses assessed. Considering the nature of the substance and the limited usage leading to environmental concentrations which are expected to be too low to measure, a definition of residue in the environment for monitoring is deemed to be unnecessary for calcium carbonate (limestone, CaCO_3).

In the area of ecotoxicology, low risk to all non-target organisms was concluded based on the exposure expected to be too low to measure.

Calcium carbonate does not meet the criteria for endocrine disruption for humans and non-target organisms as set out in points 3.6.5 and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605.

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Background

Commission Implementing Regulation (EU) No 844/2012¹, as amended by Commission Implementing Regulation (EU) No 2018/1659², (hereinafter referred to as 'the Regulation'), lays down the provisions for the procedure of the renewal of the approval of active substances, submitted under Article 14 of Regulation (EC) No 1107/2009³. This regulates for the European Food Safety Authority (EFSA) the procedure for organising the consultation of Member States, the applicant(s) and the public on the initial evaluation provided by the rapporteur Member State (RMS) and/or co-rapporteur Member State (co-RMS) in the renewal assessment report (RAR), and the organisation of an expert consultation where appropriate.

In accordance with Article 13 of the Regulation, unless formally informed by the European Commission that a conclusion is not necessary, EFSA is required to adopt a conclusion on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 within 5 months from the end of the period provided for the submission of written comments, subject to an extension of an additional 3 months where additional information is required to be submitted by the applicant(s) in accordance with Article 13(3). Furthermore, in accordance with Article 13(3a), where the information available in the dossier is not sufficient to conclude the assessment on whether the approval criteria for endocrine disruption are met, additional information can be requested to be submitted in a period of minimum 3 months, not exceeding 30 months, depending on the type of information requested.

In accordance with Article 1 of the Regulation, the RMS Spain and co-RMS Hungary received an application from Flügel GmbH for the renewal of approval of the active substance calcium carbonate. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and informed the applicant, the co-RMS (Hungary), the European Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on calcium carbonate in the RAR, which was received by EFSA on 23 October 2019 (Spain, 2019).

In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the Member States and the applicant, Flügel GmbH, for consultation and comments on 20 December 2019. EFSA also provided comments. In addition, EFSA conducted a public consultation on the RAR. EFSA collated and forwarded all comments received to the European Commission on 19 February 2020. At the same time, the collated comments were forwarded to the RMS for compilation and evaluation in the format of reporting table. In addition, the applicant was invited to respond to the comments received. The comments and the applicant's response were evaluated by the RMS in column 3.

The need for expert consultation and the necessity for additional information to be submitted by the applicant in accordance with Article 13(3) of the Regulation were considered in a telephone conference between EFSA and the RMS on 27 April 2020. On the basis of the comments received, the applicant's response to the comments and the RMS's evaluation thereof, it was concluded that additional information should be requested from the applicant, and that EFSA should conduct a joint expert consultation on the endocrine properties in the area of mammalian toxicology/ecotoxicology.

The outcome of the telephone conference, together with EFSA's further consideration of the comments, is reflected in the conclusions set out in column 4 of the reporting table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, including those issues to be considered in an expert consultation, were compiled by EFSA in the format of an evaluation table.

The conclusions arising from the consideration by EFSA, and as appropriate by the RMS, of the points identified in the evaluation table, together with the outcome of the expert consultation and the written consultation on the assessment of additional information, where these took place, were reported in the final column of the evaluation table.

¹ Commission Implementing Regulation (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 252, 19.9.2012, p. 26–32.

² Commission Implementing Regulation (EU) No 2018/1659 of 7 November 2018 amending Implementing Regulation (EU) No 844/2012 in view of the scientific criteria for the determination of endocrine disrupting properties introduced by Regulation (EU) 2018/605.

³ Regulation (EC) No 1107/2009 of 21 October 2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in February 2021.

This conclusion report summarises the outcome of the peer review of the risk assessment of the active substance and the representative formulation, evaluated on the basis of the representative uses of calcium carbonate as a repellent on deciduous and coniferous trees in forestry (field uses), as proposed by the applicant. In accordance with Article 12(2) of Regulation (EC) No 1107/2009, risk mitigation options identified in the RAR and considered during the peer review, if any, are presented in the conclusion. It should be noted that the active substance applied for is composed of mined limestone. Limestone has been permanently included in Annex IV of Regulation (EC) No 396/2005⁴ by means of Commission Regulation (EU) 2020/1565⁵. Calcium carbonate as applied for in the current peer review and limestone are considered the same compound. Therefore, calcium carbonate should be considered by risk managers for inclusion in Annex IV of Regulation 396/2005 and the maximum residue level (MRL) review according to article 12 becomes obsolete.

A list of the relevant end points for the active substance and the formulation is provided in Appendix B. In addition, the considerations as regards the cut-off criteria for calcium carbonate according to Annex II of Regulation (EC) No 1107/2009 are summarised in Appendix A.

A key supporting document to this conclusion is the peer review report (EFSA, 2021), which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The peer review report comprises the following documents, in which all views expressed during the course of the peer review, including minority views, where applicable, can be found:

- the comments received on the RAR;
- the reporting table (27 April 2020);
- the evaluation table (24 February 2021);
- the report(s) of the scientific consultation with Member State experts (where relevant);
- the comments received on the assessment of the additional information (where relevant);
- the comments received on the draft EFSA conclusion.

Given the importance of the RAR, including its revisions (Spain, 2020), and the peer review report, both documents are considered as background documents to this conclusion and thus are made publicly available.

It is recommended that this conclusion and its background documents would not be accepted to support any registration outside the European Union (EU) for which the applicant has not demonstrated that it has regulatory access to the information on which this conclusion report is based.

The active substance and the formulated product

Calcium carbonate (IUPAC) is considered by the International Organization for Standardization not to require a common name. Calcium carbonate as applied for in the current peer review and limestone are considered the same compound.

The representative formulated product for the evaluation was 'FLU17516', a gel for direct application (GD) containing 169 g/kg calcium carbonate and 120 g/kg fish oil.

The representative uses evaluated comprise applications by painting individual plants and by spraying individual plants with a low-pressure handheld sprayer, on deciduous and coniferous trees in forestry as a repellent against game browsing and fraying the antlers in Central Europe (CEU). Full details of the Good Agricultural Practices (GAPs) can be found in the list of end points in Appendix B.

Data were submitted to conclude that the representative uses of calcium carbonate proposed at CEU level result in a sufficient repellent effect following the guidance document SANCO/2012/11251-rev. 4 (European Commission, 2014).

⁴ Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1–16.

⁵ Commission Regulation (EU) 2020/1565 of 27 October 2020 amending Annexes II, III and IV to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 1,4-diaminobutane, 1-methylcyclopropene, ammonium acetate, bifentazate, chlorantraniliprole, chlormequat, cyprodinil, limestone, mandipropamid, pepper, pyridaben, repellants: blood meal, seaweed extracts and trimethylamine hydrochloride in or on certain products. OJ L 358, 28.10.2020, p. 3–29.

A data gap has been identified for a search of the scientific peer-reviewed open literature on the active substance and its relevant metabolites, dealing with side effects on the environment and non-target species and published within the 10 years before the date of submission of the dossier, to be conducted and reported in accordance with EFSA guidance on the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA, 2011a).

Conclusions of the evaluation

1. Identity, physical/chemical/technical properties and methods of analysis

The following guidance documents were followed in the production of this conclusion: European Commission (2000a,b, 2010, 2012).

The minimum purity of calcium carbonate is 950 g/kg. It originates from surface mining from natural sources. There are no relevant impurities in the active substance, specification for SiO₂ relates to the amorphous form only, with crystalline silica below the limit of detection (LOD) of 0.1%. Based on the renewal data, it is proposed to update the reference specification considering that the specification for first approval (min 995 g/kg) was considered provisional and not supported by the renewal data. An FAO specification does not exist for calcium carbonate.

A data gap was identified for the effect of low temperature on stability of the representative formulation and shelf-life storage study at ambient temperature.

Acceptable analytical methods are available for the determination of the active substance content of the formulation.

The need for methods of analysis for monitoring this compound in food of plant and animal origin and in the environment has been waived due to the nature of the compound. A method for body fluids and tissues is not available, however the need for such method can also be waived due to the nature of the compound.

2. Mammalian toxicity

Calcium carbonate was discussed at the Pesticides Peer Review Teleconference 32 in November 2020 and assessed based on the following guidance documents: European Commission (2012), EFSA PPR Panel, (2012).

With regard to the updated technical specification (see section 1), it has not been demonstrated to be covered by the batches used in the toxicological studies. However, considering the toxicity profile of the active substance and the absence of toxicologically relevant impurities (crystalline silica being below the LOD in the technical specification, see section 1), no concern is raised. Similarly, the lack of analytical methods in support of the toxicological studies was not identified as a concern.

Calcium carbonate was moderately **absorbed** (20–40%) in rats and humans, as reported in the Scientific Opinion re-evaluating the safety of calcium carbonate as food additive (E 170) (EFSA, 2011c). Based on the physico-chemical properties of the substance, the information on the available ADME studies and considering its use as a food additive, comparative *in vitro* metabolism data was not considered necessary.

Calcium carbonate is considered of low **acute toxicity** by the oral, dermal and inhalation routes, based on the use as food additive, literature data, history of safe uses, and support by summary information from REACH registration dossiers (Spain, 2020). Furthermore, the compound was neither skin irritant, eye irritant nor skin sensitiser. For the **short-term** toxicity, data from published studies (rats and mice) and from REACH registration dossiers were taken into account (no original study reports). No target organs and no signs of systemic toxicity were observed, with a relevant oral short-term no observed adverse effect level (NOAEL) of 1,000 mg/kg body weight (bw) per day from the 90-day rat study. For the short-term toxicity by inhalation, considering that local effects were observed in the lower airways at the high dose, the experts agreed that the short-term toxicity of calcium carbonate by inhalation might need to be further addressed relevant for other uses⁶ in view of possible derivation of an acceptable operator exposure concentration (AOEC) (independently of the

⁶ See Experts' consultation 2.2 from Peer Review TC32 (EFSA, 2021).

representative uses and of the representative formulation, if it cannot be justified that exposure by inhalation is not expected; see also below).

Calcium carbonate is unlikely to be **genotoxic**, and no concern was identified by the experts for the **long-term toxicity** and carcinogenicity of the compound.⁷ Regarding the **reproductive toxicity**, no adverse effects were observed on reproductive parameters in a combined repeated dose toxicity study with the reproductive and developmental toxicity screening test in rats. In a developmental rat toxicity study, a developmental NOAEL of 1,250 mg/kg bw per day was identified on the basis of increased incidence of external and skeletal foetal findings at the high dose tested (maternal NOAEL). Calcium carbonate is not considered to be teratogenic. In the absence of indications of potential neurotoxic effects in the available studies and considering the history of safe use and the chemical structure of the substance, it has been agreed that calcium carbonate has no potential for **neurotoxicity**.

