Toxicological profile for

Sugars (sucrose)

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

(2R,3R,4S,5S,6R)-2-[(2S,3S,4S,5R)-3,4-Dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol (PubChem)

1.2. Synonyms

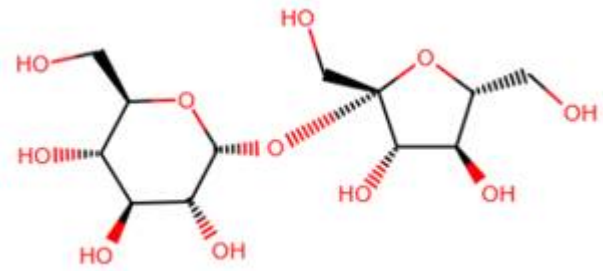
(alpha-D-Glucosido)-beta-D-fructofuranoside; AI3-09085; Amerfand; Amerfond; Beet sugar; CCRIS 2120; Cane sugar; Confectioner's sugar; D-Sucrose; EINECS 200-334-9; Fructofuranoside, alpha-D-glucopyranosyl, beta-D; Glucopyranoside, beta-D-fructofuranosyl, alpha-D; Granulated sugar; HSDB 500; Microse; NCI-C56597; NSC 406942; Rock candy; Rohrzucker; Saccharose; Saccharum; Sucralox; Sucraloxum [INN-Latin]; Sucrose; Sugar; Table sugar; UNII-C151H8M554; White sugar; alpha-D-Glucopyranoside, beta-D-fructofuranosyl-; alpha-D-Glucopyranosyl beta-D-fructofuranoside; beta-D-Fructofuranosyl alpha-D-glucopyranoside (ChemIDplus)

1.3. Molecular formula

C12H22O11 (ChemIDplus)

1.4. Structural Formula

(ChemIDplus)



1.5. Molecular weight (g/mol)

342.3 (ChemIDplus)

1.6. CAS registration number

57-50-1

1.7. Properties

1.7.1. Melting point

(°C): 185.5 (EPISuite, 2017; HSDB, 2005; PubChem); 160-180 (decomposes), 185-190, 96 (ChemSpider); 160-186 (decomposes) (PubChem)

1.7.2. Boiling point

(°C): Decomposes (PubChem); 697.1±55 (estimated) (ChemSpider); 591.59 (estimated) (EPISuite, 2017)

1.7.3. Solubility

2.1x10(6) mg/L (EPISuite, 2017; HSDB, 2005); 2000 g/L water (Merck, 2013); “Soluble to 500 mg/mL in water”, 200% (ChemSpider; NIOSH, 2018)

1.7.4. pKa

12.62 (HSDB, 2005)

1.7.5. Flashpoint

(°C): 258 (ChemSpider)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): 160-186 (HSDB, 2005); decomposes at about 160-186 (Merck, 2013); 186 (PubChem)

1.7.9. Stability

Stable in air (HSDB, 2005); stable, combustible, incompatible with strong oxidizing agents, hydrolyzed by dilute acids and by invertase (ChemSpider); fine airborne dust may explode (NIOSH, 2018)

1.7.10. Vapor pressure

5.15E-17 mmHg at 25°C (estimated) (ChemIDplus); 0 mmHg (approx.) (ChemSpider); 3.53E-016 mmHg at 25°C (estimated) (EPISuite, 2017)

1.7.11. log Kow

-3.70 (EPISuite, 2017; HSDB, 2005; PubChem); -3.67 (CIR, 2014; PubChem)

2. General information

2.1. Exposure

Occurs in low percentages in honey and maple sap. [Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 13th ed. New York, NY: John Wiley & Sons, Inc. 1997., p. 1057]

Sugar cane contains from 15-20% and sugar beet from 10-17% sucrose [Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996., p. 1517]

The quantity of sucrose synthesized by all plants on earth is estimated to be 150X10+9 t/a. [Gerhartz, W. (exec ed.). Ullmann's Encyclopedia of Industrial Chemistry. 5th ed.Vol A1: Deerfield Beach, FL: VCH Publishers, 1985 to Present., p. VA 5 (86) 83]

As taken from HSDB, 2005.

Expected maximum - exposure in humans:

Pyrolysis and thermal degradation are assumed (sec. 2.3). Therefore, maximum exposure to Sugars (Sucrose) cannot be estimated.

Cosmetics	Yes (Cosmetics Bench Ref.1996)
Food	Yes (Ash, 1995)
Environment	Yes (HSDB, 2005)
Pharmaceuticals	Yes (Martindale, 1999)
Tobacco products	In burnt part

INCI Name	SUCROSE
Description	
INN Name	sucrose

Ph. Eur. Name	
CAS #	57-50-1
EC #	200-334-9
Chemical/IUPAC Name	Sucrose
Cosmetic Restriction	
Other Restriction(s)	
Functions	HUMECTANT SKIN CONDITIONING SOOTHING
SCCS opinions	
Identified INGREDIENTS or substances e.g.	

As taken from CosIng (Cosmetic substances and ingredients database), available at https://ec.europa.eu/growth/tools-databases/cosing/

Frequency and concentration of use (in cosmetics) according to duration and type of exposure:

	# of Uses	**Max. Conc. Of Use (%)**
Totals*	738	0.001-65
Duration of use		
Leave on	423	0.001-58
Rinse off	303	0.001-65
Diluted for (bath) use	12	1-52
Exposure type		

Eye area	57	0.0035-2
Incidental ingestion	4	9-45
Incidental inhalation-spray	4; 157[a]; 84[b]	0.002; spray: 1; 0.002-2[a]
Incidental inhalation-powder	4; 84[b]	NR
Dermal contact	672	0.001-65
Deodorant (underarm)	NR	aerosol: 0.004 not spray: 0.005-0.009
Hair - Non-coloring	53	0.001-10.5
Hair - Coloring	5	NR
Nail	2	13.6
Mucous membrane	205	0.001-65
Baby products	1	NR

* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types my not equal the sum of total uses

[a] Includes products that can be sprays, but it is not known whether the reported uses are sprays

[b] Not specified whether a spray or a powder, but it is possible th use can be as a spray or a powder, therefore the information is captured in both categories.

[c]Includes products that can be powders, but it is not known whether the reported uses are powders

NR – Not reported

As taken from CIR, 2014.

Sucrose (CAS RN 57-50-1) is listed as an ingredient in inside the home, landscape/yard, personal care, pesticide and pet care products by the US Department of Health and Human Services (2019).

“About half of total sugar intake was sucrose; mean intakes were 40-50g/day for adults and children over 4 years.”

Average daily intake of sucrose by age and sex													
Aged 1.5 years and over	*National Diet and Nutrition Survey years 1-3 combined (2008/09 - 2011/12)*												
Sucrose	**Sex and age group (years)**												
	Boys		**Men**		**Girls**		**Women**		**Total**				
	4-10	11-18	19-64	65+	4-10	11-18	19-64	65+	1.5-3	4-10	11-18	19-64	65+
g/day													
Mean	46.9	55.5	46.7	45.0	46.0	44.5	38.6	37.6	29.8	46.4	50.2	42.6	40.8
Median	45.6	49.7	42.4	38.1	43.0	42.0	34.6	35.7	28.0	44.1	46.0	37.6	36.1
sd	18.5	26.8	27.7	30.0	18.0	21.4	23.3	19.3	13.5	18.2	24.9	25.9	24.8
Upper 2.5 percentile	87.1	124.4	114.8	106.3	86.4	98.8	90.8	78.0	62.0	86.4	114.3	102.9	95.5
Lower 2.5 percentile	17.0	13.6	9.0	8.5	16.7	11.9	5.9	8.1	9.7	17.0	12.4	7.0	8.2
% food and drink energy (excluding energy from alcohol)													
Mean	11.0	10.6	8.8	9.0	11.5	10.6	9.3	9.4	9.8	11.2	10.6	9.1	9.2
Median	10.8	10.2	8.1	8.1	11.2	10.3	8.9	9.5	9.5	11.0	10.2	8.5	9.0
sd	3.4	4.2	4.3	4.7	3.3	4.0	4.6	3.8	3.5	3.4	4.1	4.4	4.2
Upper 2.5 percentile	18.8	20.1	19.6	19.9	185	19.2	19.7	18.0	17.3	18.7	198	19.7	19.2
Lower 2.5 percentile	4.7	3.3	2.4	2.2	5.3	3.8	2.0	2.7	3.9	5.1	3.6	2.2	2.4

% total dietary energy (including energy from alcohol)													
Mean	11.0	10.5	8.2	8.5	11.5	10.5	8.9	9.2	9.8	11.2	10.5	8.5	8.9
Median	10.8	10.1	7.6	7.8	11.2	10.2	8.4	9.2	9.5	11.0	10.2	7.9	8.8
sd	3.4	4.1	4.2	4.6	3.3	4.0	4.4	3.8	3.5	3.4	4.0	4.3	4.2
Upper 2.5 percentile	18.8	19.7	18.8	18.4	18.5	19.1	17.9	17.3	17.3	18.7	19.3	18.7	18.3
Lower 2.5 percentile	4.7	3.3	1.9	2.0	5.3	3.8	1.8	2.7	3.9	5.1	3.5	1.9	2.0
Number of participants (unweighted)	*414*	*445*	*710*	*191*	*389*	*439*	*945*	*273*	*386*	*803*	*884*	*1,655*	*428*

As taken from SACN, 2015.

National Occupational Exposure Survey (1981 - 1983)

Estimated Numbers of Employees Potentially Exposed to Sucrose (CAS RN 57-50-1) by Occupation*

Code	Occupation Description (1980)	Total # Employees (Male & Female)	Total # Female Employees
007	FINANCIAL MANAGERS	91	
019	MANAGERS AND ADMINISTRATORS, N.E.C.	438	424
033	PURCHASING AGENTS AND BUYERS, N.E.C.	251	174
036	INSPECTORS AND COMPLIANCE OFFICERS, EXC. CONSTRUCTION	32	32
048	CHEMICAL ENGINEERS	64	3
069	PHYSICISTS AND ASTRONOMERS	1,361	836
073	CHEMISTS, EXCEPT BIOCHEMISTS	3,775	1,584

078	BIOLOGICAL AND LIFE SCIENTISTS	1,190	988
083	MEDICAL SCIENTISTS	522	385
084	PHYSICIANS	1,219	949
086	VETERINARIANS	5,182	633
095	REGISTERED NURSES	53,440	48,413
096	PHARMACISTS	10,721	6,249
099	OCCUPATIONAL THERAPISTS	84	70
103	PHYSICAL THERAPISTS	659	603
185	DESIGNERS	2,201	1,467
189	PHOTOGRAPHERS	225	44
203	CLINICAL LABORATORY TECHNOLOGISTS AND TECHNICIANS	18,784	14,811
206	RADIOLOGIC TECHNICIANS	4,502	3,365
207	LICENSED PRACTICAL NURSES	1,342	1,342
208	HEALTH TECHNOLOGISTS AND TECHNICIANS, N.E.C.	3,375	2,764
213	ELECTRICAL AND ELECTRONIC TECHNICIANS	77	
214	INDUSTRIAL ENGINEERING TECHNICIANS	34	
216	ENGINEERING TECHNICIANS, N.E.C.	4,145	2,685
223	BIOLOGICAL TECHNICIANS	3,970	1,554

224	CHEMICAL TECHNICIANS	4,749	2,156
225	SCIENCE TECHNICIANS, N.E.C.	1,803	401
235	TECHNICIANS, N.E.C.	512	183
323	INFORMATION CLERKS, N.E.C.	21	
335	FILE CLERKS	376	30
343	COST AND RATE CLERKS	9	9
364	TRAFFIC, SHIPPING, AND RECEIVING CLERKS	3,323	365
365	STOCK AND INVENTORY CLERKS	561	468
368	WEIGHERS, MEASURERS, AND CHECKERS	239	
435	WAITERS AND WAITRESSES	1,438	1,233
436	COOKS, EXCEPT SHORT ORDER	1,345	192
444	MISCELLANEOUS FOOD PREPARATION OCCUPATIONS	1,929	1,314
446	HEALTH AIDES, EXCEPT NURSING	5,825	3,651
447	NURSING AIDES, ORDERLIES, AND ATTENDANTS	7,105	4,530
449	MAIDS AND HOUSEMEN	4,242	2,178
453	JANITORS AND CLEANERS	16,614	1,725
455	PEST CONTROL OCCUPATIONS	11,665	574
458	HAIRDRESSERS AND COSMETOLOGISTS	5,900	5,446

469	PERSONAL SERVICE OCCUPATIONS, N.E.C.	601	221
486	GROUNDSKEEPERS AND GARDENERS, EXCEPT FARM	4,784	378
487	ANIMAL CARETAKERS, EXCEPT FARM	1,734	1,395
508	AIRCRAFT ENGINE MECHANICS	6,707	57
518	INDUSTRIAL MACHINERY REPAIRERS	587	
535	CAMERA, WATCH, AND MUSICAL INSTRUMENT REPAIRERS	23	
547	SPECIFIED MECHANICS AND REPAIRERS, N.E.C.	1,227	
549	NOT SPECIFIED MECHANICS AND REPAIRERS	1,543	
558	SUPERVISORS, N.E.C.	240	
563	BRICKMASONS AND STONEMASONS	17	
567	CARPENTERS	149	
575	ELECTRICIANS	91	
579	PAINTERS, CONSTRUCTION AND MAINTENANCE	31	
584	PLASTERERS	2,134	
585	PLUMBERS, PIPEFITTERS, AND STEAMFITTERS	1,974	
599	CONSTRUCTION TRADES, N.E.C.	685	
633	SUPERVISORS, PRODUCTION OCCUPATIONS	1,872	447

637	MACHINISTS	1,590	
643	BOILERMAKERS	35	
667	TAILORS	168	112
675	HAND MOLDERS AND SHAPERS, EXCEPT JEWELERS	403	
678	DENTAL LABORATORY AND MEDICAL APPLIANCE TECHNICIANS	667	21
679	BOOKBINDERS	416	29
686	BUTCHERS AND MEAT CUTTERS	2,214	374
687	BAKERS	6,358	740
688	FOOD BATCHMAKERS	8,665	5,510
695	POWER PLANT OPERATORS	175	
696	STATIONARY ENGINEERS	488	
699	MISCELLANEOUS PLANT AND SYSTEM OPERATORS	829	
709	GRINDING, ABRADING, BUFFING, AND POLISHING MACHINE OPERATORS	955	
719	MOLDING AND CASTING MACHINE OPERATORS	1,317	94
723	METAL PLATING MACHINE OPERATORS	51	26
724	HEAT TREATING EQUIPMENT OPERATORS	524	
734	PRINTING MACHINE OPERATORS	2,103	230
735	PHOTOENGRAVERS AND LITHOGRAPHERS	386	

737	MISCELLANEOUS PRINTING MACHINE OPERATORS	1,618	29
744	TEXTILE SEWING MACHINE OPERATORS	15,552	15,552
749	MISCELLANEOUS TEXTILE MACHINE OPERATORS	2,361	238
753	CEMENTING AND GLUING MACHINE OPERATORS	424	34
754	PACKAGING AND FILLING MACHINE OPERATORS	14,443	3,025
755	EXTRUDING AND FORMING MACHINE OPERATORS	318	68
756	MIXING AND BLENDING MACHINE OPERATORS	23,137	1,599
757	SEPARATING, FILTERING, AND CLARIFYING MACHINE OPERATORS	1,784	
759	PAINTING AND PAINT SPRAYING MACHINE OPERATORS	1,893	406
764	WASHING, CLEANING, AND PICKLING MACHINE OPERATORS	485	
765	FOLDING MACHINE OPERATORS	4,448	3,370
766	FURNACE, KILN, AND OVEN OPERATORS, EXC. FOOD	1,614	29
768	CRUSHING AND GRINDING MACHINE OPERATORS	907	64
769	SLICING AND CUTTING MACHINE OPERATORS	643	536
777	MISCELLANEOUS MACHINE OPERATORS, N.E.C.	7,951	3,005

779	MACHINE OPERATORS, NOT SPECIFIED	5,774	2,370
783	WELDERS AND CUTTERS	183	
785	ASSEMBLERS	2,040	143
795	MISCELLANEOUS HAND WORKING OCCUPATIONS	531	186
796	PRODUCTION INSPECTORS, CHECKERS, AND EXAMINERS	4,314	2,786
797	PRODUCTION TESTERS	130	68
798	PRODUCTION SAMPLERS AND WEIGHERS	111	
804	TRUCK DRIVERS, HEAVY	4,316	
856	INDUSTRIAL TRUCK AND TRACTOR EQUIPMENT OPERATORS	494	
859	MISCELLANEOUS MATERIAL MOVING EQUIPMENT OPERATORS	275	
869	CONSTRUCTION LABORERS	4,536	183
877	STOCK HANDLERS AND BAGGERS	1,086	
878	MACHINE FEEDERS AND OFFBEARERS	5,688	457
883	FREIGHT, STOCK, AND MATERIAL MOVERS, HAND, N.E.C.	425	
887	VEHICLE WASHERS AND EQUIPMENT CLEANERS	48	
888	HAND PACKERS AND PACKAGERS	14,554	11,539
889	LABORERS, EXCEPT CONSTRUCTION	9,745	809

TOTAL	368,912	169,962

*(1) The estimates for each occupation apply across the surveyed industries in which the agent was observed. Not all industries were surveyed, and not all agents were observed in all surveyed industries. (2) When using the estimates, standard errors associated with estimates should be considered. (3) Potential exposures to a chemical agent are categorized as actual (i.e., the surveyor observed the use of the specific agent) or tradename (i.e., the surveyor observed the use of a tradename product known to contain the specific agent). The estimates presented in the table combine both categories.

As taken from NIOSH, available at https://web.archive.org/web/20111028104042/http://www.cdc.gov/noes/noes2/81515occ.html

“Sucrose is used as a sweetener in foods and soft drinks, in the manufacture of syrups, in invert sugar, confectionery, preserves and jams, demulcent, pharmaceutical products, and caramel. Sucrose is also a chemical intermediate for detergents, emulsifying agents, and other sucrose derivatives. Sucrose is widespread in seeds, leaves, fruits, flowers and roots of plants, where it functions as an energy store for metabolism and as a carbon source for biosynthesis. The annual world production of sucrose is in excess of 90 million tons mainly from the juice of sugar cane (20%) and sugar beet (17%). In addition to its use as a sweetener, sucrose is used in food products as a preservative, antioxidant, moisture control agent, stabilizer and thickening agent.”

As taken from PubChem.

Permissible exposure limits for “particulates not otherwise regulated” (including sucrose):

Total dust – 10 mg/m3

Respirable fraction – 5 mg/m3

As taken from Cal/OSHA.

“Introduction: Sugars are major constituents and additives in traditional tobacco products, but little is known about their content or related toxins (formaldehyde, acetaldehyde, and acrolein) in electronic cigarette (e-cigarette) liquids. This study quantified levels of sugars and aldehydes in e-cigarette liquids across brands, flavors, and nicotine concentrations (n = 66).Methods: Unheated e-cigarette liquids were analyzed using liquid chromatography mass spectrometry and enzymatic test kits. Generalized linear models, Fisher's exact test, and Pearson's correlation coefficient assessed sugar, aldehyde, and nicotine concentration associations. Results: Glucose, fructose and sucrose levels exceeded the limits of quantification in 22%, 53% and 53% of the samples. Sucrose levels were significantly higher than glucose [$\chi2(1) = 85.9$, $p < .0001$] and fructose [$\chi2(1) = 10.6$, $p = .001$] levels. Formaldehyde, acetaldehyde, and acrolein levels exceeded the limits of quantification in 72%, 84%, and 75% of the samples. Acetaldehyde levels were significantly higher than formaldehyde [$\chi2(1) = 11.7$, $p = .0006$] and acrolein [$\chi2(1) = 119.5$, $p < .0001$] levels. Differences between nicotine-based and zero-nicotine labeled e-cigarette liquids were not statistically significant for sugars or aldehydes. We found significant correlations between formaldehyde and fructose (-0.22, $p = .004$) and sucrose (-0.25, $p = .002$) and acrolein and fructose (-0.26, $p = .0006$) and sucrose (-0.21, $p = .0006$). There were no significant

correlations between acetaldehyde and any of the sugars or any of the aldehydes and glucose. Conclusions: Sugars and related aldehydes were identified in unheated e-cigarette liquids and their composition may influence experimentation in naïve users and their potential toxicity. Implications:The data can inform the regulation of specific flavor constituents in tobacco products as a strategy to protect young people from using e-cigarettes, while balancing FDA's interest in how these emerging products could potentially benefit adult smokers who are seeking to safely quit cigarette smoking. The data can also be used to educate consumers about ingredients in products that may contain nicotine and inform future FDA regulatory policies related to product standards and accurate and comprehensible labeling of e-cigarette liquids." As taken from Fagan P et al. 2018. Nicotine Tob. Res. 20(8), 985-992. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29182761

"Insect-protected sugarcane that expresses Cry1Ab has been developed in Brazil. Analysis of trade information has shown that effectively all the sugarcane-derived Brazilian exports are raw or refined sugar and ethanol. The fact that raw and refined sugar are highly purified food ingredients, with no detectable transgenic protein, provides an interesting case study of a generalized safety assessment approach. In this study, both the theoretical protein intakes and safety assessments of Cry1Ab, Cry1Ac, NPTII, and Bar proteins used in insect-protected biotechnology crops were examined. The potential consumption of these proteins was examined using local market research data of average added sugar intakes in eight diverse and representative Brazilian raw and refined sugar export markets (Brazil, Canada, China, Indonesia, India, Japan, Russia, and the USA). The average sugar intakes, which ranged from 5.1 g of added sugar/person/day (India) to 126 g sugar/p/day (USA) were used to calculated possible human exposure. The theoretical protein intake estimates were carried out in the "Worst-case" scenario, assumed that 1 μg of newly-expressed protein is detected/g of raw or refined sugar; and the "Reasonable-case" scenario assumed 1 ng protein/g sugar. The "Worst-case" scenario was based on results of detailed studies of sugarcane processing in Brazil that showed that refined sugar contains less than 1 μg of total plant protein /g refined sugar. The "Reasonable-case" scenario was based on assumption that the expression levels in stalk of newly-expressed proteins were less than 0.1% of total stalk protein. Using these calculated protein intake values from the consumption of sugar, along with the accepted NOAEL levels of the four representative proteins we concluded that safety margins for the "Worst-case" scenario ranged from 6.9 × 105 to 5.9 × 107 and for the "Reasonable-case" scenario ranged from 6.9 × 108 to 5.9 × 1010. These safety margins are very high due to the extremely low possible exposures and the high NOAELs for these non-toxic proteins. This generalized approach to the safety assessment of highly purified food ingredients like sugar illustrates that sugar processed from Brazilian GM varieties are safe for consumption in representative markets globally." As taken from Kennedy RD et al. 2018. Front. Bioeng. Biotechnol. 6, 45. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29755976

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
furfural	86476630	14.21	117433747	14.57
1,4:3,6-dianhydro-alpha-D-glucopyranose	9746930	1.60	11987590	1.49
5-hydroxymethylfurfural	116650910	19.16	161950119	20.10
anhydro sugar	6258003	1.03	24229350	3.01
levoglucosan	329689990	54.16	388870326	48.26
1,6-anhydro-beta-D-glucofuranose	6735778	1.11	56899912	7.06
Total ion chromatogram	608747166	100	805794573	100

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

Ingredient CAS Number	Max. cig appln level (ppm)	Composition of pyrolysate Compound %	Max smoke (ug)
Sugar, white 57-50-1	2500	Hydroxymethylfurfurole (40.0) Furfural (32.3) Methylbenzenediol (2.4) Methyl furfural (1.9) Glycoaldehyde (1.8) Phenol (0.3) Benzene (0.2) Butanal? (0.1)	5,000 4,000 300 240 230 38 25 13

		2-Butanone? (0.1) Cresol (0.1) Styrene+? (0.1) Toluene (0.1)	13 13 13 13

Various pyrolysis conditions yielded products including acrolein, poly aromatic hydrocarbons, 5-hydroxymethylfurfural, furfural, furans, phenol and carbon monoxide (Kroller, 1967; Schlotzhauer, 1982, 1985, 1986; Bell,1966; Tomasik, 1989; Gilbert and Lindsey, 1957; Anonymous, 2001; Gager, 1971a,b). Some of the pyrolysis products have been identified as human or animal carcinogens.

“The relationship between cigarette blend sugar and acetaldehyde formed in its smoke is a matter of current regulatory interest. This paper provides a re-analysis of data from 83 European commercial cigarettes studied in the 1970s and more modern data on sugar levels and acetaldehyde yields from a series of 97 European commercial cigarettes containing both inherent sugar and in other cases inherent and added sugar. It also provides data from 65 experimental cigarette products made from single curing grades of tobacco, having a wide range of inherent sugar levels but no added sugar.

This study has shown that there is no relationship between acetaldehyde yields and blend sugar content even if a multivariate analysis is carried out taking into account Nicotine Free Dry Particulate Matter (NFDPM) as a co-factor. Such analyses should take into consideration each of the known contributory factors in order to avoid misleading conclusions.

No distinction was found between the mainstream acetaldehyde yields from dark air-cured, flue-cured or US blended style cigarettes irrespective of their sugar content after taking account of differences in NFDPM yields. Similarly, no distinction was found between mainstream acetaldehyde yields of cigarettes made from single grades of either flue-cured, sun-cured or air-cured tobaccos with no sugar added.

This work supports the conclusion that structural material in the tobacco plant is the main source of acetaldehyde in mainstream smoke after combustion during cigarette smoking.”

As taken from Cahours X et al. 2012. Beiträge zur Tabakforschung International 25(2), 381–395. Available at http://www.degruyter.com/view/j/cttr.2012.25.issue-2/cttr-2013-0917/cttr-2013-0917.xml?rskey=vlZjPi&result=6

“All the evidence obtained in our laboratories has shown that the total aldehyde yield in tobacco smoke is not related to either sugar content or the equilibrium moisture content of the tobaccos. There is, however, a relationship between particulate matter [PM(WNF)]+ and aldehyde delivery. This accounts for some 41 % of the total variation between different cigarettes. + PM(WMF) = Total particulate matter - (water + nicotine)”. As taken from Phillpotts DF et al. 1975. Beiträge zur Tabakforschung International 8(1), 7–10. Available at http://www.degruyter.com/view/j/cttr.1975.8.issue-1/cttr-2013-0348/cttr-2013-0348.xml?rskey=vlZjPi&result=7

“A series of cigarettes made from Burley tobacco containing different levels of added reducing sugar (10.5 to 17.8 %) have been examined. Compared to the control cigarette there was virtually no change in the deliveries of aldehydes and carbonyl constituents. However, an increase in the delivery of 2-furfural was observed, especially when fructose

was the added sugar: even so, the conversion efficiency was only 1-2 %. A similar increase in the delivery of 2-furfural was also observed when glucose was added to flue-cured tobacco. An additional finding was that the addition of glucose and fructose reduced the delivery of nicotine. Radioactivity balance experiments on flue-cured cigarettes with added glucose indicated that this was probably due to an increase in the nicotine filtration efficiency of the cigarette rod. Filtration studies using air-cured cigarettes demonstrated that, on addition of glucose, there was a significant increase in the nicotine filtration efficiency of the tobacco rod and that less of the available nicotine was directed into the mainstream." As taken from Thornton RE and Massey SR. 1975. Beiträge zur Tabakforschung International 8(1), 11–15. Available at http://www.degruyter.com/view/j/cttr.1975.8.issue-1/cttr-2013-0349/cttr-2013-0349.xml?rskey=vlZjPi&result=8

"A rapid, semi-micropyrolysis technique was developed and applied to materials representative of tobacco cell-wall constituents and sucrose. Glass capillary gas chromatography - mass spectrometry was used to separate and identify the major semi-volatile pyrolyzate components. Cellulose and dextrin produced a pattern of furan and cyclic ketones of potential importance to flavour and aroma of tobacco smoke. Sucrose pyrolysis resulted in the formation of substantial amounts of 2-furaldehyde and lesser quantities of substituted furans. The cell-wall biopolymer lignin was a source of phenols, but contributed little to the compounds produced in the thermal breakdown of carbohydrates." As taken from Schlotzhauer WS et al. 1985. Beiträge zur Tabakforschung International 13(2), 74–80. Available at http://www.degruyter.com/view/j/cttr.1985.13.issue-2/cttr-2013-0558/cttr-2013-0558.xml?rskey=vlZjPi&result=11

"Additives used in tobacco product manufacturing are currently in the focus of public discussions with regard to potentially increased consumer health risks on account of certain additives. In addition, a few additives are suspected to enhance the addictiveness of tobacco products. In 2006, the German Federal Ministry for Food, Agriculture and Consumer Protection (BundesministeriumfuerErnaehrung, Landwirtschaft und Verbraucherschutz, BMELV) commissioned a research project intended to provide support for the evaluation of additives and their influence on the composition and properties of cigarette mainstream smoke. In this paper the results of the study are reported. Different amounts of glycerol, cocoa powder and sucrose were added to the tobacco of two kinds of filter-ventilated King size test cigarettes with 'tar' levels of 6 mg and 10 mg per cigarette. The tobacco of the test cigarettes consisted of a commercially available blend made of Virginia, Burley and Oriental tobaccos. Machine smoking was performed according to the applicable ISO smoking regimen. Various smoke components, which are suspected to be harmful for health, were determined in mainstream smoke. Increasing levels of sucrose were correlated with an increase of the amount of formaldehyde but not of acetaldehyde in the mainstream smoke of the test cigarettes. In cigarettes with different levels of added glycerol no substantial change in smoke composition was observed. The addition of cocoa powder to tobacco resulted in a decrease of tobaccospecific N-nitrosamines in mainstream smoke. The results obtained in this study can be used as evidence for the toxicological evaluation aimed at approving or banning specific additives for tobacco product manufacturing." As taken from Hahn J and Schaub J. 2010. Beiträge zur Tabakforschung International 24(3), 100–116.Available at http://www.degruyter.com/view/j/cttr.2010.24.issue-3/cttr-2013-0889/cttr-2013-0889.xml?rskey=vlZjPi&result=18

“According to European legislation, tobacco additives may not increase the toxicity or the addictive potency of the product, but there is an ongoing debate on how to reliably characterize and measure such properties. Further, too little is known on pyrolysis patterns of tobacco additives to assume that no additional toxicological risks need to be suspected. An on-line pyrolysis technique was used and coupled to gas chromatography-mass spectrometry (GC/MS) to identify the pattern of chemical species formed upon thermal decomposition of 19 different tobacco additives like raw cane sugar, licorice or cocoa. To simulate the combustion of a cigarette it was necessary to perform pyrolysis at inert conditions as well as under oxygen supply. All individual additives were pyrolyzed under inert or oxidative conditions at 350, 700 and 1000°C, respectively, and the formation of different toxicants was monitored. We observed the generation of vinyl acrylate, fumaronitrile, methacrylic anhydride, isobutyric anhydride and 3-buten-2-ol exclusively during pyrolysis of tobacco additives. According to the literature, these toxicants so far remained undetectable in tobacco or tobacco smoke. Further, the formation of 20 selected polycyclic aromatic hydrocarbons (PAHs) with molecular weights of up to 278Da was monitored during pyrolysis of cocoa in a semi-quantitative approach. It was shown that the adding of cocoa to tobacco had no influence on the relative amounts of the PAHs formed.” As taken from Paschke M et al. 2016. Int. J. Hyg. Environ. Health 219(8), 780-791. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27622657

2.3. Ingredient(s) from which it originates

In tobacco naturally (Stedman 1968).

Sucrose is obtained by crystallization from sugar cane or sugar beet juice that has been extracted by pressing or diffusion, then clarified and evaporated (CIR, 2014).

Sucrose is the most abundant carbohydrate in the sap of land plants (CIR, 2014).

“A nonreducing disaccharide composed of GLUCOSE and FRUCTOSE linked via their anomeric carbons.”

As taken from ChemIDplus.

“Sucrose as a disaccharide (household sugar from sugar beets or sugar cane) contains the two connected components glucose and fructose in a one to-one ratio in crystalline form.”

As taken from BfR, 2018.

3. Status in legislation and other official guidance

Food	UK: Yes; EU:No; US: 21CFR 184.1854
ADI	Not listed
Codex Alim.	Not listed
C of E no.	Not listed
FEMA no.	Not listed
Cosmetics (UK)	Not listed in Schedule 1

ACGIH TLV: TWA 10 mg/m(3) (Carcinogenicity category A4 – not classifiable as a human carcinogen; TLV basis – dental erosion).

OSHA PELS: TWA 15 mg/m(3) (total dust); 5 mg/m(3) (respirable fraction).

NIOSH RELs: TWA 10 mg/m(3) (total dust); 5 mg/m(3) (respirable fraction).

As taken from ACGIH, 2019a & b.

Sucrose is listed in the US EPAInert Finder Database (2019) as approved for food and non-food use pesticide products. For food use, it is regulated under 40 CFR Part 180.950a (Tolerances and Exemptions for Pesticide Chemical Residues in Food. Tolerance exemptions for minimal risk active and inert ingredients) (US EPA, 2019a).

FDA Requirements:

Sucrose is included on the US FDA's inventory of "Substances Added to Food (formerly EAFUS)" as a nutritive sweetener and is a direct food substance affirmed as generally recognized as safe (GRAS) (21 CFR 184.1854).

Also covered under 21 CFR sections 100.130; 101.4; 101.80; 101.9; 131.112; 131.170; 131.200; 131.203; 131.206; 133.124; 133.178; 133.179; 145.134; 145.180; 145.3; 146.140; 146.141; 146.145; 146.146; 146.3; 150.160; 155.170; 155.200; 169.175; 172.810; 172.816; 172.859; 172.861; 172.880; 172.884; 173.145; 73.85.

As taken from FDA, 2019a,b.

The HSE has set a long-term exposure limit (8-hr TWA) of 10 mg/m(3) and a short-term exposure limit (15 minute) of 20 mg/m(3) (HSE, 2018).

Exposure Limits:

NIOSH REL: TWA 10 mg/m(3) (total) TWA 5 mg/m(3) (resp)
OSHA PEL: TWA 15 mg/m(3) (total) TWA 5 mg/m(3) (resp)

As taken from NIOSH, 2018.

OCCUPATIONAL EXPOSURE LIMITS:

OEL-BELGIUM: TWA 10 mg/m3, MAR2002

OEL-FRANCE: VME 10 mg/m3, FEB2006

OEL-KOREA: TWA 10 mg/m3, 2006

OEL-MEXICO: TWA 10 mg/m3;STEL 20 mg/m3, 2004

OEL-THE NETHERLANDS: MAC-TGG 10 mg/m3, 2003

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

OEL-PERU: TWA 10 mg/m3, JUL2005

OEL-UNITED KINGDOM: TWA 10 mg/m3;STEL 20 mg/m3, OCT2007

OEL IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN check ACGIH TLV;

OEL IN SINGAPORE, VIETNAM check ACGIH TLV

As taken from RTECS, 2018.

US Army Military exposure guidelines (MEGs) for Short-Term exposures to chemicals in ambient air:

1 hour Critical Air MEG	5.0E+02 mg/m3
1 hour Marginal Air MEG	5.0E+01 mg/m3
1 hr Negligible air MEG	3.0E+01 mg/m3
14 day Negligible air MEG	2.4E+00 mg/m3
8 hour Negligible air MEG	1.0E+01 mg/m3
Potential health effects	cough , irrit eyes , irrit skin , irrit upper resp sys
Target Organs	Lungs

US Army Military exposure guidelines (MEGs) for Long-Term exposures to chemicals in ambient air:

1 year Negligible air MEG	2.40E+00 mg/m3
Potential health effects	cough , irrit eyes , irrit skin , irrit upper resp sys
Target Organs	Lungs

As taken from the US EPA ACToR database, 2015.

Substance	Sucrose

CAS No.	57-50-1			
	Limit value - Eight hours		Limit value - Short term	
	ppm	mg/m³	ppm	mg/m³
Australia		10 (1)		
Belgium		10		
Canada - Ontario		10		
Canada - Québec		10		
France		10		
Ireland		10		20 (1)
New Zealand		10 (1)		
Singapore		10		
South Korea		10		
Spain		10		
USA - NIOSH		10 total dust		
		5 respirable fraction		
USA - OSHA		15 inhalable aerosol		
		5 respirable aerosol		
United Kingdom		10		20
	Remarks			

Australia	(1) This value is for inhalable dust containing no asbestos and < 1% crystalline silica.
Ireland	(1) 15 minutes reference period
New Zealand	(1) The value for inhalable dust containing no asbestos and less than 1% free silica

Substance	Dust, sugar			
CAS No.				
	Limit value - Eight hours		Limit value - Short term	
	ppm	mg/m³	ppm	mg/m³
Latvia		5		

As taken from GESTIS, 2018. Available at: http://limitvalue.ifa.dguv.de/

Sucrose is pre-registered under REACH (“envisaged registration deadline 30 November 2010”) (ECHA, 2018).

Sucrose is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2019).

Listed as a fragrance ingredient by IFRA (2016).

Sucrose is listed in the European Union inventory of cosmetic ingredients and REACH Annex IV. Annex IV “sets out substances that are exempted from the registration, evaluation and downstream user provisions of REACH as sufficient information is known about these substances that they are considered to cause minimum risk because of their intrinsic properties.” The CIR Expert Panel concluded that sucrose is safe in the present practices of use and concentration in cosmetics.

Purity specifications:

Food use: NMT 1 mg/kg arsenic; NMT 0.1 mg/kg lead; NMT 0.1% invert sugars; NMT 0.15% residue on ignition (sulfated ash); NMT 0.1% loss on drying

USP: NMT 5 ppm heavy metals; NMT 0.05% residue on ignition

As taken from CIR, 2014.

Sucrose is included on the US EPA’s list of Safer Chemical Ingredients (US EPA, 2019b).

Sucrose is listed in the US EPA Toxic Substances Control Act (TSCA) inventory, and also in the US EPA 2012 CDR and 2016 CDR Partial Exempt lists (Chemical Data Reporting Rule).

The Chemical Data Reporting (CDR) Rule requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR.

The TSCA inventory, and 2012 CDR and 2016 CDR Partial Exempt lists are available at https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

alpha.-D-Glucopyranoside, .beta.-D-fructofuranosyl (CAS RN 57-50-1) is listed in the New Zealand Inventory of Chemicals and may be used as a single component chemical under an appropriate group standard (NZ EPA, 2006).

“EFSA will provide scientific advice on the daily intake of added sugar in food by early 2020. The Authority aims to establish a science-based cut-off value for daily exposure to added sugars from all sources which is not associated with adverse health effects. The work will be carried out following a request from Denmark, Finland, Iceland, Norway and Sweden.

Added sugars from all sources comprise sucrose, fructose, glucose, starch hydrolysates such as glucose syrup, high-fructose syrup, and other sugar preparations consumed as such or added during food preparation and manufacturing.

The adverse health effects under consideration will include body weight, glucose intolerance and insulin sensitivity, type-2-diabetes, cardiovascular risk factors, as well as dental caries. In its assessment, EFSA will look at the general healthy population, including children, adolescents, adults and the elderly.

The advice will guide Member States when establishing recommendations for the consumption of added sugars and in planning food-based dietary guidelines.”

As taken from EFSA, 2017.

Guideline	Target population	Sugar fraction	Recommendation	Basis (endpoint)	Other endpoints assessed	Review method
EFSA, 2010	General population	Added sugars	Consider when setting FBDGs	Dental caries Body weight Micronutrient density	Glucose homeostasis, risk of T2DM, blood lipids, blood pressure, CVD risk	Narrative
GNS, 2012	General population	SSBs	Limit consumption	Obesity Risk of T2DM	BP/hypertension, metabolic syndrome, CHD risk, cancer	Systematic
NNR, 2012	General population	Added sugars	<10E%	Micronutrient density	Dental caries (frequency of intake), weight gain and risk of T2DM (SSBs), glucose homeostasis, blood lipids, blood pressure, CVD risk, uric acid	Systematic

SACN, 2015	General population (>2 years)	Free sugars	≤ 5E%	Energy intake	Dental caries (frequency of intake), weight gain and risk of T2DM (SSBs), blood lipids, blood pressure, CHD, glucose homeostasis	Systematic
ANSES, 2016	Adults	Total sugars	100 g/day	Fasting triglycerides	Weight gain, glucose homeostasis, blood lipids, intrahepatic lipids and risk of NAFLD, uric acid, blood pressure	Systematic
IoM, 2002	General population	Added sugars	<25E%	Micronutrient density	CHD risk, energy intake, body weight, blood lipids, cancer	Narrative
DGA, 2015	General population	Added sugars	<10E%	Micronutrient density	-	Food pattern modelling and national data on added sugars intake
WHO, 2015	General population	Free sugars	<10E% <5E% conditional	Body weight Dental caries	-	Systematic
AHA, 2016	Children	Added sugars	25 g/day ≥ 2 years Avoided < 2 years	Energy intake Adiposity Dyslipidaemia CVD risk	Micronutrient density, blood pressure, risk of NAFLD, glucose homeostasis, risk of T2DM	Narrative
ESPGHAN, 2017	Children	Free sugars	≤ 5E% ≥ 2 years (lower for < 2 years)	Dental caries Weight gain (SSBs) CVD and T2DM (fructose)	Preference for sweet taste	Narrative/ systematic

CVD: cardiovascular disease

CHD: coronary heart disease

E: energy intake

FBDG: food-based dietary guidelines

SSB: sugar-sweetened beverage

T2DM: type 2 diabetes mellitus

As taken from EFSA, 2018.

Excipients and information for the package leaflet:

Name	Route of administration	Threshold	Information for the package leaflet	Comments
Sucrose	Oral	Zero	If you have been told by your doctor that you have an intolerance to some sugars, contact your doctor before taking this medicinal product.	SmPC proposal: Patients with rare glucose-galactose malabsorption should not take this medicine.
Sucrose	Oral	5 g	Contains x g sucrose per dose. This should be taken into account in patients with diabetes mellitus.	
Sucrose	Oral liquids, lozenges and chewable tablets	Zero	May be harmful to the teeth.	Information to be included only when the medicinal product may be intended for chronic use, e.g. for two weeks or more.

As taken from EMA, 2017.

Sucrose is listed by the US EPA Office of Pesticide Programs (2019) and was first registered as an antimicrobial pesticide on 25 April 2002. In December 2017, pursuant to 40 CFR section 155.50, the US EPA formally initiated a registration review for sucrose and made the following decision: "…the Agency has made the following Proposed Interim Decision: (1) no additional data are required at this time; and (2) no change to the affected registration and its label are needed at this time. In this proposed interim decision, the Agency is making no human health or environmental safety findings associated with the EDSP screening of sucrose. The Agency's final registration review decision for sucrose will be made following completion of an EDSP FFDCA §408(p) determination" (US EPA, 2017).

Sucrose (CAS RN 57-50-1) is "not considered to pose an unreasonable risk to the health of workers and public health on the basis of the Tier I IMAP assessment" and has been "identified as low concern to human health by application of expert validated rules" (NICNAS, 2018).

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

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose
SUCROSE	BUCCAL	TABLET	57501	C151H8M554	16.6MG
SUCROSE	BUCCAL/SUBLINGUAL	TABLET	57501	C151H8M554	91MG
SUCROSE	INTRAMUSCULAR	POWDER, FOR INJECTION SOLUTION, LYOPHILIZED	57501	C151H8M554	7%
SUCROSE	INTRAMUSCULAR	SOLUTION, INJECTION	57501	C151H8M554	7.29%W/V
SUCROSE	INTRAVENOUS	INJECTION	57501	C151H8M554	19.5%
SUCROSE	INTRAVENOUS	INJECTION, EMULSION	57501	C151H8M554	54MG/1ML
SUCROSE	INTRAVENOUS	INJECTION, POWDER, LYOPHILIZED, FOR LIPOSOMAL SUSPENSION	57501	C151H8M554	90%
SUCROSE	INTRAVENOUS	INJECTION, SUSPENSION, LIPOSOMAL	57501	C151H8M554	9.4%
SUCROSE	INTRAVENOUS	POWDER, FOR INJECTION SOLUTION	57501	C151H8M554	20%
SUCROSE	INTRAVENOUS	POWDER, FOR RECONSTITUTION	57501	C151H8M554	40%
SUCROSE	INTRAVENOUS	SOLUTION, INJECTION	57501	C151H8M554	NA
SUCROSE	INTRAVENOUS	SOLUTION, LIPOSOME, INJECTION	57501	C151H8M554	8.5%
SUCROSE	IV(INFUSION)	INJECTION, LIPOSOMAL	57501	C151H8M554	1%

SUCROSE	IV(INFUSION)	POWDER, FOR INJECTION SOLUTION, LYOPHILIZED	57501	C151H8M554	30.59%W/W
SUCROSE	ORAL	CAPSULE	57501	C151H8M554	527.43MG
SUCROSE	ORAL	CAPSULE, DELAYED ACTION	57501	C151H8M554	175.14MG
SUCROSE	ORAL	CAPSULE, DELAYED RELEASE	57501	C151H8M554	48MG
SUCROSE	ORAL	CAPSULE, ENTERIC COATED PELLETS	57501	C151H8M554	140.76MG
SUCROSE	ORAL	CAPSULE, EXTENDED RELEASE	57501	C151H8M554	396.14MG
SUCROSE	ORAL	CAPSULE, HARD GELATIN	57501	C151H8M554	NA
SUCROSE	ORAL	CAPSULE, SUSTAINED ACTION	57501	C151H8M554	481.7MG
SUCROSE	ORAL	CONCENTRATE	57501	C151H8M554	4250MG/5ML
SUCROSE	ORAL	DROPS	57501	C151H8M554	755.5MG/2.5ML
SUCROSE	ORAL	EMULSION	57501	C151H8M554	NA
SUCROSE	ORAL	FOR SUSPENSION	57501	C151H8M554	3041MG/5ML
SUCROSE	ORAL	GRANULE	57501	C151H8M554	3024.2MG
SUCROSE	ORAL	GRANULE, FOR ORAL SOLUTION	57501	C151H8M554	1774.84MG/5ML

SUCROSE	ORAL	GRANULE, FOR ORAL SUSPENSION	57501	C151H8M554	11MG
SUCROSE	ORAL	GRANULE, FOR RECONSTITUTION	57501	C151H8M554	2669.8MG
SUCROSE	ORAL	GRANULE, FOR SUSPENSION	57501	C151H8M554	2942.7MG
SUCROSE	ORAL	GRANULE, FOR SUSPENSION, EXTENDED RELEASE	57501	C151H8M554	NA
SUCROSE	ORAL	LIQUID	57501	C151H8M554	6.25g/10mL
SUCROSE	ORAL	LIQUID	57501	C151H8M554	720MG/1ML
SUCROSE	ORAL	PASTILLE	57501	C151H8M554	426MG
SUCROSE	ORAL	POWDER	57501	C151H8M554	4187MG/1PKT
SUCROSE	ORAL	POWDER	57501	C151H8M554	4187MG/1SCP
SUCROSE	ORAL	POWDER, FOR ORAL SOLUTION	57501	C151H8M554	1933.3MG
SUCROSE	ORAL	POWDER, FOR ORAL SUSPENSION	57501	C151H8M554	1195.44MG/5ML
SUCROSE	ORAL	POWDER, FOR ORAL SUSPENSION	57501	C151H8M554	3643MG/1PKT
SUCROSE	ORAL	POWDER, FOR ORAL SUSPENSION	57501	C151H8M554	3643MG/1SCP
SUCROSE	ORAL	POWDER, FOR ORAL SUSPENSION	57501	C151H8M554	4636mg

SUCROSE	ORAL	POWDER, FOR RECONSTITUTION	57501	C151H8M554	2322.79MG/5ML
SUCROSE	ORAL	POWDER, FOR SOLUTION	57501	C151H8M554	1892.86MG/2GM
SUCROSE	ORAL	POWDER, FOR SUSPENSION	57501	C151H8M554	166MG/5ML
SUCROSE	ORAL	POWDER, FOR SUSPENSION	57501	C151H8M554	9700MG
SUCROSE	ORAL	SOLUTION	57501	C151H8M554	NA
SUCROSE	ORAL	SOLUTION	57501	C151H8M554	30.51%W/W
SUCROSE	ORAL	SOLUTION	57501	C151H8M554	2255MG/5ML
SUCROSE	ORAL	SOLUTION	57501	C151H8M554	21000MG/30ML
SUCROSE	ORAL	SOLUTION, ELIXIR	57501	C151H8M554	2600MG/5ML
SUCROSE	ORAL	SOLUTION, SYRUP	57501	C151H8M554	2255MG/5ML
SUCROSE	ORAL	SUSPENSION	57501	C151H8M554	44.44%W/V
SUCROSE	ORAL	SUSPENSION	57501	C151H8M554	375mg/1.25mL
SUCROSE	ORAL	SUSPENSION	57501	C151H8M554	952.42MG/5 ML
SUCROSE	ORAL	SUSPENSION	57501	C151H8M554	8500MG/5ML

SUCROSE	ORAL	SUSPENSION, DROPS	57501	C151H8M554	1250MG/2.5ML
SUCROSE	ORAL	SUSPENSION, EXTENDED RELEASE	57501	C151H8M554	0.8MG/ML
SUCROSE	ORAL	SUSPENSION, EXTENDED RELEASE	57501	C151H8M554	1350MG/5ML
SUCROSE	ORAL	SUSPENSION, LIQUID	57501	C151H8M554	1750MG/5ML
SUCROSE	ORAL	SUSPENSION, SUSTAINED ACTION	57501	C151H8M554	600MG/5ML
SUCROSE	ORAL	SUSPENSION, SUSTAINED ACTION	57501	C151H8M554	2323.3MG/1PKT
SUCROSE	ORAL	SUSPENSION, SYRUP, SUSTAINED ACTION	57501	C151H8M554	10%
SUCROSE	ORAL	SYRUP	57501	C151H8M554	2.9g/5mL
SUCROSE	ORAL	SYRUP	57501	C151H8M554	85%
SUCROSE	ORAL	TABLET	57501	C151H8M554	9700MG
SUCROSE	ORAL	TABLET (IMMED./COMP. RELEASE), UNCOATED, CHEWABLE	57501	C151H8M554	2400MG
SUCROSE	ORAL	TABLET, CHEWABLE	57501	C151H8M554	14.78MG
SUCROSE	ORAL	TABLET, COATED	57501	C151H8M554	400MG
SUCROSE	ORAL	TABLET, DELAYED ACTION, ENTERIC COATED	57501	C151H8M554	279.5MG

SUCROSE	ORAL	TABLET, DELAYED RELEASE	57501	C151H8M554	33.5MG
SUCROSE	ORAL	TABLET, EXTENDED RELEASE	57501	C151H8M554	185.07MG
SUCROSE	ORAL	TABLET, FILM COATED	57501	C151H8M554	200MG
SUCROSE	ORAL	TABLET, REPEAT ACTION	57501	C151H8M554	129.55MG
SUCROSE	ORAL	TABLET, SUGAR COATED	57501	C151H8M554	73.18MG
SUCROSE	ORAL	TABLET, SUSTAINED ACTION	57501	C151H8M554	284.54MG
SUCROSE	ORAL	TABLET, SUSTAINED ACTION, FILM COATED	57501	C151H8M554	119.12MG
SUCROSE	ORAL	TABLET, UNCOATED, LOZENGE	57501	C151H8M554	1255MG
SUCROSE	ORAL	TABLET, UNCOATED, TROCHE	57501	C151H8M554	NA
SUCROSE	ORAL	WAFER	57501	C151H8M554	NA
SUCROSE	RECTAL	LIQUID	57501	C151H8M554	NA
SUCROSE	RECTAL	SOLUTION	57501	C151H8M554	35%
SUCROSE	SUBCUTANEOUS	INJECTION	57501	C151H8M554	17%
SUCROSE	SUBCUTANEOUS	INJECTION, SUSPENSION, EXTENDED RELEASE	57501	C151H8M554	0.8MG/0.85 ML

SUCROSE	SUBCUTANEOUS	MICROCAPSULES FOR INJECTION SUSPENSION, STERILE	57501	C151H8M554	0%
SUCROSE	SUBCUTANEOUS	POWDER, FOR INJECTION SOLUTION, LYOPHILIZED	57501	C151H8M554	8%
SUCROSE	SUBCUTANEOUS	POWDER, FOR INJECTION SOLUTION, LYOPHILIZED, WITH ADDITIVES	57501	C151H8M554	4.1%
SUCROSE	SUBCUTANEOUS	SOLUTION, INJECTION	57501	C151H8M554	7%
SUCROSE	SUBLINGUAL	TABLET	57501	C151H8M554	17MG
SUCROSE	TOPICAL	OINTMENT	57501	C151H8M554	20%W/W
SUCROSE	TRANSMUCOSAL	TABLET, UNCOATED, LOZENGE	57501	C151H8M554	100.35MG

As taken from FDA (2019c).

“Consumers should ensure that their daily intake of added sugar does not exceed 10 % of their total daily intake of energy from food, including beverages. The consumption of added sugar should be even lower if possible. Therefore, an adult with energy requirements of approximately 2000 kilocalories should not consume more than 6 - 12 teaspoons of added sugar per day from all food, including beverages.”

As taken from BfR, 2018.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

ScrB (Cg2927) is a sucrose-6-phosphate hydrolase essential for sucrose utilization by Corynebacterium glutamicum (Abstract). Corynebacterium glutamicum can grow on a variety of carbohydrates from which glucose, fructose and sucrose are taken up and

phosphorylated by the phosphoenolpyruvate-dependent phosphotransferase system (PTS). Here, we show that cg2927 (scrB) encodes sucrose-6-phosphate hydrolase. The purified His-tagged protein hydrolyzed sucrose-6-phosphate and sucrose, but not sucrose-6'-phosphate. The Km value for sucrose was 190 mM while the Km for sucrose-6-phosphate was much lower, 0.04 mM. Sucrose-6-phosphate hydrolase activity was stimulated by MgSO4 and fructose-6-phosphate and was inhibited by MnCl2, CaCl2, CuSO4 and ZnSO4. A scrB deletion mutant could not grow on sucrose as the sole carbon source. In addition, growth in the absence of scrB was severely decreased when sucrose was present in addition to glucose, fructose or acetate, suggesting that higher intracellular concentrations of sucrose-6-phosphate are toxic. Transcriptional start sites in the cg2929-cg2928-scrB-ptsS locus could be revealed upstream of cg2929 and upstream of the sucrose-specific PTS gene ptsS. Of these, only ptsS showed increased expression when grown in the presence of sucrose, which was due to control by the transcriptional regulator SugR. The sucrose-6-phosphate hydrolase activity, however, was increased two- to threefold during growth in fructose- or sucrose-containing media, regardless of the presence or absence of SugR. As taken from Engels et al., FEMS Microbiol Lett. 2008, Dec; 289(1):80-9. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/19054097

Demonstration of a specific metabolic effect of dietary disaccharides in the rat (Abstract). Male Wistar rats were starved and refed diets containing either 40% carbohydrate as monosaccharides (glucose, fructose, invert sugar) or disaccharides (maltose, sucrose), or 42.2% carbohydrate as glucose. Induction of various liver enzymes and changes in total liver lipid levels by the different dietary sugars were studied. Liver enzymes measured included glucose-6-phosphate dehydrogenase (g6pd), 6-phosphogluconate dehydrogenase (6PGD), malic enzyme (ME), phosphofructokinase (PFK), L-alpha-glycerol phosphate dehydrogenase (LalphaGPD), pyruvate kinase (PK), citrate cleavage enzyme (CCE), acetyl CoA carboxylase (AcCoAC), and fatty acid synthetase (FAS). The responses in enzyme activity to diets containing glucose or invert sugar were used as the basal response. Enzyme responses to refeeding the carbohydrate diets fell into three categories: (1) enzyme activity increased both by the disaccharide configuration of the carbohydrate and by fructose (G6PD, PK, CCE, AcCoAC, FAS); (2) enzyme activity increased only by the disaccharide configuration of the carbohydrate (6PGD, ME); and (3) enzyme activity increased only by fructose (PFK, LalphaGPD). Total liver lipid level was increased both by the disaccharide configuration of the carbohydrate and by fructose. Refeeding diets containing equal molar amounts of glucose or maltose did not abolish the disaccharide effect. The data indicate that the disaccharide configuration of maltose and sucrose may have an effect at the gastrointestinal level, which causes an increased induction of certain enzymes in the liver. As taken from Michaelis OE 4th; et al. J Nutr. 1975, Sep; 105(9):1186-91. PubMed, 2010 available at

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=240012&dopt=AbstractPlus

“Sucrose …… enters intermediary metabolism and is eventually converted to CO2” (EFSA, 2012).

“Both controversy and confusion exist concerning fructose, sucrose, and high-fructose corn syrup (HFCS) with respect to their metabolism and health effects. These concerns have often been fueled by speculation based on limited data or animal studies. In retrospect, recent controversies arose when a scientific commentary was published suggesting a

possible unique link between HFCS consumption and obesity. Since then, a broad scientific consensus has emerged that there are no metabolic or endocrine response differences between HFCS and sucrose related to obesity or any other adverse health outcome. This equivalence is not surprising given that both of these sugars contain approximately equal amounts of fructose and glucose, contain the same number of calories, possess the same level of sweetness, and are absorbed identically through the gastrointestinal tract. Research comparing pure fructose with pure glucose, although interesting from a scientific point of view, has limited application to human nutrition given that neither is consumed to an appreciable degree in isolation in the human diet. Whether there is a link between fructose, HFCS, or sucrose and increased risk of heart disease, metabolic syndrome, or fatty infiltration of the liver or muscle remains in dispute with different studies using different methodologies arriving at different conclusions. Further randomized clinical trials are needed to resolve many of these issues. The purpose of this review is to summarize current knowledge about the metabolism, endocrine responses, and potential health effects of sucrose, HFCS, and fructose." As taken from Rippe JM & Angelopoulos TJ. 2013. Adv. Nutr. 4(2), 236-45. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23493540?dopt=AbstractPlus

4.2. Absorption, distribution and excretion

Sucrose urinary excretion in the rat measured using a simple assay: a model of gastroduodenal permeability (Abstract). PURPOSE: To develop a non-invasive animal model suitable for studies of altered gastroduodenal (GD) permeability, which is suggested to indicate GD damage; to validate a low cost and convenient assay for sucrose in urine, a permeability marker of GD.

METHODS: Control (n = 87) and treated male Sprague-Dawley rats were dosed orally with 1 g of sucrose. Urinary excretion of the sucrose (0-8 h) was measured indirectly by cleavage to glucose and subsequent measurement of glucose in urine using a calorimetric assay. Treated rats were administered single oral doses of 10 and 20 mg/kg indomethacin, or 42 mg/kg aspirin alone or with 0.5 mL 50% ethanol (n = 7 in each group).

RESULTS: The assay was linear within the examined range of 10-100 ug/mL sucrose. The inter and intraday variations were 7.63% and 6.89%, respectively. The urinary excretion of sucrose was complete in 8 h. In control rats the urinary excretion of sucrose exhibited a left skewed frequency distribution curve with a mean of 0.6 +/- 0.14% of the dose excreted. All treatment, with the exception of 10 mg/kg indomethacin significantly increased the GD permeability. The GD effect was found to be dose dependent and parallels those reported for humans. The rat is a suitable model for studies of GD permeability. Combined use of sucrose and 51Cr-EDTA, a marker of intestinal permeability, allows for non-invasive examination of abnormalities of the entire gut. The sucrose assay is convenient and cost effective.

CONCLUSIONS: The rat model may be useful in the preclinical screening of NSAID formulations and also in the detection of other GI abnormalities. As taken from Davies NM et al.

Pharm Res. 1995 Nov; 12(11):1733-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=8592678&query_hl=1&itool=pubmed_docsum

Urinary sucrose and fructose as biomarkers for sugar consumption (Abstract). The use of 24-hour urinary sucrose and fructose as potential biomarkers for sugars consumption was investigated in two studies of 21 healthy participants living in a volunteer suite where dietary intake was known and all specimens collected. The dose-response was assessed in 12 males using a randomized crossover design of three diets containing constant levels of 63, 143, and 264 g of sugars for 10 days each. Both sugars and sucrose intake were significantly correlated with the sum of sucrose and fructose concentration in urine (0.888; P < 0.001). To assess effects with volunteers consuming their habitual varying diets, seven males and six females were fed their usual diet (assessed beforehand from four consecutive self-completed 7-day food diaries) for 30 days under controlled conditions in the volunteer suite. The mean (+/-SD) calculated total sugars intake was 202 +/- 69 g/d, 41% from sucrose. Mean (+/-SD) urinary sucrose and fructose were 36.6 +/- 16.6 and 61.8 +/- 61.3 mg/d, respectively. The sum of sucrose and fructose in urine was significantly correlated with sugars (0.841; P < 0.001) and sucrose intake (0.773; P = 0.002). In the regression, 200 g of sugars intake predicted approximately 100 mg of sucrose and fructose in urine. The correlation between individual means of randomized 16 days of sugars intake and 8 days of sugars excretion data (as used in validation studies) remained as high as that obtained with the means of 30-day measurements and the regression estimates were very similar. Twenty-four-hour urinary sucrose and fructose could be grouped into a new category of biomarkers, predictive biomarkers, that can be used in studies determining the structure of dietary measurement error in free living individuals and to relate sugars intake to disease risk. As taken from Tasevska N et al. Cancer Epidemiol Biomarkers Prev. 2005 May; 14(5):1287-94. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=15894688&query_hl=4&itool=pubmed_docsum

The disaccharide effect of sucrose feeding on glucuronide excretion and bile concentration of injected phenolphthalein in guinea pigs (Abstract). The hypothesis tested was that feeding guinea pigs sucrose produces a more rapid concentration in the bile and excretion in the feces and urine of substances catabolized by the liver than does feeding invert sugar (50:50 mixture of glucose and fructose). Fifty male guinea pigs of the Hartley strain were divided into two groups of 25 animals each and fed for 4 wk repelleted nonpurified diet with 20% of total energy provided by sucrose or invert sugar. At the end of 4 wk all 50 animals were injected i.p. with a dose of 15 mg/kg body weight of phenolphthalein. Phenolphthalein is excreted almost quantitatively in feces. After injection all guinea pigs were housed in metabolism cages. Urine and feces were recovered and analyzed for free glucuronic acid and glucuronide content by a modified naphthoresorcinol procedure over 24 h. Guinea pigs fed sucrose produced more urine than those fed invert sugar, although there was no difference in water intake. After 24 h 15 animals in each group were killed, and the bile was sampled from their gall bladders to determine its phenolphthalein content. The remaining 10 animals in each group were held three additional days when they were killed and their bile was sampled to determine its phenolphthalein content. All biliary phenolphthalein was in conjugated form. Guinea pigs fed sucrose had less free glucuronic acid in their feces than those fed invert sugar. Feeding sucrose resulted in a higher bile conjugated phenolphthalein content 4 d after injection than

did feeding invert sugar. As taken from Ahrens RA et al. J Nutr. 1985, Feb; 115(2):288-91. PubMed, 2009 available at

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=3968593&dopt=AbstractPlus

“Sucrose …… is readily absorbed from the GI tract” (EFSA, 2012).

“Urinary sugars excretion has been proposed as a potential biomarker for intake of sugars. In this study, we compared 2 analytical methods (gas chromatography [GC] and enzymatic reactions-UV absorption) for quantifying urinary fructose and sucrose using 24-hour urine samples from a randomized crossover controlled feeding study. All samples were successfully quantified by the GC method; however, 21% and 1.9% of samples were below the detection limit of the enzymatic method for sucrose and fructose, respectively. Although the correlation between the 2 methods was good for fructose (Pearson correlation, 0.71), the correlation was weak for sucrose (Pearson correlation, 0.27). We favor the GC method because of its better sensitivity, simplicity, and the ability to quantify fructose and sucrose directly in the same run. Of the 106 samples from 53 participants with complete urine collection after 2 study diets, 24-hour urinary fructose excretion was significantly associated with fructose intake. The sum of 24-hour urinary fructose and sucrose was significantly associated with total sugars consumption. However, variation in intakes of sugars explained only a modest amount of variation in urinary sugars excretion. In the unadjusted models, fructose intake explained 24.3% of urinary fructose excretion, and intake of total sugars explained 16.3% of the sum of urinary fructose and sucrose. The adjusted models explained 44.3% of urinary fructose excretion and 41.7% of the sum of urinary fructose and sucrose. Therefore, we caution using these biomarkers to predict sugars consumption before other factors that determine urinary sugars excretion are understood.” As taken from Song X et al. 2013. Nutr. Res. 33(9), 696-703. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24034568

“BACKGROUND: Measurement error in self-reported sugars intake may be obscuring the association between sugars and cancer risk in nutritional epidemiologic studies. METHODS: We used 24-hour urinary sucrose and fructose as a predictive biomarker for total sugars, to assess measurement error in self-reported sugars intake. The Nutrition and Physical Activity Assessment Study (NPAAS) is a biomarker study within the Women's Health Initiative (WHI) Observational Study that includes 450 postmenopausal women ages 60 to 91 years. Food Frequency Questionnaires (FFQ), four-day food records (4DFR), and three 24-hour dietary recalls (24HRs) were collected along with sugars and energy dietary biomarkers. RESULTS: Using the biomarker, we found self-reported sugars to be substantially and roughly equally misreported across the FFQ, 4DFR, and 24HR. All instruments were associated with considerable intake- and person-specific bias. Three 24HRs would provide the least attenuated risk estimate for sugars (attenuation factor, AF = 0.57), followed by FFQ (AF = 0.48) and 4DFR (AF = 0.32), in studies of energy-adjusted sugars and disease risk. In calibration models, self-reports explained little variation in true intake (5%-6% for absolute sugars and 7%-18% for sugars density). Adding participants' characteristics somewhat improved the percentage variation explained (16%-18% for absolute sugars and 29%-40% for sugars density). CONCLUSIONS: None of the self-report instruments provided a good estimate of sugars intake, although overall 24HRs seemed to perform the best. IMPACT: Assuming the calibrated sugars biomarker is unbiased, this analysis suggests that measuring the biomarker in a subsample of the study population for

calibration purposes may be necessary for obtaining unbiased risk estimates in cancer association studies." As taken from Tasevska N et al. 2014a. Cancer Epidemiol Biomarkers Prev. 23(12), 2874-83. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25234237

"The substance can be absorbed into the body by inhalation and by ingestion."

As taken from PubChem.

4.3. Interactions

Moisture effects on protein-excipient interactions in spray-dried powders. Nature of destabilizing effects of sucrose (Abstract). The preparation of stable solid protein formulations presents significant challenges. Ultimately, the interactions between incorporated excipients and the pharmaceutical protein determine the formulation stability. In this study, moisture was utilized to probe the interactions between a model protein, trypsinogen, and sucrose in the solid state, following spray drying. Through investigation of the physical properties of the spray-dried formulations, we attempted to elucidate the mechanisms underlying the previously observed stabilizing and destabilizing effects of the carbohydrate during spray drying. Both dynamic and equilibrium moisture uptake studies indicated the presence of an optimal protein-sugar hydrogen bonding network. At low sucrose contents, a preferential protein-sucrose hydrogen bonding interaction was dominant, resulting in protein stabilization. However, at high carbohydrate concentrations, preferential sugar-sugar interactions prevailed, resulting in a phase separation within the formulation matrix. The preferential incorporation of the sucrose molecules in a sugar-rich phase reduced the actual amount of the carbohydrate available to interact with the protein and thereby decreased the number of effective protein-sucrose contacts. As a consequence, the protein could not be effectively protected during spray drying. We hypothesize that the observed phase separation at this sucrose concentration regime originates from its exclusion from the protein in solution before spray drying, further accompanied by preferential clustering of the sucrose molecules. As taken from Tzannis ST and Prestrelski SJ. J Pharm Sci. 1999, Mar; 88(3):360-70. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/10052996

"Hypertension remains a major health problem worldwide considering the prevalence of morbidity and mortality. Plants remain a reliable source of efficacious and better tolerated drugs and botanicals. This study was designed to investigate the effect of the chemo-profiled hydroethanolic leaf extract of Byrsocarpus coccineus in ethanol- and sucrose-induced hypertension. Groups of rats were treated orally (p.o.) with distilled water (10 ml/kg), ethanol (35%; 3 g/kg), sucrose (5-7%), and B. coccineus (100, 200, and 400 mg/kg), and nifedipine together with ethanol and sucrose separately for 8 weeks. At the end of the treatment period, blood pressure and heart rate of rats were determined. Blood was collected for serum biochemical parameters and lipid profile assessment, and the liver, aorta, kidney, and heart were harvested for estimation of in vivo antioxidants and malondialdehyde (MDA). Results obtained in this study showed that B. coccineus at the various doses administered reduced the systolic, diastolic, and arterial blood pressure

elevated by ethanol and sucrose. Also, the extract reversed the reduction in catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD) induced by ethanol and sucrose. The level of MDA was reduced compared to the ethanol- and sucrose-induced hypertensive group. With respect to lipid profile, administration of B. coccineus at the various doses reduced the levels of triglycerides, low-density lipoprotein (LDL), cholesterol, and atherogenic indices, compared to the ethanol and sucrose groups. In conclusion the hydroethanolic leaf extract of B. coccineus exerted significant antihypertensive effect and this is probably related to the antioxidant property and improvement of lipid profile observed in this study." As taken from Akindele AJ et al. 2014. J. Tradit. Complement. Med. 4(3), 177-88. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25161923

"Eating high fat chow accelerates the development of sensitization to cocaine-induced locomotion in female rats. It is not known whether consumption of sucrose or saccharin also increases sensitivity to the behavioral effects of cocaine or whether continuous (or intermittent) access to these feeding conditions is necessary to change sensitivity. Adolescent female Sprague-Dawley rats were assigned to one of seven feeding conditions from postnatal day 25 through to postnatal day 60. The rats either ate high fat (60% kcal from fat) chow and drank water or ate standard (17% kcal from fat) chow and drank either water, a 10% sucrose solution, or a 0.1% saccharin solution. The rats either had continuous access to high fat chow, sucrose, or saccharin, or had intermittent access (i.e. 2 days/week) to these substances, with access to water and standard chow on other days. As compared with standard chow, continuous (but not intermittent) access to high fat chow enhanced the development of sensitization to cocaine-induced (1-17.8 mg/kg) locomotion; drinking sucrose or saccharin (continuous or intermittent access) did not alter the development of sensitization to cocaine-induced locomotion. The impact of feeding condition on the behavioral effects of cocaine varies between sexes and across dietary composition." As taken from Serafine KM et al. 2015. Behav. Pharmacol. 26(3), 321-5. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/25485647

"Drug abuse and obesity are serious public health problems. Dopamine plays a central role in mediating the reinforcing effects of drugs and food. Prolonged use of drugs is known to alter the function and/or sensitivity of many neurotransmitter systems, including dopamine; however, the impact of consuming foods high in fat and/or sugar is less clear. These studies characterized the locomotor effects of acute and repeated cocaine in male and female C57BL/6J mice consuming 1 of 4 diets: (a) standard chow + water; (b) standard chow + 10% sucrose solution; (c) high-fat chow + water; or (d) high-fat chow + 10% sucrose solution. The acute locomotor effects of cocaine (3.2-32.0 mg/kg) were evaluated 4 weeks after initiating dietary conditions; the effects of repeated cocaine administration were evaluated after 5, 6, 7, and 12 weeks. During acute tests, mice consuming a diet high in fat and/or sucrose exhibited greater locomotor responses to cocaine than mice consuming standard chow and water, regardless of sex. Although diet-induced enhancements persisted across repeated cocaine testing, locomotor sensitization developed more rapidly in females drinking sucrose (and consuming either standard or high-fat chow) than in females consuming standard chow and water. In addition to providing evidence that consuming a diet high in fat and/or sugar enhances abuse-related effects of cocaine in ways that might increase vulnerability to abuse cocaine, these studies identified a potentially important sex-related difference in the interaction between nutrition and cocaine effects, with the impacts of sucrose consumption being greater in females than in males."

As taken from Collins GT et al. 2015. Exp. Clin. Psychopharmacol. 23(4), 228-37. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26237320

“Caffeine is a psychostimulant frequently consumed by adults and children, often in combination with high levels of sugar. Chronic pretreatment with either substance can amplify both amphetamine and cocaine-induced hyperactivity in rodents. The present study sought to elucidate whether age at the time of exposure to sugar and/or caffeine alters sensitivity to an acute illicit psychostimulant (methamphetamine, [METH]) challenge in adulthood. Adult and adolescent (Postnatal Day 35 on first day of treatment) male Sprague-Dawley rats were treated for 26 days with water, caffeine (0.6 g/L), 10% sucrose or their combination. Locomotor behavior was measured on the first and last day of treatment. Following 9-days treatment free, animals were challenged with saline (1 ml/kg, i.p.) or METH (1 mg/kg, i.p.) and locomotor activity was measured. During the treatment period, adolescent rats maintained a higher caffeine (mg/kg) dose than their adult counterparts. Adding sugar to caffeine increased adolescent consumption and the highest caffeine dose consumed was measured in these animals. Drinking sugar-sweetened caffeinated water or combination did not produce cross-sensitization to METH administration in either age group. Nevertheless, the finding that regular exposure through adolescence to caffeinated sugar-sweetened beverages could increase consumption of caffeine and sugar later in life is important, as there is a large body of evidence that has linked excess consumption of sugar-sweetened beverages to a broad range of other negative physical and mental health outcomes.” As taken from Franklin JL et al. 2017. Behav. Neurosci. 131(4), 348-358. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28714720

“Genetic variation drives phenotypic diversity and influences the predisposition to metabolic disease. Here, we characterize the metabolic phenotypes of eight genetically distinct inbred mouse strains in response to a high-fat/high-sucrose diet. We found significant variation in diabetes-related phenotypes and gut microbiota composition among the different mouse strains in response to the dietary challenge and identified taxa associated with these traits. Follow-up microbiota transplant experiments showed that altering the composition of the gut microbiota modifies strain-specific susceptibility to diet-induced metabolic disease. Animals harboring microbial communities with enhanced capacity for processing dietary sugars and for generating hydrophobic bile acids showed increased susceptibility to metabolic disease. Notably, differences in glucose-stimulated insulin secretion between different mouse strains were partially recapitulated via gut microbiota transfer. Our results suggest that the gut microbiome contributes to the genetic and phenotypic diversity observed among mouse strains and provide a link between the gut microbiome and insulin secretion.” As taken from Kreznar JH et al. 2017. Cell Rep. 18(7), 1739-1750. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28199845

“Diets containing excess carbohydrate and fat promote hepatic steatosis and steatohepatitis in mice. Little is known, however, about the impact of specific carbohydrate/fat combinations on liver outcome. This study was designed to determine whether high-energy diets with identical caloric density but different carbohydrate and fat composition have unique effects on the liver. Four experimental diets were formulated with 60%kcal carbohydrate and 20%kcal fat, each in nearly pure form from a single source: starch-oleate, starch-palmitate, sucrose-oleate and sucrose-palmitate. The diets were fed to mice for 3 or 12 weeks for analysis of lipid metabolism and liver injury. All mice developed hepatic steatosis over 12 weeks, but mice fed the sucrose-palmitate diet accumulated more hepatic lipid than those in the other three experimental groups. The exaggerated lipid accumulation in sucrose-

palmitate-fed mice was attributable to a disproportionate rise in hepatic de novo lipogenesis. These mice accrued more hepatic palmitate and exhibited more evidence of liver injury than any of the other experimental groups. Interestingly, lipogenic gene expression in mice fed the custom diets did not correlate with actual de novo lipogenesis. In addition, de novo lipogenesis rose in all mice between 3 and 12 weeks, without feedback inhibition from hepatic steatosis. The pairing of simple sugar (sucrose) and saturated fat (palmitate) in a high-carbohydrate/moderate-fat diet induces more de novo lipogenesis and liver injury than other carbohydrate/fat combinations. Diet-induced liver injury correlates positively with hepatic de novo lipogenesis and is not predictable by isolated analysis of lipogenic gene expression." As taken from Pierce AA et al. 2016. J. Nutr. Biochem. 29, 12-20. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26895660

"Studies indicate that fructose absorption is dose-dependent and facilitated by the simultaneous ingestion of glucose."

