Toxicological profile for

Starch

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

No data available to us at this time.

1.2. Synonyms

Starch, Corn; Corn Starch; Corn Starch Preparation; Cornstarch; Maize Starch; Starch, Corn [Ii]; Starch, Maize; Topical Starch; Zea Mays (Corn) Starch; (PubChem); starch; starch, unmodified; amidon; food starch, unmodified (FDA, 2022)

1.3. Molecular formula

$(C_6H_{10}O_5)n$ (NIOSH, 2019)

1.4. Structural Formula

No data available to us at this time.

1.5. Molecular weight (g/mol)

varies (NIOSH, 2019)

1.6. CAS registration number

9005-25-8

1.7. Properties

1.7.1. Melting point

(°C): Decomposes (GESTIS; NIOSH, 2019)

1.7.2. Boiling point

(°C): Decomposes (GESTIS; ICSC, 2004; NIOSH, 2019)

1.7.3. Solubility

Insoluble (NIOSH, 2019)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): Noncombustible solid, but may form explosive mixture with air, incompatible with oxidizers, acids, iodine, alkalis (NIOSH, 2019)

1.7.6. Flammability limits (vol/vol%)

Minimal explosive concentration: 50 g/m3 (NIOSH, 2019)

1.7.7. (Auto)ignition temperature

(°C): 410 (ICSC, 2004)

1.7.8. Decomposition temperature

(°C): ca. 200 (GESTIS)

1.7.9. Stability

Stable at normal temperatures and pressure; “There is a risk of a dust explosion if the following conditions are met: The substance is given in very finely distributed form (powder, dust). The substance is whirled up in sufficient quantity in the air. An ignition source is present (flame, spark, electrostatic discharge, etc.) (GESTIS)

1.7.10. Vapor pressure

0 mmHg (approximate) (NIOSH, 2019)

1.7.11. log Kow

No data available to us at this time.

2. General information

2.1. Exposure

Avena sativa starch is used as an absorbent and viscosity controlling agent; Oryza sativa starch and Solanum tuberosum starch as absorbent, binding, bulking and viscosity controlling agents; Pueraria lobata starch as an absorbent and opacifying agent; Tapioca starch as a viscosity controlling agent; Triticum vulgare starch as an abrasive, absorbent, binding, bulking and viscosity controlling agent; Zea mays starch as an abrasive, absorbent, anticaking, skin protecting and viscosity controlling agent (all CAS RN 9005-25-8); chenopodium quinoa starch (no CAS RN) as an emulsion stabilising and viscosity controlling agent, and Phaseolus radiatus seed starch (no CAS RN) as an abrasive and bulking agent in cosmetics in the EU.

As taken from Cosing (Cosmetic Substances and Ingredients Database).

Starch is listed as an ingredient used in fragrance compounds (IFRA).

Starch (CAS RN 9005-25-8) is listed as an ingredient (at given concentrations, where specified) in hobby/craft, home maintenance (1-100%, includes “old” products), inside the home (<0.1-10%), personal care (<0.5- >98%) and pesticide (1-30%, includes “old” products) products by the CPID.

According to Health Canada’s Natural Health Products (NHP) Ingredients Database, the following substances (no CAS RNs listed) are used for the indicated purposes in non-medicinal NHPs:

Arrowroot starch as a binder and disintegrant,

Metroxylon sagu (Sago palm) as a binder in oral use products,

Pea starch as a diluent and filler,

Pisum sativum (pea) starch extract as a filler,

Potato and rice starch as binders, diluents, disintegrants and glidants,

Pregelatinized starch as a binder, diluent and disintegrant,

Sorghum starch as a disintegrant,

Starch – maize as an abrasive, absorbent, binder, diluent, disintegrant, dusting powder, glidant, stabilizing agent and viscosity increasing agent – aqueous,

Starch – tapioca as a binder, diluent, disintegrant, glidant and viscosity increasing agent – aqueous,

Starch – wheat as an abrasive, absorbent, binder, bulking agent, diluent, disintegrant, glidant and viscosity increasing agent – aqueous,

Sweet potato starch as a binder, diluent and disintegrant in oral use products,

Waxy maize starch as a stabilizing agent, thickening agent and viscosity increasing agent.

Tinospora cordifolia stem starch is also listed in the NHP Database, with no further details of its function.

As taken from Health Canada, 2021

2.2. Combustion products

30g of the additive were heated at 700 degrees until the organic matter had disappeared in an airflow of 1.5l/min. The pyrolysis products were picene, benzo[a]pyrene, fluoranthene, anthracene, 4,5-methylene phenanthrene, phenanthrene quinone, anthra-quinone, pyrogallol, gallic acid, m-cresol, aliphatic olefinic hydrocarbons, water, furfurol, acetic acid, (formaldehyde) (Bell et al 1966; Kroller 1966).

2.3. Ingredient(s) from which it originates

Starch is found in many plants, including maize (corn), tapioca, wheat, rice, barley, oats, millet, lentils, potatoes, and other grains. Most starches are composed of 22-26% amylose and 74-78% amylopectin (ICSC, 2004).

Avena sativa starch is a starch obtained from the oat, Avena sativa L., Poaceae.

Oryza sativa starch is a high-polymeric carbohydrate material derived from the peeled seeds of the rice, Oryza sativa L., Poaceae.

Pueraria lobata starch is the starch obtained from the roots of Pueraria lobata, Fabaceae.

Solanum tuberosum starch is a polysaccharide obtained from the potato Solanum tuberosum L., Solanaceae.

Tapioca starch is the starch obtained from tapioca. It consists primarily of amylose and amylopectin.

Triticum vulgare starch is a high-polymeric carbohydrate material usually derived from the wheat, Triticum vulgare, Poaceae.

Zea mays starch is a high-polymeric carbohydrate material usually derived from the peeled seeds of the corn, Zea mays L., Gramineae.

Chenopodium Quinoa Starch is the starch obtained from Chenopodium quinoa, Amaranthaceae.

Phaseolus Radiatus Seed Starch is the starch obtained from the seeds of the Bean, Phaseolus radiatus L., Leguminosae

As taken from Cosing (Cosmetic Substances and Ingredients Database).

Zea Mays (Corn) Starch is a carbohydrate polymer derived from corn of various types, composed of 25% amylase and 75% amylopectin.

CIR (2011)

3. Status in legislation and other official guidance

Starch, unmodified (CAS RN 9005-25-8) is included on the FDA's list of Substances Added to Food (formerly EAFUS) and on the FDA's list of Indirect Additives Used in Food Contact Substances as an anti-caking agent or free-flow agent, drying agent, formulation aid, humectant, leavening agent, lubricant or release agent, stabilizer or thickener, and texturizer, and is covered under 21 CFR sections 137.105 (Flour); 155.130 (Canned corn); 169.150 (Salad dressing); 169.179 (Vanilla powder); 175.105 (Adhesives); 178.1010 (Sanitizing solutions); 182.90 (GRAS). As taken from FDA, 2022,2023a,c.

Generally Recognized as Safe in the US (21 CFR 182.90 – Substances migrating to food from paper and paperboard products) (FDA, 2022,c).

The 8-hour time-weighted average threshold limit values for total inhalable and respirable starch are 10 and 4 mg/m3 respectively (HSE, 2020).

Exposure Limits

NIOSH REL: TWA 10 mg/m^3 (total dust), TWA 5 mg/m^3 (respirable dust)

OSHA PEL: TWA 15 mg/m^3 (total dust), TWA 5 mg/m^3 (respirable fraction)

As taken from NIOSH, 2019; ACGIH, 2021a.

ACGIH TLV-TWA 10 mg/m3	DTLVS* The Threshold Limit Values (TLVs) and Biological Exposure Indices (BEIs) booklet issues by American Conference of Governmental Industrial Hygienists (ACGIH), Cincinnati, OH, 1996 Volume(issue)/page/year: TLV/BEI,2013
ACGIH TLV-Not classifiable as a human carcinogen	DTLVS* The Threshold Limit Values (TLVs) and Biological Exposure Indices (BEIs) booklet issues by American Conference of Governmental Industrial Hygienists (ACGIH), Cincinnati, OH, 1996 Volume(issue)/page/year: TLV/BEI,2013
MSHA STANDARD:NUISANCE PARTICULATES	DTLWS* "Documentation of the Threshold Limit Values for Substances in Workroom Air," Supplements. For publisher information, see 85INA8. Volume(issue)/page/year: 3,28,1972
OSHA PEL (Gen Indu):8H TWA 15 mg/m3, total dust	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1910.1000,1994
OSHA PEL (Gen Indu):8H TWA 5 mg/m3, respirable fraction	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1910.1000,1994
OSHA PEL (Construc):8H TWA 15 mg/m3, total dust	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1926.55,1994
OSHA PEL (Construc):8H TWA 5 mg/m3, respirable fraction	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1926.55,1994
OSHA PEL (Shipyard):8H TWA 15 mg/m3, total dust	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1915.1000,1993
OSHA PEL (Shipyard):8H TWA 5 mg/m3, respirable fraction	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402)

		Volume(issue)/page/year: 29,1915.1000,1993
	OSHA PEL (Fed Cont):8H TWA 15 mg/m3, total dust	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 41,50-204.50,1994
	OSHA PEL (Fed Cont):8H TWA 5 mg/m3, respirable fraction	CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 41,50-204.50,1994

OEL-BELGIUM: TWA 10 mg/m3, MAR2002

OEL-KOREA: TWA 10 mg/m3, 2006

OEL-NEW ZEALAND: TWA 10 mg/m3 (inspirable dust), JAN2002

OEL-PERU: TWA 10 mg/m3, JUL2005

OEL-RUSSIA: STEL 10 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.

TWA	STEL	Notations	MW	TLV Basis
10 mg/m3	-	A4 [Not classifiable as a human carcinogen]	-	Dermatitis

As taken from ACGIH, 2021b

Starch (CAS RN 9005-25-8) is not registered under REACH (ECHA).

Starch (CAS RN 9005-25-8) is listed in the US EPA InertFinder Database (2023) as approved for food and non-food use pesticide products.

Starch (CAS RN 9005-25-8) is included on the US EPA's list of Safer Chemical Ingredients with functional use: processing aids and additives (US EPA, 2023).

Starch (CAS RN 9005-25-8) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also on the US EPA 2020 CDR and 2020 CDR Full Exempt lists (Chemical Data Reporting Rule). The CDR regulation requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR.

US EPA Substance Registry Services (SRS)Starch (CAS RN 9005-25-8) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023).

OSHA PEL: This substance is considered under "particulates not otherwise regulated". TWA 10 mg/m3 (total dust), TWA 5 mg/m3 (respirable fraction)

As taken from Cal/OSHA.

Starch, corn starch, potato starch, rice starch, tapioca starch and wheat starch (all CAS RN 9005-25-8) are included on the US FDA's list of inactive ingredients for approved drug products. They are permitted for use as ingredients 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)
STARCH	BUCCAL	TABLET	9005258	NA		45 mg
STARCH	ORAL	CAPSULE	9005258	NA	430 mg	
STARCH	ORAL	CAPSULE, COATED PELLETS	9005258	NA	36.4 mg	
STARCH	ORAL	CAPSULE, DELAYED RELEASE	9005258	NA		392 mg
STARCH	ORAL	CAPSULE, EXTENDED RELEASE	9005258	NA	84.8 mg	
STARCH	ORAL	GRANULE, FOR SUSPENSION	9005258	NA	NA	
STARCH	ORAL	POWDER	9005258	NA	0.17 mg	
STARCH	ORAL	SUSPENSION	9005258	NA	20 mg/5ml	

STARCH	ORAL	SUSPENSION, EXTENDED RELEASE	9005258	NA		400 mg
STARCH	ORAL	SYRUP	9005258	NA	NA	
STARCH	ORAL	TABLET	9005258	NA		2440 mg
STARCH	ORAL	TABLET, CHEWABLE	9005258	NA		309 mg
STARCH	ORAL	TABLET, COATED	9005258	NA	210 mg	
STARCH	ORAL	TABLET, DELAYED RELEASE	9005258	NA	179.88 mg	
STARCH	ORAL	TABLET, EXTENDED RELEASE	9005258	NA	130.45 mg	
STARCH	ORAL	TABLET, FILM COATED	9005258	NA		480 mg
STARCH	ORAL	TABLET, SUGAR COATED	9005258	NA	NA	
STARCH	ORAL	WAFER	9005258	NA	4.8 mg	
STARCH	RECTAL	SUPPOSITORY	9005258	NA	NA	
STARCH	RECTAL	TABLET	9005258	NA	55 mg	
STARCH	SUBLINGUAL	TABLET	9005258	NA		225 mg
STARCH	TOPICAL	CREAM	9005258	NA	NA	
STARCH	TOPICAL	POWDER	9005258	NA	0.1 mg/mg	
STARCH 1500, PREGELATINIZED	BUCCAL	TABLET	9005258	O8232NY3SJ	16.6 mg	
STARCH 1500, PREGELATINIZED	ORAL	CAPSULE	9005258	O8232NY3SJ		5785 mg

STARCH 1500, PREGELATINIZED	ORAL	CAPSULE, COATED, EXTENDED RELEASE	9005258	O8232NY3SJ		19 mg
STARCH 1500, PREGELATINIZED	ORAL	CAPSULE, DELAYED RELEASE	9005258	O8232NY3SJ		216 mg
STARCH 1500, PREGELATINIZED	ORAL	CAPSULE, EXTENDED RELEASE	9005258	O8232NY3SJ		194 mg
STARCH 1500, PREGELATINIZED	ORAL	CONCENTRATE	9005258	O8232NY3SJ	NA	
STARCH 1500, PREGELATINIZED	ORAL	DROPS	9005258	O8232NY3SJ	NA	
STARCH 1500, PREGELATINIZED	ORAL	PASTILLE	9005258	O8232NY3SJ	NA	
STARCH 1500, PREGELATINIZED	ORAL	POWDER, FOR SUSPENSION	9005258	O8232NY3SJ		34 mg
STARCH 1500, PREGELATINIZED	ORAL	SUSPENSION	9005258	O8232NY3SJ		900 mg
STARCH 1500, PREGELATINIZED	ORAL	SUSPENSION, EXTENDED RELEASE	9005258	O8232NY3SJ		113 mg
STARCH 1500, PREGELATINIZED	ORAL	SUSPENSION/ DROPS	9005258	O8232NY3SJ		90 mg
STARCH 1500, PREGELATINIZED	ORAL	TABLET	9005258	O8232NY3SJ		1116 mg
STARCH 1500, PREGELATINIZED	ORAL	TABLET, CHEWABLE	9005258	O8232NY3SJ		180 mg

STARCH 1500, PREGELATINIZED	ORAL	TABLET, COATED	9005258	O8232NY3SJ		256 mg
STARCH 1500, PREGELATINIZED	ORAL	TABLET, DELAYED RELEASE	9005258	O8232NY3SJ		130 mg
STARCH 1500, PREGELATINIZED	ORAL	TABLET, EXTENDED RELEASE	9005258	O8232NY3SJ		184 mg
STARCH 1500, PREGELATINIZED	ORAL	TABLET, FILM COATED	9005258	O8232NY3SJ		696 mg
STARCH 1500, PREGELATINIZED	ORAL	TABLET, FILM COATED, EXTENDED RELEASE	9005258	O8232NY3SJ		149 mg
STARCH 1500, PREGELATINIZED	ORAL	TABLET, ORALLY DISINTEGRATING	9005258	O8232NY3SJ		100 mg
STARCH 1500, PREGELATINIZED	ORAL	TABLET, ORALLY DISINTEGRATING, DELAYED RELEASE	9005258	O8232NY3SJ		21 mg
STARCH 1500, PREGELATINIZED	SUBLINGUAL	TABLET	9005258	O8232NY3SJ		409 mg
STARCH 1500, PREGELATINIZED	VAGINAL	INSERT	9005258	O8232NY3SJ		147 mg
STARCH, POTATO	ORAL	TABLET	9005258	8I089SAH3T		458 mg
STARCH, POTATO	ORAL	TABLET, COATED	9005258	8I089SAH3T	1.37 mg	
STARCH, POTATO	ORAL	TABLET, EXTENDED RELEASE	9005258	8I089SAH3T	NA	
STARCH, RICE	ORAL	TABLET, EXTENDED RELEASE	9005258	4DGK8B7I3S	301 mg	

STARCH, TAPIOCA	ORAL	CAPSULE	9005258	24SC3U704I	100 mg	
STARCH, TAPIOCA	ORAL	TABLET	9005258	24SC3U704I	5 mg	
STARCH, WHEAT	ORAL	CAPSULE, EXTENDED RELEASE	9005258	79QS2MG2LP	0.75 mg	
STARCH, WHEAT	ORAL	TABLET	9005258	79QS2MG2LP	65.59 mg	
STARCH, WHEAT	ORAL	TABLET, COATED	9005258	79QS2MG2LP	NA	
STARCH, WHEAT	ORAL	TABLET, FILM COATED	9005258	79QS2MG2LP	49 mg	

As taken from FDA, 2023b

Starch (CAS RN 9005-25-8) has been “identified as low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach” and “poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment” (AICIS, 2012).

Pea starch, potato starch, rice starch, starch – maize, starch - wheat and waxy maize starch (no CAS RNs listed) are classified as natural health products (NHPs) under Schedule 1, item 2 (an extract) of the Natural Health Products Regulations (Health Canada, 2021).

Substance	CAS No.(c)	Regulatory Limits			Recommended Limits
		OSHA PEL(b)		Cal/OSHA PEL(f) (as of 11/12/2021)	NIOSH REL(g) (as of 11/15/2021)
		ppm	mg/m3(e)	8-hour TWA (ST) STEL (C) Ceiling	Up to 10-hour TWA (ST) STEL (C) Ceiling
Total dust	9005-25-8		15	10 mg/m3	10 mg/m3
Respirable fraction	9005-25-8		5	5 mg/m3	5 mg/m3

OSHA (2019)

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

No data available to us at this time.

“Starting in the mouth, food is mechanically broken down during the process of chewing while salivary amylase, secreted during mastication, initiates the chemical breakdown of starch. (…) Digestible dietary carbohydrates are mainly starch (a polymer of glucose molecules linked by alpha1-4 and alpha 1-6 glycosidic bonds), disaccharides (sucrose, lactose) and monosaccharides (glucose, fructose). Pancreatic amylase is the primary starch digestive enzyme that cleaves thea1-4 (but notthea1-6) glycosidic bonds. End products are maltose, maltotriose and a-limit dextrins, which are small glucose polymers containing a1-6 glycosidic bonds. Alpha-limit dextrins,

maltotrioses and disaccharides are digested into monosaccharides by digestive enzymes present in the brush border membrane of the small bowel: sucrase-isomaltase is involved in the digestion of a-limit dextrins and maltotriose into glucose, and in the digestion of sucrose into glucose and fructose (Boron and Boulpaep, 2016), maltase-glucoamylase in that of maltose into two molecules of glucose, (…) (Amiri and Naim, 2017). Congenital disaccharidase deficiencies are extremely rare, but lactase expression in the gut decreases drastically during childhood in approximately two-thirds of the world population, leading to adult lactose maldigestion (Storhaug et al., 2017). Digestion of dietary sugars and starch results in the release of the monosaccharides glucose, galactose and fructose at the surface of small bowel enterocytes. (…)

Monosaccharides (glucose, fructose, galactose) reaching the hepatic portal circulation are delivered to the liver and eventually entirely metabolised to CO2 and H2O.

Glucose can be metabolised in all cells of the human organism. Its metabolism involves a transport from the interstitial fluid to the cell, which is operated by a variety of non-insulin-dependent (mainly GLUT1-3), insulin-responsive (GLUT4) and sodium-glucose (SGLT1-2) membrane transporters. Intracellular glucose is initially metabolised by a member of the hexokinase enzyme family to glucose-6-phopshate (glucose-6-P) (Wilson, 2003). According to the cell type and energy status, glucose-6-P is further metabolised to pyruvate and lactate in the glycolytic pathway, to glucose-1-P and glycogen for storage or metabolised in the pentose monophosphate pathway.

Ingested glucose is already metabolised in part in the gut and liver. Hepatocytes transport glucose through non-insulin-dependent GLUT2 transporters and synthesise glucose-6-P by the enzyme hexokinase IV (also called glucokinase), whose activity is mainly dependent on glucose concentration (Iynedjian, 1993). Glycolysis in the hepatocytes is tightly regulated at the level of the enzyme phosphofructokinase, which is potently inhibited by high intracellular ATP and citrate concentrations. As a consequence, only a portion (usually 10–25%) of absorbed glucose is metabolised in hepatocytes, and the rest escapes hepatic uptake to reach the systemic circulation, where it will increase systemic glycaemia, elicit insulin secretion and stimulate insulin-dependent and non-insulin-dependent glucose disposal in the various organs and tissues (Petersen and Shulman, 2018).

The amount of glucose escaping splanchnic metabolism, thus reaching the systemic circulation and arterial blood can transiently increase blood glucose levels from ca. 5 mmol/L (fasting) to 8–10 mmol/L (postprandial). This increase elicits a marked stimulation of insulin secretion, and arterial glucose will be taken up by peripheral organs, either independently of insulin (brain) or under the control of insulin (skeletal muscle, adipose tissue) (Gerich, 1993).

Different from glucose, fructose cannot be readily phosphorylated by hexokinases and its initial metabolic steps rely on the presence of specific (GLUT5) or non-specific (GLUT2) membrane transporters (Thorens and Mueckler, 2010) and of specific fructolytic enzymes: ketohexokinase C or fructokinase, which catalyses the conversion of fructose into fructose-1-phosphate (F-1-P); aldolase B, which splits F-1-P into dihydroxyacetone-phosphate and glyceraldehyde, and triokinase, which phosphorylates dihydroxyacetone-phosphate and glyceraldehyde to glyceraldehyde-3-phosphate. Dihydroxyacetone-P and glyceraldehyde-P (triose phosphates) then join the normal glycolytic pathways.

Fructolytic enzymes are expressed in small bowel enterocytes, hepatocytes and kidney proximal tubules, which are the organs primarily involved in fructose metabolism. Part of ingested fructose is already metabolised to glucose (gluconeogenesis), lactate, glyceric acid and fatty acids in small bowel enterocytes. Any fructose escaping gut metabolism reaches the liver through the hepatic portal circulation. In hepatocytes, fructolysis, unlike glycolysis, is not inhibited by intracellular mediators such as ATP or citrate, and almost all fructose transported in liver cells is converted into triose phosphates. An excess of intracellular triose phosphates triggers the synthesis of lactate, glucose, glycogen, glycerol and fatty acids (Ter Horst and Serlie, 2017).

Very little fructose escapes gut and liver metabolism. Fructose concentrations in blood increase transiently up to about 0.5 mmol/L after ingestion of fructose-containing sugars. The fate of this systemic fructose remains unknown. Experiments with intravenous administration of fructose suggest that systemic fructose is mainly metabolised in the kidney (Mayes, 1993), gut and liver, although a portion may also be metabolised in non-fructolytic tissues using alternative metabolic pathways (Helsley et al., 2020). Fructose does not increase blood glucose and insulin concentration to any great extent."

EFSA (2022)

4.2. Absorption, distribution and excretion

Digestive and absorptive characteristic of starches determined in vitro and in vivo (Abstract). To compare the different digestive and absorptive characteristic of digestible starch (DS) and resistant starch (RS), using both in vitro and in vitro methods. Firstly, DS and RS content in four starchy samples were determined in vitro according to Englyst's method. Secondly, DS and RS digestion and absorption were evaluated by determination of four-hour blood glucose level, after direct injection of DS and RS solution into rats' duodenum. And in another animal test, the effect of RS on apparatus absorption rate of carbohydrate and nitrogen and secretion of gastric acid and pepsin was observed. In vitro, it was showed that general cornstarch was rich of DS, while 76% of potato starch was RS. In vitro, it was tested that after injection of DS into duodenum, most of DS could be absorbed in 120 min with high peak value of plasma glucose. While, RS needed more time to complete its absorption, and its apparatus absorption was up to 96%. It was also showed that RS could increase fecal nitrogen excretion, decrease nitrogen apparatus absorption rate and change gastric acid and pepsin excretion. By both in vitro and in vitro methods, RS is identified to be absorbed slowly but completely. The results partly support Englyst's method, which regards 120min as the time point to separate DS with RS in vitro. As taken from Wang Z et al. 2004 Wei Sheng Yan Jiu. 33(4), 470-2. PubMed, 2009 available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15461280&query_hl=52&itool=pubmed_docsum

"Although starch provides a large fraction of human caloric intake, there is limited information concerning the efficiency of intestinal absorption of this nutrient. Owing to the fermentation of starch by colonic bacteria, there is no quantitative test for starch absorption comparable to the fecal fat determination. The most accurate estimation of starch absorption has been obtained by intubating the terminal ileum and aspirating ileal contents following ingestion of a meal containing starch plus a nonabsorbable marker. Starch absorption is calculated from the ratio of starch:marker in the ileal aspirate relative to the ratio in the meal. Disadvantages of the technique are the requirement for ileal intubation and the possible adverse effect of intubation on the absorptive process. A more widely used technique to assess starch absorption involves measurement of breath hydrogen (H2) excretion after ingestion of starch. Malabsorbed starch is fermented by colonic bacteria with liberation of H2 that is absorbed and excreted in expired air. This test is simple and noninvasive and can provide quantitative measurements of starch malabsorption. Application of this technique has demonstrated that 5-10% of starch in wheat, potatoes, and corn is not absorbed by healthy subjects, while rice starch is nearly completely absorbed." As taken from Strocchi A, Levitt MD. Can J Physiol Pharmacol. 1991 Jan; 69(1):108-10. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=2036592&query_hl=55&itool=pubmed_docsum

"Twelve 3-day metabolic balance studies were carried out in 12 low birth weight infants fed an infant formula providing 3.5 g of corn starch per kilogram body weight per day. The mean coefficients of net absorption were 88 +/- 6% for starch, 70 +/- 14% for fat and 90 +/- 4% for nitrogen. No relationship was found between starch absorption and nitrogen or fat absorption. There was no relationship between starch absorption and the duration of starch feeding. It is concluded that the ability of young infants to digest large quantities of starch is most likely limited

resulting from low pancreatic alpha-amylase activity. Nevertheless, from a practical point of view, small amounts of starch in infant formulas can be considered not only as a thickener but also as a source of calories." As taken from Senterre J. Acta Paediatr Scand. 1980 Sep; 69(5):653-7. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=6972147&query_hl=58&itool=pubmed_docsum

"Dietary starch delivery to the colon and excretion in stools and the ability of unabsorbed carbohydrates to promote hydrogen and methane release in breath were evaluated in 6 volunteers during two 8-day periods on starch diets of 100 and 300 g, respectively. Significantly less starch was recovered from the terminal ileum by aspiration per 24 h during the low-starch period (4.1 +/- 0.3 vs. 9.5 +/- 1.1 g, mean +/- SEM, p less than 0.01). Unabsorbed glucose tended to rise during the high-starch period (2.7 +/- 0.8 vs. 1.1 +/- 0.3 g). Fecal outputs of starch, glucose, volatile fatty acids, and lactic acid were not significantly different during the two periods. Daily breath hydrogen excretion was unchanged (181.2 +/- 22.7 vs. 193.7 +/- 19.8 ml for the low- and high-starch periods, respectively), whereas breath methane excretion increased markedly in the three methane producers during the high-starch period (217.2 +/- 80.9 vs. 32.4 +/- 7.3 ml). Starch malabsorption in the healthy small intestine was moderate even with a high-starch diet and less than that previously estimated by indirect methods. Unabsorbed starch catabolism by the colonic flora does not seem to explain most of the breath hydrogen excretion." As taken from Flourié B et al. Gastroenterology. 1988 Aug; 95(2):356-63. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=3391366&query_hl=72&itool=pubmed_DocSum

"To study the short-term effect of resistant starch (RS) from retrograded high-amylose corn starch (HACS) on the excretion of bile acids and nutrients from the small bowel in humans. Seven healthy ileostomists were given a controlled, constant diet during three days. On days 2 and 3, 100 g/d of one of two test-products--drum-dried ordinary corn starch and autoclaved retrograded HACS, providing 5 and 39 g RS/d, respectively--was given, in random order. Ileostomy effluents were collected for 24 h per day and analysed for wet weight, dry weight, energy, bile acids and nutrients. In-patient study at the metabolic ward, Department of Clinical Nutrition, Sahlgrenska University Hospital, Goteborg. Consumption of retrograded HACS caused (1) a 42% lower mean excretion of cholic acid (P = 0.024); (2) a 42% lower mean wet weight concentration of bile acids (P<0.001); (3) a 70% increased excretion of dry weight (P = 0.001); and (4) a 41% increased excretion of energy (P= 0.036) compared with consumption of drum-dried ordinary corn starch. The reduced ileal excretion and concentration of cholic acid would be protective regarding colon cancer risk in addition to the increased fermentation substrate provided by RS and other energy-yielding components." As taken from Langkilde AM et al. Eur J Clin Nutr. 1998 Nov; 52(11):790-5. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=9846590&query_hl=75&itool=pubmed_docsum

"Ten men (29 to 41 years old) participated in an oral exposure study. Blood was collected after a 12-h fast. Tapioca starch (30 g) containing 0.1 g aspartame was dissolved in 150 L of water, and the solution or dispersion remained for 3 minutes in boiling water. Subjects then drank the solution 1 to 2 min later. Three tolerance tests were performed, using a crossover design, over three days. Tapioca starch produced a large, rapid increase in plasma glucose concentration, which peaked in 30 minutes and then decreased toward the basal value." As taken from CIR, 2015, available at http://www.cir-safety.org/sites/default/files/plpogu092015final.pdf

"The potential toxicity of corn starch fiber was assessed and compared to polydextrose, a commonly used bulking agent with a long history of safe use in the food supply. Groups of male and female Crl:CD(SD) rats were fed 0 (control), 1,000, 3,000, or 10,000 mg/kg-bw/day corn starch fiber in the diet for 90 days. The polydextrose reference article was offered on a comparable regimen at 10,000 mg/kg-bw/day. Following a single gavage dose of [14C]-corn starch fiber on

study day 13 or 90, the mass balance of the test article was assessed by analysis of excreta samples collected from 0 to 168 h post-dose. There were no toxicologically or biologically relevant findings in any of the test article-treated groups. The few minor differences observed between the corn starch fiber and polydextrose exposed groups were considered to be due to normal biological variation. Following [14C]-corn starch fiber dosing, nearly complete excretion of the administered dose occurred over 168 h post-dosing, with the majority excreted in the feces. The dietary no-observed-adverse-effect level of corn starch fiber after 90 days was 10,000 mg/kg-bw/day. Similar toxicity profiles for corn starch fiber and polydextrose were observed due to the structural and compositional similarities of these materials." As taken from Crincoli CM et al. 2016a. Food Chem. Toxicol. 97, 57-69. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27580979

"Starch provides the major source of caloric intake in many diets. Cleavage of starch into malto-oligosaccharides in the gut is catalyzed by pancreatic α-amylase. These oligosaccharides are then further cleaved by gut wall α-glucosidases to release glucose, which is absorbed into the bloodstream. Potential surface binding sites for starch on the pancreatic amylase, distinct from the active site of the amylase, have been identified through X-ray crystallographic analyses. The role of these sites in the degradation of both starch granules and soluble starch was probed by the generation of a series of surface variants modified at each site to disrupt binding. Kinetic analysis of the binding and/or cleavage of substrates ranging from simple maltotriosides to soluble starch and insoluble starch granules has allowed evaluation of the potential role of each such surface site. In this way, two key surface binding sites, on the same face as the active site, are identified. One site, containing a pair of aromatic residues, is responsible for attachment to starch granules, while a second site featuring a tryptophan residue around which a malto-oligosaccharide wraps is shown to heavily influence soluble starch binding and hydrolysis. These studies provide insights into the mechanisms by which enzymes tackle the degradation of largely insoluble polymers and also present some new approaches to the interrogation of the binding sites involved." As taken from Zhang X et al. 2016. Biochemistry 55(43), 6000-6009. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27756128

"Sugars (and starch, after digestion to glucose) are absorbed in the blood as monosaccharides. Disaccharides are not absorbed as such, except for traces. Glucose and galactose are transferred from the gut lumen to the enterocyte by a Sodium-Glucose-coTransporter, SGLT1. This process is driven by the extra-intracellular sodium gradient maintained by the energy-dependent Na+/K+ATPase and results in the complete absorption of glucose and galactose. Fructose is absorbed by facilitated diffusion through a GLUT5 transporter. This absorption depends on the presence of a gut lumen-intracellular fructose gradient and it is not complete. Symptoms of fructose malabsorption frequently occur in individuals with very low fructose intakes but tend to decrease overtime upon chronic exposure to fructose due to an increased expression of GLUT5. Co-ingestion of glucose with fructose potentiates fructose absorption, thus decreasing symptoms of fructose malabsorption. Intracellular glucose, galactose and fructose are transported by facilitated diffusion from the enterocyte into the hepatic portal circulation through the same transporter, GLUT2 (Wright et al., 2003). (…)
Trace amounts of disaccharides that reach the systemic circulation are excreted in the urine as such. Glucose reabsorption occurring in the kidneys is almost complete under normal conditions but depends on glycaemia. When blood glucose levels exceed about 10 mmol/L (180 mg/dL), as in uncontrolled diabetes, glucose is lost in urine. The small amounts of galactose and fructose remaining in the systemic circulation after splanchnic extraction and metabolism are filtered in primary urine and almost entirely reabsorbed by kidney tubule cells through the SGLT-1 transporter. In normal conditions, only traces of galactose and fructose appear in the urine (Gammeltoft and Kjerulf-Jensen, 1943). When a threshold level of filtered hexoses is reached, as in inherited fructokinase deficiency (essential fructosuria), fructose absorbed in the blood after ingestion is excreted as such in the urine (Tran, 2017)."

EFSA (2022)

4.3. Interactions

“Salivary amylase initiates the digestion of starch and it has been hypothesized that salivary amylase may play a role in the development of insulin resistance and type 2 diabetes. The aim was to examine the interaction between copy number variation in the salivary amylase gene AMY1 and starch intake. We studied 3,624 adults without diabetes or elevated blood glucose in the Malmö Diet Cancer cohort. We assessed the associations and interactions between starch intake, AMY1 copies and glucose homeostasis traits (i.e., fasting plasma glucose, insulin and HOMA-IR) and risk of type 2 diabetes over an average of 18 follow-up years. AMY1 copy number was not associated with glucose, insulin or HOMA-IR. We observed a significant interaction between starch intake and AMY1 copies on insulin and HOMA-IR after adjusting for potential confounders ($p < 0.05$). The inverse association between starch intake and insulin and HOMA-IR was stronger in the group with 10 or more copies (P trend < 0.001). In addition, we observed an inverse association between starch intake and type 2 diabetes in the group with 10 or more copies (p trend = 0.003), but not in the other groups. This cross-sectional observational study suggests that AMY1 copy numbers might interact with starch intake on glucose homeostasis traits. Interventional studies are required to determine whether individuals with high AMY1 copy numbers may benefit from a high starch intake.” As taken from Hamid AK et al. 2021. Front. Nutr. 7, 598850. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33490099/

5. Toxicity

5.1. Single dose toxicity

“Cornstarch is a white, taste- and odorless powder widely used for food processing, papermaking, production of industrial adhesives, and is also a component of many cosmetic products. We herein report a case of severe cornstarch inhalation in a 49-year-old male who was exposed to high amounts of cornstarch powder while unloading a cornstarch transporter system. To our knowledge this is the first report on a high-pressure cornstarch inhalation during occupational activities. This report demonstrates the initial clinical situation, the patient's symptoms, and the severe clinical course. Finally the problems during the management of this rare but life-threatening accident are discussed.” As taken from Stromps JP et al. (2010). Inhal. Toxicol. 22(9), 767-9. Abstract available at http://www.ncbi.nlm.nih.gov/pubmed/20507256

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
mouse	LD50	intraperitoneal	6600mg/kg (6600mg/kg)		Pharmaceutical Chemistry Journal Vol. 15, Pg. 139

As taken from RTECS (2013)

5.2. Repeated dose toxicity

“Objective: We investigated the associations of dietary glycemic load (GL), glycemic index (GI), carbohydrate, and fiber intake with the incidence of type 2 diabetes. Design: A prospective cohort study was conducted in 37,846 participants of the EPIC-NL (European Prospective Investigation into Cancer and Nutrition–Netherlands) study, aged 21–70 y at baseline and free of diabetes. Dietary intake was assessed with the use of a validated food-frequency questionnaire. Incident diabetes cases were mainly self-reported and verified against general practitioner records. Results: During a mean follow-up of 10 y, 915 incident diabetes cases were documented. ……. Of the carbohydrate subtypes, only starch was related to increased diabetes risk [HR: 1.25 (1.07, 1.46), P

<0.05]. All associations became slightly stronger after exclusion of energy misreporters. Conclusions: Diets high in GL, GI, and starch and low in fiber were associated with an increased diabetes risk. Both carbohydrate quantity and quality seem to be important factors in diabetes prevention. Energy misreporting contributed to a slight attenuation of associations" (Sluijs et al., 2010. Am. J. clin. Nutr. 92, 905-911). Full paper available at http://www.ncbi.nlm.nih.gov/pubmed/20685945

"A Chronic feeding study (89-weeks) on mice gave no evidence of carcinogenicity of products (Feron et al 1985)".

"The purpose of this study is the investigation of possible adverse effects of a powder formulation containing drum-dried waxy maize (DDWM) starch and Carbopol 974 P (90/10) on the nasal mucosa of rabbits and the foot mucosa of slugs after multiple administrations. In the rabbit, the effect of the formulation was measured by the release of proteins and lactate dehydrogenase (LDH) from the nasal mucosa with a new non-invasive in vivo method and also by histopathology. The mucosal toxicity of the formulation was evaluated using slugs by measuring the effect on the body weight and the amount of mucus produced during a repeated contact period. Additionally, the release of proteins, lactate dehydrogenase and alkaline phosphatase from the body wall of the slugs after a repeated treatment was measured. Twenty four hours after the powder administration to the rabbits the release of the marker molecules was comparable with the negative controls. The histopathological study showed only a slight increase of granulocytes in the epithelium. The formulation induced a higher mucus production in the slugs but no additional effects were detected on the body weight and on the release of proteins. No enzymes were released from the body wall. The results indicate that the effect of the bioadhesive powder consisting of DDWM/Carbopol 974 P (90:10, w/w) on the mucosa was negligible." As taken from Callens C et al. J Control Release. 2001 Sep 11; 76(1-2):81-91. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=11532315&query_hl=77&itool=pubmed_docsum

"Resistant starch (RS) includes the sum of starch and degradation products of starch that resist small intestinal digestion and enter the colon. This study was planned to examine the effect of resistant starch on hypolipidemic actions, blood glucose, insulin levels and humoral immune responses in healthy overweight subjects. Healthy overweight subjects (over 120% of their ideal body weights) were fed either 24 g/d of resistant corn starch (RS) or regular corn starch (CS) for 21 d with their regular meals. Although this double-blind feeding regiment resulted in no significant changes in their weights or other physical parameters for the relatively acute period of intakes, there were significant lowering effects of serum total cholesterol (P<0.05) and serum LDL-cholesterol (P<0.05) in subjects supplemented RS. Compared with the control starch group, the RS supplementation also reduced the mean fasting serum glucose concentrations (P<0.05). Resistant starch supplement resulted in the increase in serum immunoglobulin G (IgG) concentrations. Serum insulin and complement 3 (C3) were unaffected. Tested resistant starch supplementation was reported to be palatable with minimal bowel discomfort. These results suggest that RS supplementation improves the blood lipid profile and controls the blood glucose levels in healthy overweight subjects without bowel discomfort. Therefore, RS has a potential to be used as one of the promising food ingredients for reducing risk factors involved in the development of atherosclerosis and type 2 diabetes in overweight individuals. However, in order to prove RS as a novel therapeutic agent of cardiovascular diseases and diabetes, controlled trials with larger sample sizes and longer duration are warranted." As taken from Park OJ et al. J Nutr Sci Vitaminol (Tokyo). 2004 Apr; 50(2):93-9. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15242012&query_hl=79&itool=pubmed_docsum

"The effects of raw potato starch (RPS) and high amylose corn starch (HAS) on cecal digestion, lipid metabolism and mineral utilization (Ca and Mg) were compared in rats adapted to semipurified diets. The diets provided either 710 g wheat starch/100 g diet (control) alone or 510 g wheat

starch/100 g diet plus 200 g resistant starch/100 g (RPS or HAS). Compared with rats fed the control diet, significant cecal hypertrophy (240% after 7 d of the fiber consumption) and short-chain fatty acids accumulation (especially propionic and butyric acids) occurred after both resistant starch diets. Apparent Ca, Mg, Zn, Fe and Cu absorptions were similarly enhanced by RPS and HAS (50, 50, 27, 21 and 90%, respectively). Cholesterol absorption was reduced to 14% of intake in rats fed RPS or HAS compared with 47% absorption in control rats. RPS and HAS were also effective in lowering plasma cholesterol (-31 and -27%, respectively) and triglycerides (-28 and -22%, respectively). There was no effect of the diets on cholesterol in d > 1.040 kg/L lipoproteins (HDL), whereas RPS and HAS depressed cholesterol in d <1.040 kg/L lipoproteins (especially in triglyceride-rich lipoproteins). Moreover, there were lower concentrations of cholesterol (-50 and -40%, respectively) and triglycerides (-53 and -47%, respectively) in the livers of RPS- and HAS-fed rats. Thus, RPS and HAS have similar effects on intestinal fermentation, mineral utilization and cholesterol metabolism in rats." As taken from Lopez HW et al. J Nutr. 2001 Apr; 131(4):1283-9. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=11285339&query_hl=80&itool=pubmed_docsum

"Male Wistar rats were fed on a conventional diet containing normal corn starch or 6% enzyme-resistant starch originating from either raw or retrograded high-amylose corn starch. Furthermore, the diets were either cholesterol-free or contained 1% cholesterol and 0.1% cholic acid. The main objective of this study was to investigate whether the addition of enzyme-resistant starch to a rat conventional diet had any effect on cholesterol metabolism. Therefore, plasma and liver cholesterol concentrations, plasma HDL:LDL cholesterol ratios and neutral steroid and bile acid excretion were determined. No significant effect of enzyme-resistant starch feeding on plasma and liver cholesterol concentrations was found. However, consumption of raw or retrograded high-amylose corn starch resulted in a decrease in esterified and total liver cholesterol concentrations of 24 and 22%, respectively. This was accompanied by a reduction in plasma esterified and total cholesterol levels of 4% and a tendency to higher daily faecal coprostanol and total bile acid excretion." As taken from Vanhoof K, De Schrijver R. Br J Nutr. 1998 Aug; 80(2):193-8. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=9828761&query_hl=81&itool=pubmed_docsum

"1. Fructose feeding, as opposed to vegetable starch feeding, has been shown to elevate blood pressure and to decrease insulin sensitivity in normotensive rats. The long-term relevance of this is unclear, and data in hypertensive strains are scarce. 2. We studied the effects of 27 weeks of a fructose-versus a corn-starch-enriched (69.5% w/w) diet in the spontaneously hypertensive rat. 3. In both dietary groups, blood pressure increased with ageing, with no apparent difference between the diets. The fructose-fed rats gained less weight. However, even selecting fructose-fed rats that matched the weight gain in the corn starch group, did not reveal a significant elevation of systolic blood pressure over time. 4. Extracellular fluid volume was comparable in fructose-fed and corn-starch-fed rats. No effects on creatinine clearance, proteinuria or renal histology were found. Fasting values of plasma triacylgycerols and cholesterol were increased mildly after 2 weeks on the fructose diet. However, fasting glucose and insulin measured after 2 weeks, and the response to an intraperitoneal glucose load, were no different. After 23 weeks of the diets, fasting values of plasma glucose, insulin, triacylglycerols and cholesterol did not differ. There were small differences in the response of plasma glucose levels to the intraperitoneal glucose load, but the area under the curve was not different. The baseline insulin resistance present in spontaneously hypertensive rats possibly blunts the metabolic response to dietary fructose. 5. After 27 weeks, the diets were switched in cross-over design, and measurements were continued until 39 weeks. The fructose diet did not elevate systolic blood pressure in this follow-up experiment." As taken from van der Schaaf MR et al. Clin Sci (Lond). 1995 Jun; 88(6):719-25. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=7634758&query_hl=83&itool=pubmed_docsum

“In Experiment 1, adult female rats were fed, in addition to chow and water, a carbohydrate source that consisted of pure amylopectin corn starch or hydrolyzed corn starch (Polycose) in either a dry powder form or a hydrated gel form. Over the 30-day test periods, carbohydrate intake, total food intake, and body weight gain were greater with the Polycose than with the amylopectin, and greater with the gel form than with the powder form of the carbohydrates. The amylopectin gel produced overeating and overweight relative to a chow-fed control group, although the effects were less than that obtained with the Polycose gel. In a second experiment, test meals of the carbohydrate gels produced larger postmeal increases in plasma glucose than did the carbohydrate powders. There was no effect of carbohydrate type (amylopectin vs. Polycose) on the plasma glucose response. In Experiment 3, the addition of amylopectin to a Polycose gel reduced carbohydrate and total caloric intake. Both orosensory and postingestive factors may contribute to the differential food intake and body weight gains produced by the different types (Polycose vs. amylopectin) and forms (gel vs. powder) of carbohydrates.” As taken from Sclafani A et al. Physiol Behav. 1988; 42(5):409-15. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=3164867&query_hl=86&itool=pubmed_docsum

“A total of 32 Wistar rats were given 1, 10, 100 and 1000 mg glove powder (Biosorb) intraperitoneally for 4, 11, 18 or 25 days. Four control rats received physiological saline. Examination of the abdominal cavity displayed granulomatous inflammation which was clearly dose-dependent in the experimental animal, but not in the controls. A biphasic time-sequence of the granulomatous reaction was observed in those rats receiving 100 and 1000 mg Biosorb with a minimum at day 18. The mean size of the granules (8.1 microns) within the inflammatory tissue was almost identical with the size of powder granules found on the external surface of the gloves (8.8 microns). X-ray microanalysis demonstrated maize-starch additives of magnesium and aluminium. The study indicates a foreign body reaction to maize-starch. In addition, immunological factors may play a role later in the development of the disease.” As taken from Nordstrand K et al. Acta Pathol Microbiol Immunol Scand [A]. 1987 Mar; 95(2):93-8. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=3551497&query_hl=88&itool=pubmed_docsum

Type of Test	Route of Exposure	Species	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	420 g/ kg/4W (continuous)	Gastrointestinal - hypermotility, diarrhea Gastrointestinal - other changes Nutritional and Gross Metabolic - weight loss or decreased weight gain	American Journal of Physiology. 188, 550, 1957

As taken from RTECS, 2013

“The transgenic rice line (TRS) enriched with amylose and resistant starch (RS) was developed by antisense RNA inhibition of starch-branching enzymes. Cereal starch with high amylose has a great benefit on human health through its resistant starch. In order to evaluate the effect of transgenic rice on rats, the rats were fed diets containing 70% TRS rice flour, its near-isogenic rice flour or the standard diet as the control through three generations. In the present study, clinical performance, reproductive capacity and pathological responses including body weight, food consumption, reproductive data, hematological parameters, serum chemistry components, organ relative weights and histopathology were examined. Some statistically significant differences were observed in rats consuming the high amylose rice diet when compared to rats fed the near-isogenic control rice diet or the conventional (non-rice) standard diet. These differences were generally of

small magnitude, appeared to be random in nature, and were within normal limits for the strain of rat used, and were therefore not considered to be biologically meaningful or treatment related." As taken from Zhou XH et al. 2014. Food Chem. Toxicol. 74, 20-7. PubMed 2015, available at: http://www.ncbi.nlm.nih.gov/pubmed/25194626?dopt=AbstractPlus.

"In the present study, we investigated the association between dietary intake of carbohydrates and the risk of type 2 diabetes. Incident cases of diabetes (n 749) were identified and compared with a randomly selected subcohort of 3496 participants aged 40-79 years. For dietary assessment, we used 7 d food diaries administered at baseline. We carried out modified Cox proportional hazards regression analyses and compared results obtained from the different methods of adjustment for total energy intake. Dietary intakes of total carbohydrates, starch, sucrose, lactose or maltose were not significantly related to diabetes risk after adjustment for confounders. However, in the residual method for energy adjustment, intakes of fructose and glucose were inversely related to diabetes risk. The multivariable-adjusted hazard ratios (HR) of diabetes comparing the extreme quintiles of intake were 0·79 (95 % CI 0·59, 1·07; P for trend = 0·03) for glucose and 0·62 (95 % CI 0·46, 0·83; P for trend = 0·01) for fructose. In the nutrient density method, only fructose was inversely related to diabetes risk (HR 0·65, 95 % CI 0·48, 0·88). The replacement of 5 % energy intake from SFA with an isoenergetic amount of fructose was associated with a 30 % lower diabetes risk (HR 0·69, 95 % CI 0·50, 0·96). Results of the standard and energy partition methods were similar to those of the residual method. These prospective findings suggest that the intakes of starch and sucrose are not associated, but that those of fructose and glucose are inversely associated with diabetes risk. Whether the inverse associations with fructose and glucose reflect the effect of substitution of these carbohydrate subtypes with other nutrients (i.e. SFA), their net higher intake or other nutrients associated with their intake remains to be established through further investigation." As taken from Ahmadi-Abhari S et al. 2014. Br. J. Nutr. 111(2), 342-52. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/23880355?dopt=AbstractPlus

"Pectin and Solanum Tuberosum (Potato) Starch: Test diets containing 5% or 10% pectin-derived acidic oligosaccharides (pAOS). Two groups of F1 rats (from outbred strain of Wistar rats (Crl:WI(WU); number not stated). Dietary feeding with test (±7 g/kg body weight/day) and control diets for 13 weeks. To keep the total level of added test substance equal in each diet, the low-dose diet (5% pAOS) was adjusted with 5% potato starch. One control group received the standard rodent diet supplemented with 10% potato starch, and the other control group received 10% short-chain FOS (scFOS) in the diet. No treatment- related clinical signs observed, and none of the rats died . Ophthalmoscopic examination did not reveal any treatment-related ocular changes. Neurobehavioral examination and motor activity assessment did not indicate any neurotoxic potential. No relevant differences in body weight, growth rate and feed intake. Macroscopic examination at necropsy did not reveal any adverse effects. Microscopic examination revealed treatment-related histopathological changes in the urinary bladder of animals of the 10% pAOS group. One male and one female of the 5% pAOS group and one male of the control group showed diffuse hyperplasia (very slight). In addition, two males and two females of the 5% pAOS group showed simple hyperplasia in a part of the urinary bladder lining ('focal hyperplasia'). No treatment-related hyperplasia of the transitional epithelium was observed in the kidney. Administration of pAOS at dietary levels up to 10% (equivalent to 7.1 g/kg body weight/day) did not reveal any relevant effects that could be attributed to the ingestion of acidic oligosaccharides.168" As taken from CIR, 2015, available at http://www.cir-safety.org/sites/default/files/plpogu092015final.pdf

"BACKGROUND: Carbohydrate quality may be an important determinant of type 2 diabetes (T2D); however, relations between various carbohydrate quality metrics and T2D risk have not been systematically investigated. OBJECTIVE: The purpose of this study was to prospectively examine the association between carbohydrates, starch, fibers, and different combinations of these nutrients and risk of T2D in women. DESIGN: We prospectively followed 70,025 women free of cardiovascular disease, cancer, and diabetes at baseline from the Nurses' Health Study (1984-2008). Diet information was collected with the use of a validated questionnaire every 4 y. Cox regression was used to evaluate associations with incident T2D. RESULTS: During 1,484,213

person-years of follow-up, we ascertained 6934 incident T2D cases. In multivariable analyses, when extreme quintiles were compared, higher carbohydrate intake was not associated with T2D (RR = 0.98; 95% CI: 0.89, 1.08; P-trend = 0.84), whereas starch was associated with a higher risk (RR = 1.23; 95% CI: 1.12, 1.35; P-trend <0.0001). Total fiber (RR = 0.80; 95% CI: 0.72, 0.89; P-trend <0.0001), cereal fiber (RR = 0.71, 95% CI: 0.65, 0.78; P-trend <0.0001), and fruit fiber (RR = 0.79; 95% CI: 0.72, 0.85; P-trend <0.0001) were associated with a lower T2D risk. The ratio of carbohydrate to total fiber intake was marginally associated with a higher risk of T2D (RR = 1.09; 95% CI: 1.00, 1.20; P-trend = 0.04). On the other hand, we found positive associations between the ratios of carbohydrate to cereal fiber (RR = 1.28; 95% CI: 1.17, 1.39; P-trend <0.0001), starch to total fiber (RR = 1.12; 95% CI: 1.02, 1.23; P-trend = 0.03), and starch to cereal fiber (RR = 1.39; 95% CI: 1.27, 1.53; P-trend <0.0001) and T2D. CONCLUSIONS: Diets with high starch, low fiber, and a high starch-to-cereal fiber ratio were associated with a higher risk of T2D. The starch-to-cereal fiber ratio of the diet may be a novel metric for assessing carbohydrate quality in relation to T2D." As taken from AlEssa HB et al. 2015. Am. J. Clin. Nutr. 102(6), 1543-53. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26537938

"The potential toxicity of corn starch fiber was assessed and compared to polydextrose, a commonly used bulking agent with a long history of safe use in the food supply. Groups of male and female Crl:CD(SD) rats were fed 0 (control), 1,000, 3,000, or 10,000 mg/kg-bw/day corn starch fiber in the diet for 90 days. The polydextrose reference article was offered on a comparable regimen at 10,000 mg/kg-bw/day. Following a single gavage dose of [14C]-corn starch fiber on study day 13 or 90, the mass balance of the test article was assessed by analysis of excreta samples collected from 0 to 168 h post-dose. There were no toxicologically or biologically relevant findings in any of the test article-treated groups. The few minor differences observed between the corn starch fiber and polydextrose exposed groups were considered to be due to normal biological variation. Following [14C]-corn starch fiber dosing, nearly complete excretion of the administered dose occurred over 168 h post-dosing, with the majority excreted in the feces. The dietary no-observed-adverse-effect level of corn starch fiber after 90 days was 10,000 mg/kg-bw/day. Similar toxicity profiles for corn starch fiber and polydextrose were observed due to the structural and compositional similarities of these materials." As taken from Crincoli CM et al. 2016a. Food Chem. Toxicol. 97, 57-69. PubMed, 2016 available at https://www.ncbi.nlm.nih.gov/pubmed/27580979

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Wheat starch (containing gluten)	09/10/2017 Corrigendum 19/11/2018	Oral	Zero	This medicine contains only very low levels of gluten (from wheat starch)<. It is regarded as 'gluten-free'*> and is very unlikely to cause problems if you have coeliac disease. One <dosage unit> contains no more than x micrograms of gluten. If you have wheat allergy (different from coeliac disease) you should not take this medicine. [* The statement 'gluten-free' applies only if the gluten content in the medicinal product is less than 20 ppm.]	The name of the excipient on the packaging should be: 'Wheat starch'.

EMA (2022)

Introduction: Propylene glycol (PG) is usually considered safe, however, toxicity can develop with high doses or when used for prolonged periods of time. PG can be found in some medications as well as some food products. We report a case of likely PG toxicity that occurred after compulsive daily ingestion of large amounts of corn starch.
Case report: Our patient initially presented to an outside hospital (OSH) via ambulance for altered mental status. Her mental status improved after her blood sugar of 25 was corrected. On admission to OSH Emergency Department her initial vital signs included a heart rate of 115 bpm, blood pressure 113/59 mm/hg, temperature 35.8C. Pertinent labs included: sodium 119 mEq/L, bicarbonate 9 mEq/L, anion gap 29 mEq/L, creatinine 2.5 mg/dL and lactic acid 20 mEq/L. On transfer to our hospital her repeat lactic acid was 20 mEq/L, osmolar gap was 20. Her PG level, which was drawn several hours after her initial presentation, was 11 mg/dL. Our patient noted that she ingested a 16 oz. package of corn starch mixed with baking soda approximately every 2 days. Given the concerns for PG she was underwent intermittent hemodialysis. PG and lactic acid levels improved, however, she ultimately died due to complications from her hospitalization.
Discussion: PG causes toxicity through metabolism to lactic acid. While there are small amounts in food products and medications, under the right circumstances, PG can accumulate and lead to significant toxicity.

Peterson, J., et al. (2022). Propylene glycol toxicity from compulsive corn starch ingestion. The American journal of emergency medicine, 53, 286.e1–286.e3. https://doi.org/10.1016/j.ajem.2021.09.054

5.3. Reproduction toxicity

“The transgenic rice line (TRS) enriched with amylose and resistant starch (RS) was developed by antisense RNA inhibition of starch-branching enzymes. Cereal starch with high amylose has a great benefit on human health through its resistant starch. In order to evaluate the effect of transgenic rice on rats, the rats were fed diets containing 70% TRS rice flour, its near-isogenic rice flour or the standard diet as the control through three generations. In the present study, clinical performance, reproductive capacity and pathological responses including body weight, food consumption, reproductive data, hmatological parameters, serum chemistry components, organ relative weights and histopathology were examined. Some statistically significant differences were observed in rats consuming the high amylose rice diet when compared to rats fed the near-isogenic control rice diet or the conventional (non-rice) standard diet. These differences were generally of small magnitude, appeared to be random in nature, and were within normal limits for the strain of rat used, and were therefore not considered to be biologically meaningful or treatment related.” As taken from Zhou XH et al. 2014. Food Chem. Toxicol. 74, 20-7. PubMed 2015, available at: http://www.ncbi.nlm.nih.gov/pubmed/25194626?dopt=AbstractPlus.

“The objective of the present study was to investigate the effects of dietary-induced insulin enhancement during the late luteal phase on subsequent fertility of gilts. Fifty-two littermate cyclic gilts were subjected to dietary treatments where two energy sources were tested: corn starch (T1) and soybean oil (T2). The experimental diets were supposed to provide similar amounts of dietary energy, but from different sources. Gilts were fed ad libitum, starting day 8 of the estrous cycle, until the next standing heat. Blood sampling was performed in a subgroup of 20 gilts on days 14 and 21 of the cycle for analyses of glucose and insulin, and after ovulation detection until 18 h after ovulation for progesterone. All gilts were slaughtered on day 28 of pregnancy and the reproductive tracts recovered for further analysis. T1 gilts showed higher postprandial insulin peak on days 14 and 21 and lower glucose levels 4 h after feeding on day 14 ($P<0.05$), however, there were no treatment effects on plasma progesterone concentrations. Dietary energy sources did not affect average daily feed intake, body weight and backfat on day 28 of pregnancy. Estrous cycle length, estrus duration and time of ovulation were not affected by previous nutritional treatments either. T1

gilts showed higher ovulation rates, number of embryos, embryo weight and placental weight (P<0.05). There were no treatment effects on pregnancy rate, embryo survival rate and volume of amniotic fluid. A positive correlation between progesterone concentration 18 h after ovulation and ovulation rate was observed (r=0.75; P<0.01). These results suggest that it is possible to manipulate dietary insulin response in cyclic gilts and, thus, improve reproductive efficiency when feeding starch as the main energy source during the late luteal and follicular phases of the cycle." As taken from Almeida FR et al. 2014. Animal 8(2), 293-9. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24284005?dopt=AbstractPlus

"CONTEXT: Diet is proposed to contribute to androgen-related reproductive dysfunction. OBJECTIVE: This study evaluated the association between dietary macronutrient intake, carbohydrate fraction intake, and overall diet quality on androgens and related hormones, including anti-Müllerian hormone (AMH) and insulin, in healthy, regularly menstruating women. DESIGN: This was a prospective cohort study from 2005 and 2007. SETTING: The study was conducted at the University at Buffalo, western New York State, USA. PARTICIPANTS: Participants were 259 eumenorrheic women without a self-reported history of infertility, polycystic ovary syndrome (PCOS), or other endocrine disorder. MAIN OUTCOME MEASURES: A 24-hour dietary recall was administered 4 times per menstrual cycle, and hormones were measured 5 to 8 times per cycle for 1 (n = 9) or 2 (n = 250) cycles per woman (n = 509 cycles). Associations between the dietary intake of carbohydrates (starch, sugar, sucrose, and fiber), macronutrients, overall diet quality and hormones (insulin, AMH, and total and free testosterone), as well as the relationship of dietary intake with occurrences of high total testosterone combined with high AMH (fourth quartile of each), ie, the "PCOS-like phenotype," were assessed. RESULTS: No significant relationships were identified between dietary intake of carbohydrates, percent calories from any macronutrient or overall diet quality (ie, Mediterranean diet score) and relevant hormones (insulin, AMH, and total and free testosterone). Likewise, no significant relationships were identified between dietary factors and the occurrence of a subclinical PCOS-like phenotype. CONCLUSIONS: Despite evidence of a subclinical continuum of a PCOS-related phenotype of elevated androgens and AMH related to sporadic anovulation identified in previous studies, dietary carbohydrate and diet quality do not appear to relate to these subclinical endocrine characteristics in women without overt PCOS." As taken from Sjaarda LA et al. 2015. J. Clin. Endocrinol. Metab. 100(8), 2979-86. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26066675

5.4. Mutagenicity

Ames mutagenicity tests on a charred sample heated with ammonium sulphate at 600 degrees produced water which was collected and extracted with methylene chloride. The basic fraction exhibited strong mutagenicity on Salmonella typhumurium (Shibamoto 1983).

"Red meat may increase promutagenic lesions in the colon. Resistant starch (RS) can reduce these lesions and chemically induced colon tumours in rodents. Msh2 is a mismatch repair (MMR) protein, recognising unrepaired promutagenic adducts for removal. We determined if red meat and/or RS modulated DNA adducts or oncogenesis in Msh2-deficient mice. A total of 100 Msh2-/- and 60 wild-type mice consumed 1 of 4 diets for 6 months: control, RS, red meat and red meat+RS. Survival time, aberrant crypt foci (ACF), colon and small intestinal tumours, lymphoma, colonic O6-methyl-2-deoxyguanosine (O6MeG) adducts, methylguanine methyltransferase (MGMT) and cell proliferation were examined. In Msh2-/- mice, red meat enhanced survival compared to control (P<0.01) and lowered total tumour burden compared to RS (p<0.167). Msh2-/- mice had more ACF than wild-type mice (P<0.014), but no colon tumours developed. Msh2-/- increased cell proliferation (P<0.001), lowered DNA O6MeG adducts (p<0.143) and enhanced MGMT protein levels (P<0.001) compared to wild-type mice, with RS supplementation also protecting against DNA adducts (P<0.01). No link between red meat-induced promutagenic adducts and risk for colorectal cancer was observed after 6 months' feeding. Colonic epithelial changes after red meat and RS consumption with MMR deficiency will differ from normal epithelial cells." As taken from Winter JM

et al. 2014. J. Nutrigenet. Nutrigenomics 7(4-6), 299-313. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26022687

5.5. Cytotoxicity

No data available to us at this time.