Based on the low toxicity profile of the active substance and the representative uses of the product, no **toxicological reference values** (acceptable daily intake (ADI, acute reference dose (ARFD)), acceptable operator exposure level (AOEL) and acute acceptable operator exposure level (AAOEL) values) were considered necessary for calcium carbonate. The **dermal absorption** value for the representative formulation FLU17516 is 25% (default value). In the absence of toxicological reference values, non-dietary exposure estimates are not necessary. It is noted that for the representative uses and representative formulation (gel for direct application), exposure by inhalation is not expected to be significant; however, some experts agreed that, on a precautionary basis, the use of respiratory protective equipment for operators and workers may be considered at Member State (MS) level for national authorisations.

3. Residues

Standard studies according to EU/OECD guidance documents and EU data requirements have not been submitted to address the residue behaviour of calcium carbonate from the proposed use. Due to the nature of the active substance and the proposed uses, such studies are not required.

The proposed uses of calcium carbonate on deciduous and coniferous forestry trees will not result in exposure via food and feed to humans and livestock. Hence, a consumer risk assessment and the underlying data are not required.

Limestone has been permanently included in Annex IV of Regulation (EC) No 396/2005 by means of Commission Regulation (EU) 2020/1565. The inclusion in Annex IV followed the statement of EFSA for substances that do not require a review of existing MRLs (EFSA, 2019). In particular, it was proposed to retain limestone in Annex IV of Regulation (EC) No 396/2005 based on the outcome of the peer review (EFSA, 2011b). Consequently, the review of MRLs under Art 12 of Regulation (EC) No 396/2005 for limestone became obsolete. Calcium carbonate and limestone are considered the same compound in the course of this peer review. Based on the outcome of the peer review for calcium carbonate that consumer risk assessment is not considered required, calcium carbonate should be considered by risk managers for inclusion in Annex IV of Regulation 396/2005 and the MRL review according to article 12 becomes obsolete.

4. Environmental fate and behaviour

Calcium carbonate is the main component of naturally occurring sedimentary rocks composed largely of the mineral calcite (limestone). In some regions it is the water bearing rock material of groundwater aquifers.

After application (by brush coating or hand-held trigger spraying) the formulation dries and forms a protective coating. The dried formulation is not water soluble. The spray application is performed with a low-pressure hand-held sprayer with a cone nozzle leading to the formation of large droplets. Therefore, it is very likely that the environmental concentrations of the product due to spray drift are too low to measure.

Because of the method of application and the natural presence of calcium carbonate in soils, aquatic sediments and it being a groundwater aquifer material, further consideration of its fate and behaviour in the environment was concluded to be unnecessary.

⁷ See Experts' consultation 2.4 from Peer Review TC32 (EFSA, 2021).

5. Ecotoxicology

Toxicity data with the active substance were not available for any group of non-target organisms (data gap for aquatic organisms). Acute toxicity data with the representative formulation were available for honeybees. Acute toxicity data with fish, aquatic invertebrates and algae⁸ and chronic toxicity data with earthworms were available with a formulation different than the representative one. According to Regulation 283/2013⁹, acute toxicity data with active substances should always be submitted for fish, aquatic invertebrates and algae and therefore a data gap was identified. As reported in section 3 and 4, exposure to calcium carbonate following the representative uses is expected to be too low to be measured. Therefore, based on this, low risk was concluded for all non-target organisms.

6. Endocrine disruption properties

With regard to the assessment of the endocrine disruption potential of calcium carbonate for humans and non-target organisms according to the ECHA/EFSA guidance (2018), although no (eco)toxicological data are available to assess the endocrine disrupting properties, this does not appear scientifically necessary since the substance is used as food and feed additive, pharmaceutical and cosmetic substance. It has no toxic mode of action as it is used as repellent and it has a very low solubility in water. Moreover, no exposure is anticipated to non-target organisms.

Considering the above, it can be concluded that calcium carbonate does not meet the criteria for endocrine disruption for humans and non-target organisms according to points 3.6.5 and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605.

7. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments (Tables 1–4)

Table 1: Soil

Compound (name and/or code)	Ecotoxicology
Not applicable	Not triggered
Considering the nature of the substance, it being a constituent of many soils and the limited exposure from the representative uses, a definition of residue in the environment for risk assessment triggering assessment of effects data is deemed to be unnecessary for calcium carbonate (limestone)	

⁸ All the available studies have deficiencies as the test item was not analytically verified. By considering the nature of the substance, i.e. ubiquitous in nature and of very low solubility, they have been considered supportive and the endpoint set at the solubility level.

⁹ Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.

Table 2: Groundwater^(a)

Compound (name and/or code)	> 0.1 µg/L at 1 m depth for the representative uses ^(b) Step 2	Biological (pesticidal) activity/relevance Step 3a	Hazard identified Steps 3b and 3c	Consumer RA triggered Steps 4 and 5	Human health relevance
Not applicable Considering the nature of the substance and the limited exposure from the representative uses a definition of residue in the environment for risk assessment triggering assessment of effects data is deemed to be unnecessary for calcium carbonate (limestone)	Due to calcium carbonate being inorganic and its function as a repellent, the parametric drinking water limit (0.1 µg/L) is not applicable according to the regulatory framework.	Yes	Not triggered	No	Not triggered

(a): Assessment according to European Commission guidance of the relevance of groundwater metabolites (2003).

(b): FOCUS scenarios or a relevant lysimeter.

Table 3: Surface water and sediment

Compound (name and/or code)	Ecotoxicology
Not applicable Considering the nature of the substance, it being a constituent of many sediments and the limited exposure from the representative uses a definition of residue in the environment for risk assessment triggering assessment of effects data is deemed to be unnecessary for calcium carbonate (limestone)	Not triggered

Table 4: Air

Compound (name and/or code)	Toxicology
Not applicable Considering the nature of the substance and the limited exposure from the representative uses a definition of residue in the environment for risk assessment triggering assessment of effects data is deemed to be unnecessary for calcium carbonate (limestone)	Rat LC ₅₀ inhalation > 3 mg/L per 4 h (nose only) (no classification required)

LC₅₀: lethal concentration, median.

8. Particular conditions proposed to be taken into account by risk managers

Risk mitigation measures (RMMs) identified following consideration of MS and/or applicant's proposal(s) during the peer review, if any, are presented in this section. These measures applicable for human health and/or the environment leading to a reduction of exposure levels of operators, workers, bystanders/residents, environmental compartments and/or non-target organisms for the representative uses are listed below. The list may also cover any RMMs as appropriate, leading to an acceptable level of risks for the respective non-target organisms.

It is noted that final decisions on the need of RMMs to ensure the safe use of the plant protection product containing the concerned active substance will be taken by risk managers during the decision-making phase. Consideration of the validity and appropriateness of the RMMs remains the responsibility of MSs at product authorisation, taking into account their specific agricultural, plant health and environmental conditions at national level.

No particular conditions are proposed for the representative uses evaluated.

9. Concerns and related data gaps

9.1. Issues that could not be finalised

An issue is listed as 'could not be finalised' if there is not enough information available to perform an assessment, even at the lowest tier level, for one or more of the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011¹⁰ and if the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

An issue is also listed as 'could not be finalised' if the available information is considered insufficient to conclude on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

The following issues or assessments that could not be finalised have been identified, together with the reasons including the associated data gaps where relevant, which are reported directly under the specific issue to which they are related:

- None

9.2. Critical areas of concern

An issue is listed as a critical area of concern if there is enough information available to perform an assessment for the representative uses in line with the uniform principles in accordance with Article 29 (6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011, and if this assessment does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if the assessment at a higher tier level could not be finalised due to lack of information, and if the assessment performed at the lower tier level does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if, in the light of current scientific and technical knowledge using guidance documents available at the time of application, the active substance is not expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

The following critical areas of concern are identified, together with any associated data gaps, where relevant, which are reported directly under the specific critical area of concern to which they are related:

- None

9.3. Overview of the concerns identified for each representative use considered (Table 5)

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 8, has been evaluated as being effective, then 'risk identified' is not indicated in Table 5.)

¹⁰ Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

Table 5: Overview of concerns reflecting the issues not finalised, critical areas of concerns and the risks identified that may be applicable for some but not for all uses or risk assessment scenarios

Representative use		Deciduous and coniferous trees in forestry By painting individual plants and by spraying individual plants with a low-pressure handheld sprayer
Operator risk	Risk identified	
	Assessment not finalised	
Worker risk	Risk identified	
	Assessment not finalised	
Resident/bystander risk	Risk identified	
	Assessment not finalised	
Consumer risk	Risk identified	
	Assessment not finalised	
Risk to wild non-target terrestrial vertebrates	Risk identified	
	Assessment not finalised	
Risk to wild non-target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	
Groundwater exposure to active substance	Legal parametric value breached	
	Assessment not finalised	
Groundwater exposure to metabolites	Legal parametric value breached ^(a)	
	Parametric value of 10 µg/L ^(b) breached	
	Assessment not finalised	

The superscript numbers relate to the numbered points indicated in Sections 9.1 and 9.2. Where there is no superscript number, see Sections 2–7 for further information.

(a): When the consideration for classification made in the context of this evaluation under Regulation (EC) No 1107/2009 is confirmed under Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008.

(b): Value for non-relevant metabolites prescribed in SANCO/221/2000-rev. 10 final, European Commission (2003).

10. List of other outstanding issues

Remaining data gaps not leading to critical areas of concern or issues not finalised but considered necessary to comply with the data requirements, and which are relevant for some or all of the representative uses assessed at EU level. Although not critical, these data gaps may lead to uncertainties in the assessment and are considered relevant.

These data gaps refer only to the representative uses assessed and are listed in the order of the sections:

- A search of the scientific peer-reviewed open literature on the active substance and its relevant metabolites, dealing with side effects on the environment and non-target species and published within the 10 years before the date of submission of the dossier, to be conducted and reported in accordance with EFSA guidance on the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA, 2011a–c) (relevant for all representative uses evaluated).
- Further information on the effect of low temperature on stability of the representative formulation and shelf-life storage study at ambient temperature (relevant for all representative uses evaluated; see Section 1).
- Further information on the toxicity of calcium carbonate on aquatic organisms, i.e. acute toxicity data (relevant for all representative uses evaluated; see Section 5).