As taken from EMA, 2016

"BACKGROUND & AIMS: The factors that distinguish metabolically healthy obesity from metabolically unhealthy obesity are not well understood. Diet has been implicated as a determinant of the unhealthy obesity phenotype, but which aspects of the diet induce dysmetabolism are unknown. The goal of this study was to investigate whether specific macronutrients or macronutrient combinations provoke dysmetabolism in the context of isocaloric, high-energy diets. METHODS: Mice were fed 4 high-energy diets identical in calorie and nutrient content but different in nutrient composition for 3 weeks to 6 months. The test diets contained 42% carbohydrate (sucrose or starch) and 42% fat (oleate or palmitate). Weight and glucose tolerance were monitored; blood and tissues were collected for histology, gene expression, and immunophenotyping. RESULTS: Mice gained weight on all 4 test diets but differed significantly in other metabolic outcomes. Animals fed the starch-oleate diet developed more severe hepatic steatosis than those on other formulas. Stable isotope incorporation showed that the excess hepatic steatosis in starch-oleate-fed mice derived from exaggerated adipose tissue lipolysis. In these mice, adipose tissue lipolysis coincided with adipocyte necrosis and inflammation. Notably, the liver and adipose tissue abnormalities provoked by starch-oleate feeding were reproduced when mice were fed a mixed-nutrient Western diet with 42% carbohydrate and 42% fat. CONCLUSIONS: The macronutrient composition of the diet exerts a significant influence on metabolic outcome, independent of calories and nutrient proportions. Starch-oleate appears to cause hepatic steatosis by inducing progressive adipose tissue injury. Starch-oleate phenocopies the effect of a Western diet; consequently, it may provide clues to the mechanism whereby specific nutrients cause metabolically unhealthy obesity." As taken from Duwaerts CC et al. 2017. Cell. Mol. Gastroenterol. Hepatol. 4(2), 223-236. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28649594

"The aim of the present study was to determine how nicotine pre-exposure affects the elasticity of demand for intravenous cocaine and for sucrose pellets in adult male rats. In Experiment 1, demand for cocaine was assessed in rats that had nicotine in their drinking water. Nicotine pre-exposure significantly decreased rats' willingness to defend cocaine consumption as the price (measured as the number of responses per cocaine infusion) increased compared with a control group with no nicotine pre-exposure. That is, nicotine increased the elasticity of demand for cocaine infusions. Experiment 2 repeated the first experiment, but with rats working for sucrose pellets instead of cocaine. Nicotine pre-exposure had no effect on the elasticity of demand for sucrose. This pattern of results

suggests that nicotine pre-exposure can reduce the reinforcing effects of cocaine, but not sucrose, in adult male rats." As taken from Schwartz LP et al. 2018. Behav. Pharmacol. 29(4), 316-326. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29064841

5. Toxicity

5.1. Single dose toxicity

Organism	**Test Type**	**Route**	**Reported Dose (Normalized Dose)**	**Effect**	**Source**
domestic animals - goat/sheep	LDLo	oral	40gm/kg (40000mg/kg)	BEHAVIORAL: SOMNOLENCE (GENERAL DEPRESSED ACTIVITY) LUNGS, THORAX, OR RESPIRATION: RESPIRATORY STIMULATION GASTROINTESTINAL: "HYPERMOTILITY, DIARRHEA"	Nutrition Abstracts and Reviews. Vol. 30, Pg. 503, 1960.
mouse	LD50	intraperitoneal	14000mg/kg (14000mg/kg)		Pharmaceutical Chemistry Journal Vol. 15, Pg. 139, 1981.
rat	LD50	oral	29700mg/kg (29700mg/kg)	BEHAVIORAL: SOMNOLENCE (GENERAL DEPRESSED ACTIVITY) LUNGS, THORAX, OR RESPIRATION: CYANOSIS GASTROINTESTINAL: "HYPERMOTILITY, DIARRHEA"	Toxicology and Applied Pharmacology. Vol. 7, Pg. 609, 1965.

As taken from ChemIDplus.

"Probable oral lethal dose (human) above 15 g/kg more than 1 quart (2.2 lb) for 70 kg person (150 lb)."

"The acute toxicity of sucrose is very low. Most lethal dosages are in the g/kg range. ... The initial clinical signs of toxicity were hypokinesia, prostration, cyanosis, clonic-tonic convulsions, abdominal bloating, and diarrhea."

As taken from HSDB, 2005.

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Human	0.096 ug/kg	Behavioral - analgesia	APHRER Annals of Pharmacotherpy. (Harvey Whitney Books Co., POB 42696, Cincinnati, OH 45242) V. 26- 1992- Volume(issue)/page/year: 39,1029,2005
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	2 mg/kg	Endocrine - hyperglycemia	JPHSC* Journal of pharmacological sciences (Kyoto, Japan : Japanese Pharmacological Society) V.91- 2003- Volume(issue)/page/year: 104,29,2007

As taken from RTECS, 2018.

Record for alpha-D-glucopyranoside, beta-D-fructofuranosyl (CAS RN 57-50-1):

Species Scientific Name	Species Common Name	Exposure Duration Mean (Days)	Test Location	Exposure Type	Endpoint	Effect	Effect Measurement	Response Site	Conc 1 (Author)	Conc 1 Units (Author)	Observed Duration (Days)
Rattus norvegicus	Norway Rat	0.875	Lab	Diet, unspecified	NOEL	Enzyme(s)	Alanine transaminase (ALT)	Serum	6000/	mg/kg	0.875
Rattus norvegicus	Norway Rat	0.875	Lab	Diet, unspecified	NOEL	Genetics	Damage	Liver	6000/	mg/kg	0.875
Rattus norvegicus	Norway Rat	0.875	Lab	Diet, unspecified	NOEL	Biochemistry	Glutathione (reduced glutathione)	Liver	6000/	mg/kg	0.875
Rattus norvegicus	Norway Rat	0.875	Lab	Diet, unspecified	NOEL	Enzyme(s)	Ornithine decarboxylase	Liver	6000/	mg/kg	0.875

Rattus norvegicus	Norway Rat	0.875	Lab	Diet, unspecified	NOEL	Enzyme(s)	Cytochrome P-450	Liver	6000/	mg/kg	0.875

As taken from the EPA ECOTOX database (2019).

5.2. Repeated dose toxicity

“Sublethal doses for 1-to 3-day exposures produced anorexia, polydipsia, hypothermia, diarrhea, and weight loss. Death is the result of respiratory failure.”

As taken from HSDB, 2005.

Activities of antioxidant enzymes in two stages of pathology development in sucrose-fed rats (Abstract). The activities of catalase in liver, heart and kidney as well as glutathione peroxidase and superoxide dismutase in liver, heart, kidney, and serum from hypertriglyceridemic and hypertensive female and male rats were measured at 3 and 8 months of daily administration of sucrose in their drinking water. This treatment induces high levels of serum triglycerides, central obesity, moderate hypertension, hyperinsulinemia, and an increase in lipoperoxidation, among other alterations. The experimental periods were chosen on the basis of previous observations: at 3 months the level of serum triglycerides increases significantly above the normal value and remains without major changes thereafter, but the blood pressure only rises significantly at about 4 months in males and 5 months in females. So, at 8 months the rats have been subjected to abnormal conditions for 3-4 months. The effect of these and the influence of sex on levels of antioxidant enzymes were investigated. Both factors, sucrose treatment and sex, were conducive to significant changes in those variables. As taken from Baños G et al. Can J Physiol Pharmacol. 2005 Mar;83(3):278-86. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=15870842&query_hl=7&itool=pubmed_docsum

Species Common Name	Exposure Duration Mean (Days)	Test Location	Exposure Type	Endpoint	Effect	Effect Measurement	Response Site	Conc 1 (Author)	Conc 1 Units (Author)	Observed Duration (Days)
House Mouse	12	Lab	Gavage	NOEL	Mortality	Mortality	Not reported	10000	mg/kg bdwt/d	12

House Mouse	12	Lab	Gavage	NOEL	Reproduction	Pregnant females in a population	Not reported	10000	mg/kg bdwt/d	12
House Mouse	12	Lab	Gavage	NOEL	Reproduction	Resorbed embryos	Not reported	10000	mg/kg bdwt/d	12
House Mouse	14	Lab	Gavage	NOEL	Mortality	Survival	Not reported	10000	mg/kg bdwt/d	14
House Mouse	12	Lab	Gavage	NOEL	Mortality	Survival	Not reported	10000	mg/kg bdwt/d	12
House Mouse	12	Lab	Gavage	NOEL	Growth	Weight	Whole organism	10000	mg/kg bdwt/d	12
House Mouse	14	Lab	Gavage	NOEL	Growth	Weight	Whole organism	10000	mg/kg bdwt/d	14
House Mouse	12	Lab	Gavage	NOEL	Growth	Weight	Whole organism	10000	mg/kg bdwt/d	12
House Mouse	12	Lab	Gavage	NR-ZERO	Mortality	Mortality	Not reported	10000	mg/kg bdwt/d	12

As taken from the EPA ECOTOX database (2019).

Type of Test	**Route of Exposure**	**Species Observed**	**Dose Data**	**Toxic Effects**	**Reference**
TDLo - Lowest published toxic dose	Oral	Rodent - rat	2730 gm/kg/2W (continuous)	Cardiac - other changes Nutritional and Gross Metabolic - changes in metals, not otherwise specified Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - other Enzymes	LIFSAK Life Sciences. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1-8, 1962-69; V.14- 1974- Volume(issue)/page/year: 71,1303,2002
TDLo - Lowest published	Oral	Rodent - rat	700000 mg/kg/35D (continuous)	Gastrointestinal - other changes	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982-

toxic dose					Volume(issue)/page/year: 46,752,2008
TDLo - Lowest published toxic dose	Oral	Rodent - rat	1050 gm/kg/10W (intermittent)	Endocrine - hyperglycemia Endocrine - other changes Biochemical - Metabolism (Intermediary) - lipids including transport	JPHSC* Journal of pharmacological sciences (Kyoto, Japan : Japanese Pharmacological Society) V.91- 2003- Volume(issue)/page/year: 102,213,2006

As taken from RTECS, 2018.

“BACKGROUND: Although previous studies have linked intake of sugars with incidence of cancer and other chronic diseases, its association with mortality remains unknown. OBJECTIVE: We investigated the association of total sugars, added sugars, total fructose, added fructose, sucrose, and added sucrose with the risk of all-cause, cardiovascular disease, cancer, and other-cause mortality in the NIH-AARP Diet and Health Study. DESIGN: The participants (n = 353,751), aged 50-71 y, were followed for up to 13 y. Intake of individual sugars over the previous 12 mo was assessed at baseline by using a 124-item NIH Diet History Questionnaire. RESULTS: In fully adjusted models (fifth quartile compared with first quartile), all-cause mortality was positively associated with the intake of total sugars [HR (95% CI): 1.13 (1.06, 1.20); P-trend < 0.0001], total fructose [1.10 (1.04, 1.17); P-trend < 0.0001], and added fructose [1.07 (1.01, 1.13); P-trend = 0.005) in women and total fructose [1.06 (1.01, 1.10); P-trend = 0.002] in men. In men, a weak inverse association was found between other-cause mortality and dietary added sugars (P-trend = 0.04), sucrose (P-trend = 0.03), and added sucrose (P-trend = 0.006). Investigation of consumption of sugars by source showed that the positive association with mortality risk was confined only to sugars from beverages, whereas the inverse association was confined to sugars from solid foods. CONCLUSIONS: In this large prospective study, total fructose intake was weakly positively associated with all-cause mortality in both women and men, whereas added sugars, sucrose, and added sucrose intakes were inversely associated with other-cause mortality in men. In our analyses, intake of added sugars was not associated with an increased risk of mortality. The NIH-AARP Diet and Health Study was registered at clinicaltrials.gov as NCT00340015.” As taken from Tasevska N et al. 2014b. Am. J. Clin. Nutr. 99(5), 1077-88. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24552754

Sucrose (g/day) and type 2 diabetes mellitus
· No association · Limited evidence

Insufficient evidence-cohort studies

HDL-cholesterol	sucrose

Body weight	sucrose
Weight gain	sucrose

Insufficient evidence-randomised controlled trials

Fasting blood lipids	sucrose
Eating motivation	sucrose
Glycaemia	sucrose

As taken from SACN, 2015.

“The aim was to describe the exposure to excipients among neonates hospitalised in the neonatal intensive care unit (NICU) of a public hospital in Brasilia, Brazil. This was a retrospective study based on medicines that were prescribed electronically to neonates (≤28 days) who were admitted to the NICU of a hospital in Brasilia between January 1 and March 31, 2012. Excipients were identified from the medicine package leaflets and were classified according to toxicity. Seventy-nine infants received a total of 1,303 prescriptions comprising 77 formulations and 70 active drugs. Eighty-six excipients were identified, of which, 9 were harmful excipients (HE) and 48 were potentially harmful excipients (PHE). Almost all the neonates (98.7 %) were exposed to at least one HE and PHE. Preterm neonates (n = 64; 1,502 neonate days) presented high risk of exposure to polysorbate 80 (3.26/100 neonate days), sodium hydroxide (3.39), PG (3.19) and propylparaben (3.06). Full-term neonates (n = 15; 289 neonate days) presented risks in relation to phenol (4.84), ethanol (3.8) and sodium citrate (3.46). CONCLUSION: Neonates in NICUs in Brazil are exposed to a wide variety of HE and PHE with unpredictable results. Safer alternatives are needed, as well as further studies on the subject.” As taken from Souza A Jr et al. 2014. Eur. J. Pediatr. 173(7), 935-45. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24500397

“BACKGROUND: Evidence is mixed regarding sugar-sweetened beverage (SSB) intake and adiposity among adults, perhaps because of reporting bias. OBJECTIVE: The objective of this study is to determine the impact of reporting bias on any associations between increased SSB intake and overweight/obesity. DESIGN: Beverage intake and overweight/obese status (body mass index ≥ 25 kg m(-2)) were examined among adults from a dietary assessment and doubly labeled water study (n=250). Four web-based, 24-h recalls assessed dietary intake. SSB intake was categorized as no intake, 1-99 kcals per day and >99 kcals per day. Logistic regression models adjusted for total caloric intake, age, race, education and diet quality compared SSB intake with overweight/obese status. To investigate dietary self-reporting bias, analyses were replicated in a subset of 'true reporters': those with self-reported total caloric intake within 25% of total energy expenditure per doubly labeled water assessments (n=108). RESULTS: One-half of participants were overweight/obese; more overweight/obese participants consumed SSB than normal-weight participants (69% vs 47%; P<0.001). Intake of other beverages did not differ by adiposity. Less number of White participants (48%) consumed SSB compared with African-American

participants (68%; P=0.002). Compared with no intake, SSB intake up to the median intake doubled the risk of being overweight/obese (odds ratio: 2.1, 95% confidence interval: 1.0-4.3; P=0.046) and SSB intake over the median more than doubled the risk (odds ratio: 2.6, 95% confidence interval: 1.2-6.0; P=0.018). When limited to true reporters, SSB intake significantly increased the risk of being overweight/obese by nearly fourfold. CONCLUSION: Underreporting of SSB intake may be attenuating true associations of SSB intake and the risk of being overweight/obese." As taken from Emond JA et al. 2014. Int. J. Obes. (Lond). 38(4), 603-9. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/23867782

"BACKGROUND: Intake of added sugar has been shown to correlate with many human metabolic diseases, and rodent models have characterized numerous aspects of the resulting disease phenotypes. However, there is a controversy about whether differential health effects occur because of the consumption of either of the two common types of added sugar-high-fructose corn syrup (fructose and glucose monosaccharides; F/G) or table sugar (sucrose, a fructose and glucose disaccharide). OBJECTIVES: We tested the equivalence of sucrose- vs. F/G-containing diets on mouse (Mus musculus) longevity, reproductive success, and social dominance. METHODS: We fed wild-derived mice, outbred mice descended from wild-caught ancestors, a diet in which 25% of the calories came from either an equal ratio of F/G or an isocaloric amount of sucrose (both diets had 63% of total calories as carbohydrates). Exposure lasted 40 wk, starting at weaning (21 d of age), and then mice (104 females and 56 males) were released into organismal performances assays-seminatural enclosures where mice competed for territories, resources, and mates for 32 wk. Within enclosures all mice consumed the F/G diet. RESULTS: Females initially fed the F/G diet experienced a mortality rate 1.9 times the rate (P = 0.012) and produced 26.4% fewer offspring than females initially fed sucrose (P = 0.001). This reproductive deficiency was present before mortality differences, suggesting the F/G diet was causing physiologic performance deficits prior to mortality. No differential patterns in survival, reproduction, or social dominance were observed in males, indicating a sex-specific outcome of exposure. CONCLUSION: This study provides experimental evidence that the consumption of human-relevant levels of F/G is more deleterious than an isocaloric amount of sucrose for key organism-level health measures in female mice." As taken from Ruff JS et al. 2015. J. Nutr. 145(3), 434-41. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25733457

Quantitative Risk Type - Not calculated

Quantitative Risk Value - Not calculated

Product Use – Not specified

Safety Evaluation Owner - COSMOS TTC (NON-CANCER)

POD Method - NOAEL

POD Value – 7500.0 mg/kg bw/day

POD Owner - COSMOS TTC (NON-CANCER)

Critical study: RAT (Chronic Toxicity) Oral exposure for 567 day

NOAEL/LOAEL Owner - PAFA

Original NOAEL - 7500.0 mg/kg bw/day

Original LOAEL – Not established

Critical Effects – NO EFFECTS

As taken from the COSMOS database available at http://www.cosmostox.eu/what/COSMOSdb/

5.3. Reproduction toxicity

“It is well established that uncontrolled glucose conc in maternal blood are associated with elevated embryonic and fetal death and increased neonatal morbidity and mortality.”

“Sucrose produced skeletal changes in a guinea pig fetus after feeding the mother 5 to 10 g sucrose/kg body weight in the latter half of pregnancy.”

“A high resorption rate and an increased number of malformed offspring ... /were seen in/ rats fed a diet composed of 72% sucrose, 18% casein, and 5% butter plus vitamins and a salt mixture.”

“No adverse maternal or neonatal effects /were observed/ in female rats gavaged with 10 g/kg/day of sucrose in distilled water on days 8 through 12 of gestation.”

“Sucrose administered to pregnant ferrets produced litters with significantly reduced body weight, crown-rump length, and head width, length, and volume. Compared with controls and ethanol exposed offspring, sucrose exposed dams had poor reproductive outcome (increased prenatal deaths), possibly from poor utilization of sucrose as a carbohydrate source.”

As taken from HSDB, 2005.

Type of Test	**Route of Exposure**	**Species Observed**	**Dose Data**	**Sex/ Duration**	**Toxic Effects**	**Reference**
TDLo - Lowest published toxic dose	Oral	Rodent - rat	1548 gm/kg	female 21 day(s) pre-mating 1-22 day(s) after conception	Reproductive - Specific Developmental Abnormalities - Central Nervous System	IJMDAI Israel Journal of Medical Sciences. (POB 1435, Jerusalem 91013, Israel) V.1- 1965- Volume(issue)/page/year: 16,789,1980

TDLo - Lowest published toxic dose	Oral	Rodent - rat	683 gm/kg	female 1-21 day(s) after conception	Reproductive - Specific Developmental Abnormalities - hepatobiliary system Reproductive - Effects on Newborn - growth statistics (e.g.%, reduced weight gain)	AJCNAC American Journal of Clinical Nutrition. (American Soc. for Clinical Nutrition, Inc., 9650 Rockville Pike, Bethesda, MD 20814) V.2- 1954- Volume(issue)/page/year: 28,1416,1975
TDLo - Lowest published toxic dose	Oral	Rodent - rat	683 gm/kg	lactating female 21 day(s) post-birth	Reproductive - Effects on Newborn - growth statistics (e.g.%, reduced weight gain)	AJCNAC American Journal of Clinical Nutrition. (American Soc. for Clinical Nutrition, Inc., 9650 Rockville Pike, Bethesda, MD 20814) V.2- 1954- Volume(issue)/page/year: 28,1416,1975
TDLo - Lowest published toxic dose	Oral	Mammal - species unspecified	54810 mg/kg	female 15-35 day(s) after conception	Reproductive - Effects on Embryo or Fetus - fetotoxicity (except death, e.g., stunted fetus)	TJADAB Teratology, The International Journal of Abnormal Development. (Alan R. Liss, Inc., 41 E. 11th St., New York, NY 10003) V.1- 1968- Volume(issue)/page/year: 30,203,1984

As taken from RTECS, 2018.

Altered dipsogenic responses and expression of angiotensin receptors in the offspring exposed to prenatal high sucrose (Abstract). The present study determined water and salt intake as well as expression of AT(1) and AT(2) receptors in the brain and kidney in the adult offspring rats prenatally exposed to high sucrose. Following the exposure during pregnancy, water intake and salt intake at baseline levels were not changed in the adult offspring. However, after 24h water deprivation, consumption of water and salt was significantly increased compared to that of the control. Plasma sodium and osmolality levels remained the same between the offspring in the control and the exposed groups, while hematocrit was higher in the offspring exposed to prenatal high sucrose immediately following water deprivation. Density of renal AT(1) receptor protein was the same between the control and the exposed group, while AT(2) receptor protein in the kidney was significantly increased in the offspring exposed to prenatal high sucrose in association of thicker basal membrane of glomerular. In the forebrain, both AT(1) and AT(2) receptor levels were significantly increased in the offspring with history of prenatal high sucrose. In addition, water deprivation induced more c-fos expression in the central dipsogenic areas, including the paraventricular and supraoptic nuclei in the offspring exposed to prenatal high sucrose. The results suggested that prenatal high intake of sucrose may affect development of pathways in regulation of dipsogenic behavior in face of dehydration, which was associated with altered expression of AT(1) or/and AT(2) receptors in the kidney and brain.

As taken from Wu L et al. Peptides. 2011 Jan;32(1):104-11. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/20965221?dopt=AbstractPlus

Effects of different cryoprotective agents on ram sperm morphology and DNA integrity (Abstract). This study investigates the effects of glycerol, 1,2 propanediol, sucrose, and trehalose on post-thaw motility, morphology, and genome integrity of Awassi ram semen. Ejaculates of thick consistency with rapid wave motion (>+++) and >70% initial motility were pooled. Sperm were diluted to a final concentration of 1/5 (semen/extender) in 0% cryoprotectant, 6% glycerol, 6% 1,2 propanediol, 62.5 mM sucrose or 62.5 mM trehalose using a two-step dilution method. The equilibrated semen was frozen in 0.25-ml straws. Semen samples were examined for sperm motility, defective acrosomes (FITC-Pisum sativum agglutinin (FITC PSA)), DNA integrity (acridine orange staining (AO)) and apoptotic activity (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) and Caspase-3 activity) at four time points: after dilution with extender A, after cooling to 5 degrees C, after equilibration and post-thaw. Freezing and thawing procedures (cooling at 5 degrees C, dilution, equilibration, and thawing) had negative effects on motility ($P<0.001$), acrosome integrity ($P<0.001$), and DNA integrity as determined by AO ($P<0.001$) and TUNEL ($P<0.001$) assays. There were positive correlations between sperm with defective acrosomes and apoptotic (AO- and TUNEL-positive) spermatozoa. In contrast, a significant negative correlation was found between sperm motility and defective acrosomes and AO- and TUNEL positivity ($P<0.01$). The cryopreservation process acts as an apoptotic inducer in ram semen; all cryoprotectants used in the present study allowed apoptosis to some extent, with negative effects on sperm morphology and DNA integrity. The glycerol group performed better than the propanediol, sucrose, trehalose, and control groups in terms of post-thaw sperm motility but not DNA integrity. As taken from Nur Z et al. Theriogenology. 2010 Jun;73(9):1267-75. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/20172600?dopt=AbstractPlus

Effects of maternal genotype and diet on offspring glucose and fatty acid-sensing ventromedial hypothalamic nucleus neurons (Abstract). Maternal obesity accentuates offspring obesity in dams bred to develop diet-induced obesity (DIO) on a 31% fat, high-sucrose, high-energy (HE) diet but has no effect on offspring of diet-resistant (DR) dams. Also, only DIO dams become obese when they and DR dams are fed HE diet throughout gestation and lactation. We assessed glucose and oleic acid (OA) sensitivity of dissociated ventromedial hypothalamic nucleus (VMN) neurons from 3- to 4-wk old offspring of DIO and DR dams fed chow or HE diet using fura-2 calcium imaging to monitor intracellular calcium fluctuations as an index of neuronal activity. Offspring of DIO dams fed chow had approximately 2-fold more glucose-inhibited (GI) neurons than did DR offspring. This difference was eliminated in offspring of DIO dams fed HE diet. At 2.5 mM glucose, offspring of chow-fed DIO dams had more GI neurons that were either excited or inhibited by OA than did DR offspring. Maternal HE diet intake generally increased the percentage of neurons that were excited and decreased the percentage that were inhibited by OA in both DIO and DR offspring. However, this effect was more pronounced in DIO offspring. These data, as well as concentration-dependent differences in OA sensitivity, suggest that genotype, maternal obesity, and dietary content can all affect the sensitivity of offspring VMN neurons to glucose and long-chain fatty acids. Such altered sensitivities may underlie the propensity of DIO offspring to become obese when fed high-fat, high-sucrose diets. As taken from Le Foll C et al., Am J Physiol Regul Integr Comp Physiol. 2009, Nov; 297(5):R1351-7, PubMed 2012 available at http://www.ncbi.nlm.nih.gov/pubmed/19710389?dopt=AbstractPlus

D-cycloserine and early ethanol exposure in developing rats (Abstract). Pregnant rats were exposed to one of the following treatments: 20% aqueous sucrose (w/v; Control), 20% aqueous sucrose with 20 mg D-cycloserine (DCS), 20% aqueous sucrose with 5% ethanol (ETH), or 20% aqueous sucrose with both 20 mg DCS and 5% ethanol (DCS+ ETH). Treatments were delivered in 20 ml of drinking water provided daily, as pilot work had determined that this was the average daily water consumption for female rats. Treatments began on Day 10 or 11 of pregnancy and terminated on postnatal Day 10. As juveniles, offspring were tested for activity in an open field and motor coordination using a rotating rod. Ethanol and DCS+ Ethanol groups were the most active groups in the open field, and DCS and DCS+ Ethanol groups had fewer falls than the Control and Ethanol groups on the rod test. Results suggest that DCS might provide protection from ethanol's adverse effects on some developmental behaviors. As taken from Isaac WL et al. Psychol Rep. 2009 Oct;105(2):472-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/19928608?dopt=AbstractPlus

The effect of sucrose and trehalose on viability of one- and two-cell rabbit embryos (Abstract).

The effect oc sucrose and trehalose on the viability of one- and two-cell rabbit embryos was investigated. A significant decrease in the viability of one- and two-cell embryos exposed for 30 min at 20 degrees C was observed. At 38 degrees C none of the two-cell embryos in a sucrose solution survived after 30 min exposure, while approximately 50% of the embryos survvied in a trehalose solution. The cleavage rate in culture of two-cell embryos exposed both to 2.0 M or 1.45 M trehalose was significantly lower in comparison with the control group. However the survival rate after transfer of two-cell embryos exposed to 1.45 M trehalose solution at 20 degrees C remained the same as that of the control group. As taken from Smorag Z et al., (1990), Theriogenology, 1990, 33(3), pp. 741-747. PubMed 2012 available at http://www.ncbi.nlm.nih.gov/pubmed/16726770

Bourne et al (1975) linked high levels of plasma trigllycerides to sucrose fed of rats, resulting in death of rats at various stages throughout pregnancy and early lactation. Naismith and Rana (1974), showed that sucrose depressed fat synthesis in adipose tissue. Hamel et al (1986) described reproductive anomalies in pregnant mice fed with sucrose. Kavalock et al (1985) and Berdanier (1975) reported no effect of sucrose on weight reduction of females, on the birth of neonatal pups or their survival. Rosenmann et al (1974) found that proteinuria and testicular atrophy could be induced in animals if fed a 72% by weight sucrose diet.

Fritz and Hess (1968) showed no significant differences in skeletal elements or malformation of sucrose fed rats. Klotzsche (1996), Kavalock (1985) and ACGIH (1991) observed no teratogenic or embryotoxic effects. Ornoy and Cohen (1980) showed that feeding high sucrose to pregnant diabetic female rats resulted in an increased number of fetal resorptions and malformations which occurred in both diabetic and control (normal) females. Skeletal changes in guinea pig fetus and rabbits have been observed by feeding pregnant animals with sucrose (Seta, 1931; Hirata, 1936; Furukawa, 1939).

“Antenatal malnutrition could be linked to hypertension and vascular diseases in fetal origins. This study determined the influence of maternal intake of high sucrose (HS) during pregnancy on vessel tone, intracellular Ca(2+) ([Ca(2+)](i)), K(+) channels, especially large-conductance Ca(2+)-activated K(+) channels (BK), in mesenteric arteries in the offspring rats exposed to prenatal HS. Vessel tension and [Ca(2+)](i) induced by angiotensin II were

higher in the small mesenteric arteries of the HS offspring. In the vascular smooth muscle cells (VSMCs) from the HS offspring, electrophysiological studies showed depressed BK current density and depolarized membrane. Western blot showed altered expressions of BK α-subunits, AT1 and AT2 receptors in mesenteric arteries. The results suggest that decreased BK channel activity and depolarized membrane potential in the VSMCs partly contributed to the increased vessel tone and [Ca(2+)](i) in the HS offspring, adding new information for understanding mechanisms in vascular malfunctions in fetal origins, and novel insights for early prevention and treatments against such vascular diseases." As taken from Li S et al. 2013. Hypertens. Res. 36(2), 158-65. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23013887

"The consumption of refined sugars continues to pose a significant health risk. However, nearly nothing is known about the effects of sugar intake by healthy women on the oocyte or embryo. Using rhesus monkeys, we show that low-dose sucrose intake over a 6-month period has an impact on the oocyte with subsequent effects on the early embryo. The ability of oocytes to resume meiosis was significantly impaired, although the differentiation of the somatic component of the ovarian follicle into progesterone-producing cells was not altered. Although the small subset of oocytes that did mature were able to be fertilized in vitro and develop into preimplantation blastocysts, there were >1100 changes in blastocyst gene expression. Because sucrose treatment ended before fertilization, the effects of sugar intake by healthy primates are concluded to be epigenetic modifications to the immature oocyte that are manifest in the preimplantation embryo." As taken from Chaffin CL et al. 2014. Endocrinology 155(7), 2688-95. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24731100

"Excess consumption of added sugars, including sucrose and high fructose corn syrup (HFCS-55), have been implicated in the global epidemics of obesity and type 2 diabetes. This study aimed to investigate and compare the impact of maternal consumption of sucrose or HFCS-55 during pregnancy and lactation on the metabolic health of the dam and her offspring at birth. Female Albino Wistar rats were given access to chow and water, in addition to a sucrose or HFCS-55 beverage (10% w/v) before, and during pregnancy and lactation. Maternal glucose tolerance was determined throughout the study, and a postmortem was conducted on dams following lactation, and on offspring within 24 h of birth. Sucrose and HFCS-55 consumption resulted in increased total energy intake compared with controls, however the increase from sucrose consumption was accompanied by a compensatory decrease in chow consumption. There was no effect of sucrose or HFCS-55 consumption on body weight, however sucrose consumption resulted in increased adiposity and elevated total plasma cholesterol in the dam, while HFCS-55 consumption resulted in increased plasma insulin and decreased plasma non-esterified fatty acids (NEFA). Maternal HFCS-55 consumption was associated with decreased relative liver weight and plasma NEFA in the offspring at birth. There was no effect of either treatment on pup weight at birth. These findings suggest that both sucrose and HFCS-55 consumption during pregnancy and lactation have the potential to impact negatively on maternal metabolic health, which may have adverse consequences for the long-term health of the offspring." As taken from Toop CR et al. 2015. J. Dev. Orig. Health Dis. 6(1), 38-46. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25523154

"BACKGROUND: Intake of added sugar has been shown to correlate with many human metabolic diseases, and rodent models have characterized numerous aspects of the resulting disease phenotypes. However, there is a controversy about whether differential

health effects occur because of the consumption of either of the two common types of added sugar-high-fructose corn syrup (fructose and glucose monosaccharides; F/G) or table sugar (sucrose, a fructose and glucose disaccharide). OBJECTIVES: We tested the equivalence of sucrose- vs. F/G-containing diets on mouse (Mus musculus) longevity, reproductive success, and social dominance. METHODS: We fed wild-derived mice, outbred mice descended from wild-caught ancestors, a diet in which 25% of the calories came from either an equal ratio of F/G or an isocaloric amount of sucrose (both diets had 63% of total calories as carbohydrates). Exposure lasted 40 wk, starting at weaning (21 d of age), and then mice (104 females and 56 males) were released into organismal performances assays-seminatural enclosures where mice competed for territories, resources, and mates for 32 wk. Within enclosures all mice consumed the F/G diet. RESULTS: Females initially fed the F/G diet experienced a mortality rate 1.9 times the rate (P = 0.012) and produced 26.4% fewer offspring than females initially fed sucrose (P = 0.001). This reproductive deficiency was present before mortality differences, suggesting the F/G diet was causing physiologic performance deficits prior to mortality. No differential patterns in survival, reproduction, or social dominance were observed in males, indicating a sex-specific outcome of exposure. CONCLUSION: This study provides experimental evidence that the consumption of human-relevant levels of F/G is more deleterious than an isocaloric amount of sucrose for key organism-level health measures in female mice." As taken from Ruff JS et al. 2015. J. Nutr. 145(3), 434-41. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25733457

"STUDY QUESTION: Is sugar-sweetened beverage (SSB) consumption associated with age at menarche? SUMMARY ANSWER: More frequent SSB consumption was associated with earlier menarche in a population of US girls. WHAT IS KNOWN ALREADY: SSB consumption is associated with metabolic changes that could potentially impact menarcheal timing, but direct associations with age at menarche have yet to be investigated. STUDY DESIGN, SIZE, DURATION: The Growing up Today Study, a prospective cohort study of 16 875 children of Nurses' Health Study II participants residing in all 50 US states. This analysis followed 5583 girls, aged 9-14 years and premenarcheal at baseline, between 1996 and 2001. During 10 555 person-years of follow-up, 94% (n = 5227) of girls reported their age at menarche, and 3% (n = 159) remained premenarcheal in 2001; 4% (n = 197) of eligible girls were censored, primarily for missing age at menarche. PARTICIPANTS/MATERIALS, SETTING, METHODS: Cumulative updated SSB consumption (composed of non-carbonated fruit drinks, sugar-sweetened soda and iced tea) was calculated using annual Youth/Adolescent Food Frequency Questionnaires from 1996 to 1998. Age at menarche was self-reported annually. The association between SSB consumption and age at menarche was assessed using Cox proportional hazards regression. MAIN RESULTS AND THE ROLE OF CHANCE: More frequent SSB consumption predicted earlier menarche. At any given age between 9 and 18.5 years, premenarcheal girls who reported consuming >1.5 servings of SSBs per day were, on average, 24% more likely [95% confidence interval (CI): 13, 36%; P-trend: <0.001] to attain menarche in the next month relative to girls consuming ≤2 servings of SSBs weekly, adjusting for potential confounders including height, but not BMI (considered an intermediate). Correspondingly, girls consuming >1.5 SSBs daily had an estimated 2.7-month earlier menarche (95% CI: -4.1, -1.3 months) relative to those consuming ≤2 SSBs weekly. The frequency of non-carbonated fruit drink (P-trend: 0.03) and sugar-sweetened soda (P-trend: 0.001), but not iced tea (P-trend: 0.49), consumption also predicted earlier menarche. The effect of SSB consumption on age at menarche was observed in every

tertile of baseline BMI. Diet soda and fruit juice consumption were not associated with age at menarche. LIMITATIONS, REASONS FOR CAUTION: Although we adjusted for a variety of suspected confounders, residual confounding is possible. We did not measure SSB consumption during early childhood, which may be an important window of exposure. WIDER IMPLICATIONS OF THE FINDINGS: More frequent SSB consumption may predict earlier menarche through mechanisms other than increased BMI. Our findings provide further support for public health efforts to reduce SSB consumption." As taken from Carwile JL et al. 2015. Hum. Reprod. 30(3), 675-83. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25628346

"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

"The aim of this study was to analyse the characteristics of ram spermatozoa subjected to varying concentrations of sucrose, and the influence of storage temperature (22°C or 5°C) prior to vitrification. Ejaculated semen was diluted in TCFEY (tris-citric acid-fructose 20% egg yolk), and two aliquots were prepared at a final concentration of 100×106spz/ml, one maintained at room temperature (22°C) and the other at 5°C. In the first experiment, the toxicity of sucrose diluents on the sperm was analysed; sperm samples at different temperatures were diluted (1:2) in TCF-BSA 2% (control) or in the same extender supplemented with various sucrose concentrations (0.4M, 0.6M and 0.8M). The effects of vitrification were studied in the second experiment, where sperm samples were mixed with different concentrations of cryoprotectants (sucrose) and vitrified by being plunged directly into liquid nitrogen. In both experiments, the sperm quality was assessed by measuring motility, morphology, membrane functionality (HOST), viability, acrosome integrity and DNA fragmentation. The toxicity test revealed significant differences ($p \leq 0.05$) when different sucrose concentrations were used; lower total and progressive motility, normal morphology

and membrane functionality were noted when sucrose concentration was higher, compared to the control treatment. Samples maintained at room temperature showed significantly ($p \leq 0.05$) higher viability than samples stored at 5°C. In contrast, although the quality of vitrified sperm was drastically decreased in comparison with fresh sperm, sucrose was associated with greater total motility, viability and membrane functionality. This improvement was closely linked to the temperature at which the sperm had been previously maintained, showing higher values when sperm was stored at 5°C. The main conclusions to be drawn from the study are therefore that sucrose shows promising potential as a cryoprotectant, and storing samples at 5°C is linked to improved sperm quality following vitrification." As taken from Arando A et al. 2017. Anim. Reprod. Sci. 181, 175-185. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28461086

"BACKGROUND: Diabetes mellitus become an epidemic problem throughout the world. Relation of the diabetes with diet is known. Some evidence is reported about mother died and risk of diabetes in babies during the life related with gestational diabetes. This study was conducted to examine the effects of the exposure of high-dose sucrose to rats and pups during pregnancy and lactation. METHODS: The mother rats were categorized into four groups, during pregnancy and until the offspring were 1-month-old, as follows: Group 1, provided with normal drinking water; Group 2, provided with water containing 10%; Group 3, 20%; and Group 4, 30% table sugar. During the study, the weights and daily fluid consumption of the animals were recorded. At the end of the study, the changes in blood, urine, and pancreatic tissues of the rats were examined. RESULTS: The pups in the groups supplemented with sugar had more weight gain than those of the control group. Although serum glucose levels of mothers and young rats in the groups fed with sugar-containing water did not reach the diabetic limits, it was observed that these animals had statistically significantly higher blood glucose levels than those in the control group. Insulin levels were also similarly increased by an increase in the amount of sugar. Immunohistochemical studies on the mother rats showed that insulin secreted cell numbers and insulin receptors significantly decreased in some pancreatic islets in the groups supplemented with sugar. Glucagon immunoreactivity examination showed that the number of glucagon-expressing cells decreased in the rat groups supplemented with sugar. Similar and more severe findings were observed in the offspring. CONCLUSION: This study has experimentally demonstrated that high daily intake of sugar in healthy pregnancies causes adverse effects on the mother and offspring." As taken from Ozkan H et al. 2019. Biomed. Pharmacother. 110, 609-617. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30537678

5.4. Mutagenicity

"Sucrose was evaluated for its mutagenic potential in the L5178Y TK+/TK- mouse lymphoma forward mutation assay using established procedures. Three experiments were conducted in the presence of an exogenous metabolic activation system and two experiments without activation. The dose levels tested in these experiments ranged from 0-5000 ug/ml. No significant increase in toxicity or in mutant frequency was observed at any dose level up to the highest dose tested (5000 ug/ml). Thus, sucrose was negative in these tests and the highest ineffective dose tested was 5000 ug/ml."

“Sucrose failed to elicit a mutagenic response in CHO-WBL cells in tests for chromosomal aberrations, degranulation, unscheduled DNA synthesis, and many other in vitro assays.”

“The mutagenicity of sucrose was assessed in sister chromatid exchange in non human cells, in micronucleus test and chromosomal aberrations test in mammalian polychromatic erythrocytes assay but none of these assays gave relevant results. No conclusion were determined”

As taken from HSDB, 2005.

In vivo				
Species	**Test conditions**	**Endpoint**	**Result**	**Reference**
Mice (2 per sex per group)	Mice were given 0, 2, 4 or 8 g/kg bw/day on 2 consecutive days by intraperitoneal injection, and killed 6 hr after the second dose.	Micronuclei induction in the bone marrow erythrocytes	-ive	Tsuchimoto & Matter, 1981
Mice (3 or 4 Per timepoint)	Mice were given 0.2 g (about 8 g/kg bw) by intraperitoneal injection on 2 consecutive days and killed 12, 24 or 48 hr after the second dose.	Micronuclei induction in the bone marrow erythrocytes	-ive	Salamone et al. 1981

In vitro					
Test system	**Test conditions**	**Endpoint**	**Activation**	**Result**	**References**
Sucrose was tested at 17 laboratories in Salmonella typhimurium (Ames test) (strains varied – all labs used	Plate and fluctuation assays at up to 5 mg/plate or limits of toxicity or solubility (no further details)	Mutation	With and without S9	-ive (in all labs)	Bridges et al. 1981

TA98 and TA100. Some also used TA1535, TA1537 and TA1538) and Escherichia coli WP2 strains.					
Various mammalian cells in vitro	Details were not given in this summary report	SCE, UDS and cell transformation	No details given in summary report	-ive	Brookes et al. 1981
Mouse L5178Y lymphoma cells	Up to 5 mg/ml (toxicity was not achieved)	Mutation	With and without S9	-ive	Mitchell et al. 1988
Mouse L5178Y lymphoma cells	Up to 5 mg/ml (toxicity was not achieved)	Mutation	With and without S9 (from induced and uninduced rats)	-ive	Myhr & Caspary, 1988
Various assays in bacteria, yeast, rat liver cells, HeLa cells, mouse lymphoma cells, hamster kidney cells and hamster ovary cells	No details described in this secondary source (a review by the Dutch expert Committee on Updating OELs)	Mutation, DNA damage, SCEs transfomation, aneuploidy, mitotic gene conversion and crossingover	Details not given	-ive in all assays	Various references cited in Gezondheidsraad, 2000.
+ive, positive; -ive, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation					

Sucrose was not genotoxic in bacterial or yeast cells (ACGIH, 1991), or mammalian cells in vitro (McGregor et al, 1987).

Mutagenicity Studies:

Test System	MOUSE LYMPHOMA
Strain Indicator	L5178Y (TK+/TK-)
Metabolic Activation	NONE
Method	SUSPENSION/PLATE
Dose	156.2-5000 UG/ML (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results	NEGATIVE
Source	[MCGREGOR,DB, MARTIN,R, CATTANACH,P, EDWARDS,I, MCBRIDE,D AND CASPARY,WJ; RESPONSES OF THE L5178Y TK+/TK- MOUSE LYMPHOMA CELL FORWARD MUTATION ASSAY TO CODED CHEMICALS. I. RESULTS FOR NINE COMPOUNDS; ENVIRON. MUTAGEN. 9(2):143-160, 1987]
Test System	MOUSE LYMPHOMA
Strain Indicator	L5178Y (TK+/TK-)
Metabolic Activation	RAT, LIVER, S-9, AROCLOR 1254
Method	SUSPENSION/PLATE
Dose	312.5-5000 UG/ML (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results	NEGATIVE
Source:	[MCGREGOR,DB, MARTIN,R, CATTANACH,P, EDWARDS,I, MCBRIDE,D AND CASPARY,WJ; RESPONSES OF THE L5178Y TK+/TK- MOUSE LYMPHOMA CELL FORWARD MUTATION ASSAY TO CODED CHEMICALS. I. RESULTS FOR NINE COMPOUNDS; ENVIRON. MUTAGEN. 9(2):143-160, 1987]
Test System	MOUSE LYMPHOMA

Strain Indicator	L5178Y (TK+/TK-)
Metabolic Activation	RAT, LIVER, S-9
Method	SUSPENSION/PLATE
Dose	312.5-5000 UG/ML (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results	NEGATIVE
Source	[MCGREGOR,DB, MARTIN,R, CATTANACH,P, EDWARDS,I, MCBRIDE,D AND CASPARY,WJ; RESPONSES OF THE L5178Y TK+/TK- MOUSE LYMPHOMA CELL FORWARD MUTATION ASSAY TO CODED CHEMICALS. I. RESULTS FOR NINE COMPOUNDS; ENVIRON. MUTAGEN. 9(2):143-160, 1987]
Test System	MOUSE LYMPHOMA
Strain Indicator	L5178Y (TK+/TK-)
Metabolic Activation	NONE
Method	SUSPENSION/PLATE
Dose	156-5000 UG/ML (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results	NEGATIVE
Source	[MITCHELL,AD, RUDD,CJ AND CASPARY,WJ; EVALUATION OF THE L5178Y MOUSE LYMPHOMA CELL MUTAGENESIS ASSAY: INTRALABORATORY RESULTS FOR SIXTY-THREE CODED CHEMICALS TESTED AT SRI INTERNATIONAL; ENVIRON. MOL. MUTAGEN. 12(SUPPL. 13):37-101, 1988][MYHR,BC AND CASPARY,WJ; EVALUATION OF THE L5178Y MOUSE LYMPHOMA CELL MUTAGENESIS ASSAY: INTRALABORATORY RESULTS FOR SIXTY-THREE CODED CHEMICALS TESTED AT LITTON BIONETICS, INC.; ENVIRON. MOL. MUTAGEN. 12(SUPPL. 13):103-194, 1988]
Test System	MOUSE LYMPHOMA
Strain Indicator	L5178Y (TK+/TK-)

Metabolic Activation	RAT, LIVER, S-9, AROCLOR 1254
Method	SUSPENSION/PLATE
Dose	156-5000 UG/ML (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results	NEGATIVE
Source	[MITCHELL,AD, RUDD,CJ AND CASPARY,WJ; EVALUATION OF THE L5178Y MOUSE LYMPHOMA CELL MUTAGENESIS ASSAY: INTRALABORATORY RESULTS FOR SIXTY-THREE CODED CHEMICALS TESTED AT SRI INTERNATIONAL; ENVIRON. MOL. MUTAGEN. 12(SUPPL. 13):37-101, 1988][MYHR,BC AND CASPARY,WJ; EVALUATION OF THE L5178Y MOUSE LYMPHOMA CELL MUTAGENESIS ASSAY: INTRALABORATORY RESULTS FOR SIXTY-THREE CODED CHEMICALS TESTED AT LITTON BIONETICS, INC.; ENVIRON. MOL. MUTAGEN. 12(SUPPL. 13):103-194, 1988]
Test System	MOUSE LYMPHOMA
Strain Indicator	L5178Y (TK+/TK-)
Metabolic Activation	RAT, LIVER, S-9
Method	SUSPENSION/PLATE
Dose	500-5000 UG/ML (TEST MATERIAL SOLVENT: DISTILLED WATER)
Results	NEGATIVE
Source	[MITCHELL,AD, RUDD,CJ AND CASPARY,WJ; EVALUATION OF THE L5178Y MOUSE LYMPHOMA CELL MUTAGENESIS ASSAY: INTRALABORATORY RESULTS FOR SIXTY-THREE CODED CHEMICALS TESTED AT SRI INTERNATIONAL; ENVIRON. MOL. MUTAGEN. 12(SUPPL. 13):37-101, 1988][MYHR,BC AND CASPARY,WJ; EVALUATION OF THE L5178Y MOUSE LYMPHOMA CELL MUTAGENESIS ASSAY: INTRALABORATORY RESULTS FOR SIXTY-THREE CODED CHEMICALS TESTED AT LITTON BIONETICS, INC.; ENVIRON. MOL. MUTAGEN. 12(SUPPL. 13):103-194, 1988]
Test System	CHO-WBL CELLS
Strain Indicator	IN VITRO CHROMOSOMAL ABERRATIONS

Metabolic Activation	NONE
Dose	250; 275; 300; 325 MM
Dose Regimen	4 HR TREATMENT, 12-20 HR RECOVERY
Results	: POSITIVE (STRUCTURAL CHANGES); OSMOLALITY OF SOLUTION DECISIVE FACTOR
Source	[GALLOWAY,M, DEASY,DM, BEAN,CL, KRAYNAK,AR, ARMSTRONG,MJ, AND BRADLEY,MO; EFFECTS OF HIGH OSMOTIC STRENGTH ON CHROMOSOME ABERRATIONS, SISTER-CHROMATID EXCHANGES AND DNA STRAND BREAKS, AND THE RELATION TO TOXICITY; MUTAT. RES. 189(1):15-25,1987]

As taken from CCRIS, 2005.

GENE-TOX Evaluation A (pre-1980):

Species/Cell Type	Mammalian polychromatic erythrocytes
Assay Type	Micronucleus test, chromosome aberrations
Assay Code	MNTT
Results	No conclusion
Panel Report	EMICBACK/50890; MUTAT RES 123:61-118,1983

GENE-TOX Evaluation B (post-1980):

Species/Cell Type	Nonhuman
Assay Type	Sister-chromatid exchange (SCE) in vitro
Assay Code	SC2T
Results	
Panel Report	EMIC/91392; Mutat Res 297:101-180,1993

As taken from GENETOX, 1998.

Type of Test	Route of Exposure	Species Observed	Dose Data	Reference
Mutation in microorganisms		Bacteria - Salmonella typhimurium	600 ug/plate	PMRSDJ Progress in Mutation Research. (Elsevier Science Pub. Co., Inc., 52 Vanderbilt Ave., New York, NY 10017) V.1- 1981- Volume(issue)/page/year: 1,343,1981
DNA repair		Yeast - Saccharomyces cerevisiae	300 mg/L	PMRSDJ Progress in Mutation Research. (Elsevier Science Pub. Co., Inc., 52 Vanderbilt Ave., New York, NY 10017) V.1- 1981- Volume(issue)/page/year: 1,502,1981
Cytogenetic analysis		Rodent - hamster Lung	10 gm/L	ATSUDG Archives of Toxicology, Supplement. (Springer-Verlag New York, Inc., Service Center, 44 Hartz Way, Secaucus, NJ 07094) No.1- 1978- Volume(issue)/page/year: 4,41,1980
Cytogenetic analysis		Rodent - hamster Ovary	275 mmol/L	MUREAV Mutation Research. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1964- Volume(issue)/page/year: 189,15,1987

As taken from RTECS, 2018.

“Pyridoxal 5'-phosphate (PLP), the active form of vitamin B6, has been implicated in preventing human pathologies, such as diabetes and cancer. However, the mechanisms underlying the beneficial effects of PLP are still unclear. Using Drosophila as a model system, we show that PLP deficiency, caused either by mutations in the pyridoxal kinase-coding gene (dPdxk) or by vitamin B6 antagonists, results in chromosome aberrations (CABs). The CAB frequency in PLP-depleted cells was strongly enhanced by sucrose, glucose or fructose treatments, and dPdxk mutant cells consistently displayed higher glucose contents than their wild type counterparts, an effect that is at least in part a consequence of an acquired insulin resistance. Together, our results indicate that a high intracellular level of glucose has a dramatic clastogenic effect if combined with PLP deficiency. This is likely due to an elevated level of Advanced Glycation End-products (AGE) formation. Treatment of dPdxk mutant cells with α-lipoic acid (ALA) lowered both AGE formation and CAB frequency, suggesting a possible AGE-CAB cause-effect relationship. The clastogenic effect of glucose in PLP-depleted cells is evolutionarily conserved. RNAi-mediated silencing of PDXK in human cells or treatments with PLP inhibitors resulted in chromosome breakage, which was potentiated by glucose and reduced by ALA. These results suggest that patients with concomitant hyperglycemia and vitamin B6 deficiency may suffer chromosome damage. This might impact cancer risk, as CABs are a well-known tumorigenic factor.” As taken from Marzio A et al. 2014. PLoS Genet. 10(3), e1004199. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24651653

“The purpose of this study was to determine the effects of the high consumption of sucrose on the levels of DNA damage in blood, hippocampus and bone marrow of rats. Male Wistar

rats were treated for 4 months with sucrose (10% for 60 initial days and 34% for the following 60 days) in drinking water, and then, glycemia and glycated hemoglobin (A1C) were measured. Levels of DNA damage in blood and hippocampus were evaluated by the comet assay. The micronucleus test was used to evaluate chromosomal damages in the bone marrow. The sucrose treatment significantly increased ($p<0.01$) the serum glucose levels (~20%) and A1C (~60%). The level of primary DNA damage was significantly increased ($p<0.05$) in hippocampal cells (~60%) but not in peripheral blood leukocytes ($p>0.05$). Additionally, it was observed a significative increase ($p<0.05$) in the markers of chromosomal breaks/losses in bone marrow, as indicated by the micronucleus test. This is the first study that evaluated DNA damage induced by high sucrose concentration in the hippocampus and bone marrow of rats. Sucrose-induced DNA damage was observed in both tissues. However, the mechanism of sucrose toxicity on DNA remains unknown." As taken from Franke SIR et al. 2017. An. Acad. Bras. Cienc. 89(4), 2657-2662. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29267792

5.5. Cytotoxicity

"BACKGROUND: Applied radiofrequency (RF) energy induces hyperthermia in tissues, facilitating vascular perfusion This study explores the impact of RF radiation on the integrity of the luminal endothelium, and then predominately explores the impact of altering the conductivity of biologically-relevant solutions on RF-induced heating rates and cell death. The ability of cells to survive high sucrose (i.e. hyperosmotic conditions) to achieve lower conductivity as a mechanism for directing hyperthermia is evaluated. METHODS: RF radiation was generated using a capacitively-coupled radiofrequency system operating at 13.56 MHz. Temperatures were recorded using a FLIR SC 6000 infrared camera. RESULTS: RF radiation reduced cell-to-cell connections among endothelial cells and altered cell morphology towards a more rounded appearance at temperatures reported to cause in vivo vessel deformation. Isotonic solutions containing high sucrose and low levels of NaCl displayed low conductivity and faster heating rates compared to high salt solutions. Heating rates were positively correlated with cell death. Addition of sucrose to serum similarly reduced conductivity and increased heating rates in a dose-dependent manner. Cellular proliferation was normal for cells grown in media supplemented with 125 mM sucrose for 24 hours or for cells grown in 750 mM sucrose for 10 minutes followed by a 24 h recovery period. CONCLUSIONS: Sucrose is known to form weak hydrogen bonds in fluids as opposed to ions, freeing water molecules to rotate in an oscillating field of electromagnetic radiation and contributing to heat induction. The ability of cells to survive temporal exposures to hyperosmotic (i.e. elevated sucrose) conditions creates an opportunity to use sucrose or other saccharides to selectively elevate heating in specific tissues upon exposure to a radiofrequency field." As taken from Pulikkathara M et al. 2017. Converg. Sci. Phys. Oncol. 3(3), 035001. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29177085

High-throughput Assay Data

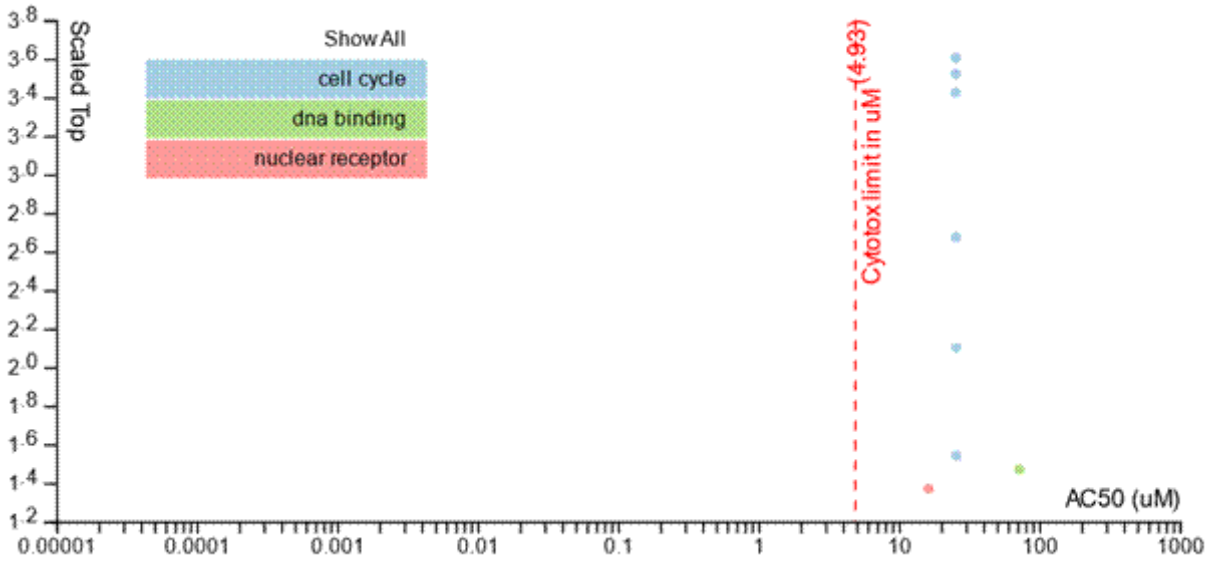
The US Environmental Protection Agency (EPA) evaluated sucrose in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard

(section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: https://comptox.epa.gov/dashboard

EPA provides the following data use considerations for ToxCast data: “The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve.”

A summary of the ToxCast assay data on sucrose is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on EPA’s CompTox Dashboard.

Figure 1



5.6. Carcinogenicity

“Sucrose was not carcinogenic, but showed tumor promoting activity in female Swiss albino mice after 18 months as 10% of a standard diet or when injected into the nape of the neck of rats and mice, 3 times/week for up to two years. The National Research Council concluded that epidemiologic and experimental evidence for carbohydrates (including sucrose) carcinogenesis was too sparse to implicate this class of compounds as having a direct role in carcinogenesis.”

As taken from HSDB, 2005.

Sucrose and IQ induced mutations in rat colon by independent mechanism (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 rats 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 background mutations 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. As taken from Hansen et al., Mutat Res. 2004, Oct 4; 554(1-2):279-86. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/15450425

Dietary glycemic load, carbohydrate, sugar, and colorectal cancer risk in men and women (Abstract). Hyperinsulinemia may explain excess colorectal cancer among individuals who are overweight or inactive. Recent studies have observed elevated colorectal cancer risk among individuals with elevated insulin levels 2 hours after oral glucose challenge or with elevated plasma C-peptide levels. The effect of consuming a high glycemic diet on colorectal risk, however, remains uncertain. Two prospective cohort studies, the Nurses' Health Study and the Health Professionals Follow-up Study, contributed up to 20 years of follow-up. After exclusions, 1,809 incident colorectal cancers were available for analyses. Dietary glycemic load (GL) was calculated as a function of glycemic index (postprandial blood glucose response as compared with a reference food), carbohydrate content, and frequency of intake of individual foods reported on food frequency questionnaires. Multivariable Cox proportional hazards models were used to adjust for potential confounders. Intakes of dietary carbohydrate, GL, overall glycemic index, sucrose, and fructose were not associated with colorectal cancer risk in women. A small increase in risk was observed in men with high dietary GL (multivariate relative risk, 1.32; 95% confidence interval, 0.98-1.79; highest versus lowest quintile), sucrose or fructose (multivariate relative risk, 1.37; 95% confidence interval, 1.05-1.78; highest versus lowest quintile of fructose, P = 0.008). Associations were slightly stronger among men with elevated body mass index (> or =25 kg/m(2)). Results among women were similar after stratifying by body mass index or physical activity. High intakes of GL, fructose, and sucrose were related to an elevated colorectal cancer risk among men. For women, however, these factors did not seem to increase the risk of colorectal cancer. As taken from Michaud DS et al. Cancer Epidemiol Biomarkers Prev. 2005 Jan; 14(1):138-47. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=15668487&query_hl=11&itool=pubmed_docsum

Sucrose, glucose and fructose have similar genotoxicity in the rat colon and affect the metabolome (Abstract). We have shown previously that a high sucrose intake increases the background level of somatic mutations and the level of bulky DNA adducts in the colon epithelium of rats. The mechanism may involve either glucose or fructose formed by hydrolysis of sucrose. Male Big Blue rats were fed 30% sucrose, glucose, fructose or potato starch as part of the diet. Mutation rates and bulky DNA adduct levels were determined in colon and liver. The concentration of short-chain fatty acids and pH were

determined in caecum, C-peptide was determined in plasma, biomarkers for oxidative damage and proliferation were determined in colon, and a metabonomic analysis was performed in plasma and urine. The sugars increased the mutation rates in colon and the bulky adduct levels in colon and liver to a similar extent. All sugars decrease the caecal concentration of acetic acid and propionic acid. The metabonomic studies indicated disturbed amino acid metabolism and decrease in plasma and urinary acetate as a common feature for all sugars and confirmed triglyceridemic effects of fructose. In conclusion, the genotoxicity may be related to the altered chemical environment in the caecum and thereby also in the colon but we found no related changes in insulin resistance or oxidative stress. As taken from Hansen M et al. Food Chem Toxicol. 2008 Feb;46(2):752-60. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/17988776

Species	**Test conditions**	**Evidence of carcinogenicity**	**Reference**
Groups of 50 rats per sex	Treated rats were given a basal diet containing 10% sucrose for about 2.5 years (the rats had also been exposed in utero, as the parents had been fed sucrose for 12 wk before mating and during pregnancy and weaning). The control group was given basal diet containing 10% maize starch.	None (a comprehensive range of tissues was examined microscopically)	Smits-Van Prooije et al. 1990
Groups of 50 mice per sex	Treated mice were given a basal diet containing 10% sucrose for 2 years. The control group was given basal diet containing 10% maize starch.	None (a comprehensive range of tissues was examined microscopically)	Smits-Van Prooije et al. 1990
Groups of 10 male rats	Rats were fed a high sugar diet (containing about 30% sucrose and 30% dextrin) or a low sugar diet (based on cornstarch and potato starch) during one, two or all three periods of a study divided into pre-initiation (weeks 1-4), initiation with a known colon carcinogen (azoxymethane) (weeks 5-6) and post-initiation (weeks 7-24). A further (control) group was fed	No convincing evidence of carcinogenic activity was seen in the rats without azoxymethane treatment. When fed prior to initiation or during initiation, the high sucrose diet did not affect the number or multiplicity of aberrant crypt foci (a	Poulsen et al. 2001

	the high sugar diet throughout, with no azoxymethane treatment	presumed preneoplastic lesion). However, such foci were increased in the colon of rats fed the high sucrose diet during the postinitiation period.	
Various studies in rats and mice	Various protocols involving feeding of sucrose to rats and mice treated with known colon carcinogens.	Enhanced development of aberrant crypt foci and/or colon tumours was reported in sucrose-fed rodents.	Stamp et al. 1993 Gaderni et al. 1991, 1994 Kristiansen et al. 1995, 1996 Luceri et al. 1996 (These are examples, others are available)

10g/kg dosage may lead to an increase in colonic cell proliferation in mice (Stamp et al, 1993). Tumor yields were similar in sucrose and corn starch fed rats (Klurfeld et al, 1984). Caderni et al (1993) drew no direct conclusion regarding a link of sucrose to colon cancer in rats.

In humans, high sucrose or sugar intakes may be risk factors in lung (De Stefani et al, 1998), breast (Favero et al, 1998) and colon (Bostick et al, 1994; Slattery et al, 1997) cancers. No relationships were found between dietary sugar and other cancers, sometimes because of insufficient data (Burley, 1997 and 1998).

A4: Not classifiable as a human carcinogen.

[American Conference of Governmental Industrial Hygienists TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati, OH, 2008, p. 53]

As taken from HSDB, 2005.

Rats and Mice: Cancer Test Summary

Rat Target Sites		Mouse Target Sites		TD_{50} (mg/kg/day)	
Male	Female	Male	Female	Rat	Mouse
no test	no test	no positive	no positive	no test	no positive

As taken from CPDB, 2007.

Coffee and tea consumption and endometrial cancer risk in a population-based study in New Jersey.

“We evaluated the role of tea and coffee and substances added (sugar/honey, creamers, and milk) on endometrial cancer risk in a population-based case-control study in six

counties in New Jersey, including 417 cases and 395 controls. Multivariate odds ratios (OR) and 95% confidence intervals (CI) were computed using unconditional logistic regression. …….. Tea consumption appeared to increase risk (OR: 1.93; 95% CI: 1.08-3.45), but after including the variables sugar/honey and cream/milk added to tea in the model, the risk estimate was attenuated and no longer statistically significant (OR: 1.77; 95% CI: 0.96-3.28 for those consuming more than one cup/day of tea compared to nonusers). ……. We found an association with adding sugar/honey to tea, with those adding two or more teaspoons/cup having an OR of 2.66 (95% CI: 1.42-4.98; p for trend <0.01) after adjusting for relevant confounders. For sugar/honey added to coffee the corresponding OR was 1.43 (95% CI: 0.81-2.55). Our results indicate that sugars and milk/cream added to coffee and tea should be considered in future studies evaluating coffee and tea and endometrial cancer risk." As taken from Bandera EV et al. Cancer Causes Control 2010 Sep;21(9):1467-73. PubMed, 2012 available at http://www.ncbi.nlm.nih.gov/pubmed/20467800

Available carbohydrates, glycemic load, and pancreatic cancer: is there a link?

"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. Overall, elevated risks for pancreatic cancer were observed in the 90th versus 10th percentile of glycemic load (hazards ratio (HR) = 1.45, 95% confidence interval (CI): 1.05, 2.00), available carbohydrate (HR = 1.47, 95% CI: 1.05, 2.06), and sucrose (HR = 1.37, 95% CI: 0.99, 1.89) intake. The positive association for available carbohydrate intake was observed during the first 4 years of follow-up (HR(<2 years) = 2.60, 95% CI: 1.34, 5.06; HR(2-<4 years) = 1.94, 95% CI: 1.06, 3.55) but not subsequently (HR = 0.86, 95% CI: 0.52, 1.44); the opposite pattern was observed for total fat and saturated fat intake. Rather than being causal, the short-term increase in pancreatic cancer risk associated with high available carbohydrate and low fat intake may be capturing dietary changes associated with subclinical disease." As taken from Meinhold CL et al. Am J Epidemiol 2010 Jun 1;171(11):1174-82. PubMed, 2012 available at http://www.ncbi.nlm.nih.gov/pubmed/20452999

"OBJECTIVES: The aim of this review is to summarize the evidence available about the association between sugar consumption, especially sucrose, and the risk of different types of cancer. METHODS: A systematic review was conducted of key reports, systematic reviews, meta-analysis as well as big prospective studies published after 2007 January 1 thru 2012 December 31 about the association between sugar consumption, especially sucrose, and the risk of cancer. RESULTS: Evidence of the association of the intake of mono and disaccharides with different types of cancer is insufficient or there is evidence of lack of association. There is only possible evidence of a positive relation between the intake of monosaccharides (fructose and glucose) and pancreatic cancer. Evidence about the association between monosaccharides intake and obesity is insufficient, as well as between the intake ofsucrose or added sugars and the risk of obesity in adults and children. There is possible evidence of a positive association between glycemic index (GI) and colorectal cancer and that there is no association between GI and the risk of endometrial cancer, breast cancer and pancreas cancer. CONCLUSION: More research is needed. Cohort

studies are especially required and randomized intervention trials would be desirable, although these are difficult in this field." As taken from Aranceta Bertrina J et al. 2013. Nutr. Hosp. 28(Suppl. 4), 94-105. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23834098

"BACKGROUND: Although previous studies have linked intake of sugars with incidence of cancer and other chronic diseases, its association with mortality remains unknown. OBJECTIVE: We investigated the association of total sugars, added sugars, total fructose, added fructose, sucrose, and added sucrose with the risk of all-cause, cardiovascular disease, cancer, and other-cause mortality in the NIH-AARP Diet and Health Study. DESIGN: The participants (n = 353,751), aged 50-71 y, were followed for up to 13 y. Intake of individual sugars over the previous 12 mo was assessed at baseline by using a 124-item NIH Diet History Questionnaire. RESULTS: In fully adjusted models (fifth quartile compared with first quartile), all-cause mortality was positively associated with the intake of total sugars [HR (95% CI): 1.13 (1.06, 1.20); P-trend < 0.0001], total fructose [1.10 (1.04, 1.17); P-trend < 0.0001], and added fructose [1.07 (1.01, 1.13); P-trend = 0.005) in women and total fructose [1.06 (1.01, 1.10); P-trend = 0.002] in men. In men, a weak inverse association was found between other-cause mortality and dietary added sugars (P-trend = 0.04), sucrose (P-trend = 0.03), and added sucrose (P-trend = 0.006). Investigation of consumption of sugars by source showed that the positive association with mortality risk was confined only to sugars from beverages, whereas the inverse association was confined to sugars from solid foods. CONCLUSIONS: In this large prospective study, total fructose intake was weakly positively associated with all-cause mortality in both women and men, whereas added sugars, sucrose, and added sucrose intakes were inversely associated with other-cause mortality in men. In our analyses, intake of added sugars was not associated with an increased risk of mortality. The NIH-AARP Diet and Health Study was registered at clinicaltrials.gov as NCT00340015." As taken from Tasevska N et al. 2014b. Am. J. Clin. Nutr. 99(5), 1077-88. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24552754

Insufficient evidence-cohort studies

Colo-rectal, colon and rectal cancer	sucrose

As taken from SACN, 2015.