5.6. Carcinogenicity

“Epidemiologic studies suggest that dietary complex carbohydrates are protective against colorectal cancer but dietary protein may increase risk. However, experimental data to support these relationships are scant. We have shown in rats that consumption of a high-protein (25% casein) diet for 4 wk resulted in a twofold increase in damage to colonocyte DNA compared with a low-protein (15% casein) diet. This was associated with thinning of the colonic mucous barrier and increased levels of fecal p-cresol. Addition of resistant starch as a high-amylose maize starch to the diet increased cecal short-chain fatty acid pools and attenuated DNA damage, suggesting protection against genotoxic agents. In humans, this could translate to altered risk of colonic cancer.” As taken from Toden S et al. Nutr Cancer. 2005; 51(1):45-51. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15749629&query_hl=93&itool=pubmed_docsum

“Although both non-starch polysaccharides (NSP) and resistant starches (RS) are included in current definitions of dietary fibre, our previous work has suggested fundamental differences in the way in which these two classes of material affect the disposition and absorption of a dietary carcinogen. The present studies explore whether different effects on carcinogen metabolism could play a role in the contrasting patterns seen previously. Groups of female Wistar rats were pre-fed for 4 weeks one of five types of defined diet (AIN-76). The control diet contained 35% maize starch and no dietary fibre. The RS-containing diets had all the maize starch substituted with either Hi-maize or potato starch. In the NSP-containing diets, 10% of the maize starch was substituted with dietary fibre in the form of either lignified plant cell walls (wheat straw) or soluble dietary fibre (apple pectin). Pre-fed rats were gavaged with the food carcinogen, [2-14C] 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), and plasma and urinary metabolites characterized using HPLC at various time intervals after administration. After 4 h gavage, plasma from rats on both RS-containing diets contained significantly higher levels of intact IQ and lower levels of the major metabolites, IQ-5-O-glucuronide and IQ-5-sulfate, as compared with plasma from the negative control group at this time. In contrast, plasma from animals on the NSP-containing wheat straw diet (and to a lesser extent the apple pectin diet) showed significantly lower levels of intact IQ, and significantly higher levels of the two major metabolites, as compared with those from the control rats. These different metabolite profiles were also reflected in different urinary excretion profiles. Urine from rats pre-fed RS-containing diets revealed significantly slower metabolite excretion as compared with urine from rats that had been given the NSP-containing diets. Western blotting methodologies also profiled differences between the effects of these two types of dietary fibre in the expression of xenobiotic metabolizing enzymes. We conclude that changes in activity and expression of xenobiotic metabolising enzymes could play a role in the contrasting effects of these two types of dietary fibre on carcinogen uptake and disposition.” As taken from Kestell P et al. J Chromatogr B Analyt Technol Biomed Life Sci. 2004 Mar 25; 802(1):201-10. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15036012&query_hl=97&itool=pubmed_docsum

“High amylose maize starch (HAS) is not digested in the small intestine and most of it reaches the large intestine. In the large intestine, HAS is fermented by intestinal bacteria, resulting in production of short-chain fatty acids (SCFA), particularly butyrate. Clostridium butyricum can utilize HAS and produce butyrate and acetate. It has been proposed that butyrate inhibits carcinogenesis in the colon. In this study, we examined the inhibitory effects of HAS and C. butyricum strain MIYAIRI588

(CBM588) on azoxymethane-induced aberrant crypt foci (ACF) formation in rats. In the group of rats administered only CBM588 spores, the concentration of butyrate in the cecum increased, but there was no decrease in the number of ACF. In the group of rats fed an HAS diet, a decrease in the number of ACF was observed, and in the group of rats administered HAS and CBM588, the number of ACF decreased significantly. In these two groups, the concentrations of acetate and propionate in intestinal contents significantly increased, but the concentration of butyrate did not change. It was found that the beta-glucuronidase activity level of colonic contents decreased significantly in the two groups of rats fed HAS. This study showed that HAS and CBM588 changed the metabolism of colonic microbiota and decreased the level of beta-glucuronidase activity, phenomena that may play a role in the inhibition of ACF formation in the rat colon." As taken from Nakanishi S et al. Microbiol Immunol. 2003; 47(12):951-8. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=14695445&query_hl=99&itool=pubmed_docsum

"In a previous study we have shown that high levels of dietary protein (as casein) result in increased levels of colonic DNA damage, measured by the comet assay, and thinning of the colonic mucus layer in rats when dietary resistant starch (RS) is negligible. Feeding RS abolishes these effects. This study aimed to establish whether a diet high in protein as cooked red meat would have similar effects and whether RS was protective. Rats were fed a diet containing 15% or 25% casein or 25% cooked lean red beef, each with or without the addition of 48% high amylose maize starch (a rich source of RS) for four weeks. As expected, high dietary casein caused a 2-fold increase in colonic DNA damage compared with a low casein diet and reduced the thickness of the colonic mucus layer by 41%. High levels of cooked meat caused 26% greater DNA damage than the high casein diet but reduced mucus thickness to a similar degree to casein. Addition of RS to the diet abolished the increase in DNA damage and the loss of colonic mucus thickness induced by either high protein diet. Cecal and fecal short chain fatty acid pools were also increased by inclusion of RS in the diet. Because DNA damage is an early step in the initiation of cancer, these findings suggest that increased DNA damage due to high dietary protein as cooked red meat or casein could increase colorectal cancer risk but inclusion of resistant starch in the diet could significantly reduce that risk." As taken from Toden S et al. Cancer Biol Ther. 2006 Mar; 5(3):267-72. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16410726&query_hl=93&itool=pubmed_docsum

"High-carbohydrate diets have been linked to pancreatic cancer risk in case-control studies, but prospective studies have shown mostly null results. The authors investigated the associations of glycemic load, glycemic index, and carbohydrate intake with pancreatic cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Dietary intake was assessed by using a self-administered questionnaire. Between 1998 and 2006 (median follow-up = 6.5 years), 266 incident, confirmed pancreatic cancers were identified among 109,175 participants. Hazards ratios and 95% confidence intervals were adjusted for sex, smoking, body mass index, and total energy." "Higher dietary intakes of glycemic load, available carbohydrates, and sucrose were associated with greater risk of pancreatic cancer, while dietary glycemic index, starch, and fructose intakes were not associated with risk in this cohort" (Meinhold et al., 2010. Am. J. Epidemiol. 171, 1174-1182). Abstract available at http://www.ncbi.nlm.nih.gov/pubmed/20452999

"Red meat may increase promutagenic lesions in the colon. Resistant starch (RS) can reduce these lesions and chemically induced colon tumours in rodents. Msh2 is a mismatch repair (MMR) protein, recognising unrepaired promutagenic adducts for removal. We determined if red meat and/or RS modulated DNA adducts or oncogenesis in Msh2-deficient mice. A total of 100 Msh2-/- and 60 wild-type mice consumed 1 of 4 diets for 6 months: control, RS, red meat and red meat+RS. Survival time, aberrant crypt foci (ACF), colon and small intestinal tumours, lymphoma, colonic O6-methyl-2-deoxyguanosine (O6MeG) adducts, methylguanine methyltransferase (MGMT) and cell proliferation were examined. In Msh2-/- mice, red meat enhanced survival compared to control ($P<0.01$) and lowered total tumour burden compared to RS ($p<0.167$). Msh2-/- mice had more ACF

than wild-type mice (P<0.014), but no colon tumours developed. Msh2-/- increased cell proliferation (P<0.001), lowered DNA O6MeG adducts (p<0.143) and enhanced MGMT protein levels (P<0.001) compared to wild-type mice, with RS supplementation also protecting against DNA adducts (P<0.01). No link between red meat-induced promutagenic adducts and risk for colorectal cancer was observed after 6 months' feeding. Colonic epithelial changes after red meat and RS consumption with MMR deficiency will differ from normal epithelial cells." As taken from Winter JM et al. 2014. J. Nutrigenet. Nutrigenomics 7(4-6), 299-313. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26022687

"A carbohydrate-rich diet results in hyperglycaemia and hyperinsulinaemia; it may further induce the carcinogenesis of colorectal cancer. However, epidemiological evidence among Chinese population is quite limited. The aim of this study was to investigate total carbohydrate, non-fibre carbohydrate, total fibre, starch, dietary glycaemic index (GI) and glycaemic load (GL) in relation to colorectal cancer risk in Chinese population. A case-control study was conducted from July 2010 to April 2017, recruiting 1944 eligible colorectal cancer cases and 2027 age (5-year interval) and sex frequency-matched controls. Dietary information was collected by using a validated FFQ. The OR and 95 % CI of colorectal cancer risk were assessed by multivariable logistic regression models. There was no clear association between total carbohydrate intake and colorectal cancer risk. The adjusted OR was 0·85 (95 % CI 0·70, 1·03, P trend=0·08) comparing the highest with the lowest quartile. Total fibre was related to a 53 % reduction in colorectal cancer risk (adjusted ORquartile 4 v. 1 0·47; 95 % CI 0·39, 0·58). However, dietary GI was positively associated with colorectal cancer risk, with an adjusted ORquartile 4 v. 1 of 3·10 (95 % CI 2·51, 3·85). No significant association was found between the intakes of non-fibre carbohydrate, starch and dietary GL and colorectal cancer risk. This study indicated that dietary GI was positively associated with colorectal cancer risk, but no evidence supported that total carbohydrate, non-fibre carbohydrate, starch or high dietary GL intake were related to an increased risk of colorectal cancer in a Chinese population." As taken from Huang J et al. 2018. Br. J. Nutr. 119(8), 937-948. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29644952

"Context: Pancreatic cancer has the highest case fatality rate of all major cancers. Objective: A systematic review using PRISMA guidelines was conducted to summarize the associations between dietary patterns and risk of pancreatic cancer. Data Sources: PubMed and Web of Science databases were searched for case-control and cohort studies published up to June 15, 2016. Study Selection: Eligible studies included a dietary pattern as exposure and pancreatic cancer incidence or mortality as outcome and reported odds ratios, hazard ratios, or relative risks, along with corresponding 95%CIs. Data Extraction: Important characteristics of each study, along with the dietary assessment instrument, the component foods or nutrients included in each dietary pattern or the scoring algorithm of a priori dietary patterns, were presented. For each dietary pattern identified, the estimate of association and the 95%CI comparing the highest versus the lowest category from the model with the most covariate adjustment were reported. Results: A total of 16 studies were identified. Among the 8 studies that examined data-driven dietary patterns, significant positive associations were found between pancreatic cancer risk and the Animal Products, Starch Rich, and Western dietary patterns, with effect estimates ranging from 1.69 to 2.40. Significant inverse relationships were found between risk of pancreatic cancer and dietary patterns designated as Fruits and Vegetables, Vitamins and Fiber, and Prudent, with effect estimates ranging from 0.51 to 0.55. Eight studies of a priori dietary patterns consistently suggested that improved dietary quality was associated with reduced risk of pancreatic cancer. Conclusions: Better diet quality is associated with reduced risk of pancreatic cancer. The associations between dietary patterns and pancreatic cancer were stronger in case-control studies than in cohort studies and were stronger among men than among women." As taken from Zheng J et al. 2017. Nutr. Rev. 75(11), 883-908. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29025004

"Carbohydrate intake affects postprandial glucose levels and insulin response, which plays a role in carcinogenesis. The relationship between carbohydrate intake, dietary glycemic index (GI) and

glycemic load (GL), and risk of renal cell carcinoma (RCC) remains unclear. We conducted a case-control study including 854 patients with newly diagnosed RCC (cases) and 1255 healthy participants (controls) recruited since 2002. GI, GL and carbohydrate intake were obtained via a validated food frequency questionnaire. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using multivariable logistic regression, adjusting for potential confounders. We found that higher GI was significantly associated with RCC risk with an OR of 1.32 (95% CI, 0.99-1.74; Ptrend = 0.026) (the highest versus the lowest quartiles). We also observed an inverse association between fiber intake and RCC risk with OR of 0.70 (95% CI = 0.50-0.99) as well as between starch intake and risk of RCC with OR of 0.65 (95% CI = 0.49-0.87). Individuals with a high-GI diet and hypertension or high body mass index (BMI) had a 2.7 times (OR = 2.67, 95% CI = 1.96-3.64) and two times (OR = 1.95, 95% CI = 1.29-2.92) higher RCC risk, respectively, than those without these factors. Our findings suggest that a high-GI diet is associated with an increased risk of RCC, whereas increased fiber and starch intakes appear to be associated with a decreased risk of RCC. We found that reducing GI levels and increasing fiber intake could be a dietary strategy to decrease RCC risk, especially for individuals with hypertension or high BMI." As taken from Zhu J et al. 2017. Carcinogenesis 38(11), 1129-1135. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28968893

"A systematic review was conducted to update evidence on the effect of total dietary starch and of replacing rapidly digestible starches (RDSs) with slowly digestible starches (SDSs) on oral health outcomes to inform updating of World Health Organization guidance on carbohydrate intake. Data sources included MEDLINE, Embase, Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, LILACS, and Wanfang. Eligible studies were comparative and reported any intervention with a different starch content of diets or foods and data on oral health outcomes relating to dental caries, periodontal disease, or oral cancer. Studies that reported total dietary starch intake or change in starch intake were included or where comparisons or exposure included diets and foods that compared RDSs and/or SDSs. The review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analyses) statement, and evidence was assessed with the GRADE Working Group guidelines. From 6,080 papers identified, 33 (28 studies) were included in the RDS versus SDS comparison: 15 (14 studies) assessed the relationship between SDS and/or RDS and dental caries; 16 (12 studies) considered oral cancer; and 2 studied periodontal disease. For total starch, 23 papers (22 studies) were included: 22 assessed the effects on dental caries, and 1 considered oral cancer. GRADE assessment indicated low-quality evidence, suggesting no association between total starch intake and caries risk but that RDS intake may significantly increase caries risk. Very low-quality evidence suggested no association between total starch and oral cancer risk, and low-quality evidence suggested that SDS decreases oral cancer risk. Data on RDS and oral cancer risk were inconclusive. Very low-quality data relating to periodontitis suggested a protective effect of whole grain starches (SDS). The best available evidence suggests that only RDS adversely affects oral health." As taken from Halvorsrud K et al. 2019. J. Dent. Res. 98(1), 46-53. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30074866

"Cancer initiation and protection mainly derives from a systemic metabolic environment regulated by dietary patterns. Less is known about the impact of nutritional interventions in people with a diagnosis of cancer. The aim of our study was to investigate the effect of a diet rich in resistant starch (RS) on cell pathways modulation and metabolomic phenotype in pancreatic cancer xenograft mice. RNA-Seq experiments on tumor tissue showed that 25 genes resulted in dysregulated pancreatic cancer in mice fed with an RS diet, as compared to those fed with control diet. Moreover, in these two different mice groups, six serum metabolites were deregulated as detected by $LC^{-}MS$ analysis. A bioinformatic prediction analysis showed the involvement of the differentially expressed genes on insulin receptor signaling, circadian rhythm signaling, and cancer drug resistance among the three top canonical pathways, whilst cell death and survival, gene expression, and neurological disease were among the three top disease and biological functions. These findings shed light on the genomic and metabolic phenotype, contributing to the knowledge

of the mechanisms through which RS may act as a potential supportive approach for enhancing the efficacy of existing cancer treatments." As taken from Panebianco C et al. 2019. Nutrients 11(4), E709. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30934731

"Background: The role that diet plays in the development of breast cancer is unclear and breast cancer continues to increase in Colombia and worldwide. The objective of this study was to assess the association between patterns of dietary consumption and the incidence of breast cancer. Methods: An ecological study was conducted in 24 geodemographic units in which 95% of the women of Colombia live. The association between breast cancer rate (dependent variable) and three dietary patterns established with factor analysis (traditional/starch, fiber/dairy and snack) was investigated using simple and multiple linear regression. The use of variables related to socioeconomic context and the duration of breastfeeding allowed for the control of possible confounding. All information was derived from concurrent national surveys or was obtained directly over a period of time close to the period during which the study was conducted. Results: There is an inverse relationship between breast cancer rate and illiteracy rate (β=-2808.3), duration of breastfeeding (β=-3354.1), adherence to traditional/fiber dietary patterns (β=-30467) and adherence to the snack dietary pattern (β=-43612). The goodness of fit for the model was R2=84%. Conclusions: Increasing the duration of breastfeeding, ensuring education to promote health and following traditional food consumption patterns, regardless of what foods are consumed, can protect against the development of breast cancer." As taken from Herrán OF et al. 2020. Int. Health 12(4), 317-324. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/31691807/

"Background: Conclusive evidence on foods, nutrients, or dietary patterns and the risk of renal cell carcinoma (RCC) is lacking in the literature. **Methods:** We considered data from an Italian hospital-based case-control study (1992-2004) on 767 incident RCC cases and 1534 controls. A posteriori dietary patterns were identified by applying principal component factor analysis on 28 nutrients derived from a 78-item food-frequency questionnaire. We estimated the odds ratios (ORs) of RCC and corresponding 95% confidence intervals (CIs) for each quartile category (compared to the lowest one) using conditional multiple logistic regression models providing adjustment for major confounding factors. **Results:** We identified four dietary patterns, named "Animal products", "Starch-rich", "Vitamins and fiber", and "Cooking oils and dressings". Higher intakes of the "Starch-rich" pattern were positively associated with RCC risk (OR = 1.38, 95% CI: 1.04-1.82 for the highest quartile, p = 0.018). The association was inverse with the "Cooking oils and dressings" pattern (OR = 0.61, 95% CI: 0.47-0.80, p < 0.001), whereas no association was found with "Animal products" and "Vitamins and fiber" patterns. **Conclusions:** Higher intakes of starch-related foods may increase RCC risk, whereas consumption of olive and seed oils may favorably influence RCC risk." As taken from Dalmartello M et al. 2020. Nutrients 12(1), 134. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31906594/

5.7. Irritation/immunotoxicity

An application of 300ug for 3 days on human skin produced only a mild irritant effect

As taken from RTECS, 2013.

"The safety profile of the ingredients used in topical dosage forms and its evaluation is an issue of utmost importance. A suitable equilibrium between safety and efficacy is crucial before promoting a dermatological product. The aim of this work was to assess the safety and biological effects of starch-based vehicles (St-BV) used in such products. The hazard, exposure and dose-response assessment were used to characterize the risk of each ingredient. The EpiSkin™ assay and human repeat insult patch tests were performed to compare the theoretical safety assessment to in vitro and in vivo data. The efficacy of the St-BV was studied using biophysical measurements in human volunteers during 28 days, showing that all ingredients and their combinations were safe for the consumer. Tissue viability determined using the EpiSkin™ testing reached values between

84.0 ± 5.0% and 98.0 ± 8.6% after application of St-BV, which were considered as non-irritant to the skin. These observations were confirmed by the in vivo studies where the St-BV did not induce any sensitization on the volunteers, being safe for human use. Moreover, St-BV increased skin hydration and microcirculation, emerging as an attractive alternative to chemical raw materials." As taken from Marto J et al. 2018. Toxicol. Appl. Pharmacol. 342, 14-21. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29407772

"Background: An essential step in ensuring the toxicological safety of cosmetic or personal care products is the evaluation of the skin sensitizing potential of product ingredients. **Objective:** We used a standardized protocol from cosmetic trade industry and consumer safety groups to evaluate the sensitization potential of ingredients in 3 commercially available cleansing conditioners. **Methods:** A total of 33 ingredients were evaluated. Each ingredient underwent (1) dermatological evaluation, (2) in silico analysis for irritation and sensitization potential, and (3) a literature evaluation to determine risk of sensitization. Consumer exposure level was compared with the weight-of-evidence no-expected sensitization induction level for the constituent. If a no-expected sensitization induction level for a specific ingredient was not available, the dermal sensitization threshold approach was used. A margin of safety was calculated for each constituent. **Results:** The margins of safety for all evaluated ingredients in the cleansing conditioners were greater than 1. **Conclusions:** This analysis indicates that exposure to the individual ingredients present in these cleansing conditioners would not be expected to induce dermal sensitization in a consumer under the examined exposure scenario." As taken from Monnot AD et al. 2019. Dermatitis 30(2), 116–128. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/30829810/

Symptoms: dermatitis, rhinorrhea (discharge of thin nasal mucus) (NIOSH, 2019).

5.8. All other relevant types of toxicity

"Corneal infiltrations after keratoplasty may be the first sign of postoperative infection. We report on our findings of unusual corneal infiltrations, which were caused by contamination of the corneal tissue with remnants of latex glove powder. The diagnosis was proved by the specific distribution pattern and the clinical course. Infiltrates of decreasing size were observed in the area of all four temporarily supporting single-stitch sutures of the transplant and at the first stitches of the permanent running corneal sutures. There is no treatment necessary, but the infiltrates have to be differentiated from infection. Improvement of operative habits and techniques can avoid this complication." As taken from Mittelviefhaus H. Ophthalmologe. 1993 Dec; 90(6):720-2. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=8124042&query_hl=102&itool=pubmed_docsum

"Background: Studies have shown conflicting associations between the salivary amylase gene (AMY1) copy number and obesity. Salivary amylase initiates starch digestion in the oral cavity; starch is a major source of energy in the diet. Objective: We investigated the association between AMY1 copy number and obesity traits, and the effect of the interaction between AMY1 copy number and starch intake on these obesity traits. Design: We first assessed the association between AMY1 copy number (genotyped by digital droplet polymerase chain reaction) and obesity traits in 4800 individuals without diabetes (mean age: 57 y; 60% female) from the Malmö Diet and Cancer Cohort. Then we analyzed interactions between AMY1 copy number and energy-adjusted starch intake (obtained by a modified diet history method) on body mass index (BMI) and body fat percentage. Results:AMY1 copy number was not associated with BMI (P = 0.80) or body fat percentage (P = 0.38). We observed a significant effect of the interaction between AMY1 copy number and starch intake on BMI (P-interaction = 0.007) and body fat percentage (P-interaction = 0.03). Upon stratification by dietary starch intake, BMI tended to decrease with increasing AMY1 copy numbers in the low-starch intake group (P = 0.07) and tended to increase with increasing AMY1 copy numbers in the high-starch intake group (P = 0.08). The lowest mean BMI was

observed in the group of participants with a low AMY1 copy number and a high dietary intake of starch. Conclusions: Our findings suggest an effect of the interaction between starch intake and AMY1 copy number on obesity. Individuals with high starch intake but low genetic capacity to digest starch had the lowest BMI, potentially because larger amounts of undigested starch are transported through the gastrointestinal tract, contributing to fewer calories extracted from ingested starch." As taken from Rukh G et al. 2017. Am. J. Clin. Nutr. 106(1), 256-262. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28539377

"Colorectal cancer (CRC) risk is modulated by diet and there is convincing evidence of reduced risk with higher non-digestible carbohydrates (NDCs) consumption. Resistant starch (RS), a NDC, positively modulates the expression of oncogenic microRNAs, suggesting that this could be a mechanism through which NDCs protect against CRC. The present study aimed to investigate the effects of supplementation with two NDCs, RS, and polydextrose (PD), on microRNA expression in the macroscopically-normal human rectal epithelium using samples from the DISC Study, a randomized, double-blind, placebo-controlled dietary intervention. We screened 1008 miRNAs in pooled post-intervention rectal mucosal samples from participants allocated to the double placebo group and those supplemented with both RS and PD. A total of 111 miRNAs were up- or down-regulated by at least twofold in the RS + PD group compared with the control group. From these, eight were selected for quantification in individual participant samples by qPCR, and fold-change direction was consistent with the array for seven miRNAs. The inconsistency for miR-133b and the lower fold-change values observed for the seven miRNAs is probably because qPCR of individual participant samples is a more robust and sensitive method of quantification than the array. miR-32 expression was increased by approximately threefold ($P = 0.033$) in the rectal mucosa of participants supplemented with RS + PD compared with placebo. miR-32 is involved in the regulation of processes such as cell proliferation that are dysregulated in CRC. Furthermore, miR-32 may affect non-canonical NF-κB signaling via regulation of TRAF3 expression and consequently NIK stabilization." As taken from Malcomson FC et al. 2017. Mol. Carcinog. 56(9), 2104-2111. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28418082

"PURPOSE: The protective function of the intestinal mucosa largely depends on carbohydrate moieties that as a part of glycoproteins and glycolipids form the epithelial glycocalyx or are secreted as mucins. Modifications of their expression can be induced by an altered intestinal microenvironment and have been associated with inflammatory disorders and colorectal cancer. Given the influence of dietary factors on the gut ecosystem, here we have investigated whether a long term feeding on a starch-rich diet can modulate the glucidic profile in the colonic mucosa of rats. METHODS: Animals were divided into two groups and maintained for 9 months at different diets: one group was fed a standard diet, the second was fed a starch-enriched diet. Samples of colonic mucosa, divided in proximal and distal portions, were processed for microscopic analysis. Conventional stainings and lectin histochemistry were applied to identify acidic glycoconjugates and specific sugar residues in oligosaccharide chains, respectively. Some lectins were applied on adjacent sections after sialidase/fucosidase digestion, deacetylation, and oxidation to characterize either terminal dimers or sialic acid acetylation. RESULTS: An increase in sulfomucins was found to be associated with the starch-enriched diet that affected also the expression of several sugar residues as well as fucosylated and sialylated sequences in both proximal and distal colon. CONCLUSIONS: Although the mechanisms leading to such a modulation are at present unknown, either an altered intestinal microbiota or a dysregulation of glycosylation patterns might be responsible for the types and distribution of changes in the glucidic profile here observed." As taken from Gabrielli MG and Tomassoni D. 2017. Eur. J. Nutr. 57(3), 1109-1121. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28393287

"Resistant starch has been studied extensively for its hypoglycemic activity, while its underlying molecular mechanism is not fully understood. In this study, we investigated the hypoglycemic effect of different doses of lotus seed resistant starch (LSRS) supplementation on type 2 diabetic mice and elucidated the molecular basis of its hypoglycemic effect. LSRS supplementation significantly reduced blood glucose level by 16.0%-33.6%, recovered serum insulin level by 25.0%-39.0% and

improved lipid metabolism disorder in the diabetic mice. The genome-wide expression patterns in pancreatic tissue were analyzed, and 511 differentially expressed genes (DEGs) were identified. The analysis results of gene ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways indicated that the protective effect of LSRS supplementation was most likely driven by modulating expression levels of various key factors involved in insulin secretion, insulin signal transmission, cell apoptosis, antioxidant activity and p53 signaling pathways." As taken from Wang Q et al. 2018. Food Chem. 264, 427-434. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29853397

"Obesity is a risk factor for many chronic diseases, and the anti-obesity effect of starch in a whole grain-like structural form (WGLSF) prepared through co-gelation with oat β-glucan and alginate was studied using high-fat (HF) induced obese male C57BL/6J mice. In vitro human fecal fermentation of WGLSF-starch showed a slower rate of fermentation and a higher production of butyric acid (132.0 μmol per 50 mg sample) when compared to the physical mixture counterpart of starch, β-glucan, and alginate (PM) (110.5 μmol per 50 mg) or β-glucan itself (96.2 μmol per 50 mg). The body weight gain of obese mice fed with a HF-WGLSF diet was significantly reduced (42.0% lower than the HF group, 30.2% lower than the physical mixture) with decreased cell size in white adipose tissue and similar levels of serum lipid profiles to the control of the low-fat (LF) group. Western blotting experiments showed the down-regulated lipogenic transcription factor of SREBP-1c and fatty acid synthase (FAS), but the lipid-oxidation related transcription factors of peroxisome proliferator-activated receptor-α (PPAR-α) and phosphorylated AMP-activated protein kinase (p-AMPK) were up-regulated. Energy metabolism analysis revealed increased lipid-sourced energy expenditure with higher heat production and respiratory exchange ratios. Consistently, the expression of hypothalamic pro-opiomelanocortin (POMC), favoring energy expenditure, was increased significantly while the neuropeptide Y (NPY) was reduced. Thus, the increased energy expenditure stimulated by starch in a whole-grain-like structural form is responsible for the reduced body weight gain of obese mice fed with a high fat-based diet." As taken from Luo K et al. 2018. Fd Funct. 9, 3755-3763. Available at https://pubs.rsc.org/en/content/articlelanding/2018/fo/c8fo00602d/unauth#!divAbstract

"The effects of resistant starch (RS) on serum cholesterol levels have been previously investigated. However, the results of those studies are inconsistent. The purpose of our meta-analysis was to determine if RS affects blood lipids based on the current literature. The methods included searching databases (PubMed, Embase, Scopus, and Cochrane Library) up to September 2017, as well as hand-searching reference lists of articles published in English. The initial search yielded 1228 articles. Of these, 14 articles (20 trials) were included in our investigation focusing on the effects of RS on total cholesterol (TC; 19 trials), triglycerides (TG; 19 trials), low-density lipoprotein cholesterol (LDL-C; 16 trials), and high-density lipoprotein cholesterol (17 trials). Methodological quality was assessed using TC, LDL-C, TGs, and high-density lipoprotein cholesterol. Pooled effects were calculated using a random-effects model. The meta-analysis of these data showed that RS supplementation has an effect on lowering TC and LDL-C (TC: mean difference, -7.33mg/dL [95% confidence interval -12.15 to -2.52mg/dL]; LDL-C: mean difference: -3.40mg/dL [95% confidence interval, -6.74 to -0.07mg/dL]). Subgroup meta-analysis revealed that a longer time (>4weeks) of RS supplementation can generate more obvious effects on TC and LDL-C levels, and higher dose (>20 g/d) of RS also had a lowering effect on TG level. Future research should focus on the relationship between RS type and cholesterol-lowering effects, and the effects on subjects of different health status or those with different baseline levels of serum lipids. Moreover, the mechanism for the cholesterol-lowering effects of RS should be further explored. In conclusion, RS can reduce serum TC and LDL-C levels, particularly when administered for a duration longer than 4 weeks." As taken from Yuan HC et al. 2018. Nutr. Res. 54, 1-11. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29914662

"Species differences between domestic cats (Felis catus) and dogs (Canis familiaris) has led to differences in their ability to digest, absorb and metabolize carbohydrates through poorly characterized mechanisms. The current study aimed to first examine biopsied small intestine,

pancreas, liver and skeletal muscle from laboratory beagles and domestic cats for mRNA expression of key enzymes involved in starch digestion (amylase), glucose transport (sodium-dependent SGLTs and -independent glucose transporters, GLUT) and glucose metabolism (hexokinase and glucokinase). Cats had lower mRNA expression of most genes examined in almost all tissues compared to dogs ($p < 0.05$). Next, postprandial glucose, insulin, methylglyoxal (a toxic glucose metabolite) and d-lactate (metabolite of methylglyoxal) after single feedings of different starch sources were tested in fasted dogs and cats. After feeding pure glucose, peak postprandial blood glucose and methylglyoxal were surprisingly similar between dogs and cats, except cats had a longer time to peak and a greater area under the curve consistent with lower glycolytic enzyme expression. After feeding starches or whole diets to dogs, postprandial glycemic response, glycemic index, insulin, methylglyoxal and d-lactate followed reported glycemic index trends in humans. In contrast, cats showed very low to negligible postprandial glycemic responses and low insulin after feeding different starch sources, but not whole diets, with no relationship to methylglyoxal or d-lactate. Thus, the concept of glycemic index appears valid in dogs, but not cats. Differences in amylase, glucose transporters, and glycolytic enzymes are consistent with species differences in starch and glucose handling between cats and dogs." As taken from Briens JM et al. 2021. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 257, 110973. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33933629/

"Background: To date there is no therapy consensus in patients with multifocal hepatocellular carcinoma (mHCC). Purpose: To compare outcome of trans-arterial chemoembolization (TACE) with degradable starch microspheres (DSM-TACE) versus selective internal radiation therapy (SIRT) in mHCC. Material and methods: In this single-center study, 36 patients without portal vein invasion, treated between May 2014 and May 2018, were enrolled retrospectively. Eighteen consecutive patients received DSM-TACE and were matched by age, gender, BCLC stage, Child-Pugh status, and tumor volume and 18 patients underwent SIRT. Overall survival (OS), progression-free survival (PFS), and local tumor control (LTC) were evaluated. Toxicity profiles for both therapies were also evaluated and compared. Results: In the entire collective, median OS was 9.5, PFS 5.0, and LTC 5.5 months. Subgroup analysis revealed an OS of 9.5 months in both groups ($P = 0.621$). PFS was 6 months for the SIRT and 4 months for the DSM-TACE cohort ($P = 0.065$). Although not significantly, LTC was lower (4 months) in the SIRT compared to the DSM-TACE cohort (7 months; $P = 0.391$). When DSM-TACE was performed ≥3 times ($n = 11$), OS increased, however without statistical difference compared to SIRT, to 11 months, PFS to 7 months, and LTC to 7 months. When DSM-TACE was performed <3 times ($n = 7$), OS, PFS, and LTC decreased (5 months, $P = 0.333$; 2 months, $P = 0.047$; 2 months, $P = 0.47$). Toxicity profiles and adverse event analysis only revealed a significant difference for nausea and vomiting (more frequent in the SIRT cohort, $P = 0.015$), while no other parameter showed a significant difference ($P > 0.05$). Conclusion: DSM-TACE might be an alternative to SIRT in multifocal HCC patients as OS, PFS, and LTC did not differ significantly and toxicity profiles seem to be comparable." As taken from Auer TA et al. 2021. Acta Radiol. 62(3), 313-321. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32498543/

"Equol is a metabolite of daidzein and has a higher biological activity than daidzein. Equol, combined with estrogen receptors, can reduce the incidence of diseases such as cardiovascular disease, osteoporosis, and breast cancer; more effectively alleviate the symptoms of perimenopausal syndrome; and improve age-related decline of the uterus and ovaries. Research has shown that food composition can greatly affect the formation of equol in the intestinal tract. In the intestines, the content of nonstarch polysaccharides that can stimulate fermentation is high, thereby allowing intestinal bacteria to quickly and completely transform the daidzein into equol. This study used Sprague Dawley (SD) rats as a model, where menopause was established through direct intragastric administration of formistan. In the 6-week-long experiment, intragastric administration of RS while feeding bean pulp reduced the body weight of postmenopausal rats, reduced the efficiency of feed utilization of rats, and increased the weight of organs such as the uterus and ovaries. Routine blood indexes showed that no adverse reactions were produced by

intragastric administration of RS. 16s rDNA sequencing further verified Lactobacillus and Clostridium XIVa, as the bacteria that converted daidzein into equol." As taken from Ge YF et al. 2020. Food Sci. Nutr. 8(8), 4055-4065. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32884687/

"According to the EASL Guidelines for the management of hepatocellular carcinoma, transcatheter arterial chemoembolization is the first-line treatment recommended for intermediate-stage HCC. Furthermore, it is widely accepted that patients beyond the Milan criteria can be considered for a liver transplant after successful downstaging to within the Milan criteria. Response to downstaging treatments significantly influences not just drop-outs, but also the rate of post-transplantation tumor recurrences. TACE with degradable starch microspheres represents an alternative to conventional TACE with lipiodol and TACE with drug-eluting beads, and it leads to transient arterial occlusion allowing lower activation of hypoxia-inducible factors and less release of vascular endothelial growth factor, a promoter of neoangiogenesis, tumor proliferation, and metastatic growth. In patients with intermediate-stage HCC and a Child-Pugh score of 8 or 9, life expectancy may be dominated by cirrhotic liver dysfunction, rather than by the tumor progression itself; hence, locoregional treatments might also be detrimental, precipitating liver dysfunction to an extent that survival is shortened rather than prolonged. Data on tolerability, toxicity, and effectiveness of DSM-TACE are limited but encouraging. Between January 2015 and October 2020, 50 consecutive patients with intermediate-stage hepatocellular carcinoma and a Child-Pugh score of 8/9, who had undergone DSM-TACE as the first-line treatment, were eligible for the study. A total of 142 DSM-TACEs were performed, with a mean number of 2.84 procedures per patient. The mean time-to-downstaging was 19.2 months, with six patients successfully downstaged. OS was about 100% at six months, 81.8% at 12 months, and 50% at 24 months. Twenty-two patients experienced adverse events after chemoembolization. The median OS and safety of DSM-TACE in this study are comparable with other published investigations in this field. Furthermore, 12% of patients were successfully downstaged. Hence, the results of the current investigation demonstrate that DSM-TACE is effective and safe in intermediate-stage HCC, achieving an interesting downstaging rate. Such data were observed in the population subset with a Child-Pugh score of 8 or 9, in which life expectancy may be determined by cirrhotic liver dysfunction, so the achievement of a balance between the safety and efficacy profile of the TACE treatment is crucial." As taken from Minici R et al. 2021. Front. Pharmacol. 12, 634087. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33897422/

"Background & aims: Circulating insulin-like growth factor-I (IGF-I) is associated with the risk of several cancers. Dietary protein intake, particularly dairy protein, may increase circulating IGF-I; however, associations with different protein sources, other macronutrients, and fibre are inconclusive. To investigate the associations between intake of protein, macronutrients and their sources, fibre, and alcohol with serum IGF-I concentrations. Methods: A total of 11,815 participants from UK Biobank who completed ≥4 24-h dietary assessments and had serum IGF-I concentrations measured at baseline were included. Multivariable linear regression was used to assess the cross-sectional associations of macronutrient and fibre intake with circulating IGF-I concentrations. Results: Circulating IGF-I concentrations were positively associated with intake of total protein (per 2.5% higher energy intake: 0.56 nmol/L (95% confidence interval: 0.47, 0.66)), milk protein: 1.20 nmol/L (0.90, 1.51), and yogurt protein: 1.33 nmol/L (0.79, 1.86), but not with cheese protein: -0.07 nmol/L (-0.40, 0.25). IGF-I concentrations were also positively associated with intake of fibre (per 5 g/day higher intake: 0.46 nmol/L (0.35, 0.57)) and starch from wholegrains (Q5 vs. Q1: 1.08 nmol/L (0.77, 1.39)), and inversely associated with alcohol consumption (>40 g/day vs <1 g/day: -1.36 nmol/L (-1.00, -1.71)). Conclusions: These results show differing associations with IGF-I concentrations depending on the source of dairy protein, with positive associations with milk and yogurt protein intake but no association with cheese protein. The positive association of fibre and starch from wholegrains with IGF-I warrants further investigation." As taken from Watling CZ et al. 2021. Clin. Nutr. 40(7), 4685-4693. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/34237695/

6. Functional effects on

6.1. Broncho/pulmonary system

“Corn starch powder is widely used for routine infant skin care as a substitute for talcum powder, as it is belived to have fewer respiratory hazards. We describe a one-month-old infant who presented to an emergency department with respiratory failure and a severe pneumonitis from aspiration of corn starch powder. The patient recovered after five days of mechanical ventilation support. We conclude that the careless use of corn starch for infant skin care can lead to accidental aspiration of this substance and severe respiratory disease.” As taken from Silver P et al. Pediatr Emerg Care.1996 Apr; 12(2):108-10. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=8859920&query_hl=92&itool=pubmed_docsum

Symptoms: cough, chest pain (NIOSH, 2019).

6.2. Cardiovascular system

“Maize starch powder, used as lubricant in surgical gloves, was administered into the pericardial cavity of rats and was found to induce granulomatosis with formation of pericardial adhesions. The effect of dextran 70 on the formation of these adhesions was investigated. It was found that intrapericardial dextran reduces the occurrence of pericardial adhesions.” As taken from Reikerås O Eur Surg Res. 1987; 19(1):62-4. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=2431912&query_hl=104&itool=pubmed_docsum

“Blood substitutes are being developed using molecular solutions of modified free hemoglobin; however, anaphylactic reactions, severe renal toxicity, and hypertension have been reported in experimental models and human beings. Hypertension remains as an obstacle to the clinical use of most blood substitutes. Several investigators suggest that this effect is due to the interaction between nitric oxide and hemoglobin into the endothelial cells; hence, prevention of hemoglobin extravasation would avoid vasoconstriction. The forms of hemoglobin likely to prevent extravasation include polymerized and encapsulated Hb. Another alternative and significantly less expensive approach is the hydroxyethyl starch Hb-polymer. The aim of the present study was to compare the effect of hydroxyethyl-starch-hemoglobin with that of stroma-free hemoglobin on the in vitro contractile activity of aortic rings isolated from adult male rats. The hemoglobin-based oxygen carrier was made using stroma-free hemoglobin prepared from outdated human red cells and conjugated with 10% hydroxyethyl starch 200-260 MW. The experiments were made in thoracic segments of the aortic rings incubated with hemoglobin, starch-hemoglobin or Ringer Krebs-Bicarbonate solution (RKB) during 30 min. Smooth muscle contraction with phenylephrine and subsequent inhibition of contraction with carbachol were performed before and after incubation with hemoglobin, starch-hemoglobin, or vehicle. Incubation with hemoglobin and starch-hemoglobin significantly increased the contractile response to phenylephrine of aortic rings compared with RKB solution. The maximal response to carbachol was significantly decreased in the aortic rings incubated with either hemoglobin or starch-hemoglobin in comparison with the RKB-incubated tissues. There were no differences between the aortic rings incubated with either hemoglobin, or starch-hemoglobin. These results show that there are no differences between the effects of stroma-free hemoglobin and starch-hemoglobin on the in vitro contractile activity of aortic rings isolated from adult male rats. Our findings do not support the hypothesis that an increase in the size of the hemoglobin molecule prevents hemoglobin extravasation, and the consequent vasoconstriction due to the scavenging of nitric oxide by stroma free hemoglobin in the cellular space between endothelium and smooth muscle.” As taken from Chávez-Negrete A et al. Artif Cells Blood Substit Immobil Biotechnol. 2004; 32(4):549-61. PubMed, 2009 available at

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15974182&query_hl=105&itool=pubmed_docsum

“SACN has recently finalised a report which considers the evidence for a role of dietary carbohydrate on colorectal disease (including cancer, irritable bowel syndrome and constipation), cardiovascular disease (insulin resistance, glycaemic response and obesity), and oral health. It concludes that carbohydrates should provide around 50% of total dietary energy, but that a greater proportion should come from foods that are lower in free sugars and higher in dietary fibre.

UK Scientific Advisory Committee on Nutrition (2015). Carbohydrates and Health. July 2015.

“Background: The carbohydrate-to-fiber ratio is a recommended measure of carbohydrate quality; however, its relation to incident coronary heart disease (CHD) is not currently known. Objective: We aimed to assess the relation between various measures of carbohydrate quality and incident CHD. Design: Data on diet and lifestyle behaviors were prospectively collected on 75,020 women and 42,865 men participating in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS) starting in 1984 and 1986, respectively, and every 2-4 y thereafter until 2012. All participants were free of known diabetes mellitus, cancer, or cardiovascular disease at baseline. Cox proportional hazards regression models were used to assess the relation between dietary measures of carbohydrate quality and incident CHD. Results: After 1,905,047 (NHS) and 921,975 (HPFS) person-years of follow-up, we identified 7,320 cases of incident CHD. In models adjusted for age, lifestyle behaviors, and dietary variables, the highest quintile of carbohydrate intake was not associated with incident CHD (pooled-RR = 1.04; 95% CI: 0.96, 1.14; P-trend = 0.31). Total fiber intake was not associated with risk of CHD (pooled-RR = 0.94; 95% CI: 0.85, 1.03; P-trend = 0.72), while cereal fiber was associated with a lower risk for incident CHD (pooled-RR = 0.80; 95% CI: 0.74, 0.87; P-trend < 0.0001). In fully adjusted models, the carbohydrate-to-total fiber ratio was not associated with incident CHD (pooled-RR = 1.04; 95% CI: 0.96, 1.13; P-trend = 0.46). However, the carbohydrate-to-cereal fiber ratio and the starch-to-cereal fiber ratio were associated with an increased risk for incident CHD (pooled-RR = 1.20; 95% CI: 1.11, 1.29; P-trend < 0.0001, and pooled-RR = 1.17; 95%CI: 1.09, 1.27; P-trend < 0.0001, respectively). Conclusion: Dietary cereal fiber appears to be an important component of carbohydrate quality. The carbohydrate-to-cereal fiber ratio and the starch-to-cereal fiber ratio, but not the carbohydrate-to-fiber ratio, was associated with an increased risk for incident CHD. Future research should focus on how various measures of carbohydrate quality are associated with CHD prevention.” As taken from AlEssa HB et al. 2018. Am. J. Clin. Nutr. 107(2), 257-267. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29529162

“Studies on the intake of different types of carbohydrates and long-term mortality are sparse. We examined the association of starch, total and each type of sugar and free sugars with the risk of total and cause-specific mortality in a cohort of the general population in Japan. Study subjects were 29 079 residents from the Takayama Study, Japan, who responded to a self-administered questionnaire in 1992. Diet was assessed by a validated FFQ at the baseline. Mortality was ascertained during 16 years of follow-up. We noted 2901 deaths (974 cancer related and 775 cardiovascular related) in men and 2438 death (646 cancer related and 903 cardiovascular related) in women. In men, intake of starch was inversely associated with total mortality after controlling for covariates (hazard ratio (HR) for the highest quartile v. lowest quartile: 0·71; 95 % CI 0·60, 0·84; Ptrend < 0·001). Intakes of total sugars, glucose, fructose, sucrose, maltose and free and naturally occurring sugars were significantly positively associated with total mortality in men (HR for the highest v. lowest quartile of total sugar: 1·27; 95 % CI 1·12, 1·45; Ptrend < 0·0001). Similar relations were observed for cardiovascular mortality and non-cancer, non-cardiovascular mortality in men. In women, there was no significant association between any type of carbohydrates and mortality except that intake of free sugars was significantly positively associated with total and non-cancer, non-cardiovascular mortality. Data suggest that the high intake of starch reduces mortality, whereas the high intake of sugars, including glucose, fructose and sucrose, increases mortality in

Japanese men." As taken from Nagata C et al. 2019. Br. J. Nutr. 122(7), 820–828. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32124712/

6.3. Nervous system

"Introduction: Psychomotor performance task is used to assess the arousal and cognitive functions of the central nervous system. Alternatively, human visual working memory reflects the capability of the individual's short-term memory. Psycho-mental stimuli are linked to the stimulation of Malondialdehyde (MDA) formations. Citicoline is a nootropic nucleotide agent with a favorable effect on the augmentation of human memory and cognitive function. Thus, the purpose of this study was to determine the effect of citicoline on human vigilance, visual working memory, and oxidative stress using healthy volunteers. Methods: 40 healthy volunteers were enrolled and divided into two groups: group A: 20 volunteers received 500mg/day starch capsule for two weeks and group B: 20 volunteers received 500mg/day citicoline capsule for two weeks. Human vigilance, visual working memory, and oxidative stress markers of each volunteer were assessed before and after citicoline and placebo intake. The obtained data were analyzed by SPSS regarding $P<0.05$ as statistically significant. Results: Placebo had no significant effect on human vigilance and visual working memory after two weeks of therapy ($P>0.05$), whereas citicoline improved most variables of psychomotor performances and working memory ($P<0.01$). Placebo significantly increased serum MDA levels from 19.44±2.11 to 29.66±3.28 nmol/mL ($P=0.0001$), while citicoline significantly decreased MDA serum levels from 19.11±2.66 to 15.63±1.33 nmol/mL ($P=0.0001$). Conclusion: Citicoline improves human psychomotor vigilance, arousal, and visual working memory with significant amelioration of oxidative stress compared with placebo." As taken from Al-Kuraishy HM and Al-Gareeb AI. 2020. Basic Clin. Neurosci. 11(4), 423-432. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33613880/

"Objectives: In this clinical trial, the effect of aqueous extract of Berberis vulgaris L. was investigated on opiate withdrawal syndrome, depression, anxiety, stress, and sleep quality in opioid addicts which were under methadone maintenance therapy. Methods: For this purpose, 52 opiate addicts were randomly selected of whom 28 received 500 mg capsules of B. vulgaris extract (treatment) and the rest received 500 mg of starch capsules (placebo), twice daily for 4 weeks. Signs and symptoms of opiate withdrawal syndrome, depression, anxiety, stress, and sleep quality were assessed through Clinical Opiate Withdrawal Scale (COWS), Pittsburgh Sleep Quality Inventory (PSQI) and Depression Anxiety Stress Scales-21 (DASS-21) questionnaires at baseline and after 7, 14, and 28 days of receiving intervention. Results: Signs and symptoms of opiate withdrawal syndrome were significantly improved in those who received the extract for 1 month compared to the placebo group. However, there were no significant differences in depression, anxiety, stress, and sleep quality scores in the treatment group compared to those in the placebo group. Conclusions: The extract of B. vulgaris root as a traditional herbal product in combination with methadone could improve the symptoms and signs of opiate withdrawal." As taken from Dabaghzadeh F et al. 2021. J. Basic Clin. Physiol. Pharmacol. Epub ahead of print. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/34147041/

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

"Portal appearance of short-chain fatty acids (SCFA) produced from fermentation of three different resistant starch (RS) sources (raw potato starch, high-amylose maize starch and retrograded high-amylose maize starch) was investigated in pigs. The catheterization technique coupled with determination of portal blood flow was used to estimate SCFA uptake by the colonic mucosa. Our hypothesis was that these three RS were not equivalent butyrate providers for the colonic mucosa and that butyrate uptake would therefore be different after in vivo fermentation of each starch. The starches induced different patterns of appearance of SCFA in the portal blood; raw potato starch was the only RS source to show a significant appearance of butyrate in the portal blood. Thus, uptake of butyrate by the colonic mucosa apparently differed between starches. This finding

suggests that butyrate uptake does not only depend on the flow of butyrate appearing in the lumen. Indeed, for unexplained reasons, utilization of butyrate by the colonic mucosa appeared to be less efficient when the butyrate was produced from fermentation of potato starch than when it was produced from fermentation of the other RS sources." As taken from Martin LJ et al. Br J Nutr. 2000 Nov; 84(5):689-96. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=11177182&query_hl=109&itool=pubmed_docsum

The protective effect of a dietary high-amylose cornstarch (HAS) against trinitrobenzene sulfonic acid (TNBS)-induced colitis was examined in rats. Rats were fed a HAS-free basal diet or, a 15% or 30% HAS supplemented diet for 10 d, and then received intracolonic TNBS to induce colitis and fed the respective diets for a further 8 d. HAS ingestion significantly protected colonic injuries as evidenced by lower colonic myeloperoxidase activity. Rats fed the HAS diet showed greater cecal short-chain fatty acid (SCFA) production than those fed the basal diet. Further, just before TNBS administration, HAS ingestion dose-dependently increased fecal and cecal mucin contents, and protein and nucleic acid contents in the colonic mucosa. HAS ingestion also reduced colonic permeability. The protective effect of HAS ingestion on TNBS-induced colitis is perhaps exerted through alterations in colonic mucosa, possibly due to cecal SCFA production." As taken from Morita T et al. Biosci Biotechnol Biochem. 2004 Oct; 68(10):2155-64. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15502362&query_hl=110&itool=pubmed_docsum

"To investigate the effect of resistant starch (RS2 and RS3) intake on colon flora of mice 32 mice were divided into four groups (8 mice/group) according to their weight randomly. Four groups of mice were given basic feed (containing only corn starch and no RS), containing 6% RS2 feed, containing 12% RS2 feed, and containing 6% RS3 feed, respectively. Feces were collected on the 1st and 29th day of the experiment, and pH of feces were measured and five feces flora (enterobacter, bifidobacteria, lactobacillus, bacteroid, and enterococcus) were detected with culture medium method. All the mice were killed on the 29th day to get caecum content. The pH of caecum content were measured and short chain fatty acid (SCFA) were detected with gas chromatography. RS2 and RS3 could all increase feces bifidobacteria ($P<0.05$) and decrease enterobacteria ($P<0.05$). RS2 could increase SCFA of ceacum content, and 12% RS2 group is more than control group ($P<0.01$), and also more than 6% RS2 group ($P<0.05$). RS could also decrease pH of feces and ceacum content of mice, and the effect of RS3 is more than RS2. Resistant starch could improve colon flora, increase its fermentable production SCFA, decrease caecum and feces pH, and then improve health." As taken from He M et al. Wei Sheng Yan Jiu. 2005 Jan; 34(1):85-7. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15862034&query_hl=112&itool=pubmed_docsum

"The inflammatory response resulting from the implantation of a medical device may compromise its performance and efficiency leading, in certain cases, to the failure of the implant. Thus, the assessment of the behavior of inflammatory cells in vitro, constitutes a key feature in the evaluation of the adverse potential, or not, of new promising biomaterials. The objectives of this study were to determine whether starch-based polymers and composites activated human neutrophils. Blends of starch with ethylene-vinyl alcohol, with cellulose acetate and polycaprolactone, as well as composites based on all these materials filled with hydroxyapatite have been studied. A lysozyme assay was adapted to examine enzyme secretion from human neutrophils incubated with different starch-based materials. Changes in the free radical and degranulation activity of the neutrophil were also determined by measuring the luminescent response of Pholasin, a photoprotein that emits light after excitation by reactive oxygen species. The amount of lysozyme secreted by neutrophils incubated with the polymers did not exhibit significant differences between the tested materials. Results were in all cases similar to those obtained for the control (polypropylene) except for one of the starch blends (corn starch with polycaprolactone reinforced with 30% (w/w) of HA). The chemiluminescence experiments showed that polymers reduce the signal produced by

activated neutrophils. Furthermore, for some polymers it was demonstrated that the phenomenon was due to an effect of the surface of the materials in cell adhesion or a simultaneous competition for the photoprotein in solution, which results in the decrease of the intensity of light emitted and detected." As taken from Marques AP et al. J Mater Sci Mater Med. 2003 Feb; 14(2):167-73. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15348489&query_hl=115&itool=pubmed_docsum

"The presence of carbohydrates and organic acids was monitored in the oral cavity over a 3-hour period following the ingestion of six foods containing cooked starch (popcorn, potato chips, corn flakes, bread stick, hard pretzel and wheat cracker) and compared to a food containing sugar (chocolate-covered candy bar). Oral fluid samples were collected at 30-min intervals from five different tooth sites from 7 volunteers using absorbent paper points. Samples were analyzed for carbohydrates and organic acids using high-performance liquid chromatography. Analytical data for each food were pooled and compared to the results of the sugar food. The amount of lactic acid produced 30 min after ingestion was highest with the potato chips and lowest with the corn flakes. Potato starch contributed more readily to oral lactic acid production than wheat or corn starch. A direct linear relationship existed between lactic acid production and the presence of oral glucose produced from starch, which occurred via the metabolites maltotriose and maltose. Oral clearance of foods containing cooked starch proceeded significantly slower than that of the sugar food, thus contributing to a prolonged period of lactic acid production." As taken from Linke HA et al. Ann Nutr Metab. 1999; 43(3):131-9. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=10545668&query_hl=117&itool=pubmed_docsum

"An investigation was carried out to determine whether variations of dietary carbohydrates could modify the colonic flora in rats. Sprague-Dawley rats were fed with two equicaloric diets based on the AIN-76 diet (American Institute of Nutrition 1977) but differing from that diet in content of carbohydrates, i.e. high sucrose (64%) of high corn starch (64%). Feeding was continued for 9 months ad libitum and no variation in weight gain was recorded among the different diets. A prevalence of aerobes, and a significant reduction in the ratio anaerobes/aerobes in the faeces of rats on the high starch diet compared with the high sucrose diet, was observed. The anaerobe genera identified included Actinomyces, Bacteroides, Bifidobacterium, Clostridium, Eubacterium, Lactobacillus and Propionibacterium. Bacteroides was the most prevalent genus in both dietary groups (51.2 and 29.5% in the faeces of rats fed the sucrose and starch diets, respectively). In contrast, clostridia were prevalent in the starch-fed group (23.8%) and less so in the sucrose diet (11.5%), as propionibacteria were prevalent in faeces of rats fed the starch diet (15.5%), and low in the sucrose diet (3.9%). The remaining genera were scarce in faeces from rats on either diet. Total short-chain fatty acids (SCFA) were significantly higher in the faeces of animals fed the starch diet compared with those fed the sucrose diet. The relative concentrations of acetic, propionic and butyric acids were not significantly different between the two dietary groups. In conclusion, high starch diet can markedly modify the composition of faecal flora and alter considerably the faecal concentration of SCFAs, compound which might have a health-promoting effect." As taken from Cresci A et al. J Appl Microbiol. 1999 Feb; 86(2):245-50. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=10063624&query_hl=119&itool=pubmed_docsum

"Administering raw corn starch can maintain normoglycemia for long periods after being ingested, thus facilitating control in patients with type I and III glycogenosis. The metabolic effects and the effects on the nutritional status of a treatment with fractionated administrations of raw starch are assessed in two patients with type I glycogenosis (ages 18 and 12 years) and one patient with type III glycogenosis (aged 13 years). In the first two cases the response was previously studied after administering a load of raw corn starch in a water suspension, in an amount similar to the estimated rate of endogenous glucose production during the fasting period (5 mg/kg/minute). The results of the overload of starch showed a normoglycemia and an absence of lactoacidosis

between 4 and 6 hours after its ingestion. The three patients were given two doses of raw corn starch (2 g/kg/dose) at 1.00 and 5.00 hours during the night. After one year of treatment, all patients showed glycemia levels at 9.00 AM that were greater than 90 mg/dl and lactic acid levels that were lower than 2.4 mmol/l. Moreover, in two of the cases there was an increase in the growth rate. In all cases the amount of the hepatomegaly decreased as did the size of the hepatic adenomas that were present in two of the cases. In patients with type I and III glycogenosis, raw corn starch can balance the results of the nightly gastric glucose infusion, both with regard to the metabolic control and with regard to the growth." As taken from Galiano-Segovia MJ et al. Nutr Hosp. 1998 Sep-Oct; 13(5):228-32. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=9830843&query_hl=122&itool=pubmed_docsum

"The first nonmilk foods that are given to infants contain high levels of starch, a fraction of which is resistant to enzyme hydrolysis. Incomplete digestion of starch may interfere with the absorption of certain minerals. A fraction of dietary starch which is resistant to in vitro enzymatic hydrolysis has been termed resistant starch. The aim of this study was to compare the intestinal apparent absorption of calcium, phosphorus, iron, and zinc in the presence of either resistant or digestible starch. Twelve 7-10-d-old piglets were fitted with a T-tube inserted into the intestine approximately 3 m distal to the duodenum. Animals received in random order 200 mL of a test meal of cooked, cooled, high amylose corn starch (16.4% resistant starch), or cooked rice starch (digestible starch) administered by an orogastric tube. Both meals contained the same amount of calcium, phosphorus, iron, and zinc. The test meal also contained tracer amounts of 59Fe and 65Zn, as well as polyethylene glycol 3350, as a nonabsorbable marker. Intestinal apparent absorption of starch was greater the meal with digestible starch (71.0 +/- 17.0%) than after the meal with resistant starch (49.2 +/- 10.3) (P<0.001). After feeding the meals with resistant and digestible starch, mineral apparent absorption was, respectively: calcium, 40.2 +/- 11.8% versus 28.1 +/- 16.4% (P<0.05); phosphorus, 73.2 +/- 14.0% versus 67.8 +/- 18% (NS); iron, 24.1 +/- 12.2% versus 12.6 +/- 10.6% (P<0.01), and zinc, 35.0 +/- 13.0% versus 30.6 +/- 8.22% (NS). In conclusion, a meal containing 16.4% resistant starch resulted in a greater apparent absorption of calcium and iron compared with a completely digestible starch meal. If this finding holds true for the whole bowel, administration of resistant starches could have a positive effect on intestinal calcium and iron absorption." As taken from Morias MB et al. Pediatr Res. 1996 May; 39(5):872-6. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=8726244&query_hl=124&itool=pubmed_docsum

"Cornflour has been used as a lubricant on surgical gloves since 1947. Serious complications have been reported after deposition of cornflour powder in the human body. The best known complication is granulomatous peritonitis, but cornflour granulomas have also been found in the heart, the kidneys and the central nervous system. Maltesercross, when seen in polarized light in the microscope, is pathognomonic. Malignancy and other granulomatous inflammations are actual differential diagnoses. Nowadays gloves free of lubricating powder are commercially available." As taken from Saatvedt K, Nordstrand K. Tidsskr Nor Laegeforen. 1992 Oct 10; 112(24):3081-2. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=1471082&query_hl=127&itool=pubmed_docsum

"Fecal bulking occurs through water holding by dietary residue as well as by enhanced bacterial mass as a result of bacterial utilization of dietary fiber. In this study, increasing the energy intake of human subjects by supplementing the carbohydrate content of the diet with corn starch increased fecal weights and fecal nitrogen content. This indicates that the carbohydrate content, more specifically the starch content, of the diet influences fecal bulking by possibly increasing available substrates for colonic bacterial proliferation which are dependent largely on undigested fiber in the colon. This may explain why high fecal weights occur in the tropics on comparable intakes of dietary fiber but on high starch intakes." As taken from Shetty PS, Kurpad AV. Am J Clin Nutr. 1986

Feb; 43(2):210-2. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=3004188&query_hl=130&itool=pubmed_docsum

"Corn starch particles are used as a surgical glove lubricant. At present there is no better alternative for this lubricant. Implantation of corn starch particles into the peritoneal cavity can induce foreign body reactions, starch peritonitis and starch granulomata, and may cause adhesions and intestinal obstruction. Starch peritonitis should be treated conservatively."

As taken from Michowitz M, Stavorovsky M, Ilie B. Postgrad Med J. 1983 Sep;59(695):593-5. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=6634562&query_hl=133&itool=pubmed_docsum

"The pH optimum of pancreatic alpha-amylase from grain-fed steers was determined to be 6.9, while that of intestinal maltase was established at 5.8. Both assays were found to be linear up to 1 hr of incubation. The V max of pancreatic amylase was determined to be pancreatic amylase was determined to be 1.15 mg of maltose monohydrate produced/hr. Activities of pancreatic and intestinal maltase were not reduced (P greater than .05) during the interval from sample collection from the animal until analysis 4 hr later when tissues were kept on ice. Twenty-four yearling Holstein steers fed either alfalfa hay at a maintenance level of metabolizable energy (ME) intake or corn at one, two or three times the maintenance ME intake level were slaughtered after being fed 106 days. The pancreas was removed alone with sections of the intestine. Specific activity of pancreatic amylase for steers fed the high level of corn was 129% of that for steers fed the alfalfa diet (P greater than .05). Intestinal maltase activity was highest in the jejunum and decreased toward the ileum. Increasing dietary starch intake resulted in no response (P greater than .05) in maltase activity at 10, 30, 50, 70, or 90% of the small intestine length. The effect of dietary starch level on dieesta pH was dependent on sampling location within the small intestine. There were no dietary effects (P greater than .05) on digesta pH for the first 10% segment of intestine distal to the pylorus. However, in all subsequent sections, digesta pH was higher steers fed the alfalfa diet than for those fed the two higher levels of grain. A calculation for estimating th amount of pancreatic amylase needed to hydrolyze starch presented to small intestine is discussed." As taken from Russell JR, Young AW, Jorgensen NA. J Anim Sci. 1981 May; 52(5):1177-82. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=6165710&query_hl=136&itool=pubmed_DocSum

"Different absorbtion level is inherent capacities for natural, resistant and hydrolized starches to regulate a volume of non-hydrolyzed starches in colon. This regulates an interaction with intestinal microflora to produce the short chain fatty acids and other bio-active compounds. The T- and B-lymphocyte receptors are targets for starches to disrupt the number and density of plasma membrane receptors CD3, CD4, and CD8. All starches regulate the expression of adhesion molecules LFA-1 and ICAM-1, as well as receptor Mac-1. Maize starch increases the level of spontaneous and ceramide-dependent apoptosis in thymic and spleen cells of experimental animals." As taken from Sotnikova EV et al. Vopr Pitan. 2002; 71(5):34-8. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=12599997&query_hl=138&itool=pubmed_docsum

"This study was designed to quantify starch digestion within the small and large bowels separately when raw potato starch (RPS) was included at 0-240 g/kg in diets fed to growing male Wistar rats. RPS was incorporated in the diets at the expense of maize starch which was expected to be almost completely digested in the small bowel. The digestibility of the maize starch was 0.99 but only 0.28 of the RPS was digested before the terminal ileum so that with increasing intakes of RPS there was a progressive increase in starch supply to the large bowel (LB). Of this starch 0.77, 0.72 and 0.73 was fermented in the large bowel when RPS constituted 80, 160 and 240 g/kg diet respectively.

With increasing RPS intake, there was a curvilinear response in molar proportion of butyrate in caecal contents with a maximum value at about 80 g RPS/kg diet. The molar proportion of acetate increased linearly, that of propionate was unchanged, whilst proportions of the minor short-chain fatty acids all declined markedly with increasing RPS intake. The novel marker Bacillus stearothermophilus spores (BSS) was compared with CrEDTA in estimation of whole-gut mean transit time (MTT) when given together in a single test meal. Whilst estimates of MTT for the two markers were strongly correlated within individual rats (r2 0.72), BSS produced estimates that were 13 h longer than those based on CrEDTA. Neither marker detected a change in MTT with increasing RPS intake but, with both, the rate constant (k1) for the 'largest mixing pool' declined significantly (P<0.001) as dietary RPS concentration was changed from 0-240 g/kg." As taken from Mathers JC et al. Br J Nutr. 1997 Dec; 78(6):1015-29. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=9497449&query_hl=141&itool=pubmed_docsum

"Male Wistar rats were meal-fed on diets containing various amounts of resistant starch in the form of raw starch (either amylomaize starch, potato starch or modified high-amylose starch) or retrograded starch (prepared from each of the starches) for 6 weeks. Two diets containing normal maize starch were fed as diets poor in resistant starch. Energy absorption (energy consumption minus faecal energy loss), growth, weight of the epididymal fat pads, serum total cholesterol and triacylglycerol concentrations and a number of intestinal and faecal variables were determined. The resistant starches affected all the variables determined except the serum total cholesterol concentration. Relationships were found between energy absorption and both growth and the weight of the fat pads, and between the weight of the fat pads and both the serum triacylglycerol concentration and the serum total cholesterol concentration. No clear differences between the effects of the two types of resistant starch (raw starch v. retrograded starch) were found except that raw potato starch hardly stimulated H2 excretion and led to lower amounts of propionic and butyric acids in the caecal contents than the other starches. The results suggest that dietary resistant starch reduces energy absorption leading to less abdominal depot fat and lower serum triacylglycerol concentrations." As taken from de Deckere EA et al. Br J Nutr. 1995 Feb; 73(2):287-98. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=7718547&query_hl=143&itool=pubmed_docsum

"Resistant starch is by definition that part of starch that escapes digestion in the small bowel. Cecal fermentation of resistant starch into short-chain fatty acids will result subsequently in a decrease in pH. Thus, resistant starch may have the same effect on colonic luminal contents and mucosa as some fiber components. We studied the effects of adding 45 g native amylomaize (Hylon-VII) to a standardized diet in 14 healthy volunteers on fermentation and colonic mucosal proliferation. Hylon-VII is a high amylose maize starch, containing 62% resistant starch. During amylomaize consumption, breath hydrogen excretion rose 85% and fecal short chain fatty acid output increased 35% (P<0.01). Excretion of primary bile acids increased and the soluble deoxycholic acid concentration decreased by 50% (P = 0.002). Subsequently, cytotoxicity of the aqueous phase of feces--as measured on a colon cancer cell line--decreased (P = 0.007). Colonic mucosal proliferation in rectal biopsies (proliferating cell nuclear antigen immunostaining) decreased from 6.7 to 5.4% (P = 0.05). We speculate that resistant starch consumption decreases colonic mucosal proliferation as a result of the decreased formation of cytotoxic secondary bile acids, which is possibly mediated through acidification of the large bowel by production of short-chain fatty acids." As taken from van Munster IP et al. Dig Dis Sci. 1994 Apr; 39(4):834-42. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=8149850&query_hl=146&itool=pubmed_docsum

"1. Male Sprague-Dawley rats were fed on either a purified, fibre-free diet or a diet in which half the maize starch was replaced with uncooked amylomaize or potato starch (equivalent to 100 or 200 g amylase-resistant starch (ARS)/kg diet respectively). Changes in short-chain fatty acids (SCFA),

pH, ammonia and a number of bacterial variables in caecal contents were then assessed. 2. Both ARS supplements decreased caecal content pH by approximately 1-2 units, with an associated reduction in ammonia concentration. Potato starch significantly decreased the concentration of SCFA in the hindgut, while amylomaize supplementation increased propionic and butyric acids but decreased the occurrence of minor, branched-chain fatty acids. 3. Caecal bacterial biotransformation activities (beta-glucosidase (EC 3.2.1.21), beta-glucuronidase (EC 3.2.1.31), reduction of p-nitrobenzoic acid, apparent ammonia formation) were consistently decreased by both ARS sources. 4. The results demonstrate that amylase-resistant carbohydrate altered toxicologically important functions in the large-intestinal flora of the rat." As taken from Mallett AK et al. Br J Nutr. 1988 Nov; 60(3):597-604. PubMed, 2009 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=3219326&query_hl=148&itool=pubmed_docsum

"PURPOSE OF REVIEW: Acute kidney injury (AKI) is a serious problem. Understanding an individual patient's risk profile may offer opportunities for prevention or early intervention. The aim of this review is to describe novel nontraditional risk factors. RECENT FINDINGS: The risk of AKI is determined by patient factors and nephrotoxic exposures. Hyperuricaemia, obesity, hypoalbuminaemia and certain genetic polymorphism have been found to be associated with an increased susceptibility to AKI, especially in surgical patients. However, there is no convincing evidence that albumin replacement or uric acid lowering ameliorates the risk. Genetic predisposition contributes to AKI in general and also drug-nephrotoxicity. The exact relationship between obesity and AKI has not been fully understood.Patients exposed to starches, chloride-rich fluids or mechanical ventilation have an increased risk of AKI. Starches in particular should be avoided in high-risk patients. Although chloride-rich fluids are associated with AKI based on observational studies, direct proof of harm is lacking. SUMMARY: Novel risk factors for AKI have been identified but more work is necessary to investigate the nature of the association. There is no evidence that correction of hyperuricaemia or hypoalbuminaemia is beneficial but high-risk exposures should be avoided in patients at risk of AKI." As taken from Varrier M & Ostermann M. 2014. Curr. Opin. Nephrol. Hypertens. 23(6), 560-9. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25162200?dopt=AbstractPlus

"Two independent clinical studies were conducted to compare the gastrointestinal (GI) tolerability of corn starch fiber, a novel dietary fiber, at up to 50 g/day (single-dose study) or 90 g/day (multiple-serving study) with a negative control (no fiber) and a positive control (50 or 90 g polydextrose, for single- and multiple-serving studies, respectively) in generally healthy study volunteers. Flatulence and borborygmus were the primary symptoms reported at the higher doses of corn starch fiber and for the positive control interventions. Bowel movements were increased over 48 h with corn starch fiber at 90 g. Thresholds for mild GI effects were established at 30 g as a single dose and 60 g as multiple servings spread over the day. Other than moderate abdominal pain and mild increased appetite in one subject at 90-g corn starchfiber, no test article-related adverse events were reported." As taken from Crincoli CM et al. 2016b. Int. J. Food Sci. Nutr. 67(7), 844-56. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27346078

"BACKGROUND/OBJECTIVES: Previous small studies have shown either no difference or a lower risk of symptomatic gallstone disease in vegetarians than in non-vegetarians. This study examined the incidence of symptomatic gallstone disease in a cohort of British vegetarians and non-vegetarians, and investigated the associations between nutrient intake and risk of symptomatic gallstone disease. SUBJECTS/METHODS: The data were analysed from 49 652 adults enroled in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Oxford study, one-third of whom were vegetarian. The linked databases of hospital records were used to identify incident cases. Risk by diet group was estimated using Cox proportional hazards models. Further analysis quantified risk by intakes of selected macronutrients. RESULTS: There were 1182 cases of symptomatic gallstone disease during 687 822 person-years of follow-up (mean=13.85 years). There was a large significant association between increasing body mass index (BMI) and risk of developing symptomatic gallstone disease (overall trend P<0.001). After adjustment for BMI and

other risk factors, vegetarians had a moderately increased risk compared with non-vegetarians (HR: 1.22; 95% CI: 1.06-1.41; P=0.006). Although starch consumption was positively associated with gallstones risk (P=0.002 for trend), it did not explain the increased risk in vegetarians. CONCLUSIONS: There is a highly significant association of increased BMI with risk of symptomatic gallstone disease. After adjusting for BMI, there is a small but statistically significant positive association between vegetarian diet and symptomatic gallstone disease." As taken from McConnell TJ et al. 2017. Eur. J. Clin. Nutr. 71(6), 731-735. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28272400

"Acute kidney injury (AKI) and sepsis carry consensus definitions. The simultaneous presence of both identifies septic AKI. Septic AKI is the most common AKI syndrome in ICU and accounts for approximately half of all such AKI. Its pathophysiology remains poorly understood, but animal models and lack of histological changes suggest that, at least initially, septic AKI may be a functional phenomenon with combined microvascular shunting and tubular cell stress. The diagnosis remains based on clinical assessment and measurement of urinary output and serum creatinine. However, multiple biomarkers and especially cell cycle arrest biomarkers are gaining acceptance. Prevention of septic AKI remains based on the treatment of sepsis and on early resuscitation. Such resuscitation relies on the judicious use of both fluids and vasoactive drugs. In particular, there is strong evidence that starch-containing fluids are nephrotoxic and decrease renal function and suggestive evidence that chloride-rich fluid may also adversely affect renal function. Vasoactive drugs have variable effects on renal function in septic AKI. At this time, norepinephrine is the dominant agent, but vasopressin may also have a role. Despite supportive therapies, renal function may be temporarily or completely lost. In such patients, renal replacement therapy (RRT) becomes necessary. The optimal intensity of this therapy has been established, while the timing of when to commence RRT is now a focus of investigation. If sepsis resolves, the majority of patients recover renal function. Yet, even a single episode of septic AKI is associated with increased subsequent risk of chronic kidney disease." As taken from Bellomo R et al. 2017. Intensive Care Med. 43(6), 816-828. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28364303

"Purpose: Given that 27-hydroxycholesterol (27HC) is the first identified endogenous selective estrogen receptor modulator, the aim of this study was to investigate the extent to which dietary or lifestyle factors impact circulating 27HC concentrations in a large-scale setting. Methods: This cross-sectional analysis included 1,036 women aged 35-65 years who served as controls in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg breast cancer case-control study. Circulating 27HC was quantified in serum using liquid chromatography/tandem mass spectrometry. Generalized linear models were used to investigate the association between 27HC concentrations and dietary habits, and lifestyle, reproductive, and anthropometric factors. Results: Higher concentrations of 27HC were observed among postmenopausal relative to premenopausal women (geometric mean 200.5 vs. 188.4 nM, p = 0.03), whereas women reporting ever full-term pregnancy had lower concentrations of 27HC relative to never (191.4 vs. 198.6; p = 0.03). Significant trends were observed showing higher concentrations with relatively high levels of physical activity (ptrend = 0.03) and alcohol consumption (ptrend = 0.01), and women currently smoking at blood collection (ptrend < 0.01). Of the investigated dietary factors, starch (ptrend < 0.01) and thiamine (ptrend < 0.01) intakes were inversely associated with 27HC. Circulating lipid concentrations were positively associated with 27HC concentrations (all ptrend < 0.01). No significant associations were found between 27HC and factors including age at blood collection, body mass index, or use of hormone therapy or cholesterol-lowering medications. Conclusion: 27HC is of increasing interest for multiple chronic disease pathways. Despite significant associations found between circulating 27HC and dietary habits, reproductive factors, and modifiable lifestyle factors, circulating cholesterol, mostly low-density lipoprotein cholesterol, accounted for the majority of the variability in circulating 27HC." As taken from Le Cornet C et al. 2020. Cancer Causes Control 31(2), 181–192. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31938951/

7. Addiction

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

8. Burnt ingredient toxicity

Tobacco smoke condensates from cigarettes containing starch 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 starch. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	19,000	Baker et al., 2004a
	-	Coggins et al, 2013
In vitro genotoxicity	19,000	Baker et al., 2004c
	-	Coggins et al, 2013
In vitro cytotoxicity	19,000	Baker et al., 2004c
	-	Coggins et al, 2013
Inhalation study	97	Gaworski et al., 1998
	19,000	Baker et al., 2004c
	-	Coggins et al, 2013
Skin painting	97	Gaworski et al., 1999

“There was no alkylating activity alteration by starch to other mainstream components (Crosthwaite et al 1979)”.