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Abbreviations

ADI	acceptable daily intake
AAOEL	acute acceptable operator exposure level
AOEC	acceptable operator exposure concentration
AOEL	acceptable operator exposure level
ARfD	acute reference dose
bw	body weight
DAR	draft assessment report
ECHA	European Chemicals Agency
EEC	European Economic Community
FAO	Food and Agriculture Organization of the United Nations
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
GAP	Good Agricultural Practice
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry

LC ₅₀	lethal concentration, median
LOD	limit of detection
MRL	maximum residue level
MS	Member State
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
RAC	regulatory acceptable concentration
RAR	Renewal Assessment Report
RBC	red blood cells
REACH	Registration, Evaluation, Authorisation of Chemicals Regulation
RMM	risk mitigation measure
RPE	respiratory protective equipment
RUD	residue per unit dose
SC	suspension concentrate
SD	standard deviation
SFO	single first-order
SMILES	simplified molecular-input line-entry system
SPG	specific protection goal
SSD	species sensitivity distribution
STMR	supervised trials median residue
t _{1/2}	half-life (define method of estimation)
TER	toxicity exposure ratio
TER _A	toxicity exposure ratio for acute exposure
TER _{LT}	toxicity exposure ratio following chronic exposure
TER _{ST}	toxicity exposure ratio following repeated exposure
TK	technical concentrate
TLV	threshold limit value
Tmax	time until peak blood levels achieved
TMDI	theoretical maximum daily intake
ToxCAST	(US EPA) Toxicity Forecaster
TRR	total radioactive residue
TSH	thyroid-stimulating hormone (thyrotropin)
TWA	time-weighted average
UDS	unscheduled DNA synthesis
UF	uncertainty factor
UV	ultraviolet
W/S	water/sediment
w/v	weight per unit volume
w/w	weight per unit weight
WBC	white blood cell
WG	water-dispersible granule
WHO	World Health Organization

Appendix A – Consideration of cut-off criteria for calcium carbonate according to Annex II of Regulation (EC) No 1107/2009 of the European Parliament and of the Council

Properties		Conclusion ^(a)
CMR	Carcinogenicity (C) Mutagenicity (M) Toxic for Reproduction (R)	Calcium carbonate is not considered to be carcinogenic, mutagenic or toxic for reproduction.
	Endocrine disrupting properties	Calcium carbonate is not considered to meet the criteria for endocrine disruption for human health and non-target organisms according to points 3.6.5 and 3.8.2 of Annex II of Regulation No 1107/2009, as amended by Commission Regulation (EU) 2018/605.
POP	Persistence Bioaccumulation Long-range transport	Calcium carbonate is not considered to be a persistent organic pollutant (POP) according to point 3.7.1 of Annex II of Regulation (EC) 1107/2009.
PBT	Persistence Bioaccumulation Toxicity	Calcium carbonate is not considered to be a persistent, bioaccumulative and toxic (PBT) substance according to point 3.7.2 of Annex II of Regulation (EC) 1107/2009.
vPvB	Persistence Bioaccumulation	Calcium carbonate is not considered to be a very persistent, very bioaccumulative substance according to point 3.7.3 of Annex II of Regulation (EC) 1107/2009.

(a): Origin of data to be included where applicable (e.g. EFSA, ECHA RAC, Regulation).

Appendix B – List of end points for the active substance and the representative formulation

Appendix B can be found in the online version of this output ('Supporting information' section):
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Safety assessment of the substance fatty acid-coated nano precipitated calcium carbonate for use in plastic food contact materials

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Abstract

The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) assessed the safety of the substance identified by the applicant as 'fatty acid-coated nano precipitated calcium carbonate'. It is intended to be used as a filler in all plastics at up to 5% for contact with acidic food and at up to 40% for contact with all other types of food. Articles made with the substance are intended for long-term storage over 6 months at room temperature and below. No information was provided on the mechanism of coating as to whether there is chemical modification of the surface and/or physical adsorption. The substance was not properly characterised, either as pristine material or when incorporated into plastic. Contrary to the non-coated material, data show that the coated material does not dissolve fully and quickly under pH conditions simulating gastrointestinal tract. Surface analysis with electron microscopy of low-density polyethylene (LDPE) samples before and after migration experiments showed major differences that indicate release of the substance from the surface after the tests with 3% acetic acid and to a lesser extent with 10% ethanol. This is consistent with measurable migrations that were up to 39 mg CaCO₃/kg when using an LDPE sample made with 5% of the substance in contact with acetic acid for 64 days at 40°C. The required data on the release of nanoparticles and on the potential toxicity of the substance in nanoform were not provided. Therefore, the Panel could not conclude on the safe use of the substance.

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Keywords: calcium carbonate, coated, fatty acids, nano, filler, food contact materials, safety assessment

Requestor: Federal public service of the Health, Food Chain Safety and Environment, Belgium

Question number: EFSA-Q-2019-00233

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Competing interests: R. Franz declared that Fraunhofer institute at which he is employed provides advisory services to private business operators active in the sector on food contact materials. In line with EFSA's Policy on Independence (https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf) and the Decision of the Executive Director on Competing Interest Management (https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf), a waiver was granted to R. Franz regarding his participation to the EFSA's Working Group on Food Contact Materials (FCMWG) in accordance with Article 21 of the Decision of the Executive Director on Competing Interest Management. Pursuant to Article 21(6) of the above-mentioned Decision, the involvement of R. Franz is authorised as a member in the FCM WG, allowing him to take part in the discussions and in the drafting phase of the scientific output, but he is not allowed to be, or act as, a chairman, a vice-chairman or rapporteur of the working group.

Note: The full opinion will be published in accordance with Article 10(6) of Regulation (EC) No 1935/2004 once the decision on confidentiality, in line with Article 20(3) of the Regulation, will be received from the European Commission. The following information have been provided under confidentiality and they are redacted awaiting the decision of the Commission: the manufacturing details, the identities and levels of impurities and some of the information on the particle and agglomerate sizes.

Legal notice: The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission).

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at <https://ess.efsa.europa.eu/doi/doiweb/doisearch>.

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† Deceased.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Before a substance is authorised to be used in food contact materials (FCM) and is included in a positive list, EFSA's opinion on its safety is required. This procedure has been established in Articles 8, 9 and 10 of Regulation (EC) No 1935/2004¹ of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.

According to this procedure, the industry submits applications to the Member States' competent authorities which transmit the applications to the European Food Safety Authority (EFSA) for their evaluation.

In this case, EFSA received an application from the federal public service of the Health, Food Chain Safety and Environment, Belgium, requesting the evaluation of the substance fatty acid-coated nano Precipitated Calcium Carbonate. The dossier was submitted on behalf of the Calcium Carbonate Association Europe (CCA Aisbl).

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 substance and to deliver a scientific opinion.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of its application for the authorisation of the substance fatty acid (saturated and unsaturated)-coated nano precipitated calcium carbonate to be used in plastic food contact materials.

Additional information was provided by the applicant during the assessment process in response to the request from EFSA sent on 30 January 2020, with addendum sent on 4 May 2021 (see Documentation provided to EFSA').

Data submitted and used for the evaluation are:

Non-toxicological data and information

- Identity and characterisation of the particles
- Description of manufacturing process of substance/FCM
- Physical and chemical properties including solubility and dissolution rate tests
- Intended uses
- Existing authorisation(s)
- Migration of the substance from low-density polyethylene (LDPE)
- Surface analysis of a LDPE substance composite before and after migration testing

Toxicological data

- Review of toxicological studies on calcium carbonate (bulk or nano form) and on the fatty acids

2.2. Methodologies

The assessment was conducted in line with the principles laid down in Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food. This Regulation underlines that applicants may consult the Guidelines of the Scientific Committee on Food (SCF) for the presentation of an application for safety assessment of a substance to be used in FCM prior to its authorisation (European Commission, 2001), including the corresponding data requirements. The dossier that the applicant submitted for evaluation was in line with the SCF guidelines (European Commission, 2001).

The methodology is based on the characterisation of the substance that is/are the subject of the request for safety assessment prior to authorisation, its impurities and reaction and degradation products, the evaluation of the exposure to those substances through migration and the definition of minimum sets of toxicity data required for safety assessment.

¹ 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.

To establish the safety from ingestion of migrating substances, the toxicological data indicating the potential hazard and the likely human exposure data need to be combined. Exposure is estimated from studies on migration into food or food simulants and considering that a person may consume daily up to 1 kg of food in contact with the relevant FCM.

As a general rule, the greater the exposure through migration, the more toxicological data is required for the safety assessment of a substance. Currently there are three tiers with different thresholds triggering the need for more toxicological information as follows:

- a) In case of high migration (i.e. 5–60 mg/kg food), an extensive data set is needed.
- b) In case of migration between 0.05 and 5 mg/kg food, a reduced data set may suffice.
- c) In case of low migration (i.e. < 0.05 mg/kg food), only a limited data set is needed.

More detailed information on the required data is available in the SCF guidelines (European Commission, 2001).

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA, 2009) and considering the relevant guidance from the EFSA Scientific Committee, such as the Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health (EFSA Scientific Committee, 2018). The update of the Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health and the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA Scientific Committee, 2021a,b) were not published at the moment of the submission of the data by the applicant. They were considered during the assessment, but not for rejecting data compliant with the version from 2018 and not with the versions from 2021.

3. Assessment

According to the applicant², the substance fatty acid-coated nano precipitated calcium carbonate is intended to be used as a filler in all types of polymers to impart strength and rigidity to the polymer, at concentrations up to 5% for contact with acidic food and up to 40% for contact with all other types of food. Articles made with the substance are intended for long-term storage over 6 months between –10°C and +30°C.

The substance was not evaluated by SCF and EFSA in the past. However, the bulk calcium carbonate (not in nanoform) and all the fatty acids used to coat the particles are authorised and listed in the Regulation (EU) No 10/2011 under either a single substance entry or a mixture. None of the calcium carbonate (FCM No. 21, calcium salts of carbonic acid) and the fatty acids have a restriction. Calcium carbonate is also authorised as food additive (E 170) and was re-evaluated by the ANS Panel in 2011 (EFSA ANS Panel, 2011).

3.1. Non-toxicological data

3.1.1. Identity of the substance³

According to the applicant, the substance is a white powder in the form of nanoparticles [REDACTED]

[REDACTED] The amounts of fatty acids added individually or in a mixture to the calcium carbonate are between 0.1% and 5% (w/w). According to the applicant, the fatty acids are aliphatic, linear, C12–C18 saturated and C16–C18 unsaturated. The Panel noted that minor fatty acids may be present in the source material. No information was provided on the mechanism of coating as to whether there is chemical modification of the surface (e.g. formation of fatty acids salts) and/or physical adsorption.

The formula weight for CaCO₃ is 100 Da and for fatty acids ranges from 200.3 Da (lauric acid) up to 284.5 Da (stearic acid).

The purity of the additive is stated to be from 96.5% to 100% (typically 97.5%). However, this was not consistent amongst the set of information provided (e.g. 85–100%, typically 95%). The main impurities in the calcium carbonate are [REDACTED]

² Technical dossier/2subm211019/Appendix B/Sections 1 and 4.

³ Technical dossier/2subm211019/Appendix B/sections 1 and 2; technical dossier/add data Sept 21/supporting letter; related annexes.

Although requested, no information was provided on possible impurities of the fatty acids.

The total surface area of the particles reported in the 'Chemical Safety Report' was 15–30 m²/g mass-specific surface area (MSSA), measured using the Brunauer, Emmett and Teller (BET) method. The particles are not porous, but they form agglomerates that have some porosity.

According to two scanning electron microscopy (SEM) images, the material consists of agglomerated primary particles that are quasi-spherical with a reported diameter of about [REDACTED]

The size of the agglomerates is reported to be around [REDACTED]. For two samples, laser diffraction (LD) determined diameters of up to [REDACTED] and D50 of [REDACTED] respectively. The identity of the tested samples was not clear and the method performance characteristics and data conversion were not provided.

For the characterisation of the size distribution of the primary particles, the applicant provided two sets of information, a 'Chemical Safety Report' and a LD analysis report. According to the 'Chemical Safety Report', the particle size (D50) ranges between 40 and 90 nm boundary composition. However, it was not supported by raw data, method of analysis or data conversion. According to the LD analysis, the particle size ranges from [REDACTED] with a D50 of [REDACTED] and a D98 of [REDACTED]. The identity of the tested samples was not clear, and the method performance characteristics and data conversion were not provided. Therefore, in accordance with the Opinions of the Scientific Committee on nanoparticle assessment (EFSA Scientific Committee, 2018, 2021a,b), the substance was considered not to be properly characterised.