"The aim of this study was to estimate the effect of diet on prostate and breast cancer (PC and BC) risks in smokers and nonsmokers and to explore the effect modification between smoking and dietary patterns. PC or BC incidence rates were assessed spatially according to tobacco exposure, age-adjusted standardization using lung cancer mortality as a proxy. Two case-control studies were carried out in Argentina (2008-2012). Participants were interviewed about their diet, smoking habits, and other lifestyle factors. Multilevel models were fitted including family history of cancer as the random intercept for the second level, and diet and lifestyle variables as covariates. Tobacco exposure was aggregated spatially. Family history of cancer significantly accounts for PC and BC. In smokers, high intake of fat meat increased PC and BC risks [odds ratio (OR) 1.56, 95% confidence interval (CI) 0.81-3.05 and OR 6.01, 95% CI 1.99-8.19, respectively]. PC and BC risks were also greater in smokers with high intakes of fatty foods (OR 1.95, 95% CI 1.09-3.50 and OR 24.2, 95% CI

0.82-7.21, respectively). Moderate intake of nonstarchy vegetables and risk of PC were inversely associated in nonsmokers (OR 0.55, 95% CI 0.20-1.48). In smoker women, BC risk was associated with sweet drink consumption (OR 2.96, 95% CI 1.10-7.92) and ethanol intake (OR 5.15, 95% CI 1.88-14.16). Spatial distributions of cancer incidence rates match those of tobacco exposure. Differential effects of diet on PC and BC risks were found in smokers and nonsmokers." As taken from Román MD et al. 2014. Eur. J. Cancer Prev. 23(4), 310-8. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24871563

"OBJECTIVE: A diet high in sugars may promote colorectal carcinogenesis, but it remains uncertain whether high intake of sugars or sucrose confers increased risk of colorectal cancer. The authors investigated the associations of sugars and sucrose intake with colorectal cancer risk in a community-based case-control study in Japan. METHODS: The study subjects comprised 816 incident cases of colorectal cancer and 815 community controls. Consumption frequencies and portion sizes of 148 food and beverage items were ascertained by a computer-assisted interview. The authors used the consumption of 29 food items to estimate sugars and sucrose intake. The odds ratios of colorectal cancer risk according to intake categories were obtained using a logistic regression model with adjustment for potential confounding variables. RESULTS: Overall, intakes of sugars and sucrose were not related to colorectal cancer risk either in men or women. The association between sugars intake and colorectal cancer risk differed by smoking status and alcohol use in men, but not in women. In men, sugars intake tended to be associated with colorectal cancer risk inversely among never-smokers and positively among male ever-smokers (interaction p=0.01). Sugars intake was associated with an increased risk among men with no alcohol consumption, but was unrelated to the risk among male alcohol drinkers (interaction p=0.02). Body mass index did not modify the association with sugars intake in either men or women. CONCLUSION: Sugars intake was associated with increased risk of colorectal cancer among smokers and non-alcohol drinkers in men selectively." As taken from Wang Z et al. 2014. Scand. J. Gastroenterol. 49(5), 581-8. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24716480

"Ovarian cancer is a leading cause of gynecological cancer death. There is a need to identify modifiable dietary risk factors for this disease. To evaluate the role of diet in ovarian cancer risk, we performed a PRISMA-directed systematic review that included prospective cohort studies with >200 cases (n = 24). Higher risk for ovarian cancer was shown for total, animal, and dairy fat (five of nine studies), as well as total nitrate and possibly total vitamin C. No associations were demonstrated for red meat, fiber, vitamin A, vitamin E, β-carotene, or folate. Vegetables were associated with lower risk in one of three studies; fruit showed no association, although risk estimates were all greater than 1.0. Isoflavones and flavonoids were associated with modestly lower risk in two studies and tea intake was associated with lower risk in one of two studies. This review suggests that no specific dietary factors are consistently associated with ovarian cancer risk. Data by tumor subtypes are limited, but suggest that differential associations by tumor subtype may exist and should be evaluated. Studies of ample sample size, varied exposure, which can better control for dietary measurement error, are needed to fully define dietary recommendations for ovarian cancer prevention." As taken from Crane TE et al. 2014. Cancer Epidemiol. Biomarkers Prev. 23(2), 255-73. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24142805

"Epidemiologic studies have shown that dietary sugar intake has a significant impact on the development of breast cancer. One proposed mechanism for how sugar impacts cancer development involves inflammation. In the current study, we investigated the impact of

dietary sugar on mammary gland tumor development in multiple mouse models, along with mechanisms that may be involved. We found that sucrose intake in mice comparable with levels of Western diets led to increased tumor growth and metastasis, when compared with a nonsugar starch diet. This effect was ascribed in part to increased expression of 12-lipoxygenase (12-LOX) and its arachidonate metabolite 12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid (12-HETE). We determined that fructose derived from the sucrose was responsible for facilitating lung metastasis and 12-HETE production in breast tumors. Overall, our data suggested that dietary sugar induces 12-LOX signaling to increase risks of breast cancer development and metastasis." As taken from Jiang Y et al. 2016. Cancer Res. 76(1), 24-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26729790

"BACKGROUND: There is limited research examining beverage habits, one of the most habitual dietary behaviors, with mortality risk. OBJECTIVE: This study examined the association between coffee, black and green tea, sugar-sweetened beverages (soft drinks and juice), and alcohol and all-cause and cause-specific mortality. METHODS: A prospective data analysis was conducted with the use of the Singapore Chinese Health Study, including 52,584 Chinese men and women (aged 45-74 y) free of diabetes, cardiovascular disease (CVD), and cancer at baseline (1993-1998) and followed through 2011 with 10,029 deaths. Beverages were examined with all-cause and cause-specific (cancer, CVD, and respiratory disease) mortality risk with the use of Cox proportional hazards regression. RESULTS: The associations between coffee, black tea, and alcohol intake and all-cause mortality were modified by smoking status. Among never-smokers there was an inverse dose-response association between higher amounts of coffee and black tea intake and all-cause, respiratory-related, and CVD mortality (black tea only). The fully adjusted HRs for all-cause mortality for coffee for <1/d, 1/d, and ≥2/d relative to no coffee intake were 0.89, 0.86, and 0.83, respectively (P-trend = 0.0003). For the same black tea categories the HRs were 0.95, 0.90, and 0.72, respectively (P-trend = 0.0005). Among ever-smokers there was no association between coffee or black tea and the outcomes. Relative to no alcohol, light to moderate intake was inversely associated with all-cause mortality (HR: 0.87; 95% CI: 0.79, 0.96) in never-smokers with a similar magnitude of association in ever-smokers. There was no association between heavy alcohol intake and all-cause mortality in never-smokers and a strong positive association in ever-smokers (HR: 1.56; 95% CI: 1.40, 1.74). Green tea and sugar-sweetened beverages were not associated with all-cause or cause-specific mortality. CONCLUSIONS: Higher coffee and black tea intake was inversely associated with mortality in never-smokers, light to moderate alcohol intake was inversely associated with mortality regardless of smoking status, heavy alcohol intake was positively associated with mortality in ever-smokers, and there was no association between sugar-sweetened beverages and green tea and mortality." As taken from Odegaard AO et al. 2015. J. Nutr. 145(3), 595-604. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25733477

"BACKGROUND: Sugar-sweetened beverage consumption raises blood glucose concentration and has been positively associated with weight gain and type 2 diabetes, all of which have been implicated in the development of biliary tract cancer (BTC). This study examined the hypothesis that sweetened beverage consumption is positively associated with risk of BTC in a prospective study. METHODS: The study population comprised 70 832 Swedish adults (55.9% men, age 45-83 years) from the Swedish Mammography Cohort and Cohort of Swedish Men who were free of cancer and diabetes and completed a food frequency questionnaire at baseline. Incident BTC case patients were ascertained through

linkage with the Swedish Cancer Register. Cox proportional hazards regression model was used to analyze the data. All statistical tests were two-sided. RESULTS: During a mean follow-up of 13.4 years, 127 extrahepatic BTC case patients (including 71 gallbladder cancers) and 21 intrahepatic BTC case patients were ascertained. After adjustment for other risk factors, women and men in the highest category of combined sugar-sweetened and artificially sweetened beverage consumption had a statistically significantly increased risk of extrahepatic BTC and gallbladder cancer. The multivariable hazard ratios for two or more servings per day (200 mL/serving) of sweetened beverages compared with no consumption were 1.79 (95% confidence interval [CI] = 1.02 to 3.13) for extrahepatic BTC and 2.24 (95% CI = 1.02 to 4.89) for gallbladder cancer. The corresponding hazard ratio for intrahepatic BTC was 1.69 (95% CI = 0.41 to 7.03). CONCLUSIONS: These findings support the hypothesis that high consumption of sweetened beverages may increase the risk of BTC, particularly gallbladder cancer." As taken from Larsson SC et al. 2016. J. Natl Cancer Inst. 108(10), djw125. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27281756

"We herein compared the effects of the chronic feeding of high-fat (HF), high-sucrose (HS), and low-fat/low-sucrose (control) diets on carcinogenesis following chronic ultraviolet B (UVB) irradiation in hairless mice. UVB irradiation-induced carcinogenesis was more prominent in HF diet-fed group than in control diet- and HS diet-fed groups. The HS diet group, as well as the HF diet one, showed tumor development and growth, increased skin matrix metalloproteinase (MMP) and blood plasminogen activator inhibitor-1 (PAI-1) levels, and decreased blood leptin and adiponectin levels after long-term UVB irradiation. These changes were smaller in the HS diet group than in the HF diet group. In addition, no difference was noted in the above changes between the control and HS diet groups. The increase induced in adipose tissue weight by the HF diet was markedly reduced by UVB irradiation. This result suggests that the abundant availability of lipids in hypertrophic adipose tissue may be related to tumor incidence and growth through increases in blood PAI-1 and skin MMP-9 expression levels and decreases in blood adiponectin levels by UVB irradiation. In conclusion, HF diet-induced hypertrophic adipose tissue is an important cancer risk factor that promotes UV irradiation-induced carcinogenesis and tumor growth." As taken from Sumiyoshi M and Kimura Y. 2016. Nutr. Cancer. 68(5), 791-803. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27046042

"Overnutrition can promote liver cancer in mice and humans that have liver damage caused by alcohol, viruses, or carcinogens. However, the mechanism linking diet to increased liver tumorigenesis remains unclear in the context of whether tumorigenesis is secondary to obesity, or whether nutrients like sugar or fat drive tumorigenesis independent of obesity. In male mice, liver tumor burden was recently found to correlate with sugar intake, independent of dietary fat intake and obesity. However, females are less susceptible to developing liver cancer than males, and it remains unclear how nutrition affects tumorigenesis in females. Herein, female mice were exposed to the liver carcinogen diethylnitrosamine (DEN) and fed diets with well-defined sugar and fat content. Mice fed diets with high sugar content had the greatest liver tumor incidence while dietary fat intake was not associated with tumorigenesis. Diet-induced postprandial hyperglycemia and fasting hyperinsulinemia significantly correlated with tumor incidence, while tumor incidence was not associated with obesity and obesity-related disorders including liver steatosis, glucose intolerance, or elevated serum levels of estrogen, ALT, and lipids. These results simplify the pathophysiology of diet-induced liver tumorigenesis by focusing attention on the role of sugar metabolism and reducing emphasis on the complex milieu associated with

obesity." As taken from Healy ME et al. 2016. Sci. Rep. 6, 22292. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26924712

"MicroRNAs (miRNAs) are small non-protein-coding RNA molecules that regulate gene expression. Diet and lifestyle factors have been hypothesized to be involved in the regulation of miRNA expression. In this study it was hypothesized that diet and lifestyle factors are associated with miRNA expression. Data from 1,447 cases of colorectal cancer to evaluate 34 diet and lifestyle variables using miRNA expression in normal colorectal mucosa as well as for differential expression between paired carcinoma and normal tissue were used. miRNA data were obtained using an Agilent platform. Multiple comparisons were adjusted for using the false discovery rate q-value. There were 250 miRNAs differentially expressed between carcinoma and normal colonic tissue by level of carbohydrate intake and 198 miRNAs differentially expressed by the level of sucrose intake. Of these miRNAs, 166 miRNAs were differentially expressed for both carbohydrate intake and sucrose intake. Ninety-nine miRNAs were differentially expressed by the level of whole grain intake in normal colonic mucosa. Level of oxidative balance score was associated with 137 differentially expressed miRNAs between carcinoma and paired normal rectal mucosa. Additionally, 135 miRNAs were differentially expressed in colon tissue based on recent NSAID use. Other dietary factors, body mass index, waist and hip circumference, and long-term physical activity levels did not alter miRNA expression after adjustment for multiple comparisons. These results suggest that diet and lifestyle factors regulate miRNA level. They provide additional support for the influence of carbohydrate, sucrose, whole grains, NSAIDs, and oxidative balance score on colorectal cancer risk." As taken from Slattery ML et al. 2016. Pharmgenomics Pers. Med. 10, 1-16. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28053552

"High sugar intake may increase cancer risk by promoting insulin-glucose dysregulation, oxidative stress, inflammation, and body adiposity, but epidemiologic evidence is unclear. Associations between dietary sugars and lifestyle-related cancer risk from longitudinal studies were evaluated. We systematically searched PubMed, Embase, and CINAHL and identified 37 prospective cohort studies (1990-2017) reporting multivariable adjusted risk estimates for dietary sugars in relation to cancer. Of 15 and 14 studies on total sugar and sucrose respectively, 11 reported a null association in relation to cancer. Of 14 studies on fructose, 8 reported null associations, and 2 reported protective and 4 reported detrimental associations. In two of five studies on added sugars, a 60-95% increased cancer risk was observed with higher intakes. In 8 of 15 studies on sugary foods and beverages, a 23-200% higher cancer risk was observed with higher sugary beverage consumption. In conclusion, most studies were indicative of a null association, but suggestive detrimental associations were reported for added sugars and sugary beverages." As taken from Makarem N et al. 2018a. Annu. Rev. Nutr. 38, 17-39. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29801420

"Background: Higher sugar consumption may increase cancer risk by promoting insulin-glucose dysregulation, oxidative stress, hormonal imbalances, and excess adiposity. This prospective study investigates the association between dietary sugars (fructose and sucrose) and sugary foods and beverages in relation to combined and site-specific (breast, prostate, colorectal) adiposity-associated cancers.Methods: The analytic sample consisted of 3,184 adults, aged 26-84 years, from the Framingham Offspring cohort. Diet data were first collected between 1991 and 1995 using a food frequency questionnaire. Intakes of fructose, sucrose, sugary foods, and sugary beverages (fruit juice and sugar-sweetened

beverages) were derived. Participants were followed up until 2013 to ascertain cancer incidence; 565 doctor-diagnosed adiposity-related cancers, including 124 breast, 157 prostate, and 68 colorectal cancers occurred. Multivariable-adjusted Cox proportional hazards models were used to evaluate associations. Tests for interaction with BMI and waist circumference were conducted.Results: No associations were observed between fructose, sucrose, sugary food consumption, and combined incidence of adiposity-related cancers or the examined site-specific cancers. While total consumption of sugary beverages was not associated with site-specific cancer risk, higher intakes of fruit juice were associated with 58% increased prostate cancer risk (HR: 1.58; 95% CI, 1.04-2.41) in multivariable-adjusted models. In exploratory stratified analyses, higher sugary beverage intakes increased overall adiposity-related cancer risk by 59% in participants with excessive central adiposity (HR: 1.59; 95% CI, 1.01-2.50; Ptrend = 0.057).Conclusions: In this cohort of American adults, higher sugary beverage consumption was associated with increased cancer risk among participants with central adiposity.Impact: These analyses suggest that avoiding sugary beverages represents a simple dietary modification that may be used as an effective cancer control strategy." As taken from Makarem N et al. 2018b. Cancer Prev. Res. (Phila.) 11(6), 347-358. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29674390

"In 1965, the Sugar Research Foundation (SRF) secretly funded a review in the New England Journal of Medicine that discounted evidence linking sucrose consumption to blood lipid levels and hence coronary heart disease (CHD). SRF subsequently funded animal research to evaluate sucrose's CHD risks. The objective of this study was to examine the planning, funding, and internal evaluation of an SRF-funded research project titled "Project 259: Dietary Carbohydrate and Blood Lipids in Germ-Free Rats," led by Dr. W.F.R. Pover at the University of Birmingham, Birmingham, United Kingdom, between 1967 and 1971. A narrative case study method was used to assess SRF Project 259 from 1967 to 1971 based on sugar industry internal documents. Project 259 found a statistically significant decrease in serum triglycerides in germ-free rats fed a high sugar diet compared to conventional rats fed a basic PRM diet (a pelleted diet containing cereal meals, soybean meals, whitefish meal, and dried yeast, fortified with a balanced vitamin supplement and trace element mixture). The results suggested to SRF that gut microbiota have a causal role in carbohydrate-induced hypertriglyceridemia. A study comparing conventional rats fed a high-sugar diet to those fed a high-starch diet suggested that sucrose consumption might be associated with elevated levels of beta-glucuronidase, an enzyme previously associated with bladder cancer in humans. SRF terminated Project 259 without publishing the results. The sugar industry did not disclose evidence of harm from animal studies that would have (1) strengthened the case that the CHD risk of sucrose is greater than starch and (2) caused sucrose to be scrutinized as a potential carcinogen. The influence of the gut microbiota in the differential effects of sucrose and starch on blood lipids, as well as the influence of carbohydrate quality on beta-glucuronidase and cancer activity, deserve further scrutiny." As taken from Kearns CE et al. 2017. PLoS Biol. 15(11), e2003460. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29161267

"PURPOSE: An association between dietary carbohydrate intake and prostate cancer (PCa) prognosis is biologically plausible, but data are scarce. This prospective cohort study examined the relation between pre-diagnostic carbohydrate intake and treatment failure following radical prostatectomy for clinically early-stage PCa. METHODS: We identified 205 men awaiting radical prostatectomy and assessed their usual dietary intake of

carbohydrates using the 110-item Block food frequency questionnaire. We also evaluated carbohydrate intake quality using a score based on the consumption of sugars relative to fiber, fat, and protein. Logistic regression analyzed their associations with the odds of treatment failure, defined as a detectable and rising serum prostate-specific antigen (PSA) or receiving androgen deprivation therapy (ADT) within 2 years. RESULTS: Sucrose consumption was associated with a higher odds and fiber consumption with a lower odds of ADT after accounting for age, race/ethnicity, body mass index, and tumor characteristics (odds ratio [OR] (95% confidence interval [CI]) 5.68 (1.71, 18.9) for 3rd vs. 1st sucrose tertile and 0.88 (0.81, 0.96) per gram of fiber/day, respectively). Increasing carbohydrate intake quality also associated with a lower odds of ADT (OR (95% CI) 0.78 (0.66, 0.92) per unit increase in score, range 0-12). CONCLUSIONS: Pre-diagnostic dietary carbohydrate intake composition and quality influence the risk of primary treatment failure for early-stage PCa. Future studies incorporating molecular aspects of carbohydrate metabolism could clarify possible underlying mechanisms." As taken from Kim K et al. 2019. Cancer Causes Control 30(3), 271-279. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30729360

"A high energy intake contributes to obesity, a risk factor for cancer. We previously reported that an excessive intake of dietary fat enhances malignant spread in mice. This study tested the hypothesis that consumption of a diet with an excessive amount of sucrose enhances metastasis. In a spontaneous metastasis model of Lewis lung carcinoma (LLC), male C57BL/6 mice were maintained on an AIN93G, a high-fat, or a high-sucrose diet for the duration of the study. Pulmonary metastases from a primary tumor, established by a subcutaneous injection of LLC cells, were quantified. There were no differences in energy intake among the 3 groups. The percent body fat mass of the high-sucrose group, while higher than that of the AIN93G group, was lower than that of the high-fat group. The number and size of lung metastases were significantly higher in the high-fat group than in the AIN93G group; these measurements in the high-sucrose group remained similar to those in the AIN93G group. Hepatic concentrations of triacylglycerols and plasma concentrations of insulin, proinflammatory cytokines (leptin, plasminogen activator inhibitor-1, and monocyte chemotactic protein-1) and angiogenic factors (vascular endothelial growth factor and tissue inhibitor of metalloproteinase-1) in the high-sucrose group were significantly lower than those in the high-fat group. In conclusion, the high-sucrose diet does not enhance spontaneous metastasis of LLC. This null effect may be due to the inadequate production of tumorigenic proinflammatory cytokines and angiogenic factors by the high-sucrose diet compared to the high-fat diet." As taken from Yan L and Sundaram S. 2018. Nutr. Res. 58, 55-61. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30340815

5.7. Irritation/immunotoxicity

Test article	Concentration/ Dose	Test population	Procedure	Results
rinse-off hair product containing 29% sucrose	diluted to 50% in distilled water 0.02 ml over 50 mm^2	102 subjects	HRIPT using 48-72 h occlusive patches for induction, and a 48-h patch at challenge	Not an irritant or sensitizer. Mean irritation index of <0.25; 16% of the subjects presented with score ≥2 reactions during induction.

“The (CIR) Panel discussed a human repeated insult patch test of a hair product that contained 29% sucrose, diluted to 50% in water, that reported irritation observed during induction. The Panel concluded that the irritation reported was likely attributable to a surfactant effect, and was not due to sucrose. Furthermore, the Panel acknowledged that sucrose and glucose are used in cosmetics at relatively high concentrations, and that data from irritation and sensitization studies at maximum use concentrations of these ingredients are lacking; however, based on the clinical experience of the Panel, there is little concern that these ingredients are irritants or sensitizers.”

As taken from CIR, 2014.

“May cause mechanical irritation” (Haz-Map, 2018).

“While numerous changes in human lifestyle constitute modern life, our diet has been gaining attention as a potential contributor to the increase in immune-mediated diseases. The Western diet is characterized by an over consumption and reduced variety of refined sugars, salt, and saturated fat. Herein our objective is to detail the mechanisms for the Western diet's impact on immune function. The manuscript reviews the impacts and mechanisms of harm for our over-indulgence in sugar, salt, and fat, as well as the data outlining the impacts of artificial sweeteners, gluten, and genetically modified foods; attention is given to revealing where the literature on the immune impacts of macronutrients is limited to either animal or in vitro models versus where human trials exist. Detailed attention is given to the dietary impact on the gut microbiome and the mechanisms by which our poor dietary choices are encoded into our gut, our genes, and are passed to our offspring. While today's modern diet may provide beneficial protection from micro- and macronutrient deficiencies, our over abundance of calories and the macronutrients that compose our diet may all lead to increased inflammation, reduced control of infection, increased rates of cancer, and increased risk for allergic and auto-inflammatory disease.” As taken from Myles IA 2014. Nutr. J. 13, 61. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24939238

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing sugars (sucrose) was 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 TPM was not increased by the

addition of sugars (sucrose) when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	39	JTI KB Study Report(s)
In vitro cytotoxicity	39	JTI KB Study Report(s)

“Both controversy and confusion exist concerning fructose, sucrose, and high-fructose corn syrup (HFCS) with respect to their metabolism and health effects. These concerns have often been fueled by speculation based on limited data or animal studies. In retrospect, recent controversies arose when a scientific commentary was published suggesting a possible unique link between HFCS consumption and obesity. Since then, a broad scientific consensus has emerged that there are no metabolic or endocrine response differences between HFCS and sucrose related to obesity or any other adverse health outcome. This equivalence is not surprising given that both of these sugars contain approximately equal amounts of fructose and glucose, contain the same number of calories, possess the same level of sweetness, and are absorbed identically through the gastrointestinal tract. Research comparing pure fructose with pure glucose, although interesting from a scientific point of view, has limited application to human nutrition given that neither is consumed to an appreciable degree in isolation in the human diet. Whether there is a link between fructose, HFCS, or sucrose and increased risk of heart disease, metabolic syndrome, or fatty infiltration of the liver or muscle remains in dispute with different studies using different methodologies arriving at different conclusions. Further randomized clinical trials are needed to resolve many of these issues. The purpose of this review is to summarize current knowledge about the metabolism, endocrine responses, and potential health effects of sucrose, HFCS, and fructose.” As taken from Rippe JM & Angelopoulos TJ. 2013. Adv. Nutr. 4(2), 236-45. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23493540?dopt=AbstractPlus

“We investigated the effects of host diet on the intestinal persistence and gene expression of Lactobacillus plantarum WCFS1 in healthy and health-compromised, 2,4,6-trinitrobenzene sulfonic acid (TNBS)-treated mice. Mice fed either a low-fat chow diet (CD) or high fat and sucrose Western diet (WD) received 10(9) L. plantarum WCFS1 cells for five consecutive days. Lactobacillus plantarum persistence was 10- to 100-fold greater in the intestines of WD-fed compared with CD-fed mice. TNBS, an intestinal irritant that induces the development of inflammatory bowel disease-like symptoms, resulted in up to a 10(4) -fold increase in L. plantarum survival in the digestive tract relative to healthy animals. Expression levels of 12 metabolic and gut-inducible L. plantarum genes were differentially affected by diet and TNBS administration. Pyrosequencing of 16S rRNA transcripts from the indigenous intestinal microbiota showed that WD resulted in significant reductions in proportions of metabolically active indigenous Lactobacillus species and increases in the Desulfovibrionaceae family. Feeding L. plantarum WCFS1 resulted in lower levels of colitis and higher concentrations of colonic IL-10 and IL-12 in WD and not CD-fed mice. Interactions between probiotics, nutritional components and the intestinal bacteria should be considered when examining for probiotic-mediated effects and elucidating mechanisms of probiotic function in the mammalian gut.” As taken from Tachon S et al. 2014. Environ. Microbiol. 16(9), 2915-26. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24118739

“OBJECTIVE: The calorie distribution of macronutrients affects individuals' health. Quantity and source of macronutrients may play major roles in waist circumference (WC) and hip

circumference (HC). This study's purpose is to investigate the association between the quantity and source of macronutrients and the change in WC and HC over 9 years. METHODS: Participants (N = 11,343) were from the Atherosclerosis Risk in Community Study. Those diagnosed with cancer or a decrease in WC or HC of 15 cm or more over 9 years were excluded. Change scores were created for anthropometrics between clinic visits over 9 years. Macronutrient intakes were assessed by a food frequency questionnaire at visit 1 and presented as a percentage of energy intake. Linear regressions were performed with quartiles of dietary components on change scores for WC and HC with controlling cofactors. Gender subgroup analysis was performed. RESULTS: A larger increase in WC was associated with higher intakes of total carbohydrates, dietary fiber, and fructose (p for trend < 0.005). A smaller increase in WC was associated with higher intakes of sucrose, total protein, animal protein, and alcohol (p for trend < 0.02). A larger increase in HC was associated with higher intakes of total carbohydrate, sucrose, fructose, animal protein, and vegetable fat (p for trend < 0.05). A smaller increase in HC was associated with higher intakes of animal fat, total fat, and total protein (p for trend < 0.05). In males, changes in WC and HC were associated with fructose, sucrose, total fat, and total protein. In females, changes in WC and HC were associated with dietary fiber, fructose, alcohol, animal protein, total protein, animal fat, and vegetable fat. CONCLUSION: Macronutrient source and quantity play a significant role in individuals' adiposity and effects on WC and HC. Overall, an increase in WC and HC was seen over the 11 years. The source and quantity of the macronutrients play a significant role in WC and HC. Further research needs to be conducted to see the exact effect that macronutrients play on WC and HC." As taken from Lofley AC and Root MM. 2017. J. Am. Coll. Nutr. 36(1), 57-63. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27797648

"BACKGROUND: The objective of this study was to compare, in an animal model, the effect of different sugar types (sucrose vs. high-fructose corn syrup 55%) consumed as 10% by weight of the diet (11.6% of daily caloric intake) on the amount of food consumed, body weight, fatty tissue deposits, concentrations of selected lipids, and atherogenic indices of blood plasma. Material and method. The experiment was carried out on 30 5-month-old Wistar male rats, fed three differ- ent diets, containing, amongst other foods, (1) ground unrefined cereal grains, (2) sucrose, (3) high-fructose corn syrup. Results. Weight gains in animals on sucrose or high-fructose corn syrup diets were higher than those con- suming basic feed, but the effect was not associated with perivisceral fat accumulation. It has been found that all the atherogenic indices (Castelli's Risk Index I, Castelli's Risk Index II, Atherogenic Index of Plasma, Atherogenic Coefficient) were statistically significantly higher in animals on a high-fructose corn syrup diet compared to both the control group and those on a sucrose diet. Conclusion. The effect of the 55% high-fructose corn syrup on the tested parameters of lipid metabolism was not equivalent to that of sucrose. Using HFCS-55 instead of sucrose has an adverse effect on blood lipid parameters, while weight gains and peri-organ fat deposits are comparable. Moreover, the obtained results confirm that tested animals were susceptible to the adverse effects of sugars added to their diet, even in small amounts. This emphasises the need to precisely control the amount of added sugars in. METHODS: nd. The objective of this study was to compare, in an animal model, the effect of different sugar types (sucrose vs. high-fructose corn syrup 55%) consumed as 10% by weight of the diet (11.6% of daily caloric intake) on the amount of food consumed, body weight, fatty tissue deposits, concentrations of selected lipids, and atherogenic indices of blood plasma. Material and method. The experiment was carried out on 30 5-month-old Wistar male rats, fed three differ- ent diets, containing, amongst other foods, (1) ground

unrefined cereal grains, (2) sucrose, (3) high-fructose corn syrup. RESULTS: Weight gains in animals on sucrose or high-fructose corn syrup diets were higher than those con- suming basic feed, but the effect was not associated with perivisceral fat accumulation. It has been found that all the atherogenic indices (Castelli's Risk Index I, Castelli's Risk Index II, Atherogenic Index of Plasma, Atherogenic Coefficient) were statistically significantly higher in animals on a high-fructose corn syrup diet compared to both the control group and those on a sucrose diet. CONCLUSIONS: The effect of the 55% high-fructose corn syrup on the tested parameters of lipid metabolism was not equivalent to that of sucrose. Using HFCS-55 instead of sucrose has an adverse effect on blood lipid parameters, while weight gains and peri-organ fat deposits are comparable. Moreover, the obtained results confirm that tested animals were susceptible to the adverse effects of sugars added to their diet, even in small amounts. This emphasises the need to precisely control the amount of added sugars in the diet." As taken from Sadowska J and Bruszkowska M. 2017. Acta Sci. Pol. Technol. Aliment. 16(2), 231-240. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28703963

"Trehalose is a natural disaccharide that is found in a diverse range of organisms but not in mammals. Autophagy is a process which mediates the sequestration, lysosomal delivery and degradation of proteins and organelles. Studies have shown that trehalose exerts beneficial effects through inducing autophagy in mammalian cells. However, whether trehalose or other saccharides can activate autophagy in keratinocytes is unknown. Here, we found that trehalose treatment increased the LC3-I to LC3-II conversion, acridine orange-stained vacuoles and GFP-LC3B (LC3B protein tagged with green fluorescent protein) puncta in the HaCaT human keratinocyte cell line, indicating autophagy induction. Trehalose-induced autophagy was also observed in primary keratinocytes and the A431 epidermal cancer cell line. mTOR signalling was not affected by trehalose treatment, suggesting that trehalose induced autophagy through an mTOR-independent pathway. mTOR-independent autophagy induction was also observed in HaCaT and HeLa cells treated with sucrose or raffinose but not in glucose, maltose or sorbitol treated HaCaT cells, indicating that autophagy induction was not a general property of saccharides. Finally, although trehalose treatment had an inhibitory effect on cell proliferation, it had a cytoprotective effect on cells exposed to UVB radiation. Our study provides new insight into the saccharide-mediated regulation of autophagy in keratinocytes." As taken from Chen X et al. 2016. Sci. Rep. 6, 28423. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27328819

"Background: The co-epidemic of obesity and type 2 diabetes is associated with increased morbidity and mortality. Genetic factors are highly involved in the development of these diseases, in the form of interactions of multiple genes within obesogenic and diabetogenic environments, such as a high fat diet. The TALLYHO/Jng (TH) mouse is an inbred polygenic model for human obesity and type 2 diabetes. In order to further develop the TH mouse as a clinically relevant model, we investigated diet dependence of obesity and type 2 diabetes in TH mice vs. C57BL/6 (B6) mice. Results: TH and B6 mice were weaned onto a standard rodent chow, semi-purified high-sucrose low-fat (HSLF), or semi-purified high-sucrose high-fat (HSHF) diet and maintained on these diets throughout the study. Despite similar fat contents in HSLF diets and chow, both B6 and TH mice responded to HSLF diets, with increases in adiposity. TH mice, but not B6 mice, exhibited significantly higher adiposity with severely aggravated glucose intolerance and hyperglycemia on HSHF diets compared to the other diets. HSLF diets also advanced diabetes in TH mice compared to chow, but it did not surpass the effects of HSHF diets. The severe glucose intolerance and

hyperglycemia in TH mice on both HSLF and HSHF diets were accompanied by significantly reduced Glut4 mRNA levels compared to B6 mice. Conclusions: The present data demonstrate that diets are important modulators of genetic susceptibility to type 2 diabetes and obesity in TH mice. The interplay between heredity and dietary environment in TH mice appears to amplify insulin resistance, contributing to severe glucose intolerance and diabetes." As taken from Parkman JK et al. 2016. Exp. Clin. Endocrinol. Diabetes 124(10), 622-629. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27437918

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human - liver tumor	200 mmol/L/24H	In Vitro Toxicity Studies - cell protein synthesis	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1-1987- Volume(issue)/page/year: 3,189,1989
IC50 - Inhibitor Concentration 50	In vitro	Human - lung tumor	>100 mg/L/48H	In Vitro Toxicity Studies - cell protein synthesis	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 155,1393,2014
IC50 - Inhibitor Concentration 50	In vitro	Rat - glioma	>100 mg/L/48H	In Vitro Toxicity Studies - cell protein synthesis	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 155,1393,2014

As taken from RTECS, 2018.

High-throughput Assay Data

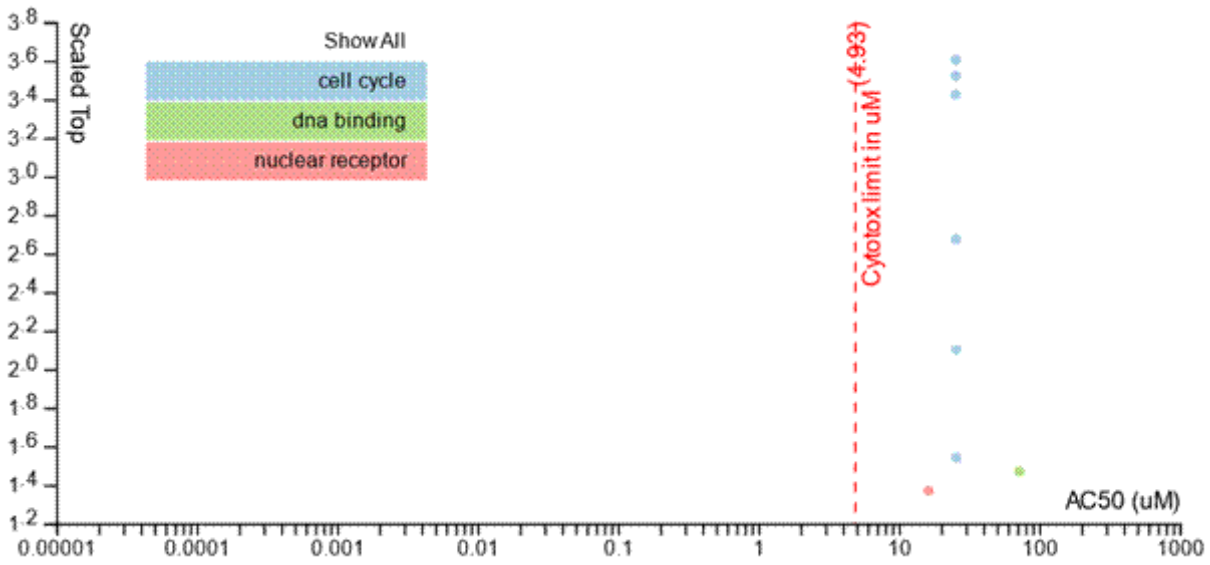
The US Environmental Protection Agency (EPA) evaluated sucrose in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: https://comptox.epa.gov/dashboard

EPA provides the following data use considerations for ToxCast data: "The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of

the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve."

A summary of the ToxCast assay data on sucrose is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on EPA's CompTox Dashboard.

Figure 1



6. Functional effects on

6.1. Broncho/pulmonary system

Workers in a sugar cube factory had lower lung function (as measured by FEV1) when exposed to undisclosed atmospheric concentrations of sugar dust, than did nonexposed workers and laboratory and office controls (Bohadana et al. 1996).

"BACKGROUND: There is limited research examining beverage habits, one of the most habitual dietary behaviors, with mortality risk. OBJECTIVE: This study examined the association between coffee, black and green tea, sugar-sweetened beverages (soft drinks and juice), and alcohol and all-cause and cause-specific mortality. METHODS: A prospective data analysis was conducted with the use of the Singapore Chinese Health Study, including 52,584 Chinese men and women (aged 45-74 y) free of diabetes, cardiovascular disease (CVD), and cancer at baseline (1993-1998) and followed through 2011 with 10,029 deaths. Beverages were examined with all-cause and cause-specific (cancer, CVD, and respiratory disease) mortality risk with the use of Cox proportional hazards regression. RESULTS: The associations between coffee, black tea, and alcohol intake and all-cause mortality were modified by smoking status. Among never-smokers there was an inverse dose-response association between higher amounts of coffee and

black tea intake and all-cause, respiratory-related, and CVD mortality (black tea only). The fully adjusted HRs for all-cause mortality for coffee for <1/d, 1/d, and ≥2/d relative to no coffee intake were 0.89, 0.86, and 0.83, respectively (P-trend = 0.0003). For the same black tea categories the HRs were 0.95, 0.90, and 0.72, respectively (P-trend = 0.0005). Among ever-smokers there was no association between coffee or black tea and the outcomes. Relative to no alcohol, light to moderate intake was inversely associated with all-cause mortality (HR: 0.87; 95% CI: 0.79, 0.96) in never-smokers with a similar magnitude of association in ever-smokers. There was no association between heavy alcohol intake and all-cause mortality in never-smokers and a strong positive association in ever-smokers (HR: 1.56; 95% CI: 1.40, 1.74). Green tea and sugar-sweetened beverages were not associated with all-cause or cause-specific mortality. CONCLUSIONS: Higher coffee and black tea intake was inversely associated with mortality in never-smokers, light to moderate alcohol intake was inversely associated with mortality regardless of smoking status, heavy alcohol intake was positively associated with mortality in ever-smokers, and there was no association between sugar-sweetened beverages and green tea and mortality.." As taken from Odegaard AO et al. 2015. J. Nutr. 145(3), 595-604. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25733477

6.2. Cardiovascular system

Characterization of sucrose-induced changes in cardiac phenotype (Abstract). The neuroendocrine factors responsible for long-term regulation of cardiac contractile performance remain ill defined. We examined influences of diet on the expression of myosin isozymes, sarcoplasmic reticulum (SR) Ca(2+) uptake and serum parameters. Dietary regimens (ad libitum feeding, intermittent fasting and 32% sucrose feeding) were used to alter the neurohumoral status of rats. Intermittent fasting decreased serum insulin levels (P<0.05) and was associated with decreased SR Ca(2+) uptake and myosin V1 proportion (P<0.05). Sucrose (32%) feeding increased myosin V1 of fasted and ad libitum fed rats (P<0.05) but had no effect on insulin or SR Ca(2+) uptake. Expression of the alpha-myosin heavy chain correlated with serum insulin. Treatment of sucrose-fed rats with the sympatholytic compound moxonidine and the hypoglycaemic compounds BM13.907 and ciglitazone partially prevented the increase in myosin V1 (P<0.05) but had no effect on SR Ca(2+) uptake and insulin. The data show that adrenergic activity and metabolic signals are important for an increase in myosin V1 in sucrose-fed rats, which can be associated with an unaltered SR Ca(2+) uptake rate. As taken from Rupp H et al. Pflugers Arch. 2002 Oct; 445(1):32-9 PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=12397384&query_hl=40&itool=pubmed_DocSum

Reducing Consumption of Sugar-Sweetened Beverages Is Associated With Reduced Blood Pressure A Prospective Study Among United States Adults

Background— Increased consumption of sugar-sweetened beverages (SSBs) has been associated with an elevated risk of obesity, metabolic syndrome, and type II diabetes mellitus. However, the effects of SSB consumption on blood pressure (BP) are uncertain.

The objective of this study was to determine the relationship between changes in SSB consumption and changes in BP among adults.

Methods and Results— This was a prospective analysis of 810 adults who participated in the PREMIER Study (an 18-month behavioral intervention trial). BP and dietary intake (by two 24-hour recalls) were measured at baseline and at 6 and 18 months. Mixed-effects models were applied to estimate the changes in BP in responding to changes in SSB consumption. At baseline, mean SSB intake was 0.9±1.0 servings per day (10.5±11.9 fl oz/d), and mean systolic BP/diastolic BP was 134.9±9.6/84.8±4.2 mm Hg. After potential confounders were controlled for, a reduction in SSB of 1 serving per day was associated with a 1.8-mm Hg (95% confidence interval, 1.2 to 2.4) reduction in systolic BP and 1.1-mm Hg (95% confidence interval, 0.7 to 1.4) reduction in diastolic BP over 18 months. After additional adjustment for weight change over the same period, a reduction in SSB intake was still significantly associated with reductions in systolic and diastolic BPs (P<0.05). Reduced intake of sugars was also significantly associated with reduced BP. No association was found for diet beverage consumption or caffeine intake and BP. These findings suggest that sugars may be the nutrients that contribute to the observed association between SSB and BP.

Conclusions— Reduced consumption of SSB and sugars was significantly associated with reduced BP. Reducing SSB and sugar consumption may be an important dietary strategy to lower BP. As taken from Chen L et al. Circulation 2010 Jun 8;121(22):2398-406. PubMed, 2012 available at http://www.ncbi.nlm.nih.gov/pubmed/20497980

Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial (Abstract).

Background: Sugar-sweetened beverages (SSBs) have unfavorable effects on glucose and lipid metabolism if consumed in high quantities by obese subjects, but the effect of lower doses in normal-weight subjects is less clear. OBJECTIVE: The aim was to investigate the effects of SSBs consumed in small to moderate quantities for 3 wk on LDL particle distribution and on other parameters of glucose and lipid metabolism as well as on inflammatory markers in healthy young men. Design: Twenty-nine subjects were studied in a prospective, randomized, controlled crossover trial. Six 3-wk interventions were assigned in random order as follows: 600 mL SSBs containing 1)40 g fructose/d [medium fructose (MF)], 2) 80 g fructose/d [high fructose (HF)], 3) 40 g glucose/d [medium glucose (MG)], 4) 80 g glucose/d [high glucose (HG)], 5) 80 g sucrose/d [high sucrose (HS)], or 6) dietary advice to consume low amounts of fructose. Outcome parameters were measured at baseline and after each intervention. Results: LDL particle size was reduced after HF by -0.51 nm (95% CI: -0.19, -0.82 nm) and after HS by -0.43 nm (95% CI: -0.12, -0.74; $P < 0.05$ for both). Similarly, a more atherogenic LDL subclass distribution was seen when fructose-containing SSBs were consumed (MF, HF, and HS: $P < 0.05$). Fasting glucose and high-sensitivity C-reactive protein (hs-CRP) increased significantly after all interventions (by 4-9% and 60-109%, respectively; $P < 0.05$); leptin increased during interventions with SSBs containing glucose only (MG and HG: $P < 0.05$). CONCLUSION: The present data show potentially harmful effects of low to moderate consumption of SSBs on markers of cardiovascular risk such as LDL particles, fasting glucose, and hs-CRP within just 3 wk in healthy young men, which is of particular significance for young consumers. This trial was registered at clinicaltrials.gov as NCT01021969. As taken from Aeberli I et al. Am J Clin

Nutr. 2011 Aug;94(2):479-85. Epub 2011 Jun 15. Available via PubMed at http://www.ncbi.nlm.nih.gov/pubmed/21677052

Sugar-sweetened beverage link to cardiovascular risk factors is unsupported (no abstract). White JS. Am J Clin Nutr. 2012 Mar;95(3):773; author reply 773-4. http://www.ncbi.nlm.nih.gov/pubmed/22350364?dopt=AbstractPlus

Sugar-sweetened beverage, sugar intake of individuals, and their blood pressure: international study of macro/micronutrients and blood pressure (Abstract).

The obesity epidemic has focused attention on relationships of sugars and sugar-sweetened beverages (SSBs) to cardiovascular risk factors. Here we report cross-sectional associations of SSBs, diet beverages, and sugars with blood pressure (BP) for United Kingdom and US participants of the International Study of Macro/Micronutrients and Blood Pressure. Data collected include four 24-hour dietary recalls, two 24-hour urine collections, 8 BP readings, and questionnaire data for 2696 people ages 40 to 59 years of age from 10 US/United Kingdom population samples. Associations of SSBs, diet beverages, and sugars (fructose, glucose, and sucrose) with BP were assessed by multiple linear regression. SSB intake related directly to BP, with P values of 0.005 to <0.001 (systolic BP) and 0.14 to <0.001 (diastolic BP). SSB intake higher by 1 serving per day (355 mL/24 hours) was associated with systolic/diastolic BP differences of +1.6/+0.8 mm Hg (both P<0.001) and +1.1/+0.4 mm Hg (P<0.001/<0.05) with adjustment for weight and height. Diet beverage intake was inversely associated with BP (P 0.41 to 0.003). Fructose- and glucose-BP associations were direct, with significant sugar-sodium interactions: for individuals with above-median 24-hour urinary sodium excretion, fructose intake higher by 2 SD (5.6% kcal) was associated with systolic/diastolic BP differences of +3.4/+2.2 mm Hg (both P<0.001) and +2.5/+1.7 mm Hg (both P=0.002) with adjustment for weight and height. Observed independent, direct associations of SSB intake and BP are consistent with recent trial data. These findings, plus adverse nutrient intakes among SSB consumers, and greater sugar-BP differences for persons with higher sodium excretion lend support to recommendations that intake of SSBs, sugars, and salt be substantially reduced. As taken from Brown IJ et al. Hypertension. 2011 Apr;57(4):695-701. Epub 2011 Feb 28. Available via PubMed at http://www.ncbi.nlm.nih.gov/pubmed/21357284?dopt=AbstractPlus

“BACKGROUND: Although previous studies have linked intake of sugars with incidence of cancer and other chronic diseases, its association with mortality remains unknown. OBJECTIVE: We investigated the association of total sugars, added sugars, total fructose, added fructose, sucrose, and added sucrose with the risk of all-cause, cardiovascular disease, cancer, and other-cause mortality in the NIH-AARP Diet and Health Study. DESIGN: The participants (n = 353,751), aged 50-71 y, were followed for up to 13 y. Intake of individual sugars over the previous 12 mo was assessed at baseline by using a 124-item NIH Diet History Questionnaire. RESULTS: In fully adjusted models (fifth quartile compared with first quartile), all-cause mortality was positively associated with the intake of total sugars [HR (95% CI): 1.13 (1.06, 1.20); P-trend < 0.0001], total fructose [1.10 (1.04, 1.17); P-trend < 0.0001], and added fructose [1.07 (1.01, 1.13); P-trend = 0.005) in women and total fructose [1.06 (1.01, 1.10); P-trend = 0.002] in men. In men, a weak inverse association was found between other-cause mortality and dietary added sugars (P-trend = 0.04), sucrose (P-trend = 0.03), and added sucrose (P-trend = 0.006). Investigation of consumption of sugars by source showed that the positive association with mortality risk was confined only to sugars from beverages, whereas the inverse association was confined to sugars from solid foods. CONCLUSIONS: In this large prospective study, total fructose

intake was weakly positively associated with all-cause mortality in both women and men, whereas added sugars, sucrose, and added sucrose intakes were inversely associated with other-cause mortality in men. In our analyses, intake of added sugars was not associated with an increased risk of mortality. The NIH-AARP Diet and Health Study was registered at clinicaltrials.gov as NCT00340015." As taken from Tasevska N et al. 2014b. Am. J. Clin. Nutr. 99(5), 1077-88. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24552754

"Hypertension remains a major health problem worldwide considering the prevalence of morbidity and mortality. Plants remain a reliable source of efficacious and better tolerated drugs and botanicals. This study was designed to investigate the effect of the chemo-profiled hydroethanolic leaf extract of Byrsocarpus coccineus in ethanol- and sucrose-induced hypertension. Groups of rats were treated orally (p.o.) with distilled water (10 ml/kg), ethanol (35%; 3 g/kg), sucrose (5-7%), and B. coccineus (100, 200, and 400 mg/kg), and nifedipine together with ethanol and sucrose separately for 8 weeks. At the end of the treatment period, blood pressure and heart rate of rats were determined. Blood was collected for serum biochemical parameters and lipid profile assessment, and the liver, aorta, kidney, and heart were harvested for estimation of in vivo antioxidants and malondialdehyde (MDA). Results obtained in this study showed that B. coccineus at the various doses administered reduced the systolic, diastolic, and arterial blood pressure elevated by ethanol and sucrose. Also, the extract reversed the reduction in catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD) induced by ethanol and sucrose. The level of MDA was reduced compared to the ethanol- and sucrose-induced hypertensive group. With respect to lipid profile, administration of B. coccineus at the various doses reduced the levels of triglycerides, low-density lipoprotein (LDL), cholesterol, and atherogenic indices, compared to the ethanol and sucrose groups. In conclusion the hydroethanolic leaf extract of B. coccineus exerted significant antihypertensive effect and this is probably related to the antioxidant property and improvement of lipid profile observed in this study." As taken from Akindele AJ et al. 2014. J. Tradit. Complement. Med. 4(3), 177-88. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25161923

"BACKGROUND: There is limited research examining beverage habits, one of the most habitual dietary behaviors, with mortality risk. OBJECTIVE: This study examined the association between coffee, black and green tea, sugar-sweetened beverages (soft drinks and juice), and alcohol and all-cause and cause-specific mortality. METHODS: A prospective data analysis was conducted with the use of the Singapore Chinese Health Study, including 52,584 Chinese men and women (aged 45-74 y) free of diabetes, cardiovascular disease (CVD), and cancer at baseline (1993-1998) and followed through 2011 with 10,029 deaths. Beverages were examined with all-cause and cause-specific (cancer, CVD, and respiratory disease) mortality risk with the use of Cox proportional hazards regression. RESULTS: The associations between coffee, black tea, and alcohol intake and all-cause mortality were modified by smoking status. Among never-smokers there was an inverse dose-response association between higher amounts of coffee and black tea intake and all-cause, respiratory-related, and CVD mortality (black tea only). The fully adjusted HRs for all-cause mortality for coffee for <1/d, 1/d, and ≥2/d relative to no coffee intake were 0.89, 0.86, and 0.83, respectively (P-trend = 0.0003). For the same black tea categories the HRs were 0.95, 0.90, and 0.72, respectively (P-trend = 0.0005). Among ever-smokers there was no association between coffee or black tea and the outcomes. Relative to no alcohol, light to moderate intake was inversely associated with all-cause

mortality (HR: 0.87; 95% CI: 0.79, 0.96) in never-smokers with a similar magnitude of association in ever-smokers. There was no association between heavy alcohol intake and all-cause mortality in never-smokers and a strong positive association in ever-smokers (HR: 1.56; 95% CI: 1.40, 1.74). Green tea and sugar-sweetened beverages were not associated with all-cause or cause-specific mortality. CONCLUSIONS: Higher coffee and black tea intake was inversely associated with mortality in never-smokers, light to moderate alcohol intake was inversely associated with mortality regardless of smoking status, heavy alcohol intake was positively associated with mortality in ever-smokers, and there was no association between sugar-sweetened beverages and green tea and mortality." As taken from Odegaard AO et al. 2015. J. Nutr. 145(3), 595-604. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25733477

"A number of prospective cohort studies have investigated the associations between consumption of sugar-sweetened beverages (SSB) and the risk of hypertension, CHD and stroke, but revealed mixed results. In the present study, we aimed to perform a dose-response meta-analysis of these prospective studies to clarify these associations. A systematic literature search was conducted using the PubMed and Embase databases up to 5 May 2014. Random- or fixed-effects models were used to calculate the pooled relative risks (RR) with 95 % CI for the highest compared with the lowest category of SSB consumption, and to conduct a dose-response analysis. A total of six prospective studies (240 726 participants and 80 411 incident cases of hypertension) from four publications on hypertension were identified. A total of four prospective studies (194 664 participants and 7396 incident cases of CHD) from four publications on CHD were identified. A total of four prospective studies (259 176 participants and 10 011 incident cases of stroke) from four publications on stroke were identified. The summary RR for incident hypertension was 1·08 (95 % CI 1·04, 1·12) for every additional one serving/d increase in SSB consumption. The summary RR for incident CHD was 1·17 (95 % CI 1·10, 1·24) for every serving/d increase in SSB consumption. There was no significant association between SSB consumption and total stroke (summary RR 1·06, 95 % CI 0·97, 1·15) for every serving/d increase in SSB consumption. The present meta-analysis suggested that a higher consumption of SSB was associated with a higher risk of hypertension and CHD, but not with a higher risk of stroke." As taken from Xi B et al. 2015. Br. J. Nutr. 113(5), 709-17. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25735740

"PURPOSE: The objective of this study is to investigate the effect of dual blockage of renin-angiotensin system (RAS) on renal kallikrein expression and inflammatory response in diabetic nephropathy (DN). METHODS: Rats were randomly divided into 5 groups with 10 rats in each group: normal control; DN model induced by high fat and high sucrose diets; and DN treated with either benazepril 10 mg/kg/d, irbesartan 30 mg/kg/d, or both. After 8-week treatment, we examined changes in the kidney histopathology, function and immunohistochemical stain of kallikrein, macrophage marker CD68, and profibrotic markers transforming growth factor- (TGF-) β and α-smooth muscle action (SMA). RESULTS: DN rats showed enlarged kidneys with glomerulosclerosis, interstitial chronic inflammation and fibrosis, and proteinuria. All the pathological damage and functional impairments were improved after the RAS blockades (all $P < 0.05$). Compared with monotherapy, combined treatment further alleviated the kidney impairments in parallel to increased tubular immunoreactivity for kallikrein and decreased immunopositive cells for CD68, TGF-β, and α-SMA. CONCLUSION: The renoprotective effects of the dual RAS blockade in diabetic nephropathy may be attributed to improved tubular kallikrein expression and interstitial

inflammatory response." As taken from Zou X et al. 2015. J. Diabetes Res. 2015, 310645. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25918729

"Previous studies have suggested that a high intake of sugar-sweetened beverages is positively associated with the risk of a coronary event. However, a few studies have examined the association between sucrose (the most common extrinsic sugar in Sweden) and incident coronary events. The objective of the present study was to examine the associations between sucrose intake and coronary event risk and to determine whether these associations are specific to certain subgroups of the population (i.e. according to physical activity, obesity status, educational level, alcohol consumption, smoking habits, intake of fat and intake of fruits and vegetables). We performed a prospective analysis on 26 190 individuals (62 % women) free from diabetes and without a history of CVD from the Swedish population-based Malmö Diet and Cancer cohort. Over an average of 17 years of follow-up (457 131 person-years), 2493 incident cases of coronary events were identified. Sucrose intake was obtained from an interview-based diet history method, including 7-d records of prepared meals and cold beverages and a 168-item diet questionnaire covering other foods. Participants who consumed >15 % of their energy intake (E%) from sucrose showed a 37 (95 % CI 13, 66) % increased risk of a coronary event compared with the lowest sucrose consumers (<5 E%) after adjusting for potential confounders. The association was not modified by the selected lifestyle factors. The results indicated that sucrose consumption higher than 15 E% (5 % of this population) is associated with an increased risk of a coronary event." As taken from Warfa K et al. 2016. Br. J. Nutr. 116(9), 1611-1620. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27774913

"The "Salt Hypothesis" is the notion that an increase in salt intake will increase blood pressure and thus increase the risk of cardiovascular disease (CVD),which has been a point of contention for decades. Despite this, numerous health organizations, dietary guidelines, and government policies advocate population-wide salt restriction. However, there is no conclusive proof that restricting salt intake reduces the risk of hypertension (HTN) and/or CVD events; sodium restriction in fact may paradoxically lead to adverse health outcomes. Importantly, another white crystal, sucrose (or table sugar) but also high-fructose corn syrup are much more detrimental food additives. Indeed, added sugars have the ability to induce hypertension via the promotion of inflammation, oxidative stress, insulin resistance, and obesity. Considering that there is no physiologic requirement for dietary carbohydrate, there is little reason to suspect adverse health consequences from cutting back on sugar. This paper reviews the evidence relating to salt and sugar on HTN and CVD. Based on our review of the scientific literature, guidelines should focus more on reducing sugar rather than salt for the prevention and treatment of HTN and its consequences." As taken from DiNicolantonio JJ and O'Keefe JH. 2016. Prog. Cardiovasc. Dis. 59(3), 219-225. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27449852

"OBJECTIVE: Exploring the pathophysiological changes in transient receptor potential vanilloid 1 (TRPV1) receptor of the trigeminovascular system in high-fat, high-sucrose (HFHS) diet-induced obesity of experimental animals. BACKGROUND: Clinical and experimental observations suggest a link between obesity and migraine. Accumulating evidence indicates that metabolic and immunological alterations associated with obesity may potentially modulate trigeminovascular functions. A possible target for obesity-induced pathophysiological changes is the TRPV1/capsaicin receptor which is implicated in the pathomechanism of headaches in a complex way. METHODS: Male Sprague-Dawley rats were fed a regular (n = 25) or HFHS diet (n = 26) for 20 weeks. At the end of the dietary

period, body weight of the animals was normally distributed in both groups and it was significantly higher in animals on HFHS diet. Therefore, experimental groups were regarded as control and HFHS diet-induced obese groups. Capsaicin-induced changes in meningeal blood flow and release of calcitonin gene-related peptide (CGRP) from dural trigeminal afferents were measured in control and obese rats. The distribution of TRPV1- and CGRP-immunoreactive meningeal sensory nerves was also compared in whole mount preparations of the dura mater. Metabolic parameters of the animals were assessed by examining glucose and insulin homeostasis as well as plasma cytokine concentrations. RESULTS: HFHS diet was accompanied by reduced food consumption and greater fluid and energy intakes in addition to increased body weight of the animals. HFHS diet increased fasting blood glucose and insulin concentrations as well as levels of circulating proinflammatory cytokines interleukin-1β and interleukin-6. In obese animals, dural application of the archetypal TRPV1 agonist capsaicin resulted in significantly augmented vasodilatory and vasoconstrictor responses as compared to controls. Diet-induced obesity was also associated with enhanced basal and capsaicin-induced CGRP release from meningeal afferents ex vivo. Except for minor morphological changes, the distribution of dural TRPV1- and CGRP-immunoreactive afferents was similar in control and obese animals. CONCLUSIONS: Our results suggest that obesity induced by long-term HFHS diet results in sensitization of the trigeminovascular system. Changes in TRPV1-mediated vascular reactions and CGRP release are pathophysiological alterations that may be of relevance to the enhanced headache susceptibility of obese individuals." As taken from Marics B et al. 2017. Headache 57(3), 441-454. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28133727

"Previous studies have shown positive effects of long-term resveratrol (RSV) supplementation in preventing pancreatic beta cell dysfunction, arterial stiffening and metabolic decline induced by high-fat/high-sugar (HFS) diet in nonhuman primates. Here, the analysis was extended to examine whether RSV may reduce dietary stress toxicity in the cerebral cortex of the same cohort of treated animals. Middle-aged male rhesus monkeys were fed for 2 years with HFS alone or combined with RSV, after which whole-genome microarray analysis of cerebral cortex tissue was carried out along with ELISA, immunofluorescence, and biochemical analyses to examine markers of vascular health and inflammation in the cerebral cortices. A number of genes and pathways that were differentially modulated in these dietary interventions indicated an exacerbation of neuroinflammation (e.g., oxidative stress markers, apoptosis, NF-κB activation) in HFS-fed animals and protection by RSV treatment. The decreased expression of mitochondrial aldehyde dehydrogenase 2, dysregulation in endothelial nitric oxide synthase, and reduced capillary density induced by HFS stress were rescued by RSV supplementation. Our results suggest that long-term RSV treatment confers neuroprotection against cerebral vascular dysfunction during nutrient stress." As taken from Bernier M et al. 2016. Aging (Albany NY) 8(5), 899-916. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27070252

6.3. Nervous system

“Cane sugar...observed to cause diarrhea, colic, inflammation of kidneys and ...petechial hemorrhages in spinal dura mater of horses”

As taken from HSDB, 2005.

Effects of acute abstinence and nicotine administration on taste perception in cigarette smokers (Abstract). We investigated the effects of short-term abstinence from smoking and acute nicotine administration on taste perception in smokers. We assessed sensitivity for salt and sucrose solutions and the self-reported intensity and pleasantness of these tastes, using a previously validated model of taste perception. This was in order to investigate mechanisms by which cigarette smoking and smoking cessation may modulate dietary behaviour. Male and female daily smokers attended a single testing session. Participants were randomised to either abstain for smoking for 12 h or smoke as usual on the morning of testing. At the testing session, participants completed subjective ratings of mood and ratings of intensity and pleasantness of salt and sucrose solutions, followed by measurement of the threshold at which these solutions could be detected on the tongue. Participants were then randomised to smoking either a nicotine-containing or denicotinised cigarette, after which they completed the same measures as previously. Our data suggest that following cigarette smoking, lower taste thresholds are obtained after smoking a denicotinised cigarette compared with a nicotinised cigarette, but among females only. This effect was not observed among males and did not differ as a function of abstinence condition. In addition, among non-abstinent smokers, females demonstrated higher taste thresholds (i.e. reduced sensitivity) for salt than males, but this sex difference was not observed among abstinent smokers. As taken from Mullings et al., J Psychopharmacol. 2009 May 7. PubMed, 2010, available at http://www.ncbi.nlm.nih.gov/pubmed/19423612

Kanaret *et al* (2004) suggested that sucrose intake may increase the analgesic properties of nicotine in animals and humans. (Kanaret *et al* 2004).

Antinociceptive effect of sucrose ingestion in the human (Abstract). Sucrose ingestion has been shown to alleviate pain and distress in rats, human infants as well as adults. Sucrose induced analgesia is related to the reward value associated with its sweet taste. The sweet taste of sucrose is a stimulus for the activation of endogenous opioid pool. The opioids in turn modulate pain perception. It has been demonstrated in a number of animal and human studies that sucrose ingestion increases the hypothalamic/CSF opioid levels. This gains support from the results obtained from naloxone challenge test, a neuro-endocrine method for assessment of endogenous opioid tone. Moreover, the analgesic effects of sucrose can be reversed by administration of opioid antagonists such as naloxone. On the other hand, long-term sucrose ingestion leads to hyperalgesia in rats and it has been hypothesized to result from a complex interaction of sucrose with the endogenous opioid system leading to a deficiency of opioids. In the present article mechanisms underlying sucrose induced analgesia including the interaction of the palatability and reward value of food with the neural substrates and its neuro-chemical basis have been reviewed in the light of both animal and human studies. In addition, clinical application of the knowledge about sucrose and its modulatory effect on the endogenous opioid system has been suggested. As taken from Bhattacharjee M, Mathur R. Indian J Physiol Pharmacol. 2005 Oct-Dec; 49(4):383-94. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=16579391&query_hl=42&itool=pubmed_docsum

Sucrose attenuates a negative electroencephalographic response to an aversive stimulus for newborns (Abstract). Reports that sweet taste calms crying in newborns and is analgesic against the pain caused by a heel lance served as the basis for this study. Electroencephalographic (EEG) activity, heart rate activity, and infants' facial behaviors were recorded before and after a noninvasive, but noxious, heelstroke (procedure from the Brazelton Neonatal Behavior Assessment Scale). In a randomized and controlled trial, 34 newborns were administered 2 mL of water or sucrose solution before the heelstroke. Frontal EEG asymmetry scores were computed, and power in the 3 to 6 Hz frequency band was analyzed. Infants who received water showed increased relative right frontal EEG activation from baseline to the post-heelstroke phase, a pattern that typifies negative affect. The EEG of infants in the sucrose group did not change. Heart rate increased rapidly in both groups during the heelstroke phase. However, after the heelstroke, the heart rate of infants who received sucrose returned to baseline, whereas the heart rate of infants who tasted water remained elevated. During the heelstroke, the infants in the water group cried and grimaced twice as long as the infants in the sucrose group. These findings add to the growing literature showing that sucrose attenuates newborns' negative response to aversive or noxious stimuli. As taken from Fernandez M et al. J Dev Behav Pediatr. 2003 Aug; 24(4):261-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=12915798&query_hl=42&itool=pubmed_DocSum

The Effects of Nicotine and Sucrose on Spatial Memory and Attention (Abstract). Both nicotine and sucrose can enhance performance on cognitive tasks. However, little is known about whether nicotine and sucrose could act jointly to augment mental performance. To investigate if there is an interaction between nicotine and sucrose on cognitive behavior, performance on a continuous performance task (CPT) and a spatial memory task was examined in 14 healthy smokers after they had drunk 8 oz of either a sucrose- or aspartame-containing beverage, and then chewed a piece of gum containing either 2 mg nicotine or no nicotine. To assess changes in mood as a function of nicotine and sucrose intake, the profile of mood states (POMS) test was administered three times during each test session. Participants made significantly more correct responses and significantly fewer incorrect responses on the CPT when they received nicotine than when they received the placebo gum. Closer analysis of the data revealed that there was an interaction between sucrose consumption and nicotine intake. Nicotine increased hits and decreased misses when participants were given the sucrose-containing beverage, but not when they were given the aspartame-containing beverage. Neither nicotine nor sucrose affected spatial memory or mood across experimental sessions. However, when data were analyzed for just the first session, participants who drank the sucrose-containing beverage performed significantly better on the spatial memory task than those who drank the aspartame-containing beverage. No gender differences in the effects of nicotine or sucrose on cognitive performance were detected. The results provide support that both nicotine and sucrose have positive effects on cognitive behavior, and that under some conditions the two variables have additive effects on performance. As taken from Hartea and Kanareka, Short Communication:; Nutritional Neuroscience, Volume 7, Issue 2 April 2004, pages 121 – 125. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/15279498

Comparison of 14C-sucrose delivery to the brain by intravenous, intraventricular, and convection-enhanced intracerebral infusion (Abstract). The authors evaluated convection-enhanced delivery (CED) of 14C-sucrose to the rat brain as a method of enhancing cerebral drug delivery and compared it with intravenous (i.v.) and intraventricular

(i.v.t.) routes of administration. Groups of rats received 14C-sucrose by bolus i.v. infusion, i.v.t. infusion for 1, 2, or 7 days at 0.17 microl/minute, or CED at rates from 0.01 to 0.5 microl/minute for periods from 1 hour to 7 days. Radioisotope distribution and concentration in tissue were analyzed using quantitative autoradiography. Intravenously administered sucrose reached the entire brain, but levels in tissue were low. After i.v.t. administration, sucrose levels in tissue were high at, and declined exponentially away from, the ventricular surface. Chronic CED administration maintained high levels of sucrose in tissue that focally were up to 10,000 times higher than in the i.v. group. The isotope distribution pattern after chronic CED infusions indicated a central component that resulted from convention and a peripheral component in gray matter that was the result of diffusion. The brain influx (0.42 microl/g/min) and diffusion constants of sucrose (2.8 x 10(-6) cm2/second) were similar to reported values. The total brain efflux constant was 0.0044 minute, whereas the blood-brain barrier (BBB) efflux constant was 0.0016 minute. There were no pathological changes in the brains after CED except those associated with cannula insertion. Sucrose, which was thought to be inert, was found to interact with brain tissue; up to 25% was bound to an unidentified tissue component. Chronic CED appears to be a potentially useful method for significantly circumventing the BBB and increasing delivery of water-soluble drugs to the brain. As taken from Groothuis DR et al. J Neurosurg. 1999 Feb; 90(2):321-31. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=9950504&query_hl=26&itool=pubmed_docsum

A diet promoting sugar dependency causes behavioral cross-sensitization to a low dose of amphetamine (Abstract). Previous research in this laboratory has shown that a diet of intermittent excessive sugar consumption produces a state with neurochemical and behavioral similarities to drug dependency. The present study examined whether female rats on various regimens of sugar access would show behavioral cross-sensitization to a low dose of amphetamine. After a 30-min baseline measure of locomotor activity (day 0), animals were maintained on a cyclic diet of 12-h deprivation followed by 12-h access to 10% sucrose solution and chow pellets (12 h access starting 4 h after onset of the dark period) for 21 days. Locomotor activity was measured again for 30 min at the beginning of days 1 and 21 of sugar access. Beginning on day 22, all rats were maintained on ad libitum chow. Nine days later locomotor activity was measured in response to a single low dose of amphetamine (0.5 mg/kg). The animals that had experienced cyclic sucrose and chow were hyperactive in response to amphetamine compared with four control groups (ad libitum 10% sucrose and chow followed by amphetamine injection, cyclic chow followed by amphetamine injection, ad libitum chow with amphetamine, or cyclic 10% sucrose and chow with a saline injection). These results suggest that a diet comprised of alternating deprivation and access to a sugar solution and chow produces bingeing on sugar that leads to a long lasting state of increased sensitivity to amphetamine, possibly due to a lasting alteration in the dopamine system. As taken from Avena NM, Hoebel BG.

Neuroscience. 2003; 122(1):17-20. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=14596845&query_hl=23&itool=pubmed_DocSum

Sugar triggers our reward-system. Sweets release opiates which stimulates the appetite for sucrose--insulin can depress it (Abstract). The consumption of sweet food has increased in Sweden, as in other Western countries. The type of food item has changed. The sweet is dominated by soft drinks. Appetite regulation for sucrose has been

described in experimental animal models. It has been found that opioids stimulate appetite for sucrose. At the same time sucrose releases endogenous opioids so that a triggering of sucrose consumption occurs. Insulin has been shown to decrease sucrose intake by blocking the opioid response. Sucrose addiction has been described in rat model. With a concentrated sucrose solution to drink an opioid dependence developed with 1) increased consumption of sucrose 2) abstinence symptoms with no sucrose and 3) anxiety with an opiate blocker. Sucrose addiction in man has not been described in the scientific literature. There is an increased liking of sweets with alcoholic persons, which may be significant to support a strongly rewarding effect of sucrose, also in man. We should limit the access to sweet foods, in particular the sweet drinks. Insulin and insulin sensitivity may be an important factor to restrict the intake of sweet food. As taken from Erlanson-Albertsson C. Lakartidningen. 2005 May 23-29; 102(21):1620-2, 1625,1627. [Article in Swedish] PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=15962882&query_hl=11&itool=pubmed_docsum

Incubation of sucrose craving: effects of reduced training and sucrose pre-loading (Abstract).