9. Heated/vapor emissions toxicity

Aerosol from heated tobacco stick(s) containing Starch 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	1.48	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	1.48	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	1.48	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that starch (CAS RN 9005-25-8) is of uncertain persistence in the environment.

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

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that starch (CAS RN 9005-25-8) is not inherently toxic to aquatic organisms and is of low ecotoxicological concern, with an experimental value for inherent toxicity of 1000 mg/L.

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

US EPA ECOTOX Database provides the following data:

Bairdiella chrysoura (Silver Perch), 4-day LD0: 5000 mg/L

Lagodon rhomboides (Pinfish), 4-day LD0: 5000 mg/L

Orthopristis chrysoptera (Pigfish), 4-day LD0: 5000 mg/L

Crassostrea virginica (American Or Virginia Oyster), 4-day LD100: 3000 mg/L

Crassostrea virginica (American Or Virginia Oyster), 4-day LD0: 1000 mg/L

“Flocculant modified soils/clays are being increasingly studied as geo-engineering materials for lake restoration and harmful algal bloom control. However, the potential impacts of adding these materials in aquatic ecological systems remain unclear. This study investigated the potential effects of chitosan, cationic starch, chitosan modified soils (MS-C) and cationic starch modified soils (MS-S) on the aquatic organisms by using a bioassay battery. The toxicity potential of these four flocculants was quantitatively assessed using an integrated biotic toxicity index (BTI). The test system includes four aquatic species, namely Chlorella vulgaris, Daphnia magna, Cyprinus carpio and Limnodrilus hoffmeisteri, which represent four trophic levels in the freshwater ecosystem. Results showed that median effect concentrations (EC50) of the MS-C and MS-S were 31-124 times higher than chitosan and cationic starch, respectively. D. magna was the most sensitive species to the four flocculants. Histological examination of C. carpio showed that significant pathological changes were found in gills. Different from chitosan and cationic starch, MS-C and MS-S significantly alleviated the acute toxicities of chitosan and cationic starch. The toxicity order of the four flocculants based on BTI were cationic starch > chitosan > MS-S > MS-C. The results suggested that BTI can be used as a quantitative and comparable indicator to assess biotic toxicity for aquatic geo-engineering materials. Chitosan or cationic starchmodified soil/clay materials can be used at their optimal dosage without causing substantial adverse effects to the bioassay battery in aquatic ecosystem.” As taken from Wang Z et al. 2016. Water Res. 97, 133-141. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26321048

“Starch from Dioscorea pyrifolia tubers was characterized for its proximate composition, physicochemical properties and toxicity. This starch contains 44.47±1.86% amylose, 4.84±0.29% moisture, 0.88±0.21% ash, 1.34±0.11% proteins and 92.73±0.48% carbohydrates. X-ray diffraction (XRD) analysis showed a type-C starch with a relative crystallinity of 23.31±2.41%. The starch granules are polyhedral, with a diameter of 2.8 to 5.6μm and average size of 3.93±1.47μm. Initial, peak and finishing gelatinization temperatures for the starch were 71.51±0.07, 75.05±0.15, and 78.25±0.18°C, respectively; the gelatinization enthalpy was 3.86±0.02J/g, and the peak height index was 1.09±0.05. Thermogravimetric analysis showed a weight loss of 85.81±0.52% and a decomposition temperature of 320.16±0.35°C, which indicated that there was good thermal stability of the starch. Fish embryo toxicity (FET) showed that the starch was not toxic and that it was suitable for food and non-food industries.” As taken from Sharlina E et al. 2017. Food Chem. 220, 225-232. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27855893

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

US EPA ECOTOX Database provides the following data:

Spec. Sci. Name **Spec. Common Name**	**Resp. Site** **Exp. Dur. (Days)**	**Media Type** **Test Loc.**	**Exp. Type** **Chem. Anal.**	**Dose#** **Res. Sample Unit**	**Endpoint** **BAF/BCF**	**Effect** **Effect Meas.**	**Signif.** **Sig. Level**	**Dose** **Dose Stat. Meth.**
Phaseolus vulgaris Bean	AB 56	NAT LAB	DA U	3		GRO BMAS	SIG 0.05-0.01	NC (0-40.9) g/1.8 kg soil
Zea mays Corn	AB 56	NAT LAB	DA U	3		GRO BMAS	SIG 0.0 5-0.01	NC (0-40.9) g/1.8 kg soil
Phaseolus vulgaris Bean	AB 56	NAT LAB	DA/ U	3		GRO BMAS	MULT 0.05-0.01	NC (0-40.9) g/1.4 kg soil
Zea mays Corn	AB 56	NAT LAB	DA/ U	3		GRO BMAS	SIG 0.05-0.01	NC (0-40.9) g/1.4 kg soil

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that starch (CAS RN 9005-25-8) is of uncertain bioaccumulative potential in the environment.

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

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13. Last audited

July 2023

PRIVILEGED AND CONFIDENTIAL

LITERATURE SEARCH AND REVIEW TOBACCO INGREDIENTS USED BY MANUFACTURERS IN THE PRODUCTION OF CIGARETTES

FINAL REPORT: 2005
For 2004 list of ingredients

Donald E. Gardner PhD, F.ATS
Susan C. Gardner PhD
Inhalation Toxicology Associates
Savannah, GA

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INTRODUCTION

In a continuing effort to improve the safety evaluation of ingredients added to tobacco, this literature review program identifies and reviews relevant abstracts and documents for information regarding potential health effects of a large number of ingredients.

This review is intended to provide an appropriate means for the continuing safety assessment of the ingredients added to tobacco. This is not intended to be a summary of all available data on a particular ingredient; rather, the aim and scope of this review is on providing the sponsors with an overview of available data regarding issues that can play a role in establishing their safe use. Results from this review can aid in (1) prioritizing for additional toxicology testing and for mechanistic studies, (2) facilitating the evaluation of any proposed modifications to cigarettes, and (3) allowing data exchange between the sponsors and the panel members.

A list of 217 ingredients was provided by Covington and Burling as representing the high-priority chemicals. These ingredients are divided into four categories:

1). New ingredients (8). For these ingredients Inhalation Toxicology Associates (ITA) searched the databases for all citations entered into the database since 1965.

2). Major ingredients (45). These are ingredients having a maximum use level (MUL) of 500 ppm or greater. For this category ITA searched the databases for relevant citations between the dates of the last search to 2004.

3). High MUL ingredients (36). This category includes ingredients whose MUL has increased by a factor of 10 or more from the prior year. For these high MUL ingredients ITA searched the databases for relevant citations between the dates of the last search to 2004.

4). Standard ingredients (128) as identified by Covington and Burling. For this category ITA searched the databases for relevant citations between the dates of the last search to 2004.

The first stage involves the collection of relevant data, including the results of *in vivo* and *in vitro* studies. The second stage involves the assessment of these data to determine the acceptability of the study and relevance of the results to the substance as a tobacco ingredient. To meet these objectives, ITA searches the databases using chemical abstract numbers for relevant citations during the dates corresponding to the category in which they are listed. ITA primarily used the American Chemical Society's Chemical Abstract Services and Dialog Database to search for information about ingredients of interest.

A series of databases were used to search for relevant national and international studies. If in the judgment of ITA, the search for a particular ingredient in any of these databases was not expected to produce relevant information, ITA was authorized to omit

the search of such database(s). ITA was also authorized to modify the literature search strategies in order to better meet the needs of the sponsors.

After the sponsors/panel members have had an opportunity to examine this 2004 report and they believe the goals and objectives of this project would benefit by including some "other" sources, ITA would be most willing to expand our coverage to seek out additional publications/reports for any specific ingredient they determine needs more coverage. If it is decided that "other" sources should be added to our list of databases in future years, we would be most pleased to add these sources to our list of databases searched. During 2004, a total of 9427 titles were retrieved of which 461 were identified as potentially relevant and their abstracts were collected and reviewed by ITA. Using the data from these abstracts, a total of 134 full text copies of relevant documents were retrieved by ITA for a more in-depth review.

As in previous years, it is appropriate to establish some generally accepted and recognized criteria that can be used in assessing the toxicological risk of ingredients in a relatively efficient manner. These guidelines are intended to expedite the safety assessment of ingredients added to tobacco. While the material examined was extensive, most of the toxicological testing of ingredients was not designed to evaluate the health hazards of ingredients in cigarette smoke, but instead focused on the hazards associated with exposure to either the pure substances or as additives in some other medium, such as food. This adds to the complexity of trying to interpret and extrapolate this data for assessing and predicting human health risk associated with exposure to those ingredients found in cigarettes. Although many of these studies were not designed to evaluate tobacco additives, the results have to be considered since they aid in providing a complete picture of the database for these chemicals.

From the large number of studies encountered, it was practical to summarize only the most specific and relevant observations. However, situations that have become controversial are dealt with in more detail. While it was appropriate that ITA considered all data and make decisions about the validity and usefulness of these data, certain research areas received lower priority and may have been excluded from further examination. Examples would be studies involving 1) the use of such ingredients in the treatment of a variety of diseases, 2) new methodologies for measurement, 3) studies addressing potential anti-microbial or pesticidal activity, 4) effects reported on plants and lower animal systems and 5) publications not in English. Even with these exclusions, ITA has provided the sponsors with a vast amount of information. Good decisions are most likely to result from integration of all available data, including those demonstrating adverse effects as well as well-designed studies indicting no effects. This was done to provide the sponsors with a broad base of published literature, and they can select from these studies the most relevant information useful in meeting their unique needs. For each ingredient where there was relevant scientific data addressing the safe use of ingredients in cigarette products, these studies are discussed below. All of the titles and abstracts retrieved have been retained, and hard copies of the most relevant papers are available upon request.

In our professional judgment, based on the literature reviewed during this time period, no information has been generated which indicates that the use of the ingredients evaluated in this review presents a hazard to the health of the consumer at the level being used, so far as can be judged by the scientific evidence available.

Thank you for providing ITA the opportunity to review this subject matter and to express an opinion regarding the health effects of these ingredients. We are available to provide further clarification or discussion if you have questions.

Donald E. Gardner PhD., Fellow ATS,
National Associate of the National Academy of Science
Susan C. Gardner PhD.
DATE: February 15, 2006
Inhalation Toxicology Associates, Inc.

INGREDIENTS REVIEW

CATEGORY: NEW INGREDIENTS

PARA-TOLUALDEHYDE
CAS: 104-87-0

Number of relevant papers: 7

GENERAL COMMENTS ON PAPERS LISTED BELOW:
The first five papers listed below provide a broad view of biological activity for a large number (239 to 464) of individual tobacco smoke constituents using an array of short-term assays. The general conclusion reached was that tobacco smoke contains a number of substances that inhibit cell growth using Ascites sarcoma cells, inhibits noradrenaline, stimulated oxidative metabolism in isolated brown fat cells, damages plasma membrane of cultured human lung fibroblasts and may be mutagenic in the Ames test. Although not directly applicable to the human exposure situation, these assays provide information on possible mechanisms involved in the interaction of specific smoke constituents and cell function.

1. Effects of tobacco smoke compounds on the ciliary activity of the embryo chicken trachea in vitro.
Pettersson B, Curvall M, Enzell CR.
Toxicology. 1982;23(1):41-55.

ABSTRACT: The ciliotoxicity of 316 individual compounds representative of the gaseous and semivolatile phases of tobacco smoke has been investigated using chicken tracheal organ cultures. When examined at 5 mM concentration and measuring the time to complete ciliostasis, 36% of the compounds were found to cause ciliostasis within 15 min, while about 50% had no visible effect on the ciliary activity during a 60-min exposure. The majority of the ciliotoxic compounds were either alkylated phenylethers, benzonitriles, benzaldehydes, phenols, benzenes, naphthalenes and indoles, or alpha, beta-unsaturated ketones and aldehydes or C6-C10 aliphatic alcohols, aldehydes, acids and nitriles. Most of the compounds classified as benzoic acids, esters, polyaromatic hydrocarbons, amines and N-heterocycles, except indoles, were found to be inactive.

COMMENTS: Comments are provided above.

2. Effects of tobacco smoke compounds on the noradrenaline induced oxidative metabolism in isolated brown fat cells.

Pettersson B, Curvall M, Enzell CR.
Toxicology. 1980;18(1):1-15.

ABSTRACT: The effect on cell metabolism of 320 individual smoke components have been investigated by measuring their inhibition of noradrenaline induced respiration in isolated hamster brown fat cells. The compounds are representative of the gaseous and semivolatile phases of tobacco smoke. The strongest inhibitors were found within the groups of aliphatic alcohols, aldehydes and acids, of alkylated phenols and indoles and of alpha, beta-unsaturated aliphatic aldehydes and ketones. Some of the aliphatic aldehydes and acids significantly increased the basal respiration of the cells, probably by acting as substrates and/or uncoupling of mitochondrial respiratory control.

COMMENTS: Comments are provided above.

3. Effect of tobacco smoke compounds on the plasma membrane of cultured human lung fibroblasts

Thelestam M, Curvall M, Enzell CR.
Toxicology. 1980;15(3):203-17.

ABSTRACT: The ability of compounds derived from tobacco and tobacco smoke to increase the permeability of the membranes of human lung fibroblasts has been studied by measuring the release of an intracellular marker after short term exposure. Of the 464 compounds tested, about 25% gave rise to severe membrane damage. The most active compounds, when divided according to functionality, were found within the groups of amines, strong acids and alkylated phenols, whereas nitriles and polycyclic aromatic hydrocarbons were found completely inactive. A pronounced effect of the chain length on the activity was observed for the aliphatic alcohols, aldehydes and acids, and all monocyclic aromatic compounds but benzonitriles and benzoic acids showed an increase in activity with increasing alkylsubstitution. It is concluded that tobacco smoke contains a number of membrane damaging substances. These membrane active compounds could not only cause direct toxic reactions but also potentiate the toxic effect by promoting the cell membrane penetration of other toxic substances in tobacco smoke.

COMMENTS: Comments are provided above.

4. Screening of tobacco smoke constituents for mutagenicity using the Ames' test

Florin I, Rutberg L, Curvall M, Enzell CR.
Toxicology. 1980;15(3):219-232.

ABSTRACT: To clarify the mutagenic activity of individual smoke components, 239 compounds, representative of the gaseous and semivolatile phases of tobacco smoke, were assayed for mutagenicity towards 4 histidine-requiring mutants of Salmonella typhimurium (TA 98, TA 100, TA 1535 and TA 1537). All compounds were tested qualitatively both with and without metabolic activation using a liver fraction (S-9) from Aroclor 1254 or methylcholanthrene induced rats. Without S-9, only 2,3-dimethylindole and 2,3,5-trimethylindole showed mutagenic activity that was not enhanced by the

metabolic activation system. 2,6-Diaminotoluene and coronene, which like the above compounds are not documented carcinogens were found to be mutagenic for strain TA 98 with S-9. Mutagenic activity was also observed for the previously known mutagens benz[a]pyrene, chrysene, benz[a]-anthracene, perylene and beta-naphthylamine, on exposure to strains TA 98 and/or TA 100 with S-9.

COMMENTS: Comments are provided above.

5. Effects of tobacco and tobacco smoke constituents on cell multiplication in vitro - CA
Pilotti A, Ancker K, Arrhenius E, Enzell C.
Toxicology. 1975 Sep;5(1):49-62.

ABSTRACT: Ascites sarcoma BP8 cells, cultured in suspension in vitro were used as a general toxicity test system for tobacco and tobacco smoke constituents. Some 250 compounds, representative of these materials, were examined by exposing cells to different concentrations of these constituents and measuring the inhibition of culture growth, which was related to corresponding effects encountered for positive standards. When employing the present cell toxicity test system possible effects of factors such as penetration, distribution and microsomal metabolism of the compounds studied, are not taken into account. The most active constituents were found to be unsaturated aldehydes and ketones, phenols and indoles. The good correlation observed between functional groups and toxicity permits, within the range of functionalities studied, prediction of the toxicity for a compound of known structure.

COMMENTS: Comments are provided above.

6. AMES SALMONELLA/MAMMALIAN MICROSOME MUTAGENICITY TEST AND REVERSE MUTATION ASSAY - E. COLI WP2 UVRA A (STANDARD PLATE TEST AND PREINCUBATION TEST) (OCT. 19, 1988)

Source: EPA/OTS; Doc #86-920000590

ABSTRACT: P-Tolualdehyde (CAS # 104-87-0) was evaluated for mutagenicity in the Ames test (strains TA1535, TA100, TA1537, TA98) with and without metabolic activation (S-9 mix) and in the Escherichia coli (WP2 uvrA) reverse mutation assay at a dose range of 20 ug - 5000 ug/plate in the standard plate test (SPT) and 4 ug - 2500 ug/plate in the preincubation test (PIT). No bacteriotoxic effect was observed with E. coli. Bacteriotoxicity was detected in all Salmonella strains detected at 2500 ug/plate (PIT) and at 5000 ug/plate (SPT). The test substance was determined to be non-mutagenetic.

COMMENTS: P-Tolualdehyde was determined to be non-mutagenic in the Ames test.

7. Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA 104
Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN.
Mutat Res. 1985 Jan-Feb;148(1-2):25-34.

ABSTRACT: Strains of Salmonella typhimurium that carry a nonsense mutation at the site of reversion detect a variety of naturally occurring and synthetic carbonyl compounds as direct-acting mutagens. TA104 is reverted efficiently by formaldehyde, alpha, beta-unsaturated aldehydes (enals), and dicarbonyl compounds, such as diacetyl and glutaraldehyde. This strain is much more sensitive to carbonyl mutagenesis than is TA100, a strain previously reported to detect aldehydes as mutagens, or any other characterized strains of Salmonella. Long-chain enals are very toxic to TA104, but addition of a reduced glutathione chase following an incubation period decreases this toxicity, thus enabling the detection of 4-hydroxy-pentenal, a homolog of the lipid peroxidation product, 4-hydroxy-nonenal, as a mutagen. This is the first report of the mutagenicity of a hydroxy-enal, a class of enals produced by lipid peroxidation. Testing conducted with strains that carry the nonsense mutation in different repair backgrounds indicates that the presence of pKM101 and the deletion of the uvrB gene facilitate the detection of enals and dicarbonyls, but not malondialdehyde, as mutagens. Since carbonyl compounds are widely distributed in foods, are generated during cellular metabolism, and are present in body fluids, they may make a significant contribution to the risk of human cancer.

COMMENTS: Additional comments not necessary, abstract satisfactory.

CITRONELLOL
CAS: 106-22-9

Number of relevant papers: 3

1. Effects of fragrance inhalation on sympathetic activity in normal adults

Haze S, Sakai K, Gozu Y.
Jpn J Pharmacol. 2002 Nov;90(3):247-53.

ABSTRACT: We investigated the effects of fragrance inhalation on sympathetic activity in normal adult subjects using both power spectral analysis of blood pressure fluctuations and measurement of plasma catecholamine levels. Fragrance inhalation of essential oils, such 19 Effects of fragrance inhalation on sympathetic activity in normal adults

COMMENTS: This study demonstrated that inhalation of fragrances can stimulate or depress sympathetic activity in human volunteers. While citronellol was not tested, it was identified as being present (27.7%) in rose oil that was tested. Inhaled rose oil significantly inhibited sympathetic activity and decreased adrenaline levels. The authors

suggest that citronellol might be involved in the modulation of sympathetic activity in normal adults.

2. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.
Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-be nzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m3 for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: Rats and hamsters were exposed by inhalation to a complex mixture of fragrances. The exposure levels were 10 to 100 fold greater than one would expect to be encountered by humans using such fragrances. None of the fragrances produced signs of toxicity following exposures up to 13 weeks. No histopathological abnormalities were reported in trachea or lungs. The results are consistent with those earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

3. Fragrance compounds and essential oils with sedative effects upon inhalation

Buchbauer G, Jirovetz L, Jager W, Plank C, Dietrich H.
J Pharm Sci. 1993 Jun;82(6):660-4.

ABSTRACT: Fragrance compounds and essential oils with sedative effects influence the motility of mice in inhalation studies under standardized conditions. A significant drop in the motility of mice was registered following exposure to these fragrances. The same results were achieved when the mice were artificially induced into overagitation by intraperitoneal application of caffeine and subsequently subjected to inhalation of fragrance compounds and essential oils. These results proved the sedative effects of these fragrants via inhalative exposure in low concentrations. Blood samples were taken from the mice after a 1-h inhalation period. Chromatographic and spectroscopic methods were used to detect and characterize the actual effective compounds after solid-phase extraction. Serum concentrations of 42 different substances, including fragrance compounds, were found in low ranges (ng/mL serum). The results contribute to the correct interpretation of the term aromatherapy (i.e., a stimulating or sedative effect on the behaviour of individuals only upon inhalation of fragrance compounds).

COMMENTS: A one-hour inhalation of citronellol showed a significant sedative effect in over-agitated (caffeine-treated) mice but not with animals without prior caffeine induction. These sedative effects were observed at low blood concentrations (2.0 ng/mL). Substances that produce such an effective may interact with lipids of cell membranes in the cortex thus indicating a direct pharmacological interaction of fragrance molecules with bodily tissue.

ETHYL HEPTANOATE
CAS: 106-30-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOAMYL FORMATE
CAS: 110-45-2

Number of relevant papers: 2

1. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs -

Yoo, Y.S. (1986)
Osaka-shi Igakkai Zasshi [J. Osaka City Medical Center], 34(3-4), 267-288

ABSTRACT: N/A

COMMENTS: This article was in Japanese and was not translated. Briefly, these investigators tested for genotoxicity in 33 synthetic flavorings used in foodstuffs. Isoamyl formate had little or no toxic effect and was considered to be negative in the assay system.

2. Primary mutagenicity screening of food additives currently used in Japan –

Ishidate, M; Sofuni, T; Yoshikawa, K;
Food Chem Toxicol 22:623-636.

ABSTRACT: Salmonella/microsome tests (Ames tests) and chromosomal aberration tests in vitro using a Chinese hamster fibroblast cell line were carried out on 190 synthetic food additives and 52 food additives derived from natural sources, all of which are currently used in Japan. Fourteen out of 200 tested in the Ames assay showed positive effects and 54 out of 242 were positive in the chromosome test. Three additives (erythorbic acid, chlorine dioxide and beet red) were positive only in the Ames test, although their mutagenic potentials were relatively weak, while 43 additives were positive only in the chromosome test. Eleven additives (calcium hypochlorite, cinnamic aldehyde, L-cysteine monohydrochloride, Food Green No. 3 (Fast Green FCF), hydrogen peroxide, potassium bromate, sodium chlorite, sodium hypochlorite, sodium nitrite, cacao pigment and caramel) were positive in both the Ames test and the chromosome test. The usefulness of such primary screening tests combining two different genetic end-points, gene mutation and chromosomal aberration, and some correlation between mutagenicity and carcinogenicity of food additives are discussed.

COMMENTS: These investigators did primary screening of over 200 food additives using both the Ames test and chromosomal aberration tests. Only a few (11) were positive in both tests. More additives were positive in the chromosome test than the Ames test indicating that chromosomal aberrations can be induced by a wider range of additives than the Ames test, and suggesting that this may indicate not only initiators of carcinogenesis but also promoters. It should be recognized that data from such short term *in vitro* tests needs further *in vivo* testing to predict carcinogenicity. The correlation between carcinogenicity and mutagenicity of additives are discussed.

HEXYL ACETATE
CAS: 142-92-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PECTIN
CAS: 9000-69-5

Number of relevant papers: 3

1. Pectin and cashew nut allergy: Cross-reacting allergens?

Rasanen L, Makinen-Kiljunen S, Harvima RJ.
Allergy. 1998 Jun;53(6):626-8.

ABSTRACT: N/A

COMMENTS: This case report indicates that exposure to pectin may cause sneezing, rhinitis, conjunctivitis and contact urticaria. Occupational sensitization with rhinitis and asthma from pectin has been previously identified. In this study, blood basophil histamine release and serum IgE were positive.

2. Occupational asthma caused by pectin inhalation during the manufacture of jam

AJ Cohen, MS Forse and SM Tarlo
Chest, Vol 103, 309-311, Copyright © 1993

ABSTRACT: We report a case of pectin-induced occupational asthma in a 35-year-old man. His job involved mixing powdered pectin into a fruit puree during the manufacture of jam. Within minutes of adding pectin, he developed coryza, rhinorrhea, coughing, and wheezing. His symptoms cleared during weekends while away from work and improved with the use of a protective facemask at work. Peak flow rates were significantly lower while at work compared with those at home, and a prick skin test with the pectin powder was positive. We conclude that pectin should be added to the list of the substances known to induce occupational asthma.

COMMENTS: This is another case report describing a pectin-induced occupational asthma. The individual exhibited positive skin testing to pectin. The authors suggested that pectin should be considered to be an allergen that causes occupational asthma.

3. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results.

Prival MJ, Simmon VF, Mortelmans KE.
Mutat Res. 1991 Aug; 260(4):321-9.

ABSTRACT: 49 substances permitted for use in food in the United States was tested for mutagenicity in the Ames Salmonella typhimurium assay and in Escherichia coli strain WP2. Four of these substances caused increases in revertant counts in S. typhimurium. Two of these four (papain and pepsin) were found to contain histidine, and therefore the results of the tests on these two substances could not be taken as demonstrating mutagenicity. The other two substances causing increases in revertant counts (hydrogen peroxide and potassium nitrite) were mutagenic. The results on one chemical, beta-carotene, were evaluated as inconclusive or questionable. The remaining 44 substances were nonmutagenic in the test systems used. It is concluded that, for those generally physiologically innocuous chemicals tested, there are very few 'false positives' in the bacterial test systems used.

COMMENTS: The Salmonella Ames test and E coli mutagenicity assays were used to evaluate the mutagenicity of a number of food ingredients. Pectin gave no evidence of

mutagenicity. In these studies, the frequency of positive results was much lower than in many other previous studies. The authors believe that this is due to the fact that the chemicals tested were almost all nontoxic to mammals and to bacteria even at relatively high doses.

CORN STARCH
9005-25-8

Number of relevant papers: 2

1. Inhaled cornstarch glove powder increases latex-induced airway hyper-sensitivity in guinea-pigs

Barbara J.; Santais M.-C.1; Levy D.A.2; Ruff F.1; Leynadier F.
Clinical & Experimental Allergy 34 (6): 978-983

ABSTRACT: Summary Background. Breathing is one of the most important modes of sensitization to natural rubber latex (NRL) for health-care workers, a group most at risk. Cornstarch powder (CSp) from medical powdered NRL gloves is known to be an allergen carrier, and sensitization to NRL can occur by inhaling airborne particles from such gloves. Objective: The aim of this study was to demonstrate, using an experimental model, which CSp may act as an adjuvant in NRL-induced airway hyper-responsiveness. Methods: Guinea-pigs were exposed to aerosolized NRL-contaminated CSp or to NRL in saline solution for 1 h every day for 2 weeks. The control groups were exposed either to CSp or to saline alone. An additional group of guinea-pigs was exposed to aerosolized ovalbumin (OVA) in saline. Three weeks after the last exposure, specific bronchial challenges were performed. In addition, Specific IgG and IgG1 in sera and thromboxane (Tx) B2 levels in bronchoalveolar lavage fluid (BALF) were measured. Results: The NRL challenge caused significant bronchospasm in the animals that had been exposed to NRL compared with those in the control groups ($P<0.02$). Guinea-pigs exposed to OVA also demonstrated a significant bronchospasm after OVA challenge ($P<0.001$). The guinea-pigs that had inhaled NRL-contaminated CSp had a significantly higher bronchoconstriction level than those that had inhaled NRL alone ($P<0.02$). Specific IgG and IgG1 were undetectable in sera from all groups, whereas significant amounts of TxB2 ($P<0.001$) were found in the lungs of the guinea-pigs exposed to NRL or OVA. Conclusion: Inhaling CSp increases the airway response to NRL. The fact that specific IgG and IgG1 were not detected might be the result of an immune response limited to the airways. This finding is supported by a significant increase of TxB2 level in the BALF of sensitized guinea-pigs.

COMMENTS: These authors previously reported that corn starch acts as an immunoadjuvant in guinea pigs that were previously sensitized to rubber latex by the IP route. This experimental model was used to determine if corn starch potentiates immunotoxicity of rubber latex through inhaling latex adsorbed onto corn starch. While the direct relevance of this study was to the health care workers who become sensitive to

rubber latex, it also indicates that breathing corn starch may induce hypersensitivity and may act as an adjuvant, resulting in increased airway responsiveness.

2. Bronchial provocation testing in the diagnosis of occupational asthma due to latex surgical gloves –

G Pisati, A Baruffini, F Bernabeo, and R Stanizzi
Eur Respir J 1994; 7: 332-336

ABSTRACT: In sensitized subjects, provocation tests to latex may induce severe systemic reactions and even anaphylactic shock. It is probable that part of the risk is due to the difficulty in grading the stimulating dose and in starting from very low levels of exposure. To identify the aetiological agent of work-related asthma in four nurses with previous allergic contact urticaria to latex surgical gloves dusted with cornstarch powder, we performed a specific bronchial provocation test study, based on exposure on three different days to nonpowdered latex surgical glove extract, powdered latex surgical glove extract and cornstarch powder extract, respectively. Extracts were nebulized in increasing concentrations in a 7 m3 challenge room, in the absence of the patients. The initial extract concentration was a tenfold dilution of the predetermined skin test end-point in the individual undergoing challenge, and the highest concentration was the undiluted extract. After exposure, the patients' forced expiratory volume in one second (FEV1) was monitored for 2 h. If FEV1 decreased by at least 15%, the next scheduled exposure was not carried out and FEV1 was monitored over a period of 24 h. Whereas nebulization of cornstarch powder extract caused no bronchial reaction in the patients, nebulization of nonpowdered latex surgical glove extract induced immediate bronchoconstriction in two subjects as an undiluted solution, and nebulization of powdered latex surgical glove extract induced immediate bronchoconstriction in all subjects at the 1:10 dilution. No systemic reaction was elicited by the bronchial provocation challenges. Our results demonstrate that airborne powder from latex gloves can be an inhalative occupational hazard. Latex, absorbed by the cornstarch powder and then airborne when gloves were handled, was the causative agent of the respiratory events in our patients. The standardized method that we used minimizes the risk of eliciting systemic reactions when performing specific bronchial provocation tests to latex.

COMMENTS: This paper describes the use of skin tests and specific bronchial challenge to determine the causative agent of asthma in four hospital nurses. The nurses were experimentally exposed to cornstarch powder alone and in combination with latex glove extract. The skin tests with powdered latex surgical gloves extract gave a positive reaction from the 1:100 dilution, whereas pure cornstarch powder did not induce any reaction. The results of the bronchial provocation test similarly demonstrated that latex was the causative agent of asthma in these patients, since bronchoconstriction was observed after the challenges with powdered and unpowdered glove extract, but not after the cornstarch powder extract alone.

L-MENTHONE
14073-97-3

Number of relevant papers: 1

1. Inhibition of Human Liver Microsomal (S)-Nicotine Oxidation by (-)-Menthol and Analogues

MacDougall JM, Fandrick K, Zhang X, Serafin SV, and Cashman JR
Chem Res Toxicol 16: 988-993

ABSTRACT: Menthol is a widely used flavoring ingredient present in mouthwash, foods, toothpaste, and cigarettes; yet, the pharmacological effects of menthol have not been widely studied. Mentholated cigarette smoking may increase the risk for lung cancer. Many African American smokers smoke mentholated cigarettes, and African Americans have a significantly higher incidence of lung cancer as compared with whites. There may be a relationship between the incidence of lung cancer and the type of cigarette smoked because the use of mentholated cigarettes by white smokers is significantly less and the incidence of lung cancer is less. The mechanism whereby (-)-menthol could increase the health risk of smoking is not known. The results of our in vitro studies herein show that (-)-menthol and synthetic congeners inhibit the microsomal oxidation of nicotine to cotinine and the P450 2A6-mediated 7-hydroxylation of coumarin. Replacement of the alcohol oxygen atom of menthol with other heteroatoms increased the potency of P450 2A6 inhibition. Thus, the K(i) value of (-)-menthol for inhibition of microsomal nicotine oxidation was 69.7 micro M but neomenthyl thiol possesses a K(i) value of 13.8 micro M. Menthylamine inhibited nicotine oxidation with a K(i) value of 49.8 micro M, but its hydroxylamine derivative gave an IC(50) value of 2.2 micro M. A series of 16 menthol derivatives and putative metabolites were procured or chemically synthesized and tested as inhibitors of P450 2A6. While highly potent inhibition of P450 2A6 was not observed for the menthol analogues examined, it is nevertheless possible that smoking mentholated cigarettes leads to inhibition of nicotine metabolism and allows the smoker to achieve a certain elevated dose of nicotine each day. This may be another example of self-medication to obtain the desired effect of nicotine.

COMMENTS: Abstract summary of the paper is adequate.

CATEGORY: HIGH MUL'S INGREDIENTS

ACETIC ACID
CAS: 64-19-7

Number of relevant papers: 2

1. On the deposition of volatiles and semivolatiles from cigarette smoke aerosols: Relative rates of transfer of nicotine and ammonia from particles to the gas phase

Seeman Jeffrey I,; Lipowicz Peter J; Piade Jean-Jacques; Poget Laurent; Sanders Edward B; Snyder James P; Trowbridge Clarence G
Chemical Research in Toxicology , Volume: 17 , Number: 8 , Page: 1020-1037

ABSTRACT: The hypothesis that elevated levels of ammonia-releasing compounds in tobacco and ammonia in mainstream (MS) smoke increase the rate and amount of nicotine evaporation from the particles of MS smoke aerosol was examined by kinetic modeling and experiments with MS cigarette smoke. Computational simulation of a kinetic mechanism describing volatile loss of nicotine, ammonia, and acetic acid from an aqueous solution was used to compute the time-dependent concentration of all species in the model. Because of the high volatility of ammonia relative to that of nicotine, variation over a wide range of initial ammonia concentration had no significant effect upon the rate of loss of nicotine from the model system. The effects of a variation in the volatile loss rate constant for ammonia and for the acid were examined. The simulations show that ammonia is lost from the model solution at a greater rate than nicotine and acid, and the loss of volatile acid has a significant role in the rate and amount of nicotine loss. Simulations with a model system undergoing a continuous steady addition of ammonia showed that high rates of ammonia addition could significantly increase the rate of nicotine volatile loss from the model solution. A series of smoking experiments was performed using blended cigarettes connected to a denuder tube. Deposition of smoke constituents can occur directly from the gas phase and by the deposition of smoke aerosol particles themselves. As nicotine exists >99% in the particle phase of MS smoke, in the absence of particle deposition, denuder tube deposition of nicotine occurs via the evaporation-deposition pathway. Solanesol, a nonvolatile tobacco and smoke terpene, was used to quantify the amount of particle deposition onto the denuder tube. The amount of ammonia deposited on the denuder tube was an order of magnitude greater than that of nicotine, showing that ammonia evaporates from the MS smoke particles much faster than does nicotine. The experimental results were supported and explained by the aqueous model simulations. Included in these experiments are cigarettes that differ in their MS smoke ammonia content by a factor of ca. five. However, an increased amount of MS smoke ammonia does not increase the rate of nicotine loss from the particles. The combined results support the conclusion that ammonia in mainstream smoke has little effect, if any, upon the rate and amount of nicotine evaporation from MS smoke particles.

COMMENTS: A computation model using chemical kinetics was employed to exam the role of volatile acids (acetic acid or formic acid) and bases (ammonia) in nicotine evaporation from smoke aerosol particles. Experimental results and model simulations indicate that ammonia and acetate evaporate from particles far faster than nicotine. Ammonia in mainstream smoke aerosol has little effect on nicotine loss in smoke particles. Increasing acid volatility increased the rate and amount of nicotine and ammonia loss. Formic acid caused a similar but slower effect than acetic acid. This paper is relevant to the effects of acetic acid as an ingredient in cigarette smoke in that it describes the theoretical effect of acetic acid on nicotine and ammonia volatility.

2. Physician diagnosed asthma, respiratory symptoms, and associations with workplace tasks among radiographers in Ontario, Canada

G M Liss, S M Tarlo, J Doherty, J Purdham, J Greene, L McCaskell, M Kerr
Occup Environ Med 2003; 60:254–261.

ABSTRACT: Background: Medical radiation technologists (MRTs) or radiographers have potential exposure to chemicals including sensitisers and irritants such as glutaraldehyde, formaldehyde, sulphur dioxide, and acetic acid. Aims: To determine the prevalence of asthma and work related respiratory symptoms among MRTs compared with physiotherapists, and to identify work related factors in the darkroom environment that are associated with these outcomes. Methods: As part of a two component study, we undertook a questionnaire mail survey of the members of the professional associations of MRTs and physiotherapists in Ontario, Canada, to ascertain the prevalence of physician diagnosed asthma, and the prevalence in the past 12 months of three or more of the nine respiratory symptoms (previously validated by Venables et al to be sensitive and specific for the presence of self reported asthma). Information on exposure factors during the past 12 months, such as ventilation conditions, processor leaks, cleanup activities, and use of personal protective equipment was also collected. Results: The survey response rate was 63.9% among MRTs and 63.1% among physiotherapists. Most analyses were confined to 1110 MRTs and 1523 physiotherapists who never smoked. The prevalence of new onset asthma (since starting in the profession) was greater among never smoking MRTs than physiotherapists (6.4% v 3.95%), and this differed across gender: it was 30% greater among females but fivefold greater among males. Compared with physiotherapists, the prevalence of reporting three or more respiratory symptoms, two or more work related, and three or more work related respiratory symptoms in the past 12 months was more frequent among MRTs, with odds ratios (ORs) (and 95% confidence intervals) adjusted for age, gender, and childhood asthma, of 1.9 (1.5 to 2.3), 3.7 (2.6 to 5.3), and 3.2 (2.0 to 5.0), respectively. Analyses examining latex glove use indicated that this was not likely to account for these differences. Among MRTs, respiratory symptoms were associated with a number of workplace and exposure factors likely to generate aerosol or chemical exposures such as processors not having local ventilation, adjusted OR 2.0 (1.4 to 3.0); leaking processor in which clean up was delayed, 2.4 (1.6 to 3.5); floor drain clogged, 2.0 (1.2 to 3.2); freeing a film jam, 2.9 (1.8 to 4.8); unblocking a blocked processor drain, 2.4 (1.6 to 3.7); and cleaning up processor chemical spill, 2.8 (1.9 to 4.2). These outcomes were not associated with routine tasks unlikely to generate exposures, such as working

outside primary workplace, loading film into processor, routine cleaning of processors, or removing processed film. Males reported that they carried out a number of tasks potentially associated with irritant exposures more frequently than females, consistent with the marked increase in risk for new onset asthma. Conclusions: These findings suggest an increase of work related asthma and respiratory symptoms shown to denote asthma among MRTs, which is consistent with previous surveys. The mechanism is not known but appears to be linked with workplace factors and may involve a role for irritant exposures.

COMMENTS: This study described a higher prevalence of asthma and work-related respiratory symptoms among medical radiation technologists as compared to other workers (physiotherapists) and attempted to identify environmental factors associated with these outcomes. Medical radiation technologists are exposed to acetic acid and other chemicals during the processing of films, however, the causative agent(s) in these work-related respiratory symptoms is currently unknown.

BENZALDEHYDE
CAS: 100-52-7

Number of relevant papers: 2

1. The GreenScreen genotoxicity assay: a screening validation programme -

Cahill PA, Knight AW, Billinton N, Barker MG, Walsh L, Keenan PO, Williams CV, Tweats DJ, Walmsley RM.
Mutagenesis. 2004 Mar;19(2):105-19

ABSTRACT: A yeast (Saccharomyces cerevisiae) DNA repair reporter assay termed the GreenScreen assay (GSA) is described. This is a novel, cost-effective genotoxicity screen, developed to provide a pre-regulatory screening assay for use by the pharmaceutical industry and in other applications where significant numbers of compounds need to be tested. It provides a higher throughput and a lower compound consumption than existing eukaryotic genotoxicity assays and is sensitive to a broad spectrum of mutagens and, importantly, clastogens. We describe a simple, robust assay protocol and a validation study. The end-point of the test reflects the typically eukaryotic chromosomes and DNA metabolizing enzymes of yeast. The capacity for metabolic activation (MA) in yeast is limited compared with the mammalian liver or its extracts, but the assay does detect a subset of compounds that would require MA in existing genotoxicity tests. The GSA detects a different spectrum of compounds to bacterial genotoxicity assays and thus, together with an in silico structure-activity relationship (SAR) screen, and possibly a high throughput bacterial screen, would provide an effective preview of the regulatory battery of genotoxicity tests.

COMMENTS: This paper describes a genotoxicity assay that measures a different end-point (DNA repair induction) using a different type of cell (yeast) than the Ames test. The

authors used this yeast assay to test over 100 compounds. In this assay benzaldehyde was positive for genotoxicity.

2. Effects of garage employment and tobacco smoking on breathing-zone concentrations of carbonyl compounds.

Zhang L; Chung FL; Boccia L; Colosimo S; Liu WL; Zhang JF.
AIHA Journal 64(3): 388-393, 2003. (26 refs.)

ABSTRACT: Exposure to carbonyl compounds may cause adverse health effects. The present study examined whether working in a garage and smoking can significantly affect personal "daily" exposure to a number of important carbonyl compounds. The study was carried out on 37 subjects including 22 garage workers (9 smokers and 13 nonsmokers) and 15 nongarage workers or so-called controls (4 smokers and 11 nonsmokers). Daily exposure was estimated using 48-hour integrated measurement of breathing-zone concentrations. The measurement involved the use of a passive carbonyl sampler and high performance liquid chromatography/fluorescence analysis technique. Each subject was measured for up to three measurement sessions. A wide range of breathing-zone concentrations (unit: microgram per cubic meter) was observed for each of the following carbonyls: formaldehyde (14.1-80.1); acetaldehyde (8.41-80.3); acetone (0.65-1096); acrolein (<0.14-3.71); propionaldehyde (1.08-14.6); crotonaldehyde (<0.13-2.80); benzaldehyde (1.79-9.91); and hexaldehyde (0.122-22.4). Statistical significance of smoking effects and working in a garage effects were assessed using SAS mixed models. The results show that the garage workers had significantly higher levels of formaldehyde and acetal dehyde than the controls, and that the smokers had significantly higher levels of acetaldehyde, propionaldehyde, and hexaldehyde, than the nonsmokers (P<.10). Garage employment and smoking appeared to increase breathing-zone concentrations of crotonaldehyde. In general, within-subject variations were smaller than between-subject variations on 48-hour averaged breathing-zone concentrations of carbonyl compounds.

COMMENTS: While the primary focus of this study was to determine exposure to 8 carbonyl compounds commonly found in a garage environment, they also examined the added effect of smoking on breathing zone concentration of these carbony compounds. While all carbonyls tested are known to be present in tobacco smoke, only acetaldehyde, propionaldehyde and hexaldehyde were found to be higher in the workers' breathing zone area of smokers as compared to nonsmokers.

BUTYRIC ACID
CAS: 107-92-6

Number of relevant papers: 1

1. Oncogenic Ras promotes butyrate-induced apoptosis through inhibition of gelsolin expression

Lidija Klampfer, Jie Huang, Takehiko Sasazuki, Senji Shirasawa, and Leonard Augenlicht
J. Biol. Chem., Vol. 279, Issue 35, 36680-36688

ABSTRACT: Activation of Ras promotes oncogenesis by altering a multiple of cellular processes, such as cell cycle progression, differentiation, and apoptosis. Oncogenic Ras can either promote or inhibit apoptosis, depending on the cell type and the nature of the apoptotic stimuli. The response of normal and transformed colonic epithelial cells to the short chain fatty acid butyrate, a physiological regulator of epithelial cell maturation, is also divergent: normal epithelial cells proliferate, and transformed cells undergo apoptosis in response to butyrate. To investigate the role of k-ras mutations in butyrate-induced apoptosis, we utilized HCT116 cells, which harbor an oncogenic k-ras mutation and two isogenic clones with targeted inactivation of the mutant k-ras allele, Hkh2, and Hke-3. We demonstrated that the targeted deletion of the mutant k-ras allele is sufficient to protect epithelial cells from butyrate-induced apoptosis. Consistent with this, we showed that apigenin, a dietary flavonoid that has been shown to inhibit Ras signaling and to reverse transformation of cancer cell lines, prevented butyrate-induced apoptosis in HCT116 cells. To investigate the mechanism whereby activated k-ras sensitizes colonic cells to butyrate, we performed a genome-wide analysis of Ras target genes in the isogenic cell lines HCT116, Hkh2, and Hke-3. The gene exhibiting the greatest down-regulation by the activating k-ras mutation was gelsolin, an actin-binding protein whose expression is frequently reduced or absent in colorectal cancer cell lines and primary tumors. We demonstrated that silencing of gelsolin expression by small interfering RNA sensitized cells to butyrate-induced apoptosis through amplification of the activation of caspase-9 and caspase-7. These data therefore demonstrate that gelsolin protects cells from butyrate-induced apoptosis and suggest that Ras promotes apoptosis, at least in part, through its ability to down-regulate the expression of gelsolin.

COMMENTS: This paper indicates a possible butyrate effect, but is not directly relevant to inhaled ingredient.

CAPRYLIC/CAPRIC TRIGLYCERIDE
CAS: 65381-09-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CARYOPHYLLENE OXIDE
CAS: 1139-30-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GAMMA-DECALACTONE
CAS: 706-14-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,5-DIMETHYLPYRAZINE
123-32-0

Number of relevant papers: 3

1. Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning –

Karen Riveles, Ryan Roza, Janet Arey and Prue Talbot
Reproductive Biology and Endocrinology 2004, 2:23 doi:10.1186/1477-7827-2-23

ABSTRACT: Our past studies have shown that cigarette smoke inhibits oviductal functioning in vivo and in vitro. The goals in this study were to identify pyrazine derivatives in cigarette smoke solutions that inhibit ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction in the hamster oviduct and to determine their lowest observable adverse effect levels (LOAELs) using in vitro bioassays.
Methods: MS smoke solutions were fractionated using solid phase extraction cartridges and the fractions were both tested on the hamster oviduct in vitro and analyzed by gas chromatography-mass spectrometry to identify individual pyrazine derivatives. Commercial pyrazine standards were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The LOAEL and efficacy were determined for each compound in the in vitro bioassays. Statistical significance was determined using the Student's t-Test where $p < 0.05$. Results: The LOAELs for the most inhibitory pyrazine derivatives in the ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction assays were as follows: for pyrazine (1 picomolar, 10 picomolar, and 1 nanomolar); for 2-methylpyrazine (1 picomolar, 10 picomolar, and 10 picomolar); and for 2-ethylpyrazine (1 picomolar, 10 picomolar, and 1 picomolar). Six of the seven pyrazine derivatives tested (pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2-methoxy-3-methylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine) were inhibitory in picomolar or nanomolar doses in all three bioassays, while the seventh derivative, 2,6-dimethylpyrazine, had LOAELs in the nanomolar to micromolar range.
Conclusion: This work shows that very low doses of pyrazines significantly inhibit proper oviductal functioning, raising questions regarding the safety of these compounds in cigarettes and other consumer products.

COMMENTS: An *in vitro* assay was used to study the effects of pyrazine and its derivatives in cigarette smoke on hamster oviductal functioning. Pyrazine derivatives had

equal or greater effects than pyrazine and inhibited ciliary beating, oocyte pickup rate and smooth muscle contraction in hamster oviductal explants at nanomolar and picomolar doses. Oocyte pickup rate was the most sensitive parameter, and derivatives with single ethyl or methyl substitutions were among the most inhibitory. The LOAEL of 2,5-dimethylpyrazine was equal to pyrazine for oocyte pickup rate (10^{-11} M) and smooth muscle contraction (10^{-9} M), but 10,000 times greater for ciliary beat frequency (10^{-8} M). For all three measurements, 2,5-dimethylpyrazine was more potent than 2,6-dimethylpyrazine. The authors suggested that these data concur with results reported from *in vivo* hamster studies and epidemiological studies that show increased risk of ectopic pregnancies and spontaneous abortions in female smokers. The authors recommend that further toxicological testing of pyrazines be conducted.

2. The influence of cigarette moisture to the chemistry of particulate phase smoke of a common commercial cigarette -

Q. Zha and S.C. Moldoveanu
Beiträge zur Tabakforschung International/Contributions to Tobacco Research Volume 21(3):184-191

ABSTRACT: This study presents the results on the influence of cigarette moisture content to the chemical composition of particulate phase smoke. Seventy-five selected compounds were monitored for the comparison of particulate phase smoke of a commercial full-flavored (FF) cigarette with three different moisture contents at 7.8%, 14.5% and 20.4%, respectively. It was demonstrated that the smoke of a dry cigarette is richer in lower molecular mass compounds than a regular cigarette. On the other hand, the smoke of a moist cigarette is richer in higher molecular mass compounds than a regular cigarette. To maximize the influence of cigarette moisture to the chemical composition, a separate set of measurements were done using only the first three puffs of smoke. The accumulation of moisture in the tobacco column of a burning cigarette may influence the smoke composition, as generated during burning. The differences between dry, regular and moist cigarettes were more obvious for the first three puffs.

COMMENTS: While this is not a health effect study, the results are interesting. These investigators compared the chemical composition of cigarette smoke from cigarettes with three moisture levels (dry-8.3%, regular-11.6%, and moist- 12.9%). The first three puffs showed the greatest differences. The nicotine content and total particulate matter (TPM) was reduced with increasing moisture. The data would indicate that the dry cigarette had a higher percentage of semi-volatile compounds in TPM. The data presents additional evidence that the cigarette moisture content significantly affects the chemistry of the particulate phase of smoke. Of the 75 compounds tested, the more volatile compounds were more affected than the less volatile compounds. Compared to the first three puffs, the particulate phase of smoke from the entire cigarette was less sensitive to the moisture content.

3. Identification of compounds in cigarette smoke that inhibit hamster oviductal functioning. -

K. Riveles, R. Roza and P. Talbot. Cell Biology & Neuroscience, UC Riverside, Riverside, CA. Poster SETAC Utah 2003.

ABSTRACT: Our past studies have shown that chemicals in cigarette smoke inhibit oviductal functioning in vivo and in vitro. The purposes of this study were to identify the individual toxicants in cigarette smoke solutions that inhibit oocyte pickup rate, ciliary beat frequency, and infundibular smooth muscle contraction and to determine their effective doses using in vitro bioassays. Solid phase extraction and gas chromatography-mass spectrometry were used to identify individual chemicals in the mainstream and sidestream cigarette smoke solutions that were active in the above assays. Pyridines, pyrazines, indoles, quinolines, and phenols were identified in the solutions of mainstream and sidestream cigarette smoke. Commercially available standards of the identified compounds were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The lowest observable adverse effect level and efficacy were determined for each compound using the oocyte pickup rate, ciliary beat frequency, and infundibular muscle contraction assays. Previously, we have shown that several pyridine compounds including 2-methylpyridine, 4-methylpyridine, 2-ethylpyridine, 3-ethylpyridine, and 4- vinylpyridine were inhibitory at picomolar concentrations in all three bioassays. Further studies have shown that compounds in the pyrazine group: 2- methylpyrazine, ethylpyrazine, 2-methoxy-3-methylpyrazine, 2, 5-dimethylpyrazine, and 2, 3, 5-trimethylpyrazine, were inhibitory in pico or nanomolar doses. Both quinoline and isoquinoline were inhibitory in picomolar doses. 5-Methylindole showed inhibition in the nanomolar range. Indole, which is found in large quantities relative to other compounds in the smoke, showed inhibition at 10-15M. The phenolic compounds were not as inhibitory as the other classes of compounds in the bioassays, although hydroquinone and 4-ethylphenol were inhibitory at nanomolar doses. This work is important because it shows that very low doses of cigarette smoke components significantly inhibit proper oviductal functioning raising questions regarding the safety of these compounds.

COMMENTS: POSTER PRESENTATION -- Paper N/A

ETHYL BUTYRATE
CAS: 105-54-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL DECANOATE
CAS: 110-38-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL HEXANOATE
CAS: 123-66-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ISOVALERATE
CAS: 108-64-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LACTATE
CAS: 97-64-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LAURATE
CAS: 106-33-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL MYRISTATE
CAS: 124-06-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OCTANOATE
CAS: 106-32-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL PHENYLACETATE
CAS: 101-97-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-ETHYL-3,(5 OR 6)-DIMETHYLPYRAZINE
CAS: 27043-05-6
CAS: 13925-07-0
CAS: 13360-65-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

5-ETHYL-3-HYDROXY-4-METHYL-2(5H)-FURANONE
CAS: 698-10-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

HEXYL PHENYLACETATE
CAS: 5421-17-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOAMYL ACETATE
CAS: 123-92-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOBUTYL CINNAMATE
CAS: 122-67-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOBUTYL PHENYLACETATE
CAS: 102-13-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALPHA-ISOBUTYLPHENETHYL ALCOHOL (BENZYL ISOBUTYL CARBINOL) (BENZENEETHANOL, ALPHA- (2-METHYLPROPYL)-)
CAS: 7779-78-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOBUTYRIC ACID
CAS: 79-31-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-,5-, OR 6-METHOXY-3-METHYLPYRAZINE
CAS: 2847-30-5

Number of relevant papers: 1

1. Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning

Karen Riveles, Ryan Roza , Janet Arey and Prue Talbot
Reproductive Biology and Endocrinology 2004, 2:23 doi:10.1186/1477-7827-2-23

ABSTRACT: Our past studies have shown that cigarette smoke inhibits oviductal functioning in vivo and in vitro. The goals in this study were to identify pyrazine derivatives in cigarette smoke solutions that inhibit ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction in the hamster oviduct and to determine their lowest observable adverse effect levels (LOAELs) using in vitro bioassays. Methods:MS smoke solutions were fractionated using solid phase extraction cartridges and the fractions were both tested on the hamster oviduct in vitro and analyzed by gas chromatography-mass spectrometry to identify individual pyrazine derivatives. Commercial pyrazine standards were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The LOAEL and efficacy were determined for each compound in the in vitro bioassays. Statistical significance was determined using the Student's t-Test where $p < 0.05$. Results: The LOAELs for the most inhibitory pyrazine derivatives in the ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction assays were as follows: for pyrazine (1 picomolar, 10 picomolar, and 1 nanomolar); for 2-methylpyrazine (1 picomolar, 10 picomolar, and 10 picomolar); and for 2-ethylpyrazine (1 picomolar, 10 picomolar, and 1 picomolar). Six of the seven pyrazine derivatives tested (pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2-methoxy-3-methylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine) were inhibitory in picomolar or nanomolar doses in all three bioassays, while the seventh derivative, 2,6-dimethylpyrazine, had LOAELs in the nanomolar to micromolar range.

COMMENTS: An *in vitro* assay was used to study the effects of pyrazine and its derivatives in cigarette smoke on hamster oviductal functioning. Pyrazine derivatives had equal or greater effects than pyrazine and inhibited ciliary beating, oocyte pickup rate and smooth muscle contraction in hamster oviductal explants at nanomolar and picomolar doses. Oocyte pickup rate was the most sensitive parameter, and derivatives with single ethyl or methyl substitutions were among the most inhibitory. The 2-methoxy-3-methylpyrazine LOAELs for oocyte pickup rate (10^{-12} M) and muscle contraction assays (10^{-12} M) were the lowest of all pyrazines tested. The LOAEL for ciliary beat frequency (10^{-9} M) was similar to the trimethyl substritured pyrazines. The authors suggested that these data concur with results reported from in vivo hamster studies and epidemiological studies that show increased risk of ectopic pregnancies and spontaneous abortions in female smokers. The authors recommend that further toxicological testing of pyrazines be conducted.

2-METHYLHEPTANOIC ACID
CAS: 1188-02-9

Number of relevant papers: 1

1. Evaluation of certain food additives and contaminants -

Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series 922, 2004 Geneva

ABSTRACT: This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives.The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants,assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (a-amylase from Bacilluslichenformis containing a genetically engineered a-amylase gene from B. licheniformis, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from Myceliophthora thermophila expressed in Aspergillus oryzae, mixed xylanase, b-glucanase enzyme preparation produced by a strain of Humicola insolens, neotame, polyvinyl alcohol, quillaia extracts and xylanase from Thermomyces lanuginosus expressed in Fusarium venenatum), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking-water (sodium dichloroisocyanurate) and contaminants (cadmium and methylmercury). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, and tolerable intakes of the contaminants considered, changes in the status of specifications and further information requested or desired.

COMMENTS: The abstract describes this report well.

2-METHYLPYRAZINE
CAS: 109-08-0

Number of relevant papers: 3

1. Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning –

Karen Riveles , Ryan Roza , Janet Arey and Prue Talbot
Reproductive Biology and Endocrinology 2004, 2:23 doi:10.1186/1477-7827-2-23

ABSTRACT: Our past studies have shown that cigarette smoke inhibits oviductal functioning in vivo and in vitro. The goals in this study were to identify pyrazine derivatives in cigarette smoke solutions that inhibit ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction in the hamster oviduct and to determine their lowest observable adverse effect levels (LOAELs) using in vitro bioassays.
Methods : MS smoke solutions were fractionated using solid phase extraction cartridges and the fractions were both tested on the hamster oviduct in vitro and analyzed by gas chromatography-mass spectrometry to identify individual pyrazine derivatives. Commercial pyrazine standards were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The LOAEL and efficacy were determined for each compound in the in vitro bioassays. Statistical significance was determined using

the Student's t-Test where $p < 0.05$. Results: The LOAELs for the most inhibitory pyrazine derivatives in the ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction assays were as follows: for pyrazine (1 picomolar, 10 picomolar, and 1 nanomolar); for 2-methylpyrazine (1 picomolar, 10 picomolar, and 10 picomolar); and for 2-ethylpyrazine (1 picomolar, 10 picomolar, and 1 picomolar). Six of the seven pyrazine derivatives tested (pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2-methoxy-3-methylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine) were inhibitory in picomolar or nanomolar doses in all three bioassays, while the seventh derivative, 2,6-dimethylpyrazine, had LOAELs in the nanomolar to micromolar range.
Conclusion: This work shows that very low doses of pyrazines significantly inhibit proper oviductal functioning, raising questions regarding the safety of these compounds in cigarettes and other consumer products.

COMMENTS: An *in vitro* assay was used to study the effects of to pyrazine and its derivatives in cigarette smoke on hamster oviductal functioning. Pyrazine derivatives had equal or greater effects than pyrazine and inhibited ciliary beating, oocyte pickup rate and smooth muscle contraction in hamster oviductal explants at nanomolar and picomolar doses. Oocyte pickup rate was the most sensitive parameter, and derivatives with single ethyl or methyl substitutions were among the most inhibitory. 2-methylpyrazine was one of the most potent derivatives in this study, causing effects at concentrations as low as 10^{-12} M. The authors suggested that these data concur with results reported from in vivo hamster studies and epidemiological studies that show increased risk of ectopic pregnancies and spontaneous abortions in female smokers. The authors recommend that further toxicological testing of pyrazines be conducted.

2. The influence of cigarette moisture to the chemistry of particulate phase smoke of a common commercial cigarette

Q. Zha and S.C. Moldoveanu
Beiträge zur Tabakforschung International/Contributions to Tobacco Research Volume 21(3):184-191

ABSTRACT: This study presents the results on the influence of cigarette moisture content to the chemical composition of particulate phase smoke. Seventy-five selected compounds were monitored for the comparison of particulate phase smoke of a commercial full-flavored (FF) cigarette with three different moisture contents at 7.8%, 14.5% and 20.4%, respectively. It was demonstrated that the smoke of a dry cigarette is richer in lower molecular mass compounds than a regular cigarette. On the other hand, the smoke of a moist cigarette is richer in higher molecular mass compounds than a regular cigarette. To maximize the influence of cigarette moisture to the chemical composition, a separate set of measurements were done using only the first three puffs of smoke. The accumulation of moisture in the tobacco column of a burning cigarette may influence the smoke composition, as generated during burning. The differences between dry, regular and moist cigarettes were more obvious for the first three puffs.

COMMENTS; While this is not a health effect study, the results are interesting. These investigators compared the chemical composition of cigarette smoke from cigarettes with three moisture levels (dry-8.3%, regular-11.6%, and moist- 12.9%). The first three puffs showed the greatest differences. The nicotine content and total particulate matter (TPM) were reduced with increasing moisture. The data would indicate that the dry cigarette had a higher percentage of semi-volatile compounds in TPM. The data presents additional evidence that the cigarette moisture content significantly affects the chemistry of the particulate phase of smoke. Of the 75 compounds tested the more volatile compounds were more affected than the less volatile compounds. Compared to the first three puffs, the particulate phase of smoke from the entire cigarette was less sensitive to the moisture content.

3. Growth and Angiogenesis Are Inhibited in Vivo in Developing Tissues by Pyrazine and Its Derivatives –

Goar Melkonian, Holly Lautenschlager, Melinda Wu, Yuhuan Wang, Cathy Tong, Karen Riveles, P. Talbot.
Toxicological Sciences Volume 75, Number 2 Pp. 393-401

ABSTRACT: Sidestream cigarette smoke solution was previously screened to identify the groups of chemicals in smoke that inhibit growth and angiogenesis in the chick chorioallantoic membrane (CAM). Pyrazine and several pyrazine derivatives were identified as a major chemical group in this screen. In the current study, purified pyrazine and six pyrazine derivatives identified in the screen were tested in dose response experiments to measure their effects on CAM growth, embryo growth, and angiogenesis. Chemicals or control medium were placed on CAMs in ovo on day 5 of development, and results were evaluated on day 6. Of the chemicals tested, pyrazine was the most potent and inhibited both CAM and embryo growth at picomolar doses. 2-ethylpyrazine and 2,3, dimethylpyrazine were inhibitory at nanomolar doses. Inhibition of growth by pyrazine was correlated with inhibition of DNA synthesis. The pattern of blood vessel development in CAMs was disturbed by micromolar doses of pyrazine and 2,3,-dimethylpyrazine. Migration of mesodermal blood vessels to the ectoderm of CAMs and their subsequent differentiation into the capillary plexus was impaired by nanomolar doses of pyrazine. In summary, these data show that pyrazine and some of its derivatives inhibit growth and certain process important in angiogenesis at very low doses. Since pyrazine and some of its derivatives are considered safe food additives, further toxicological testing of pyrazine, in particular on developing tissues, should be done to fully evaluate its safety as a consumer product additive.

COMMENTS: These authors previously presented data that both mainstream and sidestream smoke inhibit growth and angiogenesis in chick chorioallantoic membrane (CAM). The CAM is important to the chick since it serves as the respiratory organ for gaseous exchange until hatching. These studies are an extension of their earlier work in hope to identify the compound responsible for this effect. The data show that pyrazine in sidesteam smoke can inhibit CAM, embryo growth and impair angiogenesis in nano and picomolar doses. Of the pyrazines tested, 2-methylpyrazine significantly inhibited CAM

growth at $5X10^{-5}$ M but did not significantly affect embryo growth at any dose tested. The authors state that the implications of this data to human reproduction are not known.

GAMMA-OCTALACTONE
CAS: 104-50-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,3-PENTANEDIONE
CAS: 600-14-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-PHENETHYL ACETATE
CAS: 103-45-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PHENYLACETALDEHYDE
CAS: 122-78-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SODIUM BICARBONATE
CAS: 144-55-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SUCROSE OCTAACETATE
CAS: 126-14-7

Number of relevant papers: 1

1. The Contribution of Taste Bud Populations to Bitter Avoidance in Mouse Strains Differentially Sensitive to Sucrose Octa-acetate and Quinine -

St John SJ, Boughter JD Jr.
Chem Senses. 2004 Nov;29(9):775-87.