3.1.2. Physical and chemical properties of the substance⁴

The bulk calcium carbonate decomposes at temperatures greater than 825°C.

The solubilisation rate of the coated and non-coated nano calcium carbonate was tested at 37°C, both at pH 3 and 7 and at five time points (0, 30, 60, 90 and 120 min), based on an ultrafiltration test using a filter of 100 kDa pore size. Measurements of the dissolution rate were performed with 100 mg/L of the substance, corresponding to 40 mg/L of calcium. The results show that the nanoparticles do not dissolve completely and rapidly even at pH 3. After 30 min, 18% (corresponding to 7 mg/L) was dissolved at pH 3 and 5% (corresponding to 2 mg/L) at pH 7. After 120 min at pH 3, 23% was solubilised. From this data, the Panel concluded that the fatty acid-coated nano calcium carbonate is much less soluble than the non-coated nano calcium carbonate (95–98% after 30 min at pH 3). The thickness and the percentage of coating of the tested samples were not provided.

In accordance with the EFSA Scientific Committee Opinions (EFSA Scientific Committee, 2021a,b), it is concluded that the substance does not dissolve fully and quickly. Therefore, an assessment at the nanoscale was required, but was not provided although requested.

3.1.3. Characterisation and quantification of the substance after incorporation in FCM⁵

The external surface of the LDPE samples containing the substance were analysed with Electron Microscopy (backscattered SEM, SEM-energy-dispersive X-ray (EDX), and semi-quantitative SEM-EDX) before and after migration experiments lasting for 10 days at 60°C. After contact with 95% ethanol and isooctane, no significant differences were found. However, major differences, such as changes in the shape of agglomerates and the appearance of voids that indicate release of the substance from the surface were observed after the tests with 3% acetic acid and to a lesser extent with 10% ethanol. These observations are consistent with clearly measurable migration observed in 3% acetic acid and 10% ethanol (see Section 3.1.4 below).

The characterisation of the particle size distribution within the plastic by surface analysis of the cross-section was requested. In response, surface analyses of the bulk of LDPE samples containing the substance were performed, using the above-mentioned methodology before and after a migration experiment with 10% ethanol for 10 days at 60°C. Pictures show that the substance is evenly distributed throughout the selected area of the LDPE, with agglomerates in the µm range. Considering 200 primary particles, the average particle size was 73 nm (40–150 nm) before exposure and 74 nm (30–160 nm) after exposure, i.e. showing no significant difference. The images did not show whether

⁴ Technical dossier/2subm211019/Appendix B/section 2; technical dossier/add data Sept 21/supporting letter; related annexes.

⁵ Technical dossier/2subm211019/Appendix B/sections 2 and 5; technical dossier/add data Sept 21/supporting letter; related annexes.

or not the particles were fully embedded in the host polymer, for which cross section images would need to show also the physical boundaries of the composites. The thickness and the percentage of coating of the tested sample were not provided.

3.1.4. Specific migration⁶

The specific migration of calcium, expressed as calcium carbonate, from LDPE containing the substance at 5, 10, 20 and 40% into 10% ethanol, 3% acetic acid, 95% ethanol and isooctane was determined by inductively coupled plasma (ICP) with optical emission spectrometry (OES) and mass spectrometry (MS). The conditions were 10 days at 60°C. 3% Acetic acid was also tested for 64 days at 40°C. The surface to volume (S/V) ratio was 6 dm²/kg.

In the experiments carried out with LDPE containing 40% of the substance, the migration was below 0.03 mg/kg in isooctane, equal to 0.03 mg/kg in 95% ethanol, 3.4 mg/kg in 10% ethanol and 2,502 mg/kg in acetic acid (out of the scope of the request). When using LDPE made with 5% of the substance, the maximum intended for contact with acidic foods, the migration was 39 mg/kg.

Since the substance does not dissolve quickly and fully under pH conditions simulating gastrointestinal tract and the migration of the substance in nanoform was not ruled out, the following information were requested, but not provided:

- migration experiments with LDPE determining nanoparticles in simulants;
- data on polar polymers: on the particle size distribution following incorporation of the substance into the polymer(s), on the swelling of the polymers in contact with aqueous and acidic simulants and on the migration (i.e. the amount, the chemical composition and the size distribution of the released nano particles or ionic chemicals);
- data on the potential release of the substance due to mechanical stress or physical degradation (e.g. abrasion), particularly with dry foods, concluding on the amount, chemical composition and size distribution of the particles.

3.2. Toxicological data

Since the substance does not dissolve quickly and fully under pH conditions simulating gastrointestinal tract and the migration of the substance in nanoform was not ruled out, toxicological data in accordance with the Opinion of the Scientific Committee 'Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain' (EFSA Scientific Committee, 2018)⁷ were requested. Instead of data on the substance, the applicant provided toxicological data and a review of toxicological studies on calcium carbonate (bulk and nano form) and on the fatty acids, mainly referring to the REACH registration dossiers, that does not address the data requirement from the Scientific Committee. Therefore, no conclusion could be drawn on the potential toxicity of the substance in nanoform.

4. Conclusions

Based on the information provided by the applicant, the CEP Panel concluded that the substance was not properly characterised, either as pristine material or when incorporated into plastic. The substance does not dissolve rapidly and fully under conditions simulating gastrointestinal tract. The required data on the release of nanoparticles and on the potential toxicity of the substance in nanoform were not provided. Therefore, the Panel could not conclude on the safe use of the substance.

5. Documentation provided to EFSA

- 1) Initial dossier. April 2019. Submitted on behalf of CCA Aisbl.
- 2) Additional data. September 2021. Submitted on behalf of CCA Aisbl.

⁶ Technical dossier/2subm211019/Appendix B/section 5; related annexes.

⁷ For new application(s), the EFSA Scientific Committee Opinions on "Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health" and "Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles" (EFSA Scientific Committee, 2021a,b) should be considered.

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- European Commission, 2001. Guidelines of the Scientific Committee on Food for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorization.

Abbreviations

ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
BET	Brunauer, Emmett and Teller
CCA Aisbl	Calcium Carbonate Association Europe
CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
EDX	energy-dispersive X-Ray
FCM	food contact materials
ICP	inductively coupled plasma
LD	laser diffraction
LDPE	low-density polyethylene
MS	mass spectrometry
MSSA	mass-specific surface area
OES	optical emission spectrometry
REACH	registration, evaluation, authorisation and restriction of chemicals
SEM	scanning electron microscopy
SCF	Scientific Committee on Food
S/V	surface to volume
w/w	weight per weight

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Peer review of the pesticide risk assessment of the active substance limestone powder (calcium carbonate)

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Abstract

The conclusions of EFSA following the peer review of the initial risk assessments carried out by the competent authority of the rapporteur Member State Czech Republic for the pesticide active substance limestone powder (calcium carbonate) are reported. The context of the peer review was that required by Regulation (EC) No 1107/2009 of the European Parliament and of the Council. The conclusions were reached on the basis of the evaluation of the representative use of limestone powder in paste form as a repellent in forest plantations and forest tree nurseries (field uses). The reliable endpoints, appropriate for use in regulatory risk assessment, are presented. Missing information identified as being required by the regulatory framework is listed. No concerns are identified.

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Keywords: limestone, calcium carbonate, peer review, risk assessment, pesticide, repellent

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Summary

Limestone is an active substance for which, in accordance with Article 7 of Regulation (EC) No 1107/2009 of the European Parliament and of the Council, the rapporteur Member State (RMS), Czech Republic received an application from Agro Radomyšl a.s. on 11 August 2017 for approval. Complying with Article 9 of the Regulation, the completeness of the dossier was checked by the RMS and the date of admissibility of the application was recognised as being 26 July 2019.

An initial evaluation of the dossier on the active substance named limestone was provided by the RMS in the draft assessment report (DAR) and subsequently, a peer review of the pesticide risk assessment on the RMS evaluation was conducted by EFSA in accordance with Article 12 of Regulation (EC) No 1107/2009. The following conclusions are derived.

Limestone powder and calcium carbonate are considered the same substance in the course of this peer review.

The use of limestone powder (calcium carbonate) according to the representative use as a repellent to prevent browsing damage from red and roe deer and brown hare, applied in paste form manually by gloves or special forestry brush in forest plantations and forest tree nurseries (field uses), as proposed at EU level, results in a sufficient repellent efficacy.

The assessment of the data package revealed no issues that could not be finalised or that need to be included as critical areas of concern with respect to identity, physical and chemical properties and analytical methods.

In the area of mammalian toxicology, the information available for the active substance did not reveal issues that could not be finalised or critical areas of concern for the representative use as a repellent applied in paste form.

In the area of residues, issues not finalised or areas of concerns were not identified.

Limestone is a naturally occurring sedimentary rock composed largely of the mineral calcite (calcium carbonate: CaCO_3). The information available and its evaluation regarding the environmental fate and behaviour of the active substance were considered sufficient to complete the assessments necessary regarding the environmental exposure assessment at the European Union (EU) level for the representative use assessed. Considering the nature of the substance and the use pattern, environmental concentrations (except on the treated trees) are expected to be too low to measure, consequently a definition of the residue in the environment for monitoring is considered unnecessary for limestone powder (calcium carbonate).

In the area of ecotoxicology, low risk to all non-target organisms was concluded based on the low exposure in the environment and relevant food items for non-target organisms.

Limestone powder (calcium carbonate) does not meet the criteria for endocrine disruption for humans and non-target organisms according to points 3.6.5 and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605.

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Background

Regulation (EC) No 1107/2009 of the European Parliament and of the Council¹ (hereinafter referred to as 'the Regulation') lays down, *inter alia*, the detailed rules as regards the procedure and conditions for approval of active substances. This regulates for the European Food Safety Authority (EFSA) the procedure for organising the consultation of Member States (MS) and the applicant for comments on the initial evaluation in the draft assessment report (DAR), provided by the rapporteur Member State (RMS), and the organisation of an expert consultation, where appropriate.

In accordance with Article 12 of the Regulation, EFSA is required to adopt a conclusion on whether an active substance can be expected to meet the approval criteria provided for in Article 4 of the Regulation (also taking into consideration recital (10) of the Regulation) within 120 days from the end of the period provided for the submission of written comments, subject to an extension of 30 days where an expert consultation is necessary, and a further extension of up to 150 days where additional information is required to be submitted by the applicant in accordance with Article 12(3).

Limestone is an active substance for which, in accordance with Article 7 of the Regulation, the RMS, Czech Republic (hereinafter referred to as the 'RMS'), received an application from Agro Radomyšl a.s. on 11 August 2017. Complying with Article 9 of the Regulation, the completeness of the dossier was checked by the RMS and the date of admissibility of the application was recognised as being 26 July 2019.