Time-dependent increases in cue-induced reward seeking after forced abstinence were described in rats with a history of cocaine or sucrose self-administration, suggesting reward craving incubates over time. In the present study, we examined the effects of reduced training experience, or sucrose pre-loading just prior to testing, on the incubation of sucrose craving. Sucrose seeking (responding in extinction and then for a sucrose-paired cue) increased over time in groups of rats that self-administered sucrose 6 h/day for 10 days and were tested at 1, 7, or 30 days of forced abstinence. We found that groups of rats that had self-administered 2 instead of 6 h/day showed a similar profile of responding. Incubation of sucrose craving was attenuated by free access to sucrose in home cages for 17 h immediately prior to testing assessed as extinction responding on days 1 and 30. However, this sucrose pre-loading had no effect on the time-dependent increase in responding for the sucrose-paired cue. In summary, reducing the training experience had no effect on the incubation of sucrose craving and free access to sucrose had only a limited effect-attenuating extinction responding. These results illustrate the strength of the incubation of craving and further suggest long-term changes in brain motivational circuitry following sucrose self-administration. As taken from Grimm JW et al., Physiol Behav. 2005 Jan 31; 84(1):73-9. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=15642609&query_hl=21&itool=pubmed_DocSum

“BACKGROUND: Pain and natural rewards such as food elicit different behavioral effects. Both pain and rewards, however, have been shown to alter synaptic activities in the nucleus accumbens (NAc), a key component of the brain reward system. Mechanisms by which external stimuli regulate plasticity at NAc synapses are largely unexplored. Medium spiny neurons (MSNs) from the NAc receive excitatory glutamatergic inputs and modulatory dopaminergic and cholinergic inputs from a variety of cortical and subcortical structures. Glutamate inputs to the NAc arise primarily from prefrontal cortex, thalamus, amygdala, and hippocampus, and different glutamate projections provide distinct synaptic and ultimately behavioral functions. The family of vesicular glutamate transporters (VGLUTs 1-3) plays a key role in the uploading of glutamate into synaptic vesicles. VGLUT1-3 isoforms have distinct expression patterns in the brain, but the effects of external stimuli on their

expression patterns have not been studied. RESULTS: In this study, we use a sucrose self-administration paradigm for natural rewards, and spared nerve injury (SNI) model for chronic pain. We examine the levels of VGLUTs (1-3) in synaptoneurosomes of the NAc in these two behavioral models. We find that chronic pain leads to a decrease of VGLUT1, likely reflecting decreased projections from the cortex. Pain also decreases VGLUT3 levels, likely representing a decrease in projections from GABAergic, serotonergic, and/or cholinergic interneurons. In contrast, chronic consumption of sucrose increases VGLUT3 in the NAc, possibly reflecting an increase from these interneuron projections. CONCLUSION: Our study shows that natural rewards and pain have distinct effects on the VGLUT expression pattern in the NAc, indicating that glutamate inputs to the NAc are differentially modulated by rewards and pain." As taken from Tukey DS et al. 2013. Mol. Brain 6, 32. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23835161

"PURPOSE OF REVIEW: To review research that tests the validity of the analogy between addictive drugs, like cocaine, and hyperpalatable foods, notably those high in added sugar (i.e., sucrose). RECENT FINDINGS: Available evidence in humans shows that sugar and sweetness can induce reward and craving that are comparable in magnitude to those induced by addictive drugs. Although this evidence is limited by the inherent difficulty of comparing different types of rewards and psychological experiences in humans, it is nevertheless supported by recent experimental research on sugar and sweet reward in laboratory rats. Overall, this research has revealed that sugar and sweet reward can not only substitute to addictive drugs, like cocaine, but can even be more rewarding and attractive. At the neurobiological level, the neural substrates of sugar and sweet reward appear to be more robust than those of cocaine (i.e., more resistant to functional failures), possibly reflecting past selective evolutionary pressures for seeking and taking foods high in sugar and calories. SUMMARY:The biological robustness in the neural substrates of sugar and sweet reward may be sufficient to explain why many people can have difficultly to control the consumption of foods high in sugar when continuously exposed to them." As taken from Ahmed SH et al. 2013. Curr. Opin. Clin. Nutr. Metab. Care 16(4), 434-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23719144

"BACKGROUND: Ingestion of sweet food is driven by central reward circuits and restrained by endocrine and neurocrine satiety signals. The specific influence of sucrose intake on central affective and reward circuitry and alterations of these mechanisms in the obese are incompletely understood. For this, we hypothesized that (i) similar brain regions are engaged by the stimulation of sweet taste receptors by sucrose and by non-nutrient sweeteners and (ii) during visual food-related cues, obese subjects show greater brain responses to sucrose compared with lean controls. METHODS: In a double-blind, crossover design, 10 obese and 10 lean healthy females received a sucrose or a non-nutrient sweetened beverage prior to viewing food or neutral images. BOLD signal was measured using a 1.5 Tesla MRI scanner. KEY RESULTS: Viewing food images after ingestion of either drink was associated with engagement of similar brain regions (amygdala, hippocampus, thalamus, anterior insula). Obese differed from lean subjects in behavioral and brain responses rating both beverages as less tasteful and satisfying, yet demonstrating greater brain responses. Obese subjects also showed engagement of an additional brain network (including anterior insula, anterior cingulate, hippocampus, and amygdala) only after sucrose ingestion. CONCLUSIONS & INFERENCES: Obese subjects

had a reduced behavioral hedonic response, yet a greater engagement of affective brain networks, particularly after sucrose ingestion, suggesting that in obese subjects, lingual and gut-derived signaling generate less central hedonic effects than food-related memories in response to visual cues, analogous to response patterns implicated in food addiction." As taken from Connolly L et al. 2013. Neurogastroenterol. Motil. 25(7), 579-e460. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23566308

"….The aims of the present work were to study if sucrose and/or saccharin could attenuate food restriction-induced hyperactivity, weight loss, increased plasma corticosterone, and activation of brain structures involved in neuroendocrine control, energy balance, physical activity, and reward signaling in rats. Its major findings are that access to sucrose, but not to saccharin, attenuated food restriction-induced running wheel activity, weight loss, rises in plasma corticosterone, and expression of the cellular activation marker c-Fos in the paraventricular and arcuate hypothalamus and in the nucleus accumbens. These findings suggest that the energy-richness and easy availability of sucrose interrupted a fleeing-famine-like hyperactivity response. Since corticosterone mediates food restriction-induced wheel running (Duclos et al., 2009), we propose that the attenuating effect of sucrose consumption on plasma corticosterone plays a role in reduced wheel running and weight loss by lowering activation of the nucleus accumbens and arcuate hypothalamus in these animals." As taken from Duclos M et al. 2013. Psychoneuroendocrinology 38(6), 884-97. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23059205

"RATIONALE AND OBJECTIVE: There is evidence that cue-induced sucrose seeking progressively increases after cessation of oral sucrose self-administration (incubation of sucrose craving) in both adolescent and adult rats. The synaptic plasticity changes associated with this incubation at different age groups are unknown. We assessed whether incubation of sucrose craving in rats trained to self-administer sucrose as young adolescents, adolescents, or adults is associated with changes in 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA)/N-methyl-D-aspartate (NMDA) ratio (a measure of postsynaptic changes in synaptic strength) in nucleus accumbens. METHODS: Three age groups initiated oral sucrose self-administration training (10 days) on postnatal day (P) 35 (young adolescents), P42 (adolescents), or P70 (adults). They were then tested for cue-induced sucrose seeking (assessed in an extinction test) on abstinence days 1 and 21. Separate groups of rats were trained to self-administer sucrose or water (a control condition), and assessed for AMPA/NMDA ratio in nucleus accumbens on abstinence days 1-3 and 21. RESULTS: Adult rats earned more sucrose rewards, but sucrose intake per body weight was higher in young adolescent rats. Time-dependent increases in cue-induced sucrose seeking (incubation of sucrose craving) were more pronounced in adult rats, less pronounced in adolescents, and not detected in young adolescents. On abstinence day 21, but not days 1-3, AMPA/NMDA ratio in nucleus accumbens were decreased in rats that self-administered sucrose as adults and adolescents, but not young adolescents. CONCLUSIONS: Our data demonstrate age-dependent changes in magnitude of incubation of sucrose craving and nucleus accumbens synaptic plasticity after cessation of sucrose self-administration." As taken from Counotte DS et al. 2014. Psychopharmacology (Berl.) 231(8), 1675-84. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24114427

“Hypothalamic inflammation and gliosis are proposed to participate in the pathogenesis of high-fat diet-induced obesity. Because other factors and nutrients also induce weight gain and adiposity, we analyzed the inflammatory and glial responses to a sucrose (S)-enriched diet. Neonatal overnutrition (NON) exacerbates weight gain in response to metabolic challenges; thus, we compared the inflammatory response of male Wistar rats with NON (4 pups/litter) and controls (12 pups/litter) to increased S intake. At weaning rats received water or a 33% sucrose solution and normal chow ad libitum for 2 months. Sucrose increased serum IL-1β and -6 and hypothalamic IL-6 mRNA levels in NON and TNFα mRNA levels in control and NON rats, whereas NON alone had no effect. The astrocyte marker glial fibrillary acidic protein was increased by NON but decreased by S. This was associated with hypothalamic nuclei specific changes in glial fibrillary acidic protein-positive cell number and morphology. Sucrose increased the number of microglia and phosphorylation of inhibitor of -κB and c-Jun N-terminal kinase in control but not NON rats, with no effect on microglia activation markers. Proteins highly expressed in astrocytes (glutamate, glucose, and lactate transporters) were increased by NON but not S, with no increase in vimentin expression in astrocytes, further suggesting that S-induced adiposity is not associated with hypothalamic astrogliosis. Hence, activation of hypothalamic inflammatory processes and gliosis depend not only on weight gain but also on the diet inducing this weight gain and the early nutritional status. These diverse inflammatory processes could indicate a differential disposition to obesity-induced pathologies.” As taken from Fuente-Martin E et al. 2013. Endocrinology 154(7), 2318-30. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23671260?dopt=AbstractPlus

“Among the comorbidities observed in epilepsy patients depression is the most frequent one. Likewise, depression by itself is accompanied by an increased risk to develop epilepsy. Both epilepsy and depression are characterized by a high incidence of pharmacoresistance, which might be based on overactivity of multidrug transporters like P-glycoprotein at the blood-brain barrier. Using genetically modified mice in preclinical epilepsy research is pivotal for investigating this bidirectional relationship. In the present study, we used the sucrose consumption test (SCT) in the pilocarpine and the intrahippocampal kainate mouse post-status epilepticus model to reveal anhedonic behavior, i.e. hyposensitivity to pleasure, as a key symptom of depression. Mice were repetitively investigated by SCT during early epilepsy and the chronic phase of the disease, during which response to antidepressant drug treatment was assessed. SCT revealed long-lasting anhedonia in both models. Anhedonia appeared to be pharmacoresistant, as neither chronic treatment with imipramine in the pilocarpine model nor chronic treatment with fluoxetine in the kainate model could annihilate the differences in sucrose consumption between control and epileptic mice. Moreover, knock-out of P-glycoprotein did not improve the treatment effect of fluoxetine. In conclusion, our findings show for the first time that the SCT is suited for detection of depression-like behavior in mouse models of temporal-lobe epilepsy. Both models might serve as tools to further investigate the neurobiology and pharmacology of epilepsy-associated pharmacoresistant depression.” As taken from Klein S et al. 2015. Exp. Neurol. 263, 263-71. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25220610

“High energy diets have been shown to impair cognition however, the rapidity of these effects, and the dietary component/s responsible are currently unclear. We conducted two experiments in rats to examine the effects of short-term exposure to a diet rich in sugar and fat or rich in sugar on object (perirhinal-dependent) and place (hippocampal-dependent)

recognition memory, and the role of inflammatory mediators in these responses. In Experiment 1, rats fed a cafeteria style diet containing chow supplemented with lard, cakes, biscuits, and a 10% sucrose solution performed worse on the place, but not the object recognition task, than chow fed control rats when tested after 5, 11, and 20 days. In Experiment 2, rats fed the cafeteria style diet either with or without sucrose and rats fed chow supplemented with sucrose also performed worse on the place, but not the object recognition task when tested after 5, 11, and 20 days. Rats fed the cafeteria diets consumed five times more energy than control rats and exhibited increased plasma leptin, insulin and triglyceride concentrations; these were not affected in the sucrose only rats. Rats exposed to sucrose exhibited both increased hippocampal inflammation (TNF-α and IL-1β mRNA) and oxidative stress, as indicated by an upregulation of NRF1 mRNA compared to control rats. In contrast, these markers were not significantly elevated in rats that received the cafeteria diet without added sucrose. Hippocampal BDNF and neuritin mRNA were similar across all groups. These results show that relatively short exposures to diets rich in both fat and sugar or rich in sugar, impair hippocampal-dependent place recognition memory prior to the emergence of weight differences, and suggest a role for oxidative stress and neuroinflammation in this impairment." As taken from Beilharz JE et al. 2014. Brain Behav. Immun. 37, 134-41. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24309633

"Drug abuse and obesity are serious public health problems. Dopamine plays a central role in mediating the reinforcing effects of drugs and food. Prolonged use of drugs is known to alter the function and/or sensitivity of many neurotransmitter systems, including dopamine; however, the impact of consuming foods high in fat and/or sugar is less clear. These studies characterized the locomotor effects of acute and repeated cocaine in male and female C57BL/6J mice consuming 1 of 4 diets: (a) standard chow + water; (b) standard chow + 10% sucrose solution; (c) high-fat chow + water; or (d) high-fat chow + 10% sucrose solution. The acute locomotor effects of cocaine (3.2-32.0 mg/kg) were evaluated 4 weeks after initiating dietary conditions; the effects of repeated cocaine administration were evaluated after 5, 6, 7, and 12 weeks. During acute tests, mice consuming a diet high in fat and/or sucrose exhibited greater locomotor responses to cocaine than mice consuming standard chow and water, regardless of sex. Although diet-induced enhancements persisted across repeated cocaine testing, locomotor sensitization developed more rapidly in females drinking sucrose (and consuming either standard or high-fat chow) than in females consuming standard chow and water. In addition to providing evidence that consuming a diet high in fat and/or sugar enhances abuse-related effects of cocaine in ways that might increase vulnerability to abuse cocaine, these studies identified a potentially important sex-related difference in the interaction between nutrition and cocaine effects, with the impacts of sucrose consumption being greater in females than in males." As taken from Collins GT et al. 2015. Exp. Clin. Psychopharmacol. 23(4), 228-37. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26237320

"OBJECTIVE: Exploring the pathophysiological changes in transient receptor potential vanilloid 1 (TRPV1) receptor of the trigeminovascular system in high-fat, high-sucrose (HFHS) diet-induced obesity of experimental animals. BACKGROUND: Clinical and experimental observations suggest a link between obesity and migraine. Accumulating evidence indicates that metabolic and immunological alterations associated with obesity may potentially modulate trigeminovascular functions. A possible target for obesity-induced pathophysiological changes is the TRPV1/capsaicin receptor which is implicated in the pathomechanism of headaches in a complex way. METHODS: Male Sprague-Dawley rats

were fed a regular (n = 25) or HFHS diet (n = 26) for 20 weeks. At the end of the dietary period, body weight of the animals was normally distributed in both groups and it was significantly higher in animals on HFHS diet. Therefore, experimental groups were regarded as control and HFHS diet-induced obese groups. Capsaicin-induced changes in meningeal blood flow and release of calcitonin gene-related peptide (CGRP) from dural trigeminal afferents were measured in control and obese rats. The distribution of TRPV1- and CGRP-immunoreactive meningeal sensory nerves was also compared in whole mount preparations of the dura mater. Metabolic parameters of the animals were assessed by examining glucose and insulin homeostasis as well as plasma cytokine concentrations. RESULTS: HFHS diet was accompanied by reduced food consumption and greater fluid and energy intakes in addition to increased body weight of the animals. HFHS diet increased fasting blood glucose and insulin concentrations as well as levels of circulating proinflammatory cytokines interleukin-1β and interleukin-6. In obese animals, dural application of the archetypal TRPV1 agonist capsaicin resulted in significantly augmented vasodilatory and vasoconstrictor responses as compared to controls. Diet-induced obesity was also associated with enhanced basal and capsaicin-induced CGRP release from meningeal afferents ex vivo. Except for minor morphological changes, the distribution of dural TRPV1- and CGRP-immunoreactive afferents was similar in control and obese animals. CONCLUSIONS: Our results suggest that obesity induced by long-term HFHS diet results in sensitization of the trigeminovascular system. Changes in TRPV1-mediated vascular reactions and CGRP release are pathophysiological alterations that may be of relevance to the enhanced headache susceptibility of obese individuals." As taken from Marics B et al. 2017. Headache 57(3), 441-454. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28133727

"Previous studies have shown positive effects of long-term resveratrol (RSV) supplementation in preventing pancreatic beta cell dysfunction, arterial stiffening and metabolic decline induced by high-fat/high-sugar (HFS) diet in nonhuman primates. Here, the analysis was extended to examine whether RSV may reduce dietary stress toxicity in the cerebral cortex of the same cohort of treated animals. Middle-aged male rhesus monkeys were fed for 2 years with HFS alone or combined with RSV, after which whole-genome microarray analysis of cerebral cortex tissue was carried out along with ELISA, immunofluorescence, and biochemical analyses to examine markers of vascular health and inflammation in the cerebral cortices. A number of genes and pathways that were differentially modulated in these dietary interventions indicated an exacerbation of neuroinflammation (e.g., oxidative stress markers, apoptosis, NF-κB activation) in HFS-fed animals and protection by RSV treatment. The decreased expression of mitochondrial aldehyde dehydrogenase 2, dysregulation in endothelial nitric oxide synthase, and reduced capillary density induced by HFS stress were rescued by RSV supplementation. Our results suggest that long-term RSV treatment confers neuroprotection against cerebral vascular dysfunction during nutrient stress." As taken from Bernier M et al. 2016. Aging (Albany NY) 8(5), 899-916. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27070252

"High sugar consumption is a risk factor for metabolic disturbances leading to memory impairment. Thus, rats subject to high sucrose intake (HSu) develop a metabolic syndrome and display memory deficits. We now investigated if these HSu-induced memory deficits were associated with metabolic and electrophysiological alterations in the hippocampus. Male Wistar rats were submitted for 9 weeks to a sucrose-rich diet (35% sucrose solution) and subsequently to a battery of behavioral tests; after sacrifice, their hippocampi were collected for ex vivo high-resolution magic angle spinning (HRMAS) metabolic

characterization and electrophysiological extracellular recordings in slices. HSu rats displayed a decreased memory performance (object displacement and novel object recognition tasks) and helpless behavior (forced swimming test), without altered locomotion (open field). HRMAS analysis indicated a similar hippocampal metabolic profile of HSu and control rats. HSu rats also displayed no change of synaptic transmission and plasticity (long-term potentiation) in hippocampal Schaffer fibers-CA1 pyramid synapses, but had decreased amplitude of long-term depression in the temporoammonic (TA) pathway. Furthermore, HSu rats had an increased density of inhibitory adenosine A1 receptors (A1R), that translated into a greater potency of A1R in Schaffer fiber synapses, but not in the TA pathway, whereas the endogenous activation of A1R in HSu rats was preserved in the TA pathway but abolished in Schaffer fiber synapses. These results suggest that HSu triggers a hippocampal-dependent memory impairment that is not associated with altered hippocampal metabolism but is probably related to modified synaptic plasticity in hippocampal TA synapses." As taken from Lemos C et al. 2016. Neuroscience 315, 196-205. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26704636

"CONTEXT: Glucose is the main energy source for the brain, and as such, manipulation of glucose supply may affect brain function. It has been suggested that a change in blood glucose may influence mood. OBJECTIVE: The aim of this review was to investigate the potential effects of glucose and sucrose, compared with placebo, on mood. DATA SOURCES: The electronic databases PubMed and Scopus were searched. Reference lists of selected articles were checked manually. DATA EXTRACTION: Randomized controlled trials or crossover trials comparing the effects of glucose or sucrose on mood that were published up to May 2017 were eligible. Potentially eligible articles were selected independently by 2 reviewers. RESULTS: In total, 19 studies were found. Thirteen studies investigated the effects of glucose consumption compared with placebo on mood. Seven of these 13 studies found no effect of glucose on mood. The other 6 studies found small and partial effects that may also be due to other factors like palatability and expectation. Seven of the 19 studies investigated the effects of sucrose ingestion versus placebo on mood. None of these studies found a positive effect on mood, and 1 study observed an adverse effect. One of the studies investigated the effects of both glucose and sucrose. CONCLUSIONS: The results from this review show limited effects of glucose ingestion on mood and no effect of sucrose on mood." As taken from van de Rest O et al. 2018. Nutr. Rev. 76(2), 108-116. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29228399

"Depression is highly prevalent worldwide, but its etiology is not fully understood. An overlooked possible contributor to the epidemic of depression is feeding styles, particularly at early age when the brain is intensely changing. We have previously reported that unlimited sucrose consumption during adolescence leads to enduring changes in brain reward function. Here, we tested the hypothesis that sucrose consumption during adolescence would lead to a 'depressive-like' phenotype. Adolescent male rats were given unlimited access to 5% sucrose in their home cages from postnatal day 30 to postnatal day 46 and their emotional behavior was subsequently examined at adulthood. Sucrose consumption during adolescence caused anhedonia, decreased motivation for saccharin, increased immobility in the forced swim test and exacerbated anxiety-like behavior. Additionally, sucrose consumption during adolescence decreased cell proliferation in the hippocampus in adulthood. Chronic treatment with imipramine (10 mg/kg) normalized behavior and restored cell proliferation in the hippocampus of adult rats with a history of sucrose consumption during adolescence. A similar sucrose consumption starting at

adulthood only increases immobility in the forced swim test, suggesting that sucrose intake affects also adults' behavior but to a lesser degree. Overall, our findings reveal an unsuspected protracted effect of sucrose consumption on behavior and suggest that unlimited sucrose consumption during critical periods of brain development may play an important role in the etiology of reward-related disorders such as depression." As taken from Gueye AB et al. 2018. Neuropsychopharmacology 43(13), 2627-2635. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29487370

"Adult neurogenesis, a complex process by which stem cells in the hippocampal brain region differentiate and proliferate into new neurons and other resident brain cells, is known to be affected by many intrinsic and extrinsic factors, including diet. Neurogenesis plays a critical role in neural plasticity, brain homeostasis, and maintenance in the central nervous system and is a crucial factor in preserving the cognitive function and repair of damaged brain cells affected by aging and brain disorders. Intrinsic factors such as aging, neuroinflammation, oxidative stress, and brain injury, as well as lifestyle factors such as high-fat and high-sugar diets and alcohol and opioid addiction, negatively affect adult neurogenesis. Conversely, many dietary components such as curcumin, resveratrol, blueberry polyphenols, sulforaphane, salvionic acid, polyunsaturated fatty acids (PUFAs), and diets enriched with polyphenols and PUFAs, as well as caloric restriction, physical exercise, and learning, have been shown to induce neurogenesis in adult brains. Although many of the underlying mechanisms by which nutrients and dietary factors affect adult neurogenesis have yet to be determined, nutritional approaches provide promising prospects to stimulate adult neurogenesis and combat neurodegenerative diseases and cognitive decline. In this review, we summarize the evidence supporting the role of nutritional factors in modifying adult neurogenesis and their potential to preserve cognitive function during aging." As taken from Poulose SM et al. 2017. Adv. Nutr. 8(6), 804-811. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29141966

"Recent studies show that emotional and environmental stimuli promote epigenetic inheritance and influence behavioral development in the subsequent generations. Caloric mal- and under-nutrition has been shown to cause metabolic disturbances in the subsequent generation, but the incentive properties of paternal binge-like eating in offspring is still unknown. Here we show that paternal sucrose self-administration experience could induce inter-generational decrease in both sucrose and cocaine-seeking behavior, and sucrose responding in F1 rats, but not F2, correlated with the performance of F0 rats in sucrose self-administration. Higher anxiety level and decreased cocaine sensitivity were observed in Sucrose F1 compared with Control F1, possibly contributing to the desensitization phenotype in cocaine and sucrose self-administration. Our study revealed that paternal binge-like sucrose consumption causes decrease in reward seeking and induces anxiety-like behavior in the F1 offspring." As take from Le Q et al. 2017. Front. Behav. Neurosci. 11, 184. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29021748

"BACKGROUND: Obesity has reached global epidemic proportions and is associated with serious medical comorbidities and economic consequences. In this preclinical study, we characterized how the palatable diet changed food intake pattern, caloric intake, metabolic profile and hormone levels. We also evaluated the expression of dopamine D2 receptors in the rat striatum. METHODS: Male Wistar rats were fed with either high-fat or high-sucrose diet for 5 weeks according to different feeding regimes: ad libitum access or scheduled for a 2-h period each day without caloric restriction during the remainder of the day. RESULTS:

Both diets resulted in an enhancement in caloric intake and total body weight. Post-meal data showed that high-fat diet increased cholesterol, triglycerides and glucose concentrations. Animals fed on high sucrose diet were only hyperglycemic. High-fat diet schedules resulted in the enhancement of leptin concentrations, while increases in blood levels of ghrelin were noted after intermitted high-fat or continuous high-sucrose diet. Finally, we report that only ad libitum high-sucrose evoked a significant enhancement of the dopamine D2 receptor protein level and a reduction in the D2 mRNA and receptor affinity in the rat striatum. Independently of the diet type, a similar reduction in dopamine D2 receptor affinity (decrease in KD value) was found in the striatum of rats with intermittent food access. CONCLUSION: The findings provide a better understanding of eating disorders and indicate that diet composition leading to obesity induces distinct changes in dopamine D2 receptor signaling in the striatum." As taken from Rospond B et al. 2019. Pharmacol. Rep. 71(1), 1-12. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30343042

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

"Oral admin to young animals unable to digest it will cause or accent diarrheas."

"Liquid medicines, particularly those intended for children, often formulated as syrups, usually based on sucrose. Total sugar content may be up to 80% (w/v). Complicated diabetes management. Increased dental caries especially in chronically sick children (eg, asthmatics or epileptics) who frequently take liquid medicine."

"Sucrose is reported to be capable of producing dermatoses in bakers, candy makers, and related occupations."

"Sucrose has shown no toxicity to corneas of rabbits when applied for 3 to 7 hr in neutral aq soln"

"Diffuse pathological changes /in poisoned animals/ incl shrinkage, swelling and necrosis of renal tubular epithelium, arteriolitis, mild hepathitis, myocarditis, congestive encephalitis and some adrenal hypertrophy"

"Cane sugar...observed to cause diarrhea, colic, inflammation of kidneys and ...petechial hemorrhages in spinal dura mater of horses"

As taken from HSDB, 2005.

The effects of overnight fasting, feeding, or sucrose supplementation prior to necropsy in rats (Abstract). This study was designed to investigate the acute effects on routine hematology, serum biochemistry, gastrointestinal contents and weight, and liver weight and morphology due to overnight sucrose feeding of rats prior to necropsy. Groups of rats (five males and five females/group) were fasted overnight, fed chow, or fed sucrose and were euthanized approximately 17 h later. At necropsy, blood was obtained for hematology and serum biochemistry profiling, and the livers and gastrointestinal tracts were weighed and examined. The livers also were evaluated microscopically. The blood glucose and urea nitrogen concentrations and liver weights of animals fed sucrose differed significantly from those of the other groups. Alterations were more striking in males than females. Marked histological changes were present in livers from animals fed sucrose prior

to necropsy compared with fasted or chow-fed animals, and these changes were attributed to increased glycogen deposition in the sucrose-fed animals. Because of alterations in hepatic structure and function, we cannot recommend the practice of feeding sucrose to rats prior to necropsy for toxicology studies or any studies examining hepatic function. As taken from Turner PV et al. Contemp Top Lab Anim Sci. 2001 Jul; 40(4):36-40. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=11451394&query_hl=19&itool=pubmed_docsum

Hepatic adaptations to sucrose and fructose (Abstract). The liver is an important site of postprandial glucose disposal, accounting for the removal of up to 30% of an oral glucose load. The liver is also centrally involved in dietary lipid and amino acid uptake, and the presence of either or both of these nutrients can influence hepatic glucose uptake. The composition of ingested carbohydrate also influences hepatic glucose metabolism. For example, fructose can increase hepatic glucose uptake. In addition, fructose extraction by the liver is exceedingly high, approaching 50% to 70% of fructose delivery. The selective hepatic metabolism of fructose, and the ability of fructose to increase hepatic glucose uptake can, under appropriate conditions (eg, diets enriched in sucrose or fructose, high fructose concentrations), provoke major adaptations in hepatic metabolism. Potential adaptations that can arise in response to these conditions and putative mechanisms driving these adaptations are the subject of this review. As taken from Bizeau ME, Pagliassotti MJ. Metabolism 2005 Sep; 54(9):1189-201. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=16125531&query_hl=23&itool=pubmed_DocSum

“Sugar consumption, especially in the form of fructose, has been hypothesized to cause kidney disease. This review provides an overview of the epidemiologic evidence that sugar consumption increases CKD risk. Research supports a causal role of sugar in several kidney disease risk factors, including increasing serum uric acid levels, diabetes, and obesity. Sugar may also harm the kidney via other mechanisms. There is no evidence that sucrose is any safer for the kidney than high fructose corn syrup (HFCS) because both are similar in composition. To date, 5 epidemiologic studies have directly evaluated the relationship between sugar consumption (in the form of sugar-sweetened beverages) and CKD. Although most studies suggest that the risk of CKD is elevated among consumers of sugar-sweetened beverages, only 2 studies report statistically significant associations. Three studies have also examined diet soda consumption, with two reporting positive and significant associations. Confounding by unmeasured lifestyle factors may play a role in the positive results whereas poor measurement of sugar and artificial sweetener intake could explain null results. Nevertheless, the hypothesis that sugar causes kidney disease remains plausible, and alternative research designs may be needed.” As taken from Karalius VP & Shoham DA. 2013. Adv. Chronic Kidney Dis. 20(2), 157-64. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23439375?dopt=AbstractPlus

“The effect of rice protein (RP) on diabetic nephropathy in non-obese, spontaneous type 2 diabetic Goto-Kakizaki (GK) rats was investigated.GK rats at 7 weeks of age were fed 20% RP or casein (C) in standard or high-sucrose diets for 10 weeks. Plasma total cholesterol,TAG, alkaline phosphatase (ALP), adiponectin, creatinine and urinary albumin excretion (UAE) were measured and renal histology was evaluated….RP markedly suppressed the sharp increase in UAE when GK rats were fed high-sucrose diets (P<0·05), and prevented glomerular mesangial matrix expansion in the deep renal cortex near the

corticomedullary junction (P<0·05)….” As taken from Kubota M et al. 2013. Br. J. Nutr. 110(7), 1211-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23537514?dopt=AbstractPlus

“PURPOSE: The objective of this study is to investigate the effect of dual blockage of renin-angiotensin system (RAS) on renal kallikrein expression and inflammatory response in diabetic nephropathy (DN). METHODS: Rats were randomly divided into 5 groups with 10 rats in each group: normal control; DN model induced by high fat and high sucrose diets; and DN treated with either benazepril 10 mg/kg/d, irbesartan 30 mg/kg/d, or both. After 8-week treatment, we examined changes in the kidney histopathology, function and immunohistochemical stain of kallikrein, macrophage marker CD68, and profibrotic markers transforming growth factor- (TGF-) β and α-smooth muscle action (SMA). RESULTS: DN rats showed enlarged kidneys with glomerulosclerosis, interstitial chronic inflammation and fibrosis, and proteinuria. All the pathological damage and functional impairments were improved after the RAS blockades (all P < 0.05). Compared with monotherapy, combined treatment further alleviated the kidney impairments in parallel to increased tubular immunoreactivity for kallikrein and decreased immunopositive cells for CD68, TGF-β, and α-SMA. CONCLUSION: The renoprotective effects of the dual RAS blockade in diabetic nephropathy may be attributed to improved tubular kallikrein expression and interstitial inflammatory response.” As taken from Zou X et al. 2015. J. Diabetes Res. 2015, 310645. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25918729

“While numerous changes in human lifestyle constitute modern life, our diet has been gaining attention as a potential contributor to the increase in immune-mediated diseases. The Western diet is characterized by an over consumption and reduced variety of refined sugars, salt, and saturated fat. Herein our objective is to detail the mechanisms for the Western diet's impact on immune function. The manuscript reviews the impacts and mechanisms of harm for our over-indulgence in sugar, salt, and fat, as well as the data outlining the impacts of artificial sweeteners, gluten, and genetically modified foods; attention is given to revealing where the literature on the immune impacts of macronutrients is limited to either animal or in vitro models versus where human trials exist. Detailed attention is given to the dietary impact on the gut microbiome and the mechanisms by which our poor dietary choices are encoded into our gut, our genes, and are passed to our offspring. While today's modern diet may provide beneficial protection from micro- and macronutrient deficiencies, our over abundance of calories and the macronutrients that compose our diet may all lead to increased inflammation, reduced control of infection, increased rates of cancer, and increased risk for allergic and auto-inflammatory disease.” As taken from Myles IA 2014. Nutr. J. 13, 61. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24939238

“Glycyrrhizic acid (GA) ameliorates many components of the metabolic syndrome, but its potential therapeutic use is marred by edema caused by inhibition of renal 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2). We assessed whether 100 mg/kg per day GA administered orally could promote metabolic benefits without causing edema in rats fed on a high-sucrose diet. Groups of eight male rats were fed on one of three diets for 28 days: normal diet, a high-sucrose diet, or a high-sucrose diet supplemented with GA. Rats were then culled and renal 11β-HSD2 activity, as well as serum sodium, potassium, angiotensin II and leptin levels were determined. Histological analyses were performed to assess changes in adipocyte size in visceral and subcutaneous depots, as well as hepatic and renal tissue

morphology. This dosing paradigm of GA attenuated the increases in serum leptin levels and visceral, but not subcutaneous adipocyte size caused by the high-sucrose diet. Although GA decreased renal 11β-HSD2 activity, it did not affect serum electrolyte or angiotensin II levels, indicating no onset of edema. Furthermore, there were no apparent morphological changes in the liver or kidney, indicating no toxicity. In conclusion, it is possible to reap metabolic benefits of GA without edema using the current dosage and treatment time." As taken from Fernando HA et al. 2014. Nutrients 6(11), 4856-71. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25375630

"Hypertension remains a major health problem worldwide considering the prevalence of morbidity and mortality. Plants remain a reliable source of efficacious and better tolerated drugs and botanicals. This study was designed to investigate the effect of the chemo-profiled hydroethanolic leaf extract of Byrsocarpus coccineus in ethanol- and sucrose-induced hypertension. Groups of rats were treated orally (p.o.) with distilled water (10 ml/kg), ethanol (35%; 3 g/kg), sucrose (5-7%), and B. coccineus (100, 200, and 400 mg/kg), and nifedipine together with ethanol and sucrose separately for 8 weeks. At the end of the treatment period, blood pressure and heart rate of rats were determined. Blood was collected for serum biochemical parameters and lipid profile assessment, and the liver, aorta, kidney, and heart were harvested for estimation of in vivo antioxidants and malondialdehyde (MDA). Results obtained in this study showed that B. coccineus at the various doses administered reduced the systolic, diastolic, and arterial blood pressure elevated by ethanol and sucrose. Also, the extract reversed the reduction in catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD) induced by ethanol and sucrose. The level of MDA was reduced compared to the ethanol- and sucrose-induced hypertensive group. With respect to lipid profile, administration of B. coccineus at the various doses reduced the levels of triglycerides, low-density lipoprotein (LDL), cholesterol, and atherogenic indices, compared to the ethanol and sucrose groups. In conclusion the hydroethanolic leaf extract of B. coccineus exerted significant antihypertensive effect and this is probably related to the antioxidant property and improvement of lipid profile observed in this study." As taken from Akindele AJ et al. 2014. J. Tradit. Complement. Med. 4(3), 177-88. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25161923

"The purpose of this article review is to update what is known about the role of diet on non-alcoholic fatty liver disease (NAFLD). NAFLD is the most common cause of chronic liver disease in the developed world and is considered to be a spectrum, ranging from fatty infiltration of the liver alone (steatosis), which may lead to fatty infiltration with inflammation known as non alcoholic steatohepatitis While the majority of individuals with risk factors like obesity and insulin resistance have steatosis, only few people may develop steatohepatitis. Current treatment relies on weight loss and exercise, although various insulin-sensitizing medications appear promising. Weight loss alone by dietary changes has been shown to lead to histological improvement in fatty liver making nutrition therapy to become a cornerstone of treatment for NAFLD. Supplementation of vitamin E, C and omega 3 fatty acids are under consideration with some conflicting data. Moreover, research has been showed that saturated fat, trans-fatty acid, carbohydrate, and simple sugars (fructose and sucrose) may play significant role in the intrahepatic fat accumulation. However, true associations with specific nutrients yet to be clarified." As taken from Papandreou D and Andreou E. 2015. World J. Hepatol. 7(3), 575-82. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25848481

“BACKGROUND: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in developed countries. NAFLD encompasses a spectrum of diseases, ranging from hepatic steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis, and liver failure. The etiology of NAFLD remains unclear but is thought to relate to increased fatty acid flux within the liver that results in toxic fatty acid metabolite production. One source of increased fatty acid flux is fructose/sucrose-induced hepatic lipogenesis. Current treatment for NAFLD encompasses dietary modifications. However, little scientific evidence exists on which to base many dietary recommendations, especially the intake of different types of carbohydrates and fats. We hypothesized that lipid mixtures of unsaturated fatty acids would inhibit lipogenesis and subsequent hepatic steatosis induced by high carbohydrate diets. The aim of this study was to examine the effects of different complex mixtures of fatty acids upon the development of fructose/sucrose-induced hepatic steatosis. METHODS: C57BL/6 mice were randomized to normocaloric chow-based diets that varied in the type of carbohydrate (starch, sucrose, fructose). Animals in each carbohydrate group were further randomized to diets that varied in lipid type (no additional lipid, soybean oil, fish oil, olive/soybean oil, macadamia nut oil). These oils were chosen based upon their content of omega-6 polyunsaturated fatty acids, omega-3 polyunsaturated fatty acids, omega-9 monounsaturated fatty acids, or omega-7 monounsaturated fatty acids. Fatty acid flux in the liver was determine by assessing hepatic lipid content (steatosis). We also assessed fatty acid levels in the plasma and liver of the animals, hepatic lipogenesis activity, hepatic stearoyl-CoA-1 desaturase activity, and hepatic elongase activity. RESULTS: Animals consumed similar amounts of the diets and maintained normal body weights throughout the study. Both sucrose and fructose induced hepatic lipogenesis and steatosis, with fructose being more potent. All mixed lipids similarly inhibited steatosis, limiting lipid content to levels found in the control (starch) animals. Lipogenesis and stearoyl-CoA-1 desaturase activity were increased in the sucrose and fructose groups. Levels of these enzymatic processes remained at baseline in all of the lipid groups. CONCLUSION: This is the first study to compare various complex lipid mixtures, based upon dietary oils with different types of long-chain fatty acids, upon development of sucrose/fructose-induced steatosis. Both carbohydrate source and lipid content appear important for the modulation of steatosis. Moderate intake of complex lipids with high unsaturated to saturated fatty acid ratios inhibited both lipogenesis and steatosis.” As taken from Siddiqui RA et al. 2015. Nutr. Metab. (Lond.) 12, 41. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26583036

“OBJECTIVE: The objective of the present study was to investigate associations between sugar intake and overweight using dietary biomarkers in the Norfolk cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk). DESIGN: Prospective cohort study. SETTING: EPIC-Norfolk in the UK, recruitment between 1993 and 1997. SUBJECTS: Men and women (n 1734) aged 39-77 years. Sucrose intake was assessed using 7 d diet diaries. Baseline spot urine samples were analysed for sucrose by GC-MS. Sucrose concentration adjusted by specific gravity was used as a biomarker for intake. Regression analyses were used to investigate associations between sucrose intake and risk of BMI>25·0 kg/m2 after three years of follow-up. RESULTS: After three years of follow-up, mean BMI was 26·8 kg/m2. Self-reported sucrose intake was significantly positively associated with the biomarker. Associations between the biomarker and BMI were positive (β=0·25; 95 % CI 0·08, 0·43), while they were inverse when using self-reported dietary data (β=-1·40; 95 % CI -1·81, -0·99). The age- and sex-adjusted OR for BMI>25·0 kg/m2 in participants in the fifth v. first quintile was 1·54 (95 % CI 1·12, 2·12; P trend=0·003) when

using biomarker and 0·56 (95 % CI 0·40, 0·77; P trend<0·001) with self-reported dietary data. CONCLUSIONS: Our results suggest that sucrose measured by objective biomarker but not self-reported sucrose intake is positively associated with BMI. Future studies should consider the use of objective biomarkers of sucrose intake." As taken from Kuhnle GG et al. 2015. Public Health Nutr. 18(15), 2815-24. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25702697

"Various animal models of hyperlipidemia are used in research. Four rodent hyperlipidemia experimental models are examined in this study: three chronic hyperlipidemia models based on dietary supplementation with lipid or sucrose for 3 months and one acute hyperlipidemia model based on administration of the nonionic surfactant poloxamer. Neither lipid supplementation nor sucrose supplementation in Wistar rats was effective for establishing hyperlipidemia. Combining both lipid and sucrose supplementation in BALB/c mice induced hypercholesterolemia, as reflected in a considerable increase in blood cholesterol concentration, but did not produce an increase in blood triglyceride concentration. Poloxamer administration in C57BL/J6 mice produced increases in blood cholesterol and triglyceride concentrations. The authors conclude that supplementation of both lipid and sucrose in BALB/c mice was the most effective method for developing chronic hypercholesterolemia." As taken from Madariaga YG et al. 2015. Lab. Anim. (NY) 44(4), 135-40. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25793679

"Glucotoxicity and lipotoxicity are major hallmarks of metabolic disorder. High consumption of fat or carbohydrate rich food is a major risk of metabolic disorder. However, the evolution of high fat or high carbohydrate diet-induced metabolic disorder is not clear. In the study, we tried to find distinguished and common ways involved in the pathogenesis of insulin resistance induced by high fat (HF) and high sucrose (HS) diet. We found that HS diet induced mild glucose intolerance (2month), followed by a "temporary non-symptom phase" (3month), and then induced significant metabolic abnormality (4month). HF diet induced an early "responsive enhancement phase" (2month), and then gradually caused severe metabolic dysfunction (3-4month). After a mild induction of mitochondrial ROS generation (2month), HS diet resulted in a "temporary non-symptom phase" (3month), and then induced a more significant mitochondrial ROS production (4month). The impairment of mitochondrial function induced by HS diet was progressive (2-4month). HF diet induced gradual mitochondrial ROS generation and hyperpolarization. HF diet induced an early "responsive enhancement" of mitochondrial function (2month), and then gradually resulted in severe decrease of mitochondrial function (3-4month). Despite the patterns of HS and HF diet-induced insulin resistance were differential, final mitochondrial ROS generation combined with mitochondrial dysfunction may be the common pathway. These findings demonstrate a novel understanding of the mechanism of insulin resistance and highlight the pivotal role of mitochondrial ROS generation and mitochondrial dysfunction in the pathogenesis of metabolic disorder." As taken from Long Z et al. 2017. Gen. Comp. Endocrinol. 242, 92-100. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26497252

"STUDY QUESTION: Is sugar-sweetened beverage (SSB) consumption associated with age at menarche? SUMMARY ANSWER: More frequent SSB consumption was associated with earlier menarche in a population of US girls. WHAT IS KNOWN ALREADY: SSB consumption is associated with metabolic changes that could potentially impact menarcheal timing, but direct associations with age at menarche have yet to be investigated. STUDY DESIGN, SIZE, DURATION: The Growing up Today Study, a prospective cohort study of 16

875 children of Nurses' Health Study II participants residing in all 50 US states. This analysis followed 5583 girls, aged 9-14 years and premenarcheal at baseline, between 1996 and 2001. During 10 555 person-years of follow-up, 94% (n = 5227) of girls reported their age at menarche, and 3% (n = 159) remained premenarcheal in 2001; 4% (n = 197) of eligible girls were censored, primarily for missing age at menarche. PARTICIPANTS/MATERIALS, SETTING, METHODS: Cumulative updated SSB consumption (composed of non-carbonated fruit drinks, sugar-sweetened soda and iced tea) was calculated using annual Youth/Adolescent Food Frequency Questionnaires from 1996 to 1998. Age at menarche was self-reported annually. The association between SSB consumption and age at menarche was assessed using Cox proportional hazards regression. MAIN RESULTS AND THE ROLE OF CHANCE: More frequent SSB consumption predicted earlier menarche. At any given age between 9 and 18.5 years, premenarcheal girls who reported consuming >1.5 servings of SSBs per day were, on average, 24% more likely [95% confidence interval (CI): 13, 36%; P-trend: <0.001] to attain menarche in the next month relative to girls consuming ≤2 servings of SSBs weekly, adjusting for potential confounders including height, but not BMI (considered an intermediate). Correspondingly, girls consuming >1.5 SSBs daily had an estimated 2.7-month earlier menarche (95% CI: -4.1, -1.3 months) relative to those consuming ≤2 SSBs weekly. The frequency of non-carbonated fruit drink (P-trend: 0.03) and sugar-sweetened soda (P-trend: 0.001), but not iced tea (P-trend: 0.49), consumption also predicted earlier menarche. The effect of SSB consumption on age at menarche was observed in every tertile of baseline BMI. Diet soda and fruit juice consumption were not associated with age at menarche. LIMITATIONS, REASONS FOR CAUTION: Although we adjusted for a variety of suspected confounders, residual confounding is possible. We did not measure SSB consumption during early childhood, which may be an important window of exposure. WIDER IMPLICATIONS OF THE FINDINGS: More frequent SSB consumption may predict earlier menarche through mechanisms other than increased BMI. Our findings provide further support for public health efforts to reduce SSB consumption." As taken from Carwile JL et al. 2015. Hum. Reprod. 30(3), 675-83. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25628346

"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

"BACKGROUND & AIMS: The factors that distinguish metabolically healthy obesity from metabolically unhealthy obesity are not well understood. Diet has been implicated as a determinant of the unhealthy obesity phenotype, but which aspects of the diet induce dysmetabolism are unknown. The goal of this study was to investigate whether specific macronutrients or macronutrient combinations provoke dysmetabolism in the context of isocaloric, high-energy diets. METHODS: Mice were fed 4 high-energy diets identical in calorie and nutrient content but different in nutrient composition for 3 weeks to 6 months. The test diets contained 42% carbohydrate (sucrose or starch) and 42% fat (oleate or palmitate). Weight and glucose tolerance were monitored; blood and tissues were collected for histology, gene expression, and immunophenotyping. RESULTS: Mice gained weight on all 4 test diets but differed significantly in other metabolic outcomes. Animals fed the starch-oleate diet developed more severe hepatic steatosis than those on other formulas. Stable isotope incorporation showed that the excess hepatic steatosis in starch-oleate-fed mice derived from exaggerated adipose tissue lipolysis. In these mice, adipose tissue lipolysis coincided with adipocyte necrosis and inflammation. Notably, the liver and adipose tissue abnormalities provoked by starch-oleate feeding were reproduced when mice were fed a mixed-nutrient Western diet with 42% carbohydrate and 42% fat. CONCLUSIONS: The macronutrient composition of the diet exerts a significant influence on metabolic outcome, independent of calories and nutrient proportions. Starch-oleate appears to cause hepatic steatosis by inducing progressive adipose tissue injury. Starch-oleate phenocopies the effect of a Western diet; consequently, it may provide clues to the mechanism whereby specific nutrients cause metabolically unhealthy obesity." As taken from Duwaerts CC et al. 2017. Cell. Mol. Gastroenterol. Hepatol. 4(2), 223-236. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28649594

"With people aging, osteoporosis is expected to increase notably. Nutritional status is a relatively easily-modified risk factor, associated with many chronic diseases, and is involved in obesity, diabetes, and coronary heart disease (CHD), along with osteoporosis. Nutrients, such as fats, sugars, and proteins, play a primary function in bone metabolism and maintaining bone health. In Western nations, diets are generally high in saturated fats, however, currently, the nutritional patterns dominating in China continue to be high in carbohydrates from starch, cereals, and sugars. Moreover, high fat or high sugar (fructose, glucose, or sucrose) impart a significant impact on bone structural integrity. Due to diet being modifiable, demonstrating the effects of nutrition on bone health can provide an approach for osteoporosis prevention. Most researchers have reported that a high-fat diet consumption is associated with bone mineral density (BMD) and, as bone strength diminishes, adverse microstructure changes occur in the cancellous bone compartment, which is involved with lipid metabolism modulation disorder and the alteration of the bone marrow environment, along with an increased inflammatory environment. Some studies, however, demonstrated that a high-fat diet contributes to achieving peak bone mass, along with microstructure, at a younger age. Contrary to these results, others have shown that a high-fructose diet consumption leads to stronger bones with a superior microarchitecture

than those with the intake of a high-glucose diet and, at the same time, research indicated that a high-fat diet usually deteriorates cancellous bone parameters, and that the incorporation of fructose into a high-fat diet did not aggravate bone mass loss. High-fat/high-sucrose diets have shown both beneficial and detrimental influences on bone metabolism. Combined, these studies showed that nutrition exerts different effects on bone health. Thus, a better understanding of the regulation between dietary nutrition and bone health might provide a basis for the development of strategies to improve bone health by modifying nutritional components." As taken from Tian L and Yu X. 2017. Nutrients 9(5), E506. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28513571

"There is controversial information about the adverse effect of sucrose (S) or fructose (F) in the development of obesity. Thus, the purpose of the study was to evaluate the effect of S or F in a high fat diet (HF) on gut microbiota and renal oxidative stress. Rats were fed for four months with either high-fat + sucrose (HFS) or high-fat + fructose (HFF) or a control diet (C). Half of the HFS or HFF groups were maintained with the same diet and the other half were switched to the consumption of C. HFS and HFF groups increased 51% and 19% body weight, respectively, compared with the C group. Body fat mass, metabolic inflexibility, glucose intolerance, lipopolysaccharide (LPS), insulin, renal reactive oxygen species (ROS), malondialdehyde (MDA), Nadphox, and Srebp-1 were significantly higher and antioxidant enzymes and lean body mass were significantly lower in the HFS group with respect to the HF-F group. Change in the consumption of HFS or HFF to a C diet ameliorated the insulin and glucose intolerance. The type of carbohydrate differentially modified the microbiota composition, however, both groups significantly decreased C. eutactus with respect to the C group. Thus, metabolic alterations with the HFS diet had a more detrimental effect than HFF." As taken from Rosas-Villegas A et al. 2017. Nutrients 9(4), E393. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28420148

"Diets containing excess carbohydrate and fat promote hepatic steatosis and steatohepatitis in mice. Little is known, however, about the impact of specific carbohydrate/fat combinations on liver outcome. This study was designed to determine whether high-energy diets with identical caloric density but different carbohydrate and fat composition have unique effects on the liver. Four experimental diets were formulated with 60%kcal carbohydrate and 20%kcal fat, each in nearly pure form from a single source: starch-oleate, starch-palmitate, sucrose-oleate and sucrose-palmitate. The diets were fed to mice for 3 or 12 weeks for analysis of lipid metabolism and liver injury. All mice developed hepatic steatosis over 12 weeks, but mice fed the sucrose-palmitate diet accumulated more hepatic lipid than those in the other three experimental groups. The exaggerated lipid accumulation in sucrose-palmitate-fed mice was attributable to a disproportionate rise in hepatic de novo lipogenesis. These mice accrued more hepatic palmitate and exhibited more evidence of liver injury than any of the other experimental groups. Interestingly, lipogenic gene expression in mice fed the custom diets did not correlate with actual de novo lipogenesis. In addition, de novo lipogenesis rose in all mice between 3 and 12 weeks, without feedback inhibition from hepatic steatosis. The pairing of simple sugar (sucrose) and saturated fat (palmitate) in a high-carbohydrate/moderate-fat diet induces more de novo lipogenesis and liver injury than other carbohydrate/fat combinations. Diet-induced liver injury correlates positively with hepatic de novo lipogenesis and is not predictable by isolated analysis of lipogenic gene expression." As taken from Pierce AA et al. 2016. J. Nutr. Biochem. 29, 12-20. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26895660

“BACKGROUND AND PURPOSE: Thiazolidinediones (TZD) are known to ameliorate fatty liver in type 2 diabetes. To date, the underlying mechanisms of their hepatic actions remain unclear. EXPERIMENTAL APPROACH: Hepatic triglyceride content and export rates were assessed in 2 week high-sucrose-fed Wistar rats treated with troglitazone and compared with untreated high-sucrose rodent controls. Fractional de novo lipogenesis (DNL) contributions to hepatic triglyceride were quantified by analysis of triglyceride enrichment from deuterated water. Hepatic insulin clearance and NO status during a meal tolerance test were also evaluated. KEY RESULTS: TZD significantly reduced hepatic triglyceride ($P < 0.01$) by 48%, decreased DNL contribution to hepatic triglyceride ($P < 0.01$) and increased postprandial non-esterified fatty acids clearance rates ($P < 0.01$) in comparison with the high-sucrose rodent control group. During a meal tolerance test, plasma insulin AUC was significantly lower ($P < 0.01$), while blood glucose and plasma C-peptide levels were not different. Insulin clearance was increased ($P < 0.001$) by 24% and was associated with a 22% augmentation of hepatic insulin-degrading enzyme activity ($P < 0.05$). Finally, hepatic NO was decreased by 24% ($P < 0.05$). CONCLUSIONS: Overall, TZD show direct actions on liver by reducing hepatic DNL and increasing hepatic insulin clearance. The alterations in hepatic insulin clearance were associated with changes in insulin-degrading enzyme activity, with possible modulation of NO levels.” As taken from Martins FO et al. 2016. Br. J. Pharmacol. 173(2), 267-78. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26447327

“PURPOSE: Non-nutritive sweeteners are the most widely used food additives worldwide. However, their metabolic outcomes are still a matter of controversy and their effect on the thyroid activity, a key regulator of metabolism, has not been previously studied. Therefore, we aim to determine the influence of the sweet type flavour carrier on selected parameters of thyroid axis activity. METHODS: Male Sprague-Dawley rats (n = 105) were divided into 3 groups fed ad libitum for three weeks isocaloric diets (3.76 ± 0.5 kcal/g): two with the same sweet flavour intensity responded to 10% of sucrose (with sucrose-SC-and sucralose-SU) and one non-sweet diet (NS). To evaluate the post-ingested effects, animals were euthanised at fast and 30, 60, 120, 180 min after meal. RESULTS: The results obtained indicate that both the presence and the type of sweet taste flavour carrier affect thyroid axis activity both at fasting and postprandial state. Compared to diet with sucrose which stimulates thyroid axis activity, sucralose addition diminishes thyroid hormone synthesis as thyroid peroxidase (TPO) activity, plasma thyroxine (T4), and triiodothyronine (T3) concentration was lower than in SC and NS while in non-sweet diet the lowest level of hepatic deiodinase type 1 (DIO1) and the highest reverse T3 (rT3) level indicate on altered thyroid hormone peripheral metabolism. CONCLUSION: Both the presence and the type of sweet flavour carrier have a significant impact on thyroid axis activity. Our findings suggest that this organochlorine sweetener is metabolically active and might exacerbate metabolic disorders via an adverse effect on thyroid hormone metabolism.” As taken from Pałkowska-Goździk E et al. 2018. Eur. J. Nutr. 57(2), 773-782. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28040879

“MicroRNAs (miRNAs) are small non-protein-coding RNA molecules that regulate gene expression. Diet and lifestyle factors have been hypothesized to be involved in the regulation of miRNA expression. In this study it was hypothesized that diet and lifestyle factors are associated with miRNA expression. Data from 1,447 cases of colorectal cancer to evaluate 34 diet and lifestyle variables using miRNA expression in normal colorectal mucosa as well as for differential expression between paired carcinoma and normal tissue were used. miRNA data were obtained using an Agilent platform. Multiple comparisons

were adjusted for using the false discovery rate q-value. There were 250 miRNAs differentially expressed between carcinoma and normal colonic tissue by level of carbohydrate intake and 198 miRNAs differentially expressed by the level of sucrose intake. Of these miRNAs, 166 miRNAs were differentially expressed for both carbohydrate intake and sucrose intake. Ninety-nine miRNAs were differentially expressed by the level of whole grain intake in normal colonic mucosa. Level of oxidative balance score was associated with 137 differentially expressed miRNAs between carcinoma and paired normal rectal mucosa. Additionally, 135 miRNAs were differentially expressed in colon tissue based on recent NSAID use. Other dietary factors, body mass index, waist and hip circumference, and long-term physical activity levels did not alter miRNA expression after adjustment for multiple comparisons. These results suggest that diet and lifestyle factors regulate miRNA level. They provide additional support for the influence of carbohydrate, sucrose, whole grains, NSAIDs, and oxidative balance score on colorectal cancer risk." As taken from Slattery ML et al. 2016. Pharmgenomics Pers. Med. 10, 1-16. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28053552

"Genetic variation drives phenotypic diversity and influences the predisposition to metabolic disease. Here, we characterize the metabolic phenotypes of eight genetically distinct inbred mouse strains in response to a high-fat/high-sucrose diet. We found significant variation in diabetes-related phenotypes and gut microbiota composition among the different mouse strains in response to the dietary challenge and identified taxa associated with these traits. Follow-up microbiota transplant experiments showed that altering the composition of the gut microbiota modifies strain-specific susceptibility to diet-induced metabolic disease. Animals harboring microbial communities with enhanced capacity for processing dietary sugars and for generating hydrophobic bile acids showed increased susceptibility to metabolic disease. Notably, differences in glucose-stimulated insulin secretion between different mouse strains were partially recapitulated via gut microbiota transfer. Our results suggest that the gut microbiome contributes to the genetic and phenotypic diversity observed among mouse strains and provide a link between the gut microbiome and insulin secretion." As taken from Kreznar JH et al. 2017. Cell Rep. 18(7), 1739-1750. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28199845

"Barrett's esophagus (BE) is the key precursor lesion of esophageal adenocarcinoma, a lethal cancer that has increased rapidly in westernized countries over the past four decades. Dietary sugar intake has also been increasing over time, and may be associated with these tumors by promoting hyperinsulinemia. The study goal was to examine multiple measures of sugar/starches intake in association with BE. This pooled analysis included 472 BE cases and 492 controls from two similarly conducted case-control studies in the United States. Dietary intake data, collected by study-specific food frequency questionnaires, were harmonized across studies by linking with the University of Minnesota Nutrient Database, and pooled based on study-specific quartiles. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs), adjusting for age, sex, race, total energy intake, study indicator, body mass index, frequency of gastro-esophageal reflux, and fruit/vegetable intake. In both studies, intake of sucrose (cases vs. controls, g/day: 36.07 vs. 33.51; 36.80 vs. 35.06, respectively) and added sugar (46.15 vs. 41.01; 44.18 vs. 40.68, respectively) were higher in cases than controls. BE risk was increased 79% and 71%, respectively, for associations comparing the fourth to the first quartile of intake of sucrose ($OR_{Q4vs.Q1}$ = 1.79, 95% CI = 1.07-3.02, P_{trend} = 0.01) and added sugar ($OR_{Q4vs.Q1}$ = 1.71, 95% CI = 1.05-2.80, P_{trend} = 0.15). Intake of sweetened desserts/beverages was associated with 71% increase in BE risk ($OR_{Q4vs.Q1}$ = 1.71, 95% CI

= 1.07-2.73, P_{trend} = 0.04). Limiting dietary intake of foods and beverages that are high in added sugar, especially refined table sugar, may reduce the risk of developing BE." As taken from Li N et al. 2017. Eur. J. Epidemiol. 32(11), 1007-1017. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28864851

"PURPOSE: Carbohydrate intake increases postprandial insulin secretion and may affect breast density, a strong risk factor for breast cancer, early in life. We examined associations of adolescent and early adulthood intakes of total carbohydrates, glycemic index/load, fiber, and simple sugars with breast density among 182 young women. METHODS: Diet was assessed using three 24-h recalls at each of five Dietary Intervention Study in Children (DISC) clinic visits when participants were age 10-19 years and at the DISC06 Follow-Up Study clinic visit when participants were age 25-29 years. Associations between energy-adjusted carbohydrates and MRI-measured percent dense breast volume (%DBV) and absolute dense breast volume (ADBV) at 25-29 years were quantified using multivariable-adjusted mixed-effects linear models. RESULTS: Adolescent sucrose intakes and premenarcheal total carbohydrates intakes were modestly associated with higher %DBV (mean %DBVQ1 vs Q4, 16.6 vs 23.5% for sucrose; and 17.2 vs 22.3% for premenarcheal total carbohydrates, all Ptrend ≤ 0.02), but not with ADBV. However, adolescent intakes of fiber and fructose were not associated with %DBV and ADBV. Early adulthood intakes of total carbohydrates, glycemic index/load, fiber, and simple sugars were not associated with %DBV and ADBV. CONCLUSIONS: Insulinemic carbohydrate diet during puberty may be associated with adulthood breast density, but our findings need replication in larger studies. Clinical Trials Registration ClinicalTrials.gov Identifier, NCT00458588 April 9, 2007; NCT00000459 October 27, 1999. " As taken from Jung S et al. 2018. Cancer Causes Control 29(7), 631-642. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29802491

"Obesity is an important modifiable risk factor for chronic diseases. While there is increasing focus on the role of dietary sugars, there remains a paucity of data establishing the association between sugar intake and obesity in the general public. The objective of this study was to investigate associations of estimated sugar intake with odds for obesity in a representative sample of English adults. We used data from 434 participants of the 2005 Health Survey of England. Biomarkers for total sugar intake were measured in 24 h urine samples and used to estimate intake. Linear and logistic regression analyses were used to investigate associations between biomarker-based estimated intake and measures of obesity (body mass intake (BMI), waist circumference and waist-to-hip ratio) and obesity risk, respectively. Estimated sugar intake was significantly associated with BMI, waist circumference and waist-to-hip ratio; these associations remained significant after adjustment for estimated protein intake as a marker of non-sugar energy intake. Estimated sugar intake was also associated with increased odds for obesity based on BMI (OR 1.02; 95%CI 1.00-1.04 per 10g), waist-circumference (1.03; 1.01-1.05) and waist-to-hip ratio (1.04; 1.02-1.06); all OR estimates remained significant after adjusting for estimated protein intake. Our results strongly support positive associations between total sugar intake, measures of obesity and likelihood of being obese. It is the first time that such an association has been shown in a nationally-representative sample of the general population using a validated biomarker. This biomarker could be used to monitor the efficacy of public health interventions to reduce sugar intake." As taken from Campbell R et al. 2017. PLoS One 12(7), e0179508. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28723954

“Dramatic increases in obesity and diabetes have occurred worldwide over the past 30 years. Some investigators have suggested that these increases may be due, in part, to increased added sugars consumption. Several scientific organizations, including the World Health Organization, the Scientific Advisory Council on Nutrition, the Dietary Guidelines Advisory Committee 2015, and the American Heart Association, have recommended significant restrictions on upper limits of sugars consumption. In this review, the scientific evidence related to sugars consumption and its putative link to various chronic conditions such as obesity, diabetes, heart disease, nonalcoholic fatty liver disease, and the metabolic syndrome is examined. While it appears prudent to avoid excessive calories from sugars, the scientific basis for restrictive guidelines is far from settled.” As taken from Rippe JM et al. 2017. Nutr. Rev. 75(1), 18-36. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/27974597

“Compulsive binge eating is a hallmark of binge eating disorder and bulimia nervosa and is implicated in some obesity cases. Eating disorders are sexually dimorphic, with females more often affected than males. Animal models of binge-like eating based on intermittent access to palatable food exist; but, little is known regarding sex differences or individual vulnerability in these models with respect to the reinforcing efficacy of food, the development of compulsive- and binge-like eating, or associated changes in whole-body metabolism or body composition. Adolescent male (n = 24) and female (n = 32) Wistar rats were maintained on chow or a preferred, high-sucrose, chocolate-flavored diet in continuous or intermittent, extended access conditions. Body weight and composition, intake, fixed- and progressive-ratio operant self-administration, and whole body energy expenditure and respiratory exchange ratios were measured across an 11-week study period. Subgroup analyses were conducted to differentiate compulsive-like "high responder" intermittent access rats that escalated to extreme progressive-ratio self-administration performance vs. more resistant "low responders." Female rats had greater reinforcing efficacy of food than males in all diet conditions and were more often classified as "high responders". In both sexes, rats with intermittent access showed cycling of fuel substrate utilization and whole-body energy expenditure. Further, "high-responding" intermittent access female rats had especially elevated respiratory exchange ratios, indicating a fat-sparing phenotype. Future studies are needed to better understand the molecular and neurobiological basis of the sex and individual differences we have observed in rats and their translational impact for humans with compulsive, binge eating disorders.” As taken from Spierling SR et al. 2018. Physiol. Behav. 192, 3-16. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29654812

As taken from US EPA, 2017.

“Excessive sucrose intake, known as fructose toxicity, leads to fatty liver, hyperlipidemia, and metabolic syndrome. Circadian disorders also contribute to metabolic syndrome. Here, we investigated the effect of excessive sucrose intake on circadian rhythms of the small intestine, the main location of sucrose absorption, to elucidate a mechanism of sucrose-induced abnormal lipid metabolism. Male Wistar rats were fed control starch or high-sucrose diets for 4 weeks. High-sucrose diet-induced fatty liver and hypertriglyceridemia in rats. Amplitudes of PER1/2 expression oscillations in the small intestine were reduced by excessive sucrose, while gene expression of GLUT5 and gluconeogenic enzymes was enhanced. These changes would contribute to interfering in lipid homeostasis as well as adaptive responses to control fructose toxicity in rats.” As taken from Sun S et al. 2019.

Chronobiol. Int. 36(6), 826-837. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30917707

“This review will focus on the question of whether dietary sugars are a relevant determinant in the global rise of overweight and obesity in adults, adolescents, and children. Initially, the review describes the current definitions for sugars in the diet and makes reference to them while analyzing their role in overweight and obesity as well as diet-related diseases, including type 2 diabetes, cardiovascular diseases, non-alcoholic fatty liver disease and cancer. Second, it will focus particularly on sucrose and the question of whether it is the molecular composition of sucrose (glucose and fructose) or its energy content that promotes body weight gain and diet-related diseases. Finally, the review will clarify the molecular characteristics of sucrose regarding the release of the gastrointestinal glucose-dependent insulinotropic peptide (GIP) compared to other energy-providing nutrients and its relevance in metabolic diseases. Current data indicates that dietary sugars are only associated with an increase in obesity when consumed as an excess source of calories and with that an increase in the risk of diet-related diseases. Furthermore, it was shown that a diet rich in fat will stimulate GIP secretion more than a diet rich in sucrose. Taken together, current scientific evidence does not support the conclusion that dietary sugars per se are detrimental to human health.” As taken from Prinz P. 2019. Eur. J. Clin. Nutr. Epub ahead of print. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30787473/

“A high-sucrose diet (HSD) is widely known for its cariogenic effects and promotion of obesity, insulin resistance, type 2 diabetes, and cancer. However, the impact of the HSD diet on the salivary gland function as well as the level of salivary oxidative stress is still unknown and requires evaluation. Our study is the first to determine both redox balance and oxidative injury in the parotid and submandibular glands of rats fed the HSD diet compared to the control group. We have demonstrated that uric acid concentration and the activity of superoxide dismutase and peroxidase varied significantly in both the submandibular and parotid glands of HSD rats vs. the control group. However, enhanced oxidative damage to proteins, lipids, and DNA (increase in advanced glycation end products, advanced oxidation protein products, 4-hydroxynonenal, and 8-hydroxy-2'-deoxyguanosine) was observed only in the parotid glands of HSD rats. Moreover, the HSD diet also reduced the total protein content and amylase activity in both types of salivary glands and decreased the stimulated salivary flow rate. To sum up, an HSD diet reduces salivary gland function and disturbs the redox balance of the parotid as well as submandibular salivary glands. However, the parotid glands are more vulnerable to both antioxidant disturbances and oxidative damage.” As taken from Maciejczyk M et al. 2018. Nutrients 10(10), pii: E1530. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30336621

“Insulin resistance (IR) and impaired glucose tolerance (IGT) are the first manifestations of diet-induced metabolic alterations leading to Type 2 diabetes, while hypertension is the deadliest risk factor of cardiovascular disease. The roles of dietary fat and fructose in the development of IR, IGT, and hypertension are controversial. We tested the long-term effects of an excess of fat or sucrose (fructose/glucose) on healthy male Wistar-Kyoto (WKY) rats. Fat affects IR and IGT earlier than fructose through low-grade systemic inflammation evidenced by liver inflammatory infiltration, increased levels of plasma IL-6, PGE2, and reduced levels of protective short-chain fatty acids without triggering hypertension. Increased populations of gut Enterobacteriales and Escherichia coli may contribute to systemic inflammation through the generation of lipopolysaccharides. Unlike fat, fructose induces increased levels of diacylglycerols (lipid mediators of IR) in the liver, urine F2-

isoprostanes (markers of systemic oxidative stress), and uric acid, and triggers hypertension. Elevated populations of Enterobacteriales and E. coli were only detected in rats given an excess of fructose at the end of the study. Dietary fat and fructose trigger IR and IGT in clearly differentiated ways in WKY rats: early low-grade inflammation and late direct lipid toxicity, respectively; gut microbiota plays a role mainly in fat-induced IR, and hypertension is independent of inflammation-mediated IR. The results provide evidence that suggests that the combination of fat and sugar is potentially more harmful than fat or sugar alone when taken in excess." As taken from Ramos-Romero S et al. 2018. Am. J. Physiol. Endocrinol. Metab. 314(6), E552-E563. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/29351480

7. Addiction

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

"Understanding how tobacco product flavor additives, such as flavorants in electronic cigarettes, influence smoking behavior and addiction is critical for informing public health policy decisions regarding tobacco product regulation. Here, we developed a combined intraoral (i.o.) and intravenous (i.v.) self-administration paradigm in rats to determine how flavorants influence self-administration behavior. By combining i.o. flavorant delivery with fast scan cyclic voltammetry (FSCV) or i.v. nicotine self-administration in adult, male rats, we examined whether flavors alter phasic dopamine (DA) signaling and nicotine self-administration. Oral administration of 10% sucrose or 0.32% saccharin, but not 0.005% menthol, increased phasic DA release in the nucleus accumbens (NAc). Oral sucrose or saccharin, when combined with i.v. nicotine delivery, also led to increased self-administration behavior. Specifically, combined i.o. sucrose and i.v. nicotine decreased responding compared to sucrose alone, and increased responding compared to nicotine alone. In contrast, i.o. flavorants did not alter motivational breakpoint in a progressive ratio task. Oral menthol, which did not alter i.v. nicotine administration, reversed oral nicotine aversion (50 and 100 mg/L) in a two-bottle choice test. Here, we demonstrate that i.o. appetitive flavorants that increase phasic DA signaling also increase self-administration behavior when combined with i.v. nicotine delivery. Additionally, oral menthol effects were specific to oral nicotine, and were not observed with i.v. nicotine-mediated reinforcement. Together, these preclinical findings have important implications regarding menthol and sweet flavorant additive effects on tobacco product use and can be used to inform policy decisions on tobacco product flavorant regulation." As taken from Wickham RJ et al. 2018. Neuropharmacology 128, 33-42. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28943284

8. Burnt ingredient toxicity

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

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

Endpoint	**Tested level (ppm)**	**Reference**
Smoke chemistry	49,332	Carmines, 2002 & Rustemeier et al., 2002
	40,000 (No CAS)	JTI KB Study Report(s)
	80,000	Gaworski et al., 2011 & Coggins et al., 2011a
	807	Roemer et al., 2014b
In vitro genotoxicity	49,332	Carmines, 2002 & Röemer et al., 2002
	105,000	Baker et al., 2004c
	40,000 (No CAS)	JTI KB Study Report(s)
	28,100	fGLH Study Report (2010)
	80,000	Gaworski et al., 2011 & Coggins et al., 2011a
	807	Roemer et al., 2014b
In vitro cytotoxicity	49,332	Carmines, 2002 & Röemer et al., 2002
	105,000	Baker et al., 2004c
	28,100	fGLH Study Report (2010)
	80,000	Gaworski et al., 2011 & Coggins et al., 2011a
	807	Roemer et al., 2014b
Inhalation study	49,332	Carmines, 2002 & Vanscheeuwijck et al., 2002

	105,000	Baker et al., 2004c
	40,000 (No CAS)	JTI KB Study Report(s)
	80,000	Gaworski et al., 2011 & Coggins et al., 2011a
	807	Schramke et al., 2014
Skin painting	40,000 (No CAS)	JTI KB Study Report(s)
In vivo genotoxicity	807	Schramke et al., 2014

Sugars, such as sucrose or invert sugar, have been used as tobacco ingredients in American-blend cigarettes to replenish the sugars lost during curing of the Burley component of the blended tobacco in order to maintain a balanced flavor. Chemical-analytical studies of the mainstream smoke of research cigarettes with various sugar application levels revealed that most of the smoke constituents determined did not show any sugar-related changes in yields (per mg nicotine), while ten constituents were found to either increase (formaldehyde, acrolein, 2-butanone, isoprene, benzene, toluene, benzo[*k*]fluoranthene) or decrease (4-aminobiphenyl, N-nitrosodimethylamine, N-nitrosonornicotine) in a statistically significant manner with increasing sugar application levels. Such constituent yields were modelled into constituent uptake distributions using simulations of nicotine uptake distributions generated on the basis of published nicotine biomonitoring data, which were multiplied by the constituent/nicotine ratios determined in the current analysis. These simulations revealed extensive overlaps for the constituent uptake distributions with and without sugar application. Moreover, the differences in smoke composition did not lead to relevant changes in the activity in *in vitro* and *in vivo* assays. The potential impact of using sugars as tobacco ingredients was further assessed in an indirect manner by comparing published data from markets with predominantly American-blend or Virginiatype (no added sugars) cigarettes. No relevant difference was found between these markets for smoking prevalence, intensity, some markers of dependence, nicotine uptake, or mortality from smoking-related lung cancer and chronic obstructive pulmonary disease. In conclusion, thorough examination of the data available suggests that the use of sugars as ingredients in cigarette tobacco does not increase the inherent risk and harm of cigarette smoking (Roemer et al., 2012).

“The cigarette ingredients cocoa powder, glycerol, and saccharose were investigated regarding their potential effect on the resulting mainstream smoke, i.e., smoke chemistry (Hoffmann analytes), mammalian cell cytotoxicity (Neutral Red Uptake assay), and bacterial mutagenicity (Ames assay). Each ingredient was added at three concentrations to the tobacco of a 6 mg and 10 mg ‘tar’ yield experimental American blend filter cigarette (obtained under ISO/FTC smoking regime). The lowest application concentration was equivalent to the normal approximate use level of the ingredients; the highest application level was up to 5-fold higher. The resulting data were compared with the respective control cigarettes without addition of the ingredients. The addition of cocoa powder did not lead to any consistent effects on the measured mainstream smoke analytes. Neither the in vitro cytotoxicity nor the in vitro mutagenicity was affected by cocoa addition. The addition of glycerol resulted in a decrease in the delivery of several smoke constituents (generally around 20%), e.g. aldehydes, phenolics, and N-nitrosamines. Water in the particulate phase (TPM) was distinctly increased (up to +150%). The cytotoxicity of the TPM was decreased (approx. -15%). Mutagenicity was not affected. Saccharose addition consistently increased formaldehyde delivery in smoke by up to 40% and decreased tobacco-specific N-

nitrosamines by up to approximately 20%. The increase in formaldehyde is discussed in the context of the human smoker. The cytotoxicity was not affected by the addition of saccharose, while the mutagenicity of the TPM was decreased in tester strain TA98 with metabolic activation (-15%). The results are in agreement with currently available literature. Some investigations summarized in this publication are novel and have not yet been reported in the literature. Based on the total evidence, it can be concluded that the three ingredients added at their current use levels do not increase the inherent toxicity of the cigarette smoke." As taken from Roemer E et al. 2010. Beiträge zur Tabakforschung International 24(3), 117–138. Available at http://www.degruyter.com/view/j/cttr.2010.24.issue-3/cttr-2013-0890/cttr-2013-0890.xml?rskey=vlZjPi&result=5

In a water pipe transfer study, 0.003% of sucrose was transferred intact to the smoke (JTI Study Report (s)).

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing sugars (sucrose) was 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 TPM was not increased by the addition of sugars (sucrose) when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	**Tested level (ppm)**	**Reference**
In vitro genotoxicity	39	JTI KB Study Report(s)
In vitro cytotoxicity	39	JTI KB Study Report(s)

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List state that sucrose is not persistent in the environment:

Rational for P	QSAR

Media of concern leading to Categorization	Water
Experimental Biodegradation half-life (days)	Not Available
Predicted Ultimate degradation half-life (days)	8.67
MITI probability of biodegradation	0.7325
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	0.09317

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

EPISuite provides the following data:

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]: Bond Method :	4.47E-022 atm-m3/mole (4.53E-017 Pa-m3/mole)
Group Method:	Incomplete
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.590E-022 atm-m3/mole (1.611E-017 Pa-m3/mole) VP: 3.53E-016 mm Hg (source: MPBPVP) WS: 1E+006 mg/L (source: WSKOWWIN)
Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]: Log Kow used:	-3.70 (exp database)
Log Kaw used:	-19.738 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	16.038
Log Koa (experimental database):	None
Probability of Rapid Biodegradation (BIOWIN v4.10): Biowin1 (Linear Model):Biowin2 (Non-Linear Model) :Biowin3 (Ultimate Survey Model):Biowin4 (Primary Survey Model) :Biowin5	0.6284 0.0072

(MITI Linear Model) :Biowin6 (MITI Non-Linear Model):Biowin7 (Anaerobic Linear Model):	3.4844 (days-weeks) 4.2068 (days) 1.3027 0.7325 1.1858
Ready Biodegradability Prediction:	YES
Hydrocarbon Biodegradation (BioHCwin v1.01): Structure incompatible with current estimation method!	
Sorption to aerosols (25 Dec C)[AEROWIN v1.00]: Vapor pressure (liquid/subcooled):	2.24E-012 Pa (1.68E-014 mm Hg)
Log Koa (Koawin est):	16.038
Kp (particle/gas partition coef. (m3/ug)):Mackay model: Octanol/air (Koa) model:	1.34E+0062.68E+003
Fraction sorbed to airborne particulates (phi): Junge-Pankow model:	1
Mackay model:	1
Octanol/air (Koa) model:	1
Atmospheric Oxidation (25 deg C) [AopWin v1.92]: Hydroxyl Radicals Reaction: OVERALL OH Rate Constant =	114.7988 E-12 cm3/molecule-sec
Half-Life =	0.093 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	1.118 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 1 (Junge-Pankow, Mackay avg) 1 (Koa method)Note: the sorbed fraction may be resistant to atmospheric oxidation	
Soil Adsorption Coefficient (KOCWIN v2.00): Koc :	10 L/kg (MCI method)

Log Koc:	1.000 (MCI method)
Koc :	0.006079 L/kg (Kow method)
Log Koc:	-2.216 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]: Rate constants can NOT be estimated for this structure!

Volatilization from Water: Henry LC: 4.47E-022 atm-m3/mole (estimated by Bond SAR Method) Half-Life from Model River:	2.423E+018 hours (1.01E+017 days)
Half-Life from Model Lake:	2.644E+019 hours (1.102E+018 days)
Removal In Wastewater Treatment: Total removal:	1.85 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.75 percent
Total to Air:	0.00 percent

(using 10000 hr Bio P,A,S) **Level III Fugacity Model:**	Mass Amount(percent)	Half-Life(hr)	Emissions(kg/hr)
Air	1.98e-007	2.24	1000
Water	28.1	208	1000
Soil	71.8	416	1000
Sediment	0.0592	1.87e+003	0

Persistence Time: 414 hr

10.2. Aquatic toxicity

Invertebrates (Daphnia) (QSAR)

LC50: 1.38e+008 mg/l (16 days)

Aquatic plants (algae) (QSAR)

EC50: 6.02e+007 mg/l (96 hrs)

Fish (QSAR)

LC50: 1.99e+008 mg/l (96 hrs), 1.33e+008 mg/l (14 days), 2.2e+006 mg/l (96 hrs, SW

Chronic: 9.32e+006 mg/l (30 days)

As taken from Review of annex IV of regulation (EC) NO. 1907/2006 (REACH) http://ec.europa.eu/environment/chemicals/reach/pdf/6b_appendix_2.pdf

Record for alpha-D-glucopyranoside, beta-D-fructofuranosyl:

Spec. Sci. Name **Spec. Common Name**	**Exp. Type** **Chem. Anal.**	**Media Type** **Loc**	**Resp. Site** **Obs. Dur. (Days)**	**Endpoint** **BCF**	**Trend** **Eff %**	**Effect** **Effect Meas.**	**Conc (Standardized)** **Appl. Rate**	**Stat. Signif.** **Sig. Level**
Pagrus major Red Sea Bream	S U	SW LAB	0.5	NOEC	INC 100.0	MOR HTCH/	F 20 % v/v	ANOSIG <0.05
Pagrus major Red Sea Bream	S U	SW LAB	0.5	NOEC	NEF 99.3	MOR HTCH/	F 20 % v/v	ANOSIG <0.05
Acipenser baerii Long-Nosed Siberian Sturgeon	D U	FW LAB	(0.002 - 0.004)		DEC 7.0	FDB FCNS	F 0.29 M	NOSIG >0.95
Acipenser stellatus Sevruga, Stellate Sturgeon	D U	FW LAB	(0.002 - 0.004)		DEC 7.8	FDB FCNS	F 0.29 M	NOSIG >0.95

Anarhichas lupus Atlantic Wolf-Fish	D U	FW LAB	(0.002 - 0.004)		INC 6.4	FDB FCNS	F 0.29 M	NOSIG >0.95
Carassius gibelio Prussian Carp	D U	FW LAB	(0.002 - 0.004)		INC 73.8	FDB FCNS	F 0.29 M	NOSIG >0.95
Cyprinidae Minnow,Carp Family	D U	FW LAB	(0.002 - 0.004)		DEC >0-<10/	FDB FCNS	F 0.29 M	NOSIG <0.05
Cyprinidae Minnow,Carp Family	D U	FW LAB	(0.002 - 0.004)		NEF ~100/	FDB FCNS	F 0.29 M	NOSIG <0.05
Eleginus navaga Atlantic Navaga	D U	FW LAB	(0.002 - 0.004)		INC 20.8	FDB FCNS	F 0.29 M	NOSIG >0.95
Gadus ogac Greenland Cod	D U	FW LAB	(0.002 - 0.004)		DEC 14.9	FDB FCNS	F 0.29 M	NOSIG >0.95
Heros severus Banded Cichlid	D U	FW LAB	(0.002 - 0.004)		DEC 25.0	FDB FCNS	F 0.29 M	NOSIG >0.95
Leuciscus cephalus Chub	D U	FW LAB	(0.002 - 0.004)		DEC 25.6	FDB FCNS	F 0.29 M	NOSIG >0.95
Leuciscus leuciscus Dace	D U	FW LAB	(0.002 - 0.004)		INC 63.8	FDB FCNS	F 0.29 M	SIG >0.99
Liopsetta glacialis Arctic Flounder	D U	FW LAB	(0.002 - 0.004)		INC 78.6	FDB FCNS	F 0.29 M	NOSIG >0.95
Phoxinus phoxinus Minnow	D U	FW LAB	(0.002 - 0.004)		INC 33.0	FDB FCNS	F 0.29 M	NOSIG >0.95

Pungitius pungitius ssp. pungitius 10-Spined Stickleback	D U	FW LAB	(0.002 - 0.004)		DEC 15.6	FDB FCNS	F 0.29 M	NOSIG >0.95
Rutilus rutilus Roach	D U	FW LAB	(0.002 - 0.004)		INC 73.1	FDB FCNS	F 0.29 M	SIG >0.99
Salmo trutta ssp. caspius Brown Trout	D U	FW LAB	(0.002 - 0.004)		DEC 9.6	FDB FCNS	F 0.29 M	NOSIG >0.95
Salvelinus alpinus ssp. erythrinus Char	D U	FW LAB	(0.002 - 0.004)		INC 28.3	FDB FCNS	F 0.29 M	NOSIG >0.95
Salvelinus alpinus ssp. erythrinus Char	D U	FW LAB	(0.002 - 0.004)		INC 34.9	FDB FCNS	F 0.29 M	NOSIG >0.95
Sciaenops ocellatus Red Drum	S U	SW LAB	0.014		CHG	MOR HTCH	F (0.1-2) M	MULT <=0.05
Thymallus thymallus European Grayling	D U	FW LAB	(0.002 - 0.004)		DEC 6.2	FDB FCNS	F 0.29 M	SIG >0.95
Danio rerio Zebra Danio	S U	FW LAB	2	LD0		MOR MORT	A 2.5 % v/v	
Danio rerio Zebra Danio	S U	FW LAB	SM (<0 - < 0.002)			REP GREP/	F (80-240) mM	
Salmo trutta Brown Trout	D U	FW LAB	(0.002 - 0.004)		DEC >0-<200/	FDB FCNS	F 0.29 M	NOSIG <0.01
Salvelinus namaycush	D U	FW LAB			DEC 4.2	FDB FCNS	F 0.29 M	NOSIG >0.95

Lake Trout, Siscowet			(0.002 - 0.004)					
Cyprinus carpio Common Carp	D U	FW LAB	(0.002 - 0.004)		DEC 20.9	FDB FCNS	F 0.29 M	NOSIG >0.95
Ctenopharyngodon idella Grass Carp	D U	FW LAB	(0.002 - 0.004)		INC 47.7	FDB FCNS	F 0.29 M	SIG >0.99
Sander lucioperca Zander	S U	FW LAB	1			MOR/ MORT	F 60000000 ug/L	
Oncorhynchus keta Chum Salmon	D U	FW LAB	(0.002 - 0.004)		DEC 24.2	FDB FCNS	F 0.29 M	NOSIG >0.95
Zostera noltii Dwarf Eelgrass	S U	SW LAB	(0.083 - 0.417)		CHG	BCM ETHL	F 5 nM	
Lemna gibba Inflated Duckweed	S U	FW LAB	FD 7	EC10	DEC	POP NCHG	F < 100.00 mM	
Lemna gibba Inflated Duckweed	S U	FW LAB	FD 7	EC50	DEC	POP NCHG	F < 100.00 mM	

As taken from the EPA ECOTOX database.