ABSTRACT: Mice of the SWR/J (SW) strain avoid orally delivered sucrose octa-acetate (SOA), whereas the mice of the C3HeB/FeJ (C3) strain are insensitive to SOA. Mice of both strains and of a congenic strain (C3.SW) that shares more than 99% of the C3 genome, were tested in a taste-salient brief-access taste test for responses to SOA and quinine hydrochloride, before and after transection of the glossopharyngeal or chorda tympani nerve, or sham surgery. Prior to surgery, congenic SOA tasters (C3.SW(T)) were

phenotypically identical to the SW strain in avoidance of SOA, but showed a greater reduction in sensitivity after nerve transection. For quinine avoidance, which is thought to be a polygenic trait, SW mice showed the greatest sensitivity to quinine, C3 the least and C3.SW(T) mice were different from both parental strains, showing intermediate sensitivity. Nerve transections had only a moderate effect on quinine sensitivity, suggesting that both anterior and posterior taste bud fields contribute to behavioral quinine avoidance. These findings are discussed with regard to the distribution in the oral cavity of putative taste receptors for quinine and SOA and the peripheral organization of bitter taste.

COMMENTS: This paper has to do with taste receptors. Although it is not directly health-related, it may be of interest.

2,3,5,6-TETRAMETHYLPYRAZINE
CAS: 1124-11-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

TRIETHYL CITRATE
CAS: 77-93-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4-(2,6,6-TRIMETHYLCYCLOHEX-1-ENYL)BUT-2-EN-4-ONE (BETA-DAMASCONE)
CAS: 23726-91-2
CAS: 35044-68-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CATEGORY: MAJOR INGREDIENTS

GLYCEROL
CAS: 56-81-5

Number of relevant papers: 1

1. Glycerol transfer in cigarette mainstream smoke

C.Liu.
Beitrage Tabakforschung Int., 21 (2004) No. 2, pp.111-116.

ABSTRACT: Experiments have been conducted to examine the effect of different levels of blend cigarette at 36 for a 11.4 blend glycerol. For cigarettes with different designs the glycerol in NFDPM may also depend on the glycerol loading per unit rod length. The tobacco rod filtration did not change significantly within the glycerol range investigated and hence plays a relatively minor role. Significant glycerol condensation ahead of the burning coal after a puff was measured. This condensation may have implications on glycerol levels in the sidestream smoke during inter-puff smouldering.

COMMENTS: While this is not a health effect study, it does present data on the transfer of glycerol into MSS when added at higher levels than normally used as a humectant. The author's conclusion was that 1. mainstream glycerol yield increased with the blend glycerol levels, 2. the tobacco rod filtration was not significantly altered by glycerol levels, and 3. significant glycerol condensation was found ahead of the burning coal after a puff. Unfortunately, the levels of glycerol in the sidestream smoke, butt and filter were not measured.

CARBON
CAS: 7440-44-0

Number of relevant papers: 4

GENERAL COMMENTS ON CARBON AND GRAPHITE

There are numerous inhalation studies on the health effects associated with exposure to a variety of carbonaceous materials including activated carbon, graphite, coal dust, lamp black, soot, and diesel emissions. Some of these materials appear to cause tumors in rats when inhaled chronically at high concentrations. Such reports may be of interest since carbon-based particles can have absorbed onto them a variety of organic compounds including polycyclic aromatic hydrocarbons, nitroaromatic compounds, and heterocyclic compounds. The presence of similar organic substances in smoke may also be absorbed onto any carbon particles which theoretically could act synergistically or additively producing a greater response as compared to single components. Such effects may be involved in tumor development and DNA damage through both the chemical and particulate-mediated cytotoxicity responses. Particle size has a significant role in the toxicity of inhaled particles. For example, studies would indicate that ultrafine particles (<0.1 μm diameter) produce significantly greater inflammatory response than do fine particles per given mass. Such inhaled particles can lead to production of a number of mediators such as reactive oxygen and nitrogen species, cytokines, growth factors and other substances that might mediate tissue injury and contribute to the pathogenesis of pulmonary disease. Studies also suggest an excess risk of esophageal cancer, particularly squamous cell carcinomas, with exposure to carbon black combined with acid aerosols. However, the level of exposure in many of these studies was several orders of magnitude higher than one would expect from cigarette smoke inhalation.

It is important to differentiate between the types of carbon-based particles. Carbon black, for example, is manufactured under controlled conditions, while the soot-types of carbon contain numerous unwanted byproducts from the combustion of carbon-based materials. Often the terms carbon black and soot are used interchangeably. However, they are physically and chemically distinct. Soots have a much greater percentage of ash and more organic compounds can be extracted from particle surfaces.

Increases in human cancers have been attributed to high exposure to carbon black dust during working conditions and it has been classified as a possible lung and bladder carcinogen by IRAC. Carbon black is mutagenic in the Ames assay. Inhaled carbon black has also been shown to be carcinogenic in rat bioassays. For example, exposure for 24 mo to carbon black 16 hr/day, for 5 days/wk at a concentration of 2.5 or 6.5 mg/m^3 produced malignant and benign lung tumors. In such cases, clearance was impaired and particles accumulated progressively. There is evidence that lung overloading is a requisite for induction of lung tumors in this animal model. A similar range of tumor phenotypes has been reported in the lungs of rats exposed to high concentrations of diesel exhaust and coal dust. Intratracheal instillation of carbon black resulted in a dose-response neutrophil inflammation. Bronchoalveolar lavage cell population was associated with increased mutation rates in alveolar type II epithelial cells. Subchronic inhalation studies in rats did not show increases in mutation frequency at a concentration of 1.1 mg/m^3, and lung clearance was not impaired at that level of exposure.

1. Inhaled particles and lung cancer, part B: Paradigms and risk assessment -

Borm PJ, Schins RP, Albrecht C.
Int J Cancer. 2004 May 20;110(1):3-14.

ABSTRACT: Poorly soluble particles of low toxicity (PSP), such as CB, TiO(2) and coal mine dust, have been demonstrated to cause lung cancer in rodents, being most pronounced in rats. Adequate epidemiologic studies do not clearly indicate increased lung cancer rates in humans exposed to such particles. This has caused controversial positions in regulatory decisions on PSP on different levels. The present review discusses the current paradigms in rodent particle carcinogenicity, i.e., (i) role of particle overload and of persistent inflammation and (ii) fibrosis as an intermediate step in particle-induced lung cancer with regard to human risk assessment. Fibrosis, which is usually considered a precursor of lung cancer in humans, was not related to lung tumors in an animal study using 6 different particles, each at 3 dosages. Lung tumors after both inhalation and intratracheal instillation of PSP are related to particle surface dose, which forwards hazard assessment at surface-based nonoverload concentrations and a standard setting using surface as an exposure metric. The scarce data available on humans do not support the overload concept but suggest a role for persistent lung inflammation. Differences in antioxidant protection between different rodent species correlate with susceptibility to PSP-induced carcinogenicity and support the need for detailed studies on antioxidant response in humans. Apart from such bridging studies, further focus is also needed on surface chemistry and modifications in relation to their adverse biologic effects.

COMMENTS: This manuscript reviews the possible mechanism of action associated with particle-induced lung carcinogenesis. These authors attempt to explain why a number of poorly soluble particles (PSP), such as carbon black and graphite, has been shown to be carcinogenic in the rat and may or may not be carcinogenic in humans. The extrapolation of these rodent studies to humans is difficult because of lack of knowledge regarding antioxidant response, the significance of inflammation in the process of genotoxicity and proliferations in the human lung. The authors state that all inhaled particles are likely to induce tumors in the rat model if the particles are inhaled or instilled at sufficiently high doses and highly durable. While carbon has been identified as a possible carcinogen by IRAC, based on these rodent studies, these authors state that this action may be premature and needs further consideration.

2. Pulmonary and systemic effects of short-term inhalation exposure to ultrafine carbon black particles -

Gilmour PS, Ziesenis A, Morrison ER, Vickers MA, Drost EM, Ford I, Karg E, Mossa C, Schroeppel A, Ferron GA, Heyder J, Greaves M, MacNee W, Donaldson K.
Toxicol Appl Pharmacol. 2004 Feb 15;195(1):35-44.

ABSTRACT: While environmental particles are associated with mortality and morbidity related to pulmonary and cardiovascular (CV) disease, the mechanisms involved in CV health effects are not known. Changes in systemic clotting factors have been associated with pulmonary inflammation. We hypothesized that inhaled ultrafine particles result in an inflammatory response which may stimulate systemic clotting factor release. Adult male Wistar rats were exposed to either fine or ultrafine carbon black (CB) for 7 h. The attained total suspended particle concentrations were 1.66 mg/m(3) for ultrafine CB and 1.40 mg/m(3) for fine CB. Particle concentration of ultrafine particles was more than 10 times greater than that of fine particles and the count median aerodynamic diameter averaged 114 nm for the ultrafine and 268 nm for the fine carbon particles. Data were collected immediately, 16 and 48 h following exposure. Only ultrafine CB caused an increase in total bronchoalveolar lavage (BAL) leukocytes, whereas both fine (2-fold) and ultrafine (4-fold) carbon particles caused an increase in BAL neutrophils at 16 h postexposure. Exposure to the ultrafine, but not fine, carbon was also associated with significant increases in the total numbers of blood leukocytes. Plasma fibrinogen, factor VII and von Willebrand factor (vWF) were unaffected by particle treatments as was plasma Trolox equivalent antioxidant status (TEAC). Macrophage inflammatory protein-2 mRNA was significantly increased in BAL cells 48 h following exposure to ultrafine CB. The data show that there is a small but consistent significant proinflammatory effect of this exposure to ultrafine particles that is greater than the effect of the same exposure to fine CB.

COMMENTS: In this study, rats were exposed by inhalation to approximately 1.5 mg/m^3 fine and utrafine carbon black (CB) particles. Following a single 7-h exposure, the bronchoalveolar lavage (BAL) inflammatory profile was assessed at 0, 16, and 48 hours post-exposure. A total deposition of 3.9 µg particle mass in the deep lung was estimated.

The results indicate that particle size is an important determinant of pulmonary responses to CB, since exposure to ultrafine CB particles was associated with effects not seen following fine CB particle exposure. An increase in BAL cells in rats exposed to ultrafine CB was observed as well as an increase in the number of neutrophils (PMNs) in the BAL fluid and an increase in blood leukocytes. No effects on blood coagulation factors or plasma antioxidant capacity were observed. These findings are consistent with previous studies of acute human exposure to concentrated ambient particles, with the exception that an increase in blood fibrinogen was observed in humans. The authors note that difference in findings between the two studies may be due to species differences or the more heterogeneous nature of ambient particles used in the human studies.

3. Immunological biomarkers in salt miners exposed to salt dust, diesel exhaust and nitrogen oxides -

Backe E, Lotz G, Tittelbach U, Plitzko S, Gierke E, Schneider WD.
Int Arch Occup Environ Health. 2004 Jun;77(5):319-27. Epub 2004 Jun 12

ABSTRACT: Air pollutants can affect lung function and also the immune system. In a study about lung function of salt miners in relation to the complex exposure in a salt mine, we also analysed selected immunological parameters and inflammation markers in the blood of miners. Effect of salt dust, diesel exhaust, nitrogen oxides (NOx) and smoking on the biomarkers was analysed. METHODS: Blood was drawn from 286 salt miners, and the soluble intercellular adhesion molecule-1 (s-ICAM), monocyte chemotactic protein (MCP-1) and clara cell protein (CC16) were analysed by an immunoassay, blood profile was done and lymphocyte subpopulations (CD3, CD3/CD4, CD3/CD8, CD19, NK-cells, CD3/HLA-DR) were determined by flow cytometry. Salt dust was measured by two-step gravimetry (personal sampling). Diesel exhaust was measured as elemental carbon concentration by coulometry. NOx were determined by an electrochemical cell method. Differences between non-smokers, former smokers and active smokers were analysed by analysis of variance. Linear regression analysis to describe exposure-response relationships was done with regard to confounding factors [smoking, inflammatory diseases, time of blood drawing, respiratory infection and body-mass index (BMI)]. RESULTS: Significant differences between non-smokers and active smokers were found for most of the leukocyte types (e.g. granulocytes $P = 0.000$, lymphocytes $P = 0.002$, T-cells $P = 0.033$) and for some soluble parameters (ICAM $P = 0.000$, IgM $P = 0.007$, IgE $P = 0.035$). Increasing numbers of total lymphocytes, T-cells and HLA-DR positive T-cells in relation to exposure were found by linear regression analysis (e.g. for inhalable dust:total lymphocytes $P = 0.011$, T-cells $P = 0.061$, HLA-DR positive T-cells $P = 0.007$). CONCLUSION. Comparison of immunological markers in non-smokers and active smokers confirms leukocytosis and inflammation following tobacco consumption. The combined exposure of salt dust, diesel exhaust and NOx seems to influence the immune system. Together, the results suggest that the analysis of leukocytes and their subsets can complete other investigations (lung function, questionnaire) to monitor exposure-response relationships in occupational studies investigating the effect of inhaled substances. Longitudinal studies will be necessary to

determine the predictive value of the immunological changes. Copyright 2004 Springer-Verlag

COMMENTS: Immunological parameters and inflammation markers were assessed in salt mine workers exposed to complex mixtures of salt dust, nitrogen oxides and diesel exhaust. These same markers were also evaluated in relation to tobacco smoke exposure. Exposure –dependent increases in lymphocytes, T-cells and activated T-cells indicated an effect on the immune system, however, it was not possible to distinguish between the contributions of the different exposure types. The effect of exposure to these mixtures was confounded by smoking and body-mass index which contributed to alterations in the number of immunocompetent cells. Differences between smokers and nonsmokers included increases in immune cells and some soluble markers in blood. Lymphocytes and T-cells were positively correlated with the number of cigarettes smoked per day. Extrapolation of the findings of this study to the health effects of carbon as an ingredient in cigarettes is difficult.

4. Ultrafine particle deposition in subjects with asthma -

David C. Chalupa, Paul E. Morrow, Günter Oberdörster, Mark J. Utell, and Mark W. Frampton
Environmental Health Perspectives Volume 112, Number 8, June 2004

Abstract: Ambient air particles in the ultrafine size range (diameter < 100 nm) may contribute to the health effects of particulate matter. However, there are few data on ultrafine particle deposition during spontaneous breathing, and none in people with asthma. Sixteen subjects with mild to moderate asthma were exposed for 2 hr, by mouthpiece, to ultrafine carbon particles with a count median diameter (CMD) of 23 nm and a geometric standard deviation of 1.6. Deposition was measured during spontaneous breathing at rest (minute ventilation, 13.3 ± 2.0 L/min) and exercise (minute ventilation, 41.9 ± 9.0 L/min). The mean ± SD fractional deposition was 0.76 ± 0.05 by particle number and 0.69 ± 0.07 by particle mass concentration. The number deposition fraction increased as particle size decreased, reaching 0.84 ± 0.03 for the smallest particles (midpoint CMD = 8.7 nm). No differences between sexes were observed. The deposition fraction increased during exercise to 0.86 ± 0.04 and 0.79 ± 0.05 by particle number and mass concentration, respectively, and reached 0.93 ± 0.02 for the smallest particles. Experimental deposition data exceeded model predictions during exercise. The deposition at rest was greater in these subjects with asthma than in previously studied healthy subjects (0.76 ± 0.05 vs. 0.65 ± 0.10, $p < 0.001$). The efficient respiratory deposition of ultrafine particles increases further in subjects with asthma. Key words: air pollution, asthma, deposition, dosimetry, inhalation, ultrafine particles. Environ Health Perspect 112:879-882 (2004). doi:10.1289/ehp.6851 available via http://dx.doi.org/ [Online 2 March 2004]

COMMENTS: This study focused on ultrafine particle (UFP) deposition in individuals with asthma. The hypothesis was if lung dose of UFP are higher for individuals with asthma, than the health risk might also increase. Previous studies have shown that

individuals with chronic obstructive pulmonary disease have enhanced deposition of both fine and ultrafines. These results indicated that when both increased deposition fraction and minute ventilation were considered, the total number of carbon particles retained in the lung was 74% greater in subjects with asthma than healthy subjects which may make them more susceptible to respiratory disease.

GRAPHITE
CAS: 7782-42-5

Number of relevant papers: 2

1. Translocation of inhaled ultrafine particles to the brain -

Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C.
Inhal Toxicol. 2004 Jun;16(6-7):437-45.

ABSTRACT: Ultrafine particles (UFP, particles <100 nm) are ubiquitous in ambient urban and indoor air from multiple sources and may contribute to adverse respiratory and cardiovascular effects of PM (Particulate Matter). Depending on their particle size, inhaled UFP are efficiently deposited in nasal, tracheobronchial and alveolar regions due to diffusion. Our previous rat studies have shown that UFP can translocate to interstitial sites in the respiratory tract as well as to extrapulmonary organs such as liver within 4-24 hrs. post-exposure. There were also indications that the olfactory bulb of the brain was targeted. Our objective in this follow-up study, therefore, was to determine whether translocation of inhaled ultrafine solid particles to regions of the brain takes place, hypothesizing that UFP depositing on the olfactory mucosa of the nasal region will translocate along the olfactory nerve into the olfactory bulb. This should result in significant increases in that region on the days following the exposure as opposed to other areas of the CNS. We generated ultrafine elemental 13C particles (CMD = 36 nm; GSD = 1.66) from 13C graphite rods by electric spark discharge in an argon atmosphere at a concentration of 160 μg/m3. Rats were exposed for 6 hrs. and lungs, cerebrum, cerebellum and olfactory bulbs were removed 1,3,5 and 7 days after exposure. 13C concentrations were determined by isotope ratio mass spectroscopy and compared to background 13C levels of sham-exposed controls (day 0). The background corrected pulmonary 13C added as ultrafine 13C particles on day 1 post-exposure was 1.34 μg/lung. Lung 13C concentration decreased from 1.39 μg/g (day 1) to 0.59 μg/g by 7 days post-exposure. There was a significant and persistent increase in added 13C in the olfactory bulb of 0.35 μg/g on day 1 which increased to 0.43 μg/g by day 7. Day 1 13C concentrations of cerebrum and cerebellum were also significantly increased but the increase was inconsistent, significant only on one additional day of the post-exposure period, possibly reflecting translocation across the blood-brain barrier in certain brain regions. The increases in olfactory bulbs are consistent with earlier studies in non-human primates and rodents which demonstrated that intranasally-instilled solid UFP translocate along axons of the olfactory nerve into the CNS. We conclude from our study that the CNS can be targeted by airborne solid ultrafine particles and that the most likely

mechanism is from deposits on the olfactory mucosa of the nasopharyngeal region of the respiratory tract and subsequent translocation via the olfactory nerve. Depending on particle size, >50% of inhaled UFP can be depositing in the nasopharyngeal region during nasal breathing. Preliminary estimates from the present results show that ~20% of the UFP deposited on the olfactory mucosa of the rat can be translocated to the olfactory bulb. Such neuronal translocation constitutes an additional not generally recognized clearance pathway for inhaled solid UFP, whose significance for humans, however, still needs to be established. It could provide a portal of entry into the CNS for solid UFP, circumventing the tight blood-brain barrier. Whether this translocation of inhaled UFP can cause CNS effects needs to be determined in future studies.

COMMENTS: These authors report that they found significant and continuous increases of ultrafine particles in the olfactory bulb throughout a 7 day inhalation exposure. These results suggest that inhaled ultrafine carbon particles are translocated to the CNS. This provides evidence of a direct portal of entry for ultrafines into the CNS. Such evidence could indicate potential long term effects and accumulation of such particles to other regions of the CNS.

2. Inhaled particles and lung cancer, part B: Paradigms and risk assessment -

Borm PJ, Schins RP, Albrecht C.
Int J Cancer. 2004 May 20;110(1):3-14.

ABSTRACT: Poorly soluble particles of low toxicity (PSP), such as CB, TiO(2) and coal mine dust, have been demonstrated to cause lung cancer in rodents, being most pronounced in rats. Adequate epidemiologic studies do not clearly indicate increased lung cancer rates in humans exposed to such particles. This has caused controversial positions in regulatory decisions on PSP on different levels. The present review discusses the current paradigms in rodent particle carcinogenicity, i.e., (i) role of particle overload and of persistent inflammation and (ii) fibrosis as an intermediate step in particle-induced lung cancer with regard to human risk assessment. Fibrosis, which is usually considered a precursor of lung cancer in humans, was not related to lung tumors in an animal study using 6 different particles, each at 3 dosages. Lung tumors after both inhalation and intratracheal instillation of PSP are related to particle surface dose, which forwards hazard assessment at surface-based nonoverload concentrations and a standard setting using surface as an exposure metric. The scarce data available on humans do not support the overload concept but suggest a role for persistent lung inflammation. Differences in antioxidant protection between different rodent species correlate with susceptibility to PSP-induced carcinogenicity and support the need for detailed studies on antioxidant response in humans. Apart from such bridging studies, further focus is also needed on surface chemistry and modifications in relation to their adverse biologic effects.

COMMENTS: See General Comments for this paper under the Carbon Ingredient listing.

INVERT SUGAR
CAS: 8013-17-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

MAPLE SYRUP
CAS: 8029-81-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

HIGH FRUCTOSE CORN SYRUP
8029-43-4
977042-84-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORN SYRUP
8029-43-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CELLULOSE AND CELLULOSE FIBER
65996-61-4
09004-34-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SUCROSE
CAS: 57-50-1

Number of relevant papers: 3

1. Sucrose and IQ induced mutations in rat colon by independent mechanism

Hansen M, Hald MT, Autrup H, Vogel U, Bornholdt J, Moller P, Molck AM, Lindecrona R, Poulsen HE, Wallin H, Loft S, Dragsted LO.
Mutat Res. 2004 Oct 4;554(1-2):279-86.

ABSTRACT: Sucrose-rich diets have repeatedly been observed to have co-carcinogenic actions in colon and liver of rats and to increase the number of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) induced aberrant crypt foci in rat colon. To investigate a possible interaction between sucrose and IQ on the genotoxicity in rat liver and colon, we gave Big Blue ratsTM a diet containing sucrose (0%, 3.45% or 13.4% w/w) and/or IQ (70 ppm) for a period of 3 weeks. Sucrose and IQ increased the mutation frequency in the colon. The effect of combined treatments with IQ and sucrose on the mutation frequencies was additive indicating that sucrose and IQ act independently. This

was supported by the mutation spectra where sucrose expands the backgroundmutations in the colon, whereas IQ, in other studies, more specifically has induced G:C → T:A transversions. In the liver IQ increased the mutation frequency, whereas addition of sucrose reduced the effect of IQ in a dose-dependent manner. The level of bulky DNA adducts in liver and colon was increased in animals exposed to either sucrose or IQ. In animals exposed to IQ, addition of sucrose had marginal effects on the level of bulky DNA adducts. Markers of oxidative damage and DNA repair were generally unaffected by the treatments. In conclusion, sucrose and IQ in the diet induced mutations in the colon by independent mechanisms, whereas an interaction was observed in liver leading to a decrease in mutations by the combined treatment.

COMMENTS: The interaction between high doses (3.4 and 13.45%) of sucrose and 2-amino-3-methylimidazo [4,5-*f*]quinoline (IQ) which is a strong hepatic carcinogen in non-human primates) were assessed in rats using 3-week dietary exposures. The authors state that this study confirms previous reports of the mutagenic effects of sucrose in the rat colon. In the liver, they report a decrease in mutation frequencies with increased levels of sucrose however, the level of DNA adducts was increased by sucrose in both the colon and liver, possibly indicating that other factors may be influencing the mutagenic effects.

2. Assessment of the performance of the Ames II assay: a collaborative study with 19 coded compounds –

Fluckiger-Isler S, Baumeister M, Braun K, Gervais V, Hasler-Nguyen N, Reimann R, Van Gompel J, Wunderlich HG, Engelhardt G.
Mutat Res. 2004 Mar 14;558(1-2):181-97.

ABSTRACT: Nineteen coded chemicals were tested in an international collaborative study for their mutagenic activity. The assay system employedwas the Ames IIMutagenicity Assay, using the tester strains TA98 and TAMix (TA7001–7006). The test compounds were selected from a published study with a large data set from the standard Ames plate-incorporation test. The following test compounds including matched pairs were investigated: cyclophoshamide, 2-naphthylamine, benzo(a)pyrene, pyrene, 2-acetylaminofluorene, 4,4_-methylene-bis(2-chloroaniline), 9,10-dimethylanthracene, anthracene, 4-nitroquinoline-N-oxide, diphenylnitrosamine, urethane, isopropyl-N(3-chlorophenyl)carbamate, benzidine, 3,3_-5,5_-tetramethylbenzidine, azoxybenzene, 3-aminotriazole, diethylstilbestrol, sucrose and methionine. The results of both assay systems were compared, and the inter-laboratory consistency of the Ames II test was assessed. Of the eight mutagens selected, six were correctly identified with the Ames II assay by all laboratories, one compound was judged positive by five of six investigators and one by four of six laboratories. All seven non-mutagenic samples were consistently negative in the Ames II assay. Of the four chemicals that gave inconsistent results in the traditional Ames test, three were uniformly classified as either positive or negative in the present study, whereas one compound gave equivocal results. A comparison of the test outcome of the different investigators resulted in an inter-laboratory consistency of 89.5%. Owing to the high concordance between the two test systems, and the low inter-

laboratory variability in the Ames II assay results, the Ames II is an effective screening alternative to the standard Ames test, requiring less test material and labor.

COMMENTS: While there are studies reporting mutagenic effects of sucrose, this study examined 19 coded compounds and came to the conclusion that sucrose was consistently negative in the Ames II assay. The Ames II assay is a liquid microtiter modification of the traditional Ames test and is considered to be a suitable alterative to the standard type Ames plate method.

3. Sucrose consumption enhances the analgesic effects of cigarette smoking in male and female smokers

Kanarek RB, Carrington C.
Psychopharmacology (Berl). 2004 Apr;173(1-2):57-63. Epub 2004 Jan 14.

ABSTRACT: Abstract Rationale: Nicotine has analgesic actions in experimental animals and humans. Moreover, the analgesic properties of nicotine in experimental animals are increased by intake of sweet-tasting nutritive fluids. It is important to determine if the effects of diet on nicotineinduced analgesia are limited to experimental animals, or if these effects can be translated from the laboratory to clinical research situations. Objective: This study investigated whether intake of a sweet-tasting sucrose solution would enhance the pain relieving actions of nicotine, administered in the form of cigarette smoking, in male and female college-aged students. The effects of smoking and sucrose intake on mood were also examined. Method: Using the cold pressor test, pain thresholds and pain tolerance were determined in 24 male and 25 female
smokers. Each participant was tested 4 times. On 2 of the test days, participants drank a sucrose-containing beverage, and on 2 of the days, drank water. Twenty-five minutes later, participants either smoked a cigarette or did not smoke. Participants were tested 5 min later for their responses on the cold pressor test. To determine if mood was altered by smoking or sucrose intake, the Profile of Mood Scale was administered immediately preceding and following experimental manipulations. Results: Cold threshold and cold tolerance were greater when participants were allowed to smoke than when they were not allowed to smoke. While men and women responded in a similar manner to the experimental manipulations, men displayed significantly greater cold threshold and cold tolerance than women. Sucrose consumption augmented the effects of smoking on cold threshold, but not on cold tolerance. Men reported feeling significantly more vigorous and less angry, and women reported feeling significantly less tense after they had smoked than when they had not smoked. Sucrose consumption did not alter self reports of mood in either men or women. Conclusion: These findings suggest that sucrose augments the analgesic properties of nicotine in humans, as well as in experimental animals, and suggest that diet could serve as an adjunct in the control of pain.

COMMENTS: This study was designed to investigate the interactions between sucrose intake and smoking on pain sensitivity and mood in humans. Cigarette smoking led to increases in pain threshold and tolerance. Sucrose intake (28.5 g, achieved by drinking a sucrose-containing beverage) increased pain threshold when combined with smoking, but

not alone. Sucrose intake also did not affect self-reported mood. The authors speculate that both sucrose and nicotine may alter central cholinergic neurons. The relevance of this study to sucrose as an ingredient in cigarettes is minor because of the differences in sucrose exposure concentration and route of exposure.

PROPYLENE GLYCOL
57-55-6

Number of relevant papers: 2

1. NTP-CERHR Expert Panel report on the reproductive and developmental toxicity of propylene glycol -

Center for the Evaluation of Risks to Human Reproduction
Reproductive Toxicology Volume 18, Issue 4 , June 2004, Pages 533-579

Abstract: The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed. Propylene glycol was selected for evaluation by the CERHR based on its high production and widespread public exposure due to its use as an antifreeze and de-icing agent, as well as its use in paints, coatings, foods, drugs, and cosmetics. This evaluation results from the efforts of a nine-member panel of government and non-government scientists that culminated in a public expert panel meeting held February 11–13, 2003. This report has been reviewed by CERHR staff scientists and by members of the Ethylene Glycol/Propylene Glycol Expert Panel. Copies have been provided to the CERHR Core Committee, which is made up of representatives of NTP-participating agencies. This report is a product of the expert panel and is intended to (1) interpret the strength of scientific evidence that propylene glycol is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies, (2) assess the extent of human exposures to include exposures of the general public, occupational groups, and other sub-populations, (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future assessments of risk. The Expert Panel Report on Propylene Glycol will be a central part of the subsequent NTP CERHR Monograph. The monograph will include the NTP CERHR Brief, the expert panel report, and all public comments on the expert panel report. The NTP CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

COMMENTS: This paper provides a thorough review of the use, exposure, metabolism and toxicity of propylene glycol. The panel estimates 25 million pounds (2.9% of the

total consumption) of propylene glycol was used as tobacco humectant in 1999. American Industrial Hygiene Association Workplace Environmental Exposure Level guide of 50 ppm total exposure and 10mg/m^3 inhalation aerosol exposure have been determined. Propylene glycol has a short half life and very low systemic toxicity, is not mutagenic, nor developmentally toxic. Although human inhalation exposures were considered within this review (in situations such as actors exposed to theatrical fog), studies have not included propylene glycol as an ingredient in cigarettes and data available on inhalation exposure in animals are inconclusive. The panel concluded "the current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans." Potentially sensitive subpopulations include patients with impaired liver or kidney function.

2. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.
Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-be nzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m3 for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including propylene glycol (13 – 52 μg/m^3). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The

results are consistent with those earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

BROWN SUGAR
CAS: 57-50-1
SEE MAJOR INGREDIENTS

HONEY
CAS: 8028-66-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

MENTHOL AND L-MENTHOL
CAS: 89-78-1

Number of relevant papers: 6

1. On the biological properties of fragrance compounds and essential oils - UBER BIOLOGISCHE WIRKUNGEN VON DUFTSTOFFEN UND ATHERISCHEN OLEN -

Buchbauer G.
Wien Med Wochenschr. 2004 Nov;154(21-22):539-47.

ABSTRACT: In the present review the physiological and/or pharmacological properties of essential oils and of single fragrance compounds are discussed. Essential oils are known and have been used since ancient times as natural medicines. As natural products essential oils are dependent on climate and their composition varies according to conditions of soil, to solar irradiation, to harvest time, to production methods, to storage conditions and similar facts which are discussed in chapter 2 of this review. The next chapters deal with the therapeutic use of essential oils in treating diseases, disorders or ailments of the nervous system, against cancer and as penetration enhancers. For space-saving reasons, however, the manifold antimicrobial and antifungal properties of these natural products have been left out. In the last chapter, the pros and cons in the use of essential oils in therapy are also discussed.

COMMENTS: This article is in German and was not translated.

2. Mentholated cigarette smoking inhibits nicotine metabolism

Neal L. Benowitz, Brenda Herrera, and Peyton Jacob, III
Journal of Pharmacology And Experimental Therapeutics 310:1208-1215, 2004

ABSTRACT: Smoking mentholated cigarettes has been suggested to convey a greater cancer risk compared with smoking nonmentholated cigarettes. Two of the possible mechanisms by which mentholated cigarette smoking could increase risk are by increasing systemic exposure to tobacco smoke toxins and by affecting the metabolism of nicotine or tobacco smoke carcinogens. To examine these possibilities, we performed a crossover study in 14 healthy smokers, one-half of whom were African-Americans and one-half whites. Subjects were randomly assigned to smoke mentholated or nonmentholated cigarettes for 1 week, then to cross over to the other type of cigarettes for another week. Subjects were confined to a Clinical Research Center for 3 days of each week, during which time blood levels of nicotine and carbon monoxide were measured throughout the day and an intravenous infusion of deuterium-labeled nicotine and cotinine was administered to determine the rate and pathways of nicotine metabolism. The systemic intake of nicotine and carbon monoxide was, on average, not affected by mentholation of cigarettes. Mentholated cigarette smoking did significantly inhibit the metabolism of nicotine (clearance: 1289 versus 1431 ml/min, two sided, $p = 0.02$). Inhibition of nicotine metabolism occurred both by slower oxidative metabolism to cotinine and by slower glucuronide conjugation. Our data do not support the hypothesis that mentholated cigarette smoking results in a greater absorption of tobacco smoke toxins. Our finding of impaired metabolism of nicotine while mentholated cigarette smoking suggests that mentholated cigarette smoking enhances systemic nicotine exposure.

COMMENTS: This is an expansion of previous research where the authors have shown that African-Americans metabolize nicotine to its metabolite, cotine, differently as compared to whites. The authors report that when the number of cigarettes smoked per day is controlled, and the cigarettes smoked are in machine-determined yield as well as nicotine content, there is no difference in systemic nicotine and CO intake from smoking mentholated cigarettes compared to nonmentholated cigarettes. The results did not indicate that menthol accelerates nicotine metabolism, thus excluding the possibility that a more rapid metabolism of nicotine might explain a greater risk of intake of smoke and thus a greater carcinogenic risk.

3. Epidemiology of menthol cigarette use -

Giovino GA, Sidney S, Gfroerer JC, O'Malley PM, Allen JA, Richter PA, Cummings KM.
Nicotine Tob Res. 2004 Feb;6 Suppl 1:S67-81.

ABSTRACT: Approximately one-fourth of all cigarettes sold in the United States are mentholated. An understanding of the consequences, patterns, and correlates of menthol cigarette use can guide the development and implementation of strategies to reduce smoking prevalence and smoking-attributable morbidity and mortality. This paper summarizes the literature on the health effects of mentholated cigarettes and describes various patterns of use as indicated by consumption and survey data from the United States and other nations. The epidemiological literature on menthol cigarettes and cancer risk is inconclusive regarding whether these cigarettes confer a risk for cancer above that

of nonmentholated varieties. Available data indicate that mentholated cigarettes are at least as dangerous as their nonmentholated counterparts. In addition, because mentholation improves the taste of cigarettes for a substantial segment of the smoking population and appears to mask disease symptoms, this additive may facilitate initiation or inhibit quitting. Menthol market share is high in the Philippines (60%), Cameroon (35%-40%), Hong Kong (26%), the United States (26%), and Singapore (22%). Newport has become the leading menthol brand in the United States. Surveys from four nations indicate that menthol use among adult smokers is more common among females than males. Among U.S. smokers, 68.9% of Blacks, 29.2% of Hispanics, and 22.4% of Whites reported smoking a mentholated variety. Research is needed to better explain factors that may influence menthol preference, such as marketing, risk perceptions, brand formulation, and taste preferences. Such research would guide the development of potentially more effective programs and policies.

COMMENTS: This paper summarizes the literature on the health effects of mentholated cigarettes and describes various patterns of use as indicated by consumption and survey data from the United States and other nations. The epidemiological literature on menthol cigarettes and cancer risk is inconclusive regarding whether these cigarettes confer a risk for cancer above that of nonmentholated varieties.

4. Adolescent menthol smokers: Will they be a harder target for cessation? -

Eric T. Moolchan
Nicotine & Tobacco Research Volume 6, Supplement 1 (February 2004) S93–S95

ABSTRACT: Menthol smoking may influence the development of tobacco addiction and related health consequences, yet limited data on menthol smoking by youth are available. We assessed usual brand menthol preference by Baltimore-area teenage smokers applying to a smoking cessation study between September 1999 and December 2002. Of a biethnic (Black and White) sample of 593 youths (mean age~15.5¡1.4 years, 51% female, 45% African American), the overwhelming majority (93%) were menthol smokers. Menthol preference rates were highest among African American girls and lowest among White boys. Overall, a statistically significant association was found between ethnicity and menthol preference, x2 (df~1)~19.4, pv.001. This association also was observed separately for girls, x2 (df~1)~9.21, p~.0024, and for boys, x2 (df~1)~9.59, p~.0020. Menthol smoking did not vary with age in either ethnic group. These findings of overwhelming menthol preference in a treatment-seeking sample of adolescents warrant further research on the developmental trajectory, cessation, and health-related impact of menthol smoking by youth.

COMMENTS: This study compared the prevalence of menthol preference of Baltimore adolescents of different genders and ethnicities. The study found an overwhelming preference for menthol cigarettes (93%) in teenagers participating in this study. Both ethnicity and gender were significant factors associated with menthol preference. Menthol preference rates were highest in African Americans and females, and lowest in

white males. The findings of this paper were not relevant to the health effects of menthol as an ingredient in cigarettes.

5. Menthol pharmacology and its potential impact on cigarette smoking behavior -

Karen Ahijevych, Bridgette E. Garrett
Nicotine & Tobacco Research Volume 6, Supplement 1 (February 2004) S17–S28

ABSTRACT: Menthol is the only tobacco additive promoted and advertised by the tobacco industry. Although a considerable body of research has examined the effects of menthol when it is administered alone and unburned, the effects of menthol when burned in cigarette smoke are more complex because it is administered in a matrix of more than 4,000 substances. Therefore, it is difficult to isolate potential pharmacological and toxic effects of menthol when it is administered in a smoke mixture. Menthol properties include cooling and local anesthesia, as well as effects on drug absorption and metabolism, bronchodilation and respiration changes, and electrophysiology. Subjective effects of smoothness and less harshness have been identified as reasons for menthol cigarette smoking, but findings have been inconclusive regarding the effect of menthol on carbon monoxide exposure and smoking topography parameters. Gaps in the research literature and future research areas include the following: (a) What is the role of menthol in tobacco reinforcement and addiction? (b) In the absence of nicotine, is menthol reinforcing? (c) Are the pharmacological and physiological effects of menthol mediated by a menthol-specific receptor or some other central nervous system–mediated action? (d) What are the influences of menthol and menthol metabolism on the metabolic activation and detoxification of carcinogens in tobacco smoke? and (e) Do differences exist in cigarette smoking topography in relation to the interaction of ethnicity, gender, and menthol cigarette preference? Answers to these questions will help to elucidate the function of menthol in cigarettes and its impact on smoking behavior.

COMMENTS: These authors reviewed the current knowledge regarding the impact associated with smoking mentholated cigarettes. In this review, the authors attempted to extrapolate the actions of menthol as a nontobacco additive to its potential pharmacological and physiological effects in cigarettes. The authors provided their response to a number of questions that were related to addiction. CNS mediated effects, interaction with race, sex and cigarette preference were all addressed.

6. Percutaneous penetration enhancers in cigarette mainstream smoke -

Smith CJ, Perfetti TA, Garg R, Martin P, Hansch C.
Food Chem Toxicol. 2004 Jan;42(1):9-15.

ABSTRACT: Percutaneous penetration enhancers (PPEs) are chemicals used to enhance the transdermal delivery of drugs. Fifty-eight of the approximately 150 PPEs used for the transdermal delivery of drugs have been reported in cigarette mainstream smoke (MS). MS is a complex aerosol of minute liquid droplets (termed the particulate phase) suspended within a mixture of gases (CO(2), CO, NO(x), etc.) and semi-volatile

compounds. The gases and many of the semi-volatiles are termed the vapor phase. Twenty-nine of the 58 PPEs have been identified in MS vapor phase, 15 in the particulate phase and 14 in both the vapor and particulate phases. There is a tendency for MS PPEs to be hydrophobic, with 40 of the 58 compounds (69%) being either hydrophobic or strongly hydrophobic, and only 24% being hydrophilic. Many of the 4800 known constituents of MS are hydrophilic and would not be expected to readily cross cell membranes or penetrate tissue when delivered as single compounds. The in vivo effect on biological activity of the juxtaposition within the cigarette smoke aerosol of the large number of hydrophilic constituents with the 58 PPEs is currently unknown. As an initial step in understanding this potential complex interaction, the 58 PPEs in MS have been identified and a number of molecular parameters related to the ability to penetrate tissue have been calculated, including MS concentration, measured and calculated base ten logarithm of the octanol-water partition coefficient (Mlog P and Clog P), molecular volume (MgVol) and calculated molar refractivity (CMR).

COMMENTS: Percutaneous penetration enhancers (PPEs) are used by pharmaceutical industry to enhance delivery of drugs that are poorly absorbed. This paper identifies 58 PPEs, including menthol, found in cigarette mainstream smoke and calculates molecular parameters related to the ability to penetrate tissues for each. The authors concluded that the interaction of PPEs in cigarette mainstream smoke with constituents of smoke aerosol cannot be accurately predicted at this time and warrants the study whole cigarette smoke rather than MS fractions.

POTASSIUM CARBONATE
CAS: 584-08-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

RUM AND RUM EXTRACT
CAS: 90604-30-1
CAS: 977089-45-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COCOA, COCOA SHELLS, EXTRACT, DISTILLATE, POWDER, ALKALIZED, ABSOLUTE AND TINCTURE
CAS: 08002-31-1
CAS: 84649-99-0
CAS: 68916-17-6
CAS: 95009-22-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GUAR GUM
CAS: 9000-30-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PRUNE JUICE AND CONCENTRATE
CAS: 90082-87-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ALCOHOL, INCLUDING SDA-4
CAS: 64-17-5

Number of relevant papers: 7

GENERAL COMMENTS:
There are numerous papers on a wide range of health effects of drinking alcohol and/or smoking cigarettes. Exposure to one or both substances is a risk factor for possible colon and gastric cancers, abortions, diseases of the mouth and throat, gastro-reflux disease, olfactory ability etc. Few papers are available that examines the effects of inhaled ethanol. While most of the reports did not involve inhalation of the test substance, they all addressed possible synergy between tobacco and alcohol consumption. All of these studies used high levels of EtOH exposure to produce the reported effects. These studies did not mimic the route of exposure nor concentration of EtOH that would be associated with this ingredient used in cigarettes.

1. Pathology of the olfactory epithelium: Smoking and ethanol exposure -

Vent J, Robinson AM, Gentry-Nielsen MJ, Conley DB, Hallworth R, Leopold DA, Kern RC.
Laryngoscope. 2004 Aug;114(8):1383-8.

ABSTRACT: To investigate the effects of tobacco smoke on the olfactory epithelium. Cigarette smoking has been associated with hyposmia; however, the pathophysiology is poorly understood. The sense of smell is mediated by olfactory sensory neurons (OSNs) exposed to the nasal airway, rendering them vulnerable to environmental injury and death. As a consequence, a baseline level of apoptotic OSN death has been demonstrated even in the absence of obvious disease. Dead OSNs are replaced by the mitosis and maturation of progenitors to maintain sufficient numbers of neurons into adult life. Disruption of this balance has been suggested as a common cause for clinical smell loss. This current study will evaluate the effects of tobacco smoke on the olfactory mucosa, with emphasis on changes in the degree of OSN apoptosis. STUDY DESIGN: A rat model was used to assess the olfactory epithelium after exposure to tobacco smoke. METHODS: Rats were exposed to tobacco smoke alone (for 12 weeks), smoke plus dietary ethanol (for the final 5 weeks), or to neither (control). Immunohistochemical analysis of the olfactory epithelium was performed using an antibody to the active form

of caspase-3. Positive staining for this form of the caspase-3 enzyme indicates a cell undergoing apoptotic proteolysis. RESULTS: Control rats demonstrated a low baseline level of caspase-3 activity in the olfactory epithelium. In contrast, tobacco smoke exposure triggered a dramatic increase in the degree of OSN apoptosis that affected all stages of the neuronal lineage. CONCLUSIONS: These results support the following hypothesis: smell loss in smokers is triggered by increased OSN death, which eventually overwhelms the regenerative capacity of the epithelium.

COMMENTS: This study assessed the degree of olfactory sensory neuron (OSN) apoptosis in rats exposed to tobacco smoke with and without ethanol. The report indicates that apoptosis, as demonstrated by caspase-3 activation, is significant after exposure but there was no additional or synergistic effect on caspase-3 activity with ethanol ingestion. This study has little relevance to ethyl alcohol added to cigarette smoke but the authors suggest that increased apoptotic death of OSNs caused by sinusitis and aging, overwhelms the regenerative capacity of the epithelium mediating clinical olfactory loss.

2. A 2-year follow-up study of cigarette smoking and risk of dementia -

D. Juan, D. H. D. Zhou, J. Li, J. Y. J. Wang, C. Gao and M. Chen
European Journal of Neurology Volume 11 Issue 4 Page 277 - April 2004

ABSTRACT: The report focused on investigating the relationship between cigarette smoking and dementia in elderly people through prospective studies. We did a 2-year follow-up study of elderly people. A total of 2820 participants aged 60 years old and over from six communities of Chongqing agreed to take part. Dementia was diagnosed with MMSE (Mini-Mental State Examination) and DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders). Participants were classified as never smokers, past smokers, and current smokers. During follow-up, we recorded incident cases of dementia. The association of smoking and dementia was investigated using proportional hazards regression analysis. A total of 121 incident cases of dementia were detected, of which 84 (69%) were Alzheimer's disease, 17 (14%) were vascular dementia, and 21(17%) were other dementia. Compared with never smokers, current smokers had an increased risk of Alzheimer's disease (RR = 2.72; 95% CI = 1.63–5.42) and vascular dementia (RR = 1.98; 95% CI = 1.53–3.12) adjusting for age, sex, education, blood pressure, and alcohol intake. Compared with light smokers, the adjusted risk of Alzheimer's disease was significantly increased among smokers with a medium level of exposure (RR = 2.56; 95% CI = 1.65–5.52), with an even higher risk of Alzheimer's disease in the heavy smoking group (RR = 3.03; 95% CI = 1.25–4.02). Smoking was associated with the risk of dementia. This study suggests that both smoking status and amount is associated with dementia.

COMMENTS: This paper describes a follow up to a previous study of the relationship between cigarette smoking and cognitive impairment among elderly people in China. Current smoking increased the risk of dementia even after adjusting for other risk factors such as age, sex, education, blood pressure and alcohol intake. However, the risk of

Alzheimer's disease and other forms of dementia was not associated with past smoking amount. The results of this study were not relevant to the health effects of ethyl alcohol as an ingredient in cigarettes.

3. Risk factors for oral and pharyngeal cancer in young adults

Rodriguez T, Altieri A, Chatenoud L, Gallus S, Bosetti C, Negri E, Franceschi S, Levi F, Talamini R, La Vecchia C.
Oral Oncol. 2004 Feb;40(2):207-13.

ABSTRACT: Mortality from oral cancer has been rising in the young in several areas of the world until the early 1990s. We analyzed data from two case-control studies from Italy and Switzerland including 137 cases of oral and pharyngeal cancer below age 46 and 298 hospital controls. The multivariate odds ratios (OR) were 20.7 for heavy smokers and 4.9 for heavy drinkers. The combination of high tobacco and alcohol consumption led to an OR of over 48. Body mass index (OR=0.28, for the highest tertile), high consumption of coffee (OR=0.25), fresh vegetables (OR=0.39), fruit (OR=0.73) and beta-carotene (OR=0.48) were inversely related to risk. Tobacco accounted for 77% of all cancer cases in this population, alcohol for 52%, low vegetable consumption for 52%, and the combination of the three factors for 85%.

COMMENTS: The authors examined the data from two large case-control studies of oral and pharyngel cancer. This report is not relevant to inhaled ethanol since the authors' conclusions are based on use of very high levels of alcohol and an exposure route that did not mimic inhalation. However, the authors' statements regarding the risk for oral/pharyngeal cancers and smoking may be of interest. Heavy consumption of both tobacco smoke and alcohol may result in an over 48-fold increase in health risk in young people. This tobacco-related risk substantially declines within a few years and was not substantially elevated after 5 years of stopping smoking.

4. Desensitization of PKA-stimulated ciliary beat frequency in an ethanol-fed rat model of cigarette smoke exposure -

Wyatt TA, Gentry-Nielsen MJ, Pavlik JA, Sisson JH.
Alcohol Clin Exp Res. 2004 Jul;28(7):998-1004

ABSTRACT: Our previous studies have shown that the ciliary beat frequency (CBF) of cultured ciliated airway epithelial cells exposed to chronic ethanol fails to increase in response to beta-agonist stimulation. This loss of the ciliary "flight response" correlates with an ethanol-mediated desensitization of adenosine 3':5'-cyclic monophosphate-dependent protein kinase (PKA), a known regulatory component of CBF stimulation. We hypothesized that a similar ethanol-mediated desensitization of CBF would occur in vivo. METHODS: Sprague Dawley rats were fed a liquid diet containing various concentrations of ethanol for 1 or 5 weeks. Half were exposed to cigarette smoke for 12 weeks and half were sham exposed. Animals were killed and tracheal epithelial cells analyzed for CBF and PKA activity. RESULTS: Baseline CBF (approximately 6 Hz) was

unchanged in tracheal epithelial cells of rats consuming diets containing 0-36% ethanol for 5 weeks. Isoproterenol stimulated CBF to 12 to 13 Hz in the tracheal epithelial cells of control rats not administered ethanol. However, isoproterenol stimulation of CBF was blunted to 7.5 Hz in rats eating a 26% ethanol diet, and there was no stimulation of CBF in rats fed a diet containing 36% ethanol. Similarly, isoproterenol stimulated a 2- to 3-fold increase in PKA activity in control rats, but this PKA response to isoproterenol was blunted in rats fed increasing concentrations of ethanol. No isoproterenol-stimulated PKA response was observed in rats fed 36% ethanol. No ethanol-induced changes in cyclic guanosine monophosphate-dependent protein kinase or protein kinase C were observed in the rats' tracheal epithelial cells. Cigarette smoke exposure slightly elevated baseline CBF and lowered the ethanol consumption level for isoproterenol-desensitization of CBF and PKA activation to 16%. No isoproterenol desensitization was observed after 1 week of alcohol feeding. Furthermore, 36% ethanol-feeding for 1 week stimulated rat tracheal CBF and PKA. CONCLUSION: These data demonstrate that in vivo administration of ethanol to rats results in decreased ciliary beating and the desensitization of PKA. This suggests a mechanism for mucociliary clearance dysfunction in alcoholics.

COMMENTS: These authors used a rat model to study the combined effects of smoking and ingestion of EtOH to examine the role that smoking has in alcohol-related lung disease. Chronic EtOH use results in desensitization of B-agonist stimulated ciliary beat frequency (CBF), both *in vivo* and *in vitro*, but short term exposure to EtOH does not. Combining cigarette smoke exposure with ethanol further decreases CBF. It is interesting that smoke exposure alone elevated CBF.

5. Percutaneous penetration enhancers in cigarette mainstream smoke.

Smith CJ, Perfetti TA, Garg R, Martin P, Hansch C.
Food Chem Toxicol. 2004 Jan;42(1):9-15.

ABSTRACT: Percutaneous penetration enhancers (PPEs) are chemicals used to enhance the transdermal delivery of drugs. Fifty-eight of the approximately 150 PPEs used for the transdermal delivery of drugs have been reported in cigarette mainstream smoke (MS). MS is a complex aerosol of minute liquid droplets (termed the particulate phase) suspended within a mixture of gases (CO(2), CO, NO(x), etc.) and semi-volatile compounds. The gases and many of the semi-volatiles are termed the vapor phase. Twenty-nine of the 58 PPEs have been identified in MS vapor phase, 15 in the particulate phase and 14 in both the vapor and particulate phases. There is a tendency for MS PPEs to be hydrophobic, with 40 of the 58 compounds (69%) being either hydrophobic or strongly hydrophobic, and only 24% being hydrophilic. Many of the 4800 known constituents of MS are hydrophilic and would not be expected to readily cross cell membranes or penetrate tissue when delivered as single compounds. The in vivo effect on biological activity of the juxtaposition within the cigarette smoke aerosol of the large number of hydrophilic constituents with the 58 PPEs is currently unknown. As an initial step in understanding this potential complex interaction, the 58 PPEs in MS have been identified and a number of molecular parameters related to the ability to penetrate tissue have been calculated, including MS concentration, measured and calculated base ten

logarithm of the octanol-water partition coefficient (Mlog P and Clog P), molecular volume (MgVol) and calculated molar refractivity (CMR).

COMMENTS: Percutaneous penetration enhancers (PPEs) are used by pharmaceutical industry to enhance delivery of drugs that are poorly absorbed. This paper identifies 58 PPEs, including ethanol, found in cigarette mainstream smoke. The molecular parameters related to the ability to penetrate tissues were calculated for each. The authors conclude that the interaction of PPEs in cigarette mainstream smoke with constituents of smoke aerosol cannot be accurately predicted at this time and warrants the study of whole cigarette smoke rather than MS fractions.

6. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.
Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-be nzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m3 for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including ethyl alcohol (126 $\mu g/m^3$). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The

results are consistent with those earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

7. In utero exposure to tobacco and alcohol modifies neurobehavioral development in mice offspring: consideration a role of oxidative stress

Li Y, Wang H, Li JF.
Pharmacol Res 2004; 49: 467-473

ABSTRACT: Objective: To determine whether in utero tobacco and alcohol exposure induces long-term neurobehavioral alterations and whether oxidative stress/damage is a possible causal factor. Methods: Gravid mice were subjected to tobacco smoking and alcohol consumption. Their offspring were subsequently evaluated in developmental and behavioral tests. Antioxidative enzymes and erythrocyte membrane fluidity of adult offspring were measured. Results: The intrauterine tobacco and alcohol exposure has resulted in significant reduced postnatal body and organ weights accompanied by reduced gestational body weight gain in their mothers. Such exposure also induced remarkable developmental delay in neonatal reflexes and notable behavioral deficit in adulthood, namely reduced motive coordination and locomotor activity as well as impaired learning and memory abilities. Furthermore, the formation of malondialdehyde (MDA) increased significantly whereas the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (Cat) and glutathione S-transferases (GST) decreased in the cerebral cortex and liver of prenatal intoxicated offspring. The embryonic intoxication also markedly reduced erythrocyte membrane fluidity in offspring. Conclusion: Our study shows the long-term neurotoxicity associated with prenatal tobacco and alcohol exposure, and suggests that the deleterious outcome may be in relation to increased free radicals formation and oxidative stress.

COMMENTS: Pregnant mice were exposed to cigarette smoke and wine in order to examine the prenatal effects of the combined substances. Significant reductions in body weight and delayed neurobehavioral development were observed in the pups of the treated mice. The effects appeared to be long-lasting and related to reductions in the enzyme-mediated antioxidant system. However, this paper was not directly relevant to the health effects of ethyl alcohol as an ingredient in cigarettes.

LICORICE ROOT, FLUID EXTRACT AND POWDER
CAS: 68916-91-6
CAS: 08008-94-4
CAS: 97676-23-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM PHOSPHATE DIBASIC (DIAMMONIUM PHOSPHATE)
CAS: 7783-28-0

Number of relevant papers: 1

1. The effect of tobacco blend additives on the retention of nicotine and solanesol in the human respiratory tract and on subsequent plasma nicotine concentrations during cigarette smoking -

Alan K. Armitage, Michael Dixon,* Barrie E. Frost, Derek C. Mariner,* and Neil M. Sinclair
Chem. Res. Toxicol., 17 (4), 537 -544

ABSTRACT: The influence of the tobacco additives diammonium hydrogen phosphate (DAP) and urea on the delivery and respiratory tract retention of nicotine and solanesol and on the uptake of nicotine into venous blood was investigated in 10 smokers under mouth-hold and 75 and 500 mL inhalation conditions. Three cigarettes with identical physical specifications were produced from a common lamina tobacco blend. The control cigarette contained nonammoniated reconstituted tobacco sheet (RTS), whereas DAP and other ammonia compounds were added to the RTS of the second cigarette. Urea was added to the tobacco of the third cigarette. The presence of DAP or urea in the test cigarettes did not significantly influence solanesol retention within the mouth during the mouth-hold condition. Nicotine retention within the mouth during the mouth-hold condition was, however, significantly higher for the DAP cigarette (64.3 ± 10.5%) than for the urea (53.3 ± 11.3%) or control cigarette (46.3 ± 8.6%), but this did not result in an increase in nicotine uptake into venous blood. Solanesol retentions during the 75 and 500 mL inhalation volume conditions and nicotine retentions during the 75 mL inhalation volume condition were not significantly different for the three cigarette types. Although the nicotine retention approached 100% with each cigarette type during the 500 mL inhalation condition, the nicotine retention for the urea-treated cigarette (99.6 ± 0.2%) was marginally, but statistically, significant, higher than for the control (99.1 ± 0.5%) and DAP-treated cigarettes (98.8 ± 0.6%). There were no statistically significant differences between the indices of nicotine uptake into venous blood for the three cigarette types in any of the inhalation conditions.

COMMENTS: It has been postulated that certain ammonium compounds when used as a tobacco additive can increase smoke pH thus increasing the transfer of nicotine from tobacco to the smoke and increasing the "addictiveness" of nicotine. This study assesses the retention of nicotine in the respiratory tract and its uptake into the blood system under controlled inhalation conditions. These results do not indicate that the addition of diammonium hydrogen phosphate or urea resulted in an enhanced uptake of nicotine from the respiratory tract into the systemic circulation during smoking. The authors found that most of the nicotine inhaled in cigarette smoke is absorbed irrespective of the

pH and that the pH does not affect bioavailability but instead influences the perceived strength of the cigarette.

AMMONIUM ALGINATE
CAS: 9005-34-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHOCOLATE AND CHOCOLATE LIQUOR
MAJOR
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

LACTIC ACID
CAS: 50-21-5
CAS: 598-82-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PLUM JUICE, CONCENTRATE AND EXTRACT
CAS: 90082-87-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CAROB BEAN GUM, ABSOLUTE AND EXTRACT
CAS: 9000-40-2
CAS: 84961-45-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

FIG JUICE CONCENTRATE AND EXTRACT
CAS: 90028-74-3
CAS: 68916-52-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SORBITOL
CAS: 50-70-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM HYDROXIDE
CAS: 1336-21-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GLUCOSE/ DEXTROSE
CAS: 50-99-7
CAS: 492-62-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

UREA
CAS: 57-13-6

Number of relevant papers: 1

1. The effect of tobacco blend additives on the retention of nicotine and solanesol in the human respiratory tract and on subsequent plasma nicotine concentrations during cigarette smoking -

Alan K. Armitage, Michael Dixon,* Barrie E. Frost, Derek C. Mariner,* and Neil M. Sinclair
Chem. Res. Toxicol., 17 (4), 537 -544

ABSTRACT: The influence of the tobacco additives diammonium hydrogen phosphate (DAP) and urea on the delivery and respiratory tract retention of nicotine and solanesol and on the uptake of nicotine into venous blood was investigated in 10 smokers under mouth-hold and 75 and 500 mL inhalation conditions. Three cigarettes with identical physical specifications were produced from a common lamina tobacco blend. The control cigarette contained nonammoniated reconstituted tobacco sheet (RTS), whereas DAP and other ammonia compounds were added to the RTS of the second cigarette. Urea was added to the tobacco of the third cigarette. The presence of DAP or urea in the test cigarettes did not significantly influence solanesol retention within the mouth during the mouth-hold condition. Nicotine retention within the mouth during the mouth-hold condition was, however, significantly higher for the DAP cigarette (64.3 ± 10.5%) than for the urea (53.3 ± 11.3%) or control cigarette (46.3 ± 8.6%), but this did not result in an increase in nicotine uptake into venous blood. Solanesol retentions during the 75 and 500 mL inhalation volume conditions and nicotine retentions during the 75 mL inhalation volume condition were not significantly different for the three cigarette types. Although the nicotine retention approached 100% with each cigarette type during the 500 mL inhalation condition, the nicotine retention for the urea-treated cigarette (99.6 ± 0.2%) was marginally, but statistically, significant, higher than for the control (99.1 ± 0.5%) and DAP-treated cigarettes (98.8 ± 0.6%). There were no statistically significant differences between the indices of nicotine uptake into venous blood for the three cigarette types in any of the inhalation conditions.

COMMENTS: It has been postulated that certain ammonium compounds when used as a tobacco additive can increase smoke pH thus increasing the transfer of nicotine from tobacco to the smoke and increasing the "addictiveness" of nicotine. This study assesses the retention of nicotine in the respiratory tract and its uptake into the blood system under controlled inhalation conditions. These results do not indicate that the addition of

diammonium hydrogen phosphate or urea resulted in an enhanced uptake of nicotine from the respiratory tract into the systemic circulation during smoking. The authors found that most of the nicotine inhaled in cigarette smoke is absorbed irrespective of the pH and that the pH does not affect bioavailability but instead influences the perceived strength of the cigarette.

SODIUM CARBONATE
CAS: 497-19-8

Number of relevant papers: 1

1. Cancer incidence in textile manufacturing workers in Australia

Fritschi L, Lakhani R, Nadon L.
J Occup Health 2004 Nov;46(6):493-6.

ABSTRACT: N/A

COMMENTS: The study was designed to assess the associated of incidence of cancer with the likely exposure to individual chemicals in textile manufacturing workers. There were no significant increases in relative risk of cancer associated with any of the 32 substances assessed, including sodium carbonate, which had a relative risk of 1.55.

FRUCTOSE
CAS: 57-48-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DAVANA OIL
CAS: 8016-03-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

LIME OIL
CAS: 68916-84-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL 2-METHYLBUTYRATE
CAS: 7452-79-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PEPPERMINT OIL AND ABSOLUTE AND PEPPERMINT OIL TERPENELESS
CAS: 8006-90-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SPEARMINT OIL
CAS: 8008-79-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ORANGE OIL AND EXTRACT (SWEET, DISTILLED, TERPENELESS, AND SOUR/BITTER ORANGE OILS)
CAS: 8008-57-9
CAS: 68606-94-0
CAS: 68916-04-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

MOLASSES EXTRACT
CAS: 8052-35-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORIANDER EXTRACT, SEED, AND OIL
CAS: 8008-52-4
CAS: 84775-50-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL VANILLIN
CAS: 121-32-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

L-MENTHONE
CAS: 14073-97-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

VANILLIN
CAS: 121-33-5

Number of relevant papers: 1

1. Mutagens and Sensitizers-An Unequal Relationship? -

A. M. Wolfreys A1 and D. A. Basketter A1
Journal of Toxicology: Cutaneous and Ocular Toxicology Volume 23, Number 3 / 2004 197 – 205.

ABSTRACT: For some years, those involved with the safety assessment of chemicals have in one way or another considered the degree to which data on either skin sensitization potential or on carcinogenicity may inform them on the other endpoint for a particular substance. In this work, we have taken a pragmatic perspective on the question and assessed mutagens, rather than carcinogens, and sensitizers as this better reflects the potential for biological macromolecule interaction. A dataset of 100 substances, the majority of which have come under scrutiny for one reason or another during our own toxicology investigations, was interrogated. We focused on the extent to which results from the primary screen for skin sensitization correlated with the results from the two in vitro tests used as a screen for mutagenicity, namely the bacterial mutation assay and the in vitro chromosome aberration assay. Although there was some concordance between the two endpoints, as standalone methods, neither predicted the other particularly accurately, with 32% showing disagreement. It is probable that there are several critical elements missing from this top level assessment, not least an appreciation of which substances are positive in mutagenicity tests via non genotoxic mechanisms which could seriously impair such a correlation between results from the two different endpoints.

COMMENTS: This paper discusses the relationship between skin sensitizers and carcinogens. Previous data indicate that chemicals that induced allergic contact dermatitis had a 50% chance of being a rodent carcinogen. To investigate this hypothesis the authors examined *in vitro* mutagenicity screening data on 100 chemicals and compared the results with information on skin sensitization potential of these substances. In these comparisons about one-third of the chemicals that were positive in the mutagenicity screen would not be classified as skin sensitizer. Vanillin was mutagenic but was not a skin sensitizer. The author's conclusion was that neither endpoint is a reliable indicator of the other.

CHAMOMILE FLOWER OIL, EXTRACT AND ABSOLUTE
CAS: 8002-66-2
CAS: 8015-92-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CATEGORY: STANDARD INGREDIENTS

1. Percutaneous penetration enhancers in cigarette mainstream smoke -

Smith CJ, Perfetti TA, Garg R, Martin P, Hansch C.
Food Chem Toxicol. 2004 Jan;42(1):9-15.

This paper examines a number of standard ingredients including:

BENZYL ALCOHOL 100-51-6
1,3-BUTANEDIOL 107-88-0
BUTYL ACETATE 123-86-4
CARBON DIOXIDE 124-38-9
ETHYL ACETATE 141-78-6
DECANOIC ACID 334-48-5
BUTYL ALCOHOL (1-BUTANOL) 71-36-3

ABSTRACT: Percutaneous penetration enhancers (PPEs) are chemicals used to enhance the transdermal delivery of drugs. Fifty-eight of the approximately 150 PPEs used for the transdermal delivery of drugs have been reported in cigarette mainstream smoke (MS). MS is a complex aerosol of minute liquid droplets (termed the particulate phase) suspended within a mixture of gases (CO2, CO, NOx, etc.) and semi-volatile compounds. The gases and many of the semi-volatiles are termed the vapor phase. Twenty-nine of the 58 PPEs have been identified in MS vapor phase, 15 in the particulate phase and 14 in both the vapor and particulate phases. There is a tendency for MS PPEs to be hydrophobic, with 40 of the 58 compounds (69%) being either hydrophobic or strongly hydrophobic, and only 24% being hydrophilic. Many of the 4800 known constituents of MS are hydrophilic and would not be expected to readily cross cell membranes or penetrate tissue when delivered as single compounds. The in vivo effect on biological activity of the juxtaposition within the cigarette smoke aerosol of the large number of hydrophilic constituents with the 58 PPEs is currently unknown. As an initial step in understanding this potential complex interaction, the 58 PPEs in MS have been identified and a number of molecular parameters related to the ability to penetrate tissue have been calculated, including MS concentration, measured and calculated base ten logarithm of the octanol-water partition coefficient (Mlog P and Clog P), molecular volume (MgVol)and calculated molar refractivity (CMR).

COMMENTS: Percutaneous penetration enhancers (PPEs) are used by pharmaceutical industry to enhance delivery of drugs that are poorly absorbed. This paper identifies 58 PPEs found in cigarette mainstream smoke and calculates molecular parameters related to the ability to penetrate tissues for each. The authors concluded that the interaction of PPEs in cigarette mainstream smoke with constituents of smoke aerosol cannot be accurately predicted at this time and warrants the study of whole cigarette smoke rather than MS fractions.

ACETANISOLE
CAS: 100-06-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ACETIC ACID
CAS: 64-19-7
SEE HIGH MUL'S INGREDIENTS

ACETOIN
CAS: 513-86-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ACETOPHENONE
CAS:98-86-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ACETYLPYRAZINE (2-)
CAS: 22047-25-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3-ACETYLPYRIDINE (BETA-ACETYLPYRIDINE)
CAS: 350-03-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-ACETYLTHIAZOLE
CAS: 24295-03-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DL-ALANINE, L-ALANINE
CAS: 302-72-7
CAS: 56-41-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALFALFA EXTRACT
CAS: 84082-36-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALLYL HEXANOATE
CAS: 123-68-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM ALGINATE
CAS: 9005-34-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM HYDROXIDE
CAS: 1336-21-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM PHOSPHATE DIBASIC (DIAMMONIUM PHOSPHATE)
CAS: 7783-28-0
SEE MAJOR INGREDIENTS

AMYL ALCOHOL
CAS: 71-41-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMYL BUTYRATE
CAS: 540-18-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMYL FORMATE
CAS: 638-49-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMYL OCTANOATE
CAS: 638-25-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALPHA-AMYLCINNAMALDEHYDE
CAS: 122-40-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

TRANS-ANETHOLE
CAS: 4180-23-8
CAS: 104-46-1

Number of relevant papers: 1

1. Cytotoxic and xenoestrogenic effects via biotransformation of trans-anethole on isolated rat hepatocytes and cultured MCF-7 human breast cancer cells -

Nakagawa Y, Suzuki T.
Biochem Pharmacol. 2003 Jul 1;66(1):63-73.