The RMS provided its initial evaluation of the dossier on the active substance named limestone in the DAR, which was received by EFSA on 11 September 2020 (Czech Republic, 2020). The peer review was initiated on 14 January 2021 by dispatching the DAR to the MS and the applicant, Agro Radomyšl a.s., for consultation and comments. EFSA also provided comments. In addition, EFSA conducted a public consultation on the DAR. The comments received were collated by EFSA and forwarded to the RMS for compilation and evaluation in the format of a reporting table. The applicant was invited to respond to the comments in column 3 of the reporting table. The comments and the applicant's response were evaluated by the RMS in column 3.

The need for expert consultation and the necessity for additional information to be submitted by the applicant in accordance with Article 12(3) of the Regulation were considered in a telephone conference between EFSA and the RMS on 27 May 2021. On the basis of the comments received, the applicant's response to the comments and the RMS's evaluation thereof, it was concluded that additional information should be requested from the applicant, and that EFSA should conduct an expert consultation in the area of mammalian toxicology.

The outcome of the telephone conference, together with EFSA's further consideration of the comments is reflected in the conclusions set out in column 4 of the reporting table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, including those issues to be considered in an expert consultation, were compiled by EFSA in the format of an evaluation table.

The conclusions arising from the consideration by EFSA, and as appropriate by the RMS, of the points identified in the evaluation table, together with the outcome of the expert consultation and the written consultation on the assessment of additional information, where these took place, were reported in the final column of the evaluation table.

In accordance with Article 12 of the Regulation, EFSA should adopt a conclusion on whether the material named limestone in the application can be expected to meet the approval criteria provided for in Article 4 of the Regulation, taking into consideration recital (10) of the Regulation.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with MS via a written procedure in March 2022.

This conclusion report summarises the outcome of the peer review of the risk assessment on the active substance and the representative formulation evaluated on the basis of the representative use of limestone powder (calcium carbonate) as a repellent in forest plantations and forest tree nurseries (field uses) as proposed by the applicant. In accordance with Article 12(2) of Regulation (EC) No 1107/2009, risk mitigation options identified in the DAR and considered during the peer review, if any, are presented in the conclusion.

¹ Regulation (EC) No 1107/2009 of 21 October 2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

Furthermore, this conclusion also addresses the requirement for an assessment by EFSA under Article 12 of Regulation (EC) No 396/2005, provided that the active substance will be approved under Regulation (EC) No 1107/2009 without restrictions affecting the residue assessment. In the event of a non-approval of the active substance or an approval with restrictions that have an impact on the residue assessment, if any, from this conclusion might no longer be relevant and a new assessment under Article 12 of Regulation (EC) No 396/2005 will be required.

A list of the relevant end points for the active substance and the formulation is provided in Appendix B. In addition, the considerations as regards the cut-off criteria for limestone powder (calcium carbonate) according to Annex II of Regulation (EC) No 1107/2009 are summarised in Appendix A.

A key supporting document to this conclusion is the peer review report (EFSA, 2022), which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The peer review report comprises the following documents, in which all views expressed during the course of the peer review, including minority views, where applicable, can be found:

- the comments received on the DAR;
- the reporting table (27 May 2021);
- the evaluation table (25 March 2022);
- the report of the scientific consultation with MS experts (where relevant);
- the comments received on the assessment of the additional information (where relevant);
- the comments received on the draft EFSA conclusion.

Given the importance of the DAR, including its revisions (Czech Republic, 2022), and the peer review report, both documents are considered as background documents to this conclusion and thus are made publicly available.

It is recommended that this conclusion and its background documents would not be accepted to support any registration outside the European Union for which the applicant has not demonstrated that it has regulatory access to the information on which this conclusion report is based.

The active substance and the formulated product

Limestone is considered by the International Organization for Standardization not to require a common name. The technical active substance is prepared by converting the stone into a powder. The active substance, named limestone in the application for approval, consists mainly of calcium carbonate (IUPAC) and is considered the same substance as calcium carbonate recently evaluated by EFSA for use in plant protection (EFSA, 2021).

The representative formulated product for the evaluation is 'Neoponit L', a paste (PA) containing 750 g/kg limestone powder.

The representative use evaluated comprises manual applications using gloves or special forestry brush in forest plantations and forest tree nurseries as a repellent to prevent browsing damage from red and roe deer and brown hare. Full details of the Good Agricultural Practice (GAP) can be found in the list of end points in Appendix B.

Information was submitted to conclude that the use of limestone powder (calcium carbonate) according to the representative use proposed at EU level results in a sufficient repellent efficacy against the target organisms, following the guidance document SANCO/10054/2013-rev. 3 (European Commission, 2013).

Conclusions of the evaluation

1. Identity, physical/chemical/technical properties and methods of analysis

The following guidance documents were followed in the production of this conclusion: European Commission (2000a,b, 2010).

The proposed specification for limestone powder (calcium carbonate) is based on batch data from industrial plant production. The proposed minimum purity of the technical material is 974 g/kg expressed as calcium carbonate. The technical material contains silica (SiO₂), however no information was provided as to whether SiO₂ is present in its crystalline form. If present, this can lead to a

toxicological concern. As discussed further in Section 2, the lack of information on this is not relevant for the representative formulation (paste); however, this might be an issue for different types of formulations and/or equivalence check of technical materials done by MS. The material used in the (eco)toxicological assessment does not support the proposed specification (see Sections 2 and 5). FAO specifications are not available for calcium carbonate, limestone and limestone powder.

The main data regarding the identity of limestone powder (calcium carbonate) and its physical and chemical properties are given in Appendix B.

Methods of analysis are available for the determination of the active substance in the technical material and in the representative formulation. A data gap for validation data for the methods for determination of some of the impurities in the technical material was identified (see Section 10).

Methods for the analysis of residues in food and feed of plant origin, animal products, body fluids and tissues and environmental compartments are not required as residue definitions were not set.

2. Mammalian toxicity

Limestone powder (calcium carbonate) was discussed at the Pesticides Peer Review Experts' Teleconference 70 in January 2022 and assessed based on the following guidance documents: European Commission (2003, 2012), EFSA PPR Panel (2012), EFSA (2014) and ECHA (2017). Furthermore, calcium carbonate was also discussed earlier, at the Pesticides Peer Review Experts' Teleconference 32 in November 2020, in the context of another application (EFSA, 2021).

Silica is present in the technical material. The presence of significant amounts of crystalline silica with particle size below 10 μm has not been excluded (see Section 1). Should this fraction below 10 μm be present as crystalline silica at or above 0.1% w/w of the total silica, this would raise a toxicological concern. With regard to the technical specification, it has not been demonstrated to be covered by the material used in the toxicological studies. However, further information is not needed on these points considering the representative formulation (paste) and the intended application method (see also Section 1).

The toxicological risk assessment of limestone powder is mainly based on studies from the open literature, the REACH registration dossier of calcium carbonate,² the Scientific Opinion re-evaluating the safety of calcium carbonate (E 170) of the EFSA Panel on Food Additives and Nutrient Sources added to Food (EFSA ANS Panel, 2011).

Absorption of calcium from dietary calcium carbonate accounts for 20–40% in rats and humans (EFSA ANS Panel, 2011). After intestinal absorption, calcium and carbonate/bicarbonate ions enter the blood stream and distribute into the body. The majority of absorbed calcium is stored in the skeleton. Excess calcium is excreted with water via kidneys (and also via faeces and sweat) and excess carbonate is excreted as carbon dioxide via respiration. A comparative *in vitro* metabolism study was not considered necessary given (i) that calcium carbonate is essential to all life forms, (ii) its long history of safe use as a food additive and as a gastric antiacid and (iii) the available toxicokinetic (ADME) information not suggesting any significant metabolic difference between humans and other species.

Calcium carbonate has low **acute toxicity** by the oral, dermal and inhalation routes, based on the use as food additive, literature data, history of safe uses, and supported by summary information from REACH registration dossiers. Furthermore, the compound is neither a skin or eye irritant, nor a skin sensitiser.

Concerning **short-term** oral toxicity, no target organs and no signs of systemic toxicity were observed, with a relevant oral short-term no observed adverse effect level (NOAEL) of 1,000 mg/kg body weight (bw) per day from a 90-day rat study. Concerning short-term inhalation toxicity, in a 90-day inhalation toxicity study with a nanoform of calcium carbonate, treatment-related local effects were identified in the lower airways, i.e. increased lung weight accompanied by slight increases in bronchoalveolar lavage-derived inflammation and cytotoxicity biomarkers (partly reversible within 4 weeks), with a no observed adverse effect concentration (NOAEC) of 0.212 mg/L (= 212 mg/m³).

Limestone powder (calcium carbonate) is unlikely to be **mutagenic** or **clastogenic**. Aneugenic potential was not investigated but this is not considered needed considering the nature and use of the substance.

Despite the absence of studies, there is no concern for the **long-term** effects and **carcinogenicity** of the compound since both calcium and carbonate are natural constituents of the

² <https://echa.europa.eu/cs/registration-dossier/-/registered-dossier/16050/10> [Accessed: March 2022].

body and normal metabolites of humans, animals and plants, and have a long history of safe use as a source of calcium supplementation for humans.

Regarding the **reproductive toxicity**, no adverse effects were observed on reproductive parameters in a combined repeated dose toxicity study with the reproductive and developmental toxicity screening test in rats.

In a rat **developmental toxicity** study, a developmental NOAEL of 1,250 mg/kg bw per day was identified on the basis of increased incidence of external and skeletal foetal findings at the higher dose tested of 1,526.5 mg/kg bw per day (maternal NOAEL). Calcium carbonate is not considered to be teratogenic. In the absence of indications of potential **neurotoxic effects** in the available studies and considering the history of safe use and the chemical structure of the substance, it has been agreed that calcium carbonate has no potential for neurotoxicity.

Based on the representative use of the product (as a paste), and the low toxicity profile of the active substance, **toxicological reference values** (acceptable daily intake (ADI), acute reference dose (ARfD), acceptable operator exposure level (AOEL) and acute acceptable operator exposure level (AAOEL) values are considered not needed for limestone powder (calcium carbonate)).

The dermal absorption value for the representative formulation is 25% (default value). In the absence of toxicological reference values, **non-dietary exposure** estimates are not necessary. It is noted that for the representative use and representative formulation (paste), exposure by inhalation is not expected to be significant. For other potential uses triggering inhalation exposure, e.g. spray, should they be considered for national authorisations, an acceptable operator exposure concentration (AOEC) could be based on the NOAEC of 0.212 mg/L (=212 mg/m³) identified for treatment-related changes in the lower airways (increased lung weight accompanied by slight increases in bronchoalveolar lavage-derived inflammation and cytotoxicity biomarkers) in a 90-day inhalation toxicity study with a nanoform of calcium carbonate. It should however be noted that, concerning inhalation exposure, particular attention should be given to the possible presence of toxicologically relevant crystalline silica in the technical material (see Section 1).

EFSA notes that the representative formulation 'Neoponit L' contains styrene polymer, a co-formulant of potential concern in the case styrene monomers were released from the polymer. Styrene is classified as Skin Irrit. 2, Eye Irrit. 2, Acute tox 4, STOT RE1 and Repr. 2 according to Regulation (EC) No 1272/2008³. Additionally, EFSA has recently re-assessed styrene safety for use as a food contact material (EFSA CEP Panel, 2020) and concluded that a concern for genotoxicity associated with oral exposure to styrene cannot be excluded. For the representative use and the representative formulation (paste), exposure by inhalation and/or ingestion is not expected (see Section 3). For other potential uses triggering inhalation exposure, e.g. spray, consideration should be given by MS to the possible presence of styrene in the product.