The Ecological Categorization Results from the Canadian Domestic Substances List state that sucrose is not Inherently toxic to aquatic organisms:

Rational for iT	QSAR
Pivotal value for iT (mg/l)	60200000
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	199,000,000
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	60,200,000

Chronic toxicity to daphnia or algae (EC50 in mg/l) as predicted by Ecosar v0.99g	456,000
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	1.99E+008

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

“At the international workshop Aquatic Macrophyte Risk Assessment for Pesticides (AMRAP), it was noted that the EU risk assessment under the directive 91/414/EEC for herbicides, based only on algae and the monocotyledonous duckweed species Lemna sp., offers no certain protection against some growth regulating auxins. Therefore, AMRAP members proposed the introduction of the dicotyledonous water milfoil Myriophyllum as additional test species. This study was aimed to compare *toxicity* results from three test systems (TS) with varying complexity, namely Water TS, Sediment TS and Microcosm TS using Myriophyllum spicatum as test organism. As test substances, the photosynthesis inhibiting herbicide isoproturon, the growth regulating auxins fluroxypyr and 2,4-dichlorophenoxyacetic acid (2,4-D), and the non-specific acting toxicant 3,5-dichlorophenol (3,5-DCP) were chosen. It was assessed if and why the sensitivity of M. spicatum towards the four toxicants varied in the different test systems and if the addition of *sucrose* to the medium used in the Water TS had an effect on the sensitivity of Myriophyllum. All TS were suitable for detecting negative effects of toxicants with different modes of action on M. spicatum. The lowest variability of endpoints was found in the Water TS with lowest experimental complexity. For auxins, the endpoint weight did not result in robust EC50 values in all TS, whereas root related endpoints, which are also ecologically relevant, turned out to be very sensitive with low variance. *Sucrose* in the medium of the Water TS did not seem to influence the sensitivity of M. spicatum towards isoproturon and 3,5-DCP but may have increased the sensitivity of M. spicatum roots when exposed to 2,4-D. However, the findings of all TS resulted in similar risk estimations if root endpoints were not considered.” As taken from Mohr S et al. 2013. Ecotoxicol. Environ. Saf. 97, 32-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23928028

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 57-50-1:

Values used to Generate ECOSAR Profile:

Log Kow : -4.270 (EPISuite Kowwin v1.68 Estimate)

Wat Sol : 2.1E+006 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations:

Neutral Organics

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Neutral Organics :	Fish	96-hr	LC50	1.2e+008 *

Neutral Organics :	Daphnid	48-hr	LC50	3.83e+007 *
Neutral Organics :	Green Algae	96-hr	EC50	2.6e+006 *
Neutral Organics :	Fish		ChV	5.94e+006 *
Neutral Organics :	Daphnid		ChV	7.45e+005
Neutral Organics :	Green Algae		ChV	1.88e+005
Neutral Organics :	Fish (SW)	96-hr	LC50	1.46e+008 *
Neutral Organics :	Mysid	96-hr	LC50	7.58e+009 *
Neutral Organics :	Fish (SW)		ChV	3.44e+005
Neutral Organics :	Mysid (SW)		ChV	4.2e+009 *

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

“Sucrose as a carbon source in axenic tests affects plant growth and physiology. The high sucrose concentration in Organisation for Economic Co-operation and Development (OECD) guideline 238 for the submerged growing aquatic plant Myriophyllum spicatum might modify pollutant effects, thus impairing environmental risk assessment. In a factorial design experiment with axenic M. spicatum exposed to 3 sucrose concentrations (no, low, and high) with or without cadmium, growth, dry matter content, content in pigments or phenolic compounds, and elemental stoichiometry of carbon (C), nitrogen (N), and phosphorus (P) were measured. The results show that sucrose is crucial for growth but can be used at lower concentrations than currently considered. Sucrose-treated plants had higher dry matter content and C content but lower contents of chlorophyll and N. Cadmium affected the content in chlorophyll, phenolic compounds, and elemental stoichiometry. Interactive effects were observed on length growth, C and N content, and the C:N and N:P molar ratios. Remarkably, cadmium led to increased shoot length at low, but not at high, sucrose concentration. This contrasting effect might result from differences in osmotic potential caused by sucrose. Overall, the results suggest a strong effect of sucrose concentration on the growth and physiology of M. spicatum and modifications of the response to cadmium. Further studies should establish the lowest sucrose level needed to account for realistic environmental risk assessment based on the axenic OECD 238.” As taken from Nuttens A and Gross EM. 2017. Environ. Toxicol. Chem. 36(4), 969-975. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27597637

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

LC50: 1.8e+005 mg/kg dw (earthworms, 14 days) (QSAR)

No effect based on Koc and aquatic toxicity.

As taken from Review of annex IV of regulation (EC) NO. 1907/2006 (REACH) http://ec.europa.eu/environment/chemicals/reach/pdf/6b_appendix_2.pdf

Record for alpha-D-glucopyranoside, beta-D-fructofuranosyl:

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.**
Sturnus vulgaris European Starling	 0.25	NONE LAB	DR U	8	LOEL	FDB WCON	SIG <0.05	F 40 % w/v
Sturnus vulgaris European Starling	 0.25	NONE LAB	DR U	8	NOEL	FDB WCON	NOSIG <0.05	F 20 % w/v
Lilium sp. Lily	MT 90	AGR LAB	CM U	3	LOEL	DVP FORM	SIG 0.05	F 9000 mg/dm3
Solanum tuberosum Potato		NAT FIELDN	DU U	2	NOEL	DVP EMRG	ANOSIG <0.001	A 100 AI % w/w
Lilium sp. Lily	MT 90	AGR LAB	CM U	3	NOEL	DVP FORM	NOSIG 0.05	F 6000 mg/dm3

Lilium sp. Lily	WO 90	AGR LAB	CM U	3	NOEL	GRO WGHT	ANOSIG 0.05	F 9000 mg/dm3
Solanum tuberosum Potato		NAT FIELDN	DU U	2	NOEL	IMM IFCT	ANOSIG <0.001	A 100 AI % w/w
Lilium sp. Lily	BB 90	AGR LAB	CM U	3	NOEL	REP VEGR	ANOSIG 0.05	F 9000 mg/dm3
Sinapis alba White Mustard	CN 1	CUL LAB	SO	SL		BCM ETHL		NC 0.06 M
Sinapis alba White Mustard	CN 2	CUL LAB	SO	SL		BCM ETHL		NC 0.06 M
Sinapis alba White Mustard	CN 0.42	CUL LAB	SO	SL		BCM ETHL		NC 0.06 M
Plectranthus scutellarioides Coleus	RO 25	FIELDA	SO			GRO GGRO/		NC (1-20) %
Physocarpus opulifolius Ninebark	RO 55	LAB	SO	VG		GRO GGRO/		NC (1-20) %
Pisum sativum Pea	SS 0.83	CUL LAB	SO	SL		GRO SIZE		NC 2 %
Pisum sativum Pea	SS 0.75	CUL LAB	SO	SL		GRO SIZE		NC 2 %

Citrus sinensis Orange	MUL/ 25	 LAB	GM* 	 MT		GRO GGRO/		NC (2.5-15) %
Brassica rapa Bird Rape	MT 4	AQU LAB	DA U	4		GRO GRRT		F (0.1-1) %
Nicotiana glauca Tree Tobacco	RO 4	 LAB	EN U	2		PHY SUUP		F 1 mM
Plectranthus scutellarioides Coleus	RO 25	FIELDA	SO			POP ABND		NC (1-20) %
Iresine diffusa Bredinho-De-Linden	RO 23	FIELDA	SO			POP ABND		NC (1-20) %
Raphanus sativus Radish	WO 1	CUL LAB	SO	 SL		REP GERM		NC 1 1e-3 M
Raphanus sativus Radish	WO 2	CUL LAB	SO	 SL		REP GERM		NC 1 1e-3 M
Xanthium strumarium Common Cocklebur	WO 21	 LAB	SO	 MT		REP FLOR		NC 8 %
Beta vulgaris Sugar Beet	LE 0.04	CUL LAB	SO	 VG		BCM STRH		NC 10 mm
Phaseolus vulgaris Bean	LE 10	 LAB	SP	 SL		GRO BMAS		NC 10 %

Lonicera tatarica Tatarian Honeysuckle	MUL/ 32	 LAB	SO	 VG		GRO BMAS		NC (1-20) %
Varroa destructor Varroa Mite	 2	NONE LAB	EN U	6	NOEL	MOR MORT	ANOSIG 0.15	A 10 AI ug/cm2

As taken from the EPA ECOTOX database.

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 57-50-1:

Values used to Generate ECOSAR Profile:

Log Kow : -4.270 (EPISuite Kowwin v1.68 Estimate)

Wat Sol : 2.1E+006 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations:

Neutral Organics

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Neutral Organics :	Earthworm	14-day	LC50	2659.620

“Trehalose 6-phosphate (Tre6P), the intermediate of trehalose biosynthesis, has a profound influence on plant metabolism, growth, and development. It has been proposed that Tre6P acts as a signal of sugar availability and is possibly specific for sucrose status. Short-term sugar-feeding experiments were carried out with carbon-starved Arabidopsis thaliana seedlings grown in axenic shaking liquid cultures. Tre6P increased when seedlings were exogenously supplied with sucrose, or with hexoses that can be metabolized to sucrose, such as glucose and fructose. Conditional correlation analysis and inhibitor experiments indicated that the hexose-induced increase in Tre6P was an indirect response dependent on conversion of the hexose sugars to sucrose. Tre6P content was affected by changes in nitrogen status, but this response was also attributable to parallel changes in sucrose. The sucrose-induced rise in Tre6P was unaffected by cordycepin but almost completely blocked by cycloheximide, indicating that de novo protein synthesis is necessary for the response. There was a strong correlation between Tre6P and sucrose even in lines that constitutively express heterologous trehalose-phosphate synthase or trehalose-phosphate phosphatase, although the Tre6P:sucrose ratio was shifted higher or lower, respectively. It is proposed that the Tre6P:sucrose ratio is a critical parameter for the plant and forms part of a homeostatic mechanism to maintain sucrose levels within a range that is appropriate for the cell type and developmental stage of the plant.” As taken from Yadav UP et al. 2014. J. Exp. Bot. 65(4), 1051-68. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24420566

“Dietary exposure of insects to a feeding deterrent substance for hours to days can induce habituation and concomitant desensitization of the response of peripheral gustatory neurons to such a substance. In the present study, larvae of the herbivore Helicoverpa armigera

were fed on diets containing either a high, medium or low concentration of sucrose, a major feeding stimulant. The responsiveness of the sucrose-best neuron in the lateral sensilla styloconica on the galea was quantified. Results showed the response of the sucrose-best neuron exposed to high-sucrose diets decreased gradually over successive generations, resulting in complete desensitization in the 5th and subsequent generations. However, the sensitivity was completely restored in the ninth generation after neonate larvae were exposed to low-sucrose diet. These findings demonstrate phenotypic plasticity and exclude inadvertent artificial selection for low sensitivity to sucrose. No significant changes were found in the sensitivity of caterpillars which experienced low- or medium-sucrose diets over the same generations. Such desensitization versus re-sensitization did not generalise to the phagosimulant myo-inositol-sensitive neuron or the feeding deterrent-sensitive neuron. Our results demonstrate that under conditions of high sucrose availability trans-generational desensitization of a neuron sensitive to this feeding stimulant becomes more pronounced whereas re-sensitization occurs within one generation." As taken from Ma Y et al. 2016. Sci. Rep. 6, 39358. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27966640

"Liquid sugar baits are well accepted by the Argentine ant Linepithema humile and are suitable for the chemical control of this invasive species. We evaluated how sugar concentrations affect the foraging behavior of L. humile individuals. We quantified feeding variables for individual foragers (ingested load, feeding time and solution intake rate) when feeding on sucrose solutions of different concentrations, as well as post-feeding interactions with nestmates. Solutions of intermediate sucrose concentrations (10-30%) were the most consumed and had the highest intake rates, whereas solutions of high sucrose concentrations (60 and 70%) resulted in extended feeding times, low intake rates and ants having smaller crop loads. In terms of post-feeding interactions, individuals fed solutions of intermediate sucrose concentrations (20%) had the highest probability of conducting trophallaxis and the smallest latency to drop exposure (i.e. lowest time delay). Trophallaxis duration increased with increasing sucrose concentrations. Behavioral motor displays, including contacts with head jerking and walking with a gaster waggle, were lowest for individuals that ingested the more dilute sucrose solution (5%). These behaviors have been previously suggested to act as a communication channel for the activation and/or recruitment of nestmates. We show here that sucrose concentration affects feeding dynamics and modulates decision making related to individual behavior and social interactions of foragers. Our results indicate that intermediate sucrose concentrations (ca. 20%), appear to be most appropriate for toxic baits because they promote rapid foraging cycles, a high crop load per individual, and a high degree of stimulation for recruitment." As taken from Sola FJ and Josens R. 2016. Bull. Entomol. Res. 106(4), 522-9. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27063551

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that sucrose is not bioaccumulative in the environment:

Rational for B	QSAR
Empirical Log Kow	-3.7
Log Kow predicted by KowWin	-4.27
Log BAF T2MTL predicted by Gobas	0.0000540371955876
Log BCF 5% T2LTL predicted by Gobas	0.0005144646215092
Log BCF Max predicted by OASIS	0.931268065669035
Log BCF predicted by BCFWIN	0.5

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

EPISuite provides the following data:

Bioaccumulation Estimates (BCFBAF v3.01): Log BCF from regression-based method:	0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-4.2160 days (HL = 6.082e-005 days)
Log BCF Arnot-Gobas method (upper trophic):	-0.049 (BCF = 0.893)
Log BAF Arnot-Gobas method (upper trophic):	-0.049 (BAF = 0.893)
log Kow used:	

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13. Last audited

June 2019

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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123	Benzyl alcohol	00100-51-6	67
124	Benzyl benzoate	00120-51-4	69
125	Benzyl cinnamate (Propenic acid, 3-phenyl, phenylmethyl ester,2-)	00103-41-3	70
126	Benzyl Phenylacetate	00102-16-9	71
127	Benzyl Propionate	00122-63-4	71
128	Bornyl acetate	00076-49-3	71
129	1,3-Butanediol	00107-88-0	71

130	2, 3-Butanedione (Diacetyl)	00431-03-8	71
131	Butanoic acid, 3-methyl-, 4-methylphenyl ester (para-Tolyl 3-methylbutyrate) (p-Tolyl isovalerate)	55066-56-3	71
132	Butter, butter esters, and butter oil	91745-88-9 97926-23-3	71
133	Butyl acetate	00123-86-4	71
134	Butyl alcohol (1-Butanol)	00071-36-3	72
135	Butyl butyryl lactate (butoxy-1-methyl-2-oxoethyl ester butanoic acid, 2-)	07492-70-8	73
136	n-Butyl isovalerate	00109-19-3	73
137	3-Butylidenephthalide	00551-08-6	73
138	Butyric acid	00107-92-6	74
139	Caprylic/Capric triglyceride	65381-09-1	74
140	Caramel and caramel color	08028-89-5	74
141	Carbon	07440-44-0	74
142	Carbon dioxide	00124-38-9	74
143	Cardamom oleoresin, oil, extract, seed oil, and powder	08000-66-6 96507-91-4	76
144	Carob bean gum, absolute and extract	09000-40-2 84961-45-5	77
145	beta-Carotene	07235-40-7	77
146	Carrot oil, seed	08015-88-1	84
147	4-Carvomenthenol	00562-74-3	84
148	beta-Caryophyllene	00087-44-5	84
149	beta-Caryophyllene oxide	01139-30-6	84
150	Cassia bark, buds, oils, and extract	08007-80-5 84961-46-6	84
151	Castoreum, liquid, extract, tincture and absolute	08023-83-4	84
152	Celery seed oil	89997-35-3	84
153	Cellulose and Cellulose fiber	65996-61-4 09004-34-6	84
154	Chamomile flower oil, extract and absolute	08002-66-2 08015-92-7	84
155	Chicory extract	68650-43-1	85
156	Chocolate and chocolate liquor		85
157	1,8-Cineole (Eucalyptol)	00470-82-6	85
158	Cinnamaldehyde	00104-55-2	85
159	Cinnamon bark, buds, leaf, oil, and extract	08015-91-6 08007-80-5	87

160	Cinnamyl acetate	00103-54-8	87
161	Cinnamyl alcohol	00104-54-1	87
162	Cinnamyl cinnamate	00122-69-0	88
163	Citral	05392-40-5	88
164	Citric acid	00077-92-9	92
165	Citronella oil	08000-29-1	92
166	Citronellol	00106-22-9	93
167	Clary sage oil and extract	08016-63-5	93
168	Cocoa, cocoa shells, extract, distillate, powder, alkalized, absolute and tincture	08002-31-1 84649-99-0 68916-17-6 95009-22-6	93
169	Coconut oil	08001-31-8	93
170	Coffee and coffee solid extract	08001-67-0 68916-18-7 84650-00-0	93
171	Cognac white and green oil	08016-21-5	93
172	Coriander extract, seed, and oil	08008-52-4 84775-50-8	93
173	Corn starch	09005-25-8	93
174	beta-Damascone	23726-92-3 23726-91-2	94
175	Davana oil	08016-03-3	94
176	Decanal	00112-31-2	94
177	delta-Decalactone	00705-86-2	94
178	gamma-Decalactone	00706-14-9	94
179	Decanoic acid	00334-48-5	94
180	Diacetyl	00431-03-8	94
181	Diethyl malonate	00105-53-3	94
182	2,3-Diethylpyrazine	15707-24-1	94
183	2,6-Dimethoxyphenol	00091-10-1	95
184	Dimethyl benzyl carbinyl butyrate (alpha, alpha-Dimethylphenethyl butyrate)	10094-34-5	95
185	Dimethyl sulfide	00108-50-9	95
186	3,4-Dimethyl-1,2-cyclopentadione	13494-06-9	95
187	3,7-Dimethyl-1,3,6-octatriene	13877-91-3	95
188	4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one (3-Hydroxy-4,5-dimethyl-2(5h)furanone)	28664-35-9	95
189	2, 5-Dimethyl-4-hydroxy-3(2h)-furanone (4-Hydroxy-2,5-dimethyl-3(2h)furanone)	03658-77-3	95
190	3,7-Dimethyl-6-octenoic acid	00502-47-6	96

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.

SCIENTIFIC OPINION

Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre[1]

EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This Opinion of the EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) deals with the establishment of Dietary Reference Values for carbohydrates and dietary fibre. Nutritionally, two broad categories of carbohydrates can be differentiated: "glycaemic carbohydrates", i.e. carbohydrates digested and absorbed in the human small intestine, and 'dietary fibre', non-digestible carbohydrates passing to the large intestine. In this Opinion, dietary fibre is defined as non-digestible carbohydrates plus lignin. The absolute dietary requirement for glycaemic carbohydrates is not precisely known but will depend on the amount of fat and protein ingested. The Panel proposes 45 to 60 E% as the reference Intake range for carbohydrates applicable to both adults and children older than one year of age. Although high frequency of intake of sugar-containing foods can increase the risk of dental caries, there are insufficient data to set an upper limit for (added) sugar intake. Based on the available evidence on bowel function, the Panel considers dietary fibre intakes of 25 g/day to be adequate for normal laxation in adults. A fibre intake of 2 g/MJ is considered adequate for normal laxation in children from the age of one year. Although there is some experimental evidence that a reduction of the dietary glycaemic index and glycaemic load may have favourable effects on some metabolic risk factors such as serum lipids, the evidence for a role in weight maintenance and prevention of diet-related diseases is inconclusive.

KEY WORDS

Carbohydrates, dietary fibre, sugars, added sugars, glycaemic carbohydrates, oligosaccharides, starch, lignin, glycaemic index, glycaemic load, dietary requirements, blood lipids, lipid profile, glucose tolerance, insulin sensitivity, body weight, type 2 diabetes, blood pressure, cardiovascular disease, coronary heart disease, dental caries, gastrointestinal function, colorectal cancer, mineral absorption

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1 On request from the European Commission, Question No EFSA-Q-2008-467, adopted on 04 December 2009.

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3 Acknowledgement: The Panel wishes to thank for the preparation of this Opinion: Nils-Georg Asp, Wulf Becker, Henk van den Berg, Karin Hulshof, Albert Flynn, Ambroise Martin, Hildegard Przyrembel, Inge Tetens and EFSA's staff member Silvia Valtueña Martínez.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on Population Reference Intakes for the European population, including carbohydrates and dietary fibre.

Nutritionally, two broad categories of carbohydrates can be differentiated: "glycaemic carbohydrates", i.e. carbohydrates digested and absorbed in the human small intestine, and "dietary fibre", non-digestible carbohydrates passing to the large intestine.

The main glycaemic carbohydrates are monosaccharides, disaccharides, malto-oligosaccharides, and starch. In this Opinion the term "sugars" is used to cover monosaccharides and disaccharides. The term "added sugars" refers to sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing. Sugar alcohols (polyols) such as sorbitol, xylitol, mannitol, and lactitol, are usually not included in the term "sugars". However, they are partly metabolised and included in "carbohydrates" according to the European legislation.

In this Opinion, dietary fibre is defined as non-digestible carbohydrates plus lignin, including non-starch polysaccharides (NSP) – cellulose, hemicelluloses, pectins, hydrocolloids (i.e., gums, mucilages, β-glucans), resistant oligosaccharides – fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), other resistant oligosaccharides, resistant starch – consisting of physically enclosed starch, some types of raw starch granules, retrograded amylose, chemically and/or physically modified starches, and lignin associated with the dietary fibre polysaccharides.

Main dietary sources of sugars are fruits, berries, fruit juices, some vegetables, milk and milk products, and foods containing added sucrose and starch hydrolysates (e.g., glucose syrup, high-fructose syrup) such as carbonated beverages and sweets. Main dietary sources of starch are bread and other cereal products, potatoes, tubers and pulses.

Data from dietary surveys show that average carbohydrate intakes in European countries in children and adolescents varied between 43 to 58 E%, and from 38 to 56 E% in adults. Average intakes of sugars varied between 16 to 36 E% in children and adults.

Whole grain cereals, pulses, fruit, vegetables and potatoes are the main sources of dietary fibre. Average dietary fibre intakes varied from 10 to 20 g per day in young children (<10 to 12 years), from 15 to 30 g per day in adolescents, and from 16 to 29 g per day in adults. Average intakes of dietary fibre per MJ ranged from 1.7 to 2.5 g per MJ in (young) children and from 1.8 to 2.9 g per MJ in adults.

Total and glycemic carbohydrates

As energy balance is the ultimate goal, dietary reference values for carbohydrate intake cannot be made without considering other energy delivering macronutrients and will be given as percentage of total energy intake (E%). The absolute dietary requirement for glycaemic carbohydrates is not precisely known but will depend on the amount of fat and protein ingested. Generally, an intake of 50 to100 g per day will prevent ketosis. An intake of 130 g per day for both children (>1 year) and adults has been estimated to be sufficient to cover the needs of glucose for the brain. However, these levels of intake are not sufficient to meet energy needs in the context of acceptable intake levels of fat and protein.

Intervention studies provide evidence that high fat (>35 E%), low carbohydrate (<50 E%) diets are associated to adverse short- and long-term effects on body weight, although data are not sufficient to define a Lower Threshold of Intake (LTI) for carbohydrates. Similarly, high carbohydrate diets tend

to induce adverse effects on the blood lipid profile, but there is an insufficient scientific basis for setting a Tolerable Upper Intake Level (UL) for total carbohydrates. The Panel therefore comes to the conclusion that only a Reference Intake range can be given for total carbohydrate intake, partly based on practical considerations (e.g. current levels of intake, achievable dietary patterns).

Based on the above considerations the Panel proposes 45 to 60 E% as the Reference Intake range for carbohydrates. Diets with glycaemic carbohydrate contents of 45 to 60 E%, in combination with reduced intakes of fat and saturated fatty acids (SFA), are compatible with the improvement of metabolic risk factors for chronic disease, as well as with mean carbohydrate intakes observed in some European countries. This intake range applies to both adults and children older than one year of age.

Sugars

Frequent consumption of sugar-containing foods can increase risk of dental caries, especially when oral hygiene and fluoride prophylaxis are insufficient. However, available data do not allow the setting of an upper limit for intake of (added) sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by frequency of consumption, oral hygiene, exposure to fluoride, and various other factors.

The evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent for solid foods. However, there is some evidence that high intakes of sugars in the form of sugar-sweetened beverages might contribute to weight gain. The available evidence is insufficient to set an upper limit for intake of (added) sugars based on their effects on body weight.

Observed negative associations between added sugar intake and micronutrient density of the diet are mainly related to patterns of intake of the foods from which added sugars in the diet are derived rather than to intake of added sugars *per se*. The available data are not sufficient to set an upper limit for (added) sugar intake.

Although there is some evidence that high intakes (>20 E%) of sugars may increase serum triglyceride (TG) and cholesterol concentrations, and that >20 to 25 E% might adversely affect glucose and insulin response, the available data are not sufficient to set an upper limit for (added) sugar intake.

Evidence on the relationship between patterns of consumption of sugar-containing foods and dental caries, weight gain and micronutrient intake should be considered when establishing nutrient goals for populations and recommendations for individuals and when developing food-based dietary guidelines.

The Panel notes that a number of authorities have established upper limits for population average intake or individual intake of added sugars of <10 E% but others have not. Typically, such recommendations reflect a judgement of what level of sugar intake is practically achievable within the context of a nutritionally adequate diet based on known patterns of intake of foods and nutrients in specific populations. It is also noted that the average intake of (added) sugars in some EU Member States exceeds 10 E%, especially in children.

Dietary Fibre

The role of dietary fibre in bowel function was considered the most suitable criterion for establishing an adequate intake. Based on the available evidence on bowel function, the Panel considers dietary fibre intakes of 25 g per day to be adequate for normal laxation in adults. There is limited evidence to set adequate intakes for children. The Panel considers that the Adequate Intake (AI) for dietary fibre

for children should be based on that for adults with appropriate adjustment for energy intake. A fibre intake of 2 g per MJ is considered adequate for normal laxation in children from the age of one year.

The Panel notes that in adults there is evidence of benefit to health associated with consumption of diets rich in fibre-containing foods at dietary fibre intakes greater than 25 g per day, e.g. reduced risk of coronary heart disease and type 2 diabetes and improved weight maintenance. Such evidence should be considered when developing food-based dietary guidelines.

Glycaemic index and glycaemic load

Although there is some experimental evidence that a reduction of the dietary glycaemic index and glycaemic load may have favourable effects on some metabolic risk factors such as serum lipids, the evidence for a role in weight maintenance and prevention of diet-related diseases is inconclusive.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and if necessary to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on the nutrient and energy intakes for the European Community[4]. The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many Eropean Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context the EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context the EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002, the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on Population Reference Intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance the EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, poly-unsaturated fatty acids and mono-unsaturated fatty acids, *trans* fatty acids;
- Protein;

[4] Scientific Committee for Food, Nutrient and energy intakes for the European Community, Reports of the Scientific Committee for Food 31st series, Office for Official Publication of the European Communities, Luxembourg, 1993.

- Dietary fibre.

Following on from the first part of the task, the EFSA is asked to advise on Population Reference Intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, the EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

ASSESSMENT

A draft of this Opinion, agreed by the NDA Panel on 13 March 2009, was published on the EFSA website[5] for public consultation between 5 August and 15 October 2009. The draft Opinion was also discussed at a National Expert Meeting with Member States on Dietary Reference Values held in Barcelona on 7 and 8 September 2009. All the public comments received and comments from Member States that related to the remit of EFSA were assessed and the Opinion has been revised taking relevant comments into consideration. The comments received, a report on the outcome of the public consultation, and the minutes of the meeting with Member States have been published on the EFSA website.

1. Introduction

Carbohydrates are the main source of energy in most human diets. Carbohydrates are defined within European legislation (Directive 90/496/EEC) as "metabolisable carbohydrates and including polyols"[6]. Chemically, dietary fibre is also a carbohydrate (EFSA, 2007; Directive 2008/100/EC[7]).

2. Definition / category

Chemically, carbohydrates include a range of components such as polyhydroxy aldehydes, ketones, alcohols and acids, as well as their derivatives and polymers, e.g. starch and other polysaccharides. The chemical classification of carbohydrates is usually based on molecular size and monomeric composition, three principal groups being sugars (1–2 monomers), oligosaccharides (3–9 monomers) and polysaccharides (10 or more monomers) (FAO/WHO, 1998, see also Table 1). Due to the chemical diversity of carbohydrates, it is only recently that specific methods for analysis of various carbohydrates in foods have become routinely available. Therefore, carbohydrate values on labels and in food tables are often still derived "by difference" (section 2.1.3).

Nutritionally, it is important to differentiate between two broad categories of carbohydrates: those digested and absorbed in the human small intestine, providing carbohydrates to body cells and those passing to the large intestine, forming substrate for the colonic microflora (Asp, 1996; Englyst and Englyst, 2005). A FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition recommended the introduction of the concept "glycaemic carbohydrate", meaning 'providing carbohydrate for metabolism', which corresponds to the previously used term 'available carbohydrates' (FAO/WHO, 1998) and to 'carbohydrates' according to the European legislation. The nondigestible ("unavailable") carbohydrates are commonly referred to as "dietary fibre" (see 2.1.2 for definitions).

[5] http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902045161.htm

[6] Council Directive 90/496/EEC of 24 September 1990 on nutrition labelling for foodstuffs. OJ L 276, 6.10.1990, pp. 40–44.

7 Commission Directive 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions. OJ L 285, 29.10.2008, pp. 9-12.

Table 1: Main types of carbohydrates (Adapted from Asp, 1996).

Class (DP *)	Sub-group	Components	Monomers	Digestibility**
Sugars (1-2)	Monosaccharides	Glucose		+
		Galactose		+
		Fructose		+
	Disaccharides	Sucrose	Glu, Fru	+
		Lactose	Glu, Gal	+ (–) ***
		Trehalose	Glu	+
		Maltose	Glu	+
Oligosaccharides(3-9)	Malto-oligo-saccharfides	Maltodextrins	Glu	+
	Other oligo-saccharides	α-Galactosides (GOS)	Gal, Glu	–
		Fructo-oligosaccharides (FOS)	Fru, Glu	–
		Polydextrose	Glu	–
		Resistant dextrins	Glu	–
Polyols	Maltitol, sorbitol, xylitol, lactitol			+/–
Polysaccharides (>9)	Starch	Amylose	Glu	+ (–)
		Amylopectin	Glu	+ (–)
		Modified starch	Glu	–
		Resistant starch	Glu	–
		Inulin	Fru	–
	Non-starch poly-saccharides	Cellulose	Glu	–
		Hemicelluloses	Variable	–
		Pectins	Uronic acids	–
		Other hydrocolloids, e.g. gums, mucilages, β-glucans	Variable	–
Related substance		Lignin		–

* DP = Degree of polymerisation
**Denotes digestibility in the small intestine: + digestible, + (–) mainly digestible, +/- partly digestible, – non-digestible
***Lactose is poorly digested by individuals with low intestinal lactase activity
Fru = Fructose, Glu = Glucose, Gal = Galactose

2.1. Categories

2.1.1. Glycaemic carbohydrates

The glycaemic carbohydrates provide carbohydrate to body cells, mainly in the form of glucose. The main glycaemic carbohydrates are (see also Table 1):

- Glucose and fructose (monosaccharides)
- Sucrose and lactose (disaccharides)
- Malto-oligosaccharides
- Starch (polysaccharide)

In this Opinion the term "sugars" covers monosaccharides and disaccharides. In the literature, various terms are used to differentiate between sugars naturally occurring in foods, e.g. "intrinsic" sugars, and sugars and sugar preparations added to foods, e.g. "added" or "extrinsic" sugars" (IoM, 2005; DoH, 1991). In this opinion the term "added sugars" refers to sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing.

Sugar alcohols (polyols) such as sorbitol, xylitol, mannitol, and lactitol, are usually not included in the term "sugars". However, they are partly absorbed and included in "carbohydrates" according to the European legislation.

2.1.2. Dietary fibre

The term "dietary fibre" was originally defined as "that portion of food which is derived from cellular walls of plants which are digested very poorly by human beings" (Trowell, 1972). The recognition that polysaccharides added to foods, notably hydrocolloids, could have effects similar to those originating from plant cell walls led to a redefinition of dietary fibre to include "polysaccharides and lignin that are not digested in the human small intestine" (Trowell et al., 1976). The definition and delimitation of "dietary fibre" has been much debated and related both to physiological considerations and to methods that can be used for dietary fibre analysis in foods (FAO/WHO, 1998; Asp, 1995 and 1996; Englyst and Hudson, 1996; Englyst and Englyst, 2005; Englyst et al., 2007).

Non-starch polysaccharides (NSP) are the main constituents of dietary fibre and include a host of different polymers, highly variable in terms of molecular size and structure, as well as in monomeric composition. Main classes of non-starch polysaccharides are cellulose, hemicelluloses, pectins, and other hydrocolloids. Due to the structural variability, different non-starch polysaccharides may have very different physical-chemical properties, which are of key importance for their physiological effects. For example cellulose is insoluble in water, whereas pectins and hydrocolloids, e.g. guar gum and mucilages, may form highly viscous water solutions. Resistant starch is insoluble and indigestible due to its physical form or enclosure in cellular structures, whereas resistant oligosaccharides are readily soluble in water but do not form viscous solutions. The terms "soluble" and "insoluble" dietary fibre have been used in the literature to differentiate between viscous, soluble types of fibre (e.g. pectins) and insoluble components such as cellulose. The distinction was mainly based on the different physiological effects. However, this differentiation is method-dependent, and solubility does not always predict physiological effects. Therefore, FAO/WHO proposed the distinction between soluble and insoluble fibre should be phased out (FAO/WHO, 1998).

The interest in defining and quantifying dietary fibre in foods lies in the physiological effects that are associated with their consumption, which include decreased intestinal transit time and increased stool bulk, reducing blood total and/or LDL cholesterol concentrations, and reducing post-prandial blood glucose and /or insulin concentrations, among others (AFSSA 2002; NNR, 2004; IoM, 2005; GR, 2006; Mann et al., 2007). These physiological effects of dietary fibre are distinct from those of glycaemic carbohydrates.

In national and international recommendations on dietary fibre intake, the definitions are generally in accordance with and related to analysis with methods approved by the Association of Official Analytical Chemists (AOAC). Definitions differ with respect to some minor components such as fibre of animal origin and some synthetic or isolated fibre constituents (Annex 1).

The U.S. Food and Nutrition Board (FNB) defines "total dietary fiber" as the sum of "dietary fiber", consisting of non-digestible carbohydrates and lignin that are intrinsic and intact in plants, and "functional fiber", consisting of isolated, non-digestible carbohydrate components with demonstrated beneficial physiological effects in humans (IoM, 2005). The rationale behind this differentiation is that there is epidemiological evidence for beneficial effects of foods naturally high in dietary fibre, such as whole-grain cereals, some fruits and vegetables, and that dietary fibre can be regarded as a marker of such foods. The argument that the term "dietary fibre" should be restricted to non-starch polysaccharides of cell wall origin (Englyst and Englyst, 2005; Englyst et al., 2007) has a similar rationale. Consequently, according to the FNB, documentation of the beneficial effects of added, functional fibre is required for inclusion in "total dietary fibre".

The Panel notes that a major problem in making this differentiation in practice is that analytical methods cannot differentiate between "dietary fibre" and "functional fibre" once they occur mixed in a food product,

and similarly NSP from plant cell walls cannot be differentiated from added NSP with similar monomeric composition.

In view of the key importance of small-intestinal digestibility for the nutritional effects of carbohydrates, the Panel considers that dietary fibre should include all non-digestible carbohydrates. This includes non-starch polysaccharides, resistant starch, resistant oligosaccharides with three or more monomeric units and other non-digestible, but quantitatively minor components that are associated with the dietary fibre polysaccharides, especially lignin (Cho et al., 1997; AACC, 2001; AFSSA, 2002; NNR, 2004; GR, 2006). This definition is in accordance with the definition brought to step 8 in the Codex Alimentarius (Codex, 2009) and agreed by the Codex Alimentarius Committee in 2009, although the inclusion of non-digestible carbohydrates with 3 to 9 monomeric residues is so far left to the national authorities. As in the EU definition, beneficial physiological effects have to be demonstrated before addition of natural or synthetic fibre to foods (Annex 1).

The minimum chain length of three monomeric units, degree of polymeration (DP) 3, was set since undigestible oligosaccharides with DP3-9, such as fruco-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) are natural constituents of many foods, quantitatively important in e.g. artichokes and beans, respectively. There is considerable evidence of "fibre-like" effects of these oligosaccharides, such as promoting a presumably "healthy" microflora, short-chain fatty acids (SCFA)-production in the colon, and enhancement of calcium absorption from the colon. Undigestible disaccharides are not prominent constituents of normal foods and not well characterised physiologically. However, if such ingredients will be available in the future and shown to have "fibre-like" effects, there may be reasons to reconsider the DP3 limit.

For the purpose of this Opinion, dietary fibre is defined as non-digestible carbohydrates plus lignin. The Panel considers that the main types of total dietary fibre are:

- Non-starch polysaccharides (NSP) - cellulose, hemicelluloses, pectins, hydrocolloids (i.e. gums, mucilages, β-glucans).
- Resistant oligosaccharides - fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), other resistant oligosaccharides.
- Resistant starch - consisting of physically enclosed starch, some types of raw starch granules, retrograded amylose, chemically and/or physically modified starches.
- Lignin associated with the dietary fibre polysaccharides.

Methods of analysis

Current enzymatic gravimetric or enzymatic chemical methods for dietary fibre cover NSP, analytically resistant starch and lignin. However, given that dietary fibre is a mixture of chemically heterogeneous carbohydrate components, several analytical methods are currently required to measure all fractions of dietary fibre. Methods measuring NSP alone (Englyst and Hudson, 1996) give lower estimates than methods for total dietary fibre in foods containing resistant starch, and/or lignin, e.g. whole-grain flour and cereals processed in a way that generates resistant starch. On the other hand, methods determining dietary fibre, including resistant starch, measure the fraction, which includes mainly retrograded amylose, resistant to the enzymes used in the assay. Finally, resistant oligosaccharides and inulin are not included in any of the current methods for total dietary fibre, and therefore need to be measured separately and subsequently added to the total fibre estimate (Cho et al., 1997; Champ et al., 2001 and 2003).

2.1.3. Total carbohydrates

In many food composition tables and in food labelling, carbohydrates are still expressed “by difference”, which means that moisture, protein, fat and ash are analytically determined and the rest named

"carbohydrates". This non-specific procedure includes all kinds of carbohydrates regardless of their physiological and nutritional properties, as well as variable amounts of non-carbohydrate material, e.g. organic acids, lignin and polyphenols (FAO/WHO, 1998; Southgate, 1995; Asp, 1995).

2.2. Metabolism

2.2.1. Glycaemic carbohydrates

The glycaemic carbohydrates provide carbohydrates to body cells, mainly in the form of glucose. In general, only monosaccharides are absorbed in the small intestine. The enzymatic degradation of starch begins by the action of salivary amylase and is continued in the small intestine by pancreatic amylase. The degradation products – mainly maltose and oligosaccharides – are hydrolysed further to glucose by a set of enzymes, "disaccharidases", bound to the brush border membrane of the enterocytes. The same enzymes hydrolyse the dietary disaccharides. Glucose and galactose are absorbed efficiently by a secondary active carrier coupled with sodium (sodium glucose transporter 1, SGLT1), whereas fructose is absorbed by facilitated diffusion that does not involve sodium co-transport (GLUT5). The absorption of monosaccharides is regarded as the rate-limiting step.

Absorbed monosaccharides are transported to the liver and then to the systemic circulation. The cellular uptake is mediated by a number of glucose transporters (GLUT1–4), variously expressed in different tissues. Insulin is a key hormone for the uptake and metabolism of glucose. The plasma insulin concentration increases immediately after ingestion of glycaemic carbohydrates. Unlike glucose, fructose enters body cells without the need for insulin. The metabolism of fructose, therefore, favours lipogenesis more than glucose. In liver cells, fructose is phosphorylated to fructose-1-phosphate that can be converted to fatty acids, providing a route for lipogenesis in addition to that shared with glucose (via glucose/fructose-6-phosphate) (Vasankari and Vasankari, 2006). Both fructose and galactose, the latter arising from hydrolysis of lactose, are also transformed to glucose mainly in the liver.

2.2.2. Glycaemic index and glycaemic load

The concept of glycaemic index (GI) was introduced by Jenkins and co-workers in 1981, in order to rank foods in a standardised way according to their effects on blood glucose levels after a meal. The FAO/WHO Expert Consultation defined GI as the incremental area under the blood glucose response curve during 1.5–3 hours after intake of a 50 g carbohydrate portion of a test food, and expressed as a percentage of the response to the same amount of carbohydrate from a standard food taken by the same subject (FAO/WHO, 1998). Glucose or white bread is used as standard. GI values obtained with the white bread standard are typically about 40% higher than those obtained with the glucose standard which is the generally preferred standard. GI values for about 750 foods have been published (Foster-Powell et al., 2002) and recently updated with additional data to contain 2480 individual food items (Atkinson et al., 2008).

Whereas it was previously assumed that sugars are rapidly absorbed and therefore have a higher GI than polysaccharides (e.g. starch), which are slowly absorbed, a number of food-related factors have been identified to determine the GI. For instance, fructose has a low GI (30 with the white bread reference as 100) and sucrose an intermediate GI, i.e. lower than white bread (Björck et al., 2000). Starchy foods, on the other hand, can have low, intermediate or high GI, depending on their composition (amylose/amylopectin ratio) and physical/chemical state. The swelling and dissolution of starch at wet heat treatment, known as gelatinisation, is particularly important in making starch more readily accessible to digestive enzymes. Physical barriers such as in intact cereal grains, cellular structures in leguminous seeds, parboiled rice and whole fruits, and the protein network in pasta products, are food-related factors lowering the GI. Organic acids (acetic, propionic and lactic acid) decrease the glycaemic response to foods or meals, mainly due to inhibition of gastric emptying (Liljeberg and Björck, 1998). Viscous, soluble types of dietary fibre may also delay gastric emptying, in addition to their inhibitory effect on diffusion and transport in the small intestine (Brown et al., 1999; Jenkins et al., 2000).

In practice, the blood glucose response after a meal is influenced by both the GI and the amount of carbohydrate in a portion of a food. Consequently, the glycaemic load (GL) concept was introduced in 1997 to quantify the glycaemic effect of a portion of food (Salmeron et al., 1997a and 1997b). GL is defined as the amount of glycaemic carbohydrate in a food times the GI of the food/100, and the sum of individual GL values for foods and meals has been used to estimate the glycaemic load of the whole diet.

Studies have shown that the glycaemic response to a meal can be predicted from properly determined GI of the constituent foods (Wolever et al., 2006; Wolever and Jenkins, 1986; Järvi et al., 1999). However, the glycaemic response can also be influenced by the protein and fat content, and by the type and amount of beverage taken with the food (Henry et al., 2006). Flint et al. (2004) found no correlations between predicted postprandial glucose responses based on published GI values for foods and the measured glucose elevations after different breakfast meals containing 50 g available carbohydrates and varying amounts of fat and protein. This indicates that the composition and the size of a meal, i.e. in terms of energy and macronutrient content, are also important determinants for the glycaemic responses. Validated GI values for food products are needed in studies investigating effects of GI.

2.2.3. Dietary fibre

The components included in dietary fibre are by definition resistant to hydrolysis and absorption in the small intestine. They pass the upper gastro-intestinal tract and enter the colon substantially unmodified. Viscous, water-soluble fibre such as β-glucans and pectin can modify blood glucose response and total and LDL-cholesterol concentrations by interfering with digestion and absorption of glycaemic carbohydrates and cholesterol and/or bile acids, respectively. Inhibitory effects on mineral absorption, i.e. of iron, zinc and calcium, have been attributed to fibre-associated complexing compounds, notably phytic acid in cereals and leguminous seeds.

Dietary fibre components are subject to more or less extensive anaerobic fermentation by the colonic microflora. The extent of fermentation is dependent on both substrate and host factors, e.g. molecular structure and physical form of the substrate, bacterial flora and transit time. Less fermentable types of fibre, such as in lignified outer layers of cereal grain, generally have the most prominent faecal bulking effects due to their ability to bind water in the distal colon. Fermentable fibre also contributes to the faecal bulk through increased microbial mass.

Some fermentation products, such as propionic acid and butyrate, may influence also systemic metabolism, i.e. cholesterol synthesis and possibly insulin sensitivity. Fermentable dietary fibre components, including oligosaccharides that are often referred to as "prebiotics", increase Bifidobacteria and Lactobacilli which produce lactate and short-chain fatty acids such as acetate, propionate and butyrate (Gibson and Roberfroid, 1995). These fatty acids inhibit the fermentation of protein components, which could produce potentially toxic products, especially ammonia and amines. Short-chain fatty acids decrease the pH of the colonic content, which stimulates colonic absorption of minerals, notably calcium, and inhibits formation of potential co-carcinogens from bile acids. Butyrate is a main source of energy for the colonic mucosa and has effects on cell differentiation and apoptosis with possible implications for colon carcinogenesis. Acetate and propionate are absorbed from the colon and thus provide energy to the host (Cummings et al., 2004).

The absorption of fermentation products, i.e. short chain fatty acids, means that dietary fibre contributes to the energy content of the diet, but less than glycaemic carbohydrates. The contribution is variable depending on the extent of fermentation. FAO/WHO (1998) has recommended the use of an average energy factor for dietary fibre, 8 kJ or 2 kcal per g, and this recommendation has been now included in the EU nutrition labelling Directive[8].

[8] Council Directive of 24 September 1990 on nutrition labelling for foodstuffs (90/496/ECC). OJ L 276, 6.10.1990, p.40 ammended by Commission Directive 2008/100/EC of 28 October 2008.

3. Dietary sources and intake data

3.1. Dietary sources

3.1.1. Glycaemic carbohydrates

Main dietary sources of glucose and fructose are fruits, berries, fruit juices and some vegetables. Free galactose is rare in foods, except in fermented and lactase-hydrolysed milk products. Fruits, berries and juices are natural sources of sucrose, although sugar added to foods, carbonated beverages and sweets or in the household usually provides most of the dietary sucrose. More or less completely hydrolysed starch or high fructose syrup, in which about half the glucose is isomerised to fructose, are increasingly used in some countries, to replace sucrose in confectionary and carbonated drinks.

Lactose occurs exclusively in milk and milk products. Human milk has the highest lactose content of all milks, 7 g per 100 g. The lactose content in cow's milk is around 5 g per 100 g. Digestible malto-oligosaccharides originate mainly from partly hydrolysed starch.

Main dietary sources of starch are bread and other cereal products, potatoes, tubers and pulses (FAO/WHO, 1998).

3.1.2. Dietary fibre

Whole grain cereals, pulses, fruit and vegetables and potatoes are the main sources of dietary fibre. Also nuts and seeds contain high concentrations. Cellulose occurs together with hemicelluloses in cereals. The lignified outer layers are the predominant fibre source in whole-grain products. Oats and barley contain high concentrations of a water-soluble, viscous type of polysaccharide, β-glucan. Pectins, a main type of dietary fibre in fruits and vegetables, have similar properties.

3.2. Dietary intake

Typical intakes of carbohydrates and dietary fibre are presented for children and adolescents in 19 countries (Annex 2a and 2b) and for adults in 22 countries in Europe (Annex 3a and 3b). The data refer to individual based food consumption surveys, conducted from 1994 onwards. Most studies comprise national representative population samples. The data were derived from national reports and from a recently published overview (Elmadfa, 2009).

As shown in Annexes 2 and 3, there is a large diversity in the methodology used to assess individual intakes of children, adolescents and adults. Because the different methods apply to different time frames, this inevitably resulted in variance in both the quality and quantity of available data, which make direct comparability difficult. Moreover, age classifications are in general not uniform. Comparability might also be hampered by differences in food composition tables used for the conversion of food consumption data to estimated nutrient intakes (Deharveng et al., 1999). Food consumption data are prone to reporting errors and there might be a varying degree of underreporting in different surveys.

Although these differences may have an impact on the accuracy of between country comparisons and the results should be interpreted with caution, the presented data give a rough overview of the carbohydrate intake in a number of European countries. Most studies reported mean intakes and standard deviations (SD) or mean intakes and intake distributions. In most studies the contribution of carbohydrates to energy intake is based on total energy intake (including the energy from alcohol).

3.2.1. Total carbohydrates

Average carbohydrate intakes in children and adolescents in European countries varied between 41 to 58 E% (Annex 2b). Most of the reported average intakes (82%) were between 47 and 55 E%; approximately 13% were above 55%. Within population ranges varied from 38 to 49 E% (5th percentile) to 63 to 66 E% (95th percentile).

In adults average carbohydrate intakes varied from approximately 38 to 54 E% (Annex 3b). In the various age categories average intake of carbohydrates ranged from approximately 41 to 51 E% (19-34 years), 38 to 49 E% (35 to 64 years) and 40 to 53 E% (65 years and over), respectively. More than half (53%) of the reported mean values were between 45 and 50 E%; Average carbohydrate intakes of 50 E% and higher were achieved in 16% of the adult groups belonging to the age categories 19 to 34 years and 65 years and over. Within population ranges varied from 31 to 34 E% at the lower (5th percentile) to 58 to 61 E% at the upper end (95th percentile) of the distributions. Mean intakes were highest in the Czech Republic and Norway and lowest in Greece and Spain.

As shown in Annexes 2b and 3b, not all countries reported intakes of mono-, disaccharides and polysaccharides. When reported, average intakes of mono- and disaccharides varied between 23 to 36 E% in children and adolescents, with highest intakes in infants, whereas the intake of polysaccharides was between 23 and 25 E%.

In Finnish infants aged 8 to 13 months the reported average intake of sucrose was 3 to 5 E%. This amount increased in children aged 2 to3 years to approximately 10 to 12 E%. In schoolchildren and adolescents average intakes varied between 11 and 25 E%. More than half (56%) of these average intakes were between 10 and 15 E%. Available intake distributions showed that five percent of the children and adolescents had average intakes of 20 E% and above.

In adults the intake of mono and disaccharides varied between 17 to 26 E% and the intake of polysaccharides between 20 to 27 E%.

Average sucrose intake in adults varied from 6 to nearly 14 E%. Average intakes below 11 E% were only observed in the older age categories (35 to 64 years: in 94%; 65 years and over: in 79% of the group). Within population ranges varied from 1 to 4 E% at the lower (5th percentile) to 17 to 25 E% at the upper end (95th percentile) of the distribution.

3.2.2. Dietary fibre

Apart from infants and young children, average dietary fibre intakes varied between 10 to 20 g per day in young children (<10 to 12 years), and from 15 to 33 g per day in adolescents. The highest intakes were observed in German adolescents (Annex 2b). Within population ranges varied from 6 to 8 g per day (5th percentile) to 25 to 46 g per day (95th percentile). Expressed per MJ reported intakes were between 1.7 g per MJ and 2.5 g per MJ. Studies on German children followed from 6 months up to 18 years of age (data not presented) show that the energy adjusted fibre intake was highest at 1 year (3 g per MJ), thereafter declining to about 2.5 g per MJ in preschool- and school-age (Alexy et al., 2006).

In adults average dietary fibre intakes ranged from 15 to 30 g per day. For subjects aged 65 years and over, about 70% of the reported intakes were between 19 and 25 g per day. In the other age categories these percentages were 44% (19-35 years) and 42% (50 to 64 years), respectively. Within population ranges varied from 6 to 9 g per day (5th percentile) to 39-51 g per day (95th percentile). A few countries presented (also) the intake of dietary fibre per MJ. Then daily intakes ranged from 1.6 to 3.6 g per MJ in adults.

4. Overview of dietary reference values and recommendations

A number of national and international organisations have set dietary reference values (DRVs) for carbohydrates (total and/or glycaemic) as well as for dietary fibre (Table 2). Generally, reference intakes are expressed as percent of the total energy intake (E%). For fibre, intakes are expressed in grams per day and/or on an energy basis (per MJ or per 1,000 kcal).

4.1. Glycaemic carbohydrates

According to the Nordic Nutrition Recommendations (NNR, 2004) carbohydrates (including energy from dietary fibre, 8 kJ per g) should provide 50 to 60% of the total energy intake. The population goal is 55 E% from carbohydrates, which should be used for planning purposes. The intake of refined, added sugars should not exceed 10 E%. Although not explicitly stated in the report, it appears that this is a recommendation for individuals. Refined sugars include sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing. The basis for the recommendation on added sugars is to ensure an adequate intake of essential nutrients and dietary fibre, especially in children and older adults with a low energy intake.

The Health Council of the Netherlands (GR, 2001) based their recommendations for digestible carbohydrates on the 97.5 percentile of the endogenous production of glucose. At this level of carbohydrate intake the breakdown of tissue protein is minimal. Using data on average glucose production and assuming a coefficient of variation (CV) of 20%, DRVs were established for the various age and sex groups. The recommendations for glycaemic carbohydrates for adults (19 to 70 years) were calculated to be 272 to 282 g per day, corresponding to about 40 E%. For children and adolescents, recommendations are 45 to 50 E%. No upper limit for digestible carbohydrates is given. No quantitative recommendation was made for (added) sugars. The Netherlands guidelines for healthy eating did not specify recommended values for (added) sugars consistent with adequate nutrition and the prevention of chronic diseases because insufficient scientific evidence was available to support firm conclusions (GR, 2006).

In the Nutritional Recommendations for the French Population (AFSSA, 2001), the Reference Intake for total carbohydrates was set at 50 to 55 E%. It is stated that the reference intake is a consequence of the recommendations for fat (30 to 35 E%) and protein (8 to 10 E%). It is also stated that presently no carbohydrate constituents indispensable for growth and maintenance, which cannot be synthesised by humans, have been identified. Other considerations include e.g. energy density of the diet and effects on serum lipids. A carbohydrate intake above 55 E% is stated to be associated with risk of dyslipidemia (e.g. increased VLDL- and decreased HDL-cholesterol). Various considerations are given with respect to the contribution of MUFA and carbohydrates for reducing cardiovascular risk, treatment of obesity and the metabolic syndrome. Carbohydrates and MUFA should together contribute two-thirds of the total energy intake, with a range for carbohydrates of 40 to 55 E%. No quantitative recommendation was made for (added) sugars.

In the German-Austrian-Swiss recommendations (D-A-CH, 2008), the guiding value ("Richtwert") for carbohydrate intake is at least 50 E%. This value applies to populations and is based on evidence from epidemiological studies, and studies linking a high intake of (saturated) fat with cardiovascular risk factors and other diseases. It is stressed that carbohydrates should be derived from foods rich in starch and dietary fibre, and that intake of refined sugars should be limited. In order to avoid gluconeogenesis from protein (e.g. amino acids) and to inhibit lipolysis at least 25% of the energy should be supplied from carbohydrates. This percentage applies to all ages. No quantitative recommendation was made for (added) sugars.

WHO gives population nutrient intake goals (population average intakes that are judged to be consistent with the maintenance of health in a population) for preventing diet-related chronic diseases (WHO/FAO, 2003). For carbohydrates the population goal is set at 55-75 E%, including dietary fibre. This goal is the percentage of energy available after taking into account the proportion recommended as protein and fat. A recent FAO/WHO Scientific Update on carbohydrate in the human diet proposes that the range is extended

to 50 to 75 E% (Mann et al., 2007). It was proposed that the population average intake of free sugars, defined as "all monosaccharides and disaccharides added to foods, by cook or consumer, plus sugars naturally present in honey, syrups and fruit juice", should not exceed 10 E%. The basis for this goal is that high intakes of free sugars are associated with decreased nutrient density, and risk of weight gain, especially when consumed as beverages.

The UK Committee on Medical Aspects of Food Policy (DoH, 1991) set a dietary reference value (population average intake) for starches and intrinsic and milk sugars of 37 E%. This figure was based on considerations that starch and intrinsic sugars should provide the balance of dietary energy not provided by those nutrients for which the intake should be restricted, i.e. alcohol, protein, fat and non-milk extrinsic sugars. The reference value is applicable to adults and children above 2 years of age. For non-milk extrinsic sugars the population's average intake should not exceed 60 g per day or 10 E%, based mainly on the role of frequent consumption of such sugars in dental caries.

The US Food and Nutrition Board estimated the average requirement of (glycaemic) carbohydrates as 100 g per day for children and adolescents up to 18 years, as well as adults (IoM, 2005), based mainly on data regarding glucose utilisation by the brain. The RDA was set at 130 g per day, assuming a CV of 15%. The RDA corresponds to about 18 and 25 E% in adult males and females, respectively, assuming an energy intake of 2,800 and 2,100 kcal per day, respectively. The US Food and Nutrition Board also set Acceptable Macronutrient Distribution Ranges (AMDR) for total carbohydrates of 45 to 65 E% for individuals. The AMDR are based on evidence indicating a decreased risk for coronary heart disease (CHD) at low intakes of fat and high intakes of carbohydrates, and on evidence for an increased risk of obesity and its complications, including CHD, with high intakes of fat. For added sugars, although there were insufficient data to set a UL (e.g lack of evidence on dose-response for dental caries), a maximal intake level of 25 E% or less from added sugars for individuals was proposed to prevent the displacement of foods that are major sources of essential micronutrients.

Table 2: Recommended dietary intakes for adults.

	USA[a] (IoM, 2005)	Nordic Countries (NNR, 2004)	WHO (2003)	Netherlands (GR, 2001 and 2006)	France, (AFSSA, 2001)	Germany, Austria, Switzerland (D-A-CH, 2008)	Eurodiet (2000)	UK (DoH, 1991)
Protein, E%	10-35	10-20	10-15	8-11	8-10	10-11	-	9
Fat, E%	20-35	25-35	15-30	20-40 20-30/35[b]	30-35	30	< 30	33
Carbohydrates, total, E%	45-65	50-60	55-75	40[c]	50-55	> 50	> 55	47[d]
Sugars, E%	< 25[e]	< 10[e]	< 10[f]	-	-		< 4 occasions per day[g]	<10[h]
Dietary fibre, g/day	w: 25 m: 38	25-35	> 25[i]	32-45	25-30	30	> 25	18[j]
g/MJ	3.4	3		3.4		W: 3 M: 2.4	3	-

(a) AMDR: acceptable macronutrient distribution ranges, applies to individuals. AI for dietary fibre
(b) For subjects with BMI >25 or with undesireable weight gain
(c) RDA for digestible carbohydrates
(d) Intrinsic and milk sugars and starch 37 E%
(e) Refined, added sugars include sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing
(f) Free sugars, defined as all monosaccharides and disaccharides added to foods, plus sugars naturally present in honey, syrups and fruit juice
(g) Corresponds to an intake of < 10 E%
(h) Non-milk extrinsic sugars
(i) Total dietary fibre from wholegrain cereals, fruit and vegetables, 20 g NSP
(j) Refers to non-starch polysaccharides

4.2. Dietary fibre

The Health Council of the Netherlands (GR, 2006) has set guidelines for the intake of dietary fibre mainly based on the importance of dietary fibre for intestinal function and its relationship to risk of coronary heart disease. For children a gradual increase in the intake is recommended from 2.8 g per MJ at 1 to 3 years of age, 3.0 g per MJ at 4 to 8 years of age, 3.2 g per MJ at 9 to 13 years of age to 3.4 g per MJ from 14 years of age. An upper limit is not specified.

In the Nordic Nutrition Recommendations, the recommended intake of dietary fibre is set to 25 to 35 g per day in adults, i.e. approximately 3 g per MJ (NNR, 2004). An Adequate Intake of dietary fibre reduces the risk of constipation and can most likely contribute to protection against colon cancer. No recommendation is given for children due to limited evidence, but it is stated that intake of appropriate amounts of dietary fibre from a variety of foods is important for children as well and that from school age the intake should gradually increase during adolescence to reach the recommended level for adults.

In the WHO report (2003) there is no precise population goal for the intake of total dietary fibre, but at least 25 g per day should be provided from fruit, vegetables and whole-grain foods. This population goal is based on evidence linking high intake of dietary fibre (from fruit, vegetables and whole-grain foods) with decreased risk of e.g. weight gain (convincing), diabetes type 2 (probable), cardiovascular diseases (probable). The food-based recommendation was supported by the recent FAO/WHO Scientific Update on carbohydrate in the human diet (Mann et al., 2007).

In the Nutritional Recommendations for the French Population (AFSSA, 2001), an intake of dietary fibre of above 25 g per day is recommended for maintaining "a healthy colon" and to decrease the risk of colon cancer, with 30 g per day as a preferred level. Also, an increased intake of dietary fibre is stated to be advantageous in conditions such as dyslipidaemia and in diabetes mellitus type 2.

The guiding value for dietary fibre in the German-Austrian-Swiss recommendations is at least 30 g per day, corresponding to about 3 g per MJ for women and 2.4 g per MJ for men, respectively (D-A-CH, 2008). The basis for the value are studies associating increased dietary fibre intake with decreased risk of constipation, diverticulosis, colon cancer, gallstone formation, overweight, hypercholesterolaemia, diabetes mellitus type 2 and atherosclerosis.

The UK Committee on Medical Aspects of Food Policy (DoH, 1991) set a dietary reference value for non-starch polysaccharides (NSP) to 18 g per day, with an individual range of 12 to 24 g per day. The value is based on the effect of non-starch polysaccharides on bowel function and stool weight. The reference value refers to adults and is not applicable to children. An upper limit was set at 32 g per day.

The U.S. Food and Nutrition Board (IoM, 2005) set an Adequate Intake (AI) for total dietary fibre of 3.4 g per MJ (14 g per 1,000 kcal) based on the energy-adjusted median intake associated with the lowest risk of CHD in observational studies. The AI is applicable for all age and sex categories from 1 year of age. The AI corresponds to 25 g per day for women and 38 g per day for men aged 14 to 50 years, respectively.

ESPGHAN (European Society for Pediatric Gastroenterology Hepatology and Nutrition) concluded that by school age an otherwise balanced diet is likely to provide at least 10 g per day of dietary fibre, and that the intake should then gradually increase to reach the recommended level for adults during adolescence. An intake of dietary fibre too high might be a cause of inadequate energy and nutrient density to cover the needs of small children (Aggett et al., 2003).

5. Criteria (endpoints) on which to base the dietary reference values

The amount and type of carbohydrates and dietary fibre in the diet may affect both short-term and long-term metabolic responses such as serum lipids or plasma glucose and insulin concentrations, which can be

regarded as candidate criteria for establishing DRVs. Serum LDL-cholesterol has been causally related to the risk of developing cardiovascular diseases (EFSA, 2004; IoM, 2005), while serum triglycerides, LDL/HDL ratio or total cholesterol/HDL ratio have also been associated with cardiovascular disease (CVD) risk in epidemiological studies (EFSA, 2004; Austin et al., 1998). In addition to cardiovascular diseases, other long-term endpoints for establishing DRVs for both glycaemic carbohydrates and dietary fibre include body weight control, gastrointestinal function, diabetes and some cancers.

The DRVs apply to healthy populations and they are not intended as reference values for the treatment of patients with diseases or conditions like diabetes, obesity, or CVD. However, they apply to healthy subjects with signs of metabolic disturbances like impaired glucose tolerance, elevated blood pressure, serum lipids, etc.

Apart from carbohydrates and dietary fibre, the amount and type of fat and protein in the diet also influence these metabolic factors. As energy balance is the ultimate goal, it is necessary to consider macronutrients in combination and, as a consequence, on an energy basis (percent of energy intake, E%).

5.1. Total glycaemic carbohydrates

Most of the studies addressing the effects of macronutrient manipulation on health outcomes do not allow a precise differentiation between total and glycaemic carbohydrates due to limitations in the food composition data, and thus dietary intake data. However, glycaemic carbohydrates generally contribute 90 to 95% of the total energy derived from carbohydrate intake.

5.1.1. Dietary requirements

Glucose is a preferred energy source for most body cells, and can be stored as glycogen in the liver and in the muscles. The storage capacity is limited, in total to around 500 g for an adult, of which 300 to 400 g can be stored in the muscles. Liver glycogen is essential for liver functions such as detoxification by conjugation with glucuronic acid, and is used to maintain normal blood glucose concentrations between meals. Muscle glycogen is used primarily as a source of energy within the muscles.

Only cells in the central nervous system, red blood cells and some other cells dependent on anaerobic glycolysis have an absolute requirement for glucose (IoM, 2005). The body can synthesise glucose from protein and glycerol. Provided that the diet contains adequate amounts of protein (i.e. amino acids) and glycerol (as e.g. triglycerides) for *de novo* synthesis of glucose, it has been generally assumed that there is no need for dietary carbohydrates (IoM, 2005). Feeding studies in pregnant rats and dogs have, however, shown that diets devoid of carbohydrates can induce still birth, but also high mortality of the offspring and low birth weight (Romsos et al., 1981; Koski et al. 1986: Koski and Hill, 1986).

After a prolonged deficit of glucose, brain cells can adapt partially to utilise fat-derived metabolites, i.e. β-hydroxybutyric acid and acetoacetic acid. A very low carbohydrate diet, providing less than around 50 g per day, however, results in a chronically increased production and plasma concentrations of these acids, referred to as ketosis. Absence of glycogen stores has adverse effects on high-intensity energy production by muscles (Hultman et al., 1999). An intake of 50 to 100 g per day of glycaemic carbohydrates generally prevents ketosis.

In practice, diets totally devoid of carbohydrates have probably not been consumed by any population group during evolution. The diets of Greenland Eskimos and Alaskan Inuits have been reported to contain from 3 to 53 E% carbohydrates; current intakes are >40 E% (Jokelainen, 1965; Bang et al., 1980; Nobmann et al., 2005).

Ketogenic diets with very low carbohydrate contents (from 4 to 5 to 10 E%) have been used as an alternative therapy in children with epilepsy not responding to drug therapy and are a mandatory therapy for some inborn errors of metabolism (e.g. pyruvate dehydrogenase deficiency) (Keene, 2006; Klepper and Voit,

2002; Wexler et al., 1997; Vining, 1999). A number of adverse effects related to such diets have been reported including constipation, nutritional deficiencies and deaths (Wheless, 2001; Papandreou et al., 2006). Severe acidosis is another reported adverse effects of diets very low in carbohydrates consumed by adults who wanted to loose weight (IoM, 2005; Shah and Isley, 2006; Chen et al., 2006a). Methylglyoxal and its by-products accumulate during ketosis and are recognised as a potential cause of blood vessel and tissue damage (Beisswenger et al., 2005).

In conclusion, an intake of 50 to 100 g glycaemic carbohydrates per day is sufficient to avoid ketosis and 130 g per day for both children (> 1 year) and adults is estimated to cover the needs of glucose for the brain (IoM, 2005). However, these levels of intake are not sufficient to meet enegy needs in the context of acceptable intake levels of fat and protein.

5.1.2. Glucose tolerance and insulin sensitivity

The blood glucose concentration is determined by three main factors: the rate of intestinal carbohydrate uptake, the net liver uptake or release (from gluconeogenesis and glycogenolysis), and the peripheral glucose uptake, which is in turn dependent upon the insulin level and the peripheral insulin sensitivity/resistance. With a constant dietary carbohydrate load, there is a range of blood glucose responses between individuals, from low responses with a continuum to what is defined as impaired glucose tolerance (IGT) and type-2 diabetes. Physical activity has the potential to enhance insulin sensitivity and thereby decrease the glycaemic response to a meal (Borghouts and Keizer, 2000; Ivy, 1997).

Some small scale and short-term intervention studies designed to assess the effects of high carbohydrate (50 to 85%E), low fat (<25E%) vs low carbohydrate (8 to 40%E), high fat (>35%) intakes on measures of glucose tolerance or insulin sensitivity suggest that high carbohydrate intakes may improve insulin sensitivity and/or glucose tolerance both in non-diabetic and diabetic subjects (McClenaghan, 2005), although the available data are not consistent (IoM, 2005).

There are no long-term studies published specifically designed to address the impact of macronutrient (i.e. fat/carbohydrate) manipulations on glucose intolerance or insulin sensitivity under isocaloric conditions in adults. *Ad libitum* fat-reduced diets leading to higher carbohydrate intakes (from 46 E% to 55 E%) have been shown to improve glucose tolerance in IGT subjects in the context of significant weight loss (about 3kg) after one year (Swinburn et al., 2001; Mensink et al., 2003). However, no conclusions can be drawn from these studies regarding the effects of carbohydrate consumption itself, independent of weight loss, on glucose tolerance.

The same argument applies to epidemiological studies associating high fat, low carbohydrate intakes, to decreased glucose tolerance or insulin sensitivity. The extent to which those observations are confounded by body weight and/or body fat gain has not been fully elucidated (IoM, 2005).

In conclusion, and although the influence of dietary carbohydrates on glucose tolerance and insulin sensitivity is still unclear, total carbohydrate intakes of 46 to 55 E% appear to be compatible with the maintenance of a normal glucose tolerance and insulin sensitivity in healthy subjects and in subjects with signs of the metabolic syndrome.

5.1.3. Serum lipids

The effects of dietary variation in total carbohydrate intakes on LDL-cholesterol are strongly associated with the type of fat which is replaced by carbohydrates. When saturated fatty acids are kept constant, varying carbohydrate intake as a function of total fat has no effects on LDL-cholesterol concentrations (IoM, 2005).

Data from intervention studies consistently show that increasing carbohydrate intakes (in the range of about 30 to 70 E%) as an inverse function of fat (ranging from 50 to 18 E% as fat) at low intakes of saturated fatty acids (SFA, <10%E) induces a decrease in plasma concentrations of HDL-cholesterol and an increase in the

total/HDL-cholesterol ratio and TG concentrations (Sacks and Katan, 2002; EFSA, 2004; IoM, 2005). This effect is largely attenuated in the lean and physically active (IoM, 2005). Also, TG concentrations are consistently higher when SFA are replaced by carbohydrates rather than by monounsaturated fatty acids (MUFA), particularly in hypertrygliceridaemic subjects (Aro et al., 1998; Mensink et al. 2003; Appel et al. 2005; Berglund et al., 2007; Furtado et al., 2008), and the HDL-cholesterol lowering effect is more pronounced in subjects with higher HDL concentrations at baseline (Obarzanek et al., 2001). However, even if TG concentrations consistently increase with increasing carbohydrate intakes when administered in isocaloric conditions, this effect could be attenuated when carbohydrate-rich diets are consumed *ad libitum*, possibly due to a concomitant reduction in body weight (Rock et al., 2004; Retzlaff et al., 1995; Kasim-Karakas et al., 2000).

In conclusion, the adverse effects of increasing total carbohydrate intakes on the lipid profile provide a basis to set an upper bound of RI for total carbohydrates.

5.1.4. Body weight

The impact of macronutrient manipulation on body weight in the context of weight management in overweight and obese subjects may be different from the effects on prevention of weight gain in leaner persons and may depend on whether diets are administered *ad libitum* or under isocaloric conditions.

In intervention trials tightly controlling energy intake, energy expenditure, weight loss and weight maintenance are a function of energy intake rather than of the macronutrient composition of the diet (Poppitt et al., 2002; IoM, 2005; van Dam and Seidell, 2007; Nordman et al., 2006; Sacks et al., 2009). However, even when an equivalent energy intake is intended, fat-reduced diets (<35 E%) tend to be hypocaloric compared with carbohydrate-reduced diets (<50 E%) and increase long-term compliance with energy restriction, leading to slightly greater weight loss (IoM, 2005; van Dam and Seidell, 2007).

Several randomised intervention studies suggest that fat-reduced (< 25 to 30 E%), moderately high carbohydrate (> 50 E%) diets consumed *ad libitum* have the potential to prevent weight gain in normal weight subjects and produce weight loss in overweight (BMI > 25kg/m^2) individuals as compared to higher fat (> 35 E%), lower carbohydrate (40 to 50 E%) diets (IoM, 2005). However, although very-low carbohydrate diets (< 40 E%) consumed *ad libitum* may have an advantage in terms of weight loss up to 1 year over lower (< 35 E%) and very-low (10 E%) fat diets (van Dam and Seidell, 2007; Gardner et al., 2007; Shai et al., 2008), long-term weight regain tends to be higher and may not offer clear benefits in terms of long-term body weight control (van Dam and Seidell, 2007).

In some long-term (>1 year) intervention studies, dietary modifications with a shift from a habitual Western-type, relatively high fat (35 to 40 E%), moderately low carbohydrate (40 to 50 E%) diets to more carbohydrate-rich (>50 E%), fat-reduced (<30 E%) diets consumed *ad libitum* were reported to be associated with a reduced risk of weight gain or a moderate weight loss in various population groups including normal, overweight and obese subjects (IoM, 2005; Howard et al., 2006a; Lanza et al., 2001).

Results from prospective cohort studies are conflicting with respect to the relationship between carbohydrate intake and weight gain (Halkjær et al., 2006; Gaesser, 2007).

In conclusion, the adverse effects associated with high fat, low carbohydrate diets on short- and long-term body weight control provide a basis to set a lower bound of the RI for total carbohydrates.

5.1.5. Type 2 diabetes mellitus

There are no intervention studies specifically addressing the effect of carbohydrate intake on the risk of developing type-2 diabetes. In two lifestyle, long-term intervention studies including weight loss by reducing fat intake and increasing physical activity, the presumed increase in carbohydrate intake (targeted at 55 E% carbohydrates) was compatible with a lower risk for type 2 diabetes mellitus (Tuomilehto et al.

2001, Knowler et al., 2002; Lindström et al., 2003, 2006a and 2006b). Large-scale observational studies on the effects of carbohydrate intake and risk of diabetes have yielded conflicting results (IoM, 2005).

Several cohort studies have investigated the relationship between intake of total and individual carbohydrates and the risk of developing diabetes type 2 (Murakami et al., 2005; McKeown et al., 2004; Meyer et al., 2000). Generally, no or weak relations between total carbohydrate intake and diabetes risk were observed.

In conclusion, diets providing about 55 E% as carbohydrates are compatible with a lower risk for type 2 diabetes mellitus in the context of concomitant weight loss and physical activity.

5.1.6. Cardiovascular disease

As reviewed (Sacks and Katan, 2002), three dietary intervention studies reducing total fat intake, in particular saturated fat, and increasing the consumption of carbohydrate-rich foods, did not significantly reduce the risk of cardiovascular disease. However, it cannot be excluded that the duration, compliance, and sample sizes may have been insufficient to produce a reduction in coronary events. Also, in the Women's Health Initiative Dietary Modification Trial a dietary intervention that reduced total fat intake and increased intakes of carbohydrates from vegetables, fruits, and grains did not significantly reduce the risk of cardiovascular disease (Howard et al., 2006b).

Data from observational studies, and in particular from the U.S. Nurses Health Study, do not indicate any consistent relationship between total carbohydrate intake and CHD risk (Liu et al., 2000; Oh et al., 2005; Halton et al., 2006). Data from one cohort (Halton et al., 2006) showed that an increased carbohydrate intake was associated with an increased risk of total and haemorrhagic stroke in women with a BMI > 25 kg/m^2 (Oh et al., 2005). Conversely, in two large prospective cohort studies (Trichopoulou et al., 2007; Lagiou et al., 2007), low energy-adjusted carbohydrate intakes, particularly if combined with high protein intakes, were associated with a significantly higher risk of mortality from CVD.

In conclusion, data from intervention and observational studies do not show any consistent relationship between the intake of total or glycaemic carbohydrate intake and the risk of CVD. The ranges of carbohydrate intakes in the studies above vary from 30 to 70 E%.