ABSTRACT: Themetabolism and action of trans-anethole (anethole) and the estrogen-like activity of the compound and its metabolites were studied in freshly isolated rat hepatocytes and cultured MCF-7 human breast cancer cells, respectively. The incubation of hepatocytes with anethole (0.25–2.0 mM) caused a concentration- and time-dependent cell death accompanied by losses of cellular ATP and adenine nucleotide pools. Anethole at a weakly toxic level (0.5 mM) was metabolized to 4-methoxycinnamic acid (4MCA), 4-hydroxy-1-propenylbenzene (4OHPB), and the monosulfate conjugate of 4OHPB; the levels of 4OHPB sulfate and 4MCA reached approximately 20 and 200 mM within 2 hr,respectively, whereas that of free unconjugated 4OHPB was less than approximately 0.5 mM. At amoderately toxic concentration (1.0 mM), unconjugated4OHPBreached approximately 10 mM, followed by abrupt loss of 30-phosphoadenosine 50-phosphosulphate (PAPS). Based on cell viability and adenine nucleotide levels, 4OHPB was more toxic than anethole and 4MCA. The addition of 2,6-dichloro-4-nitrophenol (50 mM), an inhibitor of sulfotransferase, enhanced the anethole-induced cytotoxicity associated with losses of ATP, PAPS, and 4OHPB sulfate, and symmetrically increased the unconjugated 4OHPB concentration. 4OHPB as well as diethylstilbestrol (DES) and bisphenol A (BPA), which are known xenoestrogenic compounds, competitively displaced 17b-estradiol bound to the estrogen receptor a in a concentration-dependent manner; IC50 values of these compounds were approximately 1 _ 10_5, 1 _ 10_8 and 5 _ 10_5 M, respectively. 4OHPB also caused a concentration (10_8 to 10_6 M)-dependent proliferation ofMCF-7 cells, whereas neither anethole nor 4MCA(10_9 to 10_5 M) affected cell proliferation. However, at higher concentrations (>10_4 M), 4OHPB rather than anethole and 4MCAwas cytotoxic. These results suggest that the biotransformation of anethole induces a cytotoxic effect at higher concentrations in rat hepatocytes and an estrogenic effect at lower concentrations in MCF-7 cells based on the concentrations of the hydroxylated intermediate, 4OHPB.

COMMENTS: The toxicity of trans-anethole and its metabolites were measured in rat hepatocytes and MCF-7 cells. Concentration-dependent and time-dependent cytotoxicity was observed in rat hepatocytes at anethole exposures ranging from 0.25 – 2. mM. The hydroxylated metabolite, 4-hydroxy-1-propenylbenzene (4OHPB) and not the parent compound, induced cytotoxic and estrogenic effects. Treatment with 4OHPB resulted in decreased cell viability and loss of intracellular levels of ATP and total adenine

nucleotide pools in hepatocytes. Estrogenic activity of 4OHPB was observed based on a proliferative assay of estrogen-responsive human breast cancer cells and a concentration-dependent displacement of 17β-estradiol bound to ERα. This study suggests that anethole may become cytotoxic and estrogenic via biotransformation and highlights the importance of using *in vivo* experiments to assess anethole toxicity.

ANGELICA ROOT EXTRACT AND OIL
CAS: 84775-41-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ANISE STAR OIL
CAS: 8007-70-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ANISYL ACETATE
CAS: 104-21-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

APPLE JUICE CONCENTRATE, ESSENCE AND EXTRACT
CAS: 85251-63-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

L-ARGININE
CAS: 74-79-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ASCORBIC ACID
CAS: 50-81-7

Number of relevant papers: 1

1. Cigarette smoke effects on salivary antioxidants and oral cancer - Novel concepts

Rafael M. Nagler MD DMD PhD and Abraham Z. Reznick PhD
Isr Med Assoc J 2004 Nov;6:691-4

ABSTRACT: Oral squamous cell carcinoma is the most common malignancy of the head and neck, with a worldwide incidence of over 300,000 new cases annually [1]. The disease is characterized by a high rate of morbidity and mortality (about 50%) [1±4]. The major inducer of oral SCC is exposure to tobacco, considered to be responsible for

50±90% of cases worldwide [5±7]. The incidence of oral SCC in cigarette smokers is four to seven times higher than in non-smokers; when alcohol is also consumed this incidence is even higher. Moreover, compared with non-smokers, the higher cigarette smoke-related risk for oral SCC is manifested by a reduction in the mean age of development of the disease by 15 years [8,9]. The ``field cancerization" concept is the currently accepted explanation for the carcinogenic effect of cigarette smoke on oral mucosa [10]. According to this theory, there is a constant and direct attack of various cigarette smoke reagents on the oral epithelial cells, which gradually accumulate and cause a step-wise malignant transformation. It has been suggested that free radicals, reactive oxygen species and reactive nitrogen species in the inhaled cigarette smoke induce this gradually evolving process, initially expressed by dysplastic lesions of the mucosa, are then trans-formed into in situ carcinoma lesions and eventually result in full-blown infiltrating and metastasizing oral SCC. Further credence for the suggested role of free radicals in the pathogenesis of evolving oral SCC is found in a recent study [11] demonstrating that ROS, such as hydroxyl radical, are formed in the human oral cavity during areca quid chewing, and that the activity might cause oxidative DNA damage to the surrounding tissues. In this respect the salivary anticarcinogenic capacity, which has only recently been recognized, may be based on its antioxidant system.

COMMENTS: Aspects of the salivary defense system are discussed including antioxidant enzymes (peroxidase and superoxide dismutase) and molecules such as uric acid and ascorbic acid. Cigarette smoke has been shown to reduce activity of salivary antioxidant enzymes, but not antioxidant molecules. Salivary peroxidase activity was not affected by exposure to purified aldehydes, nicotine or ascorbic acid, but appeared to be affected by hydrogen cyanide exposure. The enzyme activity returned to pre-smoking levels after 30 minutes, presumably due to the secretion of new saliva into the oral cavity.

L-ASPARTIC ACID
CAS: 56-84-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BALSAM PERU AND OIL
CAS: 8007-00-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BEESWAX RESINOID AND ABSOLUTE
CAS: 8006-40-4
CAS: 8012-89-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BEET JUICE CONCENTRATE
CAS: 89957-90-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZALDEHYDE
CAS: 100-52-7

SEE HIGH MUL'S INGREDIENTS

BENZALDEHYDE GLYCERYL ACETAL
CAS: 1319-88-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZOIC ACID
CAS: 65-85-0

Number of relevant papers: 1

1. Controversies in toxicology assessing food additive toxicity using a cell model -

Stefanidou M; Alevisopoulos G; Chatziioannou A; Koutselinis
Veterinary and Human Toxicology, 2003 , 45/2 (103-105)

ABSTRACT: Food additives are widely used for technological purposes and their presence is often substantial daily diet. They have also been accused for various toxic reactions in humans. The toxicity of the food color tartrazine, the preservatives sodium nitrate and sodium benzoate, and the antioxidant BHT, was studied using the protozoan Tetrahymena pyriformis as a toxicological model. The 4 food additives were added to Tetrahymena cultures and DNA content of the protozoan nuclei measured by an image analysis system. These food additives caused a statistically significant increase in DNA content suggesting stimulation of the mitotic process. This system may contribute to the investigation of the cellular action of food additives, since mitogenic stimuli substantiallly alter susceptibility to chemical carcinogenesis. (32 References)

COMMENTS: These investigators tested the cytotoxic effect of 4 food additives, including sodium benzoate using a protozoan assay. Sodium benzoate activity is dependent on the concentration of undissociated benzoic acid. Some individuals exhibit allergy to benzoates. All four of the additives produced significant increase in DNA synthesis in protozoa macronucleus. The authors suggest that when this effect occurs, other cell activities are also depressed such as phagocytosis.

BENZOIN, RESIN, RESINOID, TINCTURE, GUM AND ABSOLUTE
CAS: 9000-05-9
CAS: 84012-39-5
CAS: 9000-72-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZYL ALCOHOL
CAS: 100-51-6

Number of relevant papers: 3

1. Anti-estrogenic activity of fifty chemicals evaluated by in vitro assays

Joohee Jung , Kunie Ishida and Tsutomu Nishihara ,
Life Sciences Volume 74, Issue 25 , 7 May 2004, Pages 3065-3074

ABSTRACT: We examined the anti-estrogenic activity of 50 chemicals by the yeast two-hybrid assay and detected the activity of hexachlorophene, pentachlorophenol, and vitamin K3 (menadione), in that order. These chemicals were also
observed to inhibit the transcriptional activity of 17h-estradiol in a reporter gene assay system using MCF-7 cells, estrogen receptor-positive breast cancer cells, and to bind directly to estrogen receptor a in a competitive binding assay system, although the order of the activity was slightly different among the 3 assays. These findings suggested that three of fifty chemicals could inhibit estrogen activity by competitive binding with 17h-estradiol to the estrogen receptor.

COMMENTS: The inhibitory effect of various chemicals against 17β-estradiol was assessed using the yeast two-hybrid assay. Fifty chemicals, including benzyl alcohol were tested in a range from 10^{-3} to 10^{-9} M. Only three chemicals showed inhibition of estrogenic activity. No anti-estogenic activity was reported for benzyl alcohol within the range of concentrations tested.

2. Neurologic issues with solvents

Rutchik JS, Wittman RI.
Clin Occup Environ Med. 2004 Nov;4(4):621-56, v-vi.

ABSTRACT: Organic solvents are a chemical class of compounds that are used routinely in commercial industries. They possess a low molecular weight, share a similar structure, lipophilicity, and volatility, and theyexist in liquid at room temperature. They may be grouped further into aliphatic compounds that exist in chain form, such as n-hexane,and aromatic compounds that exist in a 6-carbon ring form, such as benzene or xylene. Aliphatics and aromatics may contain a substituted halogen element and may be referred to as halogenated hydrocarbons, such as perchloroethylene, trichloroethylene,

and crbon tetrachloride. Alcohols, ketones, glycols, esters,ethers, aldehydes, and pyridines exist due to substitutions for a hydrogen group.

COMMENTS: This is a well-documented review of neurologic effects from exposure to a variety of solvents. The only discussion focusing on benzyl alcohol was that it was shown to block neuronal action potentials reversibly *in vitro* and exposure of rat nerve roots results in scattered demyelination and axonal degeneration.

3. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.
Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-be nzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m3 for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including benzyl alcohol (52 $\mu g/m^3$). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The results are consistent with those of earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

BENZYL BENZOATE
CAS: 120-51-4

Number of relevant papers: 2

1. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.
Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-be nzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m3 for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including benzyl benzoate (3 - 694 µg/m3). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The results are consistent with those of earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

2. Inhibitory effects of the essential oil from SuHeXiang Wan on the central nervous system after inhalation -

Koo BS, Lee SI, Ha JH, and Lee DU
Biological & Pharmaceutical Bulletin Vol. 27 (2004), No. 4, 515-519.

ABSTRACT: The present study was performed to evaluate the central nervous system inhibitory effects of the essential oil from SuHeXiang Wan (Storax pill), a prescription usually used for treating epilepsy in traditional Chinese medicine, on fragrance inhalation (aroma therapy). Preinhalation of the fragrance oil markedly delayed the appearance of pentylenetetrazole-induced convulsion, but showed weak activities on picrotoxin- and strychnine-induced convulsions, which implies this drug may inhibit the convulsion by GABAergic neuromodulation. This essential oil inhibited the binding of [3H]Ro15-1788, a selective antagonist for the benzodiazepine receptor and also the binding of [3H]flunitrazepam, a selective agonist for the receptor, in the presence of g-aminobutyric acid (GABA) and NaCl, showing a positive GABA shift, which suggested the strong possibility of the agonistic activity of the essential oil to the GABA/benzodiazepine receptor complex in rat cerebral cortices. Furthermore, inhalation inhibited the activity of GABA transaminase as the inhalation period was lengthened. The GABA level was signifi- cantly increased and glutamate content was significantly decreased in mouse brain by preinhalation of the essential oil. The above results suggest that the anticonvulsive effect of this essential oil can also originate from the enhancement of GABA level in the mouse brain, because convulsion depends partially on GABA concentration which can be properly preserved by inhibiting GABA transaminase. Fragrance inhalation progressively prolonged the pentobarbital-induced sleeping time as inhalation time was lengthened and inhibited brain lipid peroxidation, to which the anticonvulsive action is attributed; this also supported the above results, confirming the inhibitory effects of the essential oil of SuHeXiang Wan on the CNS via the GABAergic system.

COMMENTS: Fragrance inhalation of essential oils which make up Chinese medicinal prescriptions was shown to possess anticonvulsive and sedative properties in mouse experiments. Anticonvulsive effect of the essential oils was attributed to enhanced GABA levels and decreased lipid peroxidation in mouse brain. Benzyl benzoate was one of 10 compounds detected in the essential oils, and accounted for only 5.4% of the content of the mixture. Therefore, the relevance of this study to the health effects of benzyl benzoate as an ingredient in cigarettes is minimal.

BENZYL CINNAMATE (PROPENIC ACID, 3-PHENYL, PHENYLMETHYL ESTER,2-)
CAS: 103-41-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZYL PHENYLACETATE
CAS: 102-16-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZYL PROPIONATE
CAS: 122-63-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BORNYL ACETATE
CAS: 76-49-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

1,3-BUTANEDIOL
CAS: 107-88-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2, 3-BUTANEDIONE (DIACETYL)
CAS: 431-03-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTANOIC ACID, 3-METHYL-, 4-METHYLPHENYL ESTER (PARA-TOLYL 3-METHYLBUTYRATE) (P-TOLYL ISOVALERATE)
CAS: 55066-56-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTTER, BUTTER ESTERS, AND BUTTER OIL
CAS: 91745-88-9
CAS: 97926-23-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTYL ACETATE
CAS: 123-86-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTYL ALCOHOL (1-BUTANOL)
CAS: 71-36-3

Number of relevant papers: 1

1. Chemically induced renal tubule tumors in the laboratory rat and mouse: Review of the NCI/NTP database and categorization of renal carcinogens based on mechanistic information

Edward Lock; Gordon Hard
Critical Reviews in Toxicology, Volume 34, Number 3, May-June 2004, pp. 211-299(89)

Abstract: The incidence of renal tubule carcinogenesis in male and female rats or mice with 69 chemicals from the 513 bioassays conducted to date by the NCI/NTP has been collated, the chemicals categorized, and the relationship between carcinogenesis and renal tubule hyperplasia and exacerbation of the spontaneous, age-related rodent disease chronic progressive nephropathy (CPN) examined. Where information on mechanism or mode of action exists, the chemicals have been categorized based on their ability to directly or indirectly interact with renal DNA, or on their activity via epigenetic pathways involving either direct or indirect cytotoxicity with regenerative hyperplasia, or exacerbation of CPN. Nine chemicals were identified as directly interacting with DNA, with six of these producing renal tubule tumors at high incidence in rats of both sexes, and in some cases also in mice. Ochratoxin A was the most potent compound in this group, producing a high tumor incidence at very low doses, often with metastasis. Three chemicals were discussed in the context of indirect DNA damage mediated by an oxidative free radical mechanism, one of these being from the NTP database. A third category included four chemicals that had the potential to cause DNA damage following conjugation with glutathione and subsequent enzymatic activation to a reactive species, usually a thiol-containing entity. Two chemicals were allocated into the category involving a direct cytotoxic action on the renal tubule followed by sustained compensatory cell proliferation, while nine were included in a group where the cell loss and sustained increase in renal tubule cell turnover were dependent on lysosomal accumulation of the male rat-specific protein, 2-globulin. In a sixth category, morphologic evidence on two chemicals indicated that the renal tumors were a consequence of exacerbated CPN. For the remaining chemicals, there were no pertinent data enabling assignment to a mechanistic category. Accordingly, these chemicals, acting through an as yet unknown mechanism, were grouped as either being associated with an enhancement of CPN mechanism, were grouped as either being associated with an enhancement of CPN (category 7, 16 chemicals), or not associated with enhanced CPN (category 8, 4 chemicals). A ninth category dealt with 11 chemicals that were regarded as producing increases in renal tubule tumors that did not reach statistical significance. A 10th category discussed 6 chemicals that induced renal tumors in mice but not in rats, plus 8 chemicals that produced a low incidence of renal tubule tumors in mice that did not reach statistical significance. As more mechanistic data are generated, some chemicals will inevitably be placed in different groups, particularly those from categories

7 and 8. A large number of chemicals in the series exacerbated CPN, but those in category 7 especially may be candidates for inclusion in category 6 when further information is gleaned from the relevant NTP studies. Also, new data on specific chemicals will probably expand category 5 as cytotoxicity and cell regeneration are identified as obligatory steps in renal carcinogenesis in more cases. Additional confirmatory outcomes arising from this review are that metastases from renal tubule tumors, while encountered with chemicals causing DNA damage, are rare with those acting through an epigenetic pathway, with the exception being fumonisin B1; that male rats and mice are generally more susceptible than female rats and mice to chemical induction of renal tubule tumors; and that a background of atypical tubule hyperplasia is a useful indicator reflecting a chemically associated renal tubule tumor response. With respect to renal tubule tumors and human risk assessment, chemicals in categories 1 and 2, and possibly 3, would currently be judged by linear default methods; chemicals in category 4 (and probably some in category 3) as exhibiting a threshold of activity warranting the benchmark approach; and those in categories 5 and 6 as representing mechanisms that have no relevance for extrapolation to humans.

COMMENTS: This paper provides a review of 69 chemicals tested in the National Cancer Institute / National Toxicology Program (NCI/NTP) carcinogenicity bioassay database including butyl alcohol. The selected chemicals are those that have shown an association with renal tubule tumors in rat and/or mouse. Butyl alcohol was placed in category 5, considered "chemicals inducing renal tumors via indirect cytotoxicity and sustained tubule cell regeneration associated with $\alpha_{2\mu}$-globulin accumulation." Chemicals placed in this category have a nongenotoxic mechanism that has no relevance for extrapolation to renal tumors in humans. However, data on butyl alcohol exposure in drinking water to female rats demonstrate a dose-related increase in the severity of chronic progressive nephropathy, and an increased incidence of thyroid gland follicular cell hyperplasia and adenomas in mice. This review was focused towards oral exposures and did not address inhalation exposure of butyl alcohol.

BUTYL BUTYRYL LACTATE (BUTOXY-1-METHYL-2-OXOETHYL ESTER BUTANOIC ACID, 2-)
CAS: 7492-70-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

N-BUTYL ISOVALERATE
CAS: 109-19-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3-BUTYLIDENEPHTHALIDE
CAS: 551-08-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTYRIC ACID
CAS: 107-92-6
SEE HIGH MUL'S INGREDIENTS

CAPRYLIC/CAPRIC TRIGLYCERIDE
CAS: 65381-09-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CARAMEL AND CARAMEL COLOR
CAS: 8028-89-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CARBON
CAS: 7440-44-0
SEE MAJOR INGREDIENTS

CARBON DIOXIDE
CAS: 124-38-9

Number of relevant papers: 3

1. CO_2 induced acute respiratory acidosis and brain tissue intracellular pH: A SUP31P NMR study in swine -

Martoft L.1; Stødkilde-Jørgensen H.2; Forslid A.3; Pedersen H.D.1; Jørgensen P.F.1
Laboratory Animals, Volume 37, Number 3, 1 July 2003, pp. 241-248(8)

ABSTRACT: High concentration carbon dioxide (CO2) is used to promote pre-slaughter anaesthesia in swine and poultry, as well as short-lasting surgical anaesthesia and euthanasia in laboratory animals. Questions related to animal welfare have been raised, as CO2 anaesthesia does not set in momentarily. Carbon dioxide promotes anaesthesia by lowering the intracellular pH in the brain cells, but the dynamics of the changes in response to a high concentration of CO2 is not known. Based on 31P NMR spectroscopy, we describe CO2-induced changes in intracellular pH in the brains of . ve pigs inhaling 90% CO2 in ambient air for a period of 60 s, and compare the results to changes in arterial blood pH, PCO2, O2 saturation and HCO¡3 concentration. The intracellular pH paralleled the arterial pH and PCO2 during inhalation of CO2; and it is suggested that the acute reaction to CO2 inhalation mainly re□ ects respiratory acidosis, and not metabolic regulation as for example transmembrane □ uxes of H‡=HCO¡3 . The intracellular pH decreased to approximately 6.7 within the 60 s inhalation period, and the situation was metabolically reversible after the end of CO2 inhalation. The fast decrease in intracellular

pH supports the conclusion that high concentration CO2 leads to anaesthesia soon after the start of inhalation.

COMMENTS: The objective of this study was to assess the acute response of intracellular pH changes in brain of pigs induced by inhalation of 90% CO_2 in ambient air for a period of 60 seconds and to relate these changes to arterial blood. Intracellular pH decreased from the start of CO_2 inhalation period at a higher pace than that observed in arterial pH, and reached levels (6.7) lower than that observed in arterial pH. Reversal to pre-exposure conditions of intracellular pH was also rapid. The authors predict that the levels might have returned more slowly if the pigs had been allowed to respire freely due to CO_2 induced neuronal depression, which would slow the exhalation of CO_2. The objective of this work was to resolve questions related to animal welfare following the high concentrations of carbon dioxide used to promote pre-slaughter anaesthesia in livestock. Because of the high concentrations of CO_2 used in this study, the extrapolation to the effects of CO_2 exposure from cigarette smoke is difficult.

2. TOXICOLOGICAL EVALUATION OF HONEY AS AN INGREDIENT ADDED TO CIGARETTE TOBACCO

Mari S. Stavanja, Paul H. Ayres, Daniel R. Meckley, Betsy R. Bombick, Deborah H. Pence, Michael F. Borgerding, Michael J. Morton, Arnold T. Mosberg, James E. Swauger
Journal of Toxicology and Environmental Health, Part A, 66:1453–1473, 2003

ABSTRACT: A tiered testing strategy has been developed to evaluate the potential for new ingredients, tobacco processes, and technological developments to increase or reduce the biological activity that results from burning tobacco. In the manufacture of cigarettes, honey is used as a casing ingredient to impart both aroma and taste. The primary objective of this document is to summarize and interpret chemical and toxicological studies that have been conducted to evaluate the potential impact of honey on the biological activity of either mainstream cigarette smoke or cigarette smoke condensate. As part of ongoing stewardship efforts, cigarettes produced with honey (5% wet weight) as an alternative to invert sugar in tobacco casing material were subjected to extensive evaluation. Principal components of this evaluation were a determination of selected mainstream smoke constituent yields, Ames assay, sister chromatid exchange assay in Chinese hamster ovary cells, a 30-wk dermal tumor promotion evaluation of cigarette smoke condensate in SENCAR mice, and a 13-wk inhalation study of cigarette smoke in Sprague-Dawley rats. Comparative analytical evaluations demonstrated that the substitution of honey for invert sugar as a casing material in cigarettes had no significant impact on mainstream smoke chemistry. In addition, in vitro and in vivo studies demonstrated that cigarettes containing tobacco cased with honey had comparable biological activity to cigarettes containing invert sugar. Collectively, these data demonstrate that the use of honey as an alternative casing material in the manufactureof cigarettes does not alter the potential toxicity of cigarette smoke condensate (CSC) or cigarette smoke; therefore the use of honey as an ingredient added to cigarette tobacco is acceptable from a toxicological perspective.

COMMENTS: This paper compares the use of honey in place of invert sugar as casing material in cigarettes. No differences were observed in carbon dioxide measured in the mainstream smoke chemistry between the two cigarettes (mean = 41 - 42.2 mg/cig). No differences in toxicological endpoints were observed between the reference cigarette and those including honey. This paper has minor relevant to assessing the effects of carbon dioxide as an ingredient in cigarettes, but does conclude that the substitution of honey for invert sugar as a casing material does not significantly alter smoke chemistry.

3. Acute carbon dioxide exposure in healthy adults: evaluation of a novel means of investigating the stress response -

Kaye J.1; Buchanan F.2; Kendrick A.2; Johnson P.1; Lowry C.1; Bailey J.3; Nutt D.3; Lightman S.1 Source: Journal of Neuroendocrinology, Volume 16, Number 3, March 2004, pp. 256-264(9)

ABSTRACT: Acute hypercapnia was studied to assess its potential as a noninvasive and simple test for evoking neuroendocrine, cardiovascular and psychological responses to stress in man. A single breath of four concentrations of carbon dioxide, 5%, 25%, 35%, and 50% was administered to nine healthy volunteers in a randomized, single-blind fashion. Although no adverse effects occurred, most subjects were unable to take a full inspired vital capacity breath of 50%. In response to the remaining exposures, subjective and somatic symptoms of anxiety increased in a dose-dependent manner. Unlike 5% and 25% CO2, 35% stimulated significant adrenocorticotropic hormone and noradrenaline release at 2 min. and cortisol and prolactin release at 15 mins. following inhalation. This same dose also provoked a significant bradycardia that was followed by an acute pressor response. No significant habituation of psychological, hypothalamic-pitutary-adrenal (HPA)or cardiovascular responses following 35% CO2 was seen when this dose was repeated after 1 week. A single breath of 35% CO2 safety and reliably produced sympathetic and HPA axis activation and should prove a useful addition to currently available laboratory tests of the human stress response.

COMMENTS: While the aim of this study was to evaluate the stress response to acute CO_2, the data does indicate that the response to hypercapria in normal individuals is dose-dependent and anxiety produced is transit. Exposure to 35% CO_2 stimulated the release of cortisol, adrenocorticotropic prolactin and noradrenaline hormone but not at concentrations of 5% or 25%. A single breath of 35% CO_2 also produced a marked systolic response that was preceded by a significant and persistent bradycardia. The lower doses did not have significant effect on cardiovascular parameters or catecholamine release.

CARDAMOM OLEORESIN, OIL, EXTRACT, SEED OIL, AND POWDER
CAS: 8000-66-6
CAS: 96507-91-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CAROB BEAN GUM, ABSOLUTE AND EXTRACT
CAS: 9000-40-2
CAS: 84961-45-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CAROTENE
CAS: 7235-40-7

Number of relevant papers: 8

1. Bacterial Mutagenicity testing of 49 food ingredients gives very few positive results.

PRIVAL M J,; SIMMON V F; MORTELMANS K E
GENETIC TOXICOLOGY BRANCH, FOOD DRUG ADMINISTRATION, 200 C STREET SW, WASHINGTON, DC 20204, USA USA
Mutation Research , Volume: 260 , Number: 4 , Page: 321-330 , 1991

ABSTRACT: 49 substances permitted for use in food in the United States were tested for mutagenicity in the Ames Salmonella typhimurium assay and in Escherichia coli strain WP2. Four of these substances caused increases in revertant counts in S. typhimurium. Two of these four (papain and pepsin) were found to contain histidine, and therefore the results of the tests on these two substances could not be taken as demonstrating mutagenicity. The other two substances causing increases in revertant counts (hydrogen peroxide and potassium nitrite) were mutagenic. The results on one chemical, .beta.-carotene, were evaluated as inconclusive or questionable. The remaining 44 substances were nonmutagenic in the test systems used. It is concluded that, for those generally physiologically innocuous chemicals tested, there are very few 'false positives' in the bacterial test systems used.

COMMENTS: The Salmonella Ames test and E coli mutagenicity assays were used to evaluate the mutagenicity of a number of food ingredients. ß-carotene did not give a significant and reproducible increase in mutant counts and thus ß-carotene is classified as questionable or inconclusive rather than a nonmutagen. ß-carotene is an insoluble chemical and the Ames test is not considered to be suitable for testing insoluble substances.

2. beta-Carotene exacerbates DNA oxidative damage and modifies p53-related pathways of cell proliferation and apoptosis in cultured cells exposed to tobacco smoke condensate

Palozza P, Serini S, Di Nicuolo F, Boninsegna A, Torsello A, Maggiano N, Ranelletti FO, Wolf FI, Calviello G, Cittadini A.
Carcinogenesis. 2004 Aug;25(8):1315-25. Epub 2004 Apr 8.

ABSTRACT: Human intervention trials have suggested that supplemental b-carotene resulted in more cancer in smokers, whereas it was protective in non-smokers. However, the mechanisms underlying these effects are still unknown. The aim of this study was to evaluate the effects of an association of cigarette smoke condensate (tar) and b-carotene on DNA oxidative damage and molecular pathways involved in cell cycle progression and apoptosis in cultured cells. In RAT-1 fibroblasts, tar caused increased levels of 8-hydroxyl-20-deoxyguanosine (8-OHdG) and this effect was enhanced by the concomitant presence of b-carotene (0.5--4.0 mM) in a dose- and time-dependent manner. In contrast, b-carotene alone did not significantly modify it. Fibroblasts treated with tar alone decreased their cell growth with respect to control cells through an arrest of cell cycle progression in the G0/G1 phase and an induction of apoptosis. These effects were accompanied by an increased expression of p53, p21 and Bax and by a decreased expression of cyclin D1. In contrast, fibroblasts treated with tar and b-carotene, after an initial arrest of cell growth at 12 h, re-entered in cell cycle and were unable to undergo apoptosis at 36 h. Concomitantly, their p53 expression, after an increase at 12 h, progressively returned at basal levels at 36 h by a mechanism independent of Mdm2. Such a decrease was followed by a decrease in p21 and Bax expression and by an increase in cyclin D1 expression. Moreover, the presence of the carotenoid remarkably enhanced cyclooxygenase-2 expression induced by tar. During tar treatment, a depletion of b-carotene was observed in fibroblasts. The effects of tar and b-carotene on 8-OHdG levels, cell growth and apoptosis were also observed in Mv1Lu lung, MCF-7 mammary, Hep-2 larynx and LS-174 colon cancer cells. This study supports the evidence for potential detrimental effects of an association between b-carotene and cigarette smoke condensate.

COMMENTS: This study explores a new mechanism for carcinogenic association between ß-carotene and cigarette smoke using cultured cells exposed to a combination of ß-carotene and tar. Together, ß-carotene and tar caused significant increases in oxidative DNA damage over either alone. These effects were both dose- and time- dependent and were observed over a range of ß-carotene concentrations from 0.75 - 4 µM, which corresponds to the concentrations in serum of subjects receiving supplements in clinical trials. Exposure to these substances together resulted in increased cell growth using RAT-1 fibroblasts and a clonogenic assay. Similar results were observed when tested with a variety of human tumor cell lines. The authors conclude that pro oxidant action of ß-carotene exacerbates DNA oxidative damage caused by cigarette smoke and induce changes in p53-related pathways. At low concentrations, ß-carotene increased DNA resistance to oxidative damage.

3. Effect of alpha-tocopherol and beta-carotene supplementation on coronary heart disease during the 6-year post-trial follow-up in the ATBC study. - 2004 -

Tornwall ME, Virtamo J, Korhonen PA, Virtanen MJ, Taylor PR, Albanes D, Huttunen JK.
Eur Heart J. 2004 Jul;25(13):1171-8.

ABSTRACT: Aims To evaluate the 6-year post-trial effects of a-tocopherol and b-carotene supplementation on coronary heart disease (CHD) in the a-tocopherol, b-carotene cancer prevention (ATBC) study. Methods and results 29 133 male smokers, aged 50–69 years were randomised to receive a-tocopherol 50 mg, or b-carotene 20 mg, or both, or placebo daily for 5–8 years. At the beginning of the post-trial follow-up, 23 144 men were still at risk for a first-ever major coronary event (MCE), and 1255 men with pre-trial history of myocardial infarction (MI) were at risk for MCE. Post-trial risk for MCE (n ¼ 2059) was 0.95 (95% confidence interval 0.87–1.04) among a-tocopherol recipients compared with non-recipients, and 1.14 (1.04–1.24) among b-carotene recipients compared with non-recipients. The risk for non-fatal MI (n ¼ 993) was 0.96 (0.85–1.09) and 1.16 (1.03–1.32), and for fatal CHD (n ¼ 1066) 0.94 (0.83–1.06) and 1.11 (0.99–1.25), respectively. Among men with pre-trial MI no effects were observed in post-trial risk of MCE (n ¼ 257). Conclusion b-Carotene seemed to increase the post-trial risk of first-ever non-fatal MI but there is no plausible mechanism to support it. Our findings do not advocate the use of a-tocopherol or b-carotene supplements in prevention of CHD among male smokers.

COMMENTS: Research continues to accumulate to attempt to uncover the underlying mechanism of action of ß-carotene toxicity. High doses have been shown to increase risk of lung cancer among smokers. ß-carotene has been suggested as a singlet oxygen quencher. These investigators report on post-trial effects of ß-carotene on major coronary events such as non-fatal MI and fatal CHD. These studies indicate that ß-carotene possibly increases the post-trial risk of first ever non-fatal myocardial infarction but they failed to suggest a possible mechanism to explain this effect.

4. The enigma of beta-carotene in carcinogenesis: What can be learned from animal studies. -

Robert M. Russell
The American Society for Nutritional Sciences J. Nutr. 134:262S-268S, January 2004

ABSTRACT: ß-carotene and other carotenoids have been thought to have anti-cancer activity, either because of antioxidant activity or because of their ability to be converted to vitamin A. Nevertheless, two large scale intervention studies in humans using high doses of ß-carotene found that B-carotene supplementation resulted in more lung cancer rather than less lung cancer among smoking and asbestos exposed populations. Studies conducted in the ferret have elucidated molecular mechanisms behind this observation, in that high-dose ß-carotene and smoke exposure in these animals leads to squamous metaplasia, a pre-cancerous lesion in the lung. High dose ß-carotene in the smoke exposed animals was found to give rise to a number of transient oxidative metabolites, which include P450 enzymes that result in the destruction of retinoic acid, and diminished retinoid signaling, and enhanced cell proliferation. In addition, eccentric cleavage ß-carotene metabolites facilitate the binding of smoke derived carcinogens to DNA. In other ferret studies low dose ß-carotene smoke exposure provided mild protection against squamous metaplasia. Thus, it appears that the explanation of the

apparent paradoxical effects of ß-carotene on lung cancer is related to dose. The metabolism and breakdown of natural products should be thoroughly investigated in animal models before embarking on large scale intervention trials, particularly when using unusually high doses that greatly exceed normal dietary levels.

COMMENTS: The study used ferrets as an animal model to assess the effects of ß-carotene in smoke-exposed animals. Localized proliferation of alveolar cells and alveolar macrophages with keratinized squamous epithelium was observed in animals given high dose ß-carotene (equivalent to 30 mg/d in humans), and the most severe responses (focal proliferation of alveolar cells, squamous metaplasia, and alveolar wall destruction) were observed in those exposed to both beta carotene and smoke. Cell proliferation was observed in both groups, but highest in the lung tissue of ferrets exposed to both ß-carotene and smoke. Retinoic acid levels were lower in both smoke-exposed and ß-carotene- treated groups as compared to controls. Using *in vitro* experiments, the authors demonstrated that lower ß-carotene levels in animals exposed to smoke were due to enhanced molecular breakdown. The authors propose a mechanism by which ß-carotene breakdown products might induce P450 enzyme activity resulting in the destruction of retinoic acid, and subsequent diminished retinoid signaling. The interference of this signaling pathway results in enhanced cell proliferation in ferret lung tissue. Oxidative products of ß-carotene also facilitate binding of benzo[a]pyrene metabolites to DNA. However, these effects appear to occur at high ß-carotene doses only, and not associated with low doses (equivalent to 6 mg in humans).

5. beta-Carotene: A cancer chemopreventive agent or a co-carcinogen?

Paolini M, Abdel-Rahman SZ, Sapone A, Pedulli GF, Perocco P, Cantelli-Forti G, Legator MS.
Mutat Res. 2003 Jun;543(3):195-200.

ABSTRACT: Evidence from both epidemiological and experimental observations have fueled the belief that the high consumption of fruits and vegetables rich in carotenoids may help prevent cancer and heart disease in humans. Because of its well-documented antioxidant and antigenotoxic properties, the carotenoid ß-carotene (ßCT) gained most of the attention in the early 1980s and became one of the most extensively studied cancer chemopreventive agents in population-based trials supported by the National Cancer Institute. However, the results of three randomized lung cancer chemoprevention trials on ßCT supplementation unexpectedly contradicted the large body of epidemiological evidence relating to the potential benefits of dietary carotenoids. Not only did ßct show no benefit, itwas associated with significant increases in lung cancer incidence, cardiovascular diseases, and total mortality. These findings aroused widespread scientific debate that is still ongoing. It also raised the suspicion that ßCT may even possess co-carcinogenic properties. In this review, we summarize the current data on the co-carcinogenic properties of ßCT that is attributed to its role in the induction of carcinogen metabolizing enzymes and the over-generation of oxidative stress. The data presented provide convincing evidence of the harmful properties of this compound if given alone to smokers, or to individuals exposed to environmental carcinogens, as a micronutrient

supplement. This has now been directly verified in a medium-term cancer transformation bioassay. In the context of public health policies, while the benefits of a diet rich in a variety of fruits and vegetables should continue to be emphasized, the data presented here point to the need for consideration of the possible detrimental effects of certain isolated dietary supplements, before mass cancer chemoprevention clinical trials are conducted on human subjects. This is especially important for genetically predisposed individuals who are environmentally or occupationally exposed to mutagens and carcinogens, such as those found in tobacco smoke and in industrial settings.

COMMENTS: This document provides a review of the literature related to the protective and carcinogenic actions of ß-carotene. Although ß-carotene is known to act as an antioxidant, it can also behave as a pro-oxidant at high oxygen pressure. The author described that ß-carotene itself does not exert cell transforming activity, but enhances the bioactivity and carcinogenicity of other compounds (i.e. benzo[a]pyrene) either through an induction of metabolizing enzymes (CYP) or generation of oxidative stress. These effects were observed at realistic concentrations observed in clinical trials using ß-carotene as a dietary supplement.

6. In vitro investigations into the interaction of beta-carotene with DNA: evidence for the role of carbon-centered free radicals -

Jos C. S. Kleinjans 1*, Marcel H. M. van Herwijnen 1, Jan M. S. van Maanen 1, Lou M. Maas 1, Theo M. C. M. de Kok 1, Harald J. J. Moonen 1, and Jacob J. Briedé 1
Carcinogenesis Advance Access

ABSTRACT: Supplementation by ß-carotene has unexpectedly appeared to increase lung cancer risk among smokers. In order to explain this, it has been suggested that at high serum levels of ß-carotene, prooxidant characteristics of ß-carotene may become manifest, yieldingreactive oxygen species (ROS) and inducing oxidative DNA damage. It has further been hypothesized that cigarette smoke carcinogens such as benzo(a)pyrene (B[a]P) and/or B[a]P metabolites, may directly react with ß-carotene; furthermore, □-carotene oxidation products may have a role in the bioactivation of B[a]P analogous to the peroxide-shunt pathway of cytochrome P-450 supported by cumene hydroperoxide. The aim of this study was to assess the effects of □-carotene on the formation of B[a]P-DNA adducts and oxidative DNA damage in vitro in isolated DNA, applying as metabolizing systems rat liver and lung metabolizing fractions, and lung metabolizing fractions from smoking and non-smoking humans. We established that □-carotene in the presence of various metabolizing systems was not able to induce oxidative DNA damage (8-oxo-dG), although ß-carotene is capable of generating ROS spontaneously in the absence of metabolizing fractions. Also, we could not find an effect of ß-carotene on DNA adduct formation induced by B[a]P upon metabolic activation. We could however provide evidence of the occurrence of a carbon-centered □-carotene radical which was found to be able to interact with B[a]P, and to intercalate with DNA.

COMMENTS: This study assessed the *in vitro* effects of β-carotene concentrations comparable with serum levels obtained during human intervention trials. No induction of oxidative DNA damage or benzo(a)pyrene-DNA adduct formation was associated with β-carotene exposure in the presence of various metabolizing systems. However, the authors suggest that a carbon-centered β-carotene radical may be capable of interacting with DNA and contribute to the mutagenic effects of DNA adducts formed by carcinogens. They conclude that a complex interaction including β-carotene cancer-promoting and anti-carcinogenic properties may exist *in vivo* and requires further research.

7. Neoplastic and antineoplastic effects of beta-carotene on colorectal adenoma recurrence: Results of a randomized trial. -

Baron JA, Cole BF, Mott L, Haile R, Grau M, Church TR, Beck GJ, Greenberg ER. Journal of the National Cancer Institute. Vol. 95, No. 10. May 21, 2003

ABSTRACT: In two large, randomized prevention trials, supplementation with ß-carotene increased the risk of lung cancer. Subjects in these studies were predominantly cigarette smokers, and the adverse effects were concentrated among those who also drank alcohol. Although ß-carotene supplementation appeared not to increase the risk of cancer generally, it is not clear if smoking and/or alcohol use alters the effect of ß-carotene on carcinogenesis at sites outside the lung. Methods: We studied the effect of ß-carotene supplementation on colorectal adenoma recurrence among subjects in a multicenter double-blind, placebo-controlled clinical trial of antioxidants for the prevention of colorectal adenomas. A total of 864 subjects who had had an adenoma removed and were polyp-free were randomly assigned (in a factorial design) to receive ß-carotene (25 mg or placebo) and/or vitamins C and E in combination (1000 mg and 400 mg, respectively, or placebo), and were followed with colonoscopy for adenoma recurrence 1 year and 4 years after the qualifying endoscopy. A total of 707 subjects had two followup examinations and provided smoking and alcohol use data.Adjusted multivariate risk ratios (RRs) and 95% confidence intervals (CIs) were used to assess the effects of ß-carotene on adenoma recurrence. Results: Among subjects who neither smoked cigarettes nor drank alcohol, ß-carotene was associated with a marked decrease in the risk of one or morerecurrent adenomas (RR = 0.56, 95% CI = 0.35 to 0.89), but ß-carotene supplementation conferred a modest increase in the risk of recurrence among those who smoked (RR = 1.36, 95% CI = 0.70 to 2.62) or drank (RR = 1.13, 95% CI = 0.89 to 1.43). For participants who smoked cigarettes and also drank more than one alcoholic drink per day, ß-carotene doubled the risk of adenoma recurrence (RR = 2.07, 95% CI = 1.39 to 3.08; P for difference from nonsmoker/nondrinker RR < .001). Conclusion: Alcohol intake and cigarettesmoking appear to modify the effect of ß-carotene supplementation on the risk of colorectal adenoma recurrence.

COMMENTS: Evidence indicates that cigarette smoking plays a role in carcinogenic effects seen with ß-carotene supplementation. However, the increase in lung cancer incidence was also associated with alcohol consumption, leading to the hypothesis that alcohol intake modifies the effect of ß-carotene to increase lung cancer risk. In this clinical trial, ß-carotene supplementation was beneficial (anti-neoplastic) in subjects who

did not smoke or drink but the proneoplastic risk increased (doubled) among those who smoke and drank alcohol. The authors suggest that smoking and use of alcohol modifies the effects of ß-carotene on the risk of colorectal cancers.

8. Exposing ferrets to cigarette smoke and a pharmacological dose of beta-carotene supplementation enhance in vitro retinoic acid catabolism in lungs via induction of cytochrome P450 enzymes. -

Liu C, Russell RM, Wang XD.
J Nutr. 2003 Jan;133(1):173-9.

ABSTRACT: In our previous studies, we found lower levels of retinoic acid (RA) in the lungs of ferrets exposed to cigarette smoke and/or a pharmacological dose of ß-carotene. To determine whether this is involved in excessive catabolism of RA via cytochrome P450 (CYP) induction, we carried out in vitro incubations of RA with the lungmicrosomal fractions of ferrets with or without CYP inhibitors and antibodies against CYP. The polar metabolites(4-oxo-RA and 18-hydroxy-RA) of RA metabolism after the incubation were analyzed by HPLC. Expressions of CYP(1A1, 1A2, 2E1 and 3A1) were examined using Western blot analysis. Incubation of various concentrations of RA with the lung microsomal fraction from ferrets exposed to cigarette smoke, a pharmacological dose of ß-carotene or their combination dose-dependently increased the levels of 4-oxo-RA and 18-hydroxy-RA compared with that of the control ferrets. At all RA concentrations, this increase was the greatest in lung tissue from the combined treatment group. Furthermore, this enhanced RA catabolism was substantially (80%) inhibited by nonspecific CYP inhibitors (disulfiram and liarozole), but was partially (50%) inhibited by resveratrol (CYP1A1 inhibitor), -naphthoflavone (CYP1A2 inhibitor) and antibodies against CYP1A1 and CYP1A2. Cigarette smoke exposure and/or pharmacological doses of ß-carotene increased levels of CYP1A1 and 1A2 by three- to sixfold but not levels of 2E1 and 3A1 in ferret lung tissue. These findings suggest that low levels of RA in the lung of ferrets exposed tocigarette smoke and/or pharmacological doses of ß-carotene may be caused by the enhanced RA catabolism via induction of CYP, CYP1A1 and CYP1A2 in particular, which provides a possible explanation for enhanced lung carcinogenesis seen with pharmacological doses of ß-carotene supplementation in cigarette smokers.

COMMENTS: Earlier studies by this group reported that ferrets exposed to cigarette smoke and fed ß-carotene, had increased molecular markers of cellular proliferation and histopathological changes in lung tissue. This study examined induction of cytochrome p450 enzymes (CYP) in ferret lung by smoke exposure and pharmacological doses (equivalent to human dose of 30 mg/d) of β-carotene. CYP1A1 and CYP1A2 were markedly higher in lung tissue of ferrets exposed to smoke, β-carotene, or both as compared to controls. The authors also established links between CYP induction and retinoic acid catabolism by cigarettes and/or β-carotene. Because of the action of retinoic acid on blocking squamous metaplasia in bronchial epithelium, the authors suggest that reduced retinoic acid levels may contribute to lung carcinogenesis, in addition to the bioactivation of carcinogens due to induced cytochrome p450 enzymes.

CARROT OIL, SEED
CAS: 8015-88-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4-CARVOMENTHENOL
CAS: 562-74-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CARYOPHYLLENE
CAS: 87-44-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CARYOPHYLLENE OXIDE
CAS: 1139-30-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CASSIA BARK, BUDS, OILS, AND EXTRACT
CAS: 8007-80-5
CAS: 84961-46-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CASTOREUM, LIQUID, EXTRACT, TINCTURE AND ABSOLUTE
CAS: 8023-83-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CELERY SEED OIL
CAS: 89997-35-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CELLULOSE AND CELLULOSE FIBER
CAS: 65996-61-4
CAS: 9004-34-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHAMOMILE FLOWER OIL, EXTRACT AND ABSOLUTE
CAS: 8002-66-2
CAS: 8015-92-7
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHICORY EXTRACT
CAS: 68650-43-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHOCOLATE AND CHOCOLATE LIQUOR

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

1,8-CINEOLE (EUCALYPTOL)
CAS: 470-82-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMALDEHYDE
CAS: 104-55-2

Number of relevant papers: 2

1. Structure-Activity Relationships for the Mutagenicity and Carcinogenicity of Simple and alpha-beta Unsaturated Aldehydes - 2003 - EMBASE® - US$2.94

Benigni R, Passerini L, Rodomonte A.
Environ Mol Mutagen. 2003;42(3):136-43.

ABSTRACT: Aldehydes are important industrial compounds that are used for the synthesis of chemicals and pharmaceuticals and as solvents, food additives, and disinfectants. Because of their reactivity, aldehydes are able to interact with electron-rich biological macromolecules and adverse health effects have been reported, including general toxicity, allergenic reactions, mutagenicity, and carcinogenicity. The cost, time, and number of animals necessary to adequately screen these chemicals places serious limitations on the number of aldehydes whose health potential can be studied and points to the need of using alternative methods for assessing, at least in a preliminary way, the risks associated with the use of aldehydes. A method of choice is the study of quantitative structure–activity relationships (QSARs). In the present work, we present QSAR models for the mutagenicity and carcinogenicity of simple aldehydes and _-_ unsaturated aldehydes. The models point to the role of electrophilicity, bulkiness, and hydrophobicity in the genotoxic activity of the aldehydes and lend themselves to the prediction of the activity of other untested chemicals of the same class.

COMMENTS: Although cinnamaldehyde and citrao were found to be inactive in the NTP bioassay, there are several aldehydes that are suspected genotoxic carcinogens. These authors used QSAR analysis to determine toxicity of these two compounds based on molecular structure properties of these chemicals. Using their model, citral was described as extremely weak (well below the potency range of mutagens) and cinnamaldehyde was described as very weak.

2. Toxicology and carcinogenesis studies of microencapsulated trans-cinnamaldehyde in rats and mice -

Hooth MJ, Sills RC, Burka LT, Haseman JK, Witt KL, Orzech DP, Fuciarelli AF, Graves SW, Johnson JD, Bucher JR.
Food Chem Toxicol. 2004 Nov;42(11):1757-68.

ABSTRACT: trans-Cinnamaldehyde is a widely used natural ingredient that is added to foods and cosmetics as a flavoring and fragrance agent. Male and female F344/N rats and B6C3F1 mice were exposed to microencapsulated trans-cinnamaldehyde in the feed for three months or two years. All studies included untreated and vehicle control groups. In the three-month studies, rats and mice were given diets containing 4100, 8200, 16,500, or 33,000 ppm trans-cinnamaldehyde. In rats, feed consumption was reduced in all exposed groups. In mice, feed consumption was reduced in the highest dose groups. Body weights of all treated males were less than controls. Body weights were reduced in female rats exposed to 16,500 or 33,000 ppm and female mice exposed to 8200 ppm or greater. All rats survived to the end of the study but some male mice in the highest dose groups died due to inanition from unpalatability of the dosed feed. The incidence of squamous epithelial hyperplasia of the forestomach was significantly increased in rats exposed to 8200 ppm or greater and female mice exposed to 33,000 ppm. In mice, the incidence of olfactory epithelial degeneration of the nasal cavity was significantly increased in males and females exposed to 16,500 ppm and females exposed to 33,000 ppm. In the two-year studies, rats and mice were exposed to 1000, 2100, or 4100 ppm trans-cinnamaldehyde. Body weights were reduced in mice exposed to 2100 ppm and in rats and mice exposed to 4100 ppm. In rats, hippuric acid excretion was dose proportional indicating that absorption, metabolism, and excretion were not saturated. No neoplasms were attributed to trans-cinnamaldehyde in rats or mice. Squamous cell papillomas and carcinomas of the forestomach were observed in male and female mice but the incidences were within the NTP historical control range and were not considered to be related to trans-cinnamaldehyde exposure.

COMMENTS: Although the oral route of exposure was used in these studies, the results described are of interest. The authors selected to test and characterize the toxicity of microencapsulated trans-cinnamaldehyde because of its structural similarity to cinnamyl anthranilate and 3,4,5-trimethoxy-cinnamaldehyde, two known rodent carcinogens. In a 3-month study, both rats and mice were exposed to concentrations ranging from 4000 to 33,000 ppm. A 2 years study exposed the test animals to concentrations of 1000, 2100, 4100 ppm. As expected the forestomach was the target organ for both species. There was a significant increase in hyperplasia in both rats and mice and in mice, olfactory epithelial degeneration was reported of the nasal cavity. In the 2-year study, no neoplasms were observed but olfactory epithelial pigmentation was reported in mice.

CINNAMON BARK, BUDS, LEAF, OIL, AND EXTRACT
CAS: 8015-91-6
CAS: 8007-80-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMYL ACETATE
CAS: 103-54-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMYL ALCOHOL
CAS: 104-54-1

Number of relevant papers: 2

1. Toxicology databases and the concept of thresholds of toxicological concern as used by the JECFA for the safety evaluation of flavouring agents

Renwick AG.
Toxicol Lett. 2004 Apr 1;149(1-3):223-34.

ABSTRACT: Since 1996 the FAO/WHO Joint Expert Committee on Food Additives (JECFA) has evaluated the safety of 1259 flavouring substances, based on a decision tree that incorporates a series of thresholds of toxicological concern. Safety conclusions are based on the predicted consequences of metabolism and whether the estimated intake is above or below a threshold of toxicological concern that is relevant to that compound. Compounds are allocated to one of three structural classes, and the intake compared with a threshold of toxicological concern derived using data from chronic and sub-chronic toxicity studies on compounds in the same structural class. If the substance is predicted to be metabolised to innocuous products there is no safety concern if the intake is below the threshold, but suitable toxicity data on the compound or structural analogues are required if the intake exceeds the threshold. If the substance is not predicted to be metabolized to innocuous products, and the intake is below the appropriate threshold, safety evaluation is based on data on the compound or structural analogues. An additional threshold of 1.5 _g per day, derived from doses of investigated chemicals giving a calculated cancer risk of one in a million, is applied when appropriate toxicity data are not available.

COMMENTS: This paper addresses the concept of "threshold of toxicity" as it relates to safety assessments of flavoring agents. The decision-making process for safety evaluation is reviewed, including chemical structural class allocation, consideration of predicted metabolism, estimated intake (per capita) and a comparison of the intake with the threshold of toxicological concern. Substances structurally related to menthol were included in a summary of the application of the procedure and all 14 compounds were classified as "no safety concern". However, this assessment is directed towards additives in food and does not attempt to address inhalation exposures.

2. The FEMA GRAS assessment of cinnamyl derivatives used flavor ingredients

Adams, T.B., Cohen, S.M., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Munro, I.C., Portoghese, P.S., Smith, R.L., Waddell, W.J., and Wagner, B.M. (2004) Food and Chemical Toxicology, 42, 157-185.

ABSTRACT: This publication is the seventh in a series of safety evaluations performed by the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA). In 1993, the Panel initiated a comprehensive program to re-evaluate the safety of more than 1700 GRAS flavoring substances under conditions of intended use. Elements that are fundamental to the safety evaluation of flavor ingredients include exposure, structural analogy, metabolism, pharmacokinetics and toxicology. Flavor ingredients are evaluated individually and in the context of the available scientific information on the group of structurally related substances. Scientific data relevant to the safety evaluation of the use of cinnamyl derivatives as flavoring ingredients is evaluated.

COMMENT: This panel evaluated the safety of cinnamyl derivatives used as flavor ingredients. These compounds were reaffirmed as GRAS. Acute oral LD50 in mice and rats indicated a low level of toxicity. Reproductive/developmental studies with this compound indicted no observed effects. This panel did report that this compound was found to have inhibitory effects on platelet function. Increase inhibition of platelet aggregation correlated with increase lipophilicity of the test substance.

CINNAMYL CINNAMATE
CAS: 122-69-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CITRAL
CAS: 5392-40-5

Number of relevant papers: 5

1. Structure-Activity Relationships for the Mutagenicity and Carcinogenicity of Simple and alpha-beta Unsaturated Aldehydes -

Benigni R, Passerini L, Rodomonte A.
Environ Mol Mutagen. 2003;42(3):136-43.

ABSTRACT: Aldehydes are important industrial compounds that are used for the synthesis of chemicals and pharmaceuticals and as solvents, food additives, and disinfectants. Because of their reactivity, aldehydes are able to interact with electron-rich biological macromolecules and adverse health effects have been reported, including general toxicity, allergenic reactions, mutagenicity, and carcinogenicity. The cost, time,

and number of animals necessary to adequately screen these chemicals places serious limitations on the number of aldehydes whose health potential can be studied and points to the need of using alternative methods for assessing, at least in a preliminary way, the risks associated with the use of aldehydes. A method of choice is the study of quantitative structure–activity relationships (QSARs). In the present work, we present QSAR models for the mutagenicity and carcinogenicity of simple aldehydes and _-_ unsaturated aldehydes. The models point to the role of electrophilicity, bulkiness, and hydrophobicity in the genotoxic activity of the aldehydes and lend themselves to the prediction of the activity of other untested chemicals of the same class.

COMMENTS: Although cinnamaldehyde and citral were found to be inactive in the NTP bioassay, there are several aldehydes that are suspected genotoxic carcinogens. These authors used QSAR analysis to determine toxicity of these two compounds based on molecular structure properties of these chemicals. Using their model, citral was described as extremely weak (well below the potency range of mutagens) and cinnamaldehyde was described as very weak.

2. Toxicology and carcinogenesis studies of microencapsulated Citral in rats and mice -

Ress NB, Hailey JR, Maronpot RR, Bucher JR, Travlos GS, Haserman JK, Orzech DP, Johnson JD, Hejmancik MR.
Toxicolgical Sciences. 71, 198-206, 2003

ABSTRACT: Citral, a widely used natural ingredient, is added to foods and cosmetics as a flavoring and fragrance agent. Male and female F344/N rats and B6C3F1 mice were exposed to microencapsulated citral in the feed for 14 weeks or two years. All studies included untreated and vehicle control groups. In the 14-week studies, rats and mice were given diets containing 3900, 7800, 15,600, or 31,300 ppm citral. In rats, food consumption was reduced in the two highest dose groups. In mice an apparent increase in food consumption was observed, but was due to mice scattering the feed. Body weights of all treated animals were less than controls. All rats and four male mice were killed moribund in the high dose groups. In rats, forestomach and kidney lesions were observed. At the higher doses, lesions observed in the bone marrow, testes, and thymus in rats and in the ovary in mice were considered related to inanition and resultant moribundity. In the two-year studies, rats were exposed to 1000, 2000, or 4000 ppm citral. Body weights were reduced in the 4000 ppm rats. Mice were exposed to 500, 1000, or 2000 ppm citral. Body weights in the 1000 and 2000 ppm groups were reduced. No neoplasms were attributed to citral in rats or mice. Malignant lymphoma occurred with a positive trend and was significantly greater than controls in female mice in the 2000 ppm group. However, the incidences were within the NTP historical control range and could not be clearly related to citral administration.

COMMENTS: Citral was administered through the diet of rats and mice and evaluated for toxicity and carcinogenicity. Exposures were conducted for 14 weeks and 2 years with maximum concentrations in the diet of 31,300 ppm and 4000 ppm, respectively. The

minimum daily doses in the 2-year study were more than 10 times greater than the average daily intake in humans. Palatability issues resulted in decreased food consumption and lower weight gain in both species. Transient treatment-related hematological and serum biochemical effects were noted in rats, but were consistent with physiological responses related to decreased food and water consumption. Nephropathy with renal tubule granular casts was observed in treated male rats from the 14-week treatment, but no citral-related kidney neoplasms were observed in the 2-year study. In mice, there was an increase in the incidences of malignant lymphoma in the highest treatment groups during the 2-year study, but this incidence was low and within the historical range of control female mice fed similar diets. Extrapolation of the findings of this study to the effects of citral as an ingredient in cigarettes is difficult because of the route of exposure (diet) and the high concentrations of citral used in this study which were far above the expected exposure through cigarette smoke.

3. Classification of Diverse Organic Compounds That Induce Chromosomal Aberrations in Chinese Hamster Cells -

McElroy NR, Thompson ED, Jurs PC.
J Chem Inf Comput Sci. 2003 Nov-Dec;43(6):2111-9.

ABSTRACT: A data set of 297 diverse organic compounds that cause varying degrees of chromosomal aberrations in Chinese hamster lung cells is examined. Responses of an assay are categorized as clastogenic (>10% aberrant cells) and nonclastogenic (<5% aberrant cells). Each of the compounds is represented by calculated structural descriptors that encode topological, geometric, electronic, and polar surface features. A genetic algorithm (GA) employing a k-nearest neighbor (kNN) fitness evaluator is used to iteratively search a reduced descriptor space to find small, information-rich subsets of descriptors that maximize the classification rates for clastogenic and nonclastogenic responses. To further improve modeling, a similarity measure using atom-pair descriptors is employed to create more homogeneous data subsets. Three different data sets are examined. Results for a set of 297 compounds using the GA-kNN method were 86.5% and 80.0% correct classification in the training set and prediction set, respectively. Results for a subset of 279 compounds in model 2 are 85.7% and 85.7% for the training and prediction sets, respectively. Results for a subset of 182 compounds in model 3 are 91.5% and 94.4% for the training and prediction sets, respectively. Creating smaller, more topologically similar data sets result in improved classification rates.

COMMENTS: Predictive classification models were designed that link molecular structure of 297 organic compounds to their genotoxic potential, as determined by chromosomal aberration assays using Chinese hamster lung cells. The predictive ability of the models was examined using external data sets. Citral was predicted correctly to be nonclastogenic, defined as inducing fewer than 5% aberrant cells. The relevance of this study to citral as an ingredient in cigarette smoke is minimal except for the potential of such predictive models to be applied to effects assessments for smoke components.

4. Analysis of thresholds for carcinogenicity. -

William J. Waddell ,
Toxicology Letters Volume 149, Issues 1-3 , 1 April 2004, Pages 415-419
Proceedings of EUROTOX 2003. The XLI European Congress of Toxicology. Science for Safety

ABSTACT: Re-evaluations of large prominent studies, e.g. the ED01 study and N-nitrosodiethylamine, unequivocally have demonstrated that thresholds exist for carcinogenicity when the dose–response curves for animal studies done at high doses are calculated according to fundamental principles of chemistry. This requires dose to be on a logarithmic scale and percent tumors on a linear scale. Fifteen compounds approved by the Flavor and Extract Manufacturers Association (FEMA) expert panel as Generally Recognized As Safe (GRAS) have been reported to be carcinogenic in rodent studies. The thresholds for tumors of these flavors were at least several orders of magnitude greater than the estimated daily dose of these flavoring agents to individuals in the United States. Similarly, comparisons of thresholds of carcinogenicity of chemicals and drugs to which humans are exposed with their exposure levels suggest that experimental animals are more sensitive to arcinogenicity than humans. The animal studies should be viewed as providing evidence for the safety of these flavors and other compounds at current levels of human exposure.

COMMENTS: This author has published extensively, presenting good evidence for thresholds of carcinogenicity of flavors. This paper examines the threshold for 6 compounds, providing estimates of the current level of exposure and a safety factor for each chemical. For citral the minimum safety ratio of 407 was suggested. The authors suggest that the actual safety ratios are probably greater.

5. Safety evaluations of food chemicals by "COMPACT" 1. A study of some acyclic terpenes

Lewis DF, Ioannides C, Walker R, Parke DV.
Food Chem Toxicol. 1994 Nov;32(11):1053-9.

ABSTRACT: A group of 19 acyclic terpenes have been evaluated for potential toxicity/carcinogenicity by molecular orbital determinations of their spatial and electronic parameters, and hence prediction of their metabolic activation or detoxication by the cytochrome P-450 (CYP) superfamily of mixed-function oxidase enzymes. Previous studies have characterized the spatial dimensions of the CYP1A1, 1A2 and 2E1 enzymes, which are known to activate mutagens and carcinogens and to be involved in other mechanisms of toxicity. None of the terpenes was found to have shape or electronic parameters appropriate for metabolic activation by CYP1A1 or 1A2, and hence they are unlikely to be carcinogenic or mutagenic. Furthermore, none of these chemicals had spatial parameters critical for substrates of CYP2E, and they are therefore unlikely to induce the formation of reactive oxygen species (ROS) or to initiate or promote malignancy or toxicity by mechanisms involving ROS. However, citral, and others of

these terpenes are known to undergo metabolism to carboxylic acids that may induce CYP4, and are therefore possible inducers of hepatic peroxisomal proliferation at high dosage, which may have implications for possible hepatotoxicity.

COMMENTS: Abstract sufficient, no additional comments needed.

CITRIC ACID
CAS: 77-92-9

Number of relevant papers: 1

1. Cough reflex induced by microinjection of citric acid into the larynx of guinea pigs: New coughing model. -

Tanaka M, Maruyama K.
J Pharmacol Sci. 2003 Dec;93(4):465-70.

ABSTRACT: We developed a new coughing model that evoked coughs by microinjection of citric acid into the larynx in unanesthetized unrestrained guinea pigs; additionally, we recorded synchronous sounds and waveforms of coughing utilizing built-in microphones and a whole body plethysmograph. The coughing model was able to distinguish a coughing response from other expiratory responses, such as an expiratory reflex or a sigh, by examining the waveform of the expiratory response and the existence of sound. It was not necessary to distinguish a cough from a sneeze, since the administration site was restricted to the larynx. Microinjection of 0.4 M citric acid, total of 20 _l (10 times, 2 _l at 30-s intervals), induced coughs (27.03 _ 4.03 coughs in 10-min observation) that were stable and independent of the inhalation volume. In the inhalation studies, animals were exposed to citric acid only once, because the number of coughs remarkably decreased with repeated administration at intervals of 24 h (tachyphylaxis). However our coughing model was able to repeatedly challenge the microinjection of citric acid at an interval of 24 h. These results indicated that this coughing model was highly sensitive and correctly assessed the cough response.

COMMENTS: Using unanesthetized, unrestrained guinea pigs, these authors demonstrated that microinjection of citric acid stimulated both the larynx and the bifurcation of the trachea, inducing cough and bronchoconstriction.

CITRONELLA OIL
CAS: 8000-29-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CITRONELLOL
CAS: 106-22-9
SEE NEW INGREDIENTS

CLARY SAGE OIL AND EXTRACT
CAS: 8016-63-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COCOA, COCOA SHELLS, EXTRACT, DISTILLATE, POWDER, ALKALIZED, ABSOLUTE AND TINCTURE
CAS: 8002-31-1
CAS: 84649-99-0
CAS: 68916-17-6
CAS: 95009-22-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COCONUT OIL
CAS: 8001-31-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COFFEE AND COFFEE SOLID EXTRACT
CAS: 8001-67-0
CAS: 68916-18-7
CAS: 84650-00-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COGNAC WHITE AND GREEN OIL
CAS: 8016-21-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORIANDER EXTRACT, SEED, AND OIL
CAS: 8008-52-4
CAS: 84775-50-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORN STARCH
CAS: 9005-25-8
SEE NEW INGREDIENTS

BETA-DAMASCONE
CAS: 23726-92-3
CAS: 23726-91-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DAVANA OIL
CAS: 8016-03-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DECANAL
CAS: 112-31-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DELTA-DECALACTONE
CAS: 705-86-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GAMMA-DECALACTONE
CAS: 706-14-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DECANOIC ACID
CAS: 334-48-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIACETYL
CAS: 431-03-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIETHYL MALONATE
CAS: 105-53-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,3-DIETHYLPYRAZINE
CAS: 15707-24-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,6-DIMETHOXYPHENOL
CAS: 91-10-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIMETHYL BENZYL CARBINYL BUTYRATE (ALPHA, ALPHA-DIMETHYLPHENETHYL BUTYRATE)
CAS: 10094-34-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIMETHYL SULFIDE
CAS: 18-50-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3,4-DIMETHYL-1,2-CYCLOPENTADIONE
CAS: 13494-06-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3,7-DIMETHYL-1,3,6-OCTATRIENE
CAS: 13877-91-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4,5-DIMETHYL-3-HYDROXY-2,5-DIHYDROFURAN-2-ONE (3-HYDROXY-4,5-DIMETHYL-2(5H)FURANONE)
CAS: 28664-35-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2, 5-DIMETHYL-4-HYDROXY-3(2H)-FURANONE (4-HYDROXY-2,5-DIMETHYL-3(2H)FURANONE) 3658-77-3
and
2,3-DIMETHYLPYRAZINE 5910-89-4
STANDARD

Number of relevant papers: 1

1. The influence of cigarette moisture to the chemistry of particulate phase smoke of a common commercial cigarette –
Zha, Q; Moldoveanu S C, (Reprint)
Beitraege zur Tabakforschung International , Volume: 21 , Number: 3 , Page: 184-191, October 2004 , 2004

ABSTRACT: This study presents the results on the influence of cigarette moisture content to the chemical composition of particulate phase smoke. Seventy-five selected compounds were monitored for the comparison of particulate phase smoke of a commercial full-flavored (FF) cigarette with three different moisture contents at 7.8%, 14.5% and 20.4%, respectively. It was demonstrated that the smoke of a dry cigarette is richer in lower molecular mass compounds than a regular cigarette. On the other hand, the smoke of a moist cigarette is richer in higher molecular mass compounds than a regular cigarette. To maximize the influence of cigarette moisture to the chemical composition, a separate set of measurements were done using only the first three puffs of smoke. The accumulation of moisture in the tobacco column of a burning cigarette may influence the smoke composition, as generated during burning. The differences between dry, regular and moist cigarettes were more obvious for the first three puffs.