3. Residues

Standard studies according to EU/OECD guidance documents and EU data requirements have not been submitted to address the residue behaviour of limestone powder (calcium carbonate) from the proposed use. Due to the nature of the active substance and the proposed use, such studies are not required. The proposed use of limestone powder (calcium carbonate) as repellent which is applied to the shoots with brushes or manually (forest plantations, forest tree nurseries) will not result in exposure via food and feed to humans and livestock. Hence, a consumer risk assessment and the underlying data are not required.

Limestone powder and calcium carbonate are considered the same substance in the course of this peer review and hence the conclusions from the calcium carbonate output (EFSA, 2021) regarding the inclusion for Annex IV of Regulation (EC) No 396/2005 are applicable to limestone / limestone powder.

With regard to the five assessment criteria according to Commission guidance SANCO/11188/2013 rev. 2 (European Commission, 2015) for potential inclusion of substances in Annex IV of Regulation (EC) No 396/2005, i.e. approval as basic substance (criterion I), listed in Annex I of Regulation (EC) No 396/2005 (criterion II), having no identified hazardous properties (criterion III), natural exposure is higher than the one linked to the use as plant protection product (criterion IV) and consumer exposure is not expected considering the representative uses (criterion V), three criteria were considered to be met for limestone powder (calcium carbonate) for the following reasons:

³ Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

- Toxicological reference values are not required for limestone powder (calcium carbonate) (criterion III) (see Section 2).
- The application for the proposed use is unlikely to result in consumer exposure (criterion V). Therefore, the natural exposure from use as food item and food ingredient is higher than the one linked to the use as plant protection product (criterion IV).
- The other two criteria (criteria I and II) are not fulfilled.

It is noted that the representative plant protection product contains a co-formulant of potential concern. However, regarding the assessment of potential residues resulting from the co-formulant in the representative plant protection product (see Section 2), consumer exposure is not expected for the representative use as a repellent which is applied to the shoots with a brush or manually (in forest plantations or forest tree nurseries).

4. Environmental fate and behaviour

Limestone is a naturally occurring sedimentary rock composed largely of the mineral calcite (calcium carbonate: CaCO_3). In some regions it is the water bearing rock material of groundwater aquifers. The active substance is limestone powder (calcium carbonate). After application (by brush or glove) the formulation dries and forms a protective coating. The dried formulation is not water soluble. Because of the method of application environmental concentrations, except on the treated trees, are expected to be too low to measure. Considering the natural presence of calcium carbonate in soils and aquatic sediments and limestone being a groundwater aquifer material, further consideration of its fate and behaviour in the environment was concluded to be unnecessary.

5. Ecotoxicology

Valid toxicity data with the active substance or representative formulation were not available for any group of non-target organisms. Therefore, an assessment of the compliance of material tested with the specification was not required. The representative use of limestone powder (calcium carbonate) envisages localised application (by brush or gloves) in forest plantations/forest tree nurseries to prevent browsing damage. Upon application, the formulation dries and forms a protective coating. As reported in Sections 3 and 4, given the type of application and the properties of the active substance, the representative use is expected to result in exposure levels which are too low to measure in the environment. Additionally, it is anticipated that the exposure through food items relevant for non-target organisms would be low, considering the type of application and the properties of the active substance. Therefore, low risk was concluded for **all non-target organisms**. However, according to Regulation (EU) No 283/2013⁴, acute toxicity data with active substances should always be submitted for fish, aquatic invertebrates and algae. Therefore, a data gap was identified for these data regarding these aquatic organisms (see Section 10).

6. Endocrine disruption properties

With regard to the assessment of the endocrine disruption potential of limestone powder (calcium carbonate) for humans and non-target organisms according to the ECHA/EFSA guidance (2018), although no (eco)toxicological data are available to assess the endocrine disrupting properties, this does not appear scientifically necessary for the following reasons:

- It is a commonly occurring mineral of low solubility, though calcium and carbonate ions form in acidic aqueous media (including rainwater) due to the formation of the more soluble calcium bicarbonate;
- It is used as a repellent and therefore it has no toxic mode of action;
- Calcium carbonate is used as a food and feed additive; pharmaceutical and cosmetic substance.
- For the proposed use no exposure is anticipated to humans and non-target organisms, see Sections 3, 4 and 5.

⁴ Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.

Based on the available information, it can be concluded that limestone powder (calcium carbonate) does not meet the criteria for endocrine disruption for humans and non-target organisms according to points 3.6.5 and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605.

7. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments (Tables 1–4)

Table 1: Soil

Compound (name and/or code)	Ecotoxicology
Not applicable Considering the nature of the substance, it being a constituent of many soils and the limited environmental exposure from the representative use, a definition of residue in the environment for risk assessment triggering assessment of effects data is deemed to be unnecessary for limestone powder (calcium carbonate)	Not triggered

Table 2: Groundwater^(a)

Compound (name and/or code)	> 0.1 µg/L at 1 m depth for the representative uses ^(b) Step 2	Biological (pesticidal) activity/relevance Step 3a.	Hazard identified Steps 3b. and 3c.	Consumer RA triggered Steps 4 and 5	Human health relevance
Not applicable Considering the nature of the substance, it being a groundwater aquifer material and the limited environmental exposure from the representative use, a definition of residue in the environment for risk assessment triggering assessment of effects data is deemed to be unnecessary for limestone powder (calcium carbonate)	Due to limestone (calcium carbonate) being inorganic and its function as a repellent, the parametric drinking water limit (0.1 µg/L) for pesticides and their relevant metabolites as defined by the drinking water directive 98/83/EEC ^(c) is not applicable according to the regulatory framework	Yes	Not triggered	No	Not triggered

(a): Assessment according to European Commission guidance of the relevance of groundwater metabolites (2003).

(b): FOCUS scenarios or a relevant lysimeter.

(c): Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p. 32–54.

Table 3: Surface water and sediment

Compound (name and/or code)	Ecotoxicology
Not applicable Considering the nature of the substance, it being a constituent of many soils and the limited environmental exposure from the representative use, a definition of residue in the environment for risk assessment triggering assessment of effects data is deemed to be unnecessary for limestone powder (calcium carbonate)	Not triggered

Table 4: Air

Compound (name and/or code)	Toxicology
Not applicable	Rat LC ₅₀ inhalation > 3 mg/L per 4 h (nose only) (no classification required)
Considering the nature of the substance, it being a constituent of many soils and the limited environmental exposure from the representative use, a definition of residue in the environment for risk assessment triggering assessment of effects data is deemed to be unnecessary for limestone powder (calcium carbonate)	

LC₅₀: lethal concentration, 50%.

8. Particular conditions proposed to be taken into account by risk managers

Risk mitigation measures (RMMs) identified following consideration of MS and/or applicant's proposal(s) during the peer review, if any, are presented in this section. These measures applicable for human health and/or the environment leading to a reduction of exposure levels of operators, workers, bystanders/residents, environmental compartments and/or non-target organisms for the representative uses are listed below. The list may also cover any RMMs as appropriate, leading to an acceptable level of risks for the respective non-target organisms.

It is noted that final decisions on the need of RMMs to ensure the safe use of the plant protection product containing the concerned active substance will be taken by risk managers during the decision-making phase. Consideration of the validity and appropriateness of the RMMs remains the responsibility of MSs at product authorisation, taking into account their specific agricultural, plant health and environmental conditions at national level).

No particular conditions are proposed for the representative use evaluated.

9. Concerns and related data gaps

9.1. Issues that could not be finalised

An issue is listed as 'could not be finalised' if there is not enough information available to perform an assessment, even at the lowest tier level, for one or more of the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011⁵ and if the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

An issue is also listed as 'could not be finalised' if the available information is considered insufficient to conclude on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

The following issues or assessments that could not be finalised have been identified, together with the reasons including the associated data gaps where relevant, which are reported directly under the specific issue to which they are related:

Issues or assessments that could not be finalised were not identified.

9.2. Critical areas of concern

An issue is listed as a critical area of concern if there is enough information available to perform an assessment for the representative uses in line with the uniform principles in accordance with Article 29 (6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011, and if this assessment does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

⁵ Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

An issue is also listed as a critical area of concern if the assessment at a higher tier level could not be finalised due to lack of information, and if the assessment performed at the lower tier level does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if, in the light of current scientific and technical knowledge using guidance documents available at the time of application, the active substance is not expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

The following critical areas of concern are identified, together with any associated data gaps, where relevant, which are reported directly under the specific critical area of concern to which they are related:

Critical areas of concern were not identified.

9.3. Overview of the concerns identified for each representative use considered (Table 5)

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 8, has been evaluated as being effective, then 'risk identified' is not indicated in Table 5.)

Table 5: Overview of concerns reflecting the issues not finalised, critical areas of concerns and the risks identified that may be applicable for some but not for all uses or risk assessment scenarios

Representative use		Forest plantations Forest tree nurseries
		Manual applications by gloves or special forestry brush
Operator risk	Risk identified	
	Assessment not finalised	
Worker risk	Risk identified	
	Assessment not finalised	
Resident/bystander risk	Risk identified	
	Assessment not finalised	
Consumer risk	Risk identified	
	Assessment not finalised	
Risk to wild non-target terrestrial vertebrates	Risk identified	
	Assessment not finalised	
Risk to wild non-target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	
Groundwater exposure to active substance	Legal parametric value breached	
	Assessment not finalised	
Groundwater exposure to metabolites	Legal parametric value breached ^(a)	
	Parametric value of 10 µg/L ^(b) breached	
	Assessment not finalised	

(a): When the consideration for classification made in the context of this evaluation under Regulation (EC) No 1107/2009 is confirmed under Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008.

(b): Value for non-relevant metabolites prescribed in SANCO/221/2000-rev. 10 final, European Commission (2003).

10. List of other outstanding issues

Remaining data gaps not leading to critical areas of concern or issues not finalised but considered necessary to comply with the data requirements, and which are relevant for

some or all of the representative uses assessed at EU level. Although not critical, these data gaps may lead to uncertainties in the assessment and are considered relevant.

These data gaps refer only to the representative uses assessed and are listed in the order of the sections.

- Description and validation data for the methods used for determination of some of the impurities (metals, some inorganic elements and SiO₂) in the technical material (relevant for the representative use evaluated; see Section 1).
- Further information is needed on the toxicity of limestone powder (calcium carbonate) on aquatic organisms, i.e. acute toxicity data (relevant for the representative use evaluated; see Section 5).