5.2. Sugars

5.2.1. Nutrient density of diet

Nutrient density is the amount of nutrients in foods per unit of energy. An adequate nutrient density is essential for providing recommended intakes of nutrients, especially in individuals with a low energy intake. There is some evidence that high intakes of added sugars, particularly from low nutrient density foods, might be associated with a decrease in the nutrient density of the diet (‘nutrient dilution’) due to displacement of nutrient rich foods (van Dam and Seidell, 2007). In some EU countries, studies in children and elderly nursing home residents (Lyhne and Ovesen, 1999; Beck and Ovesen, 2002; Alexy et al., 2003a; Øverby et al., 2004; Kranz et al., 2005; Frary et al., 2004) have shown that an intake of >10 to 30 E% of added sugars (mono-, disaccharides and higher saccharides) is associated with a reduced intake of several micronutrients (e.g. calcium, iron, folate, vitamin A) and dietary fibre, especially in children and adults with energy intakes below about 8 MJ per day. Data from the U.S. indicate that nutrient density of the diet among children was negatively correlated to the intake of added sugars in the range of 10 to 25 E% or above, but that clear differences were seen mainly at very high intakes (>25 E%) (IoM, 2005), with some exceptions e.g. calcium in preschool children (Kranz et al., 2005). A systematic review of 15 cross-sectional studies comprising children and adults shows that there are insufficient and conflicting data with respect to the relation between intake of added sugars and nutrient density, with no clear evidence of micronutrient dilution or a threshold for a quantitative amount of added sugar intake for any of the micronutrients

investigated (Rennie and Livingstone, 2007). The observed inverse association of nutrient density or intake with added sugar intake may be partly explained by methodological issues, e.g. different definitions of added sugars, and confounding effects related to differences in energy intake (higher sugar intake as E% in individuals with lower total energy intake and consequently lower nutrient intake). The association between added sugar intake and micronutrient density of the diet is mainly dependent on the intake patterns of the food groups from which added sugars in the diet are derived (Rennie and Livingstone, 2007).

In conclusion, observed negative associations between added sugar intake and micronutrient density of the diet are mainly related to patterns of intake of the foods from which added sugars in the diet are derived rather than to intake of added sugars *per se*. The available data are not sufficient to set an upper limit for (added) sugar intake. Evidence on the relationship of foods containing added sugar to micronutrient density of the diet and to micronutrient intake of population groups should be considered when developing food-based dietary guidelines.

5.2.2. Glucose tolerance and insulin sensitivity

Some mainly small, short-term (4 to 6 weeks) studies have investigated the effect of sugar intake on glucose and insulin response comparing individual sugars (sucrose, glucose or fructose) or mixtures of different sugars with starch or "complex carbohydrates". The majority of these used iso-caloric diets aimed at body weight maintenance during the study. Both normal subjects and subjects with impaired insulin responses were included. The amount of sugars in the intervention diets varied from about 3 to10 E% in the "low-sugar" diets to 20 to 30 E% in the high-sugar diets. The studies are summarised in Annex 4. Two of the three studies with sucrose showed increased insulin concentrations at sucrose intakes of 18 and 33 E%, whereas one did not show any difference between diets providing 10 or 25 E% sucrose. One study showed increased glucose concentrations at sucrose intakes of 18 and 33 E%, whereas one did not show any difference between diets providing 10 or 25 E% sucrose.

In conclusion, there are limited, and mainly short-term, data on the effects of high intakes of sugars on glucose and insulin response. Most studies do not find any adverse effects at intakes of predominantly added sugars up to 20 to 25 E%, provided that body weight is maintained.

5.2.3. Serum lipids

A number of mainly small, short-term (2 to 6 weeks) studies have investigated the effect of sugar intake on serum lipids, comparing individual sugars (sucrose, glucose or fructose) or mixtures of different sugars with starch or "complex carbohydrates". The majority of studies used iso-caloric diets aiming at body weight maintenance during the study. Both normal subjects and subjects with impaired insulin responses were included. The amount of sugars in the intervention diets varied from about 3 to 10 E% in "low-sugar" diets to 20 to 30 E% in the high-sugar diets. The studies are summarised in Annex 5. In five of the seven studies, increased sugar intakes led to increases in total and LDL-cholesterol, in four, the TG-concentrations increased, but responses differed according to sex and insulin sensitivity. Effects on HDL-cholesterol were less prominent or not reported. Overall, negative effects were observed at sugar intakes > 20 E%. Only in the study by Hallfrisch et al. (1983) a significant increase in total, LDL-cholesterol and TG was seen at fructose intakes of 7.5 and 15 E%. Effects tended to be more pronounced in subjects with markers of metabolic syndrome, e.g. insulin resistance. Studies also varied with respect to the composition of the basic and intervention diets, which might have influenced the results. For example Black et al. (2006) found that a high-sucrose diet (25 E%) resulted in increases in total and LDL cholesterol by 15% and 24%, respectively, compared to the control diet (10 E% sucrose). The authors hypothesise that the higher level of SFA and lower level of PUFA in the high-sucrose diet could have contributed to the cholesterol-raising effect.

Few long-term studies of the effect of sugars on lipids have been published. In a six-month study by Saris et al. (2000), 316 obese subjects were randomised to three groups, which received either a control diet with 46 E% carbohydrates of which 24 E% was starch and 21 E% sugars, or an intervention diet with 52 to 56 E%

carbohydrates containing mainly starch (33 E% starch, 16 E% sugars) or mainly sugars (sucrose, fructose and lactose, 30 E%). All diets were given *ad libitum*. No significant changes were seen in serum lipids among the groups.

In a sub-study 46 obese subjects with the metabolic syndrome (≥3 risk factors) were randomised to receive either one of three diets *ad libitum*: a control diet with with 29 E% starch and 21 E% sugars, two fat-reduced diets, one with 53 E% carbohydrates, mainly as "complex carbohydrates" (33 E% starch and 18 E% sugars) and one high-sugar diet with 57 E% carbohydrates including 29 E% sugars (sucrose, fructose and lactose) (Poppitt et al., 2002). Thirty-nine subjects completed the study. After six months fasting serum TG was higher in the sugar group than in the "complex-carbohydrate" and control groups, respectively. Weight loss was correlated with a decrease in TG concentrations.

Smith et al. (1996) found that restriction of the intake of added sugars during six months led to reduced TG concentrations in hyper-triglyceridaemic, overweight subjects, even at relatively moderate intakes (from about 12 to 4 E% added sugars). This reduction was partly associated with an (unintentional) weight-loss of <2% of the initial body weight.

In conclusion, a number of small-size controlled, iso-caloric short-term studies (2 to 6 weeks), indicate that high intakes (>20 E%) of sugars, provided predominantly as added sucrose or fructose, may increase serum TG and LDL-cholesterol concentrations, especially in subjects with markers of the metabolic syndrome, e.g. insulin resistance. However, data on dose-response are limited, especially at intakes in the range of 5 to 20 E%, albeit one study found elevated lipid concentrations at a fructose intake of 7.5 E%. Information on the intake of total sugars is lacking in some studies. In long-term intervention studies in which diets were given *ad libitum* (<6 months) changes in blood lipids as result of diets high in sugars (about 30% E) or of sugar restriction (from 12 to 4 E%) are closely related to body weight changes. A number of dietary factors such as fatty acid composition, dietary fibre content and type may modulate the effects. There are insufficient data to set a UL for sugars based on their effects on serum lipids.

5.2.4. Other cardiovascular risk factors

In the study by Marckmann et al. (2000) nonfasting FVIIc (factor VII coagulant activity) was lower on the low-sucrose (2.5 E%) diet compared to the high-sucrose (23 E%) diet.

In a 10-week intervention study, overweight subjects (mean BMI 28kg/m^2) were given 1.3 L sucrose-sweetened or artificially sweetened soft drinks per day while otherwise eating an *ad libitum* habitual diet (Raben et al., 2002). Those subjects consuming the sugar-sweetened soft drink had a sucrose intake of 28 E% and increased their energy intake during the study. At the end of the study blood pressure was increased in this group (SBP +3.8, DBP +4.1 mmHg), while it was decreased among subjects who consumed the artificially sweetened soft drink (SBP –3.1, DBP -1.2 mmHg). Body weight (+1.6 kg) and fat mass (+1.3 kg) increased in the sugar-group, with no significant changes in the control group.

In conclusion, there are insufficient data to set a UL for sugars based on their effects on the risk factors for cardiovascular disease reported in this section.

5.2.5. Body weight

The evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent (IOM, 2005; van Dam and Seidell, 2007). Either weight loss (Saris et al., 2000) or weight maintenance (Poppitt et al., 2002) has been reported for high carbohydrate (52 to 56 E%), high-sugar (29 to 30 E%) diets as compared to control diets (49 E% as carbohydrates, 21 E% sugars) consumed *ad libitum* for six months. Epidemiological studies do not show a positive correlation between total sugar intake and obesity – rather the opposite (IoM, 2005).

There is some evidence that sugar sweetened beverages do not induce satiety to the same extent as solid forms of carbohydrate, and that high intakes of sugars in the form of sugar-sweetened beverages might contribute to weight gain (van Dam and Seidell, 2007; Mann *et al*., 2007). *Ad libitum* consumption of high sucrose diets (28 E% mainly as beverages) was found to increase body weight and fat mass as compared to lower sucrose diets with artificial sweeteners (Raben et al., 2002). In a systematic review Malik et al. (2006) included 30 studies, mainly in children and adolescents (15 cross-sectional, 10 prospective, and 5 experimental), that investigated the association between sugar-sweetened beverage intake and weight gain. The authors state that large cross-sectional studies and well-powered prospective cohort studies with long periods of follow-up show a positive association between higher intakes of sugar-sweetened beverages and weight gain and obesity in both children and adults. No data on overall effect size were included. Vartanian et al (2007) included 88 studies in a meta-analysis regarding the association between soft drink consumption and body weight. Most studies were cross-sectional (17) and longitudinal (10) and included both children and adults. The overall effect size across studies was 0.08 (expressed as change in BMI and/or body weight) ($p<0.001$). However, results from another meta-analysis of eight prospective studies and two intervention studies among children and adolescents did not show a clear quantitative relationship and suggested that there may be publication bias against studies that do not report statistically significant findings (Forshee et al., 2008). Long-term randomized controlled trials on the effects of sugar sweetened beverages on body weight are lacking (van Dam and Seidell, 2007; Johnson et al., 2009).

Fructose has been suggested to play a specific role in weight gain (Elliot et al., 2002, see 2.2.1). There are, however, few and mainly short-term controlled intervention studies in healthy subjects comparing fructose with other sugars or carbohydrate-sources and these do not allow a conclusion regarding the role of fructose in obesity (Vasankari and Vasankari, 2006).

In conclusion, the evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent for solid foods. However, there is some evidence that high intakes of sugars in the form of sugar-sweetened beverages might contribute to weight gain. The available evidence is insufficient to set an upper limit for sugars based on their effects on body weight. Evidence on the relationship of sugar-sweetened beverages and body weight should be considered when developing food-based dietary guidelines.

5.2.6. Type 2 diabetes

Evidence on the effects of sugar consumption on the risk of developing type 2 diabetes comes primarily from large prospective cohort studies. No (Colditz et al., 1992; Janket et al., 2003) or even inverse (Meyer et al., 2000) associations have been reported for total sugars and/or specific types of sugars and diabetes risk. However, consumption of sugar-sweetened beverages, and particularly if sweetened with glucose or fructose, was found to be positively associated with increased type 2 diabetes risk (Schultze et al., 2004a; Montonen et al., 2007). The available evidence is insufficient to set a UL for sugars based on their effects on type 2 diabetes risk.

5.2.7. Dental caries

Increased risk of dental caries in children is associated with a high frequency (more than about 4 times daily) of intake of cariogenic sugars (mainly sucrose, glucose, and fructose) rather than with the total amount of dietary sugars; the evidence indicates that frequent consumption of sweets and confectionery products and sugar-containing drinks is associated with a higher risk of caries (Moynihan and Petersen, 2004; DoH, 1991; IoM, 2005; Anderson et al., 2009).

Caries develops as tooth substance demineralises upon pH decrease due to fermentation of carbohydrates by tooth-colonising bacteria into different organic acids. Dental caries is an infectious disease, although sucrose and other easily fermentable sugars, e.g. glucose and fructose, play a key role (Navia, 1994; Lingstrom et al., 1997). Foods rich in starch may also contribute, especially when the starch molecule is easily available to

degradation by amylase. The acid production from lactose in dental plaque is normally low, while the fermentation of starch varies greatly and depends on the degree of gelatinisation (Lingstrom et al, 1997). Decreases in pH to well below 5.5 are considered critical for caries development in enamel (the tooth crown). In tooth roots the critical pH for demineralisation is approximately 6.5. In addition to lactic acid, sucrose fermentation produces insoluble extracellular glucose polymers leading to voluminous biofilms that favour colonisation of cariogenic streptococci on the teeth surfaces.

Dental caries prevalence has declined in many European countries during the last decades of the 20th century, but trends vary between countries and age groups (Touger-Decker and van Loveren, 2003; Schulte et al., 2006; Haugejorden and Magne Birkeland, 2006; Demertzi et al., 2006; Stecksen-Blicks et al., 2008; Pitts et al., 2006).

More recently, mainly cross-sectional studies generally find a weak or moderately strong relationship between the intake of sucrose and other sugars and caries prevalence (Burt and Pai, 2001). The impact of fluoride prophylaxis and other lifestyle variables seems to override variations in cariogenic carbohydrate intake in these studies. High intake of sugars has been associated with an increased risk of caries when oral hygiene is simultaneously poor and at a low level of fluoride prophylaxis (Danish Nutrition Council, 2003; Burt and Pai, 2001; Kleemoja-Kujala and Räsänen, 1982). However, results from a Finnish longitudinal study suggest a relationship between the intake of sucrose and sucrose containing foods and caries development during childhood among children with fluoride prophylaxis (Karjalainen et al., 2001; Ruottinen et al., 2004).

Available data do not allow the setting of an UL for sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by various other lifestyle factors (oral hygiene, exposure to fluoride, meal frequency and diet composition), heredity, illness, medication, malnutrition, and flow and composition of saliva.

In conclusion, frequent consumption of sugar-containing foods can increase risk of dental caries, especially when prophylactic measures, e.g. oral hygiene and fluoride prophylaxis, are insufficient. However, available data do not allow the setting of an UL for sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by oral hygiene, exposure to fluoride, frequency of consumption, and various other factors. Evidence on the relationship of frequency of consumption of sugar-containing foods and dental caries should be considered when developing food-based dietary guidelines.

5.3. Dietary fibre

5.3.1. Dietary requirements

Dietary fibre has not been shown to be an indispensable component of the diet. However, dietary fibre has a major role in bowel function and gastro-intestinal symptoms, such as constipation (IoM, 2005).

5.3.2. Gastrointestinal function

5.3.2.1. Adults

Dietary fibre has a major role in bowel function and gastro-intestinal symptoms, such as constipation, have been linked to low fibre intakes (IoM, 2005). Constipation has been defined as difficulty in passing stools or an incomplete or infrequent passage of hard stools (Longstreth et al, 2006). Constipation occurs in 5 to 18%

of adults in different countries with a greater percent of women and elderly affected and adversely affects the quality of life (Wald et al., 2008). It may also contribute to diverticular disease.

Both observational and experimental data show that dietary fibre is the most important dietary determinant of faecal bulk and transit time (Cummings et al., 1992, Birkett et al., 1997). Dietary fibre from cereals, fruits, and vegetables increases stool weight, which promotes normal laxation in children and adults. In general, the greater the weight of the stool and the more rapid the rate of passage through the colon the better the laxative effect (Birkett et al., 1997).

It has also been demonstrated that different kinds of dietary fibre have different bulking capacity. Dietary fibre in wheat bran and other fibre that is fairly resistant to fermentation in the large bowel has the most pronounced bulking effect (5 to 6 g per g dietary fibre) mainly due to water binding in the distal bowel, whereas more fermentable fibre provide some bulk mainly due to increased bacterial mass (Cummings, 2001).

Although there is no single accepted definition of what constitutes normal laxation, frequency of defaecation is typically about once per day on Western diets (Weaver, 1988). Haack et al. (1998) have indicated that a defecation frequency of about once per day and a transit time in the range of 2 to 3 days may be considered normal laxation. They reported that while increasing intake of fibre (provided by a mixture of fruit, vegetables, and grains) from 16 to 30 g per day increased defaecation frequency from 0.7 to 0.94 times per day, a further increase in fibre intake to 42 g per day had no further effect on defecation frequency, which remained at about once per day. There was no significant change over the range of fibre intakes in gastrointestinal transit time, which remained within the range of 2 to 3 days, or stool moisture, which remained within the range of 70 to 74%. Faecal weight increased from 109 g to 156 g and 195 g at fibre intakes of 16, 30 and 42 g per day, respectively.

Increasing dietary fibre intake from 12 to 45 g per day increased faecal weight from 69 to 184 g per day and reduced transit time from >70 h to 45 h (Stasse-Wolthuis et al., 1978). Mean transit time in UK adults has been reported as 70 h (median 60 h) (Cummings et al., 1992) in a population in which mean stool weight is about 110 g per day, with weights of less than 100 g per day in about 50% of individuals, and dietary fibre intake of about 18 g per day (Cummings et al., 1992). It has been estimated that a dietary fibre intake of 25 g per day is associated with stool weight of about 130 to 150 g per day (Cummings et al., 1992). Birkett et al. (1997) reported that adults who consumed 25 g dietary fibre in their usual diet excreted more than 150 g faeces per day.

Taken together, these data indicate that an intake of 25 g per day of dietary fibre from mixed foods (as AOAC fibre or equivalent) is compatible with an intestinal transit time of about two to three days and a defaecation frequency of 1 per day and a faecal moisture of >70% and may be considered adequate for normal laxation in adults.

5.3.2.2. Children

There is evidence that constipation is a common problem also during childhood (e.g. Loening-Baucke, 1993) and that there is an inverse relationship with dietary fibre intake (Edwards and Parrett, 2003).

There are few data relating intake of dietary fibre to normal laxation in children. Data from long-term intervention and observational studies can, however, give information on fibre intakes that are compatible with adequate growth and development and at the same time provide pre-requisites for good health. Results from the Finnish STRIP-study indicate that a fibre intake corresponding to 2-2.5 g per MJ is compatible with normal growth and development. The fibre intake among German children was already at one year somewhat higher (3 g per MJ) and there are no reports of adverse effects related to the fibre intake.

In conclusion, dietary fibre intake of 2 g per MJ should be adequate for normal laxation in children based on the dietary fibre intake that is considered adequate for normal laxation in adults (25 g, equivalent to 2 to 3 g

per MJ for daily energy intakes of 8 to 12 MJ) and taking into account that energy intake relative to body size in children is higher than in adults.

5.3.3. Glucose tolerance and insulin sensitivity

Few intervention studies have investigated the effects of fibre intake on measures of glucose tolerance or insulin sensitivity.

In the two-year intervention study by Mensink et al. (2003) conducted in subjects with impaired glucose tolerance (IGT), glucose tolerance improved in the intervention group compared to the control group. Dietary fibre intake was 3.1 to 3.3 g per MJ in the intervention group compared to 2.7 g per MJ in the control group.

A number of cohort studies have found that intake of fibre and fibre rich foods such as wholegrain cereals correlated favourably with measures of glucose tolerance or insulin sensitivity. Liese et al. (2005) found that dietary fibre intake was associated positively with insulin sensitivity and inversely with fasting insulin, but not with acute insulin response, in 979 adults with normal or impaired glucose tolerance. In the baseline data of an intervention study by Lau et al. (2005) the intake of dietary fibre was inversely associated with the probability of having insulin resistance (assessed by the homeostasis model assessment of insulin resistance, HOMA-IR) among middle-aged, healthy adults. Similar results were observed in another cross-sectional study on subjects at high risk of type 2 diabetes (relatives of patients with type 2 diabetes) (Ylönen et al., 2003).

In conclusion, increasing intakes of foods rich in dietary fibre are associated with reduced risk of impaired glucose control. Dietary fibre intakes associated with favourable effects are >2.6 g per MJ and about 30 g per day, although the contribution of dietary fibre *per se* to this effect remains to be established.

5.3.4. Serum lipids

A meta-analysis including 67 intervention studies showed that intakes of 2 to 10 g per day of viscous, soluble fibre (e.g. pectin, oat bran, guar gum, psyllium) were associated with a small, but significant, decrease in total cholesterol (-0.045 mmol/L per gram of dietary fibre) and LDL-cholesterol (-0.057 mmol/L per gram) (Brown et al., 1999). The effects have been confirmed in subsequent studies in both hypercholesterolaemic subjects (Jenkins et al., 2002), and normocholesterolaemic subjects (Berg et al., 2003; Aller et al., 2004), but not in the study by Chen et al. (2006b). Controlled intervention studies have generally shown that fasting TG concentrations are not affected by fibre intake (Queenan et al., 2007; Beer et al., 1995; Behall et al., 2004; Anderson et al., 1995; Braaten et al., 1994; van Horn et al., 1991).

Certain kinds of fibre, especially soluble, viscous types, can, however, reduce post-prandial hyperlipidaemia (Lairon, 2001). These effects are related to diminished cholesterol and/or bile acid absorption (Andersson, 1996) and possibly also to products of colonic fermentation. Effects on lipid metabolism of resistant starch and resistant oligosaccharides demonstrated in experimental animals have so far not been reproduced in man.

In conclusion, viscous types of dietary fibre may contribute to reducing total and LDL-cholesterol concentrations. The effects are limited at amounts usually consumed from foods.

5.3.5. Blood pressure

In a meta-analysis Whelton et al. (2005) evaluated 25 randomised controlled trials with respect to the effect of dietary fibre intake on blood pressure. The difference in fibre intake between intervention and control groups ranged from 3.8 to 12.5 g per day, with a median difference of 10.7 g per day. In eight studies fibre was given as a supplement, otherwise fibre was provided as foods (15 studies, cereal, fruit, fruit/vegetables, cereal/fruit, cereal/vegetables/fruit), pectin (1 study) or guar gum (1 study). Study duration varied from 2 to

26 weeks. Overall, dietary fibre intake was associated with a small, but significant, reduction in diastolic blood pressure (-1.6 mmHg) and a non-significant reduction in systolic BP (-1.1 mmHg). A significant reduction in both systolic and diastolic blood pressure was observed in trials conducted in patients with hypertension (SBP -5.9 mmHg, DBP -4.2 mmHg) and in trials including both normotensive and hypertensive subjects with a duration of the intervention of 8 weeks or longer (SBP -3.1 mmHg, DBP -2.6 mmHg). A further meta-analysis by Streppel et al. (2005) showed similar results. Whether the effect is related to the intake of dietary fibre *per se*, to the consumption of other nutrients in fibre-rich food products with blood-pressure lowering effects, or both, was not addressed.

In conclusion, small, but rather consistent, effects on blood pressure have been observed for diets rich in fibre from e.g. cereals, fruit and vegetables, although the contribution of dietary fibre *per se* to this effect remains to be established.

5.3.6. Body weight

5.3.6.1. Adults

Reviews of randomised trials have shown weight loss in a majority of studies with no differences between fibre types or between fibre occurring in foods or in supplements (Pereira and Ludwig, 2001; Howarth et al., 2001). This led to the conclusion by the World Health Organisation (WHO) that the evidence for a protective effect against weight gain and obesity of high dietary intake of NSP (dietary fibre) was convincing (WHO/FAO, 2003) and that an intake of at least 25 g total dietary fibre per day from wholegrain cereals, fruit and vegetables would be desireable. Results from seven prospective cohort studies show an inverse relationship between weight gain and baseline intake or change in fibre intake among adults during follow-up periods up to 12 years (Lairon, 2007; Koh-Banerjee et al., 2004). However, Iqbal et al. (2006) found no significant relationship between fibre intake at baseline and subsequent weight change during a 5-year follow-up among 30 to 60 year old men and women.

In the Finnish Diabetes Prevention study an increase in dietary fibre intake was associated with a sustained weight reduction (>5%) (Lindström et al., 2006a). The odds ratio for sustained weight loss at year 3 of the study (1 to 3 year follow up) was 2.04 (95% CI: 1.05 to 3.95) for subjects in the third quartile (3.1 to 3.7 g per MJ) and 2.67 (95%CI: 1.26 to 5.65) for subjects with a fibre intake in the upper quartile (>3.7 g per MJ), compared to subjects in the lowest quartile (< 2.6 g per MJ).

5.3.6.2. Children

In the Finnish intervention study STRIP (Special Turku Coronary Risk Factor Intervention Project) children were given dietary advice from the age of 7 to 8 months and have been followed up to 14 years of age (Niinikoski et al., 2007). These children grew and developed normally (Kaitosaari et al., 2003). Data on fibre intake are available for children up to 7 years of age. Mean intake of fibre varied from 9.2 g per day at 13 months of age to 11.8 g per day at 5 years of age (Lagström et al., 1999). The energy adjusted fibre intake was between 1.9 and 2.5 g per MJ at 13 months of age, 1.8 and 2.3 g per MJ at 3 years of age and 1.7 and 2.4 g per MJ at 5 years of age. There were no differences in body weight or growth in relation to fibre intake. At 7 years of age fibre intake was, depending on dietary pattern, between 12.3 and 15.5 g per day, corresponding to 1.9 and 2.4 g per MJ, respectively (Räsänen et al., 2002).

Studies on children eating mixed diets do not indicate adverse effects on growth due to high fibre intake. On the other hand, there are studies indicating that dietary fibre intake can contribute to lower the risk of obesity (Edwards and Parrett, 2003).

In conclusion, increased intake of dietary fibre, both from naturally fibre-rich foods and added fibre or fibre supplements, has been shown to be related to improved weight maintenance in adults and sustained weight reduction in overweight subjects. Estimated intakes associated with this effect in adults are in the order of

>25 g dietary fibre per day (from wholegrain cereals, fruit and vegetables) and >3.1 g total fibre per MJ. Results of intervention and observational studies in children indicate that a fibre intake corresponding to 2 to 3 g per MJ is compatible with normal growth and development.

5.3.7. Colorectal cancer

The effect of dietary fibre on faecal bulk has been an important parameter in setting recommended dietary intakes for dietary fibre. Intake in adults necessary to obtain a faecal bulk related to a minimal risk of intestinal disorders, particularly colon cancer, has been estimated to be 26-34 g per day (Cummings et al.,1992), 35 to 45 g per day (Spiller and Spiller, 2001) and 32 to 40 g per day (Monro, 2004).

The fermentation of dietary fibre by the colonic microflora has been recognised as important for colonic health and might have systemic metabolic effects through absorption of fermentation products. One of the fermentation products, butyric acid, is of special interest in relation to colon cancer since it is a main source of energy for colonocytes and has effects on cell differentiation, apoptosis and inflammatory processes (Cummings et al., 2004).

A large number of both *in vitro* and *in vivo* studies have provided mechanistic support for protective effects of dietary fibre against colon cancer (e.g., effects on faecal enzymes, secondary co-carcinogenic bile salt metabolites, etc.). Furthermore, there is a relationship between fibre intake and faecal bulk, and further between colon cancer and faecal bulk (Cummings et al., 1992; Birkett et al., 1997). Stool outputs of 150 g per day or more, normally obtained at a dietary fibre intake of at least 25 g per day, have been associated with lower prevalence of colon cancer (Cummings et al., 1992 and 2004).

A number of mainly small-size clinical intervention studies have been performed using polyp recurrence and rectal cell proliferation as surrogate markers for colon cancer (IoM, 2005). A pooled analysis of two larger intervention studies comprising 3,209 subjects with colorectal adenomas showed that increased intakes of fibre were associated with a significantly decreased risk of adenoma recurrence among men, but not in women, after 3 to 4 years (Jacobs et al., 2006). However, the strategy to increase fibre intake differed between studies (wheat bran supplement vs dietary intervention aimed at decreasing fat intake and increasing intake of fibre, fruit and vegetables).

Recent epidemiological studies have, however, given inconsistent results regarding a protective effect of dietary fibre against colorectal cancer (WCRF/AICR, 2007; Otani et al., 2006; Park et al., 2005; Bingham et al., 2003 and 2005). In the EPIC (European Prospective Investigation into Cancer and Nutrition) study comprising more than 0.5 million people from ten different European countries, dietary fibre intake from foods was inversely related to the incidence of large bowel cancer with an adjusted relative risk of 0.75 (95% CI 0.59 to 0.95) for the highest versus the lowest quintile of intake and 0.58 (0.41 to 0.85) after adjustment for more detailed dietary data. The association with colon cancer was strengthened with longer follow-up. However, the association with rectal cancer was no longer significant (Bingham et al., 2005).

In the World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR) report 16 cohort studies were reviewed and of these, eight could be included in a meta-analysis (WCRF/AICR, 2007). The analysis showed a statistically significant risk reduction of colorectal cancer and the overall relative risk was 0.90 (95% CI: 0.84 to 0.97) for a 10 g per day increase in fibre intake. However, a previous pooled analysis of 13 prospective cohort studies did not show a statistically significant protective effect after adjusting for known risk factors (Park et al., 2005). The WCRF/AICR concluded that foods containing dietary fibre might protect against colorectal cancer, albeit residual confounding in the studies could not be excluded.

In a nested case-control study Peters et al. (2003) found that high intakes of dietary fibre were associated with a lower risk of colorectal adenomas (polyps), especially regarding fibre from grains and cereals and from fruits.

In conclusion, dietary fibre is the most important dietary factor for faecal bulk and regular bowel movements, and might reduce the risk of colon cancer. Previous estimates of fibre intakes in adults necessary to achieve minimal risk of intestinal disorders, particularly colon cancer, range from 26 to 45 g per day. One meta-analysis of prospective cohort studies found a 10% decrease in the risk of colorectal cancer for each 10 g per day increase of dietary fibre intake. However, a consistent relation has not been shown in all cohort studies.

5.3.8. Type 2 diabetes mellitus

In the Finnish Diabetes Prevention Study an increase in dietary fibre intake was associated with a reduced risk of developing diabetes type 2 in subjects with IGT (Lindström et al. 2006b). The adjusted hazards ratios were 0.50 (95% CI: 0.28 to 0.89), 0.71 (0.40 to 1.23) and 0.38 (0.19 to 0.77) for subjects with a fibre intake in the second (2.6 to 3.1 g per MJ per day), third (3.1 to 3.7 g per MJ per day) and upper quartile (>3.7 g per MJ per day), respectively compared to subjects in the reference quartile (<2.6 g per MJ per day). The lowest risk was observed among subjects with both a high fibre (above median, >3.1 g per MJ per day) and low fat (below median, <33.2 E%) intake.

Several prospective cohort studies have investigated the relationship between dietary fibre intake and risk of type 2 diabetes. Most studies have found a decrease in risk with increasing intakes of cereal fibre (Murakami et al., 2005; Krishnan et al., 2007; Schulze et al., 2007) and whole grains (de Munter et al., 2007). Fewer studies have found a significant risk reduction with total dietary fibre intake (Salmerón et al., 1997a and 1997b; Meyer et al., 2000; Montonen et al., 2003). In the latter studies, total dietary fibre intakes of about 25 to 40 g per day have been consistently associated with a significantly lower risk of developing type 2 diabetes compared to fibre intakes of about 12 to 16 g per day.

In conclusion, dietary fibre intakes reported to be associated with a reduced risk for type 2 diabetes are >2.6 g per MJ or about 25 to 30 g per day, although the contribution of dietary fibre *per se* to this effect remains to be established.

5.3.9. Cardiovascular disease

In a meta-analysis of 10 prospective cohort studies an increase in the energy-adjusted fibre intake of 10 g per day was associated with a 14% lower risk of all coronary events (fatal and non-fatal myocardial infarction (MI)) and with a 27% lower risk of coronary death (Pereira et al., 2004). The results also indicate that a reduced relative risk was seen for subjects with an energy-adjusted fibre intake of >24 g per day compared to subjects in the reference category (18 to <21 g per day). The U.S. Food and Nutrition Board used data from three cohort studies regarding coronary heart disease (CHD) as a basis for setting an AI for total dietary fibre (IoM, 2005). The AI (14 g per 1000 kcal, 3.4g per MJ) was derived from the upper quintiles of energy-adjusted intake of dietary fibre.

In conclusion, there is epidemiological evidence for a protective effect of dietary fibre intake >24g per day on cardiovascular disease risk.

5.4. Glycaemic index and glycaemic load

5.4.1. Glucose tolerance and insulin sensitivity

Short- to medium-term (2 weeks to 6 months) intervention studies have shown that diets with reduced GI can improve markers of metabolic control in diabetes type 1 and 2 (Opperman et al., 2004).

A few intervention studies have investigated the role of GI or GL in healthy subjects in relation to the risk of developing IGT or impaired insulin sensitivity, while controlling for dietary fibre intake.

In a 10-week controlled intervention study (Sloth et al., 2004), healthy overweight subjects (BMI: 25 to 30 kg/m^2) were given fat-reduced (22 E%) diets with 57 to 58 E% as total carbohydrates *ad libitum* with either high or reduced GI, but with otherwise similar nutrient composition including dietary fibre content. Both groups lost weight. No significant differences between the groups with respect to metabolic markers such as blood glucose, insulin concentrations or insulin resistance (HOMA-IR) were observed. GI differed by 24 units. Similarly, Philippou et al., (2009) did not observe an effect of low (50) vs high (64) GI diets on insulin sensitivity or beta cell function assessed by HOMA-IR and HOMA-β, respectively, while controlling for the amount of dietary fibre intake (11 vs 13 g per day), whereas Frost et al., (1998) showed a significant increase in insulin sensitivity assessed by a short intravenous glucose tolerance test after consumption of a low GI diet (67 to 71) compared to a high GI diet (87 to 89) both comparable for the amount of dietary fibre (19 vs 18g per day).

In other intervention studies investigating the effects of low and high GI diets consumed *ad libitum* on glucose tolerance or insulin sensitivity, dietary fibre intakes are consistently higher in the low GI diet group and do not generally show an effect of low-GI diets on insulin sensitivity (De Rougemont et al., 2007; Wolever and Mehling, 2002; Wolever and Mehling, 2003; Bouche et al., 2002; Brynes et al., 2003). In an intervention study by Clapp and Lopez, (2007), insulin resistance assessed by the HOMA-IR and the quantitative insulin-sensitivity check (QUICKI) indexes significantly decreased after consumption of a low-GI diet (59) compared to a high GI diet (92) in women. Dietary fibre intake was not reported in the study. In an observational study including 979 adults, Liese et al. (2005) found no relation between GI, GL or carbohydrate intake and measures of insulin sensitivity, insulin secretion, and adiposity in adults with normal or impaired glucose tolerance. Data from the same study showed that average fasting glucose, 2 h plasma glucose after an oral glucose tolerance test and glycated haemoglobin (HbA1c) concentrations were not related to either GI or GL calculated from food frequency questionnaires (and GL adjusted for total energy intake), neither at baseline nor at a 5-year follow-up examination (Mayer-Davis et al., 2006). Also Lau et al. (2005) did not find any association between GI or high GL and insulin resistance assessed by HOMA-IR among 5,675 healthy adults aged 30 to 60 years.

In conclusion, results from observational and mainly short-term intervention studies with controlled diets have given conflicting results with respect to the importance of GI for blood glucose control and insulin sensitivity. The data available do not allow setting a DRV for GI/GL based on this outcome.

5.4.2. Serum lipids

Intervention studies with controlled diets have given conflicting results with respect to effects of GI/GL on serum lipids. This conflict might be due to methodological problems in designing experimental diets that are similar in dietary composition except for GI. In the well-controlled 10-week intervention study by Sloth et al. (2004) in healthy overweight subjects, who received a fat-reduced diet (21 to 23 E% fat, 57 to 58 E% carbohydrates) with either a high- or low-GI, there were no significant differences between the groups with respect to TG and HDL-cholesterol concentrations. However, a 10% reduction in LDL-cholesterol concentrations was obtained in the group that received the low-GI diet. These findings are supported by a meta-analysis of 15 intervention studies comparing effects of low-GI diets with high-GI diets on serum lipids and other risk factors for coronary heart disease (Kelly et al., 2004).

Some cross-sectional epidemiological studies have indicated that diets with a high GI or GL are associated with unfavourable effects on serum lipids (Augustin et al., 2002). For example Liu et al. (2001) found that fasting plasma TG concentration in 185 healthy postmenopausal women was positively related to GL, especially in overweight and obese subjects. GL was also inversely associated with non-fasting HDL-cholesterol concentration. However, there was no association with the energy adjusted total carbohydrate intake. In a cohort study of 355 healthy adults 35 to 65 years old, Oxlund and Heitmann (2006) found that dietary GI was directly related to changes in total and LDL-cholesterol concentrations in men, but not in women, after six years follow-up. No significant relationships were seen for HDL-cholesterol or TG concentrations. Associations were weak and generally confined to some subgroups, e.g. age categories.

In conclusion, effects on serum cholesterol concentrations are largely dependent on the amount and proportion of fatty acids in the diet, but diets with a low GI may contribute to lowering LDL cholesterol. However, the data available are insufficient to set a DRV for GI/GL based on their effects on serum lipids.

5.4.3. Body weight

A systematic review of 6 selected controlled intervention studies including in total 202 overweight or obese subjects showed that weight reduction was about 1 kg greater in subjects allocated to a diet with reduced GI or GL compared to controls (Thomas et al., 2007). Study duration varied between 6 weeks to 6 months. The studies used different designs, e.g. *ad libitum*/energy restriction, low-GI/low-GL or combinations of these factors. Also macronutrient composition varied widely among the studies. The differences in GI between intervention and control diets varied considerably, from 4 to 7 units to 25 to 30 units. In the three studies that used an *ad libitum* design no significant differences in final body weight were found (Bouché et al., 2002; Sloth et al., 2004; Ebbeling et al., 2005). Only one study (Sloth et al. 2004) compared diets with similar nutrient composition, with no significant difference in weight change after 10 weeks. Other short- to medium-term (12 weeks to 4 months) intervention studies in which diets were administered *ad libitum* to mainly overweight and obese subjects have not shown differences in weight change related to GI (Wolever and Mehling, 2002 and 2003; Aston et al., 2008).

Controlled intervention studies of longer duration (1 to 1.5 years) do not show major differences in weight change in overweight/obese subjects related to GI/GL (Ebbeling et al., 2007; Das et al., 2007) or normal/overweight subjects (Sichieri et al., 2007).

Results from cohort studies regarding the relation between GI and/or GL on body weight are equivocal (Gaesser, 2007; McMillan-Price et al., 2006, McMillan-Price and Brand-Miller, 2006; Sloth and Astrup, 2006; Thorsdottir and Birgisdottir, 2005).

In conclusion, available studies do not allow a firm conclusion with respect to effects of diets with different GI and/or GL on body weight, neither to set a DRV for GI/GL based on this outcome. Most studies have been short-term and there are few controlled studies with *ad libitum* food intake. The difference in GI or GL between intervention and control has varied between studies and diets have also differed in other nutritional aspects, e.g. dietary fibre intake and/or energy density. In studies comparing low GL with high GL, macronutrient composition has differed between high and low GL diets, whereas GI may or may not have differed depending on the strategy used for GL manipulation (changes in carbohydrate content, in GI of carbohydrate containing foods, or both).

5.4.4. Type 2 diabetes mellitus

Prospective cohort studies comparing diets with different GI or GL have shown conflicting results with respect to the risk of developing type 2 diabetes (Salmerón et al., 1997a; Salmerón et al., 1997b; Meyer et al., 2000; Hu et al., 2001; Stevens et al., 2002; Hodge et al., 2004; Schulze et al., 2004b; Krishnan et al,. 2007; Mosdøl et al., 2007; Sahyoun et al., 2008; Halton et al., 2008). A meta-analysis including eight studies found an increased risk of diabetes when comparing highest to lowest quintiles of both GI and GL (Barclay et al., 2008). However, the studies by Krishnan et al. (2007), Sahyoun et al. (2008) and Mosdøl et al. (2007), which showed no or an inverse relationship, were not included. Adjustment for dietary factors was limited to dietary fibre intake.

In conclusion, few observational studies indicate that diets with either low GI or low GL might be associated with a decreased risk of developing type 2 diabetes, but data are inconsistent and do not allow setting a DRV for GI/GL on the basis of type 2 diabetes risk.

5.4.5. Cardiovascular disease

Data from the Nurses Health Study show a positive association of GL with risk of cardiovascular events (MI, CHD deaths, Liu et al., 2000; Halton et al., 2006) or stroke in overweight women (Oh et al. 2005). A study among elderly men did not observe any relation between GI and CVD risk (van Dam et al., 2000). GL was not considered in the study.

In a prospective study of 36,246 Swedish men aged 45 to 79 years without diabetes or prior cardiovascular disease, dietary GI and dietary GL were not associated with ischaemic cardiovascular disease or mortality after 6 years follow-up (Levitan et al., 2007a). However, a weak trend for a greater risk of haemorrhagic stroke with increasing GL was observed. In a subsequent study of 4,617 men aged 45 to 79 years with prior cardiovascular disease, dietary GI and GL were not associated with cardiovascular or all-cause mortality after 6 years follow-up (Levitan et al., 2007b). In a recent systematic review of the evidence supporting a causal link between dietary factors and CHD, high GI and high GL were among those showing the strongest associations with increased risk of CHD (Mente et al., 2009).

In conclusion, a few observational studies indicate that diets with high GL might be associated with an increased risk of CVD, but data are inconclusive and do not allow setting a DRV for GI/GL on the basis of CVD risk.

5.4.6. Colorectal cancer

A meta-analysis of 4 case-control and 7 prospective cohort studies found a significantly increased pooled risk of colorectal cancer for the upper compared to the lower quintile of GL (Gnagnarella et al., 2008). There was, however, large heterogeneity between studies and risk estimates were not significant for prospective studies.

In conclusion, the data available do not allow setting a DRV for GI/GL on the basis of colorectal cancer risk.

6. Data on which to base dietary reference values

6.1. Total and glycaemic carbohydrates

Reference values for the intake of glycaemic carbohydrate have to take into account the amount of energy to be provided when reference intakes for protein and fat intake have been met.

The absolute dietary requirement for glycaemic carbohydrates is not precisely known but will depend on the amount of fat and protein ingested. Generally an intake of 50 to 100 g per day will prevent ketosis. An intake of 130 g per day for both children (>1 years) and adults has been estimated to be sufficient to cover the needs of glucose for the brain. This intake corresponds to about 18 and 25 E% in adult males and females assuming an energy intake of 2,800 and 2,100 kcal per day (11,700 and 8,780 kJ per day), respectively. However, these levels of intake are not sufficient to meet energy needs in the context of acceptable intake levels of fat and protein.

Intervention studies provide evidence that high fat, low carbohydrate diets consumed *ad libitum* are associated with an increase in body weight, but data are insufficient to define an LTI for carbohydrates. High carbohydrate diets tend to induce adverse effects on the lipid profile, but there is an insufficient scientific basis for setting an UL for total carbohydrates. The Panel therefore comes to the conclusion that only a Reference Intake Range can be given for total carbohydrate intake, partly based on practical considerations (e.g. current levels of intake, achievable dietary patterns) (see section 3).

Based on the above considerations the Panel proposes 45 to 60 E% as the Reference Intake Range for carbohydrates. Diets with glycaemic carbohydrate contents of 45 to 60 E%, in combination with reduced intakes of fat and SFA, are compatible with the improvement of metabolic risk factors for chronic disease,

as well as with mean carbohydrate intakes observed in some European countries. This intake range applies to both adults and children older than one year of age.

6.2. Sugars

Frequent consumption of sugar-containing foods can increase risk of dental caries, especially when prophylactic measures, e.g. oral hygiene and fluoride prophylaxis, are insufficient. However, available data do not allow the setting of an UL for (added) sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by oral hygiene, exposure to fluoride, frequency of consumption, and various other factors.

The evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent for solid foods. However, there is some evidence that high intakes of sugars in the form of sugar-sweetened beverages might contribute to weight gain. The available evidence is insufficient to set an upper limit for sugars based on their effects on body weight.

Observed negative associations between added sugar intake and micronutrient density of the diet are mainly related to patterns of intake of the foods from which added sugars in the diet are derived rather than to the intake of added sugars *per se*. The available data are not sufficient to set an upper limit for (added) sugar intake.

Most short-term intervention studies on the effects of high intakes of sugars on glucose and insulin response do not find adverse effects at intakes of predominantly added sugars up to 20 to 25 E%, provided that body weight is maintained. Although there is some evidence that high intakes (>20 E%) of sugars may increase serum TG and cholesterol concentrations, and that intakes >20-25 E% might adversely affect glucose and insulin response, the available data are not sufficient to set an upper limit for (added) sugar intake.

Evidence on the relationship of patterns of consumption of sugar-containing foods to dental caries, weight gain and micronutrient intake should be considered when establishing nutrient goals for populations and recommendations for individuals and when developing food-based dietary guidelines.

The Panel notes that some authorities have established upper limits for population average intake or individual intake of added sugars of <10 E% but others have not (see Section 4). Typically, these nutrient recommendations reflect a judgement of what level of sugar intake is practically achievable within the context of a nutritionally adequate diet based on known patterns of intake of foods and nutrients in specific populations. It is also noted that the average intake of (added) sugars in some EU Member States exceed 10 E%, especially in children.

6.3. Dietary fibre

The role of dietary fibre in bowel function was considered the most suitable criterion for establishing an adequate intake. Based on the available evidence on bowel function, the Panel considers dietary fibre intakes of about 25 g per day to be adequate for normal laxation in adults.

The Panel notes that there is evidence in adults of benefit to health associated with consumption of diets rich in fibre-containing foods at dietary fibre intakes greater than 25 g per day, e.g. reduced risk of coronary heart disease and type 2 diabetes and weight maintenance.

The Panel considers that the AI for dietary fibre for children should be based on that for adults (25 g, equivalent to 2 to 3 g per MJ for daily energy intakes of 8 to 12 MJ) with appropriate adjustment for energy intake. 2 g per MJ is considered to be adequate for normal laxation in children. Table 3 shows AI for fibre in children of different ages based on average energy intakes. Available evidence indicates that a fibre intake corresponding to 2 to 2.5 g per MJ is compatible with normal growth and development in children.

6.4. Glycaemic index and glycaemic load

Although there is some support for a role of GI and GL in the treatment of type-2 diabetes and some evidence suggesting that lowering GI and GL may have favourable effects on some metabolic risk factors such as serum lipids, the evidence regarding their role in the prevention of diet-related diseases is still inconclusive.

CONCLUSIONS

Total and glycaemic carbohydrates

The Panel considers that there is insufficient scientific basis for setting a Lower Threshold Intake (LTI), a Population Reference Intake (PRI), an Adequate Intake (AI) or a Tolerable Upper Intake Level (UL) for total carbohydrates.

Based on the effects of carbohydrates (and fat) intakes on body weight and blood lipids, while taking into account practical considerations (e.g. current levels of intake, achievable dietary patterns), the Panel proposes 45 to 60 E% as the Reference Intake range for carbohydrates. This intake range applies to both adults and children from one year of age.

Sugars

Available data do not allow the setting of a Tolerable Upper Intake Level for total or added sugars, neither an Adequate Intake nor a Reference Intake range.

Dietary fibre

The role of dietary fibre in bowel function was considered the most suitable criterion for establishing an adequate intake. Based on the available evidence on bowel function, the Panel considers dietary fibre intakes of 25 g per day to be adequate for normal laxation in adults. There is limited evidence to set adequate intakes for children. The Panel considers that the AI for dietary fibre for children should be based on that for adults with appropriate adjustment for energy intake. A fibre intake of 2 g per MJ is considered adequate for normal laxation in children from the age of one year.

The Panel notes that there is evidence in adults of benefit to health associated with consumption of diets rich in fibre-containing foods at dietary fibre intakes greater than 25 g per day, e.g reduced risk of coronary heart disease and type 2 diabetes and improved weight maintenance. Such evidence should be considered when developing food-based dietary guidelines.

Glycaemic index and glycaemic load

The evidence regarding the role of the “gylcaemic index” and the “glycaemic load” in prevention of diet-related diseases is still inconclusive.

Table 3. Summary of Dietary Reference Values for carbohydtares and dietary fibre.

Category	Adults	Children
Total carbohydrates, E% (RI)	45-60	45 to 60 (from 1 year of age)
Dietary Fibre, g/day (AI)	25	10 (from 1 to 3 years) 14 (from 4 to 6 years) 16 (from 7 to 10 years) 19 (from 11 to 14 years) 21 (from 15 to 17 years)

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ANNEXES

ANNEX 1 DEFINITIONS OF DIETARY FIBRE IN RECOMMENDATIONS OF FIBRE INTAKE

Source	Definition
Commission Directive 2008/100/EC[9]	For the purposes of this Directive "fibre" means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories: - edible carbohydrate polymers naturally occurring in the food as consumed; - edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence; - edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence."
SACN, 2008	Material that is resistant to digestion and absorption in the small intestine and that has a demonstrable physiological effect potentially associated with health benefits in the body, such as increasing stool bulk, decreasing intestinal transit time or decreasing post prandial glycaemia. This includes NSP and soluble fibre. Inclusion of other components in the definition would require physiological effects to be demonstrated.
FAO/WHO (Mann et al., 2007)	Intrinsic plant cell wall polysaccharides, NSP. Dietary fibre should reflect the health benefits of a diet rich in fruits, vegetables and whole grains.
GR, 2006	Carbohydrates, compounds analogous to carbohydrates, and lignin and related substances that are not digested or absorbed in the human small intestine. These include: Polysaccharides other than starch, and indigestible oligosaccharides: e.g. cellulose, hemicelluloses such as arabinoxylans, arabinogalactans and xyloglucans, pectin, fructans and some oligosaccharides (inulin, fructo-oligosaccharides, oligofructose), galacto-oligosaccharides and xylo-oligosaccharides, gums and mucilages (for some population groups: lactose) Compounds analogous to carbohydrates: indigestible dextrins (mainly from potatoes and maize), synthetic carbohydrates and their derivatives, polydextrose, methylcellulose, hydroxypropyl methylcellulose, etc. ndigestible starch. Lignin. Substances that occur in products containing lignin or polysaccharides other than starch: wax, cutin, saponins, suberins, tannins.
IoM 2005	Dietary fibre: non-digestible carbohydrates and lignin that are intrinsic and intact in plants, e.g. cellulose, pectin, gums, hemicellulose, b-glucans, and fibres contained in oat and wheat bran, plant carbohydrates that are not recovered by alcohol precipitation (e.g. inulin, oligosaccharides, and fructans), lignin, and some resistant starch. Excluded are non-digestible mono- and disaccharides and polyols, some resistant starch, non-digestible animal carbohydrates. Functional fibre: isolated, non-digestible carbohydrate components that have beneficial physiological effects in humans. May be isolated or extracted using chemical, enzymatic, or aqueous steps. Synthetically manufactured (DP $\geq$3) or naturally occurring isolated oligosaccharides and manufactured resistant starch are included. Naturally occurring polysaccharides or oligosaccharides usually extracted from their plant source that have been modified (e.g. to a shorter polymer length or to a different molecular arrangement) and animal derived non-digestible carbohydrates are included. Excluded are non-digestible mono-

[9] Commission Directive 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions. OJ L 285, 29.10.2008, pp. 9-12

Source	Definition
	and disaccharides and polyols, some resistant starch, non-digestible animal carbohydrates. Total dietary fibre: sum of Dietary and Functional fibre.
AFSSA, 2001	Dietary constituents not digested by the enzymes of the gastrointestinal tract, mainly plant-derived constituents, e.g. cellulose, hemicellulose, lignin, gums, alginates, carragenans, resistant starch. Minor dietary components such as oligosaccharides (fructo-oligosaccharides, a-galactosides, lactulose), constituents produced by micro-organisms (xanthanes), constituents of crustaceans (e.g. chitine, chitosane) are also included.
D-A-CH, 2008	Dietary fibre comprises those components of vegetable food which are not degraded by physiological enzymes of the human gastrointestinal tract. Dietary fibre, except for lignin, stands for indigestible carbohydrates such as cellulose, hemicellulose, pectin etc. Resistant starch and indigestible oligosaccharides such as oligofructose and oligosaccharides of the raffinose family (raffinose, stachyose, verbascose in pulses) are included.
NNR, 2004	Dietary fibre recommendation refers to dietary fibre naturally occurring in plant foods as measured by AOAC methods for total dietary fibre.
Codex proposal, Alinorm 09/32/26, 2009	Dietary fibre means carbohydrate polymers[1] with ten or more monomeric units[2], which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories: : Edible carbohydrate polymers naturally occurring in the food as consumed, carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities, synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities Properties: Dietary fibre generally has properties such as: • Decrease intestinal transit time and increase stools bulk • fermentable by colonic microflora • Reduce blood total and/or LDL cholesterol levels • Reduce post-prandial blood glucose and /or insulin levels. [1] When derived from a plant origin, dietary fibre may include fractions of lignin and/or other compounds when associated with polysaccharides in the plant cell walls and if these compounds are quantified by the AOAC gravimetric analytical method for dietary fibre analysis : Fractions of lignin and the other compounds (proteic fractions, phenolic compounds, waxes, saponins, phytates, cutin, phytosterols, etc.) intimately "associated" with plant polysaccharides are often extracted with the polysaccharides in the AOAC 991.43 method. These substances are included in the definition of fibre insofar as they are actually associated with the poly- or oligo-saccharidic fraction of fibre. However, when extracted or even re-introduced into a food containing non digestible polysaccharides, they cannot be defined as dietary fibre. When combined with polysaccharides, these associated substances may provide additional beneficial effects (pending adoption of Section on Methods of Analysis and Sampling). [2] Decision on whether to include carbohydrates from 3 to 9 monomeric units should be left to national authorities.
DoH,1991	NSP (non alpha-glucan polysaccharides): cellulose, non-cellulose polysaccharides (pectins, glucans, arabinogalactans, arabinoxylans, gums, mucilages, inulin, guar, chitin.

ANNEX 2A POPULATION, METHODS AND PERIOD OF DIETARY ASSESSMENT IN CHILDREN AND ADOLESCENTS IN EUROPEAN COUNTRIES

Country	Population	Dietary method	Year of survey	Reference
AT	Boys and girls aged 7-9 years	3-day record	2007	Elmadfa et al., 2009
	Boys and girls aged 10-14 years	3-day record	2007	Elmadfa et al., 2009
	Boys and girls aged 14-19 years	24-hour recall	2003-2004	Elmadfa et al., 2009
BE	Boys and girls aged 2.5-3 years	3-day record	2002-2003	Huybrechts and DeHenauw, 2007
	Boys and girls aged 4-6.5 years	3-day record	2002-2003	Huybrechts and DeHenauw, 2007
	Boys and girls aged 13-15 years	7-day record	1997	Matthys et al., 2003
	Boys and girls aged 15-18	2x 24-hour recall	2004	De Vriese et al., 2006
CZ	Boys and girls aged 4-6 years	2x 24-hour recall	n.a.[1]	Tláskas, Hrstková.(unpublished data) (In: Elmadfa, 2009)
	Boys and girls aged 7-9 years	2x 24-hour recall	n.a.	Tláskas, Hrstková. (unpublished data) (In:Elmadfa, 2009)
DE	Infants aged 12 months	3-day record	1989-2003	Hilbig and Kersting, 2006
	Children aged 18 months	3-day record	1989-2003	Hilbig and Kersting, 2006
	Children aged 2 years	3-day record	1989-2003	Hilbig and Kersting, 2006
	Children aged 3 years	3-day record	1989-2003	Hilbig and Kersting, 2006
	Boys and girls aged 6 years	3-day record	2006	Mensink et al., 2007
	Boys and girls aged 7-9 years	3-day record	2006	Mensink et al., 2007
	Boys and girls aged 10-11 years	3-day record	2006	Mensink et al., 2007
	Boys and girls aged 12 years	Dietary history (over the last 4 weeks)	2006	Mensink et al.,2007
	Boys and girls aged 13-14 years	Dietary history (over the last 4 weeks)	2006	Mensink et al.,2007
	Boys and girls aged 15-17 years	Dietary history (over the last 4 weeks)	2006	Mensink et al.,2007
DK	Boys and girls aged 1-3 years	7-day record	1995	Andersen et al., 1996
	Boys and girls aged 4-5 years	7-day record	2000-2002	Lyhne et al., 2005
	Boys and girls aged 6-9 years	7-day record	2000-2002	Lyhne et al., 2005
	Boys and girls aged 10-13 years	7-day record	2000-2002	Lyhne et al., 2005
	Boys and girls aged 14-17 years	7-day record	2000-2002	Lyhne et al., 2005
FI	Infants aged 8 months	3-day record	1999	Lagstrom, 1999
	Children aged 3 years	4-day record	1999	Lagstrom, 1999
	Children aged 4 years	4 day record	1999	Lagstrom, 1999
	Children aged 4 years	3-day record	2008	Kyttälä et al., 2008
	Children aged 6 years	3-day record	2008	Kyttälä et al., 2008
FR	Boys and girls aged 4-6 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009 (In: Elmadfa, 2009)

	Boys and girls aged 7-9 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009 (In: Elmadfa, 2009)
	Boys and girls aged 10-14 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009 (In: Elmadfa, 2009)
	Boys and girls aged 15-18 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009 (In: Elmadfa, 2009)
GR	Boys and girls aged 4-5 years	3-day record+24-hour recall / 3-day record	2003-2004	Manios et al., 2008
HU	Boys and girls aged 11-14 years	3x 24-hour recall	2005-2006	Biro et al.2007 (In: Elmadfa, 2009)
IE	Boys and girls 5-8 years	7-day record	2003-2004	Irish Universities Nutrition Alliance, National Irish Children's Food Survey. www.iuna.net
	Boys and girls 9-12 years	7-day record	2003-2004	Irish Universities Nutrition, Alliance National Irish Children's Food Survey. www.iuna.net
IT	Boys and girls 4-6 years	7-day record	n.a	D'Amicis, 2000
	Boys and girls 7-9 years	7-day record	n.a	D'Amicis, 2000
	Boys and girls 10-14 years	7-day record	n.a	D'Amicis, 2000
	Boys and girls 15-18 years	7-day record	n.a	D'Amicis, 2000
NL	Infants aged 9 month	2-day record (independent days)	2002	Boer et al., 2006
	Infants aged 12 monts	2-day record (independent days)	2002	Boer et al., 2006
	Children aged 18 months	2-day record (independent days)	2002	Boer et al., 2006
	Boys and girls aged 2-3 years	2-day record (independent days)	2005-2006	Ocke et al., 2008
	Boys and girls aged 4-6 years	2-day record (independent days)	2005-2006	Ocke et al., 2008
	Boys and girls aged 7-9 years	2-day record	1997-1998	Hulshof et al., 1998
	Boys and girls aged 10-12 years	2-day record	1997-1998	Hulshof et al., 1998
	Boys and girls aged 13-15 years	2-day record	1997-1998	Hulshof et al., 1998
	Boys and girls aged 16-19 years	2-day record	1997-1998	Hulshof et al., 1998
NO	Children aged 2 years	Food Frequency Questionnaire	1998-1999	Lande and Andersen, 2005
	Boys and girls aged 4 years	4-day record	2000	Øverby and Andersen, 2002
	Boys and girls aged 9 years	4-day record	2000	Øverby and Andersen, 2002
	Boys and girls aged 13	4-day record	2000	Øverby and Andersen, 2002
	Boys and girls aged 16-19 years	Food Frequency Questionnaire	1997	Johansson and Solvoll, 1999
PL	Boys and girls aged 4-6 years	24-hour recall	2000	Szponar et al, 2000 (unpublished data) (In: Elmadfa, 2009)
	Boys and girls aged 7-9 years	24-hour recall	2000	Szponar et al, 2000 (unpublished data) (In: Elmadfa, 2009)
	Boys and girls aged 10-14 years	24-hour recall	2000	Szponar et al, 2000 (unpublished data) (In: Elmadfa, 2009)
	Boys and girls aged 15-18 years	24-hour recall	2000	Szponar et al, 2000 (unpublished data) (In: Elmadfa, 2009)
PT	Boys and girls aged 7-9 years	24-hour recall	2000-2002	Moreira et al., 2005
	Boys and girls aged 13 years	24-hour recall	2000-2002	Moreira et al., 2005

SI	Boys and girls aged 14-17 years	Food Frequency Questionnaire	n.a.	Fidler Mis et al. (unpublished data) (In: Elmadfa, 2009)
ES	Boys and girls aged 10-14 years	2x 24-hour recall	2002-2003	Serra Majem and Ribas, 2007; Serra Majem et al., 2006 and 2007 (In: Elmadfa, 2009)
	Boys and girls aged 15-18 years	2x 24-hour recall	2002-2003	Serra Majem and Ribas, 2007; Serra Majem et al., 2006 and 2007 (In: Elmadfa, 2009)
SE	Boys and girls aged 4 years	4-day record	2003	Enghardt-Barbieri et al., 2006
	Boys and girls aged 8-9 years	4-day record	2003	Enghardt-Barbieri et al., 2006
	Boys and girls aged 11-12 years	4-day record	2003	Enghardt-Barbieri et al., 2006
UK	Boys and girls aged 4-6 years	7-day record	1997	Gregory et al., 2000
	Boys and girls aged 7-10 years	7-day record	1997	Gregory et al., 2000
	Boys and girls aged 11-14 years	7-day record	1997	Gregory et al., 2000
	Boys and girls aged 15-18 years	7-day record	1997	Gregory et al., 2000

[1]n.a. = not available

ANNEX 2B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG CHILDREN AGED ~1-3 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Infants and Toddlers																				
DE	12 mo	432	52.2	6.0																
	18 mo	478	49.8	6.8																
	2	458	49.4	6.6																
	3	427	50.3	6.3																
FI	8 mo	215	58.0	11.0								3.0	2.0		6.7	2.9				
	13 mo	449	54.0	10.0								5.0	3.0		9.0	2.9				
	2	398	50.0	11.0								10.0	4.0		9.7	2.8				
	3	359	51.0	10.0								11.0	5.0		10.9	3.2				
NL	9 mo	333	58.0	4.2	52.6-63.3	36.3	5.5	29.5-43.4										2.4	0.7	1.6-3.2
	12 mo	306	57.4	4.1	52.1-62,7	35.7	4.9	28.6-41.0										2.5	0.5	1.8-3.2
	18 mo	302	57.5	3.9	52.5-62.6	36.3	5.2	29.8-43.0										2.5	0.5	2.9-3.2
NO	2	172	53.3	5.6								11.7	5.8		13.6	5.2				
Pre-school children																				
Males																				
BE	2.5-3	102	54.2	5.2		31.6	5.2		22.6	3.4					14.6	3.4				
DK	1-3	129	51.0									11.0			15.0					
NL	2-3	313	58.0		49-66										13.0		8-19	2.3		1.5-3.2
Females																				
BE	2.5-3	95	52.9	5.4		29.7	5.4		23.0	4.1					13.0	2.8				
DK	1-3	149	50.0									11.0			14.0					
NL	2-3	313	58.0		51-65										12.0		8-17	2.3		1.5-3.1

[1]SE; [2] P2.5-P97.5

ANNEX 2B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG CHILDREN AGED ~4-6 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
BE	4-6.5	236	54.2	4.5		31.4	5.2		22.7	3.3					14.6	3.3				
CZ	4-6	641	56.0	5.8																
DE	6	106	53.3	6.2	41.8-63.6										15.7	4.1	9.9-24.1			
DK	4-5	82	50.0	4.1	43.0-56.0							12.0	4.1	6.0-20.0	17	4.6	10.0-24.0	2.2	0.5	1.5-3.1
FI	4	307	53.0									13.7			9.6	3.2				
	6	364	53.0									13.0			11.4	3.7				
FR	4-6	164	49.8	0.8[1]											11.8	0.5[1]				
GR	4-5	356	44.8	6.5																
IT	4-6	21	50.0	5.5											14.9	4.7				
NL	4-6	327	56.8	0.9	48.0-64.0							13.0	2.0		14	3	9.0-20.0	2.2		1.5-3.0
NO	4	206	53.0	5.0								15.0	5.0		12.0	5.0				
PL	4-6	82	57.0	7.9								18.5	5.8		16.8	5.8				
SE	4	302	54.2	4.8	46.5-62.4	28.6						13.8	4.6	7.0-21.6	12.0	3.0	7.0-17.0	1.8	0.5	1.1-2.7
UK	4-6	184	51.6	4.3	43.0-59.4															
Females																				
BE	4-6.5	228	54.9	4.1		31.3	5.1		23.4	3.4					13.9	3.2				
CZ	4-6	446	56.0	5.8											15.3	4.7				
DE	6	102	53.3	5.2	41.8-63.6										15.8	4.7	8.6-24.7			
DK	4-5	116	50.0	4.1	43.0-56.0							12.0	3.9	7.0-20.0	16	4.5	9.0-22.0	2.2	0.5	1.5-3.0
FI	4	307	53.0									13.6			9.4	3.0				
	6	349	53.0									13.8			10.3	3.3				
FR	4-6	162	48.6	0.5[1]											11.5	0.3[1]				
GR	4-5	389	45.2	6.4																
IT	4-6	17	50.3	4.9											15.8	3.9				
NL	4-6	312	57.0	4.0	50.0-64.0										13	3	8.0-17.0	2.0		1.4-2.8
NO	4	185	54.0	6.0								16.0	6.0		12.0	6.0				
PL	4-6	84	55.6	7.5								17.9	6.0		14.6	5.9				
SE	4	288	53.4	5.1	45.4-61.6	28.4						13.7	4.5	6.9-21.2	11.0	3.0	7.0-17.0	1.8	0.4	1.2-2.6
UK	4-6	171	51.4	5.0	42.1-60.5[2]															

[1]SE; [2] P2.5-P97.5

ANNEX 2B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG CHILDREN AGED ~7-9 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
AT	7-9	146	51.4	6.8								16.6	7.1		15.0	5.9				
CZ	7-9	940	53.4	6.7																
DE	7-9	321	53.2	6.1	43.6-63.1										17.5	5.3	10.6-26.2			
DK	6-9	174	51.0	3.9	44.0-57.0							13.0	5.2	6.0-22.0	18.0	5.8	11.0-28.0	2.0	0.5	1.3-2.9
FR	7-9	160	49.3	0.5[1]											13.5	0.4[1]				
IE	5-8	145	52.4	4.8	44.5-60.2															
IT	7-9	29													18.5					
NL	7-9	104	52.7	6.6	38.0-63.6	29.6	7.0	18.0-40.5	23.0	4.0	17.0-30.0				17.0	6.0	9.0-25.0	2.0	0.6	1.2-3.0
NO	9	402	54.0	6.0								16.0	6.0		16.0	7.0				
PL	7-9	101	56.3	7.9								17.3	5.6		19.6	6.8				
PT	7-9	1541	48.6	7.8								22.5	7.1		20.2	8.1				
SE	8-9	444	53.0	4.8	45.3-60.5	25.7						12.5	4.3	5.7-20.2	14.0	4.0	8.0-22.0	1.7	0.4	1.2-2.4
UK	7-10	256	52.4	4.1	44.2-60.5															
Females																				
AT	7-9	134	52.2	7.0								18.0	6.9		14.3	4.4				
CZ	7-9	765	53.4	6.7																
DE	7-9	308	54.2	6.7	43.1-66.1										16.8	5.4	10.0-26.2			
DK	6-9	157	51.0	4.3								13.0	4.7	7.0-21.0	17.0	4.5	10.0-25.0	2.1	0.5	1.4-2.9
FR	7-9	14	48.5	0.7[1]											12.2	0.4[1]				
IE	5-8	151	51.5	4.6	43.4-59.6															
IT	7-9	21													15.2	5.0				
NL	7-9	134	52.0	7.3	39.6-63.7	29.5	7.2	18.4-41.5	22.4	4.3	15.7-30.2				15.0	5.0	7.0-23.0	1.9	0.5	1.2-2.8
NO	9	408	55.0	6.0								18.0	6.0		14.0	6.0				
PL	7-9	103	55.5	7.7								16.4	6.2		17.4	6.7				
PT	7-9	1503	48.3	7.9								21.8	7.1		19.4	8.2				
SE	8-9	445	53.3	4.9	44.7-61.1	25.5						12.6	4.2	6.1-19.4	13.0	4.0	8.0-19.0	1.8	0.4	1.2-2.6
UK	7-10	226	51.3	4.3	42.6-59.9[2]															

[1]SE; [2] P2.5-97.5

ANNEX 2B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG CHILDREN AGED ~10-14 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
AT	10-14	248	50.8	7.5								17.6	8.2		15.1	6.1				
BE	13-15	74	49.1	4.6		24.3	4.9		24.8	4.4		19.0	5.9					1.8	0.5	
DE	10-11	199	53.2	6.4	43.0-64.5										17.9	6.0	9.0-28.8			
	12	114	52.0	5.4	42.1-60.9										25.3	9.4	12.6-46.1			
	13-14	214	51.7	6.2	43.1-62.5										27.7	12.2	11.8-50.1			
DK	10-13	145	52.0	4.7	44.0-60.0							14.0	5.7	7.0-25.0	18.0	6.3	9.0-28.0	1.9	0.4	1.3-2.6
FR	10-14	160	48.1	0.4[1]											15.2	0.4[1]				
HU	11-14	124	50.3	5.6								11.9	5.6		20.8	5.9				
IE	9-12	148	52.5	5.3	44.6-61.1															
IT	10-14														21.6	7.6				
NL	10-12	112	51.5	6.4	40.5-61.2	27.4	7.2	18.2-35.4	24.0	4.8	16.8-30.5				19.0	6.0	10.0-29.0	2.1	0.6	1.1-3.1
	13-15	137	51.2	5.8	41.8-60.9	27.0	6.2	16.8-37.0	24.1	3.7	17.9-30.1				22.0	7.0	11.0-34.0	2.0	0.5	1.0-3.0
NO	13	590	55.0	7.0								18.0	8.0		16.0	8.0				
PL	10-14	202	53.9	8.0								15.2	5.8		24.6	10.0				
PT	13	987	52.3	5.7								24.2	6.0		25.4	10.3				
SE	11-12	517	52.4	5.6	43.0-61.8	23.4						11.7	5.0	3.9-19.9	13.0	4.0	7.0-21.0	1.7	0.4	1.1-2.5
ES	10-14	66	41.0	4.2								16.1	3.3		18.5	1.6				
UK	11-14	237	51.7	4.6	42.5-59.8															
Females																				
AT	10-14	239	52.1	8.0								16.8	7.3		13.7	4.3				
BE	13-15	89	49.1	5.4		24.3	5.0		24.8	4.6		15.8	5.2					2.0	0.6	
DE	10-11	198	53.1	7.2	43.6-66.4										17.7	5.5	9.3-27.2			
	12	103	52.8	6.6	42.2-65.4										25.0	10.9	11.0-46.5			
	13-14	230	52.7	6.1	44.0-63.3										24.4	8.8	12.6-38.9			
DK	10-13	131	52.0	4.7								14.0	5.0	7.0-23.0	15.0	5.0	8.0-26.0	1.9	0.5	1.3-2.7
FR	10-14	144	48.0	0.4[1]											13.8	0.3[1]				
HU	11-14	111	51.7	5.4								12.5	5.9		20.1	6.7				
IE	9-12	148	52.5	5.3	44.6-61.1															
IT	10-14	47													16.8	3.9				
NL	10-12	124	52.1	6.2	41.2-61.7	28.5	6.2	18.2-38.2	23.6	4.2	16.9-30.1				17.0	6.0	9.0-28.0	2.0	0.6	1.2-3.0
	13-15	117	50.3	6.5	40.2-62.6	26.4	7.2	12.3-37.8	23.8	4.6	17.5-32.7				18.0	6.0	9.0-30.0	2.1	0.7	1.1-3.3
NO	13	515	55.0	6.0								19.0	7.0		14.0	7.0				
PL	10-14	202	54.0	7.7								15.5	6.0		20.9	8.7				

PT	13	1053	52.6	6.2					25.4	7.0		25.2	10.6						
SE	11-12	499	53.2	5.5	44.2-62.3	24.7			12.9	5.0	5.5-22.2	12.0	4.0	6-19		1.8	0.4	1.2-2.5	
ES	10-14	53	41.6	3.3					16.0	2.5		17.5	1.1						
UK	11-14	238	51.2	5.2	42.2-62.8[2]														

[1]SE; [2] P2.5-97.5

ANNEX 2B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG CHILDREN AGED ~15-18 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates E%			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
AT	14->19	1527	46.1	9.9								16.1	8.9		15.6	7.1				
BE	15-18	405	49.5	4.4																
DE	15-17	294	49.6	6.5											26.1	10.7				
DK	14-17	86	50.0	6.1	41-59							13.0	7.2	2-27	19.0	6.0	9-28	1.8	0.5	1.1-2.6
FR	15-18	181	48.7	0.6[1]											16.9	0.8[1]				
IT	15-18	52													23.9	9.1				
NL	16-18	142	49.5	6.6	38.7-59.9	24.9	7.4	14.5-35.7	24.5	4.8	16.5-32.6				24.0	11.0	8-42	2.1	0.7	1.0-3.3
NO	16-19	92	53.7									15.1			26.0					
PL	15-18	174	50.7	7.4								12.5	5.0		32.6	12.5				
SI	15-18	1010	57.0	9.0								11.0	4.0		33.0	21.0				
ES	15-18	61	39.7	4.7								14.8	3.4		18.9	1.8				
UK	15-18	179	50.5	5.4	39.9-60.5															
Females																				
AT	14->19	1422	47.3	10.3								15.7	8.7		13.8	6.0				
BE	15-18	401	50.6	5.4																
DE	15-17	317	52.7	6.4											23.1	8.3				
DK	14-17	117	52.0	5.6	45-63							14.0	6.1	5-23	15.0	5.2	8-24	1.9	0.5	1.2-2.6
FR	15-18	222	48.8	0.7[1]											12.7	0.3[1]				
IT	15-18	47													17.6	4.7				
NL	16-18	129	50.3		37.6-61.1	26.2		12.8-37.5	24.0		16.2-32.9				19.0		8-20	2.1		1.1-3.6
NO	16-19	62	54.6									11.7			21.0	8.0				
PL	15-18	175	54.2	8.5								14.1	5.7		23.0	8.9				
SI	15-18	1214	57.0	8.0								13.0	4.0		27.0	18.0				
ES	15-18	57	38.6	3.7								15.4	2.9		16.2	2.0				
UK	15-18	210	50.6	5.6	39.9-64.0[2]															

[1]SE; [2] P2.5-97.5

ANNEX 3A POPULATION, METHODS AND PERIOD OF DIETARY ASSESSMENT IN ADULTS IN EUROPEAN COUNTRIES.