COMMENTS: While this is not a health effect study, the results are interesting. These investigators compared the chemical composition of cigarette smoke from cigarettes with three moisture levels (dry-8.3%, regular-11.6%, and moist- 12.9%). The first three puffs showed the greatest differences. The nicotine content and total particulate matter (TPM) was reduced with increasing moisture. The data would indicate that the dry cigarette had higher percentage of semi-volatile compounds in TPM. The data presents additional evidence that the moisture content in cigarette significantly affects the chemistry of the particulate phase of smoke. Of the 75 compounds tested the more volatile compounds were more affected than the less volatile compounds. Compared to the first three puffs, the particulate phase of smoke from the entire cigarette was less sensitive to the moisture content.

3,7-DIMETHYL-6-OCTENOIC ACID (CITRONELLIC ACID)
CAS: 502-47-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALPHA,PARA-DIMETHYLBENZYL ALCOHOL
CAS: 536-50-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,5-DIMETHYLPYRAZINE
CAS: 123-32-0
SEE HIGH MUL'S INGREDIENTS

DODECAHYDRO-3A,6,6,9A-TETRAMETHYLNAPHTHO (2,1-B)FURAN (1,5,5,9-TETRAMETHYL-13-OXATRICYCLO(8.3.0.0(4,9))TRIDECANE)
CAS: 3738-00-9
CAS: 6790-58-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DELTA-DODECALACTONE
CAS: 713-95-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GAMMA-DODECALACTONE
CAS: 2305-05-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ACETATE
CAS: 141-78-6

Number of relevant papers: 1

1. Subchronic inhalation neurotoxicity studies of ethyl acetate in rats. -

Christoph GR, Hansen JF, Leung HW.
Neurotoxicology. 2003 Dec;24(6):861-74.

ABSTRACT: Rats were exposed to 0, 350, 750 or 1500 ppm of ethyl acetate by inhalation for 6 h per day, 5 days per week for 13 weeks. Functional observational battery (FOB) and motor activity tests occurred on non-exposure days during weeks 4, 8 and 13, after which tissues were microscopically examined for neuropathology. A subset of rats was monitored during a 4-week recovery period. Exposure to 750 and 1500 ppm, diminished behavioral responses to unexpected auditory stimuli during the exposure session and appeared to be an acute sedative effect. There were no signs of acute intoxication 30 min after exposure sessions ended. Rats exposed to 750 and 1500 ppm had reduced body weight, body weight gain, feed consumption, and feed efficiency, which fully or partially recovered within 4 weeks. Reductions in body weight gain and feed efficiency were observed in male rats exposed to 350 ppm. The principal behavioral effect of subchronic exposure was reduced motor activity in the 1500 ppm females, an effect that was not present after the 4-week recovery period. All other FOB and motor activity parameters were unaffected, and no pathology was observed in nervous system tissues. Operant sessions were conducted in another set of male rats preconditioned to a stable operant baseline under a multiple fixed ratio–fixed interval (FR–FI) schedule of food reinforcement. FR response rate, FR post-reinforcement pause duration, and the pattern of FI responding were not affected during or after the exposure series. In contrast, within-group FI rate for the treatment groups increased over time whereas those of the controls decreased. A historical control group, however, also showed a similar pattern of increase, indicating that these changes did not clearly represent a treatment related effect. Results from these studies indicate a LOEL of 350 ppm for systemic toxicity based on the decreased body weight gain in male rats, and a LOEL of 1500 ppm for neurotoxicity based on the transient reduction in motor activity in female rats. In conclusion, there was no evidence that subchronic exposure up to 1500 ppm ethyl acetate produced any enduring neurotoxic effects in rats.

COMMENTS: A large number of behavioral and neuropathological endpoints were measured by these investigators (37 functional observational battery tests, 2 motor activity and 5 operant tests). These studies suggest a LOEL of 350 ppm for decrease in body weight and a 1,500 ppm for reduction in motor activity. Even at this high concentration the authors reported no persistent adverse effect.

ETHYL ALCOHOL, INCLUDING SDA-4
CAS: 64-17-5
SEE MAJOR INGREDIENTS CATEGORY

ETHYL BENZOATE
CAS: 93-89-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL BUTYRATE
CAS: 105-54-4
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL CINNAMATE (PROPENIC ACID,3-PHENYL-,ETHYL ESTER,2-)
CAS: 103-36-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL DECANOATE
CAS: 110-38-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4-ETHYL GUAIACOL (4-ETHYL-2-METHOXY-PHENOL)
CAS: 2785-89-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL HEPTANOATE
CAS: 106-30-9
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL HEXANOATE (ETHYL CAPROATE)
CAS: 123-66-0
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ISOVALERATE
CAS: 108-64-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LACTATE
CAS: 97-64-3
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LAURATE
CAS: 106-33-2
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LEVULINATE
CAS: 539-88-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL MALTOL
CAS: 4940-11-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL 2-METHYLBUTYRATE
CAS: 7452-79-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL METHYL PHENYLGLYCIDATE
CAS: 77-83-8
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL MYRISTATE
CAS: 124-06-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL NONANOATE
CAS: 123-29-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OCTADECANOATE
CAS: 111-61-5
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OCTANOATE
CAS: 106-32-1
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OLEATE
CAS: 111-62-6
NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

INGREDIENTS USED IN MIXTURE STUDIES TOBACCO SMOKE STUDIES

GENERAL COMMENTS:
In addition to studies that looked at individual ingredients, there were a few studies specifically designed to evaluate the potential effects of a large number of ingredients commonly added to cigarettes. The studies were unique in that all ingredients were tested by adding them in groups to a single research cigarette. The strength of this research is that these studies were specifically designed to determine (1) the effect of pyrolysis on the toxicity of the ingredients, (2) if new toxic substances were produced, (3) if the mixture of ingredients acts in a synergistic manner that might increase the toxicity of the inhaled smoke and (4) if these substances produced any identifiable new target organ toxicity not associated with cigarette smoke from cigarettes without the added ingredients. Well-established *in vitro* tests were included to identify mutagenicity (Ames test), cytotoxicity (neutral red uptake assay), carcinogenicity (two-stage mouse dermal assay), as well as a 90-day rat inhalation study. To complement these studies the chemical composition of the mainstream smoke from cigarettes with and without the added ingredients were also determined. These studies indicated that the addition of these chemicals, even at exaggerated levels, did not increase the bacterial mutagenicity, cytotoxicity nor the pathological response to the inhaled cigarettes with ingredients as compared to control cigarettes. When the results were compared to reference cigarettes without ingredients, the tests would indicate that the presence of these ingredients did not alter the biological activity. This model system represents a realistic model capable of detecting potential interactions among ingredient pyrolysis products together with various constituents known to be present in cigarette smoke. A number of the ingredients being reviewed in this report were included in these mixture studies. A list of these ingredients can be found below.

These extensive reviews would indicate that no chemical nor biological evidence has been presented to support the claim that ingredients added to cigarettes modifies the chemistry or biology activity of inhaled tobacco smoke.

The following ingredients were tested as a mixture added to cigarettes. Relevant mixture and review papers are listed below:

CITRONELLOL	**CAS: 106-22-9**
PARA-TOLUALDEHYDE	**CAS: 104-87-0**
ETHYL HEPTANOATE	**CAS: 106-30-9**
ISOAMYL FORMATE	**CAS: 110-45-2**
HEXYL ACETATE	**CAS: 142-92-7**
PECTIN	**CAS: 9000-69-7**
CORN STARCH	**CAS: 9005-25-8**
L-MENTHONE	**CAS: 14073-97-3**
ACETIC ACID	**CAS: 64-19-7**
ENZALDEHYDE	**CAS: 100-52-7**
BUTRIC ACID	**CAS: 107-92-6**
BETA-CARYOPHLLENE OXIDE	**CAS: 1139-30-6**
GAMMA-DECALACTONE	**CAS: 706-14-9**
2,5-DECALACTONE	**CAS: 123-32-0**
ETHYL BUTYRATE	**CAS: 105-54-4**
ETHYL DECANOATE	**CAS: 110-38-3**
ETHYL HEXANOATE	**CAS: 123-66-0**
ETHYL ISOVALERATE	**CAS: 108-64-5**
ETHYL LACTATE	**CAS: 97-64-3**
ETHYL LAURATE	**CAS: 106-33-2**
ETHYL MYRISTATE	**CAS: 124-06-1**
ETHYL OCTANOATE	**CAS: 106-32-1**
ETHYL PHENYLACETATE	**CAS: 101-97-3**
5-ETHYL-3-HYDROXY-4METHYL-2(5H)-FURANONE	**CAS: 698-10-2**
ISOAMYL ACETATE	**CAS: 123-92-2**
ISOBUTYL CINNAMATE	**CAS: 122-67-8**
ISOBUTYL PHENYLACETATE	**CAS: 102-13-6**
ISOBUTYRIC ACID	**CAS: 79-31-2**
2-METHYLPYRAZINE	**CAS: 109-08-0**
GAMMA-OCTALACTONE	**CAS: 104-50-7**
2,3-PENTANEDIONE	**CAS: 600-14-7**
2-PHENETHYL ACETATE	**CAS: 103-45-7**
PHENYLACETALDEHYDE	**CAS: 122-78-1**
SODIUMBICARBONATE	**CAS: 144-55-8**
2,3,5,6-TETRAMETHYLPYRAZINE	**CAS: 1124-11-4**
TRIETHYL CITRATE 77-93-0	
4-(2,6,6-TRIMETHYLCYCLOHEX-1-ENY) BUT-2-4- ONE (BETA-DAMASCONE)	**CAS: 23726-91-2; 35044-68-9**
GLYCEROL	**CAS: 56-81-5**
INVERTED SUGAR	**CAS: 8013-17-0**
CELLULOSE AND CELLULOSE	

FIBER	**CAS: 65996-61-4; 9004-34-6**
PROPYLENE GLYCOL	**CAS: 57-55-6**
METHOL AND L-MENTHOL	**CAS: 89-78-1; 216-51-5**
ETHYL ALCOHOL, INCLUDING SDA-4	**CAS: 64-17-5**
CHOCHOLATE AND CHOCOLATE LIQUOR	**CAS: N/A**
LACTIC ACID	**CAS: 50-21-5; 598-82-3**
SORBITOL	**CAS: 50-70-4**
AMMONIUM HYDROXIDE	**CAS: 1336-21-6**
GLUCOSE/DEXTROSE	**CAS: 50-99-7; 492-62-6**
SODIUM CARBONATE	**CAS: 497-19-8**
ETHYL 2-METHYLBUTYRATE	**CAS: 7452-79-1**
VANILLIN	**CAS: 121-33-5**

Each of the papers listed below, except for papers 7, 8, 9, and 10, has been reviewed and evaluated in previous review documents and will not be repeated here. The new papers have extensive abstracts fully defining the goals and conclusions reached by the authors.

1. Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results.
Food and Chemical Toxicology. Volume 40, Issue 1, pp. 77-91, January, 2002
E.L. Carmines et al

2. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke.
AUTHORS: K. Rustemeiera, R. Stabberta, H.-J. Haussmanna, E. Roemera, E.L. Carmines.
SOURCE: Food and Chemical Toxicology.Vol. 40, Issue 1, pp. 93-104,January, 2002

3. Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity.
AUTHORS: E. Roemera, F.J. Tewesa, T.J. Meisgena, D.J.Veltela, E.L. Carmines.
SOURCE. Food and Chemical Toxicology.Vol. 40, Issue 1, pp.105-111, January, 2002

4. Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity.
AUTHORS: P.M. Vanscheeuwijcka,*, A. Teredesaib, P.M. Terpstraa, J. Verbeecka, P. Kuhlb, B. Gerstenbergb, S. Gebelb, E.L. Carmines.
PUBLICATION SOURCE: Food and Chemical Toxicology, Volume 40, Issue 1, pp. 113-131, January, 2002

5. Toxicologic evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice.
AUTHORS:C. L. Gaworski, J. D. Hecka, M. B. Bennetta and M. L. Wenk.
PUBLICATION SOURCE. Toxicology.Volume 139, Issues 1-2, 29 November 1999, Pages 1-17

6. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters.
AUTHORS: Fukayama Mark Y(a); Easterday Otho D; Serafino Patricia A; Renskers Kevin J ; North-Root Helen; Schrankel Kenneth R
SOURCE: Toxicology Letters 111(1-2 p 175-187 Dec. 20, 1999

7. The pyrolysis of tobacco ingredients -

Baker, R.R.;Bishop, L.J.
Journal of Analytical and Applied Pyrolysis, Volume 71, Issue 1, 1 March 2004, Pages 223-311

ABSTRACT: Relationships between tobacco components and smoke products are complex and often difficult to unravel. Pyrolysis experiments have commonly been used to establish such relationships. However, unless they are performed under dynamic conditions that are relevant to those that occur during tobacco burning, results can be obtained which have little resemblance to those obtained during cigarette smoking. The relevance of pyrolysis experiments to the behaviour of tobacco ingredients in a burning cigarette is considered. Based on the temperature, heating rate, oxygen levels and gas flow conditions that occur inside the burning zone of a cigarette, together with a review of relevant pyrolysis and smoking experiments, a set of pyrolysis conditions has been developed that approximates those occurring in the pyrolysis region of the burning cigarette. The conditions include heating the sample at 30 °C s−1 from 300 to 900 °C under a flow of 9% oxygen in nitrogen. Experiments on the pyrolytic behaviour of eleven relatively volatile substances under these conditions give results that are in good agreement with results from thirteen published studies in which cigarettes incorporating labelled versions of the substances were smoked. Subsequently, 291 single-compound tobacco ingredients have been pyrolysed under this set of conditions, most of which are relatively volatile. This enables the behaviour of these ingredients in a burning cigarette to be estimated in terms of intact transfer to mainstream smoke versus pyrolytic decomposition. It is predicted that almost a third of the substances would transfer to mainstream smoke at least 99% intact, and almost two-thirds would transfer 95% intact. Where pyrolytic decomposition does occur, the products are listed together with an estimate of the levels in smoke that would arise from the ingredient.

8. The effect of tobacco ingredients on smoke chemistry. Part I: Flavourings and additives

Baker RR; da Silva JRP; Smith G
Food and Chemical Toxicology 42(Supplement S): S3-S37, 2004. (34 refs.)

ABSTRACT: The effects of 450 tobacco ingredients added to tobacco on the forty-four "Hoffmann analytes" in mainstream cigarette smoke have been determined. These analytes are believed by regulatory authorities in the USA and Canada to be relevant to smoking related diseases. They are based on lists published by D. Hoffmann and co-workers of the American Health Foundation in New York. The ingredients comprised 431 flavours, 1 flavour/solvent, 1 solvent, 7 preservatives, 5 binders, 2 humectants, 2 process aids and I filler. The cigarettes containing mixtures of the ingredients were smoked using the standard ISO smoking machine conditions. The levels of the "Hoffmann analytes" in the smoke from the test cigarettes containing the ingredient mixture were compared to those from control cigarettes without the ingredients. In practice, flavouring ingredients are typically added to tobacco that also contains casing ingredients and reconstituted tobacco materials. In order to keep the tobacco mixtures as authentic as possible, three comparisons have been made in this study. These are: (a) control cigarette containing a typical US blended, cased tobacco incorporating reconstituted tobacco versus test cigarettes that had flavouring ingredients added to this tobacco; (b) control cigarette containing tobacco only versus test cigarettes with the tobacco cased and incorporating flavourings; (c) control cigarette containing tobacco only versus test cigarette incorporating additives made in an experimental sheet material. The significances of differences between the test and control cigarettes were determined using both the variability of the data on the specific occasion of the measurement, and also taking into account the long-term variability of the analytical measurements over the one-year period in which analyses were determined in the present study. This long-term variability was determined by measuring the levels of the 44 "Hoffmann analytes" in a reference cigarette on many occasions over the one-year period of this study. The ingredients were added to the experimental cigarettes at or above the maximum levels used commercially by British American Tobacco. The effect of the ingredient mixtures on total particulate matter and carbon monoxide levels in smoke was not significantly different to the control in most cases, and was never more than 10% with any ingredient mixture. It was found that, in most cases, the mixtures of flavouring ingredients (generally added in parts per million levels) had no statistically significant effect on the analyte smoke yields relative to the control cigarette. Occasionally with some of the mixtures, both increases and decreases were observed for some smoke analyte levels relative to the control cigarette. These differences were generally up to about 15% with the mixtures containing flavouring ingredients. The significance of many of the differences was not present when the long-term variability of the analytical methodology was taken into account. For the test cigarettes with ingredient mixtures containing casing ingredients, there were again no significant changes in smoke analyte levels in most cases. Those changes that were observed are as follows. Decreases in smoke levels were observed with some ingredient mixtures for most of the tobacco specific nitrosamines (up

to 24%), NOx, most of the phenols (up to 34%), benzo[a]pyrene, and some of the aromatic amines and miscellaneous organic compounds on the "Hoffmann list". Increases were observed for some test cigarettes in smoke ammonia, HCN, formaldehyde and lead levels (up to 24%).The significance of the ammonia and lead increases was not present when the long-term variability of the analytical methodology was taken into account. The yields of some carbonyl compounds in smoke were increased in one comparison with an additives mixture containing cellulosic components; in particular, formaldehyde was increased by 68%. This was the largest single change seen in any smoke analyte level in this study. These carbonyls are produced from the pyrolysis of cellulosic and other polysaccharide materials, present in the additives mixture. With this test cigarette, all tobacco specific nitrosamines, phenols, semi-volatile bases, NO, and some aromatic amines and miscellaneous organic compounds on the "Hoffmann list" were decreased, by up to 22%. The significance of many of these differences remained even when the long-term variability of the analytical methodology was taken into account. The levels of all other "Hoffmann analytes" in the smoke were not significantly different to those of the control cigarette. With the exception of the determinations of "tar", nicotine and carbon monoxide, there are currently no internationally recognised standard methods for measurement of the other "Hoffmann analytes". Each laboratory uses its own methods and there are large laboratory-to-laboratory variations, as well as variations over time in a given laboratory. Therefore, it is important that in any comparison of smoke analytes amongst different cigarettes, all the analytes should be measured in the same laboratory and at the same time. This was the case in the present study and all the methods have been validated internally.

9. The effect of tobacco ingredients on smoke chemistry. Part II: Casing ingredients

Baker RR, Pereira da Silva JR, Smith G.
Food Chem Toxicol. 2004;42 Suppl:S39-52.

This is the second part of a study in which the effects of adding a range of ingredients to tobacco on the chemistry of cigarette mainstream smoke are assessed. The examination of smoke chemistry has concentrated on those constituents in smoke that regulatory authorities in the USA and Canada believe to be relevant to smoking-related diseases. In this part of the study the effects of 29 casing ingredients and three humectants have been assessed at the maximum levels typically used on cigarettes by British American Tobacco. This brings the total number of ingredients assessed in Parts I and II of this study to 482. The casing ingredients were added at levels of up to 68 mg on the cigarettes. Their effects on smoke constituents were generally larger than the effects of flavouring ingredients, which were added at parts per million levels. Many of the casing ingredient mixtures either had no statistically significant effect on the level of the analytes investigated in smoke relative to a control cigarette, or they produced decreases of up to 44% in some cases. Those analytes that were increased in smoke are highlighted in this paper. The largest increases were for formaldehyde levels, up to 26 microg (73%) in one case, observed from casing mixtures containing sugar. This is most likely due to the generation of formaldehyde by pyrolysis of sugars. Occasional small increases were also observed for other analytes. However, the statistical significance of many of these

increases was not present when the long-term variability of the analytical method was taken into account. The significance and possible reasons for the increases are discussed.

10. An overview of the effects of tobacco ingredients on smoke chemistry and toxicity -

Baker RR, Massey ED, Smith G.
Food Chem Toxicol. 2004;42 Suppl:S53-83.

ABSTRACT: This paper presents an overview of a series of studies designed to assess the influence of 482 tobacco ingredients on cigarette smoke chemistry and toxicity. The studies are: pyrolysis of the ingredients; influence of the ingredients on smoke constituents believed by regulatory authorities to be relevant to smoking-related diseases ("Hoffmann analytes"); influence of the ingredients on in vitro genotoxicity and cytotoxicity of smoke partiulate matter; and influence of the ingredients on the inhalation toxicity of smoke. The present paper brings the salient features of these studies together. A pyrolysis technique has been developed which, as far as practicably possible, mimics the combustion conditions inside a burning cigarette. The results from 291 single-substance ingredients indicate that almost a third would transfer out of the cigarette burning zone at least 99% intact (i.e. less than 1% pyrolysis), and almost two thirds would transfer at least 95% intact. Of the ingredients that underwent some degree of pyrolysis, a few "Hoffmann analytes" were detected amongst the pyrolysis products of 19 ingredients. Taking into account maximum use levels, their maximum pyrolysis levels were generally small and often insignificant compared to the levels typically present in smoke. Possible exceptions were acetaldehyde and benzene from the pyrolysis of malic acid. However, subsequent smoke chemistry studies indicated that the maximum levels predicted from pyrolysis of this involatile substance were overestimated, suggesting that malic acid does not undergo complete pyrolysis in the burning cigarette and/or generates acetaldehyde and benzene at similar rates to that of tobacco on a per weight basis. When added to tobacco, many of the ingredient mixtures produced no significant effect on the levels of many of the "Hoffmann analytes" in smoke, while some produced increases or decreases relative to the relevant control cigarettes. The study has concentrated on the increases. Many of the differences were found to be not significant when the long-term variability of the analytical methodology was taken into account. However, even taking this into account, the smoke formaldehyde levels in two of the test cigarettes were significantly increased relative to their controls, by up to 26 microg (73%). These increases are likely to be due to the pyrolysis of sugars, cellulose and other polysaccharide materials. The activity of smoke particulate matter from cigarettes containing tobacco ingredients has been determined with three in vitro bioassays, two for genotoxicity and one for cytotoxicity. These were the Ames test, the mammalian cell micronucleus assay, and the neutral red uptake cytotoxicity assay. Within the sensitivity and specificity of these bioassays, the specific activity of the cigarette smoke particulate matter was not changed by the addition of ingredients to the cigarette. Three 90-day sub-chronic inhalation studies have been undertaken and histopathological and histomorphometric assessments made within the respiratory tracts of animals exposed to smoke from cigarettes containing the various ingredient mixtures and their control

cigarettes. The response due to tobacco smoke exposure was not distinguishable between the test and control cigarettes, indicating that the presence of the ingredients had made no discernable differences to the type and severity of the treatment-related changes.

RELEVANT REVIEWS & INTERESTING PAPERS

1. Evaluation of certain food additives and contaminants -

Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives
WHO Technical Report Series 922, 2004 Geneva

GENERAL COMMENT: In this document, examples of additives that were reviewed include citric acid, 2 methylheptanoic, citral, citronellol and much more.

ABSTRACT: This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives.The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants,assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (a-amylase from Bacillus lichenformis containing a genetically engineered a-amylase gene from B. licheniformis, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from Myceliophthora thermophila expressed in Aspergillus oryzae, mixed xylanase, b-glucanase enzyme preparation produced by a strain of Humicola insolens, neotame, polyvinyl alcohol,quillaia extracts and xylanase from Thermomyces lanuginosus expressed in Fusarium venenatum), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking-water (sodium dichloroisocyanurate) and contaminants (cadmium and methylmercury). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, and tolerable intakes of the contaminants considered, changes in the status of specifications and further information requested or desired.

COMMENTS: This is a massive report that one needs to be aware of since this Committee had access to documents called Technical Data Sheets, which were prepared using new or existing food additives and which had not been published because the detailed information on manufacturing processes described therein could be commercially sensitive. These documents, however, also contain valuable information, which was not made public, on chemical and technological approaches.

The Committee recognized the need for a working definition of the term "flavouring agent" and recommended that such a definition be agreed at a future meeting. At its present meeting, the Committee noted that a range of regulatory definitions of "flavouring" and similar terms exist in different countries and concluded that any definition would need to be elaborated in an international forum, such as the Codex Alimentarius Commission. The Committee reiterated the criteria that need to be met for an individual flavouring agent to be evaluated by the existing Procedure for the Safety Evaluation of Flavouring Agents: • The substance should be chemically defined, such that at least 95% of the commercially used material consists either of the named chemical, or of the named chemical and identified secondary constituents. The substance is added to food for flavouring purposes, including the generation of active flavouring substances during storage or processing of the food. • There is a valid estimate of current exposure to the named substance and, if appropriate, its breakdown or reaction products. Some substances that have a use as flavouring agents may have been evaluated previously by the Committee in relation to other food additive functions. The use of such a substance, or its breakdown or reaction products, as a flavouring agent is included in the relevant, previously-established ADI.

2. Human functional neuroimaging in nicotine and tobacco research: Basics, background, and beyond - 2004 –

F. Joseph McClernon and David G. Gilbert
Nicotine & Tobacco Research Volume 6, Number 6 : 941 - 959

ABSTRACT: Modern functional neuroimaging techniques allow nicotine and tobacco researchers to investigate the neurobiological basis of addiction in humans. We introduce the methods and measures of the following neuroimaging techniques: Electroencephalography and event-related cortical potentials, positron emission tomography, and functional magnetic resonance imaging. We outline strengths and limitations across modalities and describe new and emerging technologies. We provide summaries of recent neuroimaging findings in the field of nicotine and tobacco research for neurochemistry, smoking and nicotine administration, craving and cue-reactivity, cognitive and affective information processing, and tobacco withdrawal. We address limitations of studies to date and identify opportunities for future research.

3. **Effect of exposure to secondhand smoke on markers of inflammation: the ATTICA study –**

Demosthenes B. Panagiotakos PhDa, , , Christos Pitsavos MD, PhDa, Christina Chrysohoou MD, PhDa, John Skoumas MDa, Constadina Masoura MDa, Pavlos Toutouzas MD, PhDa and Christodoulos Stefanadis MD, PhDa
The American Journal of Medicine Volume 116, Issue 3 , 1 February 2004, Pages 145-150

ABSTRACT: We sought to investigate the effect of secondhand smoke exposure on inflammatory markers related to cardiovascular disease. Methods. During 2001 to 2002,

we randomly selected a stratified (age-sex) sample of adults without clinical evidence of cardiovascular disease. Exposure to secondhand smoke (>30 minutes per day and ≥1 day per week) was recorded. Multivariate regression analysis was used to evaluate the effects of exposure to secondhand smoke on levels of C-reactive protein, fibrinogen, homocysteine, and oxidized low-density lipoprotein (LDL) cholesterol, and on white blood cell count. Results. One hundred and thirty-seven (38%) of the 357 men who had never smoked and 211 (33%) of the 638 never-smoking women reported current exposure to secondhand smoke. Compared with those who were not exposed to secondhand smoke, those exposed more than 3 days per week had higher white blood cell counts (by 600 cells per μL; P = 0.02), as well as higher levels of C-reactive protein (by 0.08 mg/dL; P = 0.03), homocysteine (by 0.4 μmol/L; P = 0.002), fibrinogen (by 5.2 mg/dL; P = 0.4), and oxidized LDL cholesterol (by 3.3 mg/dL; P = 0.03), after adjusting for several potential confounders. Conclusion: Our findings suggest another pathophysiological mechanism by which exposure to secondhand smoke is associated with the development of atherosclerosis.

4. **Influence of smoking and sinus on the prevalence and incidence of type 2 diabetes amongst men: the northern Sweden MONICA study**

M. Eliasson1,2, K. Asplund2, S. Nasic2 & B. Rodu3
Journal of Internal Medicine Volume 256 Issue 2 Page 101 - August 2004

ABSTRACT: To explore the effect of smoking and smokeless tobacco, 'snus', on the risk of type 2 diabetes. Design. Population-based cross-sectional and prospective follow-up study in northern Sweden. Subjects. A total of 3384 men, aged 25–74 years, who participated in the MONICA study in 1986, 1990, 1994 or 1999, 1170 of whom had an oral glucose tolerance test. In 1999, 1757 men from previous cohorts returned for re-examination. Main outcome measures. We compared the prevalence of type 2 diabetes or pathological glucose tolerance (PGT) amongst tobacco users to that of nonusers at entry into the study and at follow-up, using odds ratios. Results. Compared with never users, the ageadjusted risk of prevalent clinically diagnosed diabetes for ever smokers was 1.88 (CI 1.17–3.0) and for smokers 1.74 (0.94–3.2). Corresponding odds ratios for snus users were 1.34 (0.65–2.7) and 1.18 (0.48–2.9). We found no increased risk of prevalent PGT in snus users or smokers. Former smokers and snus users had an insignificantly increased risk for PGT. Compared with nonusers, the age-adjusted risk of developing clinically diagnosed diabetes during follow-up was 4.63 (1.37–16) in consistent exclusive smokers, 3.20 (1.16–8.8) in ex-smokers and no cases in consistent snus users. The risk of PGT during follow-up was not increased in consistent tobacco users but evident, although not statistically significant, in those who quit snus during the follow-up period, 1.85 (0.60–5.7). Adjustment for physical activity and alcohol consumption did not change the major findings. Conclusions. The risk of diabetes for snus users was not significantly increased. Smoking was associated with prevalent and incident cases of diabetes. Ex-tobacco users tended towards more PGT.

COMMENTS: This paper describes an epidemiological study comparing the effects of smoking and smokeless tobacco use on type 2 diabetes. The study confirmed previous

findings that smoking is a risk factor for type 2 diabetes, but did not find a similar association with the use of smokeless tobacco.

5. Chemically induced renal tubule tumors in the laboratory rat and mouse: Review of the NCI/NTP database and categorization of renal carcinogens based on mechanistic information

Edward Lock; Gordon Hard
Critical Reviews in Toxicology, Volume 34, Number 3, May-June 2004, pp. 211-299(89)

Abstract: The incidence of renal tubule carcinogenesis in male and female rats or mice with 69 chemicals from the 513 bioassays conducted to date by the NCI/NTP has been collated, the chemicals categorized, and the relationship between carcinogenesis and renal tubule hyperplasia and exacerbation of the spontaneous, age-related rodent disease chronic progressive nephropathy (CPN) examined. Where information on mechanism or mode of action exists, the chemicals have been categorized based on their ability to directly or indirectly interact with renal DNA, or on their activity via epigenetic pathways involving either direct or indirect cytotoxicity with regenerative hyperplasia, or exacerbation of CPN. Nine chemicals were identified as directly interacting with DNA, with six of these producing renal tubule tumors at high incidence in rats of both sexes, and in some cases also in mice. Ochratoxin A was the most potent compound in this group, producing a high tumor incidence at very low doses, often with metastasis. Three chemicals were discussed in the context of indirect DNA damage mediated by an oxidative free radical mechanism, one of these being from the NTP database. A third category included four chemicals that had the potential to cause DNA damage following conjugation with glutathione and subsequent enzymatic activation to a reactive species, usually a thiol-containing entity. Two chemicals were allocated into the category involving a direct cytotoxic action on the renal tubule followed by sustained compensatory cell proliferation, while nine were included in a group where the cell loss and sustained increase in renal tubule cell turnover were dependent on lysosomal accumulation of the male rat-specific protein, 2-globulin. In a sixth category, morphologic evidence on two chemicals indicated that the renal tumors were a consequence of exacerbated CPN. For the remaining chemicals, there were no pertinent data enabling assignment to a mechanistic category. Accordingly, these chemicals, acting through an as yet unknown mechanism, were grouped as either being associated with an enhancement of CPN mechanism, were grouped as either being associated with an enhancement of CPN (category 7, 16 chemicals), or not associated with enhanced CPN (category 8, 4 chemicals). A ninth category dealt with 11 chemicals that were regarded as producing increases in renal tubule tumors that did not reach statistical significance. A 10th category discussed 6 chemicals that induced renal tumors in mice but not in rats, plus 8 chemicals that produced a low incidence of renal tubule tumors in mice that did not reach statistical significance. As more mechanistic data are generated, some chemicals will inevitably be placed in different groups, particularly those from categories 7 and 8. A large number of chemicals in the series exacerbated CPN, but those in

category 7 especially may be candidates for inclusion in category 6 when further information is gleaned from the relevant NTP studies. Also, new data on specific chemicals will probably expand category 5 as cytotoxicity and cell regeneration are identified as obligatory steps in renal carcinogenesis in more cases. Additional confirmatory outcomes arising from this review are that metastases from renal tubule tumors, while encountered with chemicals causing DNA damage, are rare with those acting through an epigenetic pathway, with the exception being fumonisin B1; that male rats and mice are generally more susceptible than female rats and mice to chemical induction of renal tubule tumors; and that a background of atypical tubule hyperplasia is a useful indicator reflecting a chemically associated renal tubule tumor response. With respect to renal tubule tumors and human risk assessment, chemicals in categories 1 and 2, and possibly 3, would currently be judged by linear default methods; chemicals in category 4 (and probably some in category 3) as exhibiting a threshold of activity warranting the benchmark approach; and those in categories 5 and 6 as representing mechanisms that have no relevance for extrapolation to humans.

COMMENTS: This paper provides a review of 69 chemicals tested in the National Cancer Institute / National Toxicology Program (NCI/NTP) carcinogenicity bioassay database. The selected chemicals are those that have shown an association with renal tubule tumors in rat and/or mouse, and was focused on oral exposures.

6. Cigarette smoking exacerbates chronic alcohol-induced brain damage: A preliminary metabolite imaging study -

Durazzo TC, Gazdzinski S, Banys P, Meyerhoff DJ.
Alcohol Clin Exp Res. 2004 Dec;28(12):1849-60.

ABSTRACT: Cigarette smoking is common among alcohol-dependent individuals. Nevertheless, previous research has typically not accounted for the potential independent or compounding effects of cigarette smoking on alcohol-induced brain injury and neurocognition. METHODS: Twenty-four 1-week-abstinent recovering alcoholics (RAs; 14 smokers and 10 nonsmokers) in treatment and 26 light-drinking controls (7 smokers and 19 nonsmokers) were compared on measures of common brain metabolites in gray matter and white matter of the major lobes, basal ganglia, midbrain, and cerebellar vermis, obtained via multislice short-echo time proton magnetic resonance spectroscopic imaging. Smoking and nonsmoking RAs were also contrasted on measures of neurocognitive functioning, as well as laboratory markers of drinking severity and nutritional status. RESULTS: Chronic alcohol dependence, independent of smoking, was associated with lower concentrations of frontal N-acetylaspartate (NAA) and frontal choline-containing compounds, as well as lower parietal and thalamic choline. Smoking RAs had lower NAA concentrations in frontal white matter and midbrain and lower midbrain choline than nonsmoking RAs. A four-group analysis of covariance also demonstrated that chronic cigarette smoking was associated with lower midbrain NAA and choline and with lower vermian choline. In smoking RAs, heavier drinking was associated with heavier smoking, which correlated with numerous subcortical metabolite abnormalities. The 1-week-abstinent smoking and nonsmoking RAs did not differ

significantly on a brief neurocognitive battery. In smoking RAs, lower cerebellar vermis NAA was associated with poorer visuomotor scanning speed and incidental learning, and in nonsmoking RAs lower vermis NAA was related to poorer visuospatial learning and memory. CONCLUSIONS: These human in vivo proton magnetic resonance spectroscopic imaging findings indicate that chronic cigarette smoking exacerbates chronic alcohol-induced neuronal injury and cell membrane damage in the frontal lobes of RAs and has independent adverse effects on neuronal viability and cell membranes in the midbrain and on cell membranes of the cerebellar vermis. Higher smoking levels are associated with metabolite concentrations in select subcortical structures. Greater consideration of the potential effects of comorbid cigarette smoking on alcohol-induced brain damage and other diseases affecting the central nervous system is warranted.

7**. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: A review of agents and causative mechanisms**

Urmila Nair, Helmut Bartsch and Jagadeesan Nair
Mutagenesis vol. 19 no. 4 pp. 251-262, July 2004

ABSTRACT: In south-east Asia, Taiwan and Papua New Guinea, smoking, alcohol consumption and chewing of betel quid with or without tobacco or areca nut with or without tobacco are the predominant causes of oral cancer. In most areas, betel quid consists of a mixture of areca nut, slaked lime, catechu and several condiments according to taste, wrapped in a betel leaf. Almost all habitual chewers use tobacco with or without the betel quid. In the last few decades, small, attractive and inexpensive sachets of betel quid substitutes have become widely available. Aggressively advertised and marketed, often claimed to be safer products, they are consumed by the very young and old alike, particularly in India, but also among migrant populations from these areas world wide. The product is basically a ¯avoured and sweetened dry mixture of areca nut, catechu and slaked lime with tobacco (gutkha) or without tobacco (pan masala). These products have been strongly implicated in the recent increase in the incidence of oral submucous ®brosis, especially in the very young, even after a short period of use. This precancerous lesion, which has a high rate of malignant transformation, is extremely debilitating and has no known cure. The use of tobacco with lime, betel quid with tobacco, betel quid without tobacco and areca nut have been classi®ed as carcinogenic to humans. As gutkha and pan masala are mixtures of several of these ingredients, their carcinogenic affect can be surmised. We review evidence that strongly supports causative mechanisms for genotoxicity and carcinogenicity of these substitute products. Although some recent curbs have been put on the manufacture and sale of these products, urgent action is needed to permanently ban gutkha and pan masala, together with the other established oral cancer-causing tobacco products. Further, education to reduce or eliminate home-made preparations needs to be accelerated.

COMMENTS: Well-marketed and conveniently packaged commercial preparations containing chewing tobacco with various combinations of lime, betel quid and areca nut have popularized the use of these products in Asia. The authors summarize available

evidence of the carcinogenic potential of these mixtures, and suggest a ban on products such as gutkha and pan masala.

8. **Alcohol, acetaldehyde, and digestive tract cancer**

SALASPURO, M. Alcohol, acetaldehyde, and digestive tract cancer. In: Nutrition and alcohol, pp. 393-411. Boca Raton, CRC Press, 2004.
Book Chapter

ABSTRACT N/A

COMMENTS: This monograph reviews the health issues associated with use of alcohol and states that cancer risk is dose-dependent and alcohol and smoking is synergistic, producing a greater effect together than either alone. Moderate smoking without drinking and moderate drinking without smoking had a slight or negative effect on esophageal cancer risk. But simultaneous exposure to the same moderate amounts increased risk 12 to 19-fold in men and women respectively.

OSHA comments from the January 19, 1989 Final Rule on Air Contaminants Project extracted from 54FR2332 et. seq. This rule was remanded by the U.S. Circuit Court of Appeals and the limits are not currently in force.

STARCH

CAS: 9005-25-8; Chemical Formula: $(C_6H_{10}O_5)_n$

The former OSHA limit for starch was 15 mg/m^3 as an 8-hour TWA, the Agency's generic limit for all particulates. The ACGIH has a TLV-TWA of 10 mg/m^3 for starch as total dust that contains no asbestos and less than 1 percent crystalline silica. The proposed total particulate PEL was 10 mg/m^3; however, in the final rule, OSHA is retaining a total particulate limit of 15 mg/m^3 for starch. Starch is a white, odorless powder.

Exposure to high concentrations of starch dust may result in impaired vision, or may cause injury to the mucous membranes or skin. Injury may also result from the vigorous skin-cleansing procedures necessary for the complete removal of starch (ACGIH 1986/Ex. 1-3). NIOSH, the only commenter on starch, has not substantively reviewed its health effects (Ex. 8-47, Table N4).

OSHA is retaining both the 8-hour TWA total particulate PEL of 15 mg/m^3 and the 5-mg/m^3 respirable particulate limit for starch. The Agency concludes that these limits will control the significant risk of eye, skin, and other physical irritation that may result from exposure to high levels of starch in the workplace.

Substance	*ID Code*	*Rpt No.*	*Year*	*Conclusion**	*21 CFR Section*
Bleached Starch	9005-25-8	115	1979	1	There is no CFR citation.

SCOGS Opinion:

The digestibility of unmodified cereal and tapioca starches used commercially as food ingredients, both raw and after cooking, is almost complete. Potato and arrowroot starches are less completely digested when fed raw but their digestibility is similar to that of the cereal starches after cooking. Pregelatinized starches (dried, cooked starches) generally are highly digestible. Consumption of excessive quantities, pounds per day, of raw starch has resulted in obesity and iron-deficiency anemia in human subjects. Most of the foods to which starch is added by the food industry are cooked in processing or are cooked before serving. Moreover, the total quantity of unmodified and pregelatinized starch added to processed foods is insignificant compared to the natural starch content of the American dietary, some of which is eaten in its native form in raw vegetables. No adverse effects have been attributed to these starches as added food ingredients. It is suggested, however, that specifications for food grade unmodified starches be developed in order to distinguish them from the starches that are used in non-food applications.

In light of the foregoing, the Select Committee concludes that: There is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo (also called grain sorghum starch), rice, potato, tapioca or arrowroot starch that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future.

There is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo, rice, potato, tapioca or arrowroot starch that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are substances migrating to food from paper and paperboard packaging.

There is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo, rice, potato, tapioca, or arrowroot starch that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are substances migrating to food from cotton and cotton fabrics used in dry food packaging.

**** denotes Type of Conclusion 1, 2, 3, 4, or 5. Definitions of conclusion types can be found at the end of this report..***

SCOGS-115

EVALUATION OF THE HEALTH ASPECTS OF STARCH AND MODIFIED STARCHES AS FOOD INGREDIENTS

1979

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

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Federation of American Societies
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9650 Rockville Pike
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This report, by a group of qualified scientists designated the Select Committee of GRAS Substances (SCOGS), provides an independent evaluation of the safety of starch and modified starches as food ingredients when used in foods at present or projected future levels.

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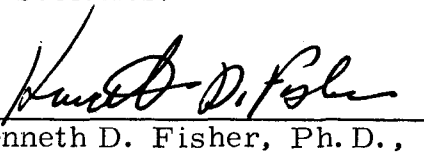
NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U. S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.



Kenneth D. Fisher, Ph. D., Director
Life Sciences Research Office
FASEB

CONTENTS

I. INTRODUCTION

This report concerns the health aspects of using starch and modified starches as food ingredients. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1973*. To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the Life Sciences Research Office staff. In addition, announcement was made in the Federal Register on June 16, 1978 (43 FR 26132) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information and views on using starch and modified starches as food ingredients or, in lieu of an oral hearing presentation, to submit a written statement. Two organizations submitted written statements in lieu of oral hearing presentations: Corn Refiners Association, Inc., 1001 Connecticut Avenue, N.W., Washington, D.C., and CPC International, Inc., International Plaza, Englewood Cliffs, N.J. No other requests were received and a hearing was not held.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (2) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA (2) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

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*The document (PB-241 956/2) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that its conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on starch and modified starches and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

This report evaluates the health aspects of the cereal starches [specifically cornstarch, waxy maize starch, high amylose cornstarch, wheat starch, milo starch (also called grain sorghum starch), and rice starch], potato starch, tapioca starch and arrowroot starch as direct food ingredients and as substances migrating to food from paper and paperboard products and cotton and cotton fabrics used in food packaging. The chemical modifications of these starches given in the Food Chemicals Codex (3) also are evaluated as food ingredients.

Starch is a polymer of glucose and is the carbohydrate reserve of plants. Native starch in tubers, roots, seeds, fruits and in the pith of some plants, occurs as minute granules varying in size (about 3 to 100 microns diameter in commercial starches) and shape depending on the plant source. The principal sources of commercial starch are corn, wheat, rice, grain sorghum, potato, cassava, arrowroot and sago palm (4, 5). Starch is produced commercially by extraction from seeds and tubers of plants by wet-milling processes in which the starch is liberated by grinding aqueous slurries of the raw material. Sulfur dioxide is used to aid in the separation of starch from the protein matrix of corn and grain sorghum; inhibit the action of oxidative enzymes that discolor potato starch and, in case of tapioca starch, to aid in starch settling, improve color and inhibit bacterial action (6-8).

Nonstarch constituents in cornstarch, the major starch produced in the United States, are moisture, 10 to 11 percent; fat (total, by acid hydrolysis) 0.65 percent; protein, 0.25 to 0.30 percent; ash, 0.08 percent; and oil (CCl_4 extraction) 0.02 percent (6). Good quality potato starch has the approximate composition: ash, 0.35 percent; nitrogen, trace; fat, practically nil; and cold water solubles, 0.1 percent. Potato starch is unusual in that it contains 0.06 to 0.1 percent phosphorus. Phosphorus is present as dihydrogen orthophosphate groups esterified to the amylopectin fraction (7).

The common starches contain two polysaccharide components, amylose and amylopectin. Amylose is a linear polymer containing about 200 to 2,000 D-anhydroglucose units (32,000 to 320,000 mol. wt.) joined by α-1,4 bonds. Amylopectin is a highly branched molecule that consists of linear α-1,4 linked segments branched through α-1,6 bonds at intervals of 15 to 25 anhydroglucose units. Molecular weights of the amylopectins of the common starches are in the multimillions (4, 5). The relative proportions of amylose and amylopectin are characteristic of any particular plant species. Cornstarch contains about 27 percent amylose; potato, 22 percent and tapioca, 17 percent. Genetic variants of corn, grain sorghum and rice, the waxy cereal grains, are composed almost entirely of amylopectin (9). Another genetic variant of corn, amylomaize, contains starch having 50 to 80 percent amylose (10).

Starch molecules in the granule are held together by hydrogen bonding. A preferred orientation of the amylose molecules and linear segments of the amylopectin molecules is manifest in birefringence properties of the granule. When a slurry of starch in water is heated beyond a critical temperature (62°C for cornstarch) the granules swell to many times their original size. This process, referred to as gelatinization, is accompanied by a loss of granule birefringence, increase in optical clarity and abrupt rise in viscosity of the solution. Some molecules leach out of the swollen granules which eventually reach maximum hydration, rupture and collapse as heating is continued to 95°C, yielding a dispersion of granule fragments, molecular aggregates and free molecules. As this occurs, the viscosity decreases and tends to stabilize. On cooling, the viscosity increases and the system may set to an opaque gel depending on the starch source and concentration. Starch gels show syneresis on standing. If the starch gel is frozen and thawed, the effect is magnified. Upon slow cooling of starch dispersions, the amylose molecules tend to associate and become insoluble, a process called retrogradation (5, 11).

Unmodified starches are primarily used in foods as thickening or gelling agents and processing aids. Corn or wheat starch may be used to thicken foods where the opacity of the starch dispersions and their gelling and syneresis characteristics are not a serious disadvantage. Ordinary cornstarch, for example, is used where a gel structure is required in custard and cream fillings. Waxy corn and tapioca starches may be used in canned foods to provide temporary viscosity and keep ingredients uniformly suspended during filling operations. However, for a large number of food applications, modified starches have been introduced because of their superior properties (5, 11).

Both unmodified and modified starches are widely used in the manufacture of paper and paperboard products. Unmodified starches, generally in the form of gelatinized starch pastes prepared at the paper mill, are added to the wood pulp slurry in the beater to serve as an internal binder and strengthen the sheet formed on the paper machine. In some mills, raw starch is added dry to the pulp furnish. However, to be effective the wet paper sheet must reach temperatures sufficient to gelatinize the starch on the dryer. Pregelatinized starches are used in mills where a dry additive is needed because of lack of starch cooking facilities (12).

Starch pastes are applied as a surface sizing to the formed paper sheet to improve appearance and erasibility, inhibit ink penetration and to form a hard surface for writing or printing. Native starches are enzyme-converted to a low viscosity for this application. Chemically modified starches such as oxidized starch also are used for this purpose. Starch also serves as an adhesive in paper coatings to bond pigment particles to each other and to the paper. Most commonly used pigments are clay, calcium carbonate and titanium dioxide. Coating improves the appearance and printability of the paper. Oxidized and enzyme-converted starches are commonly used as coating adhesives (12).

In the textile industry, pastes of unmodified cornstarch are used in sizing of warp yarns to improve strength and abrasion resistance and reduce fuzz on the yarn prior to the weaving operation. Starch is used primarily in the sizing of cotton yarns and is removed from most fabrics subsequent to weaving. The high biochemical oxygen demand (BOD) of starch wastes from cotton mills has encouraged the use of chemically modified starches and other sizing materials having lower BOD's in recent years (13).

Cornstarch is generally recognized as safe (GRAS) by FDA as a food ingredient (14). Starch, modified, appears on the list of substances presumed GRAS by FDA that was included in the NAS/NRC survey of industry on the use of food chemicals generally recognized as safe (15). Use of specified modified starches in food is regulated under CFR 172.892 (2).

Unmodified starch, cornstarch, pregelatinized starch and acid-modified starch are considered GRAS (2) as substances migrating to food from paper and paperboard products used in food packaging [21 CFR 182.90]. Cornstarch, potato starch, tapioca starch and wheat starch are cited among the GRAS substances that may migrate to food from cotton and cotton fabrics used in dry food packaging [21 CFR 182.70]. Modified starches used as food ingredients that are also used in the manufacture of paper and paperboard are hypochlorite-oxidized, acid-modified, and enzyme-modified starches (2, 12, 16, 17). Starch modified by amylolytic enzymes was considered in another report of the Select Committee (18) and will not be evaluated in this report. Regulations applicable to additional chemically modified starches that are used in paper and paperboard intended for food packaging and for such products that will contact food, are given in 21 CFR 178.3520.

Specifications for modified food starch are given in the Food Chemicals Codex (3). Upper limits of impurities specified are arsenic, 3 ppm; heavy metals (as Pb), 40 ppm; lead, 5 ppm and sulfur dioxide, 80 ppm. Additional specifications for specific starch modifications are given in Table I. These specifications are essentially identical with those given for food starch-modified in 21 CFR 172.892. No specifications are given in the Food Chemicals Codex for food grade unmodified starches.

The following types of chemical treatments are used in making modified starches for use as food ingredients:

1. Degradation of the starch molecule by acid hydrolysis or moderate oxidation in order to reduce the viscosity of starch pastes and permit them to be used at higher concentrations than would be possible with native starches. Acid-modified starches (Table I, product 1) are made by acid-catalyzed (hydrochloric or sulfuric acid) hydrolysis of water suspensions of granular starches at nongelatinizing temperatures. When the desired reduction in

TABLE I

Specifications for Modified Food Starches (3)

Product	Starch treatment	Residuals limitation
1. Acid-modified starch	Hydrochloric and/or sulfuric acid	
2. Bleached starch	Active oxygen obtained from hydrogen peroxide, and/or peracetic acid, not to exceed 0.45 percent of active oxygen Ammonium persulfate, not to exceed 0.075 percent, and sulfur dioxide, not to exceed 0.05 percent Chlorine, as sodium hypochlorite, not to exceed 0.0082 pounds (3.72 g) of chlorine per pound (454 g) of dry starch	
	Potassium permanganate, not to exceed 0.2 percent	Not more than 50 ppm of residual manganese (as Mn)
	Sodium chlorite, not to exceed 0.5 percent	
3. Oxidized starch	Chlorine as sodium hypochlorite, not to exceed 0.055 pounds (25 g) of chlorine per pound (454 g) of dry starch	
4. Starch acetate	Acetic anhydride or vinyl acetate	Not more than 2.5 percent of acetyl groups introduced into finished product
5. Starch sodium succinate	Succinic anhydride, not to exceed 4 percent	
6. Starch sodium octenyl succinate	1-octenyl succinic anhydride, not to exceed 3 percent	
7. Starch aluminum octenyl succinate	1-octenyl succinic anhydride, not to exceed 2 percent, and aluminum sulfate not to exceed 2 percent	
8. Starch phosphate	Monosodium orthophosphate	Not more than 0.4 percent of residual phosphate (calculated as P)

TABLE I continued

Product		Starch treatment	Residuals limitation
9.	Distarch phosphate	Phosphorus oxychloride, not to exceed 0.1 percent Sodium trimetaphosphate	Not more than 0.04 percent residual phosphate (calculated as P)
10.	Phosphated distarch phosphate	Sodium tripolyphosphate and sodium trimetaphosphate	Not more than 0.4 percent residual phosphate (calculated as P)
11.	Acetylated distarch phosphate	Phosphorus oxychloride, not to exceed 0.1 percent, followed by either acetic anhydride, not to exceed 8 percent, or vinyl acetate, not to exceed 7.5 percent	Not more than 2.5 percent of acetyl groups introduced into finished product
12.	Hydroxypropyl distarch phosphate	Phosphorus oxychloride, not to exceed 0.1 percent, and propylene oxide, not to exceed 10 percent	Not more than 5 ppm of residual propylene chlorohydrin
13.	Hydroxypropyl starch	Propylene oxide, not to exceed 25 percent	Not more than 5 ppm of residual propylene chlorohydrin
14.	Oxidized hydroxypropyl starch	Chlorine, as sodium hypochlorite, not to exceed 0.055 pounds (25 g) of chlorine per pound (454 g) of dry starch; active oxygen obtained from hydrogen peroxide, not to exceed 0.45 percent; and propylene oxide not to exceed 25 percent	Not more than 5 ppm of residual propylene chlorohydrin
15.	Acetylated distarch adipate	Adipic anhydride, not to exceed 0.12 percent, and acetic anhydride	Not more than 2.5 percent of acetyl groups introduced into finished product
16.	Distarchoxy propanol	Acrolein, not to exceed 0.6 percent	
17.	Distarch glycerol	Epichlorohydrin, not to exceed 0.3 percent	

TABLE I continued

Product		Starch treatment	Residual limitation
18.	Acetylated distarchoxy propanol	Acrolein, not to exceed 0.6 percent; vinyl acetate not to exceed 7.5 percent	Not more than 2.5 percent of acetyl groups introduced into finished product
19.	Hydroxypropyl distarch glycerol	Epichlorohydrin, not to exceed 0.1 percent, combined with propylene oxide, not to exceed 10 percent	Not more than 5 ppm of residual propylene chlorohydrin
		Epichlorohydrin, not to exceed 0.1 percent, followed by propylene oxide, not to exceed 25 percent*	Not more than 5 ppm of residual propylene chlorohydrin
20.	Acetylated distarch glycerol	Epichlorohydrin, not to exceed 0.3 percent, and acetic anhydride	Not more than 2.5 percent of acetyl groups introduced into finished product
21.	Succinyl distarch glycerol	Epichlorohydrin, not to exceed 0.3 percent and succinic anhydride, not to exceed 4 percent	
22.	Gelatinized starch	Sodium hydroxide, not to exceed 1 percent	

*This specification is given in 21 CFR 172.892.

viscosity has been achieved, the acid is neutralized and the granular starch is filtered, washed, and dried. The basic reaction is hydrolysis of glucosidic bonds of starch molecules and reduction in molecular size. Acid-modified starches are characterized by the fluidity of their pastes as measured under standard conditions. Commercial products range in fluidity from less than 10 to about 90. Molecular weights of amylose and amylopectin fractions of a commercial 90-fluidity starch were about 30,000 as determined by osmotic pressure measurements (17, 19).

Dextrins are modified starches produced by dry heating or roasting starch with or without an acid or alkaline catalyst. Hydrolysis of glucosidic bonds and/or transglucosidation reactions occur, their relative extent depending on reaction conditions. The dextrins were considered in another report of the Select Committee (20) and will not be evaluated in this report.

2. Treatment with minimal quantities of oxidizing agents to produce bleached starches (Table I, product 2). Reagents used are hydrogen peroxide, peracetic acid, ammonium persulfate, sodium hypochlorite, potassium permanganate and sodium chlorite. Oxidation removes color due to traces of pigments such as xanthophyll and carotene. Color is objectionable in applications where starch is added as a moisture sorbing and fluidifying agent for dry powders such as confectioners sugar. Agents used for bleaching also help reduce the microbiological count to levels necessary for certain applications. Treatments are made on aqueous suspensions of starch so that color bodies solubilized by bleaching may be removed by filtering and washing (21). Excess oxidant is neutralized by reduction with sodium bisulfite or sulfur dioxide prior to final filtration, washing and drying (19).

Oxidized starches are made by treatment of aqueous suspensions of starch granules with chlorine, as sodium hypochlorite, at levels up to 5.5 percent of the dry starch weight (Table I, product 3). On completion of the oxidation the reaction mixture is neutralized with acid; bisulfite solution or sulfur dioxide is added to destroy any unspent oxidant, and the reaction mixture is diluted with water. The product is recovered on a filter, washed with water and dried. A limited number of starch hydroxyl groups are converted to carboxyl and carbonyl groups with concomitant scission of the starch chain. Introduction of carboxyl and carbonyl groups reduces the tendency of starch solutions to retrograde and their pastes to gel. The number of carboxyl and carbonyl groups introduced may be about 1 per 30 to 200 anhydroglucose units, the values within this range depending on the particular starch and the reaction conditions (16, 19).

3. Reacting starch with monofunctional reagents to introduce substituents on the hydroxyls by esterification or etherification. Introduction of substituent groups such as acetate, succinate, octenyl succinate, phosphate, and hydroxypropyl into the molecule (Table I, products 4-8, 13) reduces the

tendency of starch to associate in solution, lose clarity and form gels (5, 22). Extent of substitution is low in all derivatives (Table I) except hydroxypropyl starch which may contain up to 15 percent or possibly more hydroxypropyl groups. Polyelectrolyte properties result from the introduction of the phosphate and the succinate ester groups.

4. Cross-linking modification by treating starch in the granule form with very small amounts of difunctional agents capable of reacting with hydroxyl groups of two different molecules within the granule (Table I, products 9, 15-17). Cross-linking stabilizes the viscosity of starch pastes by maintaining the integrity of gelatinized, swollen granules at high temperatures, in acid solutions and under shear conditions. Flow properties and clarity of pastes also are improved for certain applications. Cross-linking reactions are normally run on aqueous suspensions of granular starch at temperatures below gelatinization. A cross-linking reagent that does not react with starch is hydrolyzed under conditions of the reaction and largely removed by washing (5, 23). The residuals limitation of 0.04 percent phosphorus for distarch phosphate (Table I, product 9) corresponds to one phosphate cross-link for about every 500 anhydroglucose units.

5. Reaction of cross-linked starches with monofunctional reagents to introduce substituent groups by esterification or etherification (Table I, products 10-12, 18-21). These starches are used as thickeners in canned pie fillings, retorted puddings and similar applications where stability at high processing temperatures and prolonged low temperature storage are needed (5). A survey of infant foods in 1970 indicated that about 80 percent of strained and junior dinners, high meat dinners, desserts and fruits contained cross-linked starches or their monofunctional esters and ether derivatives (24). However, a 1977 survey indicated that only 65 percent of infant foods contained a modified starch at that time (25).

6. Gelatinization of starch by physical or chemical treatment. Pregelatinized (i.e., gelatinized prior to incorporation into a food product) starches are commonly prepared in the starch industry by feeding moist starch cake, a starch slurry, or a cooked starch paste to the rolls of a drum dryer which operates at temperatures above the glatinization temperature of the starch. The dry gelatinized product is classified by screening to give particle sizes best suited to various applications. Chemically modified starches also may be pregelatinized by this process (26).

Specifications for pregelatinized starch prepared by physical treatment are not included in the Food Chemicals Codex (3) but gelatinized starch prepared by treatment with alkali is included as a modified food starch (Table I, product 22). Depending on the conditions of treatment, hydrolytic scission of glucosidic bonds and/or oxidation of hydroxyl groups may occur in addition to gelatinization of the granule (16). It appears that alkaline

gelatinized starch is not a commercial product in the United States. However, sodium hydroxide is used by the industry as a catalyst in chemical modification of starch and for neutralization and washing of starch and modified starches (27).

The Food Chemicals Codex (3) specifications in Table I place upper limits on the content of acetyl and phosphate groups that are permitted in the corresponding food starch derivatives. Similar limitations on the content of introduced chemical groups are not specified for the other derivatized food starches. It appears that the conditions specified for starch treatment are insufficient to define the extent of reaction with the various reagents. Levels of unreacted phosphate reagent are limited for starch phosphate derivatives by the residuals limitation on total phosphorus. To the extent that bleached and oxidized starches are treated with sulfur dioxide or sulfite to neutralize excess oxidant, the limitation of 80 ppm on residual sulfur dioxide for specified modified starches is a residuals limitation for these products. A further limitation on residuals for permanganate bleached starches is given by the specification of no more than 50 ppm residual manganese. The specification that modified starches shall have a pH range between 3.0 and 9.0 places a residuals limitation on acids in acid-modified starches and base in sodium hydroxide-gelatinized starches. Residual levels are not specified for vinyl acetate nor the reaction byproduct, acetaldehyde, in starch acetate or acetylated distarch phosphate, derivatives for which vinyl acetate is permitted as an acetylating reagent. Neither are residual reactant limitations specified for the starch succinate derivatives or for distarchoxy propanols. However, manufacture of the latter starches was discontinued many years ago; residual acrolein levels were less than 1 ppm (27a). Propylene oxide will react with chloride ion present in the reaction mixture to form propylene chlorohydrins. Specifications for hydroxypropyl starch derivatives place a limitation of 5 ppm on residual propylene chlorohydrin; however, present commercial starches modified with propylene oxide contain less than 1 ppm total chlorohydrins using a test sensitive to about 0.1 ppm (28). Epichlorohydrin, the cross-linking agent used in producing distarch glycerols will react with water in the presence of alkali used to catalyze the reaction with starch to produce 3-chloro-1,2-propanediol and glycerol; in the presence of trace amounts of chloride ion, it will react to produce 1,2-dichloro,3-propanol and the isomeric compound, 1,3-dichloro,2-propanol (29). No residual limitations are specified for these compounds. Present evidence indicates that epichlorohydrin cross-linked starches contain less than 0.05 ppm residual epichlorohydrin, the sensitivity limit of present analytical methods, and less than 3 ppm total of the chlorinated propanols. Industry has, however, discontinued manufacture of epichlorohydrin cross-linked starches (28).

III. CONSUMER EXPOSURE DATA

Starch, mainly as a component of cereal products and vegetables, supplies about 20 percent of the energy content of the average American diet. In the years from 1971 through 1974, the available food supply provided about 180 g of starch per capita per day (30).

According to Agricultural Statistics (31), the annual per capita consumption of wet-milled cornstarch as food, 1968-1974, as estimated from disappearance data was 1.9 pounds (0.86 kg) or about 400 million pounds (182 million kg) total. Both unmodified and modified cornstarch are included in these figures. Breakdown of sales of cornstarch, modified and unmodified, to the food industry in 1971 shows 160 million pounds (73 million kg) sold to grocers as packaged starch and 95 million pounds (43 million kg) sold to brewers (4). This leaves 145 (66 million kg) of the 400 million pounds of cornstarch for use as a food ingredient by food processors. Total food usage of potato, wheat, tapioca (cassava), sago and arrowroot starches in 1971 was estimated to be 100 million pounds (45 million kg) (4) which added to the figure for cornstarch gives a total of 245 million pounds (111 million kg) for all starches used by the food industry in 1971. A survey of the food industry by a National Research Council (NRC) subcommittee (15) indicated 1970 usage of modified starch by food processors was 120 million pounds (54 million kg), based on poundage data adjusted to take into account the subcommittee's estimate that about 60 percent of the total poundage used was included in their survey. Assuming modified starch usage also was 120 million pounds in 1971, subtraction of modified starch usage from total starch usage leaves 125 million pounds (57 million kg) as the quantity of unmodified starches used in 1971 by food manufacturers. On a per capita daily basis, this amounts to 0.7 g, a quantity small in comparison to the 180 g of unmodified starch available per capita as a component of foods in the average diet. A recent NRC subcommittee survey indicated that 1975 usage of modified starch by the food industry was 170 million pounds (77 million kg) corresponding to a per capita daily usage of 1 g (32).

The NRC subcommittee survey (15) provided information on the level of addition of modified starch to foods in several food categories as given in Table II. Information on specific modified starches and unmodified starch usage was not requested. However, data were volunteered by three or fewer companies on levels of addition of several unmodified starches to some of the food categories. Because of the limited information base, these data are not included in this report.

The NRC subcommittee estimated the possible average daily intake of modified starches from Market Research Corporation of America data on the mean frequency of eating foods by food category, U.S. Department of

TABLE II

Level of Addition of Modified Starches to Foods by Food Category (15)

Food Category	Starch, modified Weighted mean percent
Baked goods, baking mixes	1.62
Grain products, such as pastas or rice dishes	7.47
Fats and oils	1.77
Milk products	0.01
Cheese	1.00
Processed fruits, juices and drinks	2.20
Meat products	1.50
Poultry products	1.46
Egg products	3.49
Fish products	2.73
Processed vegetables, juices	1.09
Condiments, relishes, salt substitutes	0.94
Soft candy	8.29
Sugar, confections	2.38
Gelatins, puddings, fillings	3.02
Soups, soup mixes	1.22
Snack foods	1.81
Nuts, nut products	1.20
Reconstituted vegetable proteins	1.36
Gravies, sauces	1.70
Dairy products analogs	3.91
Chewing gum	0.42
Baby food processed fruit	4.10
Baby food processed vegetables	4.32
Baby food puddings	4.53
Baby food soups, mixes	3.25
Baby food meat dinners	3.16
Baby food combination dinners	3.00

Level of addition of modified starches is the weighted mean of the levels reported by manufacturers as their usual addition to one or more products in a food category. For discussion of weighted mean see text, also Section X and Exhibit 50 of Reference (15).

Agriculture data on mean portion size in these categories, and the assumption that all food products within a category contained modified starch at the level shown in Table II. Such an assumption is likely to lead to overestimates of intake. The NRC subcommittee has recognized that in most cases its calculations of possible intakes are overstated, often by considerable margins. This is probably the case with respect to modified starches. For the 2 to 65+ year-old age group the estimated possible average daily intake was 11.8 g as contrasted with the per capita daily usage of 0.7 g of modified starch by the food industry estimated from poundage data. The possible daily intakes given for the 0 to 5 and 6 to 11 month-old age groups are in good agreement with 1970 survey data obtained from one-day dietary histories of 430 infants between one and 14 months of age which indicated that the average daily intake of modified starch for this age group was about 5.7 g or about 2.3 percent (about 0.7 g per kg body weight) of the total caloric intake (24). The maximum intake of modified food starch by any infant in the survey was 35 g per day, about 16 percent of that infant's caloric intake. Modified food starches supplied more than 10 percent of the caloric intake for only four of the 430 infants. Cross-linked starches, esterified cross-linked starches and etherified cross-linked starches were consumed in the proportions of 10:5:1.

From a food consumption survey conducted in 1974, high and low values for the intake of modified food starches were estimated from the average food consumption of infants and the range in content of modified starches in the categories of foods eaten (33). Low values ranged from 2.2 percent (about 0.7 g per kg body weight) of the caloric intake for one-month-old infants to 3.7 percent (about 1 g per kg) for those 12 months old; high values ranged from 4.0 percent (about 1.3 g per kg) at one month to 6.2 percent (1.6 g per kg) at 12 months. Highest value was 7.1 percent (about 1.7 g per kg) of caloric intake for infants 6 months old.