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Abbreviations

a.s.	active substance
AAOEL	acute acceptable operator exposure level
ADI	acceptable daily intake
AOEC	acceptable operator exposure concentration
AOEL	acceptable operator exposure level
AR	applied radioactivity
ARfD	acute reference dose
bw	body weight
DAR	draft assessment report
DAT	days after treatment
ECHA	European Chemicals Agency
EEC	European Economic Community
FAO	Food and Agriculture Organization of the United Nations
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
GAP	Good Agricultural Practice
MS	Member State
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
RMM	risk mitigation measure
SMILES	simplified molecular-input line-entry system
WHO	World Health Organization

Appendix A – Consideration of cut-off criteria for limestone powder (calcium carbonate) according to Annex II of Regulation (EC) No 1107/2009 of the European Parliament and of the Council

Properties		Conclusion ^(a)
CMR	Carcinogenicity (C)	Limestone powder (calcium carbonate) is not considered to be mutagenic, carcinogenic, or toxic for reproduction according to points 3.6.2, 3.6.3 and 3.6.4 of Annex II of Regulation (EC) 1107/2009
	Mutagenicity (M)	
	Toxic for Reproduction (R)	
Endocrine disrupting properties		Limestone powder (calcium carbonate) does not meet the criteria for endocrine disruption for humans and non-target organisms according to points 3.6.5 and 3.8.2 of Annex II of Regulation No 1107/2009, as amended by Commission Regulation (EU) 2018/605.
POP	Persistence	Limestone powder (calcium carbonate) is not considered to be a persistent organic pollutant (POP) according to point 3.7.1 of Annex II of Regulation (EC) 1107/2009
	Bioaccumulation	
	Long-range transport	
PBT	Persistence	Limestone powder (calcium carbonate) is not considered to be a persistent, bioaccumulative and toxic (PBT) substance according to point 3.7.2 of Annex II of Regulation (EC) 1107/2009
	Bioaccumulation	
	Toxicity	
vPvB	Persistence	Limestone powder (calcium carbonate) is not considered to be a very persistent, very bioaccumulative substance according to point 3.7.3 of Annex II of Regulation (EC) 1107/2009
	Bioaccumulation	

(a): Origin of data to be included where applicable (e.g. EFSA, ECHA RAC, Regulation).

Appendix B – List of end points for the active substance and the representative formulation

Appendix B can be found in the online version of this output ('Supporting information' section):
<https://doi.org/10.2903/j.efsa.2022.7315>



SECTION 1: IDENTIFICATION

1.1. Product Identifier

Product Form: Substance

Product Name: Limestone

CAS-No.: 1317-65-3

Synonyms: Aggregate, AG Lime, Barn Lime, Cover Stone, Flex Base, Manufactured Sand, Mineral Filler Screening and Crushed Stone

1.2. Intended Use of the Product

Use of the Substance/Mixture: Construction

1.3. Name, Address, and Telephone of the Responsible Party

Company

Wiregrass Construction Company, Inc.

1830 Hartford Hwy

Dothan, AL 36301

334.699.6800

wiregrassconstruction.com

1.4. Emergency Telephone Number

Emergency Number : 256.759.1835

SECTION 2: HAZARDS IDENTIFICATION

2.1. Classification of the Substance or Mixture

Not classified

2.2. Label Elements

GHS-US Labeling

No labeling applicable according to 29 CFR 1910.1200.

2.3. Other Hazards

Exposure may aggravate pre-existing eye, skin, or respiratory conditions.

2.4. Unknown Acute Toxicity (GHS-US)

No data available

SECTION 3: COMPOSITION/INFORMATION ON INGREDIENTS

3.1. Substance

Name : Limestone

CAS-No. : 1317-65-3

Name	Synonyms	Product Identifier	%	GHS US classification
Limestone	Chalk / Limestone (A noncombustible solid characteristic of sedimentary rock. It consists primarily of calcium carbonate.) / Natural calcium carbonate / Marble / Calcium carbonate / Limestone (sedimentary rock) / Calcite / Limestone ground / Acetate, 4-methyl-2-propyl-2H-tetrahydropyran-4-yl / Ground limestone	(CAS-No.) 1317-65-3	100	Not classified

Full text of H-phrases: see section 16

3.2. Mixture

Not applicable

SECTION 4: FIRST AID MEASURES

4.1. Description of First-aid Measures

First-aid Measures General: Never give anything by mouth to an unconscious person. If you feel unwell, seek medical advice (show the label where possible).

First-aid Measures After Inhalation: When symptoms occur: go into open air and ventilate suspected area. Obtain medical attention if breathing difficulty persists.

First-aid Measures After Skin Contact: Remove contaminated clothing. Drench affected area with water for at least 5 minutes. Obtain medical attention if irritation develops or persists.

First-aid Measures After Eye Contact: Rinse cautiously with water for at least 5 minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Obtain medical attention if irritation develops or persists.

First-aid Measures After Ingestion: Rinse mouth. Do NOT induce vomiting. Obtain medical attention.

Limestone

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4.2. Most Important Symptoms and Effects Both Acute and Delayed

Symptoms/Injuries: Not expected to present a significant hazard under anticipated conditions of normal use.

Symptoms/Injuries After Inhalation: Prolonged exposure may cause irritation.

Symptoms/Injuries After Skin Contact: Prolonged exposure may cause skin irritation.

Symptoms/Injuries After Eye Contact: May cause slight irritation to eyes.

Symptoms/Injuries After Ingestion: Ingestion may cause adverse effects.

Chronic Symptoms: None known.

4.3. Indication of Any Immediate Medical Attention and Special Treatment Needed

If exposed or concerned, get medical advice and attention. If medical advice is needed, have product container or label at hand.

SECTION 5: FIRE-FIGHTING MEASURES

5.1. Extinguishing Media

Suitable Extinguishing Media: Water spray, fog, carbon dioxide (CO₂), alcohol-resistant foam, or dry chemical.

Unsuitable Extinguishing Media: Do not use a heavy water stream. Use of heavy stream of water may spread fire.

5.2. Special Hazards Arising From the Substance or Mixture

Fire Hazard: Not considered flammable but may burn at high temperatures.

Explosion Hazard: Product is not explosive.

Reactivity: Hazardous reactions will not occur under normal conditions. Reacts with acids forming carbon dioxide.

5.3. Advice for Firefighters

Precautionary Measures Fire: Exercise caution when fighting any chemical fire.

Firefighting Instructions: Use water spray or fog for cooling exposed containers.

Protection During Firefighting: Do not enter fire area without proper protective equipment, including respiratory protection.

Hazardous Combustion Products: None expected under normal conditions of use.

SECTION 6: ACCIDENTAL RELEASE MEASURES

6.1. Personal Precautions, Protective Equipment and Emergency Procedures

General Measures: Avoid prolonged contact with eyes, skin and clothing. Avoid breathing dust.

6.1.1. For Non-Emergency Personnel

Protective Equipment: Use appropriate personal protective equipment (PPE).

Emergency Procedures: Evacuate unnecessary personnel.

6.1.2. For Emergency Personnel

Protective Equipment: Equip cleanup crew with proper protection.

Emergency Procedures: Upon arrival at the scene, a first responder is expected to recognize the presence of dangerous goods, protect oneself and the public, secure the area, and call for the assistance of trained personnel as soon as conditions permit. Ventilate area.

6.2. Environmental Precautions

Prevent entry to sewers and public waters.

6.3. Methods and Materials for Containment and Cleaning Up

For Containment: Contain solid spills with appropriate barriers and prevent migration and entry into sewers or streams.

Methods for Cleaning Up: Clean up spills immediately and dispose of waste safely. Recover the product by vacuuming, shoveling or sweeping. Transfer spilled material to a suitable container for disposal. Contact competent authorities after a spill.

6.4. Reference to Other Sections

See Section 8 for exposure controls and personal protection and Section 13 for disposal considerations.

SECTION 7: HANDLING AND STORAGE

7.1. Precautions for Safe Handling

Precautions for Safe Handling: Avoid breathing dust. Avoid prolonged contact with eyes, skin and clothing. Wash hands and other exposed areas with mild soap and water before eating, drinking or smoking and when leaving work.

Hygiene Measures: Handle in accordance with good industrial hygiene and safety procedures.

7.2. Conditions for Safe Storage, Including Any Incompatibilities

Technical Measures: Comply with applicable regulations.

Storage Conditions: Keep container closed when not in use. Store in a dry, cool place. Keep/Store away from direct sunlight, extremely high or low temperatures and incompatible materials.

Incompatible Materials: Strong oxidizing agents. Acids. Magnesium. Aluminum.

7.3. Specific End Use(s)

Construction

Limestone

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SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1. Control Parameters

For substances listed in section 3 that are not listed here, there are no established exposure limits from the manufacturer, supplier, importer, or the appropriate advisory agency including: ACGIH (TLV), AIHA (WEEL), NIOSH (REL), or OSHA (PEL).

Limestone (1317-65-3)		
USA NIOSH	NIOSH REL (TWA)	10 mg/m ³ (total dust) 5 mg/m ³ (respirable dust)
USA OSHA	OSHA PEL (TWA) [1]	15 mg/m ³ (total dust) 5 mg/m ³ (respirable fraction)

8.2. Exposure Controls

Appropriate Engineering Controls

: Suitable eye/body wash equipment should be available in the vicinity of any potential exposure. Ensure adequate ventilation, especially in confined areas. Ensure all national/local regulations are observed.

Personal Protective Equipment

: Gloves. Protective clothing. Protective goggles.



Materials for Protective Clothing

: Chemically resistant materials and fabrics.

Hand Protection

: Wear protective gloves.

Eye and Face Protection

: Chemical safety goggles.

Skin and Body Protection

: Wear suitable protective clothing.

Respiratory Protection

: If exposure limits are exceeded or irritation is experienced, approved respiratory protection should be worn. In case of inadequate ventilation, oxygen deficient atmosphere, or where exposure levels are not known wear approved respiratory protection.

Other Information

: When using, do not eat, drink or smoke.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

9.1. Information on Basic Physical and Chemical Properties

Physical State

: Solid

Appearance

: Angular gray, white and tan particles ranging in size from a powder to a boulder

Odor

: No odor

Odor Threshold

: No data available

pH

: No data available

Evaporation Rate

: 0 (n-butyl acetate = 1)

Melting Point

: No data available

Freezing Point

: No data available

Boiling Point

: No data available

Flash Point

: No data available

Auto-ignition Temperature

: No data available

Decomposition Temperature

: No data available

Flammability (solid, gas)

: No data available

Vapor Pressure

: No data available

Relative Vapor Density at 20°C

: No data available

Relative Density

: No data available

Specific Gravity

: 2.6 - 2.75

Solubility

: Water: 0 g/l

Partition Coefficient: N-Octanol/Water

: No data available

Viscosity

: No data available

9.2. Other Information No additional information available

SECTION 10: STABILITY AND REACTIVITY

10.1. Reactivity: Hazardous reactions will not occur under normal conditions. Reacts with acids forming carbon dioxide.

10.2. Chemical Stability: Stable under recommended handling and storage conditions (see section 7).

Limestone

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- 10.3. Possibility of Hazardous Reactions:** Hazardous polymerization will not occur.
- 10.4. Conditions to Avoid:** Direct sunlight, extremely high or low temperatures, and incompatible materials.
- 10.5. Incompatible Materials:** Strong oxidizing agents. Acids. Magnesium. Aluminum.
- 10.6. Hazardous Decomposition Products:** Thermal decomposition may produce: Carbon oxides (CO, CO₂). Calcium oxides.