Country	Population	Dietary method	Year of survey	Reference
AT	Males and females aged 19-64 years	24-hour recall	2007	Elmadfa et al., 2009
	Males and females aged 65 and over	3-day record	2007	Elmadfa et al., 2009
BE	Males and females aged 19-59 years	2x 24-hour recall	2004	De Vriese et al, 2006
	Males and females aged 60-75 years	2x 24-hour recall	2004	De Vriese et al, 2006
	Males and females aged 75+ years	2x 24-hour recall	2004	De Vriese et al, 2006
CZ	Males and females aged 19-64 years	n.a.	n.a.[1]	Cifkova and Skodova, 2004
DE	Males and females aged 35-64 years	24-hour recall	1996-1998	Linseisen et al., 2003
	Males and females aged 19-64 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and females aged 19-24 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and females aged 25-34 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and females aged 35-50 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and females aged 51-64 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and females aged 65-80 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and Females aged 65 and over	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
DK	Males and females aged 18-74 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 18-24 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 25-34 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 35-44 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 45-54 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 55-64 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 65-74 years	7-day record	2000-2002	Lyhne et al., 2005
EE	Males and females aged 19-65 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 19-34 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 35-49 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 50 and over	24-hour recall	1997	Pomerleau et al., 2001
FI	Males and females aged 25-64 years	3-day record	2002	Paturi et al., 2008
	Males and females aged 65-74 years	4-day record	2002	Paturi et al., 2008
FR	Males and females aged 19-64 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009. (In: Elmadfa, 2009)
	Males and females aged 65-75 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009. (In: Elmadfa, 2009)

GR	Males and females aged 19-64 years	FFQ + 24-hour recall in sub group	1994-1999	Greek cohort EPIC study. (In: Elmadfa, 2009)
	Males and females aged 65 and over	FFQ	1994-1999	Greek cohort EPIC study. (In: Elmadfa, 2009)
HU	Males and females aged 11-14 years	3-day record	2003-2004	Rodler et al. 2005; Zajkás et al., 2007; Bíró et al., 2007 (In: Elmadfa, 2009)
	Males and females aged 18-59	3-day record	2003-2004	Rodler et al. 2005; Zajkás et al., 2007; Bíró et al., 2007 (In: Elmadfa, 2009)
	Males and females aged 60 and over	3-day record	2003-2004	Rodler et al. 2005; Zajkás et al., 2007; Bíró et al., 2007 (In: Elmadfa, 2009)
IE	Males and females 18-64 years	7-day record	1997-1999	Irish Universities Nutrition Alliance, North/South Ireland Food Consumption Survey. www.iuna.net
	Males and females 18-35 years	7-day record	1997-1999	Irish Universities Nutrition Alliance, North/South Ireland Food Consumption Survey. www.iuna.net
	Males and females 36-50 years	7-day record	1997-1999	Irish Universities Nutrition Alliance ,North/South Ireland Food Consumption Survey. www.iuna.net
	Males and females 51-64 years	7-day record	1997-1999	Irish Universities Nutrition, Alliance North/South Ireland Food Consumption Survey. www.iuna.net
IT	Males and females 19-64 years	7-day record	n.a.	D'Amicis, 2000
	Males and females aged 65 and over	7-day record	n.a.	D'Amicis, 2000
LT	Males and females 19-64 years	24-hour recall	2007	Unpublished data (In: Elmadfa, 2009)
LV	Males and females 19-64 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 19-34 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 35-49 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 50 and over	24-hour recall	1997	Pomerleau et al., 2001
NL	Males and Females aged 19-64 years	2-day record	1997-1998	Hulshof et al., 1998
	Males and Females aged 65 and over	2-day record	1997-1998	Hulshof et al., 1998
	Males and females aged 19-30 years	2x 24-hour recall	2003	Hulshof and Ocké, 2005
NO	Males and females aged 19-64 years	FFQ	1997	Johansson and Sovoll, 1999
	Males and females aged 65 and over	FFQ	1997	Johansson and Sovoll, 1999
PL	Males and females aged 19-64 years	24-hour recall	2000	Szponar et al., 2000 unpublished data (In: Elmadfa, 2009)
	Males and females aged 65 and over	24-hour recall	2000	Szponar et al., 2000 unpublished data (In: Elmadfa, 2009)
PT	Males and females aged 18+ years	FFQ	n.a.	EPIPorto study (In: Elmadfa, 2009)
	Males and females aged 65 and over	FFQ	n.a.	EPIPorto study (In: Elmadfa, 2009)

RO	Males and females aged 19-64 years	personal interview	2006	National Synthesis 2006 (In: Elmadfa, 2009)
	Males and females aged 65 and over	personal interview	2006	National Synthesis 2006 (In: Elmadfa, 2009)
ES	Males and females aged 18-64 years	24-hour recall	2002-2003	Serra Majem et al., 2007
	Males and females aged 65-75 years	24-hour recall	2002-2003	Serra Majem et al., 2007
SE	Males and females aged 18-74 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 17-24 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 25-34 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 35-44 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 45-54 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 55-64 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 65 and over	7-day record	1997-1998	Becker and Pearson, 2002
UK	Males and females aged 19-64 years	7-day record	2000-2001	Henderson et al., 2003
	Males and females aged 19-24 years	7-day record	2000-2001	Henderson et al., 2003
	Males and females aged 25-34 years	7-day record	2000-2001	Henderson et al., 2003
	Males and females aged 35-49 years	7-day record	2000-2001	Henderson et al., 2003
	Males and females aged 50-64 years	7-day record	2000-2001	Henderson et al., 2003
	Males and females aged 65+ years	4-day record	1994-1995	Finch et al., 1998

[1]n.a. = not available

ANNEX 3B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG ADULTS AGED ~19-65 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
AT	19-64	778	42.5	10.5								8.7	6.4		19.5	9.9				
BE	19-59	413	45.2	5.3		24.1	4.8		19.4	5.7										
CZ	19-64	1094	53.9	12.0																
DE	19-64	4912	45.3	8.4																
DK	18-74	1467	43	6.8	35.0-53.0[1]							8.0		3-15[1]	21.0		12.0-32.0[1]			
EE	19-64	900	42.7	14.0																
FI	25-64	730	47.1	8.8								9.7	5.9		24.0	11.0		2.7	1.1	
FR	19-64	852	43.4	0.3[2]											18.7	0.4[2]				
GR	19-64	8365	37.9	5.9																
HU	>18	473	45.0	6.6								7.6	5.2		24.2	6.6				
IE	18-64	662	43.5	6.4	32.6-54.3										23.2	8.5	12.1-38.9			
IT	19-64	660													21.8	6.5				
LT	19-65	849	38.9	9.3								10.8	5.6		20.9	12.4				
LV	19-65	1065	42.2	11.8																
NL	19-64	1836	44.2	7.5																
NO	19-64	1050	51.0	6.0								9.0	6.0		25.0	10.0				
PL	19-64	1106	48.0	8.4								11.0	5.2		29.7	11.4				
PT	19-64	917	47.0	6.8											23.5	9.0				
RO	19-64	177	43.3	9.4																
SE	18-74	589	46	6.0	37.0-56.0							9.0	4.0	3-16	18.0	7.0	9.0-29.0	1.8	0.5	1.1-2.7
ES	18-64	718	40.5			16.6			23.2						19.2					
UK	19-64	833	47.7	6.0	35.9-59.8[4]															
Females																				
AT	19-64	1345	46.0	10.6								10.9	6.6		20.1	9.3				
BE	19-59	460	46.9	6.2		21.4	6.3		24.3	4.1										
CZ	19-64	1094	53.9	12.0												9.0				
DE	19-64	6016	48.7	7.4																
DK	18-74	1684	47	6.6	39.0-55.0[1]							9.0		4-17[1]	19.0		11.0-28.0[1]			
EE	19-65	1115	47.3	12.6																
FI	25-64	846	50.2	8.3								10.5	5.1		21.0	9.0		3.2	1.3	
FR	19-64	1499	44.4	0.2[2]											15.7	0.2[2]				
GR	19-64	12034	39.5	5.4																
HU	>18	706	48.0	5.8								8.6	4.8		21.7	5.6				
IE	18-64	717	46.6	5.6	37.4-56.6										17.4	5.9	9.3-27.5			
IT	19-64	801													18.9	6.1				

LT	19-64	1087	42.9	10.3																
LV	19-64	1235	44.6	11.9																
NL	19-64	2112	44.7	7.9																
NO	19-64	1146	51.0	6.0							9.0	6.0			21.0	8.0				
PL	19-64	1334	51.8	9.1							13.7	6.6			19.7	7.9				
PT	19-64	1472	50.1	5.9											23.7	9.4				
RO	19-64	341	43.6	9.7																
SE	18-74	626	47	5.0		20.0					9.0	4.0	4-16		16.0	5.0	9-27		2.1	0.5	1.3-3.1
ES	18-64	895	40.7			18.5		21.0							16.9					
UK	19-64	891	48.5	6.7	37.4-61.5[3]															

[1]P10-P90; [2] SE; [3] P2.5-P97.5

ANNEX 3B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG ADULTS AGED ~19-34 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
DE	19-24	510	46.7	0.35[1]	34.8-60.4										24.6	0.55[1]	10.1-51.4			
	25-34	690	46.1	0.29[1]	34.4-59.4										25.8	0.44[1]	10.5-46.1			
DK	18-25	146	47.0	5.8	39.0-58.0							12	6.5	3-25	19.0	7.3	10.0-32.0	1.8	0.6	1.1-2.9
	25-34	272	46.0	5.4	37.0-55.0							11	5.7	3-20	20.0	6.9	11.0-32.0	1.9	0.5	1.2-2.8
EE	19-34	396	41.8	131																
IE	18-35	253	42.7	6.2	31.9-53.3										22.6	8.5	11.7-38.5			
LV	19-34	337	41.3	11.2																
NL	19-30	352	47.5	4.3	40.4-54.6	23.8	5.2	15.6-32.8							22.7	6.1	13.6-33.4	2.0	0.5	1.2-2.9
SE	17-24	67	49.0	5.0	40.0-57.0							11	5.0	4-21	16.0	7.0	6.0-29.0	1.6	0.4	1.0-2.5
	25-34	128	47.0	6.0	38.0-57.0							10	4.0	4-17	18.0	6.0	9.0-28.0	1.7	0.5	1.1-2.5
UK	19-24	108	49.0	6.3	38.0-63.2															
	25-34	219	47.7	5.8	35.2-58.3															
	Females																			
DE	19-24	510	51.3	0.34[1]											21.7	0.39[3]	8.7-37.1			
	25-34	972	50.0	0.23[1]											24.0	0.31	11.9-41.1			
DK	18-25	213	50.0	5.5	42.0-61.0							13	6.9	4-26	16.0	4.5	9.0-22.0	2.0	0.6	1.2-3.2
	25-34	315	49.0	5.4	40.0-57.0							12	5.8	4-23	17.0	4.5	10.0-25.0	2.1	0.7	1.2-3.3
EE	19-34	459	46.4	13.0																
IE	18-35	269	46.6	5.1	39.0-55.7										16.1	5.1	8.8-25.7			
LV	19-34	342	44.3	12.1																
NL	19-30	398	49.3	5.5	40.5-58.4	25.6	6.1	16.3-36.3							17.0	4.5	10.2-25.0	2.2	0.6	1.3-3.3
SE	17-24	70	50.0	6.0	39.0-61.0							11	4.0	5-18	15.0	5.0	7.0-24.0	1.9	0.5	1.2-2.9
	25-34	132	48.0	5.0	40.0-56.0							10	4.0	5-16	15.0	4.0	9.0-23.0	1.9	0.5	1.2-2.8
UK	19-24	104	49.1	8.3	36.5-62.7[2]															
	25-34	210	48.7	5.8	36.6-61.9[2]															

[1]SE; [2] P2.5-P97.5

ANNEX 3B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG ADULTS AGED ~35-64 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
DE	35-64[1]	1013	40.2	9.9		17.6			22.4			8.8	6.0		21.8	11.5				
	35-64[2]	1032	38.5	9.4		17.4			20.8			8.9	5.7		21.9	9.2				
	35-50	2079	45.1	0.17[3]	32.0-58.0										27.3	0.26[3]	11.9-48.2			
	51-64	1633	44.4	0.19[3]	32.4-57.2										27.4	0.28[3]	12.4-48.2			
DK	35-44	330	43.0	5.6	34.0-53.0							8.0	4.8	2-18	22.0	7.6	11-34	2.0	0.5	1.3-2.9
	45-54	312	42.0	6.6	31.0-53.0							7.0	4.4	1-15	22.0	9.0	10-39	2.2	0.6	1.3-3.4
	55-64	242	42.0	6.9	31.0-53.0							6.0	4.1	1-14	21.0	8.3	10-37	2.2	0.7	1.2-3.4
EE	35-49	319	43.6	15.5																
	50+	185	43.1	13.0																
IE	36-50	236	43.3	6.3	32.2-53.9										23.6	8.1	12.8-38.8			
	51-64	173	45.2	6.8	32.9-55.9										17.3	7.1	9.1-31.5			
LV	35-49	372	42.6	11.8																
	50+	356	43.2	12.4																
SE	35-44	143	45.0	5.0	38.0-54.0							8.0	4.0	3-14	18.0	7.0	9.0-30.0	1.8	0.4	1.2-2.5
	45-54	18	46.0	6.0	36.0-56.0							8.0	4.0	3-16	19.0	7.0	9.0-34.0	1.9	0.6	1.2-2.8
	55-64	68	47.0	5.0	39.0-55.0							7.0	4.0	3-14	18.0	5.0	12.0-29.0	2.0	0.4	1.5-2.8
UK	35-49	253	47.5	5.9	36.0-59.9[4]															
	50-64	253	47.4	6.2	35.6-59.5[4]															
Females																				
DE	35-64[1]	1078	43.0	10.2		21.5			21.1			10.9	6.6		19.6	9.0				
	35-64[2]	898	43.9	10.6		23.3			20.1			12.1	7.3		19.4	8.2				
	35-50	2694	48.0	0.14											24.7	0.19	11.5-42.9			
	51-64	1840	47.7	0.18											26.1	0.24	12.7-44.2			
EE	35-49	376	47.4	12.4																
	50+	280	48.5	12.3																
DK	35-44	359	47.0	5.9	38.0-57.0							9.0	5.4	3-18	15.0	5.0	8.0-26.0	2.3	0.7	1.4-3.5
	45-54	370	46.0	6.1	36.0-56.0							7.0	4.1	2-15	15.0	5.2	8.0-24.0	2.5	0.6	1.6-3.6
	55-64	263	46.0	6.5	35.0-56.0							8.0	4.1	2-14	16.0	5.6	9.0-27.0	2.6	0.7	1.6-3.9
IE	36-50	286	44.7	6.1	33.9-54.1										18.2	5.3	10.7-28.5			

	51-64	162	47.5	6.2	37.3-57.6						18.2	6.2	8.8-28.2				
LV	35-49	396	43.4	11.7													
	50+	496	45.7	12.0													
SE	35-44	132	46.0	6.0	37.0-54.0			9.0	3.0	4-14	16.0	4.0	9.0-22.0	2.0	0.5	1.4-2.9	
	45-54	153	47.0	5.0	39.0-55.0			8.0	3.0	4-14	17.0	5.0	10.0-27.0	2.2	0.5	1.4-3.3	
	55-64	81	48.0	5.0	40.0-57.0			8.0	4.0	4-13	18.0	5.0	10.0-28.0	2.4	0.5	1.7-3.5	
UK	35-49	318	48.6	6.8	35.3-62.4[4]												
	50-64	259	48.1	6.7	32.2-59.4[4]												

[1]Cohort Heidelberg; [2]Cohort Potsdam; [3]SE ; [4] P2.5-P97.5;

ANNEX 4 EFFECTS OF SUGAR INTAKE ON GLUCOSE AND INSULIN RESPONSE IN CONTROLLED INTERVENTION STUDIES IN ADULTS.

Study	Study design	Subjects	Total fat, E%	CHO, E%	Sugars, E%	Results
Reiser et al. 1979	6-week cross-over, iso-energetic	10 men, 9 women, 35-55 y	42	43	30 E% sucrose or wheat starch	Fasting serum glucose and insulin higher on sucrose diet
Reiser et al 1981	6-week cross-over, iso-energetic	24 adults with impaired insulin response	42	44	5, 18 or 33 E% sucrose or starch	Fasting and postprandial insulin higher on 18 or 33 E% sucrose diets
Swanson et al. 1992	4-week cross-over, iso-energetic	14 healthy adults	32-34	51-52	20 E% as fructose or starch (< 3 E% fructose)	No sign. differences between the diets in mean values of hemoglobin A1c, serum glycosylated albumin, fasting plasma glucose, peak postprandial plasma glucose, or integrated plasma glucose response.
Bantle et al., 2000	6-week cross-over, iso-energetic	12 men, 12 women (BMI <32)	30	55	17 E% fructose or glucose. Total sugars (glucose, fructose, sucrose and lactose) about 21 E% in both diets	Lower postprandial plasma glucose serum insulin, and daylong insulin concentrations on fructose diet
Black et al. 2006	6 wk cross-over, iso-energetic	13 W/M 33 y, BMI: 26.6	33	55	10 or 25 E% sucrose	No difference in glucose serum insulin or peripheral insulin resistance

ANNEX 5 EFFECTS OF SUGAR INTAKE ON SERUM LIPIDS IN CONTROLLED INTERVENTION STUDIES IN ADULTS

Study	Study design	Subjects	Total fat (E%)	CHO (E%)	Sugars (E%)	Results
Short-term						
Hallfrisch et al., 1983	5-week cross-over	12 men hyper-insulinemic 12 with normal insulin response			0, 7.5, and 15 E% fructose replacing starch	Total and LDL-chol +5-7% (sign) in both groups on 7.5 E% and 15 E% fructose diets. Fasting TG +30% and +61%(sign) on 7.5 and 15 E% fructose diets, respectively
Reiser et al. 1989	5-week cross-over	10 hyper-insulemic and 11 normal men	36	51	20 E% as either added fructose or high-amylose cornstarch	Fasting TG (+46%), total chol (+11%) in the hyperinsulinemics, and +20% and +8%, respectively, in normal men on the fructose diet. LDL-chol (+12%) in the normal men on the fructose diet
Swanson et al., 1992	4-week cross-over	14 healthy subjects	30	55	20 E% added fructose or starch	No difference in fasting serum TG. Total and LDL chol +9% and +11% (sign), respectively, on fructose diet
Bantle et al. 2000	6-week cross-over	12 men and 12 women BMI < 32			17 E% as fructose or glucose, Total sugars 21E%	Increased fasting and daylong TG in men, but not in women, on fructose diet. Total and LDL-chol higher on day 28, but not at the end of the study period on the fructose diet.
Marckmann et al. 2000	2 wk cross-over *ad libitum*.	20 W, 21-52 y BMI:	28-29	59	2.5 or 23 E% sucrose	Fasting and nonfasting TG, total and LDL-chol lower on the low-sucrose diet.
Black et al. 2006	6 wk cross-over, iso-energetic	13 W/M 33 y, BMI: 26.6			10 or 25 E% sucrose	Total and LDL chol +15% and +24%, respectively on high sucrose diet. HDL-chol and TG unchanged.
Erkkila et al. 2007	8 wk	34			Increased sucrose intake from 7-9 to 15 E% (40 g/day)	No significant effects on lipid concentrations
Long-term						
Saris et al. 2000	6-month parallel, *ad libitum*	316 obese			Control: 46 E% CHO (21 E% sugars) Intervention diets: 52–56 E% CHO, starch (16 E% sugars) or high-sugar (sucrose, fructose and lactose, 30 E%).	No significant changes were seen in serum lipids among the groups
Poppitt et al., 2002	6 months	46 obese subjects with the metabolic syndrome			3 diets, "complex carbohydrate", 21 or 29 E% sugars (as sucrose, fructose and lactose)	Fasting TG higher in 29 E% sugar group than in the "complex-carbohydrate" and 21 E% sugar diet. Weight loss correlated with a decrease in TG concentrations.

GLOSSARY / ABBREVIATIONS

Added sugars	Term used to describe sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing
AFSSA	Agence Française de Sécurité Sanitaire des Aliments
AICR	American Institute of Cancer Research
AI	Adequate Intake
AMDR	Acceptable Macronutrient Distribution Ranges
AOAC	Association of Official Analytical Chemists
BMI	Body Mass Index
CHD	Coronary Heart Disease
CV	Coefficient of Variation
DMFT	Decayed, Missing and Filled Teeth in the permanent teeth
dmft	Decayed, missing and filled teeth in the primary teeth
CVD	Cardiovascular Disease
DoH	Department of Health
DP	Degree of Polymerisation
DRVs	Dietary Reference Values
EC	European Commission
EFSA	European Food Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FFQ	Food Frequency Questionnaire
FNB	U.S. Food and Nutrition Board
FOS	Fructo-oligosaccharides
GI	Glycaemic Index

GL	Glycaemic Load
GLUT	Glucose Transporter
GOS	Galacto-oligosaccharides
HDL-cholesterol	High Density Lipoprotein-cholesterol
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
HOMA-ß	Homeostasis Model Assessment of ß-cell function
IGT	Impaired Glucose Tolerance
IoM	U.S. Institute of Medicine of the National Academy of Sciences
LDL-cholesterol	Low Density Lipoprotein-cholesterol
LTI	Lower Threshold Intake
Metabolic syndrome	Cluster of cardiovascular risk factors including clinical measures such as waist circumferences, blood pressure, triglycerides, high-density lipoproteins, blood glucose, and insulin sensitivity. Usually three criteria are needed for the diagnosis of Metabolic Syndrome
MI	Myocardial Infarction
MUFA	Monounsaturated Fatty Acids
NSP	Non-Starch Polysaccharides
NNR	Nordic Nutrition Recommendations
PRI	Population Reference Intake
QUICKI	Quantitative Insulin-Sensitivity Check Index
RI	Reference Intake ranges for macronutrients
SCF	Scientific Committee on Food
SCFA	Short-Chain Fatty Acids
SD	Standard Deviation
SFA	Saturated Fatty Acids
SGLT 1	Sodium Glucose Transporter 1
STRIP	Special Turku Coronary Risk Factor Intervention Project
Sugars	Term conventionally used to describe mono- and disaccharides
Sugar	Term used to describe sucrose
TG	Triglycerides

UL	Tolerable Upper Intake Level
US	United States
VLDL	Very low Density Lipoproteins
WCFR	World Cancer Research Fund
WHO	World Health Organization

STATEMENT OF EFSA

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Protocol for the scientific opinion on the Tolerable Upper Intake Level of dietary sugars

European Food Safety Authority (EFSA)

Abstract

In June 2016, EFSA received a mandate from the national food competent authorities of five European countries (Denmark, Finland, Iceland, Norway and Sweden) to provide a dietary reference value (DRV) for sugars, with particular attention to added sugars. A draft protocol was developed with the aim of defining as much as possible beforehand the strategy that will be applied for collecting data, appraising the relevant evidence, and analysing and integrating the evidence in order to draw conclusions that will form the basis for the Scientific Opinion on sugars. As EFSA wished to seek advice from stakeholders on this draft protocol, the NDA Panel endorsed it for public consultation on 12 December 2017. The consultation was open from 9 January to 4 March 2018. A technical meeting with stakeholders was held in Brussels on 13 February 2018, during the consultation period. After consultation with stakeholders and the mandate requestors, EFSA interprets this mandate as a request to provide scientific advice on an Tolerable Upper Intake Level (UL) for (total/added/free) sugars, i.e. the maximum level of total chronic daily intake of sugars (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans. The assessment concerns the main types of sugars (mono- and disaccharides) found in mixed diets (i.e. glucose, fructose, galactose, sucrose, lactose, maltose and trehalose) taken through the oral route. The health outcomes of interest relate to the development of metabolic diseases and dental caries. The final version of the protocol was endorsed by the EFSA Panel on Dietetic Products, Nutrition and Allergies on 28 June 2018.

Keywords: sugars, tolerable upper intake level, adverse effects, metabolic diseases, dental caries, protocol

Requestors: National Food Agency, Sweden; Finnish Food Safety Authority, EVIRA, Finland; National Food Institute (DTU), Denmark; Norwegian Scientific Committee for Food Safety, Norway; Icelandic Food and Veterinary Authority, Iceland

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1. Introduction and scope of the protocol

This document outlines the protocol for the Scientific Opinion on the Tolerable Upper Intake Level of dietary sugars of the EFSA Panel on Nutrition, Dietetic Products and Allergies (NDA Panel), supported by the ad-hoc Working Group (WG) on sugars. This draft protocol has been developed with the aim of defining as much as possible beforehand the strategy that will be applied for collecting data (i.e. which data to use for the assessment and how to identify and select them), appraising the relevant evidence, and analysing and integrating the evidence in order to draw conclusions that will form the basis for the Scientific Opinion.

The protocol has been developed following the principles and process illustrated in the EFSA PROMETHEUS project (PROmoting METHods for Evidence Use in Scientific assessments) (EFSA, 2015a).

A draft of this protocol was open for public consultation from 9 January to 4 March 2018. The public consultation included a technical meeting with stakeholders held in Brussels on 13 February 2018. The draft protocol has been amended in view of the comments received. All comments received have been addressed and published in a technical report (EFSA, 2018).

2. Background and rationale of the mandate

In June 2016, the national food competent authorities of five European countries (Denmark, Finland, Iceland, Norway and Sweden) sent a request to the European Food Safety Authority (EFSA) in order to provide a dietary reference value (DRV) for sugars, with particular attention to added sugars, on the basis of most recent scientific evidence. After discussing the mandate at its plenary meeting on 22–23 September 2016, the NDA Panel asked for some clarifications to the requestors, particularly regarding the type of DRV to be established, the exposure of interest, the target population and the health outcomes to be considered. In February 2017, the requestors clarified that they were interested in a science-based cut-off value for a daily exposure to added sugars from all sources (i.e. sucrose, fructose, glucose, starch hydrolysates such as glucose syrup, high-fructose syrup and other isolated sugar preparations used as such or added during food preparation and manufacturing) which is not associated with adverse health effects. The target population for the assessment was defined as the general healthy population, including children, adolescents, adults and the elderly. The requestors also clarified that the request relates to an update of the EFSA's Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre (EFSA NDA Panel, 2010a) in relation to the effects of added sugars on nutrient density, glucose tolerance and insulin sensitivity, serum lipids, other cardiovascular risk factors (blood pressure), body weight, type 2 diabetes and dental caries in adults and children.

In the EFSA's 2010 opinion, the term 'added sugars' referred to sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing.

With regard to the effects of added sugar intake, the NDA Panel reached the following conclusions on the outcomes assessed:

- Micronutrient density of the diet: observed negative associations between added sugars intake and micronutrient density of the diet are mainly related to patterns of intake of the foods from which added sugars in the diet are derived rather than to the intake of added sugars per se. The available data are not sufficient to set an upper limit for (added) sugars intake.
- Glucose and insulin response: there are limited, and mainly short-term, data on the effects of high intakes of sugars on glucose and insulin response. Most studies do not find any adverse effects at intakes of predominantly added sugars up to 20–25% of total energy (E%), provided that body weight is maintained.
- Serum lipids: although there is some evidence that high intakes (> 20 E%) of sugars may increase serum triglycerides and cholesterol concentrations, the available data are not sufficient to set an upper limit for (added) sugar intake.
- Body weight: the evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent for solid foods. However, there is some evidence that high intakes of sugars in the form of sugar-sweetened beverages (SSBs) might contribute to weight gain. The available evidence is insufficient to set an upper limit for sugars based on their effects on body weight.

- Type 2 diabetes: controversial findings on the association between total sugars and/or specific types of sugars and diabetes risk were reported in large prospective cohort studies. However positive associations were found between SSBs and increased type 2 diabetes risk. The available evidence was found insufficient to set a Tolerable Upper Level of Intake (UL) for sugars based on their effects on type 2 diabetes risk.
- Dental caries: available data do not allow the setting of a UL for (added) sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by oral hygiene, exposure to fluoride, frequency of consumption and various other factors.

The NDA Panel concluded that the available data did not allow the setting of a UL for total or added sugars, neither an Adequate Intake (AI) nor a Reference Intake range (RI). However evidence on the relationship between patterns of consumption of sugar-containing foods and dental caries, weight gain and micronutrient intake should be considered when establishing nutrient goals for populations and recommendations for individuals and when developing food-based dietary guidelines (FBDG).

3. Terms of reference as provided by the mandate requestor

The request is for scientific assistance in line with Regulation (EC) No 178/2002 in assessing a DRV for added sugars, which would benefit risk managers and substantially support their work with dietary guidelines and nutrient recommendations if they could base their advices on an up-to-date assessment by EFSA.

To this end, EFSA has been requested to update its Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre published in 2010 (EFSA NDA Panel, 2010a), on the basis of the most recent scientific evidence, in order to derive a science-based cut-off value for a daily exposure to added sugars which is not associated with adverse health effects.

The mandate requestor clarified that the intake of interest is added sugars from all sources, i.e. sucrose, fructose, glucose, starch hydrolysates such as glucose syrup, high-fructose syrup and other isolated sugar preparations used as such or added during food preparation and manufacturing. The health outcomes of interest are those already addressed in the EFSA 2010 opinion, i.e. micronutrient density of the diet, glucose tolerance and insulin sensitivity, serum lipids, other cardiovascular risk factors (blood pressure), body weight, type 2 diabetes and dental caries in adults and children.

To address this mandate, EFSA is requested to consider published reports from national and international bodies/authorities addressing the health effects of added sugars, as well as systematic reviews and meta-analysis published since 2010 on this topic.

4. Background information

To address this mandate EFSA is requested to consider, as background information and sources of data, published reports from national and international authorities/bodies addressing the health effects of sugars, as well as systematic reviews and meta-analysis published since 2010 on this topic.

An overview of the most recent existing DRVs and recommendations issued by other national and international authorities/bodies can be found in Appendix A. These publications will be used as sources of individual studies meeting the inclusion criteria for the present assessment through the scrutiny of their reference list.

A scoping literature search for systematic reviews and meta-analysis addressing the health effects of sugars or any of its dietary sources published in English since 2009 has also been performed. The list of the references identified and their main characteristics (e.g. exposure and endpoints of interest) can be found in Appendix B. These systematic reviews and meta-analysis will be used in two ways:

a) As sources of individual studies meeting the inclusion criteria for the present assessment through the scrutiny of their reference list;
b) As starting point for the literature searches to be carried out in the context of this assessment, whenever appropriate (see Section 9.2).

Data on the most recent national recommendations on sugars consumption in European countries will also be gathered through a questionnaire to National Competent Authorities (Appendix C).

5. Interpretation of the Terms of Reference

5.1. Definition of the exposure

Different terms and definitions have been used by researches and risk managers for dietary sugars. Among these:

i) *Sugars (total),* which are glycaemic carbohydrates composed of 1–2 monomers found in food. The main types of sugars found in mixed diets are the monosaccharides glucose, fructose and galactose, and the disaccharides sucrose, lactose, maltose and trehalose (FAO/WHO, 1998; EFSA NDA Panel, 2010a).
ii) *Added sugars*, which include sugars (mono- and disaccharides) used as ingredients in processed and prepared foods and sugars eaten separately or added to foods at the table. This definition was first applied in the 2000 US Dietary Guidelines for Americans (USDA/HHS, 2000), and then by the IoM (2005), EFSA (EFSA NDA Panel, 2010a) and some European countries (Nordic Council of Ministers, 2014).
iii) *Free sugars*, which include monosaccharides (glucose, fructose and galactose) and disaccharides (sucrose, lactose, maltose and trehalose) added to foods by the manufacturer, cook, or consumer plus sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates. This term has been used by the World Health Organization (WHO, 2003, 2015).
iv) *Non-milk extrinsic (NME) sugars*, defined as sugars not located within the cellular structure of a food, such as those found in fruit juice, honey and syrups, and those added to processed foods, excluding lactose in milk. The term originated from the UK Department of Health (1989) as opposed to *intrinsic sugars*, which are those located within the cellular structure of a food (e.g. naturally found in fruits and vegetables). On its assessment of 2015, the UK Scientific Advisory Committee on Nutrition (SACN) (2015) adopted the WHO definition of *free sugars* to provide recommendations on sugar intakes.

This assessment concerns the main types of sugars (mono- and disaccharides) found in mixed diets (i.e. glucose, fructose, galactose, sucrose, lactose, maltose and trehalose) taken through the oral route only. Sugar alcohols (polyols), other substances used as sugar replacers and other mono- or disaccharides present in the diet in marginal amounts, are not included in the term 'sugars' for the purpose of this assessment.

To allow comparability with previous assessments done by EFSA and by other bodies, total sugars, added sugars and free sugars as defined above will be addressed in this protocol.

5.2. Objectives of the risk assessment

This mandate is a request for EFSA to update its Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre published in 2010 (EFSA NDA Panel, 2010a) with respect to (added) sugars, and therefore interpreted as a request in the context of establishing ULs for nutrients for the general European population. The principles for the application of risk assessment to nutrients in general, and for deriving ULs in particular, have been described elsewhere (SCF/EFSA NDA Panel, 2006; EFSA NDA Panel, 2010b).

EFSA interprets this mandate as a request to provide scientific advice on an UL for (total/added/free) sugars, i.e. the maximum level of total chronic daily intake of sugars (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans. 'Tolerable intake' in this context connotes what is physiologically tolerable and is a scientific judgement as determined by assessment of risk, i.e. the probability of an adverse effect occurring at some specified level of exposure.

ULs may be derived for various life stage groups in the population. The UL is not a recommended level of intake. If there are no, or insufficient, data on which to base the establishment of a UL, as it was the case in 2010 for total or added sugars (EFSA NDA Panel, 2010a), an indication should be given on the highest level of chronic daily intake (from all sources) where there is reasonable confidence in data on the absence of adverse effects (i.e. a science-based cut-off value for a daily exposure which is not associated with adverse health effects). If there are no, or insufficient, data on which to base the establishment of a UL or a cut-off value for (total/added/free) sugars from all sources because the evidence available relates to one or few sources only, or to a particular type of sugar (e.g. fructose, glucose, sucrose), and the extrapolation of the results to (total/added/free) sugars from all sources is found to be unjustified, scientific advice could be provided on quantitative

intakes in relation to one or few sugar sources only, and/or in relation to one type of sugar only (e.g. fructose, glucose, sucrose) (Figure 1).

The characterisation of the risk (SCF/EFSA NDA Panel, 2006) includes a description of the scientific uncertainties associated with the UL estimates in order to indicate the degree of scientific confidence that can be placed in these estimates. It will also include an estimate of intake for population groups as well as an indication of the margin between actual intakes and the UL, and an indication of circumstances, if any, in which risk is likely to arise. If a UL for (total/added/free) sugars cannot be established, other values could be used instead to characterise the risk (Figure 1).

It is out of the scope of this assessment to address possible beneficial health effects of sugars or of particular dietary sources of sugars.

The outcome of the assessment is expected to assist Member States and health professionals in establishing nutrient goals for populations and recommendations for individuals, and when developing FBDG.

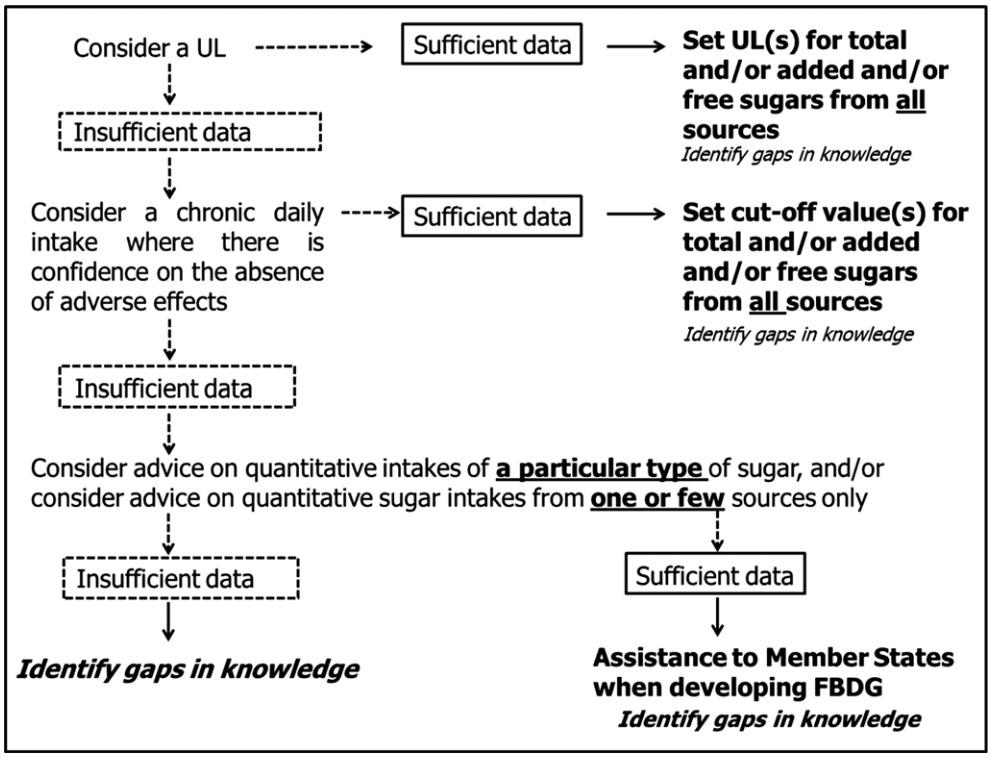


Figure 1: Step-wise process to provide scientific advice on total/added/free sugars

5.3. Target population

Adverse effects of nutrients are influenced by physiological changes and common conditions associated with growth and maturation that occur during an individual's lifespan. Therefore, where necessary, and to the extent possible, ULs are derived for each separate life-stage group, e.g. infants, children, adults, the elderly and women during pregnancy or lactation. Even within relatively homogenous life-stage groups, there is a range of sensitivities to adverse effects, e.g. sensitivity is influenced by body weight and lean body mass.

The derivation of ULs for the general population, divided into various life-stage groups, accounts for normally expected variability in sensitivity due to e.g. ethnicity, dietary habits, nutritional status, physical activity level (PAL) and metabolic risk profile. This includes individuals within the general population at risk of chronic disease (e.g. with excess body weight, elevated blood pressure, blood glucose and/or blood lipids but not on pharmacological treatment for the condition). It excludes, however, subpopulations with distinct vulnerabilities due to genetic predisposition or other considerations (e.g. individuals with inborn errors of carbohydrate metabolism) because including these would result in ULs which are significantly lower than are needed to protect most people against adverse effects of high intakes. Subpopulations needing special protection (e.g. individuals on

pharmacological treatment for obesity, diabetes, hypertension, dyslipidaemia, chronic liver or renal diseases) are better served through the use of public health screening, health care providers, or other individualised strategies.

The following groups will be considered *a priori*:

- Infants ≥ 4 to < 12 months
- Toddlers (young children) ≥ 1 to < 3 years
- Other children ≥ 3 to < 10 years
- Adolescents ≥ 10 to < 18 years
- Adults ≥ 18 to < 65 years
- Elderly adults ≥ 65 years
- Pregnant women

The age ranges may be modified by the NDA Panel depending on the available data, e.g. children may be further categorised according to the type of dentition (primary – milk or secondary – permanent) in relation to dental caries.

For this assessment, it is anticipated that lactating women will be considered together with other women in child-bearing age.

Infants < 4 months of age will be excluded from the assessment on the assumption that they are exclusively fed with breast milk or breast milk substitutes (EFSA NDA Panel, 2009).

5.4. Adverse effects and endpoints

The assessment will focus on possible adverse effects of sugars intake on endpoints selected based on the scope of the mandate, on previous assessments done by other bodies (Appendix A), on the systematic reviews available (Appendix B) and on the comments received through the public consultation (EFSA, 2018).

Both disease endpoints and other endpoints will be addressed. Disease endpoints are considered to be the most direct, or applicable, to the assessment. Other endpoints are relevant but less direct, and can include upstream indicators, risk factors, intermediate endpoints or measures related to disease endpoints.

The disease endpoints of interest are:

a) Incidence/severity of dental caries
b) Incidence of metabolic diseases:

   i) Obesity,
   ii) Type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus (GDM),
   iii) Hypertension,
   iv) Dyslipidaemia,
   v) Cardiovascular diseases (CVDs),
   vi) Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NAFLD and NASH),
   vii) Gout.

Other endpoints of interest are:

a) Measures of body fatness and body fat distribution; birth weight-related endpoints,
b) Ectopic fat deposition (e.g. muscle, liver),
c) Measures of glucose homoeostasis,
d) Blood pressure,
e) Blood lipids,
f) Uric acid,

The outcome variables that will be investigated in relation to the above-mentioned endpoints are depicted in Section 9.1.

6. Identification of the assessment subquestions

The identification of the assessment subquestions follows the principles of risk assessment for nutrients (SCF/EFSA NDA Panel, 2006). The assessment subquestions are illustrated in Table 1.

For **hazard identification**, human studies provide the most relevant data and, when they are of sufficient quality and extent, are given the greatest weight. Owing to the wealth of human studies

available on sugars intake and that extrapolation from animal models to humans is subject to considerable uncertainties, the Panel considers that evidence for adverse effects of sugars on humans should be obtained from human studies (**subquestions 4 and 5**).

Decisions on whether structural or functional alterations observed in human studies represent adverse effects or changes of little or self-limiting biological importance will be based on scientific judgement and will be taken on a case-by-case basis. In this context, it will be important to determine:

a) the causal significance of an exposure–effect association indicated by observational studies, primarily for disease endpoints. Criteria for judging this include demonstration of a temporal relationship, consistency, strength of association, a dose–response relationship (a biological gradient), specificity, biological plausibility and coherence; and
b) the clinical significance of an intake–effect relationship indicated by intervention studies primarily on other (than disease) endpoints. For energy-containing macronutrients, such as sugars, it will be important to determine how such relationships relate to energy-wise comparable intakes of other energy-containing macronutrients which are reasonably expected to replace the macronutrient under assessment in the diet. For sugars, the Panel considers that other glycaemic carbohydrates (e.g. starch) are the most appropriate comparison (EFSA NDA Panel, 2010a). Isocaloric comparisons with other macronutrients, however, may provide additional information which could be considered in the assessment.

Relevant experimental data (animal models, *in vitro* data) and knowledge of the molecular or cellular events underlying the adverse effect (mode of action) can assist in dealing with problems of data interpretation (**subquestion 6**).

Hazard characterisation includes a dose–response assessment which addresses the relationship between (total/added/free) sugars intake (dose) and adverse effect (in terms of intake and severity). Only human studies will be considered for that purpose (**subquestions 4 and 5**). The selection of the most appropriate or critical data set(s) for deriving the UL will be made on the basis of data quality, taking into account the related uncertainties and the possibility to derive quantitative estimates. Data on bioavailability (digestion, absorption and metabolism) will be considered to explain any apparent differences in dose response between different types, forms and sources of sugars (**subquestion 3**). Critical data set(s) should document the route of exposure and magnitude and duration of intake, and the intake that does not produce adverse effects as well as the intake which produces adverse effects. If more than one data set is found to be suitable and the UL that can be derived from each of them differs (e.g. for different disease endpoints), scientific advice will be provided for each of them separately. If there are no, or insufficient, data on which to base the establishment of a UL, an indication will be given on the highest level of intake of (total/added/free) sugars where there is reasonable confidence in data on the absence of adverse effects (Figure 1).

Risk characterisation includes a description of the scientific uncertainties associated with the UL estimates (or alternative estimates as the case may be; Figure 1) to indicate the degree of scientific confidence that can be placed in these estimates. Where data are available, it also includes an estimate of intake (from occurrence data and food consumption data; **subquestions 1 and 2**) for population groups as well as an indication of the margin between actual intakes and the UL, and an indication of circumstances, if any, in which risk is likely to arise.

Table 1: Assessment subquestions to be answered

Number	Subquestion
1	What are the levels of (total/added/free) sugars in foods and beverages in Europe?
2	What is the distribution of intakes of (total/added/free) sugars from all dietary sources (and by food source) by population group?
3	What are the digestion, absorption and metabolism of different types of sugars from different sources in humans?
4	What is the relationship between the intake of (total/added/free) sugars and metabolic diseases (disease endpoints and other endpoints) in the target population?
5	What is the relationship between the intake of (total/added/free sugars) and dental caries in the target population?
6	Which could be the potential mode(s) of action underlying the adverse effects (if any) of (total/added/free) sugars intake?

7. Methods to answer subquestions 1 and 2

7.1. Levels of sugars in foods and beverages in Europe

7.1.1. Development of a food composition database for total sugars

Since only total sugars are subject to mandatory labelling in Europe and there are no analytical methods to distinguish between added/free sugars and other sugars present in foods, available data on total sugars will be used as starting point to estimate the levels of added and free sugars in foods and beverages. To that end, a food composition database for total sugars will be developed first.

Data on total sugars will be extracted from the EFSA's food composition database, which was compiled as a deliverable of the procurement project 'Updated food composition database for nutrient intake' (Roe et al., 2013). The aim of the project was to provide EFSA with an updated food composition database covering approximately 1,750 food entries in the EFSA FoodEx2 classification system[1] with additional FoodEx2 facet descriptors, and to expand the data set to include harmonised information on the most common composite recipes of European countries and harmonised information on food supplements. In case no country-specific data were available for certain food codes, data compilers borrowed compatible data from other countries and/or from similar foods.

The EFSA's food composition database contains raw data for energy, macro- and micronutrients from national food composition databases up to 2012 provided by fourteen national food database compiler organisations. Within the EFSA's food composition database, 12 countries provided data on total sugars covering about 1290 FoodEx2 codes. The number of values for total sugars available for a given FoodEx2 code is variable.

For the purpose of this Scientific Opinion, a single European food composition database for total sugars will be developed from the information available in the national food composition databases. To that end, an outlier analysis will be performed to identify any value which deviates from the others for a given food code, which could be either wrongly attributed values (data cleaning) or values describing real variability in total sugars content. For food codes for which no outliers can be identified, the mean will be taken as a unique value.

The outlier assessment will prioritise foods with a high content of total sugars and foods largely consumed by one or more population subgroups. The expected variability in the total sugars content will decrease as the FoodEx2 level increases. Such variability will inform decisions regarding the level of FoodEx2 at which values for total sugars need to be attributed, together with data on the frequency of consumption gathered from the EFSA food consumption database. In this context, the Mintel Global New Products Database (GNPD),[2] an online database which monitors product introductions in consumer markets of packaged goods worldwide, will be used to check if the variability detected in the EFSA's food composition database regarding the total sugars content of manufactured foods reflects real variability of products in the market, and also to decide whether more than one scenario needs to be considered for risk characterisation regarding one or more food groups, and at which FoodEx2 level (i.e. whether two extreme values (lowest and highest), rather than a mean value, will need to be assigned to a food code to evaluate the impact of this variability on total sugars intake) (Figure 2).

A similar process has been undertaken to build the EFSA's food composition database for a number of vitamins and minerals in order to support EFSA's scientific opinions on DRVs for nutrients.

7.1.2. Development of food composition databases for added and free sugars

A European food composition database for added sugars in foods and beverages will be developed taking into account the 10-step methodology described by Louie et al. (2015) for added sugars and adapted by FSANZ[3] to determine the amount of added sugars in foods in the AUSNUT 2010–2013 food nutrient database. Although the definition of added sugars (a component of free sugars) in the

[1] https://www.efsa.europa.eu/en/data/data-standardisation

[2] The Mintel GNPD contains information on over two million food and beverage products, of which more than 800,000 are or have been available on the European food market. Mintel started covering European Union's food markets in 1996. Twenty out of the 28 EU member countries and Norway are present in the Mintel GNPD. The database provides the compulsory ingredient information presented in the labelling of products and the nutritional facts when available on the labels, which provide information about the use of sugars as ingredients and about the total sugar content of foods. http://www.mintel.com/global-new-products-database

[3] http://www.foodstandards.gov.au/science/monitoringnutrients/ausnut/foodnutrient/Pages/Determining-the-amount-of-added-sugars-and-free-sugars-in-foods-listed-in-the-AUSNUT-201113-dataset.aspx

Australian code includes maltodextrin and similar products, a decision was made by FSANZ not to capture these ingredients in the data set of added sugars (and therefore neither in the data set of free sugars) to maintain consistency with the definition of sugars used in nutrition labelling and with international food composition database practice where total sugars have been defined as being only mono- and di-saccharides. The same approach will be followed by EFSA for the same reasons.

The food composition database for added sugars will be developed for all FoodEx2 codes for which a consumption has been reported in the EFSA Comprehensive Food Consumption Database (see Section 7.2.1) in combination with the relevant FoodEx2 facet descriptors included in the EFSA FoodEx2 classification system (e.g. sugar free facet), using as starting point the food composition database for total sugars (Section 7.1.1). The step-wise methodology described by Kibblewhite et al. (2017) will be adapted to develop the food composition database for free sugars by applying the definition given in Section 5.1 of this protocol as strictly as possible. Databases for added and free sugars will be developed in close consultation with the mandate requestor (Figure 2). All the steps of the process will be thoroughly documented.

7.2. Estimates of intake of total, added and free sugars from all dietary sources

Estimates of intake of total, added and free sugars from all dietary sources will be obtained using data from the EFSA Comprehensive Food Consumption Database in combination with the food composition databases for total, added and free sugars (Sections 7.1.1 and 7.1.2).

7.2.1. The EFSA Comprehensive Food Consumption Database

Food consumption data from the EFSA Comprehensive Food Consumption Database (hereinafter referred as Comprehensive Database) will be used in order to assess the intake of free sugars. The Comprehensive Database provides a compilation of existing national information on food consumption at individual level. It was first established in 2010 (EFSA, 2011; Huybrechts et al., 2011; Merten et al., 2011). The latest version of the Comprehensive Database, updated on 26 April 2018, contains results from 61 different dietary surveys carried out in 25 different Member States.

Within the dietary surveys, subjects are classified in different age groups as follows:

1) Infants: 1–11 months old
2) Toddlers: ≥ 1 year to < 3 years old
3) Other children: ≥ 3 years to < 10 years old
4) Adolescents: ≥ 10 years to < 18 years old
5) Adults: ≥ 18 years to < 65 years old
6) Elderly: ≥ 65 years to < 75 years old
7) Very elderly: ≥ 75 years old

Data from infants 4 to 11 months old only will be considered in this assessment.

Overall, the Comprehensive Database is the most complete and detailed collection of food consumption data currently available in the EU. Consumption data were collected using single or repeated 24- or 48-h dietary recalls or dietary records covering from three to 7 days per subject. Surveys with only one observation day per subject, or which used food frequency questionnaires (FFQ) for data collection, were excluded. Owing to the differences in the methods used for data collection, direct country-to-country comparisons can be misleading. Detailed information on the different dietary surveys included in the Comprehensive Database is shown on the EFSA website,[4] including the number of subjects and days available for each age group. If new food consumption surveys become available during the assessment and up to December 2018, the most recent survey for a given country and age group will be used.

The linking between the foods consumed and the food composition databases for total, added and free sugars (Sections 7.1.1 and 7.1.2) will be done through the FoodEx2 system (EFSA, 2015b).

7.2.2. Sugars intake calculation

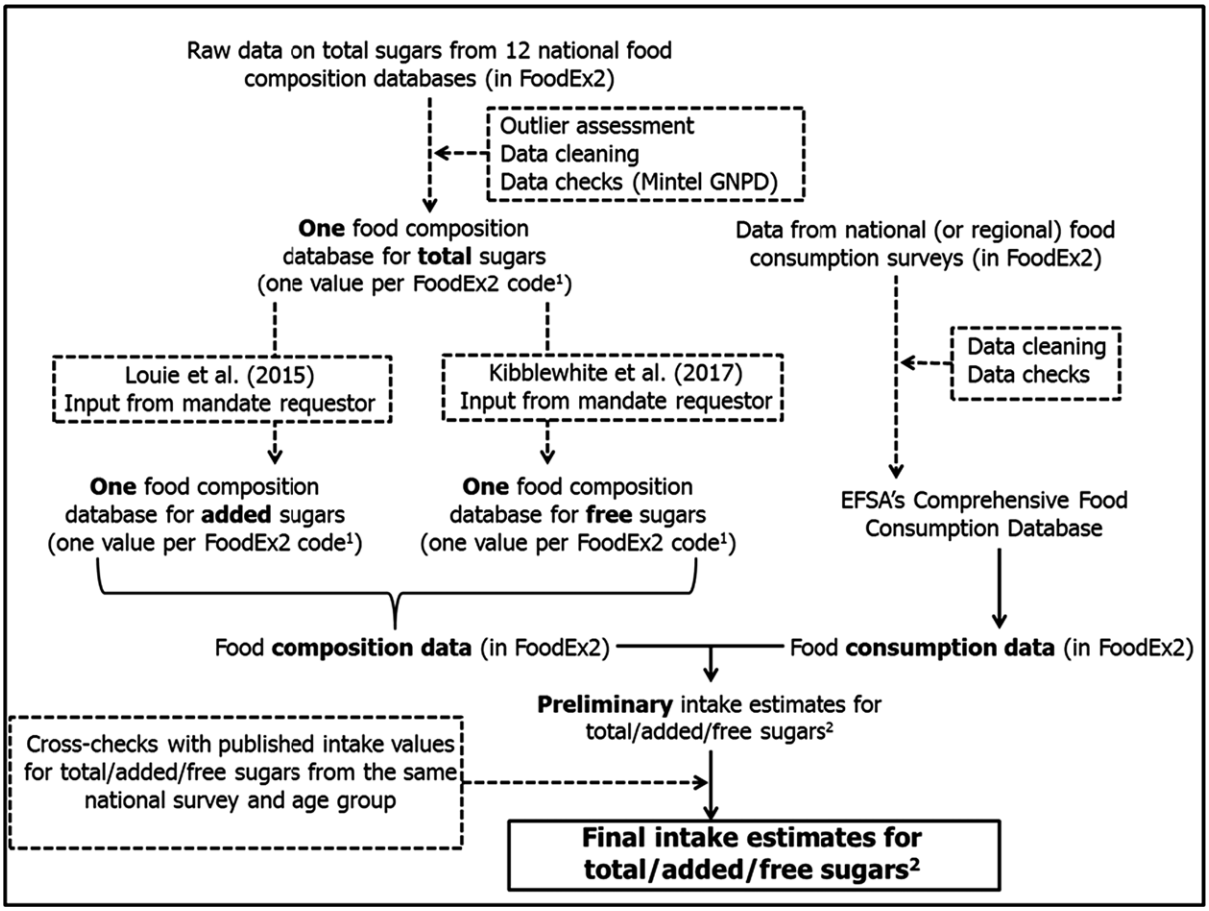
The intake of total/added/free sugars (in grams per day) will be calculated at individual level by multiplying the average daily consumption for each food or food group with the corresponding

[4] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

concentration of total/added/free sugars, summing up the respective intakes throughout the diet. The intake of total/added/free sugars will also be expressed as percentage of total energy intake (%E) and as percentage of non-alcohol energy intake (non-alcohol E%). In line with the Guidance of EFSA for the Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment (EFSA, 2011), chronic total/added/free sugars intake calculations will be performed only for subjects with at least two reporting days. In this context, chronic total/added/free sugars intake refers to the arithmetic mean of all reporting days available for the same subject. The intake will be modelled using the SAS software (SAS Enterprise Guide 5.1, 2013). The mean as well as the 5th, 50th and 95th percentiles of intake will be derived for each survey and age group (and sex group, if appropriate), respectively. Data on the contribution of different food groups to (total/added/free) sugars intake by survey and age group will also be presented.

Different intake scenarios could be considered in the intake calculation process, especially if more than one value for total/added/free sugars is assigned to one or more FoodEx2 codes in the food composition database (Figure 2).

To evaluate the accuracy of the results obtained, these will be compared with published intake values for total/added/free sugars from the same survey data set and age group, whenever available. These data will be retrieved through a questionnaire to the National Competent Authorities of European countries (Appendix C).



Legend to Figure 2: 1) Two values for a given FoodEx2 code may be assigned when both the observed variability in sugars content and the frequency of consumption are high, so that different intake scenarios could be considered; 2) In grams per day and as E% per country and population group.

Figure 2: Steps that will be followed to estimate intakes of total, added and free sugars

8. Method to answer subquestion 3

In order to address the digestion, absorption and metabolism of different types of free sugars from different food matrices in humans, background information will be gathered by the WG experts and EFSA staff through a narrative review. Recent textbooks, authoritative reviews and research papers retrieved through searches in bibliographic databases, and selected on the basis of their relevance, will be used as sources of information.

9. Methods to answer subquestions 4 and 5

Subquestions 4 and 5 will be answered by performing systematic reviews and, possibly, dose–response meta-analyses if the available data allow doing so. The conceptual framework for the systematic reviews on sugars intake in relation to disease and other endpoints is shown in Figure 3.

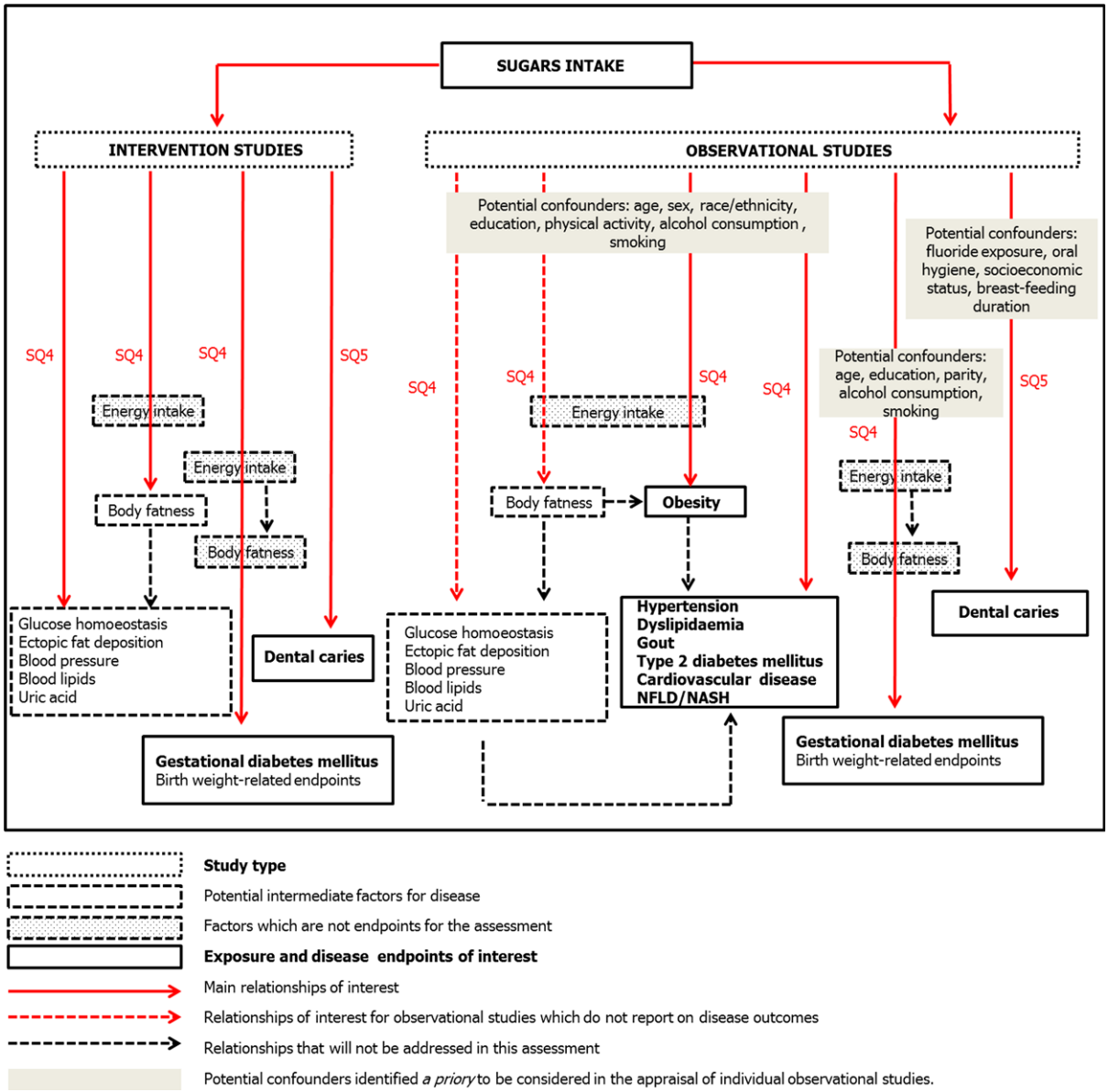


Figure 3: Conceptual framework for the systematic reviews on sugars intake in relation to disease endpoints and other endpoints

9.1. Eligibility criteria for study selection

The selection of human studies relevant to subquestions 4 and 5 will be performed using the eligibility criteria described in Table 2.

For subquestion 4, the minimum study duration for the inclusion of intervention studies has been selected by considering the time generally required for the stabilisation of the endpoints assessed following a nutritional intervention. The minimum study duration for the inclusion of observational studies for subquestion 4, and for the inclusion of intervention and observational studies for subquestion 5, is based on the minimum time estimated to be needed for the disease to develop in individuals free of the disease at baseline (expert judgement).

Regarding the study location, no limits are applied. It is acknowledged, however, that the background diet may affect the relationship between the intake of sugars and the endpoints being addressed, and that major differences in the background diet may limit the extrapolation of the results obtained outside Europe to the European population. This aspect will be considered when synthesising the evidence (Section 9.6).

Table 2: Eligibility criteria for human studies to address subquestions 4 and 5

INTERVENTION STUDIES		
Study design	In	Randomised controlled trials Non-randomised, comparative studies of interventions[(a)]
	Out	Single-arm intervention studies with no control group
Study duration	In	Depending on the endpoints addressed, as follows: Measures of body fatness ≥ 6 weeks Ectopic fat deposition ≥ 2 weeks Measures of glucose homoeostasis: ≥ 1 week to ≥ 12 weeks[(b)] Blood pressure, blood lipids and uric acid ≥ 4 weeks Dental caries: ≥ 1 year for primary dentition and ≥ 18 months for permanent dentition Pregnancy endpoints: any duration
	Out	Studies of shorter duration
Study location	In	Any location
Population	In	Adults (≥ 18 years) and children (4 months to < 18 years) from the general population, including overweight or obese subjects, subjects at risk of disease (e.g. with impaired glucose tolerance, impaired fasting glucose, NAFLD), and subjects with one or more features of the metabolic syndrome which are not on pharmacological treatment during the intervention
	Out	Studies targeting individuals with a disease (except for obesity), either untreated or under pharmacological/surgical treatment for the disease, or individuals on a therapeutic diet, including weight-loss diets Studies in individuals under physical training programs (e.g. athletes, military), except for dental caries
Intervention/control	In	Studies aiming at: – isocaloric exchanges of sugars with other glycaemic carbohydrates (e.g. starch) – isocaloric exchanges of sugars with macronutrients other than glycaemic carbohydrates (e.g. protein, fat) – isocaloric exchanges of different types of sugars (e.g. fructose vs glucose) – replacing sugar-containing foods with sugar-reduced or sugar-free foods (either under controlled or free-living conditions) – a quantitative change (increase, decrease) in sugars intake from one or more sources vs usual diet or no advice (either under controlled or free-living conditions)
	Out	Studies aiming at: – a quantitative change in sugars intake in the context of energy-restricted diets aiming at weight loss – studies not providing sufficient information to allow quantitative estimates of sugars intake, whether total or from one or more dietary sources (e.g. studies reporting only on the frequency of consumption of one or more dietary sources of sugars with unknown sugars content)

		– interventions targeting simultaneous changes in dietary/lifestyle factors other than the amount of sugars intake from one or more sources relative to the control group (e.g. advice on teeth brushing or physical exercise provided to the intervention or control group only)
Endpoints of interest	In	*Body fatness and ectopic fat deposition* – Measured body weight, BMI, waist circumference, sagittal diameter – Body fat measured by neutron activation analysis (NAA), imaging techniques (DXA, MRI, CT), hydrostatic weighing, or air displacement plethysmography. – VAT assessed by imaging techniques (CT, MRI) – Ectopic fat deposition in muscle or the liver assessed by CT, MRI, magnetic resonance spectroscopy (MRS) or in biopsies *Glucose homoeostasis* – Dynamic indices of insulin sensitivity and/or beta-cell function calculated from measures of plasma glucose, serum insulin and C-peptide (when available) during clamp tests (hyperinsulinaemic-euglycaemic, hyperglycaemic), frequently sampled intravenous glucose tolerance tests (FSIGT), standard oral glucose tolerance test (OGTT), the continuous infusion of glucose with model assessment (CIGMA), or insulin suppression tests – Fasting plasma glucose, fasting serum insulin and static indices of insulin sensitivity and/or beta-cell function thereof (e.g. HOMA, QUICKI) – Indices of blood glucose control (HbA1c, fructosamine) *Blood pressure* – SBP and DBP (point ambulatory or home BP, 24-h BP) *Blood lipids* – total-c, LDL-c, HDL-c, VLDL-c, TG, apoB100, apoA1 and ratios thereof *Liver* – NAFLD/NASH activity scores as defined by the authors *Uric acid* – Uric acid concentrations in blood *Dental caries* – Indices of dental caries measured by a trained observer *Pregnancy endpoints* – Birth-weight related endpoints (e.g. birth weight, small for gestational age, large for gestational age) as defined by the authors – Incidence of GDM as defined by the authors
	Out	– Self-reported body weight, BMI, waist circumference, sagittal diameter – Body composition assessed by BIA or skinfold thickness – Dental caries self-reported or reported by parents; other endpoints (e.g. amount of dental plaque; plaque pH) – Studies not including at least one of the endpoints listed above
Language	In	Full-text document in English
	Out	Articles with the full text in another language
Publication year	In	Up to March 2018
Publication type	In	Primary research studies (i.e. studies generating new data) reported in full-text articles Primary research studies reported in letters to editors if the information provided is sufficient to allow a scientific evaluation of the results Systematic reviews and meta-analyses[(c)]

	Out	Narrative reviews, expert opinions, editorials and letters to editors not reporting on primary data Meetings' abstracts and posters Conference proceedings PhD theses Grey literature
OBSERVATIONAL STUDIES		
Study design	In	Prospective, longitudinal, observational (prospective cohort and nested case-control) studies
	Out	Retrospective case–control studies Cross-sectional studies Ecological studies Case studies/case series
Study duration	In	≥ 18 months for dental caries for permanent dentition ≥ 1 year follow-up for all other disease endpoints, including dental caries for primary dentition Pregnancy endpoints: any duration
	Out	Studies with a shorter follow-up
Study location	In	Any location
Population	In	Adults (≥ 18 years) and children (4 months to < 18 years) from the general population Studies targeting individuals at risk of disease (e.g. with impaired glucose tolerance, impaired fasting glucose, the metabolic syndrome, overweight, NAFLD) not on pharmacological treatment for the condition Studies in which prevalent cases of the disease endpoint of interest at baseline were excluded for data analysis
	Out	Studies targeting individuals with a disease (except for obesity), either untreated or under dietary or pharmacological/surgical treatment for the disease Studies in which prevalent cases of the disease endpoint of interest at baseline were not excluded for data analysis
Exposure	In	– Studies comparing different levels of sugars intake from one or more sources – Studies providing sufficient information to allow quantitative estimates of sugars intake, whether total or from one or more dietary sources (e.g. in absolute amounts, as %E, as non-alcohol %E) *Eating conditions*: *ad libitum* *Method to quantify sugars intake (one or more of the following)*: – 24-h urinary excretion of fructose and sucrose – Food records (at least 2 reporting days) – Diet recalls (at least 2 reporting days) – Dietary history – FFQs – Dietary interview in combination with one or more of the above
	Out	– Studies not providing sufficient information to allow quantitative estimates of sugars intake, whether total or from one or more dietary sources (e.g. studies reporting only on the frequency of consumption of one or more dietary sources of sugars with unknown sugar content) *Eating conditions*: under dietary controlled conditions prior to the dietary intake assessment *Method to assess intake of sugars*: – Any other method
Endpoints of interest	In	*Disease endpoints*: – Incidence of obesity as defined by the authors – Incidence of T2DM as defined by the authors – Incidence of hypertension as defined by the authors

		– Incidence of dyslipidaemia as defined by the authors – Incidence of stroke [haemorrhagic (intracerebral, subarachnoid) and/or ischaemic; fatal and/or non-fatal] – Incidence of coronary heart disease (fatal and/or non-fatal) – Incidence of myocardial infarction (fatal and/or non-fatal) – Incidence of congestive heart failure – Incidence of cardiac death – Incidence of fatal and/or non-fatal cardiovascular events (composite endpoint) – Other endpoints of fatal and/or non-fatal cardiovascular events as defined by the authors – Incidence of NAFLD or NASH as defined by the authors – Incidence of non-alcoholic liver fibrosis/cirrhosis/liver failure as defined by the authors – Incidence of gout – Indices of dental caries measured by a trained observer *Other endpoints*: – Incidence of overweight/high waist circumference as defined by the authors – Incidence of impaired fasting glucose – Incidence of hyperuricaemia – Endpoints as defined above for human intervention studies – Self-reported body weight and related endpoints – Body fat assessed by skinfold thickness or BIA *Pregnancy endpoints*: – As defined above for human intervention studies
	Out	– Self-reported waist circumference – Overall mortality – Dental caries self-reported or reported by parents; other endpoints (e.g. amount of dental plaque; plaque pH) – Studies not reporting at least one of the endpoints listed above
Language	In	Full-text document in English
	Out	Articles with the full text in another language
Publication year	In	Up to March 2018
Publication type	In	Primary research studies (i.e. studies generating new data) reported in full-text articles Primary research studies reported in letters to editors if the information provided is sufficient to allow a scientific evaluation of the results Systematic reviews and meta-analyses(c)
	Out	Narrative reviews, expert opinions, editorials and letters to editors not reporting on primary data Meetings' abstracts and posters Conference proceedings PhD theses Grey literature

NAFLD: non-alcoholic fatty liver diseases; DXA: dual-energy X-ray absorptiometry; MRI: magnetic resonance imaging; CT: computed tomography; VAT: visceral adipose tissue; HOMA: homeostasis model assessment; QUICKI: Quantitative insulin sensitivity check index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; VLDL-c: very low-density lipoprotein cholesterol; GDM: gestational diabetes mellitus; BMI: body mass index; BIA: Bioelectrical impedance analysis; FFQ: Food frequency questionnaire; NASH: non-alcoholic steatohepatitis.

(a): Prospective studies that compare the effects of two or more interventions which did not use randomisation to allocate individuals or clusters to the comparison groups.

(b): For dynamic indices of insulin sensitivity and/or beta-cell function ≥ 1 week; for static indices and fructosamine ≥ 4 weeks; for HbA1c ≥ 12 weeks.

(c): Systematic reviews, including meta-analyses, on this topic that will be identified during the process of literature screening will be collected for the purpose of reviewing the reference list but will not be considered to contribute to the final number of studies considered eligible unless they also contain original data.

9.2. Literature searches for studies meeting the eligibility criteria

For subquestions 4 and 5, an extensive literature search will be performed in bibliographic databases. Sources of grey literature and databases of theses/dissertations will not be searched.

The bibliographic databases listed in Table 3 will be searched in order to identify relevant studies.

Table 3: Bibliographic databases to be searched for relevant studies

Database	Platform	Types of studies
Cochrane Library. Cochrane Central Register of Controlled Trials (CENTRAL)	Wiley	Intervention studies
Cochrane Library. Cochrane Database of Systematic Reviews (CDSR)	Wiley	Systematic reviews
Cochrane Library. Database of Abstracts of Reviews of Effects	Wiley	Systematic reviews
Embase	Elsevier	Systematic reviews, intervention studies, observational studies
PubMed	NLM	Systematic reviews, intervention studies, observational studies
Scopus	Elsevier	Systematic reviews, intervention studies, observational studies

For subquestion 4, literature searches will be performed by type of endpoint. Previous systematic reviews with similar review questions, similar or broader inclusion criteria and appropriate search strategies were identified during the scoping searches (see Appendix B). Therefore, date limits will be applied to the searches for subquestion 4 (by endpoint) and subquestion 5 using these systematic reviews as starting point whenever possible (Table 4). Studies published before these dates will be retrieved by hand-searching the reference lists of the systematic reviews (Appendix B) and from existing reports by other authorities/bodies (Appendix A). No retrospective date limits will be applied for endpoints for which no existing systematic review can be taken as starting point.

Table 4: Date limits applied to the searches and systematic reviews used as sources of relevant studies

Subquestion	Endpoints	Date limit	Systematic review
4	Adipose tissue	Intervention and observational studies: December 2011	Te Morenga et al. (2013)
4	Blood pressure	Interventions: August 2013	Te Morenga et al. (2014)
		Observational studies: no date limit	
4	Blood lipids	Interventions: August 2013	Te Morenga et al. (2014)
		Observational studies: no date limit	
4	All other endpoints	Intervention and observational studies: no retrospective date limit	
5	Dental caries	Intervention and observational studies: November 2011	Moynihan and Kelly (2014)

Date limits might be changed should new systematic reviews on the topic be identified which are considered to adequately cover the relevant literature. Existing systematic reviews (Appendix B) with narrower inclusion criteria regarding either the exposure (e.g. limited to SSBs) or the study duration (e.g. (Sonestedt et al., 2012; SACN, 2015)) will be hand searched.

The search terms that will be used for the exposure and the various endpoints of interest are depicted in Appendix D. The specific search strategies for each database will be developed at a later stage. The performance of the search strings will be tested against the reference lists of the systematic reviews shown in Appendix B.

The output from the searched databases, including all indexed fields per hit (e.g. title, authors, abstract), will be exported into separate Endnote® files, allowing a count of the individual hits per database. All the studies included in the above-mentioned systematic reviews will be added to specific Endnote® libraries. Files will then be combined and duplicate records will be removed.

The files obtained will be transferred into DistillerSR® Web-Based Systematic Review Software (Evidence Partners, Ottawa, Canada) for the selection procedure (see Section 9.3).

9.3. Study selection process

The whole selection process will be performed with DistillerSR®. Studies to be included in the review will be selected using a two-step selection procedure:

1) **Screening of title and abstract** to identify potentially relevant studies that will be included for full-text screening, applying the selection criteria described in Section 9.1. If the information contained in the title or abstract is not relevant to the research objectives, the article will not be selected for full-text assessment. During the screening process, studies will be categorised into two groups corresponding to the two subquestions that are the objectives of these systematic reviews.
   This step will be conducted by WG experts and/or EFSA staff in duplicate, and by using machine learning techniques where/when appropriate. The results of the use of machine learning will be evaluated by a reviewer and documented. If the title and/or abstract do not mention the endpoints of interest for the assessment, but the words 'adverse effects' or 'side effects' are mentioned, the paper will be included to check if these effects have any relation to the endpoints of interest. If there are doubts or divergences which cannot be resolved between the two reviewers, the full article will be screened.

2) **Screening of full text** to assess if the article is relevant to the risk assessment.
   This step will be conducted by WG experts and/or EFSA staff, in duplicate, for the references retrieved. Possible divergences will be discussed by the whole WG on sugars, in case these would highlight the need for amendments to the inclusion/exclusion criteria. The content of the full text will be checked against the inclusion and exclusion criteria established in the protocol.

Possible divergences or doubt for inclusion of domain-specific articles will be discussed together with the relevant expert from the WG, also in case these would highlight the need for amendments to the inclusion/exclusion criteria.

Articles reporting solely on digestion, absorption or metabolism (i.e. without reporting on the endpoints of interest), or reporting only on endpoints other than those listed in Section 9.1, will not be included in this research subquestions but will be flagged for subquestions 3 and 6, where appropriate.

Screeners will be trained using written documentation on study eligibility. Eligibility criteria will be pilot tested on a subset of records, and refined if prone to misinterpretation. The results of the different phases of the study selection process will be reported in a flowchart as recommended in the PRISMA statement on preferred reporting items for systematic reviews and meta-analyses (Moher et al., 2009).

9.4. Data extraction from included studies

Data will be extracted from the studies using predefined forms that comprise data on the characteristics of the studies (e.g. study design), their key-elements (e.g. population, intervention/exposure, comparator, outcomes (endpoints), setting and duration), results, aspects related to the internal validity of the studies (e.g. confounders, randomisation) and funding source.

Studies for which the information provided in the publication(s) does not allow a full scientific evaluation by the WG experts (e.g. studies with missing or ambiguous information) will be excluded at this step. The reasons for exclusion will be clearly indicated.

The data will be extracted in the original units of measurement, which will be subsequently harmonised to allow data analysis. The authors may be contacted to retrieve additional data if needed.

Regarding the intervention/exposure, data on the food source(s) of sugars, on the type of sugars consumed (e.g. fructose, glucose, sucrose) and on the frequency of sugars consumption will be extracted from the included studies whenever available. For observational studies, data on disease endpoints (dichotomous variables, i.e. incidence of hypertension, incidence of type 2 diabetes) will be extracted first whenever available. Data on other endpoints (continuous variables, e.g. blood pressure, fasting glucose) will only be extracted if data on corresponding disease endpoints are not reported (Figure 3).

If a full-text document reports on more than one study, the individual studies will be identified at this step to allow for data extraction at individual study level. If a single study is reported in more than one publication, different publications reporting on the same study (e.g. on different outcomes

(endpoints), at different time points) will be identified at this step. Data will be extracted for the last observation available for each endpoint (corresponding to the longest intervention or follow-up) for data analysis. Exceptions to this rule will be duly justified (e.g. on the basis of attrition and sample size, compliance with the intervention, other factors).

Clear instructions for extracting data will be developed. The data extraction forms will be created in DistillerSR® (Evidence Partners, Ottawa, Canada) and/or Excel, and pilot tested on a subset of studies. The piloting will also be used to identify sources of contextual (i.e. related to the key elements of the studies) heterogeneity. The forms and instructions will be refined if needed.

Data will be extracted from each individual study by one EFSA staff or one WG expert. In the piloting phase, extracted data will be validated by another EFSA staff or WG expert, in order to identify sources of possible errors. The data extraction will be then conducted by one EFSA staff/WG expert. Data quality checks will be performed for each study (Section 9.6).