A 1977 survey of infant food and food starch manufacturers disclosed that only three modified starches were being used in infant foods: distarch phosphate (level, >0.1 but <5.5 percent), acetylated distarch phosphate (level, >1 but <5.5 percent), and acetylated distarch adipate (level, >1 but <7 percent) (25). Infant formulas were reported to contain >0.5 but <3.0 percent distarch phosphate. However, only one infant formula listed in the Physicians' Desk Reference (34) is reported to contain modified starch in this concentration range; the level in this formula is given as 1 percent. Some special dietary formulas for feeding infants with particular medical problems contain more than 3 percent of a modified starch (34). During the second month of life, the 50th percentile for energy intake is estimated to be 565 kcal per day and the 90th percentile is estimated to be 680 kcal per day (35). Assuming the entire energy were provided by a formula containing 1 percent distarch phosphate and supplying 67 kcal per 100 ml, intakes of the modified food starch would be 8 and 10 g per day, respectively (approximately 1.8 and 2.1 g per kg per day).

From a food consumption survey conducted in April, 1977, intake of modified food starches was estimated from the average food consumption of 151 infants and the content of modified starches in the infant foods eaten (25). About 35 percent of the infant foods reported used contained no modified starch and about one-fifth of the infants had diets containing no modified starch. Overall average daily intake was 2.9 g (S.D. 6.1 g). Average daily intake ranged from 0.55 g (S.D. 0.789) for 0 to 3-month-old infants to 5.1 g (S.D. 5.1 g) for infants 8 months old. Maximum intakes for individuals in these age groups were 3.1 and 15.8 g (0.6 and 1.7 g per kg), respectively. The latter was the highest daily intake of any infant in the survey and represented 7.8 percent of that infant's caloric intake. About 50 percent of the infants surveyed had modified starch intakes (g per kg body weight) in the range 0 to 0.19; 17 percent, 0.20 to 0.39; 10 percent, 0.40 to 0.59; 9 percent 0.60 to 0.79; 13 percent, 0.80 to 1.39; and 2 percent, 1.40 to 1.79.

The NRC subcommittee survey provided no data on the use of starches or modified starches in paper and paperboard used in food packaging or in cotton and cotton fabrics used in dry food packaging. Russell (36), however, has estimated that approximately 3 billion pounds of cornstarch products, including unmodified and modified starches, were used in industrial or non-food applications in 1972. About 90 percent of this quantity was starch products used in the paper and paperboard industry. The fraction of these products that was used for packaging foods was not estimated. Breakdown of cornstarch sales for industrial applications by type of product is given in Table III. It should be noted that unmodified starch sold to the paper industry is largely processed before use by enzymatic hydrolysis or steam-jet cooking to reduce its molecular size and viscosity. The quantities of the principal starches sold to the textile industry in 1972 were estimated to be (in million pounds): unmodified starch, 83; acid-modified, 170; and high-amylose, 22 for a total of 275 million pounds.

TABLE III

Sales of Cornstarch Products for Industrial Applications in 1972 (36)

Starch product	Millions of pounds	g/capita/day*
Unmodified starch	1,817	10.8
Pregelatinized starches	100	0.6
Acid-modified starch	340	1.9
Oxidized starch	166	1.0
Dextrins	150	0.9
Cationic starches	100	0.6
All others, including hydroxyethyl starch	420	2.6
TOTAL	3,093	

*Calculated on basis of the U.S. population of 208 million in 1972.

Total per capita sales on a per day basis (Table III), and thus the maximum possible consumption of nonchemically derivatized starches (unmodified, pregelatinized and acid-modified) in paper and paper products was 13 g in 1972. This quantity is small compared to the quantity available (180 g) as a natural component of the foods in the average American diet. Per capita daily sales of the chemically modified starches was 2.6 g or less. As in the cases of the unmodified starches, only a very small fraction of this quantity could be expected to migrate into foods from paper or paper products used in food packaging.

Per capita sales on a per day basis of unmodified and acid-modified starches to the textile industry was 1.2 g. Because these starches are principally used as a sizing that is removed after the looming operation, only a small residual would likely remain in cotton or cotton fabrics used in dry food packaging (36).

IV. BIOLOGICAL STUDIES

Unmodified Starches and Pregelatinized Starches

Digestion and absorption

Cooked (gelatinized) native starch is digested within the intestinal lumen by α-amylase secreted by the pancreas. The products are maltose, maltotriose and α-limit dextrins containing five to nine glucose residues and one or more α-1,6 branching links. The brush border of the intestinal mucosa contains an α-dextrinase, glucoamylase and maltase that convert the products of intraluminal digestion into glucose. Only the monosaccharide glucose is transported through the intestinal wall into the bloodstream (37). Comprehensive discussions of the metabolism of glucose are given in standard biochemistry textbooks (38, 39).

Raw corn, waxy maize, wheat, rice and tapioca starches in diets containing 63.7 percent starch were 98 percent digested by weanling rats in 28-day feeding experiments whereas digestibilities of raw potato, arrowroot and sago starches in the same concentrations were 51, 80, and 65 percent, respectively. Utilization of starches as evidenced by weight gains at 28 days paralleled digestibility coefficients. Destruction of granule structure of potato starch by gelatinization increased digestibility to 96 percent (40). Digestibility of high amylose cornstarches containing from 35 to 67 percent amylose as determined *in vitro* by treatment with pancreatin was not directly associated with amylose content but was related to the genetic background of the corn variety (41). Digestibilities in rats of unmodified high amylose cornstarches having 50, 63, and 77 percent amylose were 71, 77, and 66 percent, respectively (42). Digestibility of high-amylose starch ingested as a component of corn meal muffins made with high amylose corn meal was 88 percent in a human subject (43). Microscopic examination showed that the starch was not gelatinized in baking the muffins. Although starch in the meal analyzed 70 percent amylose, starch granules recovered from the feces contained only 47 percent amylose. It has been suggested that susceptibility of granular starches to enzymatic attack is related to the size and number of pores in their granule structure which permit entry of enzyme molecules (44).

Pancreatic amylase activity in human infants is relatively low during the first few months of life and digestion of starch is slower than in older infants. Auricchio *et al.* (45) reported an α-amylase activity of 3.9 to 7.7 units per ml in duodenal fluids of four infants $2\frac{1}{2}$ to 5 months old whereas that of six infants 8 to 13 months of age was 48 to 73 units per ml. Average degree of polymerization of the saccharides in duodenal fluid of the younger groups of infants collected 2 to 4 hours after ingestion of amylopectin was 7 as compared to 3.3 for the older group. During balance periods of 3 days, De Vizia *et al.* (46) found that wheat, tapioca, corn, rice, and potato starch in amounts of 45 and 85 g per m^2 of body surface per day were almost completely absorbed

(98 percent) in 1- and 3-month-old infants when fed as cooked flours. Known quantities of starch were fed and the fecal content of lactic acid, glucose, dextrins, and starch was measured. Only minimal rises in glucose concentration in the blood were observed after feeding a thin-boiling (acid-modified) waxy maize starch to 3-day-old infants by nipple which suggests slow hydrolysis and/or absorption of the starch (47). Mean blood glucose levels did not rise above the base line by more than 10 mg per 100 ml in infants 4 to 60 days of age after ingesting a 10 percent solution of a thin-boiling waxy sorghum starch (48). However, Hlavon and Klušaček (49) reported peak increases in blood glucose of 24, 8, 22, 18 and 34 mg per 100 ml after intragastric administration of 2 g rice starch (in 20 percent suspensions) per kg body weight to groups of 15 to 28 infants aged 1, 3, 5, 30 and 180 days, respectively. They state that the differences among age groups were not statistically significant. Similar studies with potato and cornstarch showed no significant differences in digestibilities among starches.

Acute toxicity

No reports were available on the acute toxicity of raw or cooked unmodified starches.

Short-term studies

Raw, unmodified corn, wheat, rice, tapioca and waxy maize starch gave normal weight gains, protein efficiency ratios (PER) and cecal weights when fed for 28 days to Wistar rats, 50 g initial body weight, in diets containing either 6 or 15 percent casein and 77 or 66 percent starch. Feed consumption of animals receiving the 6 percent protein diet containing raw potato starch was relatively high compared to that of animals fed the other starches; cecal weights also were relatively high but feed efficiency and PER were not significantly different. Feed efficiency and PER were relatively low in animals fed raw potato starch in the 15 percent protein diet but were normal when fed pregelatinized (dried, gelatinized) potato starch in the same basal diet. Unmodified arrowroot starch resulted in inferior weight gain and PER on both low and high protein diets; PER was normal for diets containing pregelatinized arrowroot starch but weight gain remained relatively low (50).

Raw cornstarch fed to 61 mice for 4 weeks at a 71 percent level produced fewer lesions of the duodenal mucosa than did glucose or sucrose fed to a similar number of animals (51).

In a study by Harper et al. (52), groups of five or six male weanling Wistar rats were given diets containing 18 percent casein and 73.6 percent of either unmodified potato starch, autoclaved potato starch, glucose, sucrose or dextrin for 4 to 6 weeks. Food intake was limited to 10 g per day. Weight gains were similar for all groups except the one fed unmodified potato starch.

Bulky white feces of animals in this group were composed largely of undigested starch granules. There also was loss of dietary protein in the feces. In another study, rats grew as well on raw wheat starch or cornstarch as on sucrose but raw potato starch was poorly utilized. All diets contained 74 percent carbohydrate. Grinding, heating in an oven at 145°C or autoclaving moistened starch at 120°C improved the utilization of potato starch (53).

Weanling pigs (breed not stated) fed diets containing high levels of pearl cornstarch and low levels of protein (raw cornstarch containing 7 to 10 percent gelatinized starch) developed a high incidence of esophagogastric ulcers within 80 days (54). Frequency of occurrence of ulcers in groups of seven or eight pigs fed 89, 77, 64, or 48 to 58 percent starch in their diets was 6/7, 8/8, 6/8, and 1/8, respectively. Protein contents of these diets were 0, 5, 10, and 12 to 16 percent in the order listed. In view of previous studies which indicated that diets containing gelatinized whole corn or gelatinized corn endosperm were associated with ulcers in swine, the investigators compared cornstarch flour (no heat treatment) with pearl cornstarch in a second feeding experiment with 8-week-old Duroc and Duroc x Yorkshire pigs. Incidence of ulcers in the two groups was similar. Other dietary and stress factors also have been associated with esophagogastric ulcers in swine (55-58).

Complulsive ingestion of raw starch as well as clay is not uncommon among certain segments of the population (59-62). As much as 2 pounds, generally laundry starch, may be ingested per day. On the basis of 14 percent moisture content, this provides 3160 calories. The chief symptoms are obesity and iron-deficiency anemia. In some cases enlargement of the parotid glands has been observed and one patient who consumed 3 to 4 pounds of starch per day developed a starch gastrolith (59).

Seven adult male subjects were given low fat diets containing 500 g of raw cornstarch or sucrose daily for a 25-day period (63). The only adverse effects noted by the subjects ingesting the starch diet were flatus and borborygmi. Serum lipids fell and serum transaminase rose. The authors suggested the latter might reflect a degree of liver damage.

Fifteen hyperlipoproteinemic patients were fed formula diets containing 50 percent of the calories as carbohydrate, 35 percent as fat and 15 percent as protein for two 28-day periods. Substitution of 40 percent of the calories as sucrose for wheat starch resulted in significant increases in levels of serum cholesterol, phospholipid and triglyceride in all patients (64).

Special studies

Animal feeding experiments indicate that diets containing starch as the principal carbohydrate component are less cariogenic than similar diets in which starch is replaced by sucrose, glucose or fructose (65-70).

Orland et al.(71) reported that a dextrin (uncharacterized) was less cariogenic than sucrose when fed to rats as the carbohydrate component in semi-synthetic purified diets. Although less cariogenic than sucrose, roll-dried (gelatinized) cornstarch and roll-dried wheat starch (67) were found to be more cariogenic to rats than the corresponding uncooked starches. However, others have reported no difference in the incidence of carious lesions in rats fed cooked and raw wheat starches (70).

No studies designed to test the carcinogenicity, teratogenicity, or mutagenicity of unmodified starches were available to the Select Committee.

Acid-modified Starch

Digestion and metabolism

Acid-modified wheat starch in diets containing 63.7 percent starch was 97.8 percent digested by weanling rats in 28-day feeding experiments. Digestibility and utilization of acid-modified wheat starch as measured by weight gains did not differ significantly from the values found for unmodified wheat starch (40).

Short-term studies

An acid-modified waxy cornstarch (80 fluidity) contributed about 25 percent of the calories in the control diets fed to Pitman-Moore miniature pigs in two studies of chemically modified food starches (72, 73). The pigs were weaned at 3 days of age and fed the starch-containing diets for 25 days. Serum concentrations of cholesterol and triglyceride were markedly lower in the starch-fed pigs than in sow-reared pigs (74), reflecting the lower cholesterol content and highly unsaturated character of the fat in the starch diets. Urea levels in the serum of pigs fed the starch diets (26 mg per 100 ml) were higher than those in sow-reared pigs (18 mg per 100 ml) although protein levels in the diets were similar. All organs, including ceca, appeared to be grossly normal when inspected at autopsy. The liver weight, expressed as a percentage of body weight, was slightly less than that found at 28 days in sow-reared pigs but the weights of other organs expressed as a percentage of body weight were comparable.

Acute toxicity

Attempts to demonstrate an acute toxicity level for starch in rats resulted in gastric rupture. No deaths resulted from the intragastric administration of a 60 percent paste of a soluble acid-hydrolyzed potato starch in volumes of 50 to 100 ml per kg body weight but larger doses produced gastric rupture. Larger total daily doses could be tolerated if 50 to 100 ml volumes were administered every $2\frac{1}{2}$ hours (75).

Special studies

Orland et al. (71) reported that a dextrin (uncharacterized) was less cariogenic than sucrose when fed to rats as the carbohydrate component in semipurified diets.

Bleached Starches

No toxicity data on the bleached starches were available to the Select Committee. All agents listed in Table I for the production of bleached starches are oxidizing agents and, although the mechanism of reaction may differ, at sufficiently high concentrations all react with starch to introduce carboxyl and/or carbonyl groups (76). However, few such groups are introduced because of the low level of oxidant required to bleach and/or reduce the microbial count of starch.

A second consideration concerns the possible toxicity of the reduced form of the oxidants that may remain in the bleached starch. For hydrogen peroxide, peracetic acid, ammonium persulfate and sulfur dioxide, permanganate, sodium hypochlorite and sodium chlorite, the reduced forms include water, acetic acid, ammonium sulfate, manganous sulfate and sodium chloride. Residual sulfite and sodium sulfate may also be present in some of these starches. Because the bleached starches are washed, residual levels of reduction products will be much less than that predicted by the stoichiometry of the reaction. Health aspects of acetates, sulfates, manganese, sodium chloride and sulfites are evaluated in other reports of the Select Committee (77-81).

Hypochlorite Oxidized Starches

Digestion, metabolism and short-term feeding studies

In vitro digestibilities of two commercial hypochlorite-oxidized cornstarch samples were compared with that of unmodified cornstarch in experiments in which 1 percent solutions of the cooked starches were treated with saliva or U.S.P. pancreatin. A "lightly" oxidized starch containing 0.42 percent carboxyl groups was 84.8 percent digested by pancreatin and 98.8 percent digested by saliva as compared to the unmodified starch control. The moderately oxidized sample (0.84 percent carboxyl groups) was 88.4 and 97.6 percent digested by pancreatin and saliva, respectively (82, 83).

Digestibility of a commercial oxidized wheat starch in 45 to 60 g rats as determined by analyses of the food ingesta and fecal excretions over a 28-day feeding experiment was equal to that of unmodified wheat starch fed at the same levels (63.7 percent) in a similar diet (40). Extent of oxidation of the commercial oxidized starch was not stated.

Digestibility and caloric values of three starches of different degrees of oxidation were similar to those of regular (unmodified) cornstarch when fed to adult rats at a level of 62 percent in the diet (Table IV). No gross pathological changes were observed on autopsy after 10 days on the test diets (83). Caloric values equal to that of unmodified cornstarch also were found for two commercial oxidized cornstarches in 21-day feeding experiments in which weanling rats were fed 5 g of a basal diet plus 1 or 2 g of the oxidized starches or regular cornstarch. One commercial oxidized starch was oxidized by 6 percent (wt/wt) chlorine as hypochlorite and contained 0.9 percent carboxyl groups; the other was treated with 2.5 percent (wt/wt) chlorine as hypochlorite and contained 0.3 percent carboxyl groups. Caloric value of laboratory-prepared oxidized starch treated with two equivalents of hypochlorite (43.2 percent chlorine) was much lower than the control starch as shown by a weight gain of 19 g as compared to 34 g for rats fed the control starch. Autopsies showed that rats fed the heavily oxidized starch had a marked dilation of the colon. Animals fed the commercially oxidized starches appeared normal (84).

In a 90-day feeding study an oxidized starch, 0.9 percent carboxyl, prepared by treating cornstarch with 5.5 percent chlorine as hypochlorite, was fed at dietary levels of 5, 10, or 25 percent (about 4, 8, or 20 g per kg body weight) to groups of 15 male and 15 female weanling albino rats (85). The oxidized starch was substituted for an equal amount of cornstarch in the control diet. Growth and food intake showed no significant differences between test groups and controls nor were there significant differences in hematological indices, biochemical blood values and urine composition which could be attributed to starch treatment. Organ-to-body weight ratios of control and test groups were not significantly different for the heart, kidneys, liver, spleen, brain, gonads, thymus and thyroid. Relative weights of the adrenals were significantly higher in females fed 5 and 10 percent oxidized starch but not in the group fed the diet containing 25 percent oxidized starch. The relative cecum weight was slightly increased at the 25 percent level in females only. Histopathological examination of the lungs, salivary glands, prostate, epididymus, uterus, urinary bladder, thoracic aorta, esophagus, stomach, duodenum, ileum, cecum, colon, pancreas and mesenteric lymph nodes revealed no pathological changes that could be attributed to the ingestion of the oxidized starch. Nephrocalcinosis in the corticomedullary region was seen in 7/15 female controls and in 10/15 females fed the oxidized starch at the 25 percent level. No nephrocalcinosis was observed in 15 males receiving the high level diet nor in 15 control males. However, one male in the high dose group showed a "bud-like structure consisting of loose connective tissue covered with transitional connective tissue extending from the outer medulla into the renal calyx."

TABLE IV

Digestibility and Caloric Values of Hypochlorite Oxidized Starches (83)

Starch	Chlorine Percent *	Carboxyl Percent	Digestibility Percent	Caloric Value
Regular (unmodified)	0.	0.	100.	100.
Lightly oxidized	3.9	0.57	97.0	114.7
Moderately oxidized	4.5	0.80	96.9	99.6
Moderately oxidized	5.5	0.90	96.5	110.9

* Percent chlorine, in form of sodium hypochlorite, in reaction mixture based on dry starch weight.

Starch Acetate

Digestion and absorption

In vitro digestibilities of two acetylated cornstarches, 1.50 and 2.41 percent acetyl contents, as determined by digestion with amyloglucosidase were 84 and 69 percent of that of unmodified cornstarch. In contrast, *in vivo* digestibility in rats of an acetylated cornstarch, 1.8 percent acetyl content, was 106 percent that of pregelatinized cornstarch (86). The digestibility of starch acetate containing 1.98 percent acetyl groups by pancreatin and porcine mucosal enzymes was 90 percent of that of the unmodified starch (87).

Groups of 10 male and 10 female weanling rats were fed semipurified diets containing 25 or 50 percent (about 50 or 100 g per kg) starch acetate (1.98 percent acetyl) as replacement for an equal amount of pregelatinized potato starch in the basal diet (88). After 7 days, 4 percent cellulose was added and the diets were fed for 3 more days. Weight gains were slightly lower and fecal dry matter higher in rats fed starch acetate at the 50 percent level as compared to the controls. A slight diarrhea was observed in the test animals fed the 50 percent level; the severity was not decreased by the addition of cellulose to the diet.

Short-term studies

Groups of 10 male Sprague-Dawley rats were fed for 28 days diets containing 60 percent (about 50 g per kg) acetylated starches (plant source not stated) having 1.24, 2, 2.56, or 3.25 percent acetyl groups. Vinyl acetate was the acetylating agent used. Weight gain was reduced in groups receiving starch acetates with more than 2 percent acetylation as compared to control animals but feed efficiency was unaffected. Diarrhea occurred in animals fed acetylated starches containing 2 percent or higher acetyl content and there was noticeable cecal enlargement in these animals. No tissue damage or inflammation was observed in association with the diarrhea (89).

Acetylated potato starch (1.36 percent acetyl groups) was fed for 13 weeks to groups of 10 male and 10 female rats (strain not stated) at levels 15 and 45 percent (about 10 and 30 g per kg) of the diet (90). Growth and hematological parameters were not significantly affected. Relative weights of the liver, kidney, adrenal and pituitary were generally lower than those of the control animals. Cecal weights of males were higher than those of controls and ceca were distended in animals fed acetylated starch at the 15 and 45 percent levels. No histopathological changes were observed that were attributed to starch acetate in the diets.

A potato starch acetate containing 1.98 percent acetyl groups was fed for 8 weeks to groups of 10 male and 10 female weanling rats (Wistar derived) at 25 and 50 percent levels (about 35 and 70 g per kg) in a practical diet (88). Growth rates did not differ significantly from those of controls. No diarrhea was observed. Cecal weights were higher in animals fed the modified starch and were most pronounced at the 50 percent feeding level. However, histological examination showed no abnormalities of the ceca.

Twelve human volunteers consumed on each of 4 consecutive days 60 g (about 1 g per kg) of starch acetate (starch source not stated) containing 1.98 percent acetyl groups. No effect was noted on the frequency of defecation and on the amount of feces, fecal water or its lactic acid content. No adverse effects were observed (91).

Long-term studies

In a 2-year study, a precooked acetylated potato starch, acetyl content 1.98 percent, was fed to groups of 30 male and 30 female rats at 5, 10, and 30 percent levels (about 2.5, 5, and 15 g per kg) in the diet (92, 93). The modified starch replaced an equal quantity of precooked potato starch in the basal diet. Body weights of male rats fed the 30 percent level were significantly lower than those of the controls at 76 weeks but not at 104 weeks. There was no diarrhea. Survival, hematological parameters, urine composition and organ weights, were unaffected in the test animals with the exception of cecal weights which were greater in animals fed 10 and 30 percent modified starch than those of the controls. It was concluded that cecal enlargement had little if any toxicological significance since it was present throughout the 2-year study but did not result in any relevant microscopic changes in the tissues. Histological examination revealed that suburothelial deposits of calcium accompanied by hyperplasia of the epithelium lining the renal pelvis occurred slightly more frequently in the highest dose group than in controls (Table V). Increased incidence did not occur in females and it was suggested that incidence in males might be related to the occurrence of the parasite Trichosomoides crassicauda in the urinary tract. Similar renal changes have been observed in rats fed diets containing 60 percent lactose (97), 15 percent sodium alginate (98), 0.8-0.9 percent magnesium oxide (99), a nonsteroid anti-inflammatory agent (100), or excess sodium chloride after uninephrectomy (101). A similar pathological process of unknown etiology also has been described in Sprague-Dawley rats (102).

TABLE V

Incidence of Pelvic Nephrocalcinosis in Rats Fed Seven Chemically Modified Starches for Two Years According to Severity of Lesions and Level in Diet

Modified starch	Severity of lesion	Incidence of lesion. ( )=No. animals examined Percent of modified starch in diet *				
		0	5	10	30	62
CIVO/TNO Studies						
Acetylated distarch phosphate (ref. 92, 94)	slight	1 (59)	4 (57)	2 (56)	5 (58)	
	moderate	0	2	3	5	
Acetylated diamylopectin phosphate (ref. 92, 94)	slight	same control as above	6 (55)	4 (56)	3 (56)	
	moderate		0	1	1	
Starch acetate (ref. 92, 93)	slight	2 (58)	0 (57)	2 (57)	3 (57)	
	moderate	1	1	1	1	
Hydroxypropyl distarch glycerol (ref. 92, 93)	slight	same control as above	2 (58)	0 (59)	4 (56)	
	moderate		0	0	3	
Phosphated distarch phosphate (ref. 92, 122)	slight	1 (57)	4 (57)	0 (58)	9 (57)	
	moderate	0	0	0	1	
TOTALS		5/174	19/284	13/286	35/284	
IFFA/CREDO Studies**						
Acetylated distarch glycerol (ref. 96)	discrete	12				22
	marked	8 (49)				6 (51)
	severe	4				3
Acetylated distarch adipate (ref. 96)	discrete	same control as above				12
	marked					10 (49)
	severe					9
TOTALS		24/49				62/100

*5, 10, 30 and 62% modified starch in the diet correspond to daily intakes of about 2.5, 5, 15 and 30 g/kg, respectively.

**The data tabulated do not include incidence of hyperplasia of uroepithelium without calcification.

Potato starch acetylated with vinyl acetate to an acetyl content of 1.6 to 2.5 percent, and a control pregelatinized potato starch were fed to groups of 75 male and 75 female albino SPF mice, Swiss Random strain, for 89 weeks at dietary levels of 55 percent (about 80 g per kg body weight) (103). Other groups were fed diets containing 55 percent lactose or 25 percent sodium alginate. In week 80 ten mice of each sex and group were necropsied and a comprehensive microscopic examination of tissues was made. A similar examination was made of all survivors after week 89. Tissues of all animals found dead or killed when moribund were removed and examined if autolysis was not too far advanced.

Mean body weights of the treated mice did not differ significantly from those of the controls, with the exception of significant decreases in week 16, 20, 40 and 72 in males and in week 84 in females. Incidence of loose stools was no greater in the group fed starch acetate than in the control group. Death rate in the treated group was normal for the strain of mice fed but was abnormally high for males in the control group between weeks 39 and 65. At necropsy about 50 percent of the latter animals had an hemorrhagic myocarditis. Hematological indices, fasting glucose and blood urea nitrogen, were within normal limits in the test animals. Male mice, but not female mice, receiving the experimental diet had a higher content of amorphous material in their urine than controls. Analyses of urine sediment indicated about 95 percent protein; the remainder was phosphates, carbohydrate and possibly silica. Metal ions present included sodium, calcium, magnesium and potassium. No significant differences in organ:body weight ratios occurred between animals fed starch acetate and the controls except for an increased ratio for the cecum and colon of both sexes and a slight, but statistically significant, increase in ratio for the kidneys of females. Twenty-five to 35 percent of the females in the test group had a trichobezoar in their stomach as compared to 10 percent of the female controls. Incidence in males in the test and control groups was similar and no higher than 5 percent.

Overall incidence of very slight and slight calcareous deposits in the renal pelvis that occurred in males in the test group (9/74) was greater than in the control group (0/73). However, this finding could not be tested for statistical significance because of early deaths of males in the control group. In mice that survived for a period of at least 79 weeks, frequencies were 7/49 and 0/28, respectively, a difference not statistically significant. Incidence of intratubular calcareous deposits was significantly greater in males in the test group (25/49) that survived 79 weeks or more than in like animals in the control group (5/28). In females, incidence in the two groups was 13/56 and 11/58, respectively. A slight but not statistically significant increase in calcareous material and in thickened submucosa and epithelium in the urinary bladder occurred in males fed the test diet as compared with controls. Similar renal changes occurred in mice fed the diet containing lactose. No evidence of hyperplasia of the epithelium was found. The investigators concluded that the renal and urinary bladder changes observed had little, if any, toxicological significance.

Special studies

Acetylated potato starch (1.98 percent acetyl content) was fed at the 10 percent dietary level (about 5 g per kg) in a three-generation study using groups of 10 male and 20 female rats for the P, F_1 and F_2 generations to produce two successive litters in each generation by mating at week 12 and 20 after weaning (92, 93). Ten males and 10 females of the F_{3b} generation were kept for 3 weeks after weaning and then sacrificed for histopathological studies. The P, F_{1b} and F_{2b} mothers were examined for implantation sites. No adverse effects were noted in fertility, litter size, resorption quotient, preweaning mortality or growth rate of pups. No gross or histological changes were noted in rats of the F_{3b} generation that were attributable to acetylated starch in the diet.

Starch Sodium Succinate

Absorption and metabolism

Groups of ten weanling male albino rats (strain not stated) were fed, for 4 weeks, a basal diet to which was added 1.5 or 3.0 g (about 20 or 40 g per kg of body weight) of either cornstarch or cornstarch sodium succinate; for comparison, sucrose was fed daily at levels of 0, 0.75, 1.5, 3.0, and 4.5 g (104). Assuming a caloric value of 4 kcal per g for sucrose, values of 3.78 and 3.82 kcal per g (dry basis) were estimated from the growth data for the starch succinate and cornstarch, respectively. All of the rats on the starch succinate diet were normally active and appeared in good health during the 4-week period.

Short-term studies

Weanling albino rats (strain not stated) in groups of three males and three females each were fed ad libitum, for 10 weeks, diets in which the entire carbohydrate portion (70 percent or about 60 g per kg of body weight) was provided by cornstarch sodium succinate or cornstarch (105). Average weight gain and feed efficiency of animals fed the starch succinate diet did not differ significantly from those of the control animals. Blood hemoglobin values were in the normal range. In another experiment by the same laboratory (105) similar groups of rats were pair fed the two starch diets for 10 weeks. Weight gains, feed efficiency and hemoglobin levels did not differ significantly. In both experiments, the animals were robust and healthy and no manifestations of toxicity were observed.

Long-term studies

No reports were available to the Select Committee.

Special studies

No reports on studies of mutagenicity, teratogenicity or carcinogenicity were available to the Select Committee.

Starch Sodium Octenyl Succinate

Digestion and metabolism. Short-term studies

Groups of 10 weanling male albino rats (strain not stated) were fed 2.74 g of a basal diet and 1.5 or 3.0 g (about 20 or 40 g per kg body weight) of unmodified cornstarch or cornstarch sodium octenyl succinate daily for 4 weeks (106). Growth rates of test and control animals did not differ significantly and caloric availability was not depressed in the modified starch. All rats were normally active and appeared in good health during the 4-week period.

In an 8-week feeding experiment, groups of six male and six female weanling albino rats received a ration containing 35 percent (about 30 g per kg body weight) cornstarch sodium octenyl succinate or unmodified cornstarch (107). The test group grew at a slower rate but efficiency of food utilization was equal to that of the control group. Blood cell counts, hemoglobin and blood sugar of test animals at 8 weeks were similar to those of control animals. Serum non-protein nitrogen was lower but was within the normal range. One animal each in the control and test groups died during the experiment. All other rats behaved and appeared healthy and normal throughout the experiment.

Long-term studies

No long-term studies were available to the Select Committee.

Special studies

No studies on reproduction, mutagenicity, teratogenicity or carcinogenicity were available to the Select Committee.

Starch Aluminum Octenyl Succinate

Digestion and metabolism. Short-term studies

Groups of ten weanling male albino rats (strain not stated) were fed, for 4 weeks, 2.74 g of basal diet supplemented with 1.5 or 3.0 g (about 20 or 40 g per kg of body weight) daily of either thin-boiling (acid-modified) starch or the aluminum octenyl succinate derivative of this starch (185).

Caloric value of the test starch as measured by weight gain was equal to the control starch. All of the rats were normally active and appeared in good health throughout the 4-week period. Growth was continuous and there were no deaths.

Groups of six male and six female weanling albino rats (strain not stated) were fed for 8 weeks a basal diet containing 35 percent cornstarch or diets in which 1, 10, or 25 percent (about 1, 10, and 25 g per kg body weight) of cornstarch aluminum octenyl succinate replaced an equal quantity of cornstarch (107). No adverse effects were observed on growth, food consumption, efficiency of food utilization, blood cell count, hemoglobin, sugar or non-protein nitrogen.

Long-term studies

No long-term studies were available to the Select Committee.

Special studies

No studies on reproduction, mutagenicity, teratogenicity, or carcinogenicity were available to the Select Committee.

Monostarch Phosphate

As noted in the Background Information section, potato starch contains 0.06 to 0.1 percent phosphorus present as phosphate ester groups. This may be compared with the residual limitation (Table I) of not more than 0.4 percent phosphorus in starch phosphates prepared by reaction with sodium orthophosphate. The phosphate groups are present in the dibasic form (108).

Digestion and metabolism

Wheat starch phosphate and bleached wheat starch phosphate were hydrolyzed at a faster rate than unmodified wheat starch by alpha amylase in vitro (109).

^{32}P-labeled starch phosphate, disodium phosphate and sodium pyrophosphate were administered by gavage to female Sprague-Dawley rats; urine was collected at 5, 22, and 47 hours and feces at 22 and 47 hours thereafter (109). At 47 hours blood samples were collected and the animals were sacrificed. Percentages of the ^{32}P dose from starch phosphate found in the liver, kidneys, blood plasma, bone and total quantity excreted in feces and urine were similar to those for disodium phosphate and indicate that phosphorus in the form of starch phosphate is metabolized in a manner similar to that of the inorganic phosphate compound.

Short-term studies

Groups of six (experiment 1) and 20 (experiment 2) weanling male Sprague-Dawley rats were fed starch phosphate (starch source not stated) for 4 weeks at levels of 1, 5, and 10 percent (about 1, 5, and 10 g per kg body weight) in a semipurified diet (109). Efficiency of feed conversion was similar to that of control animals. Weights of testes, liver, thymus, adrenal, spleen and kidneys were not affected in experiment 1. Autopsy of all animals of experiments 1 and 2 revealed no abnormalities.

Long-term studies

No long-term studies were available to the Select Committee.

Special studies

No studies on the mutagenicity, carcinogenicity or teratogenicity of monostarch phosphates were available to the Select Committee.

Distarch Phosphate

Digestion and metabolism

In vitro amyloglucosidase digestibility of waxy maize starch cross-linked by reaction with 0.035, 0.070, and 0.100 percent phosphorus oxychloride was 96 to 98 percent of that of the unmodified starch. Both starches were gelatinized in water, then digested with enzyme for 16 hours at 50 to 55°C (110). Potato starch modified by reaction with 0.05 or 0.1 percent phosphorus oxychloride was degraded to the same extent as the unmodified starch by in vitro treatment for 1 hour with pancreatin after gelatinization in boiling water (95). In similar experiments, the in vitro digestibility of gelatinized distarch phosphate, prepared by reaction of milo starch with sodium trimetaphosphate, by salivary, pancreatic or intestinal amylase was equal to that of the gelatinized unmodified starch as measured by liberation of reducing sugars after 15 minutes (111). Dextrose equivalent values and distribution of saccharides, DP 1 through DP 6 in solutions of potato starch, waxy cornstarch, and two distarch phosphates were similar after conversion at 10 percent concentration with alpha amylase for 3 hours at 92°C (112). The distarch phosphates were prepared by treatment of waxy maize starch with 0.085 percent phosphorus oxychloride or 0.5 percent sodium trimetaphosphate. Further hydrolysis of the alpha amylase treated starches with glucoamylase for 2-64 hours at 60°C gave similar values for dextrose equivalent and quantities of dextrose, maltose and maltotriose liberated. In another experiment the in vitro pancreatic digestibility of milo starch modified by reaction with trimetaphosphate was about 80 percent that of unmodified starch as measured by degradation after 20 minutes' digestion (113). However, in vitro digestibilities of these starches in rats were similar. Groups of 10

weanling Sprague-Dawley rats were fed 1, 2, or 4 g (about 20, 40, or 80 g per kg body weight) of unmodified or trimetaphosphate-modified cornstarch for 10 days. Weight gains were similar to controls at each level of supplementation (114).

Caloric value of distarch phosphate prepared by treating milo starch with trimetaphosphate was the same as that of the unmodified starch when measured by weight gains of 50 g rats fed for 7 days on 4 g basal diets supplemented with 0.9 or 3.6 g of the two starches (115). In a similar study, two samples of commercial distarch phosphate (0.5 to 0.9 degrees of substitution) prepared by treating cornstarch with trimetaphosphate were fed to weanling Wistar-Purdue rats for 21 days at levels of 1 or 2 g daily added to a 5 g basal diet (84). Weight gains were similar to those of the control animals. Necropsy of one animal fed the higher level of modified starch showed no gross adverse effects. Caloric value of distarch phosphates prepared by treating waxy maize starch with 0.03 or 0.1 percent phosphorus oxychloride did not differ significantly from that of untreated waxy maize starch when fed for 6 weeks at the 52 percent level (about 60 g per kg of body weight) as the sole carbohydrate source in the diet of six male and six female weanling rats (116).

Acute toxicity

No deaths occurred after the administration of single oral doses of 50 percent aqueous suspensions of distarch phosphate (prepared from white milo, a waxy starch) to groups of 10 female mice (dose, 19 g per kg body weight), 10 female rats (35 g per kg), two guinea pigs (18 g per kg), two rabbits (10 g per kg), and two cats (9 g per kg). Gross autopsy findings of mice and rats conducted 16 days after treatment were negative. Microscopic examination of kidney and liver tissues of the rabbits, cats and guinea pigs showed no abnormalities (117).

Short-term studies

In a 90-day subacute toxicity test, groups of 25 male and 25 female Sprague-Dawley weanling rats were fed diets containing 0.20, 1.0, or 5.0 percent (about 0.2, 0.8, or 4 g per kg) distarch phosphate prepared by treating white milo starch with sodium trimetaphosphate (118). Blood and urine studies were conducted at 45 and 90 days of testing. Blood studies were done individually on five males and five females of the highest dietary group. No abnormalities were observed in hematological parameters or urinalyses of the test animals. Body weight gains and organ-body weight ratios showed only a few, randomly distributed, intergroup differences, none of which was attributed to starch ingestion. Gross pathologic findings among test animals were comparable to those observed among control animals and no adverse histopathologic changes attributed to the test starches were reported.

Groups of 10 male and 10 female rats received 5, 15, or 45 percent (about 4, 12, or 36 g per kg of body weight) of two types of distarch phosphate (0.085 and 0.128 percent esterified phosphate) in their diet for 90 days (119). No abnormalities compared to controls were seen in regard to general appearance, behavior, mortality, food consumption, hematology, serum chemistry and urinalysis which could be attributed to the test starches. No diarrhea or increased cecal weights were observed. Gross and histopathologic examination revealed no abnormalities attributable to the distarch phosphate fed.

Groups of three male and three adult female beagles were fed for 90 days a standard dog chow supplemented daily with 0.05, 0.25, or 1.25 g per kg of body weight of distarch phosphate (trimetaphosphate-treated white milo starch) administered in gelatin capsules (120). Hematological studies and urinalyses were conducted at the inception and conclusion of the feeding period and also after 45 days for the dogs fed the highest level of distarch phosphate. No significant abnormalities were observed. Mean body weight gains and organ-body weight ratios of the test animals did not differ significantly from the controls. Gross and histopathologic examination revealed no abnormalities attributable to the test substance.

Groups of eight 3-day-old Pitman-Moore miniature pigs were fed formula diets containing acid-modified waxy starch or distarch phosphate prepared by treatment of the acid-modified starch with 0.08 percent (dry weight basis) phosphorus oxychloride (72). Starch provided 24 percent of the calories in the diet and each diet was fed for 25 days. Body weight gains were similar for test and control animals. The distarch phosphate diet had no statistically significant effects on organ weights expressed as a percentage of body weight. Serum cholesterol, triglyceride, calcium, phosphorus, alkaline phosphatase, urea nitrogen, total protein, albumin and globulin levels were similar for the test and control animals.

Long-term studies

No long-term studies were available to the Select Committee.

Special studies

No studies on mutagenicity, carcinogenicity or teratogenicity of distarch phosphates were available to the Select Committee.

Phosphated Distarch Phosphate

Digestion and metabolism

In vitro pancreatic digestibility of phosphated distarch phosphate prepared from cornstarch was about 80 percent that of unmodified corn-

starch as measured by liberation of reducing sugars after 20 minutes' digestion (113). Digestibility of phosphated distarch phosphate from potato starch by in vitro pancreatin and porcine intestinal amylase also was reduced compared to the unmodified starch (87). In vivo digestibility and utilization, however, of phosphated distarch phosphate prepared from milo starch was similar to that of the unmodified starch as measured by weight gain of weanling rats fed a basal diet supplemented with 1, 2, or 4 g daily of unmodified or modified starch for 10 days (114).

Short-term studies

Groups of 10 male and 10 female weanling rats (strain not stated) were fed a diet initially containing 10 percent (about 30 g per kg) phosphated distarch phosphate (prepared from white milo starch) but increasing stepwise to 35 percent (about 70 g per kg) on the 13th day (121). At 60 days, urine and blood samples were collected and the animals were sacrificed and subjected to complete necropsy. Average weight gain of females was slightly but significantly lower than controls ($p=0.05$). Urine of test animals was negative for presence of reducing substances, protein and microscopic elements and hematological parameters were in the normal range. Kidney- and liver-body weight ratios were significantly lower for males in the test group; these were believed, however, to be coincidental rather than related to the ingestion of the test starch. Histopathological findings for test animals were comparable to those for controls.

Groups of 25 female Sprague-Dawley weanling rats were fed diets containing 0.2, 1.0, or 5.0 percent (about 0.2, 0.8, or 4 g per kg) phosphated distarch phosphate prepared from white milo starch for 90 days (118). No abnormalities were observed in hematological studies or urinalyses of the test animals. Body weight gains did not differ significantly from the control animals. Organ-body weight ratios showed no differences attributed to starch ingestion. Gross pathology of the test animals was similar to that of the controls. No adverse histopathological changes attributed to the phosphated distarch phosphate were observed.

Groups of 10 male and 10 female weanling rats (Wistar derived) were fed diets containing 25 or 50 percent (about 25 or 50 g per kg) phosphated distarch phosphate (0.3 percent P) prepared from potato starch for 8 weeks (88). Body weight gains were similar to those of control animals. No diarrhea occurred at either test level. Cecal weight was slightly increased in male rats at the 25 percent level but there was no consistent effect on females at either test level.

In a 90-day subacute toxicity test, groups of three male and three female beagles were fed a standard dog chow supplemented daily with 0.05, 0.25, or 1.25 g of phosphated distarch phosphate per kg of body weight (120). The modified starch was prepared from white milo starch and was admin-

istered in gelatin capsules. No effects were observed on blood components, urinalyses or liver function employing the sulfobromophthalein test. Mean body weight gains and organ-body weight ratios of the test animals did not differ significantly from the controls. Gross and histopathologic examination revealed no abnormalities attributed to the test substance.

Groups of eight 3-day-old Pitman-Moore miniature pigs were fed formula diets containing acid-modified waxy starch or phosphated distarch phosphate prepared by treatment of the acid-modified starch with 4.8 percent sodium tripolyphosphate and 0.59 percent sodium metaphosphate, both on a dry weight basis (72). Starch provided 24 percent of the calories (33 percent of the formula, dry weight basis) in the diet and was fed for 25 days. Body weight gain was similar for test and control animals. The test diet had no statistically significant effect on organ-body weight ratios. Clinical blood chemistry analyses were similar for test and control animals as were liver and carcass composition.

Twelve human volunteers ingested on each of 4 consecutive days 60 g (about 1 g per kg) of phosphated distarch phosphate containing 0.35 percent introduced phosphorus. No adverse effects were observed. No effect was noted on the frequency of defecation or on the amount of feces, fecal water or the lactic acid content of the feces (91).

Long-term studies

Groups of 30 male and 30 female weanling Wistar rats were fed for 2 years a basal diet containing 30 percent precooked waxy milo starch which was replaced in part or in total by 5, 10, or 30 percent (about 3, 5 or 15 g per kg) phosphated distarch phosphate (92,122). The modified starch was prepared by cross-linking white milo starch with sodium trimetaphosphate to 0.04 percent introduced phosphorus and esterified with sodium tripolyphosphate to a total of 0.35 percent bound phosphorus. Significant growth retardation did not occur and food efficiencies were comparable to those of the control animals at all stages. Diarrhea did not occur. Hematological indices showed no significant abnormalities or treatment related differences between test and control groups at any stage of growth. Biochemical analyses of blood and serum showed no evidence of dose-related responses to the test starch. Analysis of urine samples collected at intervals from each dietary group showed no effect of the modified starch on pH, sugar, protein, occult blood, ketones or sediment. There were no statistically significant changes in organ weight except for an increased kidney-body weight ratio for females on the 30 percent test diet. No gross pathological changes were observed which could be attributed to ingestion of the modified starch. No effect was seen on tumor incidence. Non-neoplastic lesions were randomly distributed among test and control animals with the possible exception of a kidney abnormality which consisted mainly of focal hyperplasia of the renal papillary and pelvic epithelium accompanied

by calcified patches in the underlying tissues. The hyperplastic and calcified tissues often protruded into the renal pelvis and were located most often in the papilla near the junction of the papillary and pelvic epithelium. The lesion occurred to a slight degree in nine animals (5/28 males and in 4/29 females) fed the 30 percent test diet and to a moderate degree in one male on the control diet (Table V). There was not, however, a distinct relationship with feeding level. The authors considered the toxicological significance of the lesions doubtful. Reports on the occurrence of similar renal changes in rats fed other types of diets are noted in the section Starch Acetates, Long-term studies (p. 26).

Special studies. Reproduction and lactation

Groups of 10 male and 20 female weanling CIVO rats were fed, for three generations, a diet containing 10 percent (about 5 g per kg) phosphated distarch phosphate and 20 percent precooked waxy maize starch (92). The modified starch was the same as described in the preceding section. Rats were mated (P, F_1, and F_2 generations) at weeks 12 and 20 after weaning. The second litter of each generation was used to produce the next generation. The F_{3b} generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F_{1b}, and F_{2b} parents were used for counting implantation sites. Body weights did not differ among groups in successive generations and no treatment-related differences in mortality were observed between the test groups and controls. No adverse effects were noted regarding mortality in utero (resorption quotient), litter size, weight of pups, preweaning mortality or growth rate of pups. No gross or histological changes attributable to feeding the modified starch was observed.

Acetylated Distarch Phosphate

Digestion

In vitro digestibilities by pancreatin and porcine intestinal amylase of acetylated distarch phosphates (prepared from potato starch), 1.6 and 2.3 percent acetyl contents, were 93 and 69 percent, respectively, of that of unmodified starch (87).

Short-term studies

Groups of 10 male and 10 female weanling Wistar rats were fed, for 8 weeks, practical type diets containing 25 or 50 percent (about 30 or 60 g per kg) acetylated distarch phosphate (88). The test starch was cross-linked by treatment of potato starch with 0.02 percent phosphorus oxychloride and acetylated with 8 percent acetic anhydride (2.3 percent acetyl

content). Weight gains were similar to those of control animals fed diets containing 50 percent precooked waxy maize starch. During week 5 slight diarrhea occurred in male rats receiving the 50 percent dietary level. At sacrifice, cecal weights of both male and female rats were greater than those of controls but microscopic examination revealed no differences from controls. In a second experiment by the same investigators (88), acetylated distarch phosphate was fed at the 25 and 50 percent levels in a semipurified diet for 10 days. At day 7, cellulose was added to all diets at a level of 4 percent. Moderate diarrhea occurred at the 50 percent dietary level of the test substance. Addition of cellulose did not reduce the diarrhea.

Groups of four male and four female pigs were fed diets containing 35 or 70 percent acetylated distarch phosphate for $14\frac{1}{2}$ weeks (123). Growth rate and food consumption were satisfactory. Three animals in the higher dietary group died during the test without evidence of cause of their death. Hematology, blood chemistry and urinalysis showed no treatment-related abnormalities nor did organ weight, gross and histopathological evaluations. One pig in each of the test diet groups showed evidence of neurological malfunction; the animal in the 70 percent dietary level group died; the one in the lower dietary level recovered. No histological evidence of nervous system abnormality was observed in these two nor in any other animal.

Long-term studies

Groups of 30 male and 30 female weanling Wistar rats were fed for two years diets containing 5, 10, or 30 percent (about 3, 5, or 15 g per kg) acetylated distarch phosphate replacing an equal amount of precooked potato starch in the control diet (92, 94). The test starch was potato starch cross-linked with 0.02 percent phosphorus oxychloride and acetylated with 8 percent acetic anhydride (acetyl content 2.33 percent). Body weight gains and food efficiencies were similar to those of control animals. Hematological indices showed no significant abnormalities or treatment-related differences between test and control groups. Blood and serum analyses showed no evidence of dose-related responses to the test starch. Analyses of pooled urine samples collected at intervals from each dietary group showed no effect of the modified starch. There were no statistically significant changes in organ weight although rats of both sexes fed 30 percent of the test starch in their diet and males fed the 10 percent diet had greater cecal weights than the controls. Histopathological lesions were randomly distributed among test and control animals with the possible exception of a kidney abnormality which consisted mainly of focal hyperplasia of the renal papillary and pelvic epithelium accompanied by calcified patches in the underlying tissues. There was not a distinct relationship between incidence of the lesion and feeding level (Table V). The authors considered its toxicological significance doubtful. Reports on the occurrence of similar renal changes in rats fed other types of diets are noted in the section on Starch Acetates, Long-term studies (p. 26).

A similar protocol was followed in a 2-year rat (Wistar strain) feeding study with acetylated diamylopectin phosphate (92, 94). Amylopectin (presumably from potato starch) was cross-linked with 1.2 percent phosphorus oxychloride (0.043 percent introduced phosphorus) and acetylated with 4.5 percent vinyl acetate (acetyl content, 1.6 percent). Findings were similar to those reported in the preceding paragraph for acetylated distarch phosphate. Incidence of pelvic nephrocalcinosis in the rats at necropsy is reported in Table V.

Special studies

Twelve human subjects ingested on each of 4 successive days 60 g (about 1 g per kg of body weight) of acetylated distarch phosphate of either 1.6 or 2.33 percent acetyl content (91). No adverse effects were noted including effect on frequency of defecation and on the amount of feces, fecal water and the lactic acid content of the feces.

Groups of 10 male and 20 female weanling Wistar rats were fed for three generations, a diet containing 10 percent (about 10 g per kg) acetylated distarch (potato) phosphate (2.33 percent acetyl) and 20 percent precooked potato starch (92). Rats were mated (P, F_1 and F_2 generations) at weeks 12 and 20 after weaning. The second litter of each generation was used to produce the next generation. The F_{3b} generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F_{1b}, and F_{2b} parents were used for counting implantation sites. Body weights did not differ among groups in successive generations and no treatment-related differences in mortality were observed between the test groups and controls. No adverse effects were observed on the resorption quotient, litter size, weight of pups, preweaning mortality or growth rate of pups. No gross or histological changes attributed to feeding the modified starch were noted.

Hydroxypropyl Distarch Phosphate

Digestion and metabolism

In vitro digestibility of gelatinized hydroxypropyl distarch phosphate (prepared from tapioca starch, molar substitution 0.045) by hog pancreatic alpha amylase or fungal amylase was about 80 percent of that of gelatinized tapioca starch (124).

Groups of five weanling male Sprague-Dawley rats were fed 5 g per day of a basal diet to which was added 1 oe 3 g of hydroxypropyl distarch phosphate (125). The test-modified starch was prepared by treating tapioca starch with 8 percent propylene oxide and 0.1 percent phosphorus oxychloride. Caloric value of the modified starch as measured by 7-day weight gains was equal to that of the unmodified control starch.

In another evaluation of the caloric value of hydroxypropyl distarch phosphates, groups of ten weanling male albino rats (strain not stated) were fed, for 10 days, 5 g daily of a basal diet to which 1, 2, or 4 g (about 20, 40, and 80 g per kg of body weight) of the modified starches was added (126). The test starches were prepared by reaction of cornstarch with 0.0123 percent phosphorus oxychloride and 3, 6, or 8 percent propylene oxide (hydroxypropyl group degree of substitution (D.S.), 0.085, 0.173, and 0.23, respectively). Caloric utilization relative to the unmodified starch control decreased slightly with increasing D.S. of the modified starch; relative caloric value for the D.S. 0.23 starch was 0.93. Diarrhea occurred when rats consumed 2 g or more of D.S. 0.23 starch or 4 g of the starches of lesser D.S.

Short-term studies

A hydroxypropyl distarch phosphate (composition not stated) prepared from cornstarch was fed ad libitum at 17, 34, 51, and 68 percent dietary levels (about 20, 40, 60, and 80 g per kg body weight) for 28 days to groups of 10 male weanling albino rats (126). Weight gains and feed efficiencies were reduced at the two highest dietary levels of the test substance. Cecum-body weight ratios were increased at all dietary levels and the increase was dose-related. No histological abnormalities were observed in the heart, liver, spleen, kidney or cecum.

Groups of 15 male and 15 female weanling FDRL-Wistar rats were fed, for 90 days, diets containing 5, 10, or 25 percent (about 4, 8, and 20 g per kg) hydroxypropyl distarch phosphate, replacing an equal quantity of unmodified cornstarch in the control diet (127). The test starch was prepared by reaction of cornstarch with 10 percent (w/w) propylene oxide and 0.1 percent phosphorus oxychloride. There were no deviations from control values with respect to growth, gains in body weight, food intakes, or efficiencies of food utilization, with the exception of a slight decrease in feed efficiency in males fed 25 percent levels of the treated starch. Hematologic, biochemical parameters and urinalyses were similar to those of control animals. No treatment-related response was observed in any organ weight except the cecum which showed a marked enlargement with contents in place. However, only males on the 25 percent diet had empty ceca significantly heavier than those of controls. Certain sections of the ceca appeared thinner but were cytologically normal. The only pathological finding of possible significance was calcareous deposits within the renal pelvis and/or the pelvic epithelium; deposits occurred only in rats of the test groups. Incidence in females at the 5, 10, and 25 percent dietary levels was 7/15, 9/15, and 11/15, respectively; in males incidence was 4/15 at each level. Focal renal tubular calcification appeared somewhat more severe than expected in the control animals; however, incidence in test (7/30 at each dietary level) and control groups (5/30) was similar.

In another 90-day study (128), groups of 15 male and 15 female weanling rats (Wistar derived) were fed pregelatinized hydroxypropyl distarch phosphate at dietary levels of 0, 5, 10, or 25 percent (about 0, 4, 8, and 20 g per kg). The modified starch was prepared by treating cornstarch with 0.1 percent phosphorus oxychloride and 5 percent propylene oxide. The product contained 96 ± 6 ppm phosphorus and less than 5 ppm propylene chlorohydrin. Degree of hydroxypropyl substitution was 0.07. Growth, food consumption, food efficiency, hematology, blood chemistry and urinalysis of test animals were comparable to those of controls fed pregelatinized cornstarch. Water content of feces was slightly higher at the 10 and 25 percent dietary levels of the test substance, but diarrhea did not occur. Organ-body weight ratios of the testicles and adrenals of males in the 25 percent group were slightly decreased ($p = 0.05$). The relative cecum weight, both filled and empty, was distinctly increased in both sexes at the 25 percent dietary level. Very slight or slight calcareous deposits were identified microscopically in the intercortico-medullary area of the kidneys in 11/15 females at the highest dietary level as compared with 2/15 in female controls and 0/15 for test males. The investigators did not consider the findings to have toxicological significance in view of a similar incidence usually encountered in female Wistar control rats of similar age in other studies. No other histopathological abnormalities were observed.

Long-term studies

Potato starch cross-linked with 0.1 percent phosphorus oxychloride and etherified with 5 percent propylene oxide (D.S. of treated starch, 0.075; propylene chlorohydrin content, 4.3 ppm) and a control pregelatinized potato starch were fed to groups of 75 male and 75 female weanling SPF mice of the Swiss Random strain for 89 weeks at dietary levels of 55 percent (about 80 g per kg body weight) (103). Other groups were fed diets containing 55 percent lactose or 25 percent sodium alginate. In week 80, ten mice of each sex and group were decapitated and necropsied and a comprehensive microscopic examination of tissues was made. A similar examination was made of all survivors after week 89. Tissues of all animals found dead or killed when moribund were removed and examined if autolysis was not too far advanced. Death rate in the group fed hydroxypropyl distarch phosphate was normal for the strain of mice fed but was abnormally high for males in the control group between weeks 39 and 65. At necropsy about 50 percent of the control animals that died in this period had an hemorrhagic myocarditis. Loose stools were seen in about 12 percent of the males and 5 percent of the females fed the test diet as compared to about 4 percent of males and 3 percent of females given the control diet. Body weights of males in the treated group were significantly reduced from week 16 to 48 and in females from week 40 to termination as compared to controls. Hematological indices and levels of fasting blood glucose and urea nitrogen were within normal limits. Males, but not females, fed the hydroxypropyl distarch phosphate diet had a greater amount of amorphous material in their urine than did control animals. Infrared analysis of

the sediment from the test animals indicated that it was nearly 100 percent protein. Mean relative weights (g per 100 g body weight) of the ceca and colons of the treated animals were greater than those of control animals. Frequency of intratubular calcareous deposits in the kidneys of mice that survived for at least 78 weeks was significantly greater in treated males (25/52) than in control males (5/28). Incidence of very slight calcareous deposits in the renal pelvis also was greater in treated (13/52) than in control males (0/28). A slight but not statistically significant increase in calcareous material and in thickened submucosa and epithelium in the urinary bladder occurred in treated males. Mitotic activity of the urothelium was not treatment related and there was no evidence for hyperplasia in the urinary bladder epithelium. There was no evidence for treatment related neoplastic changes. The investigators concluded that the renal and urinary bladder changes observed had little, if any, toxicological significance.

Special studies. Effect of modified starch on iron retention

Groups of 6 to 11 weanling Holtzman rats were fed for 25 to 28 days semipurified diets containing 35 percent (about 50 g per kg body weight) unmodified tapioca starch or hydroxypropyl distarch phosphate (molar substitution 0.045) prepared from tapioca starch (129). The starches were added to the diets in uncooked (experiment 1) or cooked (experiment 2) form; in a third experiment, the effect of cooking the entire diet was studied. One group of rats fed each type starch received no iron in their diet. On day 16 to 19, iron retention was estimated by whole-body counter assay after administering $^{59}FeCl_3$ in 5 ml of a solution containing 30 μg $FeCl_3$ and 1 g of the uncooked starch (experiment 1), 3.75 μg $FeCl_3$ and 0.125 g cooked starch (experiment 2), and 3.75 μg $FeCl_3$ and 0.35 g of cooked diet (experiment 3). Weight gains, hemoglobin levels and ^{59}Fe retention in rats fed iron-adequate diets were not affected by the starch source. Cooking the starch tended to reduce the iron retention. Rats fed the low-iron diets showed no differences in hemoglobin level that could be attributed to the type of starch that was fed. Iron retention was not affected by the type of starch when uncooked starch was used; however, retention was lower for cooked hydroxypropyl distarch phosphate (36 percent of dose) than for cooked unmodified starch (74 percent). For the cooked whole diets, respective retentions were 50 and 60 percent.

No studies on mutagenicity, carcinogenicity, teratogenicity or reproduction were available to the Select Committee.

Hydroxypropyl Starch
Oxidized Hydroxypropyl Starch

Digestion, absorption and metabolism

In vitro pancreatin digestibility of two hydroxypropyl wheat starches, 0.1 and 0.41 D.S. was similar to that of unmodified wheat starch

(130). The high D.S. hydroxypropyl starch (treated with 25 percent propylene oxide) had been further modified by oxidation with 0.055 pounds sodium hypochlorite per pound of dry starch and 0.45 percent active oxygen from hydrogen peroxide. In contrast Leegwater and Luten (131) reported the pancreatin digestibility of a hydroxypropyl starch, D.S. 0.04, as 80 percent of that of unmodified starch. ^{14}C-labeled hydroxypropyl cornstarch, D.S. 0.12, prepared using labeled propylene oxide, was administered to a male rat by stomach tube (132). Over the next 50 hours, 92 percent of the radioactivity was excreted in the feces and 3.6 percent in the urine. The investigator attributed the radioactivity in the urine to propylene glycol in the test starch.

Ten 2-month-old male Wistar rats were fed, for 3 to 5 days, diets containing 56 percent of unmodified, precooked potato starch or hydroxypropyl starch (D.S. 0.025) or a 2:5 mixture of hydroxypropyl starch (D.S. 0.047) and unmodified potato starch, or a 1:6 mixture of precooked potato starch and hydroxypropyl starch (D.S. 0.106) (133). Alcohol-soluble, ether-insoluble residue in the feces per 100 g starch ingested was 0.8, 6.6, 11.6, and 19.1 g for starches of D.S. 0, 0.025, 0.047, and 0.106, respectively, indicating that digestibility of hydroxypropyl starches decreased with increasing degree of substitution. The major component of the feces residue was identified as hydroxypropylmaltose; evidence also indicated the presence of dihydroxypropylmaltose and dihydroxypropylmaltotetraose (133). The hydroxypropylmaltose derivative was identified as 4-O-{2-O-[(RS)-2-hydroxypropyl]-α-D-glucopyranosyl}-D-glucopyranose (134).

Acute toxicity

Groups of five male and five female Sprague-Dawley rats (about 150 and 110 g body weight, respectively) received by stomach tube doses of oxidized hydroxypropyl starch (D.S. 0.41) at dosage levels of 1, 3.16, or 10 g per kg of body weight (135). No deaths resulted. The animals exhibited normal appearance and behavior for 7 days following treatment at which time they were sacrificed. At autopsy, female rats at the highest dosage level exhibited congested kidneys; all other animals showed no significant gross pathology.

Short-term studies

Groups of 10 male and 10 female Sprague-Dawley weanling rats were fed diets containing 2, 5, 10, or 25 percent (about 2, 4, 8, and 20 g per kg of body weight) of oxidized hydroxypropyl (wheat) starch (D.S. 0.41) for 13 weeks (136). One male and one female rat from the 2 percent dietary-level group died from respiratory illness during the test period. Animals fed the diet containing 25 percent of the test compound exhibited mild diarrhea; this condition was not observed among animals at the lower dietary levels. Body weight gains were lower in the male rats fed diets containing

10 and 25 percent oxidized hydroxypropyl starch but only at the higher dietary level was the growth depression statistically significant. Food utilization of males and females at the 25 percent level tended to be lower than that of control animals. Hematologic and urinalysis data for test group animals were similar to those for the control group rats. No significant gross pathologic changes were observed that could be attributed to ingestion of the test compound. Liver- and kidney-body weight ratios for animals in the 25 percent dietary-level groups were significantly higher than controls but absolute weights were not significantly different. Complete histologic studies revealed no changes which were attributable to the ingestion of the oxidized hydroxypropyl starch.

Feron et al. (98) fed groups of 10 male and 10 female rats (strain not stated) diets containing 5, 15, or 45 percent (about 4, 12, or 35 g per kg body weight) hydroxypropylated acid-modified potato starch (D.S. 0.042) for 90 days. Weight gains of male rats fed the 15 and 45 percent level diets were consistently but not significantly less than those of the controls. Diarrhea occurred in the 45 percent level group but diminished during the final weeks of the experiment. Male rats fed 15 and 45 percent-level diets showed cecal enlargement whereas enlargement in females occurred only at the higher level. The enlarged ceca showed no evidence of inflammation or histological abnormalities. Hematological findings were comparable for the test and control groups. No pathological changes were observed on microscopic examination of the major organs.

Special studies

Application of hydroxypropyl starch (D.S. 0.1 or 0.41) in powdered form, or the 0.1 D.S. product in aqueous solution, produced mild irritation in rabbits' eyes (135).

Two hundred ten human subjects were patch-tested with moistened patches of powdered hydroxypropyl (wheat) starch (D.S. 0.1), allowing the patch to remain in contact with the skin for 72 hours, followed by a challenge exposure 2 weeks later (137). There was no greater irritation from the test material than from the control wheat starch and no evidence of sensitization on reexposure. There was no evidence of irritation in 23 human subjects patch-tested by the Repeat Insult Patch Test in which the subject was given nine 24-hour exposures to hydroxypropyl starch (D.S. 0.1) at intervals of 2 to 3 days (138). Challenge exposure 1 week after the last exposure gave no evidence of sensitization.

Acetylated Distarch Adipate

Digestion, absorption and metabolism

In vitro amyloglucosidase digestibility of acetylated distarch adipate prepared by treatment of waxy maize starch with 0.15 percent adipic anhydride as a 1:3 adipic:acetic mixed anhydride was 98 percent of unmodified waxy starch (110). In vitro studies of the hydrolysis of acetylated distarch adipate with pancreatin showed that the adipic acid ester linkages are not split whereas the acetate ester bonds are hydrolyzed (139).

Young male adult rats were administered by stomach tube suspensions of acetylated starch adipate prepared using ^{14}C-labeled adipic acid. A physical mixture of ^{14}C-labeled adipic acid and unmodified starch was administered to controls. Within 4 hours after dosing, 70 percent of the radioactivity administered as the free acid was recovered in carbon dioxide in the respired air whereas only 12 percent of the activity administered as the modified starch was recovered. After 25 hours 99.3 percent of the activity of the free adipic acid was recovered in the respired air, 5.8 percent in the urine and none in the feces, gastrointestinal tract, or carcass. In case of the labeled starch, 70.5 percent of the activity was recovered in the respired air, 7.2 percent in the urine, 24.5 percent in the feces and none in the carcass (139).

Caloric value of acetylated distarch adipate as measured by growth rate was equal to that of the control starch in 28-day feeding studies in which groups of 10 weanling male albino rats were fed a basal diet containing 1.5 or 3.0 g of modified or control starch, or 0.75 to 4.5 g of sucrose supplement. The modified starch was prepared by treatment of a thin-boiling waxy maize starch with 0.2 percent adipic anhydride and 5.5 percent acetic anhydride (140).

Short-term studies

Groups of 15 male and 15 female FDRL rats were fed a diet containing 50 percent (about 40 g per kg body weight) of acetylated distarch adipate or thin-boiling starch for 90 days (141). The modified starch was a thin-boiling waxy maize starch treated with 0.12 percent adipic acid and 10.5 percent acetic anhydride based on weight of starch. Weight gain of males in the test group was moderately, but significantly lower (15 percent) than males in the control group. Food intake and food efficiency also were lower in males in the test group. Full and empty cecal weights of both sexes fed the modified starch were significantly greater than those of animals in the control group. No differences were observed between groups in respect to hematology, blood chemistry, urinalysis, liver and kidney weights, or gross and histopathological evaluations. Corticomedullary junction

nephrocalcinosis was present in rats given either the treated or the control starch but renal pelvic nephrocalcinosis was not reported.

Long-term studies

Groups of 30 male and 30 female Sprague-Dawley rats were fed, for 2 years, diets containing 62 percent (about 30 g per kg body weight) of acetylated distarch adipate or cooked unmodified starch (starch source not stated) (142). Ten additional rats of each sex were included in the test and control groups but were necropsied at 90 days. Weight gains in test and control groups did not differ at 3 months but gain in the test group was about 15 percent lower at 100 weeks. Hematological parameters did not vary outside normal limits. Although several blood biochemical parameters of the test animals were statistically significantly different from those of the controls, only the increased serum glutamic oxalacetic transaminase (SGOT) level could be considered outside normal values. Organ-body weight ratio but not absolute weights of several organs of both sexes differed significantly from those of control animals. The investigators did not consider these differences to have biological significance. Histological findings at 24 months were hyperkeratosis of the forestomach and, in the livers of females, an increase of giant multinuclear cells. However, mean age of the females in the test group was almost 1 month greater than that of the control group because of earlier deaths in the latter group; for this reason the investigators considered the two groups not comparable and attached no toxicological significance to the observation (142). A "blind" reexamination of the stomach tissue sections by three pathologists did not demonstrate any significant differences between the test and control animals. It also was concluded that the giant multinuclear cells observed in the livers of female rats were linked with senescence and were not related to the ingestion of modified starch (143).

Kidney lesions characterized by focal hyperplasia of the epithelium lining the urinary space accompanied by calcified deposits attached to the lining epithelium were present in both treated and control animals. Authors concluded that there was no significant difference in the severity and frequency of the lesions in the two groups (142). The same conclusion was reached in a subsequent reexamination (Table V) of the kidney section (96). Reports on the occurrence of similar renal alterations in rats fed other types of diets are noted in the section Starch Acetates, Long-term studies (p. 26).