SECTION 11: TOXICOLOGICAL INFORMATION

11.1. Information on Toxicological Effects

Acute Toxicity (Oral): Not classified

Acute Toxicity (Dermal): Not classified

Acute Toxicity (Inhalation): Not classified

Skin Corrosion/Irritation: Not classified

Serious Eye Damage/Irritation: Not classified

Respiratory or Skin Sensitization: Not classified

Germ Cell Mutagenicity: Not classified

Carcinogenicity: Not classified

Reproductive Toxicity: Not classified

Specific Target Organ Toxicity (Single Exposure): Not classified

Specific Target Organ Toxicity (Repeated Exposure): Not classified

Aspiration Hazard: Not classified

Symptoms/Injuries After Inhalation: Prolonged exposure may cause irritation.

Symptoms/Injuries After Skin Contact: Prolonged exposure may cause skin irritation.

Symptoms/Injuries After Eye Contact: May cause slight irritation to eyes.

Symptoms/Injuries After Ingestion: Ingestion may cause adverse effects.

Chronic Symptoms: None known.

SECTION 12: ECOLOGICAL INFORMATION

12.1. Toxicity

Ecology - General : Not classified.

12.2. Persistence and Degradability

Limestone (1317-65-3)	
Persistence and Degradability	Not established.

12.3. Bioaccumulative Potential

Limestone (1317-65-3)	
Bioaccumulative Potential	Not established.

12.4. Mobility in Soil No additional information available

12.5. Other Adverse Effects

Other Information : Avoid release to the environment.

SECTION 13: DISPOSAL CONSIDERATIONS

13.1. Waste Treatment Methods

Waste Disposal Recommendations: Dispose of contents/container in accordance with local, regional, national, and international regulations.

Ecology - Waste Materials: Avoid release to the environment.

SECTION 14: TRANSPORT INFORMATION

The shipping description(s) stated herein were prepared in accordance with certain assumptions at the time the SDS was authored, and can vary based on a number of variables that may or may not have been known at the time the SDS was issued.

14.1. In Accordance with DOT Not regulated for transport

14.2. In Accordance with IMDG Not regulated for transport

14.3. In Accordance with IATA Not regulated for transport

SECTION 15: REGULATORY INFORMATION

15.1. US Federal Regulations

Limestone (1317-65-3)	
Listed on the United States TSCA (Toxic Substances Control Act) inventory - Status: Active	

Limestone

Safety Data Sheet

According to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

15.2. US State Regulations

Limestone (1317-65-3)

U.S. - New Jersey - Right to Know Hazardous Substance List

U.S. - Pennsylvania - RTK (Right to Know) List

U.S. - Massachusetts - Right To Know List

SECTION 16: OTHER INFORMATION, INCLUDING DATE OF PREPARATION OR LAST REVISION

Date of Preparation or Latest Revision : 10/04/2021

Other Information : This document has been prepared in accordance with the SDS requirements of the OSHA Hazard Communication Standard 29 CFR 1910.1200

GHS Full Text Phrases:

This information is based on our current knowledge and is intended to describe the product for the purposes of health, safety and environmental requirements only. It should not therefore be construed as guaranteeing any specific property of the product.

SDS US (GHS HazCom)

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.3
Revision Date 13.09.2021
Print Date 08.10.2022**SECTION 1: Identification of the substance/mixture and of the company/undertaking****1.1 Product identifiers**

Product name : Calcium carbonate

Product Number : C6763

Brand : SIGALD

REACH No. : 01-2119486795-18-xxxx

CAS-No. : 471-34-1

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 sheetCompany : Merck Life Science UK Limited
New Road
The Old Brickyard
GILLINGHAM
Dorset
SP8 4XT
UNITED KINGDOM

Telephone : +44 (0)1747 833-000

Fax : +44 (0)1747 833-313

E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +44 (0)870 8200418 (CHEMTREC)

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.

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula	: CCaO_3
Molecular weight	: 100.09 g/mol
CAS-No.	: 471-34-1
EC-No.	: 207-439-9

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

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact

Wash off with soap and plenty of water.

In case of eye contact

Flush eyes with water as a precaution.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water.

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

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture

Calcium oxide

5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information

No data available

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Avoid dust formation. Avoid breathing vapors, mist or gas.
For personal protection see section 8.

6.2 Environmental precautions

No special environmental precautions required.

6.3 Methods and materials for containment and cleaning up

Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on protection against fire and explosion

Provide appropriate exhaust ventilation at places where dust is formed.

Hygiene measures

General industrial hygiene practice.

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions

Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

Keep in a dry place. hygroscopic

Storage class

Storage class (TRGS 510): 13: Non 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

Component	CAS-No.	Value	Control parameters	Basis
calcium carbonate	471-34-1	TWA	10 mg/m ³	UK. EH40 WEL - Workplace Exposure Limits
	Remarks	For the purposes of these limits, respirable dust and inhalable dust are those fractions of airborne dust which will be collected when sampling is undertaken in accordance with the methods described in MDHS14/4 General methods for sampling and gravimetric analysis or respirable, thoracic and inhalable aerosols. The COSHH definition of a substance hazardous to health includes dust of any kind when present at a concentration in air equal to or greater than 10 mg.m ⁻³ 8-hour TWA of inhalable dust or 4 mg.m ⁻³ 8-hour TWA of respirable dust. This means that any dust will be subject to COSHH if people are exposed to dust above these levels. Some dusts have been assigned specific WELs and exposure to these must comply with the appropriate limits. Most industrial dusts contain particles of a wide range of sizes. The behaviour, deposition and fate of any particular particle after entry into the human respiratory system, and the body response that it elicits, depend on the nature and size of the particle. HSE distinguishes two size fractions for limit-setting purposes termed 'inhalable' and 'respirable'. Inhalable dust approximates to the fraction of airborne		

		material that enters the nose and mouth during breathing and is therefore available for deposition in the respiratory tract. Respirable dust approximates to the fraction that penetrates to the gas exchange region of the lung. Fuller definitions and explanatory material are given in MDHS14/4. Where dusts contain components that have their own assigned WEL, all the relevant limits should be complied with. Where no specific short-term exposure limit is listed, a figure three times the long-term exposure limit should be used.		
		TWA	4 mg/m ³	UK. EH40 WEL - Workplace Exposure Limits
		For the purposes of these limits, respirable dust and inhalable dust are those fractions of airborne dust which will be collected when sampling is undertaken in accordance with the methods described in MDHS14/4 General methods for sampling and gravimetric analysis or respirable, thoracic and inhalable aerosols. The COSHH definition of a substance hazardous to health includes dust of any kind when present at a concentration in air equal to or greater than 10 mg.m⁻³ 8-hour TWA of inhalable dust or 4 mg.m⁻³ 8-hour TWA of respirable dust. This means that any dust will be subject to COSHH if people are exposed to dust above these levels. Some dusts have been assigned specific WELs and exposure to these must comply with the appropriate limits. Most industrial dusts contain particles of a wide range of sizes. The behaviour, deposition and fate of any particular particle after entry into the human respiratory system, and the body response that it elicits, depend on the nature and size of the particle. HSE distinguishes two size fractions for limit-setting purposes termed 'inhalable' and 'respirable'. Inhalable dust approximates to the fraction of airborne material that enters the nose and mouth during breathing and is therefore available for deposition in the respiratory tract. Respirable dust approximates to the fraction that penetrates to the gas exchange region of the lung. Fuller definitions and explanatory material are given in MDHS14/4. Where dusts contain components that have their own assigned WEL, all the relevant limits should be complied with. Where no specific short-term exposure limit is listed, a figure three times the long-term exposure limit should be used.		
		TWA	10 mg/m ³	UK. EH40 WEL - Workplace Exposure Limits
		TWA	4 mg/m ³	UK. EH40 WEL - Workplace Exposure Limits

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).

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: Dermatrill® (KCL 740 / Aldrich Z677272, Size M)

Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: Dermatrill® (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.

Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure

No special environmental precautions required.

SECTION 9: Physical and chemical properties**9.1 Information on basic physical and chemical properties**

a) Appearance	Form: powder Color: white
b) Odor	No data available
c) Odor Threshold	No data available

d) pH	8.0
e) Melting point/freezing point	800 °C
f) Initial boiling point and boiling range	No data available
g) Flash point	No data available
h) Evaporation rate	No data available
i) Flammability (solid, gas)	No data available
j) Upper/lower flammability or explosive limits	No data available
k) Vapor pressure	No data available
l) Vapor density	No data available
m) Density	2.93 g/mL at 25 °C - lit.
Relative density	No data available
n) Water solubility	insoluble
o) Partition coefficient: n-octanol/water	No data available
p) Autoignition temperature	No data available
q) Decomposition temperature	No data available
r) Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
s) Explosive properties	No data available
t) Oxidizing properties	No data available

9.2 Other safety information

No data available

SECTION 10: Stability and reactivity

10.1 Reactivity

No data available

10.2 Chemical stability

Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

Exposure to moisture may affect product quality.

10.5 Incompatible materials

Strong oxidizing agents, Acids, Magnesium, Aluminum

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

LD50 Oral - Rat - female - > 2,000 mg/kg
(OECD Test Guideline 420)

LC50 Inhalation - Rat - male and female - 4 h - > 3 mg/l
(OECD Test Guideline 403)

LD50 Dermal - Rat - male and female - > 2,000 mg/kg
(OECD Test Guideline 402)

Skin corrosion/irritation

Skin - reconstructed human epidermis (RhE)
Result: No skin irritation - 15 min
(OECD Test Guideline 439)

Serious eye damage/eye irritation

Eyes - Bovine cornea
Result: No eye irritation - 4 h
(OECD Test Guideline 437)

Respiratory or skin sensitization

Local lymph node assay (LLNA) - Mouse
Result: negative
(OECD Test Guideline 429)

Germ cell mutagenicity

Test Type: Ames test
Test system: Escherichia coli/Salmonella typhimurium
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 471
Result: negative
Test Type: Mutagenicity (mammal cell test): chromosome aberration.
Test system: Human lymphocytes
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 473
Result: negative
Test Type: In vitro mammalian cell gene mutation test
Test system: mouse lymphoma cells
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 476
Result: negative

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

Repeated dose toxicity - Rat - male and female - Oral - 48 Days - NOAEL (No observed adverse effect level) - 1,000 mg/kg

RTECS: FF9335000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Hazardous properties cannot be excluded but are unlikely when the product is handled appropriately.

Handle in accordance with good industrial hygiene and safety practice.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	semi-static test LC50 - Oncorhynchus mykiss (rainbow trout) - > 100 mg/l - 96 h (OECD Test Guideline 203)
Toxicity to daphnia and other aquatic invertebrates	static test EC50 - Daphnia magna (Water flea) - > 100 mg/l - 48 h (OECD Test Guideline 202)
Toxicity to algae	static test ErC50 - Desmodesmus subspicatus (green algae) - 14 mg/l - 72 h (OECD Test Guideline 201)
Toxicity to bacteria	static test EC50 - activated sludge - > 1,000 mg/l - 3 h (OECD Test Guideline 209)

12.2 Persistence and degradability

Biodegradability	aerobic - Exposure time 28 d Result: 90 % - Readily biodegradable. (OECD Test Guideline 301B)

12.3 Bioaccumulative potential

Bioaccumulation is unlikely.

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 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

Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging

Dispose of as unused product.

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

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

Further information

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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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