Special studies. Reproduction test

Groups of 10 male and 10 female Sprague-Dawley rats were fed for three generations 62 percent (about 30 g per kg body weight) acetylated distarch adipate (144). Each generation (P, F_1 and F_2) produced two successive litters with a 12 to 15 week period between matings. The

second litter was used to produce the next generation. Following weaning of F_{2b} litters, the F_{1b} parents were sacrificed and autopsied. The F_{3b} generation was kept for 10 weeks to observe weight changes of all the young, then sacrificed and autopsied. No adverse effects were observed on fertility, litter size, weight of pups, mortality and postnatal growth. Liver weight increased in both absolute and relative value in the F_1 animals. However, no macroscopic anomalies were found. Gross and histopathologic findings of F_{3b} animals revealed no differences between test and control animals.

Distarchoxy Propanol

Short-term studies

Diets containing 60 percent (about 60 g per kg body weight) of starch treated with 0.15, 0.30, 0.45, or 0.60 percent acrolein (w/w starch) were fed to groups of 10 weanling Sprague-Dawley rats for 28 days (89). Weight gains, feed efficiencies, and cecal weights of the test animals did not differ significantly from controls fed native starch. No diarrhea was observed.

Acetylated Distarchoxy Propanol

Digestibility

Digestibilities of four starches (source not stated) each cross-linked by treatment with 0.20 percent (w/w) acrolein and containing 0.46, 1.77, 2.20, and 3.50 percent acetyl groups, respectively, were determined by comparison of weights of feces from rats fed the treated starches with those fed unmodified starches in a similar basal diet. Results indicated that digestibilities of modified and unmodified starches were similar. This result was supported by similar weight gains and feed efficiencies observed for the two groups (89).

Short-term studies

Twenty-eight-day rat feeding studies were conducted with starches (source not stated) cross-linked by treatment with 0.15, 0.30, 0.45, or 0.60 percent acrolein and, for each level of acrolein, acetylated by reaction with 4.5, 6.0, 7.5, or 9.0 percent (w/w) vinyl acetate (89). Acetyl contents of the acetylated starches were determined by analysis. The starches were fed at the 60 percent dietary level to groups of 10 weanling Sprague-Dawley rats. Weight gains of groups fed starches treated with 9 percent vinyl acetate (3.23 percent acetyl content) were significantly ($p=0.05$) lower than the control animals fed unmodified starch. Groups fed starches treated with 4.5, 6.0, 7.5, or 9.0 percent vinyl acetate developed enlarged ceca and diarrhea, the incidence rates of which were significantly and progressively higher as acetyl content increased. Incidence of diarrhea increased

from 9 percent at the low level of vinyl acetate treatment to 72 percent in the groups fed starch treated with 9 percent vinyl acetate. At necropsy, cecal and large intestinal contents of all rats fed the acetylated starches were acidic. Those of the control animals were alkaline.

Groups of 10 pigs (breed not stated) were fed diets containing 66 percent unmodified starch or starch treated with 0.2 percent (w/w starch) acrolein and acetylated (2.5 percent acetyl content) with vinyl acetate (89). During a 28-day feeding period, the pigs fed the acetylated distarchoxy propanol grew slower and consumed more feed per pound of weight gain than those fed unmodified starch. The pigs fed the treated starch also exhibited a mild degree of diarrhea during the first 2 weeks of feeding after which they became adapted to the starch.

Special studies

Groups of 10 weanling Sprague-Dawley rats were fed diets containing 60 percent of acetylated distarchoxy propanol (3.4 percent acetyl groups; cross-linked with 0.13 percent acrolein) or the unmodified starch (starch source not stated) for 6 weeks (89). At necropsy, the microflora of the small and large intestines of animals fed the treated starch contained significantly fewer Escherichia coli and yeasts, but a greater number of Lactobacilli. The cecal contents were acidic. The authors noted that an acidic condition usually is associated with diarrhea. No significant histopathological changes were found in the liver, kidney, cecum, or large and small intestines of the rats fed the test starch.

In a two-generation study, groups of 20 female and 10 male Sprague-Dawley weanling rats were fed diets containing 45.7 percent acetylated distarchoxy propanol (2.5 percent acetyl groups; cross-linked with 0.20 percent acrolein) or the unmodified starch (source not stated) for 105 days (89). At this time four males were each paired with three females for breeding. The number of births, percentage survival and weanlings in the treated starch group exceeded those of the unmodified starch group. Weanlings in the modified starch group were fed the modified starch for 1 year. Weight gains and feed efficiencies were greater than those of the control animals. No diarrhea or deaths were observed in either group.

Distarch Glycerols

Digestion and metabolism

In vitro digestibility by amyloglucosidase of distarch glycerol was 98.2 percent of that of unmodified starch; both starches were gelatinized by heating at 100°C for 20 minutes prior to enzymatic hydrolysis (110). The modified starch was prepared by treatment of maize starch with 0.3 percent epichlorohydrin (w/w starch).

Caloric values of unmodified starch and two distarch glycerol samples, prepared by treatment of waxy maize starch with 0.07 percent and 0.5 percent epichlorohydrin (w/w starch), respectively, were determined by feeding the starches (3 g or about 25 g per kg) to groups of 10 weanling, male FDRL rats and comparing growth response after 28 days to that obtained by feeding the basal diet supplemented with graded sucrose levels (145, 146). Differences in caloric availability of the treated and unmodified starch were not significant.

Short-term studies

Groups of 10 male and female weanling FDRL rats were fed, for 90 days, diets containing 71 percent (about 60 g per kg body weight) of unmodified waxy maize starch or waxy maize starch treated with 0.07 or 0.5 percent epichlorohydrin (146). Food intake and weight gains of the test groups were similar to those of animals fed the control starch. No adverse effects of feeding the test starches were observed on hematology, blood non-protein nitrogen levels, urinary parameters, or organ-body weight ratios of the liver, kidneys and adrenals. Gross pathological observations revealed no differences between the modified starch group and the controls.

Hydroxypropyl Distarch Glycerol

Digestion and metabolism

In vitro digestibility by pancreatin and porcine intestinal mucosa of a hydroxypropyl distarch glycerol (D.S. 0.04) was 86 percent of that of the unmodified starch (87).

Short-term studies

Groups of 25 male and 25 female Charles River rats, 105 to 150 g body weight, were fed, for 90 days, diets containing 1.0 or 5.0 percent (about 0.6 or 3 g per kg) hydroxypropyl distarch glycerol prepared by treating tapioca starch with 10 percent propylene oxide and 0.1 percent epichlorohydrin (w/w starch) (147). Growth, feed consumption and terminal body weights for the test rats were similar to controls. No adverse effects were noted on the hematological values, blood sugar, blood urea nitrogen, serum glutamic-pyruvic transaminase or urinary parameters. Organ-body weight ratios were within the normal range. Thyroid-body weight ratio for the test rats and for negative control rats (chow diet) were significantly lower than the value for the rats receiving tapioca flour. Thyroid tissue sections were histologically similar for the positive controls and the highest dose-level test rats. Neither gross nor histopathologic examination changes attributable to ingestion of the test compound were detected.

Groups of 10 male and 10 female weanling rats (Wistar-derived) were fed practical type diets containing 25 or 50 percent (about 30 or 60 g per kg) of hydroxypropyl distarch glycerol, replacing an equal quantity of unmodified starch in the control diet (88). The modified starch was prepared by treating potato starch with 0.1 percent epichlorohydrin and 5 percent propylene oxide. Weight gains at 8 weeks were similar to those of control rats fed diets containing 50 percent maize starch. Moderate diarrhea occurred in male and female rats fed 50 percent modified starch and a slight diarrhea was observed at the 25 percent dietary level. At sacrifice, cecal weights of both male and female rats were greater than those of controls but microscopic examination revealed no differences from controls. In a second experiment by the same investigators (88) the modified starch was fed at the 25 and 50 percent levels in a semipurified diet for 10 days. Diarrhea was more marked than in animals fed the practical diet. At day 7, cellulose was added to all diets at a level of 4 percent. This did not reduce the diarrhea. Hair loss was pronounced in both sexes fed modified starch at the 50 percent level and was slight at the 25 percent level.

In a 90-day subacute toxicity study (148) groups of 10 male and 10 female rats were fed diets containing 5, 10, or 30 percent (about 4, 8, and 25 g per kg) of hydroxypropyl distarch glycerol prepared by treating potato starch with 0.1 percent epichlorohydrin and 5 percent propylene oxide (w/w starch). No definite diarrhea was observed at any test level. Weight gains and food efficiencies of the test groups did not differ significantly from those of controls. Hematological parameters, blood biochemical values and urinalyses were within the normal range. Cecal weights were increased in test animals at the 30 percent dietary level but no histopathologic changes were observed in the enlarged ceca. Gross and histopathologic examination of other tissues of animals in the highest dose group did not show changes attributable to ingestion of the modified starch.

Groups of four male and four female young adult beagles were fed for 90 days diets containing 1 or 5 percent hydroxypropyl distarch glycerol (about 0.3 or 2 g per kg body weight) prepared by treating tapioca starch with 0.1 percent epichlorohydrin and 10 percent propylene oxide (w/w starch) (149). Body weights of test animals were maintained or slightly increased except for one dog on the high level diet which had been ill (nature of illness not stated) and lost 1.5 kg (about 10 percent). No changes in hematology, blood biochemistry or urinalysis attributable to the test compound were noted. Slightly elevated kidney-body weight and testes-body weight ratios were found for the dog that had been ill during the study. Thyroid weights and thyroid-body weight ratios for two females on the 5 percent level of the test diet were slightly elevated as compared to the controls. Gross and microscopic pathologic examination showed no changes attributable to ingestion of the modified starch.

In a second 90-day subacute toxicity study with dogs (150), groups of four male and four young adult female beagles were fed diets containing 10 percent (about 4 g per kg body weight) cornstarch or hydroxypropyl distarch glycerol prepared by treating cornstarch with 0.1 percent epichlorohydrin and 25 percent propylene oxide. Animals were normal in appearance, behavior, appetite and elimination. No significant weight difference between test and control animals was observed. Food consumptions were comparable. Hematologic and blood chemical findings, urinalyses and organ-body weight ratios showed no test compound-related effects. Gross and microscopic pathologic findings showed no abnormalities.

Groups of eight 3-day-old Pitman-Moore miniature pigs were fed liquid diets containing 28.8 percent (dry basis) hydroxypropyl distarch glycerol prepared by treating a thin-boiling waxy maize starch with 0.009 percent epichlorohydrin and 4.2 percent (dry weight basis) propylene oxide (72). Hydroxypropyl group degree of substitution was about 0.06. Growth rates were similar during a 25-day test period. At sacrifice, blood biochemical values including hemoglobin, cholesterol, triglycerides, calcium, phosphorus, alkaline phosphatase, urea nitrogen, total protein, albumin, and globulin, were similar for treated and control animals. Relative organ weights as well as carcass and liver composition were similar for test and control animals.

Twelve adult human volunteers consumed 60 g hydroxypropyl distarch glycerol on each of 4 successive days. No adverse effects were observed nor were there abnormalities in frequency of defecation, quantity of feces, fecal water or its lactic acid content (91).

Long-term studies

In a 2-year study, hydroxypropyl distarch glycerol was fed to groups of 30 male and 30 female weanling CIVO rats at 5, 10, and 30 percent (about 3, 5, and 15 g per kg body weight) dietary levels, replacing an equal amount of precooked control potato starch (92, 93). The test starch was a potato starch cross-linked with 0.1 percent epichlorohydrin and etherified with 5 percent propylene oxide (D.S. 0.04 to 0.05). Body weight gains of females fed the 30 percent dietary level were significantly lower than those of control animals; females at this dietary level also showed a slight reduction in hemoglobin concentration but the values were within the normal range. Blood chemical findings and urinalysis showed no evidence of a dose-related response to the test substance. Cecal weight was increased at the 30 percent dietary level in males and at the 10 and 30 percent levels in females. No change in cecal tissue was found by microscopic examination. No distinct compound-related gross or microscopic pathologic changes were observed in any of the organs examined. Renal calcification accompanied by focal hyperplasia of the pelvic epithelium was more marked in

males receiving the 30 percent test diet than in the control animals (Table V). However, because there was no distinct relationship with either the feeding level, or with the type of chemically modified starch among the five fed in the study (Table V, CIVO/TNO Studies, p. 27), the investigators considered the toxicological significance doubtful. Reports on the occurrence of similar renal changes in rats fed other types of diets are noted in the section Starch Acetates, Long-term studies (p. 26).

Special studies on reproduction

Groups of 10 male and 20 female weanling CIVO rats (Wistar-derived) were fed, for three generations, a diet containing 10 percent (about 5 g per kg body weight) hydroxypropyl distarch glycerol and 20 percent precooked potato starch (92). The test starch was potato starch which had been cross-linked with 0.1 percent epichlorohydrin and etherified with 5 percent propylene oxide (w/w starch). Rats were mated (P, F_1 and F_2 generations) at weeks 12 and 20 after weaning. The second litter of each generation was used to produce the next generation. The F_{3b} generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. Implantation sites were counted in the P, F_{1b} and F_{2b} parents. Body weights did not differ among groups in successive generations and no treatment-related differences were observed in the test groups. No adverse effects were observed regarding resorption quotient, litter size, weight of pups, preweaning mortality or growth rate of pups. No gross or histological changes attributable to feeding the modified starch were observed.

In a modified 13-week subacute one-generation reproduction study, cornstarch and hydroxypropyl distarch glycerol (25 percent propylene oxide, 0.1 percent epichlorohydrin-treated cornstarch) were fed at a dietary level of 10 percent to Charles River rats from 2 weeks prior to mating of the F_{1a} generation until the sacrifice of the parental females for the caesarean delivery of the F_{1b} generation (151). Twenty-five F_{1a} males and 25 F_{1a} females were selected and placed on the 90-day study at the 10 percent dietary level. The number of conceptions, litters, live births, still births and preweaning deaths, weights of pups at 24 hours and at weaning for the F_{1a} litters, were comparable for the test and control groups. Necropsies on 10 male and 10 female pups of the F_{1a} litter at weaning revealed no gross or microscopic pathologic changes attributable to the test compound. Skeletal and visceral examination of F_{1b} fetuses delivered by caesarean section on day 18 or 19 did not reveal any compound-related abnormalities. Test and control groups were comparable in respect to number and placement of implantation and resorption sites and number and weight of live and dead fetuses. Terminal necropsy of the P_1 animals did not reveal any consistent compound-related alterations. Females had kidney- and spleen-body weight ratios that were significantly greater (15 and 20 percent, respectively) than those of the controls. However, microscopic examina-

tion revealed no abnormalities. Physical appearance, behavior, growth and food consumption of the F_{1a} test and control groups fed 90 days were comparable. Survival was 100 percent in both groups. Hematological determination and urine determinations were within normal limits and were comparable between the groups. Kidney-body weight ratios were significantly greater for test animals of both sexes than for controls and the thyroid-body weight ratio was significantly less. However, gross and microscopic examination of the kidneys and thyroids and other organs showed no pathological alterations which could be attributed to ingestion of the modified starches.

Acetylated Distarch Glycerol

Digestibility and metabolism

In vitro amyloglucosidase digestibilities of two gelatinized acetylated distarch glycerol samples, 1.16 and 2.58 percent acetyl content, were 80.4 and 67.6 percent, respectively, of that of gelatinized cornstarch (86). Digestibility of cornstarch cross-linked by the same treatment with epichlorohydrin, but not acetylated, was 98.2 percent of that of the control.

Caloric values of two acetylated distarch glycerol preparations, one treated with 0.1 percent epichlorohydrin and 5.5 percent acetic anhydride and the other with 0.3 percent epichlorohydrin and 5.5 percent acetic anhydride, were estimated from the 28-day growth response of groups of 10 weanling albino rats (strain not stated) fed low-calorie basal diets to which 1.5 or 3 g (about 12 or 25 g per kg body weight) of the test starch was added (152). Comparison was made with the growth response of similar groups fed the basal diet to which graded amounts of sucrose were added. All animals were normally active and in good health. Caloric availability of the modified and control starches was similar and in no instance was the caloric estimate from the high dose less than that from the lower one.

Short-term studies

Groups of 15 male and 15 female weanling FDRL rats were fed, for 90 days, semipurified diets containing 50 percent (about 40 g per kg body weight) of thin-boiling starch or acetylated distarch glycerol prepared by treating the thin-boiling waxy maize starch with 0.3 percent epichlorohydrin and 10.5 percent acetic anhydride (w/w starch) (153). Because diarrhea was observed in the test group, the level of cellulose flour was increased to 4 percent from the second week on after which diarrhea disappeared. Weight gain in males of the test group was significantly lower (13 percent) than that of the control group but weight gains of females in the two groups showed no difference. Food intake and efficiency of utilization were slightly but not significantly different in the test group than in

controls of the same sex. Blood chemical and hematologic analyses, urinalysis, organ weights, and gross and histological examinations were normal.

Two groups of 3-day-old Pitman-Moore miniature pigs were given liquid diets containing 32.8 percent (dry basis) thin-boiling waxy maize starch or this starch after treatment with 0.05 percent epichlorohydrin and acetic anhydride to an acetyl content of 1.1 percent (73). Each diet was fed ad lib for 25 days. Growth was less for pigs fed the modified starch diet but feed intake was about 20 percent less than that of the control group which was attributed to the viscous nature and relative unpalatability of the diet. Cecum-body weight ratios were greater for animals in the control group. Withthe exception of higher (10 percent) serum concentrations of inorganic phosphorus in pigs fed the modified starch, no differences due to treatment were observed in any of the serum chemical values.

Long-term studies

In a 2-year feeding study, groups of 30 male and 30 female Sprague-Dawley rats were fed diets containing 62 percent (about 30 g per kg body weight) of acetylated distarch glycerol or cooked unmodified starch (142). Ten additional rats of each sex were included in the test and control groups, but were necropsied at 90 days. No gross or histopathologic abnormalities were observed in these animals. Weight gains of males and females fed the test diet for 100 weeks were 13 and 20 percent lower than controls of the same sex. Organ-body weight ratios, but not absolute weights, of the brain and kidneys of both sexes and the heart of males differed significantly from those of control animals. The investigators did not consider these differences to have biological significance. Hematological parameters measured after 3, 12, and 24 months of treatment did not show variations outside normal limits; among biochemical parameters measured after these periods of treatment, only the level of SGOT at 24 months in the test group appeared to be outside (higher) normal limits. Incidence of tumors in the treated animals did not differ significantly from that in the control groups. Kidney lesions characterized by focal hyperplasia of the epithelium lining the urinary space accompanied by calcified deposits on the lining epithelium were present in both control and treated animals. The authors concluded that there was no significant difference qualitatively or quantitatively in the lesions in the two groups (142). The same conclusion was reached in a subsequent reexamination (96) of the kidney sections (Table V).

Abnormal histological findings in the test animals at 24 months were hyperkeratosis of the forestomach and, in females, an increase of giant multinuclear cells in the liver. The investigators considered the first finding to be of no toxicological significance to man and the second to be of doubtful significance because the mean age of the treated group was

almost 1 month older than that of the control group, and the animals were not truly comparable, as a result of earlier deaths of the controls (142). A "blind" reexamination of the stomach tissue sections by three pathologists did not demonstrate any significant differences between the test and control animals. It also was concluded that the giant multinuclear cells observed in livers of female rats were linked with senescence and were without relationship to the ingestion of modified starch (143).

Special studies. Reproduction test

Groups of 10 male and 10 female Sprague-Dawley rats were fed for three generations 62 percent (about 40 g per kg body weight) acetylated distarch glycerol (144). Each generation (P, F_1, F_2) produced two successive litters with a 12- to 15-week period between matings. The second litter was used to produce the next generation. Following weaning of the F_{2b} litters, the F_{1b} parents were sacrificed and autopsied. The F_{3b} generation was kept for 10 weeks to observe weight changes of all the young, then sacrificed and autopsied. No adverse effects were observed on fertility, litter size, weight of pups, mortality and postnatal growth. Liver weight increased in both absolute and relative values in the F_1 animals; however, no macroscopic anomalies were found. Gross and histopathologic examination of F_{3b} animals revealed no differences between test and control animals.

Succinyl Distarch Glycerol

Short-term studies

In a 90-day subacute feeding study, groups of 15 male and 15 female weanling albino rats were fed semisynthetic diets containing 50 percent succinyl distarch glycerol (about 50 g per kg) or control starch (154). The test starch was prepared by treating an acid-modified waxy maize starch with 0.3 percent epichlorohydrin and 4 percent succinic anhydride (w/w starch). Diarrhea occurred in some of the rats in the test group during the first week and the level of cellulose flour in the diet was increased from 2 to 4 percent, which corrected this condition. Growth of females of test and control groups was equivalent, but that of males of the test group was significantly lower ($P<0.01$) than that of the control group. Food efficiency of males of the test group also was significantly lower. Clinical examinations showed no treatment-related effects on the hematological or blood biochemical values. Urine of males of both test and control groups was alkaline. Cecal weights of rats of both sexes in the test group were significantly greater than those of the control group. Kidney and liver weights were normal and comparable in animals fed the test and control diets. Microscopic examination of tissue sections revealed no abnormalities attributable to the test diet. The kidneys of a number of female rats fed the

control (7/15) and test (8/15) diets exhibited varying degrees of apparent calcification in the region of the corticomedullary junction. This condition did not occur in males.

Long-term and special studies

No reports on long-term studies, mutagenicity or teratogenicity were available to the Select Committee.

Sodium Hydroxide Gelatinized Starch

No information on the biological properties of starches gelatinized by treatment with 1 percent sodium hydroxide was available to the Select Committee.

Biological Properties of Possible Residues in Chemically Derivatized Starches

Propylene oxide. The oral LD_{50} (14 days) of propylene oxide in male Wistar rats, 90 to 120 g body weight, was 1.14 g per kg body weight; in guinea pigs, 250 to 300 g body weight, it was 0.69 g per kg (155). Repeated oral doses of 0.1 or 0.2 g per kg body weight administered as a 10 percent solution in olive oil to groups of five young adult female rats, 5 days a week for 24 days, produced no toxic effects (156). In vapor inhalation studies, groups of three to 17 rats, eight guinea pigs, and one rabbit of each sex and one female monkey were exposed 7 hours daily, 5 days a week, to 457 ppm propylene oxide vapor. Male rats received 79 exposures in 112 days, female rats 138 exposures in 198 days, both male and female guinea pigs 110 exposures in 157 days, and rabbits and monkey 154 exposures in 218 days. The monkey and rabbits showed no adverse effects including gross and histopathological findings. Irritation of eyes and respiratory passages occurred in the rats and guinea pigs; a slight growth depression and increase in lung weights also were observed for the guinea pigs. All four species tolerated 102 ppm propylene oxide without effect for 183 to 198 days.

Reverse mutations were induced in macroconidia of the purple adenine requiring mutant 38701 of Neurospora crassa after treatment with 0.5 M propylene oxide in water for 15 minutes (157). Propylene oxide also induced recessive lethal mutations in Drosophila melanogaster (158).

Groups of 12 rats were administered by subcutaneous injection 1.5 g per kg total dose of propylene oxide in water or arachis oil over a period of 325 days. Sarcomata occurred at the site of injection after 507 to 739 days in eight mice injected with the arachis oil solution; and in three mice treated with the water solution after 158 to 732 days (159).

Propylene chlorohydrin. Propylene oxide reacts with chloride ion in foods to form propylene chlorohydrin (160). Two isomers are formed, 1-chloro-2-propanol and 2-chloro-1-propanol and both isomers have been identified in foods treated with propylene oxide (161). Propylene chlorohydrin has been found in starches modified by hydroxypropylation. Wesley et al. (160) found that volatilization reduced the ethylene chlorohydrin content of food about 50 percent after prolonged cooking in an open vessel but the combination of high temperature and prolonged time of cooking in a closed autoclave caused little change in the ethylene or propylene chlorohydrin content.

Groups of 10 male and 10 female 5-week-old rats were fed for 25 weeks diets to which 0, 0.1, 0.25, 0.5, and 1.0 percent propylene chlorohydrin (75:25 mixture of the 1-chloro and 2-chloro isomers) was added (162). However, analysis of the 1.0 percent-level diet showed loss by volatilization during 20 minutes' mixing in an open mixer reduced the level to 0.36 percent. After 7 days' exposure of this diet to laboratory conditions, the level of propylene chlorohydrin was 0.0838 percent (about 90 mg per kg body weight at week 1) of which 32 percent was the 2-chloro isomer. Growth rates of animals fed the 0.5 and 1.0 percent dietary levels were lower than those of controls but food consumption was less and food efficiency was similar to those of controls. Organ-body weight ratios were comparable to those of control animals. No adverse effects were observed on hematological values, mortality, gross or histopathologic findings.

In another test (162) propylene chlorohydrin was administered to groups of 10 male and 10 female 8-week-old rats by stomach tube in doses of 25, 50, 75, and 100 mg per kg of body weight daily for 22 weeks. The dose for the high level group was increased to 150 mg per kg in the 11th week, to 200 mg per kg in the 14th week and to 250 mg per kg in the 16th week. Weight gain in the high dietary level group was moderately depressed in males and slightly depressed in females while the dose was not above 150 mg per kg. Both sexes lost weight when the dose was increased to 200 mg per kg. All rats died within 3 weeks after increasing the dose to 250 mg per kg; only one death had occurred on the high level diet before the 16th week. Weight gain was slightly, but not significantly, depressed in both sexes given doses of 75 mg per kg daily. The liver-body weight ratio was increased at the 75 mg per kg dose for both sexes and for males at the 25 mg per kg dose. However, no hematological changes or gross or histopathologic changes were observed in treated rats at dose levels of 75 mg per kg or less. Microscopic examination of tissues of the rats dosed at higher levels was not conducted.

Oral LD_{50} of propylene chlorohydrin in the rat was reported as 218 mg per kg body weight. No deaths occurred in dogs administered 150 mg per kg orally; 1/7 deaths occurred at 200 mg per kg and 6/6 deaths resulted from oral administration of 250 or 300 mg per kg (162).

Propylene chlorohydrin (75 percent 1-chloro-2-propanol and 25 percent 2-chloro-1-propanol) was mutagenic to Salmonella typhimurium TA 1530 but not for TA 1538 when tested in agar containing 1.1 to 22 mg propylene-chlorohydrin per plate (163).

Epichlorohydrin. The oral LD_{50} of epichlorohydrin in male ICR mice and male Sprague-Dawley rats was 240 and 260 mg per kg of body weight, respectively. Administered intraperitoneally, the LD_{50} ranged from about 120 to 170 mg per kg for mice, rats, guinea pigs, and rabbits. Cause of death in acute experiments is generally attributed to depression of the respiratory center. Epichlorohydrin produces extreme irritation when tested intradermally, dermally or intravascularly in rabbits (164).

In a subacute toxicity study, groups of 12 male Sprague-Dawley rats received 0, 11, 22, or 56 mg epichlorohydrin per kg body weight in cottonseed oil by intraperitoneal injection 3 days per week for 12 weeks. There was a dose-related decrease in hemoglobin values, segmented neutrophils were increased in the high dose group and lymphocytes were decreased in the two highest dose groups (164).

Sprague-Dawley rats, 240 to 260 g body weight, became infertile after daily oral administration of 15 mg epichlorohydrin per kg body weight. The effect was reversible within 1 week. Histological examination of testes, epididymides, prostates and seminal vesicles after 12 days of treatment showed no differences compared with untreated controls (165).

Epichlorohydrin did not induce dominant lethal mutations in ICR/Ha Swiss mice when 150 mg per kg was administered intraperitoneally (166). Reverse mutations were induced in macronconidia of the purple adenineless mutant 38701 of Neurospora crassa by treatment for 30 minutes in a 0.15 molar aqueous solution of epichlorohydrin (157). Dissolved in ethanol and adjusted to pH 7.2, epichlorohydrin induced reverse mutations from tryptophan dependence to tryptophan independence when tested in Escherichia coli strain B/r (167). Epichlorohydrin was four- to five-fold less mutagenic than the polyfunctional alkylating agent tris(1-aziridinyl)-phosphine oxide when tested on human lymphocytes in vitro at concentrations of 10^{-4} to 10^{-11} M. The 10^{-4} M concentration was toxic and only the 10^{-5} M concentration increased significantly the number of cells with chromosomal aberrations and number of aberrations and breaks per hundred cells (168).

Sarcomas at the site of injection occurred in two of 50 6- to 8-week-old female ICR/Ha Swiss mice given weekly subcutaneous injections of 1 mg epichlorohydrin in 0.05 ml tricaprylin for 300 days (169). In a later test, 1 mg epichlorohydrin in 0.05 ml tricaprylin was administered weekly by subcutaneous injection to 50 6- to 8-week-old female ICR/Ha Swiss mice for 580 days. Median survival time was 486 days; six animals developed

sarcomas at the site of injection and one had a local adenocarcinoma. One of 50 mice had a local sarcoma in the control group given tricaprylin only (170).

In mouse-skin initiation-promotion experiments, 30 female ICR/Ha mice were injected with 2 mg epichlorohydrin in 0.1 ml acetone followed 14 days later by applications of 2.5 μg phorbol myristate acetate in 100 μl acetone three times a week until the experiment terminated at 385 days. Nine mice developed skin papillomas (first one observed at 92 days) and one developed a skin carcinoma. Three of 30 control animals treated with phorbol myristate acetate alone developed papillomas, the first being observed at 224 days (170).

Preliminary reports (171, 172) on rat inhalation studies with epichlorohydrin in which 40 rats were exposed to a concentration of 100 ppm for 6 hours daily for 30 days identified squamous cell cancers of the nasal epithelium in three animals that died at 460, 490, and 596 days and a squamous cell papilloma in a fourth animal that died at 391 days. In another study in which groups of rats were exposed at concentrations of 10, 30, and 100 ppm, two of 12 rats, alive after an unstated period of exposure to 100 ppm epichlorohydrin, had developed a mass on the nose suspected to be cancerous.

In 2-year rat feeding studies carried out with two starches cross-linked with epichlorohydrin, hydroxypropyl distarch glycerol (5, 10, and 15 percent levels in the diet) and acetylated distarch glycerol (62 percent in the diet), no evidence of carcinogenicity of the treated starches was observed (92, 142).

3-Chloro-1, 2-propanediol. This chlorohydrin is formed by the reaction of epichlorohydrin with water and may be present in trace amounts in epichlorohydrin cross-linked starches. The oral LD_{50} of this chlorohydrin in rats is reported as 150 mg per kg body weight and in mice 160 mg per kg (173). Like epichlorohydrin, 3-chloro-1, 2-propanediol produces reversible male infertility in rats (174). Minimum effective oral dose for young, mature Spartan and Upjohn male rats was approximately 6 to 7 mg per kg of body weight. No change was detected in sperm numbers or motility. Return to fertility was complete within 1 week post-treatment. Histological examination of the testis and epididymis from the males treated with one to two times the minimum effective dose for 8 to 49 days showed no deviations from normal. However, doses five times the minimum effective dose produced a lesion in the caput epididymis of the rat.

1, 2-Dichloropropanol and 1, 3-dichloropropanol. These chlorohydrins may be formed by reaction of epichlorohydrin with traces of chlorides present in starch or the reaction medium. The oral LD_{50}'s for

these compounds in rats are 90 mg per kg for the 1, 2-isomer (173) and 0.11 ml (150 mg) per kg for the 1, 3-isomer (175).

Vinyl acetate. The oral LD_{50} for vinyl acetate in rats is reported as 2.92 g per kg body weight (173).

Vinyl acetate was nonmutagenic when tested in the Salmonella/microsome test using tester strains TA 1535, TA 1537, TA 100 and TA 98 both with and without the S-9 fraction of rat liver homogenate (176). No tumors were detected in Sprague-Dawley rats exposed to atmospheres containing 2,500 ppm vinyl acetate 4 hours per day, 5 days per week, for 12 months (177).

Acetaldehyde. Acetaldehyde is a byproduct of the reaction of vinyl acetate with starch. Only traces are expected to remain in starch after washing starch acetylated by treatment with vinyl acetate. Acetaldehyde occurs naturally as a constituent of many fruits and vegetables including grapefruit (juice, 1.45 ppm), oranges (juice, 1 to 10 ppm), grapes (0.36 to 1.8 ppm), pears (3 to 119 ppm), broccoli (2 to 15 ppm), and peas (2.4 ppm) (178).

The oral LD_{50}'s for acetaldehyde in rats and mice are reported as 1.9 and 1.2 g per kg of body weight, respectively (173).

Twenty hybrid rats received subcutaneous injections, one to two times per week, of 0.5 to 1.0 ml of 0.5 percent solutions of acetaldehyde for a total of 26 to 41 injections each followed by injections of 1.0 to 1.5 ml of 1 percent solutions, also 1 to 2 times per week, for a total of 40 to 50 injections. Four rats developed spindle cell sarcomas near the site of injection within 554 days, the duration of the experiment (179).

Acrolein. The oral LD_{50} for acrolein in the rat is 46 mg per kg and in the rabbit, 7 mg per kg of body weight (173). Acrolein was mutagenic in Drosophila (180) and S. typhimurium, strains TA 1538 and TA 98 (181) but was nonmutagenic in the dominant lethal assay in the mouse when doses of 1.5 or 2.2 mg per kg body weight were administered intraperitoneally (166). Injected subcutaneously in 15 partly inbred albino mice (a strain susceptible to subcutaneous sarcoma) at weekly intervals for 24 weeks, doses of 0.2 mg acrolein in 0.1 cc of sesame oil were not carcinogenic (182). Two of 15 albino S strain mice developed skin papillomas within 33 weeks after 10 weekly applications of a 0.5 percent acrolein solution in acetone (12.6 mg total dose) (179).

Succinic anhydride. Injected subcutaneously in rats, the TDL_{o} (lowest toxic dose) of succinic anhydride was 2.6 g per kg (173). The compound was not mutagenic in the Salmonella test using strains TA 100, TA 98,

TA 1535, and 1537 with the S-9 fraction of liver homogenate (176). However, the chemical toxicity of succinic anhydride limited the dose that could be tested. Six rats, each weighing 100 g, were injected subcutaneously with arachis oil containing 2 mg succinic anhydride twice weekly for 65 weeks (183). Three rats developed local tumors at the site of injection after 93, 104, and 106 weeks, respectively.

Incidence of Kidney Stones in Age Group 0 to 20

The occurrence of uroliths in rats fed high levels of chemically derivatized starches in long-term feeding experiments raised the question whether an increased incidence of kidney stones may have occurred since the introduction of these starches in baby foods in the 1950's. Winters (184) examined the available information on the incidence of stones in the U. S. population group age 0 to 20. It was concluded that there was no evidence of increased incidence in this age group over the past 10 to 20 years and that no effects of specific dietary components have been shown to be etiologically related or even correlated with the occurrence of stones in children of this country.

V. OPINION

Unmodified and Pregelatinized Starches
(pages 18-21 in report)

The digestibility of unmodified cereal and tapioca starches used commercially as food ingredients, both raw and after cooking, is almost complete. Potato and arrowroot starches are less completely digested when fed raw but their digestibility is similar to that of the cereal starches after cooking. Pregelatinized starches (dried, cooked starches) generally are highly digestible. Consumption of excessive quantities, pounds per day, of raw starch has resulted in obesity and iron-deficiency anemia in human subjects. Most of the foods to which starch is added by the food industry are cooked in processing or are cooked before serving. Moreover, the total quantity of unmodified and pregelatinized starch added to processed foods is insignificant compared to the natural starch content of the American dietary, some of which is eaten in its native form in raw vegetables. No adverse effects have been attributed to these starches as added food ingredients. It is suggested, however, that specifications for food grade unmodified starches be developed in order to distinguish them from the starches that are used in non-food applications.

In light of the foregoing, the Select Committee concludes that:

> There is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo (also called grain sorghum starch), rice, potato, tapioca or arrowroot starch that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future.
>
> There is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo, rice, potato, tapioca or arrowroot starch that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are substances migrating to food from paper and paperboard packaging.
>
> There is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo, rice, potato, tapioca or arrowroot starch that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are substances migrating to food from cotton and cotton fabrics used in dry food packaging.

Modified Starches

Lack of intake data for individual starches is common to all of the modified starches. Consequently, in evaluating their safety as a food ingredient, it was assumed that the per capita total daily consumption of modified starches, about 17 mg per kg body weight, applied to each individual starch. Similarly, it was assumed that the maximum daily intake, 1.7 g per kg, reported for the modified starches used in infant foods (distarch phosphate, acetylated distarch phosphate, and acetylated distarch adipate) also represented the possible intake for each of these modified starches.

An observation common to the six modified starches for which long-term rat feeding experiments have been conducted, and noted with another modified starch in 90-day studies, was the occurrence of renal lesions with similar morphology in all cases. To minimize repetition, it is convenient to discuss this renal alteration and its significance at this point. The renal alteration consisted mainly of focal hyperplasia of the renal papillary and pelvic epithelium accompanied by calcified patches in the underlying tissues or calcified deposits attached to the lining epithelium. The condition occurred in some control animals and, although there was no clear dose-response relationship, highest incidence appeared at the highest dietary levels of the modified starches. The renal lesion does not appear to be related to the chemical composition of the modified starches as evidenced by the different derivatives which are associated with this condition. Similar lesions have been reported in rats fed high levels of lactose, sodium alginate, magnesium oxide and in uninephrectomized rats fed high levels of sodium chloride. A similar pathological process of unknown etiology also has been described in Sprague-Dawley rats. Hyperplastic changes and mineral deposits have been associated with the presence of the urinary tract parasite, Trichosomoides crassicauda, frequently found in rats. The mineralization observed in case of the modified starches did not appear to be associated with formation of kidney or bladder calculi although occurrence of microlithiasis was not excluded. In long-term studies with mice fed two modified starches at a single relatively high dietary level (80 g per kg body weight) a slightly increased incidence of intratubular nephrocalcinosis, concrements in the renal pelvic space and urinary bladder stones was observed in males of the treated groups. No evidence of hyperplasia of the epithelium was found. High early mortality of males in the control group complicated evaluation of the findings.

Although there is no evidence that the use of modified food starches in baby foods has increased the incidence of kidney or ureter calculi in infants or young adults that have eaten such foods, or that other adverse effects have resulted, it would be desirable to undertake studies, in due time, to determine the toxicological significance of the renal alterations observed in animal feeding studies. In any experiments repeated with rats, care should be taken to insure parasite free animals. Because mineral balance in the diet may be

a factor contributing to the renal lesion in rats, this dietary factor deserves consideration. Other species should be studied to find whether they also exhibit similar kidney lesions when fed one or more of the modified starches at levels (g per kg body weight) comparable to, and above, those of infants that receive processed baby foods.

The conditions given for starch treatment in the specifications for several of the modified food starches are insufficient to define the extent of reaction with the respective reagents. It is suggested that limitations be placed on the content of groups introduced by monofunctional reagents as is done in the case of the acetyl and phosphate derivatives.

Acid-modified starches (page 21-22 in report)

Hydrolysis of glucosidic linkages occurs during acid modification of starch resulting in molecular fragmentation similar to that which occurs in the production of glucose syrups and maltodextrins. The extent of hydrolysis is limited in the acid-modified starches and is comparable to that of the maltodextrins, the main difference being that the granular form is retained in the acid-modified starches. No adverse effects were noted in feeding acid-modified starch to 3-day-old pigs nor in 90-day rat feeding tests. The evidence indicates that the acid-modified starches are without hazard as food ingredients.

Based on consideration of the available evidence, the Select Committee concludes that:

> There is no evidence in the available information on acid-modified starches that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future.
>
> There is no evidence in the available information on acid-modified starches that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are substances migrating to food from paper and paperboard packaging.
>
> There is no evidence in the available information on acid-modified starches that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are substances migrating to food from cotton and cotton fabrics used in dry food packaging.

Bleached starches (page 22 in report)

The principal change in starch effected by the approved bleaching treatments is the removal of color due to traces of plant pigments. Bleaching treatments also help to reduce the microbiological count in starches. Although no feeding studies with bleached starches have been reported, no adverse effects would be expected because the permitted concentrations of the bleaching agents, all oxidants, are so low that few, if any, carboxyl or carbonyl groups would be introduced to affect the biological properties of bleached starches.

In light of the foregoing, the Select Committee concludes that:

> There is no evidence in the available information on bleached starches that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future.

Hypchlorite oxidized starches (pages 22-24 in report)

Digestibility and caloric value of starch oxidized with the maximum permitted level of sodium hypochlorite, as determined in rat feeding tests, were similar to those for unmodified starch. No adverse effects were observed in gross pathology or histopathology, hematology, serum chemistry or urinalyses in 90-day feeding tests when the oxidized starch was fed at levels an order of magnitude higher than the per capita consumption of all modified starches as indicated by available consumption data. However, starch treated with about eight times the permitted level of hypochlorite caused diarrhea and marked growth depression and cecal enlargement in rats in 21-day feeding studies, demonstrating the adverse effects of starches containing high levels of oxidized groups. In view of this indication of possible toxicity of starch oxidized with permitted levels of hypochlorite when ingested at high levels of intake, and the lack of long-term chronic toxicity tests and data on actual intake levels, the Select Committee recommends that information on either one or both of the latter be obtained in order to adequately evaluate the safety of hypochlorite oxidized starch as a direct food ingredient. This modified starch is not currently used in infant foods.

On basis of the above considerations, the Select Committee concludes that:

> While no evidence in the available information on sodium hypochlorite oxidized starch demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.

Starch acetate (pages 25-29 in report)

Although the in vitro rate of digestion of acetylated starches is lower than that of the corresponding unmodified starch, no difference in digestibility was found in rat feeding experiments with starches containing 2 percent acetyl groups which is near the maximum permitted level of 2.5 percent. No adverse effects were observed in short-term animal feeding studies except for enlargement of the ceca and slight diarrhea in some studies at high intake levels (50 g per kg body weight). Cecal enlargement also was observed in long-term feeding studies with rats and mice but, as in the short-term studies, no cecal tissue abnormalities were found and the enlargement is not considered a significant finding. No adverse effects were noted in fertility, litter size, resorption quotient, preweaning mortality or growth rate of pups in a three-generation rat feeding study of starch acetate. In the 2-year feeding study, suburothelial deposits of calcium accompanied by hyperplasia of the epithelium lining the renal pelvis occurred slightly more frequently in test males at the highest treatment level than in control animals. Slightly increased incidence of intratubular nephrocalcinosis and concrements in the renal pelvic space of males were observed in a 79-week mice feeding study but hyperplasia did not occur. The toxicological significance of the renal changes observed in these studies needs clarification. This modified starch is not currently used in infant foods.

Based on the foregoing considerations, the Select Committee concludes that:

> There is no evidence in the available information on starch acetate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.

Starch succinates (pages 29-31 in report)

The nutritional properties of starch sodium succinate, starch sodium octenyl succinate and starch aluminum octenyl succinate are similar to those of unmodified starch as indicated by caloric values and growth rates observed in animal feeding experiments. No adverse effects were noted on growth rate or on hematology in short-term rat feeding experiments. However, these experiments were of less than 90 days duration and no gross or histopathological examinations were made. The evidence available is insufficient to answer questions concerning the possible chronic toxicity of these succinates particularly in view of the lack of information on their consumption levels. The Select Committee considers it desirable to undertake long-term animal feeding studies with these modified starches. Information on consumption levels by the U.S. population is also needed. These modified starches are not currently used in infant foods.

In view of the foregoing, the Select Committee concludes that:

> While no evidence in the available information on starch sodium succinate, starch sodium octenyl succinate and starch aluminum octenyl succinate demonstrates a hazard to the public when they are used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.

Starch phosphates (pages 31-37 in report)

Because of their close structural relationship, monostarch phosphate, distarch phosphate and phosphated distarch phosphate are considered as a group. Digestibilities of these modified starches were similar to those of the corresponding unmodified starches as measured by caloric values and growth rates determined in rat feeding experiments. No differences were noted between distarch phosphates cross-linked by treatment with trimetaphosphate or phosphorus oxychloride. No significant gross or histological changes or dose-related responses in clinical chemical indices were observed in 90-day rat feeding studies with distarch phosphate or phosphated distarch phosphate. Neither were adverse effects observed in a 3-generation reproduction and lactation study with phosphated distarch phosphate. A possible exception in the 2-year rat feeding study was a kidney abnormality which consisted mainly of focal hyperplasia of the renal papillary and pelvic epithelium accompanied by calcified patches in the underlying tissues. Incidence of the lesion was not distinctly dose related and, as discussed earlier, is considered to be of doubtful toxicological significance.

Other than the occurrence of the renal changes which are of doubtful biological significance, no adverse effects were found in the long-term feeding experiments with phosphated distarch phosphate. Because of the relationship in structure, the results for this starch derivative would appear also to apply to distarch phosphate. Studies with monostarch phosphate have shown that the phosphorus in the ^{32}P-labeled starch derivative administered orally to rats is metabolized in a manner similar to the phosphorus in radiolabeled disodium phosphate, a substance evaluated in another report of the Select Committee. Thus, the monophosphate ester group would be expected to be removed in the digestion of phosphated distarch phosphate, presenting fragments of starch chains cross-linked by phosphate linkages such as would be present in distarch phosphate.

In view of the foregoing evidence, the Select Committee concludes that:

> There is no evidence in the available information on monostarch phosphate, distarch phosphate, and phosphated distarch phosphate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.

Acetylated distarch phosphate (pages 37-39 in report)

Although in vitro rate of digestion of acetylated distarch phosphate by pancreatin or porcine intestinal amylase was less than that of unmodified starch, this was not reflected in growth rate or feed efficiency in animals fed diets containing high levels of this starch derivative. Slight diarrhea and increased cecal weights were noted in short-term rat feeding experiments at a dietary level of 60 g per kg body weight, an intake level much greater than the highest indicated current consumption levels. No cecal tissue changes were observed. No significant effects related to treatment with the possible exception of a renal alteration were observed in long-term rat feeding studies of acetylated distarch phosphate and acetylated diamylopectin phosphate. The former derivative was acetylated with acetic anhydride and the latter with vinyl acetate. The renal alteration consisted mainly of focal hyperplasia of the renal papillary and pelvic epithelium accompanied by calcified patches in the underlying tissues. There was not a distinct relationship between incidence of lesions and feeding level for either acetylated distarch phosphate or acetylated diamylopectin phosphate. The toxicological significance of this renal alteration needs clarification.

On the basis of the foregoing, the Select Committee concludes that:

> There is no evidence in the available information on acetylated distarch phosphate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.

Hydroxypropyl distarch phosphate (pages 39-42 in report)

A reduction of 7 percent in caloric value as compared with unmodified starch in a 10-day rat feeding test was demonstrated for hydroxypropyl distarch phosphate prepared by treating a phosphate cross-linked cornstarch with 8 percent propylene oxide. However, no reduction in growth rates or feed efficiences were found in 90-day rat feeding studies with hydroxypropyl distarch phosphate prepared by treatment with 5 or 10 percent propylene oxide. Increased cecal weights were observed in both studies at dietary levels of 20 g per kg but no tissue abnormality was found. The only possible pathologic change noted was calcareous deposits within the renal pelvis and pelvic epithelium in rats fed the starch treated with 10 percent propylene oxide. In a 79-week feeding test with mice, a slightly increased incidence of intratubular nephrocalcinosis, concrements in the renal pelvic space and urinary bladder stones were observed in males. As discussed in the introduction to the opinion on modified starches, this renal alteration is considered to be of doubtful biological importance, but the Select Committee suggests that experiments should be undertaken in due time to clarify its toxicological significance.

As pointed out in the opinion on hydroxypropyl starch, treatment of starch with propylene oxide to introduce hydroxypropyl groups may also result in the formation of propylene chlorohydrin by reaction with chloride ions that may be present. Propylene chlorohydrin has been shown to be mutagenic to Salmonella typhimurium TA 1530 but not to TA 1538. Short-term (22-week) rat feeding experiments have revealed no pathologic changes at dietary levels up to 75 mg propylene chlorohydrin per kg, nor have long-term feeding experiments with hydroxypropyl distarch glycerol shown an increase in tumor incidence in rats. A long-term mice feeding study with hydroxypropyl distarch phosphate containing 4.3 ppm propylene chlorohydrin revealed no increased incidence of neoplastic lesions. Intake of propylene chlorohydrin was about 350 μg per kg body weight. This would be about four orders of magnitude greater than per capita human exposure assuming that hydroxypropylated starches are the only modified starches used in processed foods. In view of the mutagenicity of propylene chlorohydrin in the Ames test, however, the Select Committee suggests that the limits on this residue be reduced to the lowest level consistent with feasible manufacturing practice and that long-term feeding studies be undertaken with graded levels of propylene chlorohydrin to clarify whether the mutagenic activity observed in a bacterial system is an indication of potential mutagenic activity in animals. This modified starch is not currently used in infant foods.

Based on the foregoing considerations, the Select Committee concludes that:

While no evidence in the available information on hydroxypropyl distarch phosphate demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.

Hydroxypropyl starch and oxidized hydroxypropyl starch (pages 42-44 in report)

Incomplete digestibility of hydroxypropyl starch was demonstrated by the isolation of hydroxypropyl maltose in feces of rats fed this modified starch. This residue increased as the degree of hydroxypropylation of the starch was increased. Other experiments in which labeled hydroxypropyl starch was fed indicated that over 90 percent of the hydroxypropyl groups were excreted in the feces. Thus high degrees of substitution as permitted by the present specifications for hydroxypropylated food starches may reduce the digestibility and caloric value of the starch significantly. Usage of starches of high hydroxypropyl content as compared to a more completely digestible starch could result in a significant reduction of caloric value of the food. It is suggested that a limit be placed on the content of hydroxypropyl groups introduced into a hydroxypropylated modified starch.

Hematological findings were normal and no pathological changes were observed in the major organs in 90-day rat feeding studies of starch containing 1.53 percent hydroxypropyl groups. Weight gains were consistently but not significantly lower than controls at dietary levels of 12 g per kg and above, and diarrhea was observed at 35 g per kg. In a similar study with an oxidized hydroxypropyl starch containing 14.9 percent hydroxypropyl groups, no pathological findings were reported. However, growth rate and food efficiency were reduced at dietary levels of 8 and 20 g per kg and the depression in growth rate was significant at the higher dietary level. Diarrhea also occurred at the 20 g per kg level. Because this starch was both oxidized (i.e. carboxyl and carbonyl groups introduced) and hydroxypropylated, the growth depression and diarrhea cannot be attributed solely to either treatment.

In the hydroxypropylation of starch by treatment with propylene oxide, propylene chlorohydrin may be formed by reaction with chloride ions that may be present. Short-term (22-week) experiments in which propylene chlorohydrin were administered to rats by gavage showed no hematological or histopathologic changes in treated animals at dose levels of 75 mg per kg, the highest dose level at which histological examination of rats was made. However, the liver-body weight ratio was increased at the 25 mg per kg dose level for males and at the 75 mg per kg dose levels for both sexes. Propylene chlorohydrin was mutagenic to Salmonella typhimurium TA 1530 but not for TA 1538 when tested in agar containing 1.1 mg propylene chlorohydrin per plate. Present specifications for hydroxypropyl starches permit 5 ppm of residual propylene chlorohydrin in the product. Long-term rat feeding experiments with hydroxypropyl distarch glycerol etherified by treatment of starch with 5 percent propylene oxide showed no increase in tumors as compared to control animals. Three generation reproduction and lactation studies with

the same hydroxypropylated starch revealed no adverse effects. No increase in neoplastic lesions was observed in long-term studies with mice fed hydroxypropyl distarch phosphate at a level providing about 350 μg propylene chlorohydrin per kg body weight. This is four orders of magnitude greater than per capita human exposure assuming that hydroxypropylated starches are the only modified starches used in processed foods. Although no adverse effects have been observed which have been attributed to residual propylene chlorohydrin, the Select Committee suggests that the limits on this residue be reduced to the lowest level consistent with feasible manufacturing practice, and that long-term animal feeding studies be undertaken with graded levels of propylene chlorohydrin to clarify whether the mutagenic activity observed in a bacterial system is an indication of potential for similar activity in animals. This modified starch is not currently used in infant foods.

In view of the foregoing considerations, the Select Committee concludes that:

> While no evidence in the available information on hydroxypropyl starch and oxidized hydroxypropyl starch demonstrates a hazard to the public when they are used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.

Acetylated distarch adipate (pages 45-47 in report)

Studies with radio-labeled acetylated distarch adipate in the rat showed that adipic acid entered the metabolic pool more slowly but followed normal pathways for free adipic acid. Body weight gains were about 15 percent lower than controls but no significant pathological changes were observed in 90-day and 2-year rat feeding studies at dietary levels of 40 and 30 g per kg body weight, respectively. Kidney lesions, characterized by focal hyperplasia of the epithelium lining the urinary space accompanied by calcified deposits in the lining of the epithelium, were observed in both treated and control animals but there was no significant difference in severity or frequency of the lesions in the two groups. The Select Committee considers these lesions to be of doubtful biological importance but suggests that studies be undertaken in due time to determine their toxicological significance. Cecal enlargement occurred in both feeding studies but without associated histopathological change and is considered to have no toxicological significance.

Based on the foregoing evidence, the Select Committee concludes that:

> There is no evidence in the available information on acetylated distarch adipate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.

Distarchoxy propanols (pages 47-48 in report)

Two modified starches, distarchoxy propanol and acetylated distarchoxy propanol are included in this group. Twenty-eight-day feeding studies with distarchoxy propanol prepared by treatment with up to the maximum permitted level of acrolein showed no adverse effect on weight gains, feed efficiencies or cecal weights when fed at relatively high levels (60 g per kg) compared to probable human consumption. Similar studies with acetylated distarchoxy propanol prepared by acetylation with vinyl acetate and cross-linking with acrolein showed a significant reduction in growth rate and a high incidence of diarrhea with products containing 3 percent or more acetyl groups. However, no adverse effects were observed on reproduction or lactation in dams or weight gains or feed efficiencies of the pups in a two-generation study of the acetylated derivative (2.5 percent acetyl groups) fed at a level of 45 g per kg to the weanling rats. Both generations were fed for 1 year. Thus it appears advisable to limit the acetyl content of these starches to 2.5 percent; this level is consistent with the permitted treatment with a maximum of 7.5 percent vinyl acetate. Some evidence indicates that acrolein, the cross-linking agent used in preparing distarchoxy propanols, is mutagenic indicating that residues of acrolein in the modified starches should be reduced to minimum feasible levels. Long-term feeding studies on distarchoxy propanols also are suggested since those conducted have been limited to 1 year duration and no histopathological or other examinations have been reported. However, it is understood that manufacture of these modified starches was discontinued several years ago.

In view of the foregoing the Select Committee concludes that:

> While no evidence in the available information on distarchoxy propanol and acetylated distarchoxy propanol demonstrates a hazard to the public should they be used at former levels and in the manner formerly practiced, uncertainties exist requiring that additional studies should be conducted.

Distarch glycerols (pages 48-56 in report)

Included in this group of modified starches are distarch glycerol, hydroxypropyl distarch glycerol, acetylated distarch glycerol, and succinyl distarch glycerol. No adverse effects were observed in short-term rat feeding studies with distarch glycerols cross-linked by treatment with almost twice the permitted level of epichlorohydrin (0.5 percent as compared to 0.3 percent) and fed at levels (60 g per kg) much greater than probable human intake.

Cecal enlargement appeared to be the only significant change observed in short-term rat feeding studies with hydroxypropyl distarch glycerols in which the modified starch was fed at levels up to 25 g per kg; no significant changes were noted in similar studies with dogs fed at levels up to 4 g per kg. In a long-term rat feeding study weight gain was reduced in females at the highest feeding level but necropsy revealed no change except for cecal enlargement and renal calcification accompanied by focal hyperplasia of the pelvic epithelium. No histological abnormality was associated with the former change and it is considered to have no toxicological significance. The latter condition was most marked in males but was not clearly dose related. A three-generation reproduction study revealed no adverse effects of feeding this modified starch.

Short-term and long-term rat feeding studies with acetylated distarch glycerol revealed no abnormalities in gross or histopathological findings. No adverse effects were observed in a three-generation reproduction test. Growth rate was somewhat reduced in males in the short-term study at the high dietary level fed (40 g per kg) but not at the level (30 g per kg) fed in the long-term study. Kidney lesions characterized by focal hyperplasia of the epithelium lining the urinary space accompanied by calcified deposits on the lining of the epithelium were present in both control and treated animals. The Select Committee considers these lesions observed with both the hydroxypropyl and acetyl distarch glycerols to be of doubtful biological importance but suggests that studies be undertaken in due time to determine their toxicological significance.

A 90-day feeding study of succinyl distarch glycerol showed no abnormalities attributable to diets in blood analyses, gross or histopathological findings. Growth rate was somewhat reduced in males but this result is of doubtful significance in view of the high dietary level (50 g per kg) fed. However, the Select Committee considers that additional information should be obtained on the chronic toxicity of succinyl distarch glycerol or the related modified starch, sodium starch succinate.

Although there is no evidence of adverse effects resulting from unreacted epichlorohydrin or its reaction by-products in the distarch glycerols, the

mutagenic properties of epichlorohydrin and the indication that it induces cancer of the respiratory system in animals on exposure by inhalation, and local sarcomata by subcutaneous injection, suggests that this substance may also be carcinogenic when ingested in food. Although analyses reported for starches cross-linked with epichlorohydrin showed no detectable residue and two long-term feeding studies of the treated starches gave no evidence of carcinogenicity, it would be prudent to discontinue use of such cross-linked starches in foods until animal feeding experiments with graded levels of epichlorohydrin have been carried out. It is understood that industry has voluntarily adopted this precaution.

On basis of the above evidence, the Select Committee concludes that:

> The evidence on distarch glycerol, hydroxypropyl distarch glycerol, acetylated distarch glycerol, and succinyl distarch glycerol is insufficient to determine that the adverse effects reported are not deleterious to the public health should they be used at former levels and in the manner formerly practiced.

Sodium hydroxide gelatinized starch (page 56 in report)

No information was available to the Select Committee on the biological properties of starch gelatinized with 1 percent sodium hydroxide. The lack of specification of temperature and time of treatment with sodium hydroxide makes it difficult to assess what chemical changes may occur in addition to the physical disruption of the granule structure. Such specifications should be developed.

Based on the foregoing considerations, the Select Committee concludes that:

> In view of the deficiency of relevant biological studies and product specifications, the Select Committee has insufficient data upon which to base an evaluation of starch gelatinized with sodium hydroxide when it is used as a food ingredient.

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The Select Committee expresses its appreciation to the following organizations who contributed information and data:

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National Starch and Chemical Corporation, 10 Finderne Ave., Bridgewater, N.J. 08807.

A.E. Staley Manufacturing Company, 2200 Eldorado St., Decatur, Ill. 62525.

Corn Products, Moffett Technical Center, P.O. Box 345, Argo, Ill. 60501.

Stein, Hall and Co., Inc., 605 Third Ave., New York, N.Y. 10016.

American Maize-Products Company, 113th Street and Indianapolis Blvd., Hammond, Ind. 46326.

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Report submitted by:

July 30, 1979
Date

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances

Version: 2
Effective: 01/08/2020
Reviewed/Revised: 01/04/2022

SAFETY DATA SHEET

Section I Product Identification

Product Name: MSP Potato Starch

Manufacturer Details: MSP Starch Products Inc.
Box 850, 10 Fredrick St.
Carberry, MB R0K0H0

Emergency Number: 204-834-2702

Section 2 Hazard Identification

Not classified for physical or health hazards by GHS.

Hazards not otherwise classified – Combustible Dust
May form combustible dust with certain concentration of air.

Precautionary Statements:
Inhalation - Treat as nuisance dust.
Skin Contact – Drying of skin, no adverse effects expected
Eye Contact – Irritant, no adverse effects expected

HMIS RATINGS:
Health Hazard: 0
Fire Hazard: 1
Reactivity Hazard: 0

Section 3 Composition/Information on Ingredients

Ingredient	CAS No.	Percent	Chemical Formula
Potato Starch	9005-25-8	99+%	$C_6H_{10}O_5$

Section 4 First Aid Measures

Inhalation:	Remove to fresh air. Seek medical attention if breathing is difficult.
Skin Contact:	Wash with soap and water. Seek medical advice if irritation develops.
Eye Contact:	Rinse/flush eye(s) gently, as needed. Seek medical attention if irritation persists.
Ingestion:	Rinse mouth thoroughly. Do not induce vomiting. Seek medical attention if irritation or discomfort persists.

Section 5 Fire Fighting

Fire:	Automatic ignition temperature >380 C.
Explosion:	Fine dust dispersed in the air and the presence of an ignition source has the potential for a dust explosion.
Fire Extinguishing Media:	Use water spray and/or ABC extinguisher.

Protective Equipment: Use NIOSH-approved respiratory protection/breathing apparatus.

Additional Precautions: Move products/material away from fire or keep cool with water spray. Use spark proof tools and explosion proof equipment.

Section 6 Accidental Release

Remove all ignition sources. Wear appropriate PPE. Clean up in a manner that does not disperse dust and use equipment that does not spark (ie. Avoid using compressed air). Vacuum when possible. Dust deposits should not be allowed to accumulate. Ensure adequate ventilation.

Section 7 Handling and Storage

Store is a cool dry place and protect from damage. Store in an odor free area. Store away from heat source. Do not expose to open flame or sparks. Empty containers may contain dust residues and should be handled with care to prevent the possibility of a dust explosion.

Section 8 Exposure Controls and PPE

Airborne Exposure limits:

OSHA PEL:	15mg/m^3 respirable fraction
ACGIH TVL:	10mg/m^3 total dust (no asbestos and <1% crystalline silica)

Ventilation System: Where dust levels may exceed airborne limits, exhausting of dust is recommended.

Personal Respirators: Use NIOSH approved respirators when levels are above airborne exposure limits and engineering controls are not feasible.

Section 9 Physical and Chemical Properties

Appearance and odor:	Dry white powder, bland odor
Boiling point:	N/A
Specific gravity:	1.45 – 1.6
Melting point:	N/A
% volatile:	N/A
Vapor pressure:	N/A
Evaporation rate:	N/A
Vapor density:	N/A
Water solubility:	Negligible
pH:	5.5 – 7.5 (typical)

Section 10 Stability and Reactivity

Stability:	Stable under normal conditions of use and storage
Reactivity:	Non-reactive under normal conditions
Hazardous Decomposition:	Heavy black acrid smoke, Carbon Oxides (CO, CO2)
Hazardous Polymerization:	Does not occur
Incompatibilities:	Strong oxidizers. Strong acids. Strong bases.
Conditions to avoid:	Flames, heat, and ignition sources

Section 11 Toxicological Information

Carcinogenicity: This material is not considered a carcinogen by the National Toxicology Program, the International Agency for Cancer Research, or OSHA. LD50/LC50: No information found

Section 12 Ecological Information

Environmental Fate: Information not found
Environmental Toxicity: Information not found

Section 13 Disposal Considerations

Waste Disposal Recommendation:
It is the responsibility of the waste generator to properly characterize all waste materials according to applicable regulatory entities. Consult provincial and local regulations regarding the proper disposal.

Section 14 Transport Information

Not Regulated

Section 15 Regulatory Information

CAS No.: 9005-25-8
Chemical Weapons Convention: No
TSCA 12(b): No
CDTA: No

Section 16 Other

MSP Starch Products Inc. provides this information contained herein in good faith but make no representation as to its comprehensiveness or accuracy. The information contained in this bulletin is, to our knowledge, true and accurate. However, since the conditions of use are beyond our control, we disclaim all liability in connection with the use of the information, recommendations, or suggestions contained herein. Furthermore, nothing contained herein shall be construed as a recommendation to use any product in conflict with existing patents covering any material or its use.

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.4
Revision Date 09.02.2023
Print Date 08.05.2023
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name	:	Starch, soluble
Product Number	:	S9765
Brand	:	Sigma-Aldrich
REACH No.	:	A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No.	:	9005-25-8

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich Chemie GmbH Industriestrasse 25 CH-9471 BUCHS
Telephone	:	+41 81 755 2511
Fax	:	+41 81 756 5449
E-mail address	:	technischerservice@merckgroup.com

1.4 Emergency telephone

Emergency Phone #	:	+41 43-508-2011 (CHEMTREC) +41 44-251-5151 (Tox-Zentrum) 145(Tox Info Suisse)

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

No hazard pictogram, no signal word, no hazard statement(s), no precautionary statement(s) required

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

Molecular weight	:	342,30 g/mol
CAS-No.	:	9005-25-8
EC-No.	:	232-679-6

No components need to be disclosed according to the applicable regulations.

SECTION 4: First aid measures

4.1 Description of first-aid measures

If inhaled
After inhalation: fresh air.

In case of skin contact
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact
After eye contact: rinse out with plenty of water. Remove contact lenses.

If swallowed
After swallowing: make victim drink water (two glasses at most). Consult doctor if feeling unwell.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Water Foam Carbon dioxide (CO2) Dry powder

Unsuitable extinguishing media
For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Carbon oxides
Combustible.
Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters

In the event of fire, wear self-contained breathing apparatus.

5.4 Further information
none

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Advice for non-emergency personnel: Avoid inhalation of dusts. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert. For personal protection see section 8.

6.2 Environmental precautions
No special precautionary measures necessary.

6.3 Methods and materials for containment and cleaning up
Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Tightly closed. Dry.

Storage class
Storage class (TRGS 510): 11: Combustible Solids

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection
This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).
Full contact
Material: Nitrile rubber

Minimum layer thickness: 0,11 mm
Break through time: 480 min
Material tested:KCL 741 Dermatril® L

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).
Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0,11 mm
Break through time: 480 min
Material tested:KCL 741 Dermatril® L

Respiratory protection
required when dusts are generated.
Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.
Recommended Filter type: Filter type P2

The entrepeneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure
No special precautionary measures necessary.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Physical state	solid
b)	Color	white
c)	Odor	No data available
d)	Melting point/freezing point	Melting point/range: 256 - 258 °C
e)	Initial boiling point and boiling range	No data available
f)	Flammability (solid, gas)	No data available
g)	Upper/lower flammability or explosive limits	No data available
h)	Flash point	Not applicable
i)	Autoignition temperature	No data available
j)	Decomposition temperature	No data available
k)	pH	No data available

l)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
m)	Water solubility	No data available
n)	Partition coefficient: n-octanol/water	No data available
o)	Vapor pressure	No data available
p)	Density	0,14 g/cm3
	Relative density	No data available
q)	Relative vapor density	No data available
r)	Particle characteristics	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	none

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
The following applies in general to flammable organic substances and mixtures: in correspondingly fine distribution, when whirled up a dust explosion potential may generally be assumed.

10.2 Chemical stability
The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions
Violent reactions possible with:
Strong oxidizing agents

10.4 Conditions to avoid
no information available

10.5 Incompatible materials
No data available

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
Oral: No data available
Inhalation: No data available
Dermal: No data available

Skin corrosion/irritation
Skin - Human
Result: Mild skin irritation - 3 h
Remarks: (RTECS)

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
No data available

Carcinogenicity
No data available

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Substances which occur in nature

Inhalation of the dusts should be avoided as even inert dusts may impair respiratory organ functions.

However, when the product is handled appropriately, hazardous effects are unlikely to occur.

Handle in accordance with good industrial hygiene and safety practice.

SECTION 12: Ecological information

12.1 Toxicity

No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties
Product:

Assessment	:	The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects
No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

SECTION 14: Transport information

14.1 UN number

ADR/RID: -	IMDG: -	IATA: -

14.2 UN proper shipping name

ADR/RID:	Not dangerous goods
IMDG:	Not dangerous goods
IATA:	Not dangerous goods

14.3 Transport hazard class(es)

ADR/RID: -	IMDG: -	IATA: -

14.4 Packaging group

ADR/RID: -	IMDG: -	IATA: -

14.5 Environmental hazards

ADR/RID: no	IMDG Marine pollutant: no	IATA: no

14.6 Special precautions for user
No data available
Further information
Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of

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