9.5. Appraisal of the internal validity of the included studies

The risk of bias (RoB) of a given study in relation to a specific outcome (endpoint) refers to the risk of systematic errors in the design, recruitment, data collection or analysis that results in a mistaken estimation of the true effect of the exposure on the outcome.

The internal validity or RoB of each individual study included in the assessment will be appraised using a customised version of the OHAT/NTP RoB tool, which is suitable for both intervention and observational studies.[5] This tool was developed based on guidance from the Agency for Healthcare Research and Quality (Viswanathan et al., 2012), the Cochrane risk-of-bias tool for non-randomised studies of interventions (Sterne et al., 2014), the Cochrane Handbook (Higgins and Green, 2011), CLARITY Group at McMaster University (CLARITY, 2013) and other sources. The OHAT/NTP RoB tool was developed to provide a parallel approach to the evaluation of the RoB in the context of hazard identification for human risk assessment of chemicals, and to facilitate consideration of RoB across evidence streams (i.e. human, animal and mechanistic studies) with common terms and categories for RoB rating. For this assessment, the use of the tool will be limited to the aspects relevant to intervention and prospective observational studies in humans.

For each study, the appraisal will be done at outcome level, because for the same study the design and conduct may affect the RoB differently depending on the endpoints measured. Each study will be appraised by two independent experts from the WG ('the reviewers'). Possible discrepancies will be discussed by the whole WG. If upon further discussion the WG cannot reach an agreement on a RoB rating for a particular domain, the more conservative judgment (the highest RoB) will be selected.

The OHAT/NTP RoB tool outlines 10 RoB questions, grouped in 6 bias domains (selection, confounding, performance, attrition/exclusion, detection and selective reporting) - plus 'other sources of bias' -, which help identify the practices that may introduce bias (Table 5). Each RoB question addresses aspects relevant to specific study designs, i.e. 8 questions apply to intervention studies and 7 questions apply to prospective observational (cohort and nested case–control) studies (Table 5). Reviewers are required to answer RoB questions by applying a 4-level rating scale (Figure 4).

The RoB questions and rating instructions provided in the tool will be tailored to the specific subquestions illustrated in this protocol.

Table 5: Extracted from OHAT/NTP RoB tool (source: OHAT Handbook – 9 January 2015)[(a)]

Bias Domains and Questions	Controlled intervention*	Observational
Selection Bias		
1. Was administered dose or exposure level adequately randomized?	**X**	
2. Was allocation to study groups adequately concealed?	**X**	
3. Did selection of study participants result in appropriate comparison groups?		**X**
Confounding Bias		
4. Did the study design or analysis account for important confounding and modifying variables?		**X**

[5] https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf

Bias Domains and Questions	Controlled intervention*	Observational
Performance Bias		
5. Were the research personnel and human subjects blinded to the study group during the study?	**X**	
Attrition/Exclusion Bias		
6. Were outcome data complete without attrition or exclusion from analysis?	**X**	**X**
Detection Bias		
7. Can we be confident in the exposure characterization?	**X**	**X**
8. Can we be confident in the outcome assessment?	**X**	**X**
Selective Reporting Bias		
9. Were all measured endpoints reported?	**X**	**X**
Other Sources of Bias		
10. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	**X**	**X**

*: Includes studies in humans with a controlled exposure including randomised controlled trials and non-randomised intervention studies.
(a): https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookjan2015_508.pdf

++	**Definitely Low** risk of bias	There is direct evidence of low risk-of-bias practices (May include specific examples of relevant low risk-of-bias practices)
+	**Probably Low** risk of bias	There is indirect evidence of low risk-of bias practices OR it is deemed that deviations of low risk-of bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias
-/NR	**Probably High** risk of bias	There is indirect evidence of high risk-of-bias practices OR there is insufficient information (e.g. not reported or "NR") provided about relevant risk-of bias practices
--	**Definitely High** risk of bias	There is direct evidence of high risk-of-bias practices (May include specific examples of relevant high risk-of-bias practices)

Figure 4: Answer format for the RoB questions (source: OHAT/NTP RoB tool)[5]

The OHAT/NTP RoB tool encourages judging the direction of bias, when possible. Empirical evidence about the direction of bias is discussed for each of the RoB questions. If there is no clear rationale for judging the likely direction of bias, reviewers are invited to simply outline the evidence and not to attempt a guess. This approach will be followed.

Once customised, the tool will be created in the review management software DistillerSR® to allow web-based appraisal of the studies.

Specific elements identified *a priori* and that will be considered in the assessment of confounding and biases related to the exposure and outcome characterisation are discussed below.

9.5.1. Consideration of potential confounders

When assessing the RoB of studies concerned with causality (e.g. which investigate the effect of an exposure on disease), confounding should be addressed regardless of the study design. When present, confounding may lead to a biased estimate of the effect of exposure on disease, either closer to the null (resulting in underestimation of the exposure effect) or departing from the null, and can even reverse the apparent direction of the effect.

There are several requirements for a factor to actually act as a confounder, as described by McNamee (McNamee, 2003) and illustrated below. The factor must:

1) be a cause of the disease, or a surrogate measure of the cause, in unexposed people; factors satisfying this condition are called 'risk factors'; and

2) be correlated, positively or negatively, with exposure in the study populations. If the study population is classified into exposed and unexposed groups, this means that the factor has a different distribution (prevalence) in the two groups; and
3) not be affected by the exposure. When a factor is an intermediate factor in the relationship between the exposure and the disease, it is affected by both the exposure and the disease, or shares a common cause with the disease which is in turn affected by the exposure, such factor cannot be considered a confounder.

Whereas criteria 1 and 3 can be judged based on current (expert) knowledge of external empirical evidence (e.g. prior research), criteria 2 can only be judged from internal evidence (i.e. evidence from the study under evaluation with respect to the RoB). Therefore, whether potential confounders identified *a priori* on the basis on current knowledge can actually confound the association between exposure and disease in a particular study by making the study groups not comparable in terms of disease risk is to be addressed on a case-by-case basis, in the context of each particular study.

The OHAT/NTP RoB tool does not include a separate question for confounding in human intervention studies because randomisation and allocation concealment should adequately address the issue of confounding. It recognises, however, that in some cases appropriate procedures for randomisation and allocation concealment may fail in accounting for confounding. For example, in the context of this assessment, confounding could be a concern if there are important differences among study groups in baseline characteristics which are risk factors for the outcome (endpoint) but not affected by the exposure (e.g. age, sex, PAL). In accordance with the OHAT/NTP guidance, for intervention studies where confounding is strongly suspected despite the fact that randomisation and allocation concealment are rated at 'probably low' or 'definitely low risk of bias', confounding will be addressed under 'other potential threats to internal validity' (OHAT/NTP, 2015).

In observational studies, potential confounding can be reduced by good design and appropriate statistical analysis (control for measured confounders), although it is acknowledged that the problem of confounding cannot be resolved fully in non-randomised designs. The aim of controlling for confounders in observational studies is to allow comparability of the study groups for the risk of disease which is not affected by the exposure, so that any observed difference in risk among groups can be attributed to differences in the exposure of interest.

Being sugars an energy-containing nutrient, an increase in energy intake and/or an increase in body fatness could be considered intermediate factors in the causal pathway between high sugars intake and adverse effects related to glucose homoeostasis, ectopic fat deposition, blood pressure, blood lipids and uric acid, which in turn could mediate the effect of high sugars intake on the incidence of hypertension, dyslipidaemia, gout, T2DM mellitus, CVD and NFLD/NASH (Figure 3). An increase in energy intake and/or an increase in body fatness could also be considered intermediate factors in the causal pathway between high sugars intake and adverse pregnancy outcomes (endpoints). All these intermediate factors could potentially be affected by the exposure and therefore do not meet the criteria to be considered as confounders (McNamee, 2003), but rather as a potential source of over-adjustment bias. The extent to which an effect of sugars intake on the endpoints of interest (except obesity and dental caries) may be mediated by changes in energy intake and/or body fatness will be explored in the synthesis of the evidence (Section 9.6). In addition, there are dietary factors known to be risk factors for one or more of the above-mentioned diseases, such the intake of sodium, potassium, saturated and *trans* fatty acids, or dietary fibre. However, these may or may not be associated with both the exposure and the endpoint within a given study, and therefore, they will be not considered as potential confounders *a priori*, but rather in the context of individual studies.

Based on recent publications, the Panel identified *a priori* an indicative list of potential factors that could confound the relationship between the intake of sugars and measures of body fatness, ectopic fat deposition, glucose homoeostasis, blood lipids, blood pressure and uric acid, and the relationship between the intake of sugars and incidence of overweight/obesity, hypertension, dyslipidaemia, gout, T2DM, cardiovascular disease and liver disease: age, sex, race/ethnicity, education (or education of the parents for studies in children), smoking habits, physical activity, alcohol consumption.

The Panel also identified *a priori* an indicative list of potential factors that could confound the relationship between the intake of sugars and dental caries: fluoride exposure (e.g. water fluoride, use of fluoride toothpaste, supplements), oral hygiene practices, socioeconomic status and breast feeding duration for studies on young children. Frequency of sugars consumption has been highly correlated to the amount of sugars consumed in observational studies and it is likely to be affected by the exposure, so that it will not be considered as a potential confounder in observational studies in relation to dental caries.

As for pregnancy outcomes, the Panel identified *a priori* the following factors that could potentially confound the relationship between the intake of sugars and birthweight-related endpoints or the incidence of GDM: parity, maternal age, education, alcohol consumption and smoking.

When assessing RoB in observational studies, the reviewers will consider, for each study, whether these factors can confound the association on a case-by-case basis. Additional confounders may be identified by the reviewers. The reviewers will consider whether the confounding variables were measured reliably and consistently within each study and whether the design and/or the data analysis adequately accounted for potential confounding (e.g. multivariable analysis, stratification).

9.5.2. Confidence in the exposure characterisation

The exposure of interest for the assessment is the daily intake of total/added/free sugars from all dietary sources. It is acknowledged however, that few of the available individual studies investigating the health effects of dietary sugars may have used the definition of added or free sugars as described in this protocol (Section 5.1) to characterise the intervention/exposure. In this context, the confidence in the exposure characterisation that will be assessed in relation to the RoB of individual studies refers to the confidence on the quantitative estimates for the sugar fraction that is being investigated in the study, and not the extent to which the exposure investigated on each study reflects the intake of total/added/free sugars from all dietary sources as defined in this protocol. The latter aspect will be discussed when integrating and weighing the evidence in light of the identified uncertainties to provide scientific advice on total/added/free sugars (see Figure 1 and Section 12).

In assessing RoB, reviewers will consider the risk of errors in the estimate of sugar intake for individuals and related risks of misclassification of individuals according to their exposure. The accuracy of sugar intake estimates may be affected by (i) the method (or combination of methods) used to assess the sugar fraction of interest for the study (e.g. 24-h urinary excretion of fructose and sucrose vs dietary records vs diet recalls vs FFQs; specificity of FFQs for the exposure of interest; validation; number of days recorded); (ii) the accuracy of 24-h urine collections and the accuracy of reporting dietary intakes (e.g. self vs dietitian assisted compilation of FFQs); (iii) systematic changes in habitual diet prior to the intake assessment. The reviewers will consider the resulting risk of misclassification in appraising the studies.

9.5.3. Confidence in the outcome assessment

Confidence in the outcome (endpoint) requires valid, reliable and sensitive methods to assess the outcome applied consistently across groups (OHAT/NTP, 2015). Outcome misclassification or measurement error may be unrelated to the exposure (non-differential) or related to the exposure (differential).

Factors that will be considered by the reviewers while assessing bias in relation to the outcome assessment include: (1) the objectivity of the outcome assessment, (2) the consistency in measurement of endpoints, and (3) the blinding of the outcome assessors (for knowledge of the exposure) (OHAT/NTP, 2015).

9.5.4. Summarising the internal validity of each individual study

Each study will be reported using a tabular summary form which will include the key elements of the study and a summary of the results of the critical appraisal.

When all the studies have been summarised in this way, the WG will consider whether and how to combine the scores from the RoB questions at the level of individual studies. The WG may consider using an algorithm to combine the scores in a weighted or unweighted manner: if so, the rationale for the chosen algorithm will be documented. Alternatively, the RoB scores may be kept separate for each RoB question and taken into account in the synthesis of evidence. The results of the RoB assessment will be taken into account in the weight of evidence assessment and uncertainty analysis (Sections 11 and 12).

9.6. Synthesis of the evidence

Data from included individual studies will be considered separately for each of study design and for each endpoint to derive single lines of evidence (Figure 3).

Information on inclusion criteria, RoB assessment and endpoints as extracted from the individual studies will be summarised in evidence tables. Data quality checks will be performed for each study. For each variable, the proportion of missing observations will be assessed; range checks will be carried out for all included variables to ensure that all values are reasonable; categorical variables will be

tabulated and key variables will be cross-tabulated to check for internal consistency. For intervention studies, results from intention-to-treat analyses will be preferred over per-protocol analyses if both are reported. In the case of missing data, flexible and transparent strategies will be pursued, such as requesting missing data from the authors, re-doing the analysis or placing the original results in adequate context according to the feasibility and adequacy of these approaches on a per-study basis. Effect estimates such as relative risks and odds ratios for dichotomous variables for disease endpoints, and differences in means for continuous variables for other endpoints along with measures of their statistical precision (usually 95% confidence intervals) will be extracted from the studies and reported in the assessment.

Whenever data allow for a meaningful quantitative synthesis of the evidence, effect estimates from intervention studies (for all endpoints; by study design) and observational studies (for disease endpoints) will be pooled separately and assessed through meta-analysis and dose–response meta-analysis, using fixed- and random-effects models as appropriate (Orsini et al., 2012; Crippa and Orsini, 2016; Discacciati et al., 2017). Dose–response meta-analysis is a statistical technique that aims to characterise the smooth and gradual change in non-linear responses along the range of a quantitative exposure using aggregated data from several studies. Within intervention studies, effect estimates will be pooled separately by study design (e.g. isocaloric exchange of sugars with other macronutrients, changes in sugars intake under free-living conditions). For observational studies, the suitability of unadjusted and adjusted models will be considered case-by-case for data analysis depending on the level of control for potential confounders and the risk of over-adjustment bias.

In addition to the meta-analyses stratified by study design, subgroup/stratified analyses will be performed according to age, gender, changes in body fatness (except for obesity and dental caries), type of dentition (for dental caries), type of sugar/food source/frequency of consumption, country or continent, and other factors suspected or known to modify the association between sugars intake and the endpoints assessed. Subgroup and sensitivity analyses will also be performed according to the RoB of the included studies, the degree of control for confounding, the methodology and quality of the exposure assessment, duration of follow-up for both the intervention and the observational studies, and possibly by the source of study funding. If needed, sensitivity analyses will be performed to evaluate the robustness of the findings and the possible influence of different biases on the summary pooled effect estimates (Arah et al., 2008; Rothman et al., 2012; Corbin et al., 2017). Influence analysis will be carried out by examining whether removal of single studies influences the results of the meta-analyses, and the reasons underlying such an influence on the effect estimates, if any (Rothman et al., 2012).

Statistical heterogeneity across study-specific findings will be taken into account in the statistical model and evaluated by visual inspection of forest plots and the I2 statistic (Higgins and Thompson, 2002; Higgins et al., 2003), and an attempt will be made to identify its sources.

The possibility of publication bias will be investigated using one or more of the following approaches: (a) visual inspection of funnel plots to investigate the association between study size and effect size (Altman, 1986); (b) Egger's regression test (Egger et al., 1997; Sterne and Egger, 2005); and (c) trim-and-fill analysis (Duval and Tweedie, 2004; Rothstein et al., 2005) following the approach of Peters Jaime et al. (2007).

If there are studies that are relevant but cannot be included in meta-analyses (e.g. due to differences in study design), their contribution to the assessment will be integrated with the results of the meta-analysis by a weight of evidence approach (Section 11). If none of the relevant studies for an effect are suitable for meta-analysis, evidence synthesis for that effect will be performed by a weight of evidence approach (Section 11).

9.7. Plans for updating the literature searches and dealing with newly available evidence

The literature searches performed as detailed above (Section 9.2) will be repeated approximately 3 months before the planned date of endorsement of the draft opinion by the Panel. Databases and keywords will be those of the original searches. Date limits will be defined based on the cut-off date of the preceding searches. The papers retrieved by these additional searches will be screened for relevance applying the same criteria.

Relevant studies will be reviewed by the WG experts and their contribution to the assessment will be integrated (with the results of a meta-analysis or otherwise) by a weight of evidence approach (Section 11), but will not be considered for inclusion in any meta-analysis.

10. Methods to answer subquestion 6

In order to address the mode(s) of action for possible adverse health effects of (total/added/free) sugars identified in subquestions 4 and 5, background information will be gathered by the WG experts and EFSA staff through a narrative review. Recent textbooks, authoritative reviews and research papers retrieved through searches in bibliographic databases, and selected on the basis of their relevance, will be used as sources of information. Articles reporting solely on digestion, absorption or metabolism that are identified/retrieved during the screening of the full text in the context of the systematic reviews described in Section 9 will also be considered, where appropriate. The mode of action by which sugars can contribute to the development of dental caries (subquestion 5), however, is considered to be well known.

11. Methods for integrating and weighing the evidence to set a UL for sugars

Integration of evidence will be performed at a number of levels and by different methods, according to what is appropriate given the available evidence (Figure 5). For subquestions 4 and 5, the following integration steps may be needed:

- Where appropriate, different studies (by study design) for the same endpoint will be combined by meta-analysis.
- Results from meta-analysis will be integrated with evidence from other relevant studies on the same endpoint (if there are any that could not be included in the meta-analysis) by a weight of evidence approach.
- For endpoints where meta-analysis is not feasible, relevant studies will be integrated by a weight of evidence approach.
- Where appropriate, results for different endpoints of the same type (e.g. disease and other endpoints relating to the same chronic disease) may be integrated by a weight of evidence approach.

The outcome of subquestions 4 and 5 will be integrated with the endpoints of subquestions 3 and 6 by a weight of evidence approach. The results of this will form the Panel's conclusions on the levels of intake for total/added/free sugars (UL or otherwise; Figure 1): this may result in more than one level of intake, depending on whether there is material variation between sugar fraction, effects and/or population groups.

The results of subquestion 1 (levels of total/added/free sugars in foods and beverages) will be integrated by calculation with food consumption data to address subquestion 2, resulting in intake assessments for the European population.

Risk characterisation will be performed by comparing results of the intake assessment (from subquestions 1 and 2) with the levels of intake (UL or otherwise; Figure 1) for total/added/free sugars (from subquestions 3, 4, 5 and 6) (Figure 5).

In several of the steps described above, integration will be performed by a weight of evidence approach using expert judgement. The methods will vary, depending on the evidence to be integrated and the specific considerations involved. In each case, the principles of EFSA's guidance on weight of evidence will be applied (EFSA Scientific Committee, 2017). Evidence will be organised into lines of evidence, where helpful. Relevance, reliability (including the RoB evaluations described in earlier sections) and consistency will be taken into account when weighing the evidence. Formal (EFSA, 2014) or semi-formal (EFSA Scientific Committee, 2018) methods for expert knowledge elicitation (EKE) will be used where appropriate. Detailed protocols will be established for each stage of the weight of evidence process before it is performed. Each stage of the process will be documented, including the reasons for any deviations from the protocol.

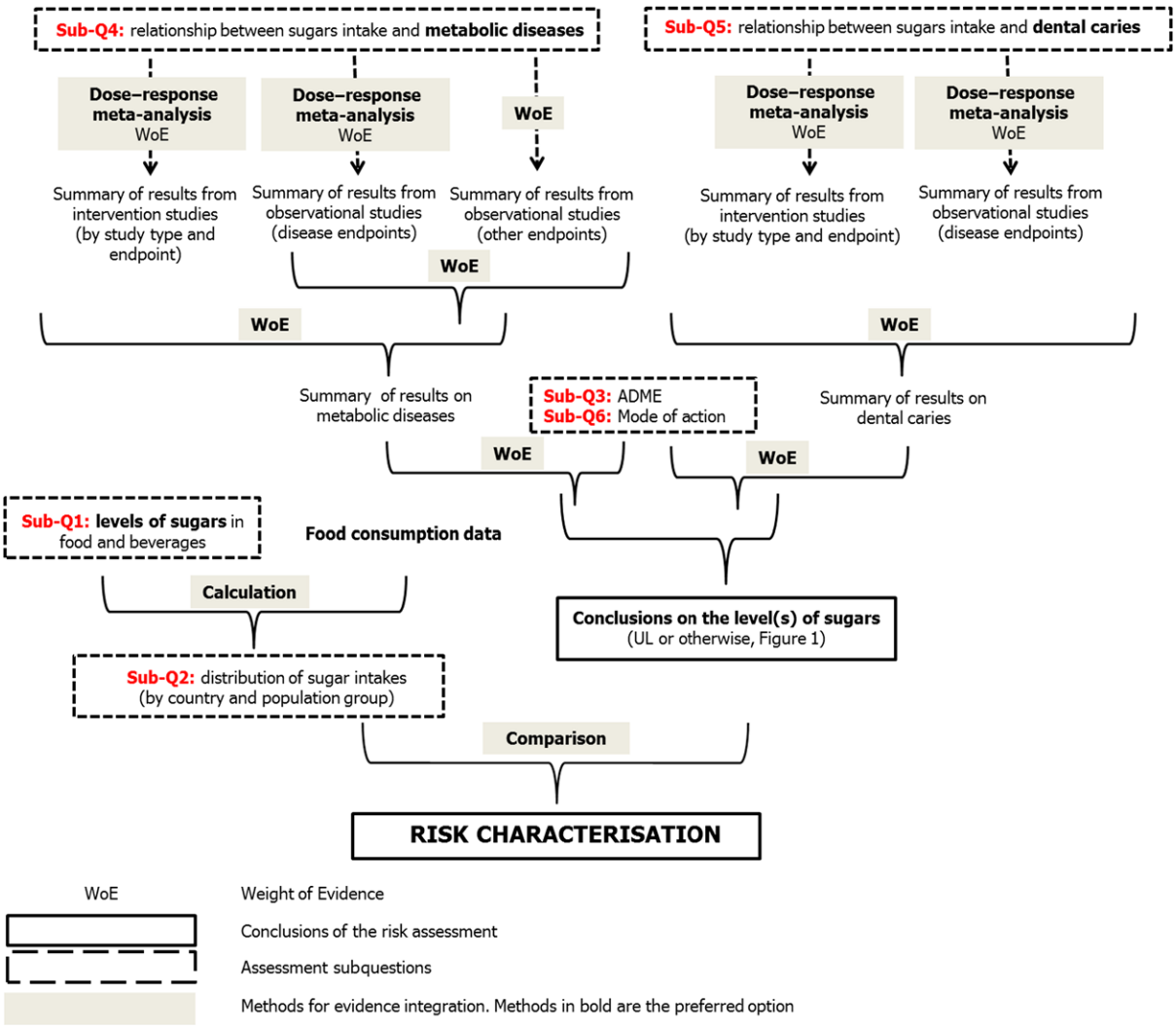


Figure 5: Conceptual framework for evidence integration

12. Evaluating the uncertainty in the body of evidence

Uncertainties in the estimates of total/added/free sugar intake in European countries may arise from inaccuracies in mapping food consumption data according to the FoodEx2 classification, from analytical errors or from errors in estimating the levels of total sugars in the national food composition tables, from errors in attributing levels of added/free sugars to foods from their content of total sugars, and from replacing missing values by values of similar food groups in the added/free sugars intake estimation process. These uncertainties may, in principle, result in both too high and too low estimates of total/added/free sugars intake.

For disease and other endpoints, once the individual studies are appraised for internal validity and after synthesising the evidence for each endpoint, line of evidence (i.e. intervention studies separately from observational studies; intervention studies by design) and subquestion, the uncertainties in the body of evidence will be identified, including factors such as the consistency of results, the precision of effect/association estimates and/or dose–response models, the internal and external validity (directness, generalisability, applicability) of the included studies, and gaps in knowledge.

Uncertainty analysis will be performed following approaches recommended by EFSA (EFSA Scientific Committee et al., 2018) for case-specific assessments. Uncertainty affecting each subquestion will be identified, and taken into account when evaluating the overall uncertainty for the main endpoints of the assessment: UL for the intake of total/added/free sugars (or otherwise; Figure 1) and risk characterisation. The overall uncertainty will be evaluated by expert judgement using either formal or semi-formal EKE methods (EFSA, 2014; EFSA Scientific Committee, 2018). For the UL of total/added/

free sugars (or otherwise; Figure 1), the weight of evidence (Section 11) and uncertainty analysis may be addressed together in a single EKE procedure. Detailed protocols cannot be specified in advance, but will be established for each stage of the uncertainty analysis before it is performed. Each stage of the process will be documented, including the reasons for any deviations from the protocol.

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Abbreviations

AHA	American Heart Association
AI	adequate intake
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
AUSNUT	Australian food and nutrient database
BIA	bioelectrical impedance analysis
BMI	body mass index
BP	blood pressure
CHD	coronary heart disease
CIGMA	Continuous infusion of glucose with model assessment
CT	computed tomography
CVD	cardiovascular disease
DRV	dietary reference value
DBP	diastolic blood pressure
DXA	dual-energy X-ray absorptiometry
EKE	expert knowledge elicitation

ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FBDG	food-based dietary guidelines
FFQ	food frequency questionnaire
FoodEx2	Standardised food classification and description system developed by EFSA
FSANZ	Food Standards Australia New Zealand
FSIGT	Frequently sampled intravenous glucose tolerance tests
GNPD	Global New Products Database
GNS	German Nutrition Society
HDL-c	high-density lipoprotein cholesterol
HFCS	high-fructose corn syrups
HOMA	homeostasis model assessment
IoM	Institute of Medicine
LDL-c	low-density lipoprotein cholesterol
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAFLD	non-alcoholic fatty liver diseases
NAA	neutron activation analysis
NASH	non-alcoholic steatohepatitis
NDA	EFSA Panel on Nutrition, Dietetic Products and Allergies
NME	non-milk extrinsic
NTP	National toxicology program
OGTT	oral glucose tolerance test
OHAT	Office of health assessment and translation
PAL	physical activity level
PC	prospective cohort studies
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROMETHEUS	PROmoting METHods for Evidence Use in Scientific assessments
QUICKI	Quantitative insulin sensitivity check index
RCT	randomised controlled trial
RI	Reference Intake range
RoB	risk of bias
SBP	systolic blood pressure
SACN	UK Scientific Advisory Committee on Nutrition
SSB	sugar-sweetened beverage
SSD	sugar-sweetened soft drinks
T2DM	type 2 diabetes mellitus
TG	triglycerides
Total-c	total cholesterol
UL	Tolerable upper level of intake
VAT	visceral adipose tissue
VLDL-c	very low-density lipoprotein cholesterol
WG	Working Group
WHO	World Health Organization

Appendix A – Overview of dietary reference values and recommendations

In 2010, in the context of setting dietary reference values for carbohydrates and dietary fibre, the European Food safety Authority (EFSA NDA Panel, 2010a) concluded that the available data **did not allow the setting of a UL for total or added sugars**, neither an Adequate Intake (AI) nor a Reference Intake range (RI). However, evidence on the relationship between patterns of consumption of sugar-containing foods and **dental caries**, **weight gain** and **micronutrient intake** should be considered when establishing nutrient goals for populations and recommendations for individuals and when developing food-based dietary guidelines (FBDG).

The evidence-based Guideline of the German Nutrition Society (GNS) on carbohydrate intake and prevention of nutrition-related diseases (Hauner et al., 2012) recommended reducing the consumption of SSBs, but did **not provide a quantitative limit for sugar intake** or any components of this. The basis for this recommendation was probable evidence that high consumption of SSBs increases the risk of obesity and type 2 diabetes in adults, and the high consumption of SSBs particularly among adolescents and young adults in Germany.

The Nordic Nutrition Recommendations (Nordic Council of Ministers, 2014) limited the intake of **added sugars** (sucrose, fructose and starch hydrolysates) to **< 10% of the total energy intake** for the general population to ensure adequate intakes of micronutrients and dietary fibre (**micronutrient density of the diet**), which was found particularly important for children and persons with a low energy intake. It was also recommended to limit the consumption of SSBs because associated with an increased **risk of type 2 diabetes** and **excess weight gain**, and to avoid frequent consumption of sugar-containing foods to reduce the **risk of dental caries**.

The UK Scientific Advisory Committee on Nutrition (SACN, 2015) recommended that the average population intake of **free sugars** (all monosaccharides and disaccharides added to foods by the manufacturer, cook or consumer, plus sugars naturally present in honey, syrups and unsweetened fruit juices) should not exceed **5% of total energy intake** for age groups from 2 years upwards. Evidence from intervention studies showing that increasing sugars intake **increases energy intake** in individuals consuming an *ad libitum* diet and that SSBs are linked to **weight gain** in children and adolescents, and evidence from prospective cohort studies showing that the consumption of sugars is associated with increased **risk of dental caries** and intake of SSBs are associated with an **increased risk of type 2 diabetes** mellitus were at the basis of this recommendation.

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) issued an opinion on the establishment of recommendations on sugar intake (ANSES, 2016). The opinion focused on the metabolic effects of sugars in food, whether naturally present or added, and their involvement in the development of chronic diseases (metabolic diseases, cancer and cardiovascular diseases). ANSES set an upper intake limit for **total sugars** of **100 g/day** for the adult healthy population, which excludes lactose and galactose naturally present in milk and dairy products. The upper intake limit was calculated from the minimum daily consumption of fructose (50 g) for which a significant increase in **blood concentrations of triglycerides** was observed in intervention studies, and considering that an intake of 50 g of fructose corresponds to an intake of 100 g of sucrose.

The Institute of Medicine of the US National Academy of Sciences (IoM, 2005) concluded that there was **insufficient evidence to set a UL for added sugars** (sugars and syrups that are added to foods during processing or preparation). However, a maximal intake level of **≤ 25% of total energy intake** was suggested to prevent the displacement of foods that are major sources of essential micronutrients (**micronutrient density of the diet**).

The 2015–2020 Dietary Guidelines for Americans (HHS/USDA, 2015) recommended that individuals aged 2 years and older should derive **< 10% of total energy intake** from **added sugars** in order to achieve healthy eating patterns within calorie limits (**micronutrient density of the diet**). This recommendation was based on food pattern modelling and national data on intakes of calories from added sugars.

The World Health Organization (WHO) appraised the evidence available on the effects of **free sugars** on the risk of non-communicable diseases in adults and children, with a particular focus on **weight gain** and **dental caries** (WHO, 2015). The WHO recommended reducing the intake of free sugars to **< 10% of total energy intake** in both adults and children, with a **conditional** recommendation to reduce it further to **< 5% of total energy intake**.

Finally, two professional associations have issued recommendations on sugar intake for children only (up to 18 years of age).

The American Heart Association (AHA) reviewed the scientific evidence on the cardiovascular health effects of **added sugars** in **children** (Vos et al., 2016). Strong evidence was found to support an association between the intake of added sugars and **increased cardiovascular disease risk** in children through **increased energy intake**, **increased adiposity** and **dyslipidaemia**. AHA provided recommendations that **added sugars** (all sugars used as ingredients in processed and prepared foods and sugars eaten separately or added to foods at the table) should be consumed up to a **maximum amount** of **25 g per day** by **children > 2 years** of age and **avoided** by **children < 2 years** of age.

In 2017, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition (Fidler et al., 2017), reviewed the scientific evidence on the relationship between sugars intake and: (a) the development of sweet taste or flavour preference, and (b) health outcomes. The Committee concluded that a preference for sweet taste is driven by interplay of many factors and that the association between high consumption of SSBs in early and late childhood could not be demonstrated to be causal. Building on the conclusions reached by WHO (2015), SACN (2015) and the AHA (Vos et al., 2016) regarding the effect of sugars intake and different health outcomes in paediatric populations, the Committee recommended that intakes of **free sugars** should be reduced and minimised with a desirable **upper limit of < 5% energy intake in children and adolescents** aged 2–18 years. This represents 15–28 g of free sugars for girls and 16–37 g for boys. Intakes should be **even lower in infants and toddlers < 2 years**.

A tabulated overview of these recommendations is given in Table A.1.

Table A.1: Summary of existing recommendations on sugar intake

Guideline	Target population	Sugar fraction	Recommendation	Basis (endpoint)	Other endpoints assessed	Review method
EFSA NDA Panel (2010a)	General population	Added sugars	Consider when setting FBDGs	Dental caries Body weight Micronutrient density	Glucose homoeostasis, risk of T2DM, blood lipids, blood pressure, CVD risk	Narrative
German Nutrition Society (2012)	General population	SSBs	Limit consumption	Obesity Risk of T2DM	BP/hypertension, metabolic syndrome, CHD risk, cancer	Systematic
Nordic Council of Ministers (2014)	General population	Added sugars	< 10E%	Micronutrient density	Dental caries (frequency of intake), weight gain and risk of T2DM (SSBs), glucose homoeostasis, blood lipids, blood pressure, CVD risk, uric acid	Systematic
Health Council of the Netherlands (2015)	General population	SSBs	Limit consumption	Obesity Risk of T2DM	–	Systematic
SACN (2015)	General population (>2 years)	Free sugars	≤ 5E%	Energy intake	Dental caries (frequency of intake), weight gain and risk of T2DM (SSBs), blood lipids, blood pressure, CHD, glucose homoeostasis	Systematic
ANSES (2016)	Adults	Total sugars	100 g/day	Fasting triglycerides	Weight gain, glucose homoeostasis, blood lipids, intrahepatic lipids and risk of NAFLD, uric acid, blood pressure	Systematic
IoM (2005)	General population	Added sugars	< 25E%	Micronutrient density	CHD risk, energy intake, body weight, blood lipids, cancer	Narrative
HHS/USDA (2015)	General population	Added sugars	< 10E%	Micronutrient density	–	Food pattern modelling and national data on added sugars intake
WHO (2015)	General population	Free sugars	< 10E% < 5E% conditional	Body weight Dental caries	–	Systematic

Guideline	Target population	Sugar fraction	Recommendation	Basis (endpoint)	Other endpoints assessed	Review method
American Heart Association (2016)	Children	Added sugars	25 g/day ≥ 2 years Avoided < 2 years	Energy intake Adiposity Dyslipidaemia CVD risk	Micronutrient density, blood pressure, risk of NAFLD, glucose homoeostasis, risk of T2DM	Narrative
ESPGHAN (2017)	Children	Free sugars	≤ 5E% ≥ 2 years (lower for < 2 years)	Dental caries Weight gain (SSBs) CVD and T2DM (fructose)	Preference for sweet taste	Narrative/systematic

FBDG: food-based dietary guidelines; T2DM: type 2 diabetes mellitus; CVD: cardiovascular disease; SSB: sugar-sweetened beverage; CHD: coronary heart disease; NAFLD: non-alcoholic fatty liver diseases.

Appendix B – Systematic reviews and meta-analysis on the relationship between added/free sugars and their sources and surrogate/disease endpoints

A scoping literature search was performed to identify systematic reviews and meta-analysis published in English since 2009 addressing the health effects of added sugars/non-milk extrinsic sugars/free sugars or any of its dietary sources.

The full list of references identified is reported in Table B.1 together with the specific exposure and outcome(s) of interest. All reviews for which the exposure of interest was added, free or total sugars from all dietary sources are presented in Table B.2. Based on the inclusion criteria identified for subquestions 5 and 6 (see Section 9.1), reviews having the same or wider inclusion criteria were used as a basis to update or build new literature searches (see Section 9.2).

Table B.1: Overview of systematic reviews and meta-analysis published since 2009 on the relationship between sugars and their sources and endpoints of interest

Reference	Endpoint (population subgroup)	Exposure
Anderson et al. (2009)	Caries (adults and children)	Sucrose including sucrose-based carbonated soft drinks, baked goods, sweets and table sugar, as added to other foods and drinks
Auerbach et al. (2017)	Obesity (children)	Fruit juice
Avery et al. (2015)	Obesity (children)	SSBs
Bucher Della Torre et al. (2016)	Obesity (children)	SSBs
Chiavaroli et al. (2015)	Blood lipids (adults and children)	Fructose
Chiu et al. (2014)	NAFLD (adults)	Fructose
Chung et al. (2014)	Liver health (adults)	Fructose
Crowe-White et al. (2016)	Obesity (children)	Fruit juice
Fattore et al. (2017)	Blood lipids, blood pressure (adults)	Free sugars (fructose, sucrose and glucose)
Gibson (2008)	Obesity (adults and children)	Sugar-sweetened soft drinks (SSD)
Gibson et al. (2013)	Blood lipids, blood pressure, glucose metabolism (adults)	Sucrose
Greenwood et al. (2014)	Risk of T2DM	SSD
Huang et al. (2014)	Risk of CVD	SSBs
Imamura et al. (2015)	Risk of T2DM	SSB, fruit juice
Jayalath et al. (2014)	Blood pressure	Fructose-containing sugar (high-fructose corn syrup, sucrose and fructose)
Jayalath et al. (2015)	Blood pressure	SSBs containing free or bound fructose
Kaiser et al. (2013)	Obesity (adults and children)	SSBs
Kelishadi et al. (2014)	Blood lipids, blood pressure, glucose metabolism	Fructose

Reference	Endpoint (population subgroup)	Exposure
Keller and Bucher Della Torre (2015)	Blood lipids, Blood pressure, glucose metabolism, risk of CVD	SSBs
Kim and Je (2016)	Blood pressure	SSBs and artificially sweetened beverages
Ma et al. (2016)	Obesity (adults and children)	Fructose, glucose, SSB, HFCS
Malik et al. (2010)	Risk of T2DM	SSBs
Malik et al. (2013)	Obesity (adults and children)	SSBs
Malik et al. (2014)	Blood pressure	SSBs
Mattes et al. (2011)	Obesity (adults and children)	SSBs
Moynihan and Kelly (2014)	Dental caries (adults and children)	Total sugars, free sugars, added sugars, sucrose, NME sugars
Perez-Morales et al. (2013)	Obesity (children)	SSBs
SACN (2015)	Blood lipids, blood pressure, dental caries, glucose metabolism, obesity, risk of CVD, risk of T2DM	Total carbohydrate, sugars reported as a nutrient (fructose, sucrose, lactose, glucose), table sugar and other extrinsic sugars (syrups), food format (solid vs. liquid, which includes SSBs)
Singh et al. (2011)	Risk of gout (adults)	SSBs, fructose
Sonestedt et al. (2012)	Blood lipids, blood pressure, glucose metabolism, risk of CVD, risk of T2DM	SSBs, sugars, sucrose and fructose
Te Morenga et al. (2013)	Obesity (adults and children)	Free sugars
Te Morenga et al. (2014)	Blood lipids, blood pressure	Sugar (sucrose) or free sugars
David Wang et al. (2014)	Blood lipids	Fructose
Wang et al. (2014)	Glucose metabolism (adults)	Fruit juice
Xi et al. (2014)	Risk of T2DM	Fruit juice
Xi et al. (2015)	Blood pressure, risk of CVD	SSBs
Zheng et al. (2015)	Obesity (adults and children)	SSBs

SSB: sugar-sweetened beverage; NAFLD: non-alcoholic fatty liver diseases; T2D: type 2 diabetes mellitus; CVD: cardiovascular disease; HFCS: high-fructose corn syrup.

Table B.2: Selected systematic reviews with research question partially or completely overlapping with the present work

Systematic review	Subquestion 4					Subquestion 5
	Fattore et al. (2017)[(a)]	**SACN (2015)**	**Sonestedt et al. (2012)**	**Te Morenga et al. (2013)**	**Te Morenga et al. (2014)**	**Moynihan and Kelly (2014)**
Endpoints	BP, blood lipids and body weight	All	Glucose metabolism, BP, blood lipids, risk of T2D, risk of CVD	Obesity	BP, blood lipids	Caries
Databases	PubMed/MEDLINE, EMBASE, Cochrane Library Hand search	Medline MEDLINE In-Process & Other Non-Indexed Citations Embase CAB Abstracts ISI Web of Science BIOSIS The Cochrane Library Hand search	PubMed SveMed+	OVID Medline, Embase PubMed, CiNAHL, Scopus, Web of Science	OVID Medline, Embase PubMed, CiNAHL, Scopus, Web of Science Grey literature Hand search	MEDLINE, EMBASE, Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, LILACS, CNKI, Wanfang South African Department of Health databases
Search dates	Up to 22 October 2015	1990–December 2010	January 2000–October 2010Update November 2010–December 2011	Up to December 2011	1960–August 2013	1950–November 2011
Language limit	English	English	English or a Nordic language	English	English	No limit
Study type	Intervention studies	RCT PC	RCT PC	RCT PC	RCT	Intervention, cohort, population or cross-sectional
Study duration	≥ 2 weeks	RCT > 6 weeks (≥ 3 days for energy intake/satiety) PC > 3 years	RCT ≥ 4 weeks (drop out ≤ 50%) PC ≥ 4 years	RT ≥ 2 weeks PC ≥ 1 year	RCT≥ 2 weeks	
Population	Adults	Adults/children	Adults/children	Adults/children	Adults/children	Adults/children

BP: blood pressure; T2D: type 2 diabetes; CVD: cardiovascular disease; RCT: randomised controlled trial; PC: prospective cohort studies.
(a): Not used as primary source of information as the endpoints were assessed under isocaloric conditions.

Appendix C – Questionnaire to National Competent Authorities of European countries

NAME: ☐

COUNTRY: ☐

ALIGFFILIGLIATION: ☐

E MAIL: ☐

DATE: ☐

- EFSA has been requested to provide a scientific opinion on the Tolerable Upper Intake Level of dietary sugars. The assessment concerns the main types of sugars (mono- and disaccharides) found in mixed diets (i.e. glucose, fructose, galactose, sucrose, lactose, maltose and trehalose). Sugar alcohols (polyols), other substances used as sugar replacers, and other mono- or disaccharides present in the diet in marginal amounts, are not included in the term "sugars" for the purpose of this assessment. In this context, EFSA will address:
  - **Total sugars:** i.e. the monosaccharides glucose, fructose, and galactose, and the disaccharides sucrose, lactose, maltose and trehalose present in foods;
  - **Added sugars:** sugars used as ingredients in processed and prepared foods and sugars eaten separately or added to foods at the table; and
  - **Free sugars:** added sugars plus sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates.

In the context, EFSA would like, through this questionnaire, to gather information on the following:

a) Data available from national food consumption surveys (or regional food consumption surveys e.g. targeting specific population groups such as infants or pregnant women) on the intake of **total/added/free sugars**;
b) National food composition data on **total, added and free sugars** if available, together with a description of the methods used to estimate **added** and **free sugars** in foods;
c) National dietary recommendations on the amount of sugars (whether total, added or free) to be consumed in the diet, if available.

To answer this questionnaire, please tick the relevant boxes. If you have doubts or queries in relation to the compilation of this questionnaire, please contact us at: nda@efsa.europa.eu

1) Please specify the last national food consumption survey(s) carried out in your country, indicating the year (or time frame) in which the survey was conducted, the method used for data collection (food diaries, food records, 24-h dietary recalls, other), the number of days in which data was collected for each subject, and the age group(s) included in the survey. Regional food consumption surveys should only be indicated if they targeted specially infants (up to 12 months of age) or pregnant women. Food consumption surveys using food–frequency questionnaires for data collection should not be included.

Name of survey	Type of survey (National/regional)	Year (range)	Method for data collection	Number of days/subject	Age groups included

Add as many rows as needed.

2) Please indicate whether there are publications available (in any language) on the intake of total sugars, added sugars, and/or free sugars in your country at national level (i.e. from the most recent national food consumption surveys that you have listed in question 1):

Author/year	Total sugars	Added sugars	Free sugars
	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no

Add as many rows as needed.

Please provide the full list of references, as well as the full text if available to you ☐

Note: *Should you wish to share data not published yet but soon to be (i.e. on a confidential basis/embargo until publication), please specify the foreseen/assumed date of publication, and note that only data published by March 2019 will be accepted. Please also specify if you have anything against sharing unpublished data with the Experts of the Working Group on Sugar, for the purposes of this risk assessment.*

If NO publications are available on the intake of total sugars, added sugars, and/or free sugars in your country at national level → please go directly to question 5.

3) The national food composition database used to calculate the intake of total sugars, added sugars, and/or free sugars in the afore-mentioned publications contains data on:

Total sugars ☐ yes ☐ no

Glucose ☐ yes ☐ no

Fructose ☐ yes ☐ no

Sucrose ☐ yes ☐ no

Lactose ☐ yes ☐ no

Other sugars ☐ yes ☐ no. If yes, please specify ☐

Please provide the link to a website where the database can be downloaded from or a contact address/details of the person(s) responsible for the maintenance/update of the database ☐

4) Your national food composition database contains information on:

Added sugars ☐ yes ☐ no

Free sugars ☐ yes ☐ no

If the answer to any of the above is yes, please specify in detail the methodology that has been used to estimate the content of added sugars and/or free sugars in foods ☐

5) Does your country have some type of national recommendations on the amount of sugars to be consumed in the diet?

☐ yes ☐ no

If the answer is no → please go directly to question 9.

6) Please specify the national dietary recommendations to limit the amount of sugars consumed in the diet available in your country, indicating the type of recommendation (e.g. in the context of setting dietary reference values for nutrients, in the context of developing Food–Based Dietary Guidelines (FBDG)) and who was involved in their development (government bodies, scientific societies, industry, non-profit organisations, other), and the year in which the recommendation was issued. Please provide a link to the full text of the recommendation, if available. If more than one national recommendation is available in your country (e.g. established by different bodies; FBDGs targeting only specific age groups), use as many rows as needed.

a) National dietary recommendation: ☐ Body/organisation ☐ Year ☐

b) National dietary recommendation: ☐ Body/organisation ☐ Year ☐

c) National dietary recommendation: ☐ Body/organisation ☐ Year ☐

7) National recommendations on the consumption of dietary sugars (total, added and/or free) are directed to:

Please note that age ranges below are only indicative

☐ General population
☐ Old adults (>65 years)
☐ Adults (18-65 years)
☐ Adolescents (14-18 years)
☐ Others ☐
☐ Infants (up to 12 mo)
☐ Young children (1-3 years)
☐ Pre-school children (3-6 Years)
☐ Schoolchildren (6-14 years)
☐ Pregnant women
☐ Lactating women

8) Which diet-related health problems were considered when developing recommendations for the intake of total, added and/or free sugars consumption in your country? Please tick as many as needed:

☐ Cardiovascular diseases
☐ Dyslipidaemia
☐ Hypertension
☐ Diabetes
☐ Nutrient deficiencies
☐ Others (please specify) ☐
☐ Cognitive impairement
☐ Dental caries
☐ Overweight/obesity
☐ Cancer
☐ Adverse pregnancy outcomes
☐ None

9) Please add any general or specific comment, you might have: ☐

We kindly ask you to send back the filled survey by e-mail to: nda@efsa.europa.eu

Thank you very much for your participation in this survey

Appendix D – Exposure and endpoints search terms for subquestions 4 and 5

Subject index terms, where available, will be combined with free-text terms. Free-text terms will be searched in title and abstract fields in Embase and PubMed; and in title, abstract and keyword fields in the Cochrane Library databases and Scopus.

Exposure

Free sugars		
Subject index terms[6]		**Free-text terms[7] (Cochrane Library, Embase, PubMed, Scopus)**
MeSH (Cochrane Library, PubMed)	**Emtree (Embase)**	
"Monosaccharides"[Mesh:noexp] "Glucose"[Mesh:noexp] "Fructose"[Mesh] "Galactose"[Mesh] "Disaccharides"[Mesh:noexp] "Sucrose"[Mesh:noexp] "Lactose"[Mesh] "Trehalose"[Mesh] "Maltose"[Mesh] "Dietary Sugars"[Mesh] "Dietary Sucrose"[Mesh] "High Fructose Corn Syrup"[Mesh] "Honey"[Mesh] "Molasses"[Mesh] "Carbonated Beverages"[Mesh] "Energy Drinks"[Mesh] "Fruit and Vegetable Juices"[Mesh] "Beverages/adverse effects"[Mesh] "Candy"[Mesh] "Chocolate"[Mesh]	'sugar intake'/exp 'glucose intake'/exp 'fructose intake'/exp 'lactose intake'/exp 'sugar'/exp 'monosaccharide'/de 'glucose'/exp 'fructose'/exp 'galactose'/exp 'disaccharide'/de 'sucrose'/exp 'maltose'/exp 'lactose'/exp 'trehalose'/exp 'syrup'/exp 'honey'/exp 'molasses'/exp 'sweetened beverage'/exp 'soft drink'/exp 'energy drink'/exp 'sports drink'/exp 'fruit and vegetable juice'/exp 'carbonated beverage'/exp 'confectionary'/de 'sugar confectionary'/exp	Sugar* OR Sucrose* OR Fructose* OR Galactose* OR Lactose* OR Trehalose* OR Maltose* OR Glucose* + dieta* OR diete* OR diet OR diets OR intake* OR consum* OR feed* OR food OR foods OR supplement* Disaccharide* Di saccharide* Monosaccharide* Mono saccharide* Simple carbohydrate* Refined carbohydrate* Syrup* Honey Candy Candies Sweet Sweets Sweetened Pastr* Confection* Patisserie Soft + drink* OR beverage* Softdrink* Fizzy + drink* OR beverage* Carbonated + drink* OR beverage* Soda + drink* OR beverage Energy + drink* OR beverage* Sports + drink* OR beverage* SSBs OR SSDs + beverage OR drink SSB OR SSD + beverage OR drink Juice* Smoothie*

[6] [Mesh] indicates that the MeSH term will be exploded, including in the search the terms below in the MesH hierarchy if available. [Mesh:noexp] indicates that the MesH term will not be exploded, the terms below in the MeSH hierarchy will not be searched. /exp indicates that the Emtree term will be exploded, including in the search terms below in the Emtree hierarchy if available. /de indicates that the Emtree term will not be exploded, the terms below in the Emtree hierarchy will not be searched.

[7] Asterisk symbol '*' indicates truncation. Plus sign '+' indicates the search terms will be linked with the Boolean operator AND.

Endpoints

Adipose tissue		
Subject index terms		**Free-text terms (Cochrane Library, Embase, PubMed, Scopus)**
MeSH (Cochrane Library, Pubmed)	**Emtree (Embase)**	
"Adipose Tissue"[Mesh:noexp] "Abdominal Fat"[Mesh:noexp] "Intra-Abdominal Fat"[Mesh] "Subcutaneous Fat, Abdominal"[Mesh] "Subcutaneous Fat"[Mesh] "Body Weights and Measures"[Mesh:noexp] "Body Fat Distribution"[Mesh] "Adiposity"[Mesh] "Body Mass Index"[Mesh] "Body Size"[Mesh:noexp] "Body Weight"[Mesh: noexp] "Body Weight Changes"[Mesh] "Weight Gain"[Mesh] "Weight Loss"[Mesh]Overweight[Mesh] Obesity[Mesh:noexp] "Obesity, Morbid"[Mesh] "Pediatric Obesity"[Mesh] "Obesity, Abdominal"[Mesh] "Sagittal Abdominal Diameter"[Mesh] "Waist Circumference"[Mesh] "Body Composition"[Mesh] "Body Constitution"[Mesh:noexp]	'adipose tissue'/de 'abdominal fat'/exp 'abdominal subcutaneous fat'/exp 'intraabdominal fat'/exp 'body fat'/exp 'body fat distribution'/exp 'fat pad'/exp 'weight, mass and size'/de 'body weight'/de 'lean body weight'/exp 'body weight change'/exp 'body weight fluctuation'/exp 'body weight gain'/de 'body weight loss'/exp 'body weight variation'/exp'obesity'/exp 'body mass'/exp 'body size'/exp 'sagittal abdominal diameter'/exp 'waist circumference'/exp 'body composition'/de 'body distribution'/exp 'body constitution'/exp	Adipos* Fat pad Fat pads Body fat* Fatty tissue* Body size Abdominal fat Intra-abdominal fat Intraabdominal fat Fat distribut* Ectopic fat Waist circumference* Abdominal diameter Obese* Obesi* Obeso* Overweight* Weight + gain OR loss OR chang* OR reduc* OR maint* OR watch* OR variation OR control* OR Body OR lean Body mass Bmi Body composition* Body constitution*

Glucose homeostasis		
Subject index terms		**Free-text terms (Cochrane Library, Embase, PubMed, Scopus)**
MeSH (Cochrane Library, Pubmed)	**Emtree (Embase)**	
"Diabetes Mellitus, Type 2"[Mesh]"Hyperinsulinism"[Mesh:noExp] "Insulin Resistance"[Mesh] "Metabolic Syndrome"[Mesh] "Blood Glucose"[Mesh] "Insulin/blood"[Mesh] "Hyperglycemia"[Mesh] "Glucose Intolerance"[Mesh] "Carbohydrate Metabolism"[Mesh] "Glycated Hemoglobin A"[Mesh] "Fructosamine"[Mesh] "Metabolic Diseases"[Mesh:NoExp]	'non insulin dependent diabetes mellitus'/exp'hyperinsulinism'/exp 'hyperinsulinemia'/exp 'insulin resistance'/exp 'metabolic syndrome X'/exp 'glucose blood level'/exp 'insulin'/exp AND 'blood'/exp 'hyperglycemia'/exp 'glucose intolerance'/exp 'hemoglobin A1c'/exp 'fructosamine'/exp 'fructosamine blood level'/exp 'metabolic disorder'/de	Diabet* + type 2 OR type II OR type2 OR typeii Late OR adult* OR matur* OR slow* OR stabl* + onset + diabetesNon-insulin-depend* + diabetes Noninsulin depend* + diabetes Hyperinsulinism Hyperinsulinemia Insulin + resistan* OR sensitivity OR tolerance OR intolerance OR control OR fasting Metabolic syndrome Glucose + tolerance OR intolerance OR fasting OR blood Hyperglycemia* Glycated OR Glycosylated + Hemoglobin OR haemoglobin Hemoglobin A Haemoglobin A Hemoglobin A1c Haemoglobin A1C Hemoglobin Aic Haemoglobin AiC HbA1c HbA(1c) HbA1 HbA 1c Hb A1c Hb a 1c Fructosamine + blood OR serum OR plasma

Cardiovascular system		
Subject index terms		**Free-text terms (Cochrane Library, Embase, PubMed, Scopus)**
MeSH (Cochrane Library, Pubmed)	**Emtree (Embase)**	
"Cardiovascular Diseases"[Mesh] "Stroke"[Mesh] "Hypertension"[Mesh] "Prehypertension"[Mesh] "Atherosclerosis"[Mesh] "Ischemic Attack, Transient"[Mesh] "Heart Diseases"[Mesh] "Myocardial Ischemia"[Mesh] "Angina Pectoris"[Mesh] "Acute Coronary Syndrome"[Mesh] "Myocardial Infarction"[Mesh] "Non-ST Elevated Myocardial Infarction"[Mesh] "ST Elevation Myocardial Infarction"[Mesh] "Coronary Disease"[Mesh] "Cardiovascular System"[Mesh] "Blood Pressure"[Mesh] "Cholesterol"[Mesh:noExp] "Cholesterol, HDL"[Mesh] "Cholesterol, LDL"[Mesh] "Cholesterol, VLDL"[Mesh] "Dyslipidemias"[Mesh:noExp] "Hyperlipidemias"[Mesh:noExp] "Hypercholesterolemia"[Mesh] "Hyperlipoproteinemias"[Mesh] "Lipids "[Mesh] "Triglycerides"[Mesh] "Lipoproteins"[Mesh:NoExp] "Apolipoproteins"[Mesh]	'cardiovascular disease'/exp 'coronary artery disease'/exp 'cerebrovascular accident'/exp 'atherosclerosis'/exp 'transient ischemic attack'/exp 'heart infarction'/exp 'non ST segment elevation myocardial infarction'/exp 'ST segment elevation myocardial infarction'/exp 'acute coronary syndrome'/exp 'abnormal blood pressure'/de 'hypertension'/exp 'prehypertension'/exp 'heart disease'/exp 'angina pectoris'/exp 'heart death'/exp 'congestive heart failure'/exp 'cardiovascular system'/exp 'blood pressure'/exp 'cholesterol'/de 'cholesterol ester'/exp 'high density lipoprotein cholesterol'/exp 'low density lipoprotein cholesterol'/exp 'very low density lipoprotein cholesterol'/exp 'cholesterol metabolism'/exp 'disorders of cholesterol metabolism'/exp 'dyslipidemia'/exp 'hyperlipidemia'/exp 'hypercholesterolemia'/exp 'hypertriglyceridemia'/exp 'lipid blood level'/exp 'cholesterol blood level'/exp 'triacylglycerol blood level'/exp 'triacylglycerol'/exp	CV disease* CVD CVDs CHD CHDs Cardiovascular OR coronary OR heart OR cardiac + disease* OR disorder* OR event* OR risk* OR complication* OR outcome* OR morbidit* OR mortalit* OR death* OR failure* Stroke* Cerebrovascular accident* Apoplex* Acute coronary syndrome Angina* Stenocardia Heart muscle OR cardiac muscle OR myocardial OR myocardium OR cardiac OR coronary OR heart OR transient OR cardiomyophath* + ischemi* OR ischaem* Myocardial infarct* Heart attack* STEMI NSTEMI Blood pressure Arterial pressure Diastolic Systolic Blood pressure Prehypertens* Hypertens* Atherosclero* LDL-C HDL-C Cholesterol Hypercholesterol* Hypertriglycer*

	'lipoprotein'/de 'apolipoprotein B100'/exp 'apolipoprotein A1'/exp	Dyslipidemi* Dyslipoproteinemi* Hyperlipidemia* Hyperlipemi* Lipidemi* Lipemi* Hyperlipoprotein* Lipid Lipids Lipoprotein* Triglycerid* triacylglycerol Fasting TG Apolipoprotein* ApoB100 ApoB Apo B Apo B100 ApoA1 ApoA ApoAi Apo A Apo A1 Apo Ai

Liver function		
Subject index terms		**Free-text terms (Cochrane Library, Embase, PubMed, Scopus)**
MeSH (Cochrane Library, Pubmed)	**Emtree (Embase)**	
"Fatty Liver"[Mesh:noexp] "Non-alcoholic Fatty Liver Disease"[Mesh] "Liver Cirrhosis"[Mesh:NoExp] "Liver Failure"[Mesh]	'liver fat'/exp 'fatty liver'/de 'nonalcoholic fatty liver'/exp 'liver cirrhosis'/exp 'liver fibrosis'/exp 'liver failure'/exp	Fatty liver NAFLD Steatohepatiti* Steatohepatiti* NASH Steatos* Fat liver accumul* Cirrhos* OR Fibros* OR failure* OR insufficienc* + liver OR Hepatic

Dental caries		
Subject index terms		**Free-text terms**
MeSH (Cochrane Library, Pubmed)	**Emtree (Embase)**	**(Cochrane Library, Embase, PubMed, Scopus)**
"Oral health"[Mesh] "Dental Caries"[Mesh] "Cariogenic Agents"[Mesh] "DMF Index"[Mesh] "Diet, Cariogenic"[Mesh]	'dental health'/exp 'dental caries'/exp 'cariogenic agent'/exp 'cariogenic diet'/exp 'caries assessment'/exp 'DMF index'/exp 'DMFS index'/exp 'DMFT index'/exp	Oral health Dental health Caries Carious Cariogen* Dental OR teeth OR tooth OR root + decay* OR white spot* OR cavit* DMF DMFT DMFS DFT DEFT DEFS

Pregnancy outcomes		
Subject index terms		**Free-text terms**
MeSH (Cochrane Library, Pubmed)	**Emtree (Embase)**	**(Cochrane Library, Embase, PubMed, Scopus)**
"Birth Weight"[Mesh] "Fetal Macrosomia"[Mesh] "Fetal Weight"[Mesh] "Gestational Age"[Mesh] "Infant, Low Birth Weight"[Mesh] "Infant, Small for Gestational Age"[Mesh] "Infant, Very Low Birth Weight"[Mesh] "Infant, Extremely Low Birth Weight"[Mesh] "Diabetes, Gestational"[Mesh] "Pregnancy in Diabetics"[Mesh]	'birth weight'/exp 'high birth weight'/exp 'low birth weight'/exp 'small for date infant'/exp 'very low birth weight'/exp 'extremely low birth weight'/exp 'fetus weight'/exp 'gestational age'/exp 'tall stature'/de 'macrosomia'/exp 'large for gestational age'/exp 'pregnancy diabetes mellitus'/exp	Weight + birth OR newborn* OR new born* OR nenonat* OR fetal OR foetal OR fetus OR foetus Size + birth OR newborn* OR new born* OR neonat* OR fetal OR foetal OR fetus OR foetus Macrosomia* Macrosomatia* SGA OR LGA + infant* OR neonat* OR newborn* OR child* OR baby OR babies Gestational age OR gestation age OR gestational time OR gestation time "small for date" OR "large for date" + infant* OR neonat* OR newborn* OR child OR children OR baby OR babies Gestational OR pregnant* OR pregnanc* + diabetes DGM

Substance	*ID Code*	*Rpt No.*	*Year*	*Conclusion**	*21 CFR Section*
Sucrose	57-50-1	69	1976	2	184.1854

SCOGS Opinion:

Sucrose is the standard of naturally occurring sweetness, joining other nutrients usually carbohydrate in nature, that comprise a group of palatable foodstuffs known to be relatively efficient sources of energy, simple in composition and rapidly metabolizable for utilization and storage. Sucrose has been used routinely since antiquity to improve the palatability of food preparations.

By all conventional tests, sucrose is a substance of extremely low acute toxicity. Consumption of sucrose in large amounts or at frequent intervals contributes to the development of dental caries. Over consumption of sucrose probably contributes to obesity and possibly results in dietary imbalances and in modification of lipid metabolism which potentiates coronary heart disease. Tenuous relationships beteween sucrose ingestion and diabetes mellitus and other disieases also have been suggested. The possibility that sucrose may be involved in such deleterious effects continues to stir controversy, as is evident by the size of the scientific and popular literature on sugar in the human diet and the appearance of new research findings and concepts. Consequently, broad generalizations based upon the inconclusive evidence now available must be made and viewed with caution.

One of the important facts is that sucrose is both a significant natural constituent of food and a major additive to foods and beverages. It is commonly used as such by the consumer and added by food processors as a component of various foods. While per capita consumption of sucrose has changed little in the United States over the past 50 years, it is also true that about 70 percent of the per capita intake is now contributed by processed foods. This situation makes it difficult to exercise individual choice in the selection of a low sucrose diet.

Unlike most other foods, sucrose furnishes virtually only energy. While sucrose makes a substantial contribution to dietary caloric needs, in excessive amounts its effect on the intake of other nutrients may result in nutritional imbalances and, at least marginal, dietary deficiencies. Since over 15 percent of the per capita caloric intake of the population in the United States is from sucrose, it is likely that some individuals may eat enough to exclude adequate amounts of other foods that furnish required nutrients.

Findings linking ingestion of sucrose with diabetes are essentially circumstantial. There is no plausible evidence that sucrose, except as it is a non-specific source of excessive calories, is related to the disease. In those experiments in which impaired glucose tolerance was measured, highly distorted dietary patterns and excessive sucrose intakes were required.

The experimental evidence associating sucrose with cardiovascular disease is also less than clear. It seems likely that

the observed hyperlipidemic effects of hight levels of sucrose in the diet of animals and man are due primarily to its relatively rapid rate of hydrolysis and absorption and that any differences between the metabolism of its hydrolytic products, glucose and fructose, are of questionable significance. There is no evidence that ingestion of sucrose in the concentrations that occur in the average diet causes significant elevations in blood cholesterol or other lipids. Furthermore, it would appear that the primary dietary factors involved in cardiovascular disease are the nature and amount of fat in the diet. Thus, the role of sucrose in cardiovascular disease appears to be secondary although it may represent a potentiating factor in its etiology.

Of all the carbohydrates tested, sucrose is among the most cariogenic. Individuals who assiduously avoid consumption of sucrose because of an inborn error of metabolism- fructose intolerance- generally have little or no dental caries. However, dental caries can and do occur in people who have never used sugar or processed foods. Various factors affect the cariogenicity of sucrose and other foods. These include frequently and duration of exposure, age of the subject, and stickiness of the sugar or materials with which it is consumed. Honey and figs, for example, are highly cariogenic and pregelatinized starches also are conductive to the development of dental caries. The significant effects of between-meal eating in the frequency and severity of dental caries has been demonstrated. Protection against dental caries is facilitated by limitation of the frequency of consumption of sucrose and other cariogenic foods. Informing the consumer of the sugar content of foods by appropriate labeling could lead to judicious selection of sweetened foods. Choices could be made easier with a greater selection of less sugared foods in the market place.

In light of all of the foregoing, the Select Commitee concludes that:

1. Reasonable evidence exists that sucrose is a contributor to the formation of dental caries when used at levels that are now current and in the manner now practiced.
2. Other than the contribution made to dental caries, there is no clear evidence in the available information on sucrose that demonstrates a hazard to the public when used at the levels that are now current and in the manner now practiced. However, it is no possible to determine without additional data, wether an increase in sugar consumption that would result if there were a significant increase in the total of sucrose, corn sugar, *corn syrup, *and invert sugar, *added to foods would constitute a dietary hazard.

*Health aspects of corn sugar (dextrose), corn syrup, and invert sugar are evaluated in a report of the Select Committee.

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.

SUCROSE

CAS: 57-50-1; Chemical Formula: $C_{12}H_{22}O_{11}$

The former OSHA 8-hour TWA limit for sucrose was 15 mg/m^3 as total particulate, the Agency's generic limit for all particulates. The ACGIH includes sucrose in its grouping of particulates that "do not produce significant organic disease or toxic effect when exposures are kept under reasonable control" (ACGIH 1986/Ex. 1-3) and has a TLV-TWA limit of 10 mg/m^3 for sucrose as total particulate containing no asbestos and less than 1 percent quartz; this is also the limit OSHA proposed for this substance. The final rule, however, retains the 15-mg/m^3 total particulate and the 5-mg/m^3 respirable fraction TWA limits for sucrose, which is found in the form of white crystals.

Exposure to excess levels of sucrose dust can cause skin and eye irritation, interference with vision, and distraction from the task at hand.

OSHA is retaining the 8-hour total particulate TWA of 15 mg/m^3 for sucrose and is also retaining the 5-mg/m^3 respirable fraction limit. The Agency concludes that these limits protect exposed workers against the significant risk of physical irritation.

Sucrose

(CAS No: 57-50-1)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

No. 2000/15OSH/140 The Hague, November 9, 2004

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1 Introduction

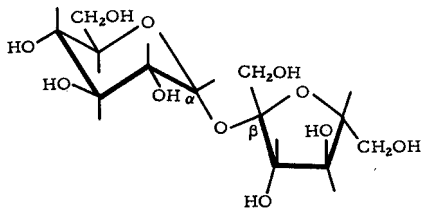
The present document contains the assessment of the health hazard of sucrose by the Committee on Updating of Occupational Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by MA Maclaine Pont, M.Sc. (Wageningen University and Research Centre, Wageningen, the Netherlands).

In April 1999, literature was searched in the databases Medline, Toxline, and Chemical Abstracts, starting from 1966, 1981, and 1937, respectively, and using the following key words: saccharose; sucrose; α-D-glucopyranoside, ß-D-fructofuranosyl-; and 57-50-1.

In July 2000, the President of the Health Council released a draft of the document for public review. No comments were received.

An additional search in Toxline and Medline in September 2004 did not result in information changing the committee's conclusions.

2 Identity

name	:	sucrose
synonyms	:	saccharose; α-D-glucopyranoside, ß-D-fructofuranosyl-; beet sugar; cane sugar; confectioner's sugar; ß-D-fructofuranoside, α-D-glucopyranosyl-; sugar
molecular formula	:	$C_{12}H_{22}O_{11}$
structural formula	:	
CAS number	:	57-50-1

3 Physical and chemical properties

molecular weight	:	342.30
melting point	:	decomposes at 160-186°
boiling point	:	-
flash point	:	-
vapour pressure	:	negligible
solubility in water	:	very soluble
log $P_{octanol/water}$	:	-3.70 (experimental); -4.27 (estimated)
conversion factors	:	not applicable

Data from ACG92, NLM04, http://www.syrres.com/esc/est_kowdemo.htm.

Sucrose is a disaccharide composed of D-glucosyl and D-fructosyl moieties. It forms hard, white, dry crystals, lumps, or powder. It has a sweet taste and is odourless (ACG92).

Sucrose is a normal ingredient of the human diet. The human body can metabolise the compound and use its energy. A high intake can cause caries. Sucrose is present in large quantities in sugar beet and sugar cane. Occupational exposure occurs mainly in sugar factories and sugar refineries. Certain, not well-known circumstances in storage can cause severe explosions of the dust (Kop39).

4 Uses

Sucrose is used as a sweetening agent and food. It is the starting material in the fermentative production of ethanol, butanol, glycerol, and alcoholic beverages. It is used in pharmacy as a preservative, in the plastics and cellulose industry, and in the manufacture of ink and transparent soaps (ACG92).

5 Biotransformation and kinetics

Based on physical and chemical constants, Barratt calculated a human skin permeability coefficient of 5.25 x 10^{-6} cm/hour (Bar95). The committee concludes that skin penetration of sucrose is negligible.

The absorption of sucrose from animal lung was investigated by intratracheal (cannula) administration of ^{14}C-labelled compound as an aerosol or as a solution. Following administration of an aerosolised solution containing 1 mM sucrose

and 100 mM *p*-aminohippuric acid (mass mean aerodynamic diameter: 2.55±0.04 µm; geometric standard deviation: 2.49±0.02 µm) to anaesthetised rats, mice, and rabbits, for 5 minutes, the time to absorb 50% of the sucrose administered was calculated to be 41.5, 11.5, and 69.3 minutes, respectively (Sch86). When injected dissolved in Krebs-Ringer phosphate solutions, the absorption half-lives were 84-87, 23.6, and 173 minutes in rats, mice, and rabbits, respectively. The finding that the absorption rates of injected solutions in rats were directly proportional to the concentration over a range of 0.1-100 mM suggested that sucrose was absorbed by simple diffusion (Enn72, Sch83, Sch86).

6 Effects and mechanism of action

Human data

Dermal effects

Skin problems have been observed in sugar artists. These workers form decorative objects from sugar, heated to around 53ºC. The main skin problems at the hands were increased sweating, seen in 20 of a group of 30 (67%), and burning with erythema and blistering, seen in 12 out of 30 (40%). Four suffered from a relapsing type of chronic eczema (13.3%). The authors conclude that skin problems in sugar artists are mainly heat related and that allergic contact dermatitis is rare (Ban96).

Also, in a group of 71 female confectioners exposed to flour dust and aerosols of sugar, starch, egg powder, nuts, cocoa, cacao, and chocolate, the incidence of allergic reactions to sugar, measured with a skin prick test, was low: 2% (Zus94).

Respiratory effects

In a sugar cane production plant in Nigeria, no increase in respiratory diseases was found among the workers (n=335), compared with a control group (n=300) consisting of male inhabitants of the same area, who never worked in the sugar factory, viz., mainly traders, office workers, and teachers. On the contrary, the frequency of occurrence of cough alone, cough with sputum, morning phlegm, nasal catarrh, and chest pain was higher in the control group than in the exposed group ($p<0.05$). No data on exposure levels were presented (Tan96).

Pathogenic spores can be present in the air of sugar-processing plants (For89). On the other hand, the number of spores of the bacteria

Thermoactinomyces sacchari in 2 Australian cane sugar mills was too low to cause bagassosis (a form of allergic alveolitis) (Daw96).

In the beet sugar industry, workers are also overexposed to calcium oxide, carbon monoxide, respirable coal dust, and sulphur dioxide. In the packaging area, the sugar dust concentration exceeded the TLV of 10 mg/m^3 for nuisance dust (Man90).

There was a high prevalence of acute respiratory symptoms in confectioners (n=71) during the work shift, especially cough, dyspnoea, eye burning, and dryness of the throat and nose. However, no significant association with immunological tests was found (Zus94). Therefore, the committee concludes that there was no evidence of sensitisation.

Workers exposed to sugar dust in a sugar-cube manufactory (n=40) had significantly lower forced expiratory volume in one second (FEV_1) compared with workers not involved in sugar-cube manufacturing (n=98; p=0.02) or with the 'pooled remaining groups' (non-exposed workers, laboratory and office workers; n=116; p=0.009) after adjustment for smoking. Forced vital capacity (FVC), FEV_1/FVC, and the maximal flow at 50% of the FVC (V_{max50}) were not affected. The proportion of subjects complaining of cough and/or phlegm was higher in the exposed group (28%) than in the non-exposed group (16%); however, the difference was not statistically significant, even after comparison between exposed workers and the 'pooled remaining groups' after adjusting for smoking in a logistic regression (p=0.25). The sugar dust concentration was not measured (Boh96).

A single case of occupational asthma has been reported in a 33-year-old worker in a beet sugar-processing plant. In a bronchoprovocation test, the patient responded positively to mouldy but not to fresh beet sugar pulp (Ros92).

Dental effects

Caries was already recognised as an occupational disease at the beginning of the 20th century. It occurred among bakers, confectioners, and workers in the chocolate and honey biscuit industry. In these occupations, there was not only exposure to sugar, but also to flour. Kunert visited 150 workshops in Wroclaw (Poland; at that time Germany), and examined the dental health status of 726 workers. Part of these workers were divided into 4 categories of expected low to high exposure to sugar: I: bakers, baking only brown and white bread (n=67), II: bakers not further specified, having little exposure to sugar (n=67), III: confectioners within bakeries (n=28), and IV: confectioners (n=104). Further, there were 3 control groups: millers (n=70), shoemakers (n=51), and butchers

(n=44). The percentages of healthy teeth in the exposed groups decreased from 69.1, 55.1, 42.4% to 36.9%, respectively, and those of caries increased from 14.0, 20.5, 24.2% to 28%, respectively. In the control groups, incidences of healthy teeth were 62.5, 70.2, and 71.8%, respectively, and of caries 9.6, 9.7, and 9.3%, respectively. The concentration of sugar dust in the air was not measured (Kun01). The committee could not make statistical calculations because of lack of individual data.

A statistically significant increase in caries was found in a group of workers of a biscuit-production line (n=49) when compared with a control group of employees not exposed to sugar or flour dust (n=74) after age adjustment (73.0% vs. 60.7%; $p<0.05$). No increase in caries was found in a group of workers of a sweet- or bakery-production line (n=117 and 58, respectively). The levels of airborne sugar dust were 3 mg/m^3 or lower in 25/27 samples. In the 2 other samples, sugar concentrations were 18 and 21 mg/m^3. Masalin et al. concluded that these low sugar dust concentrations do not represent a danger to dental health, and, therefore, other factors must be the cause of the increase in dental caries. Since caries is multifactorial in nature, it is impossible to give a specific threshold value above which caries might appear. Consumption levels of 2.5-3.0 g/day or of 15 kg/year might be 'cut-off' points for high-caries and low-caries incidence. The average sugar consumption of American and European children is between 110 and 160 g/day (Mas88, Mas90).

In a group of 59 workers in a chocolate factory in Denmark, one third claimed to have had trouble with their teeth (many cavities, gingival bleeding). Upon examination, the workers had lost more teeth, had more untreated dental decay, and a higher caries incidence than a control group consisting of shipyard workers matched with respect to age, urbanisation, education, occupation, shift work, and dental care. Similar results were found when chocolate workers who confessed often consuming chocolate at work (about 25%) were excluded from analyses. Before starting the examination, high organic dust levels of 16-205 mg/m^3 were found in two departments, but the proportion of sucrose was not reported. From his studies, Petersen considered chocolate workers at high risk for dental problems (Pet84a, Pet84b).

A single case of dental caries in a worker in the confectionary industry has been described more recently (Par95).

The human data indicate that the teeth may be the target 'organ' following occupational exposure to sucrose. However, it is not clear to what extent additional oral intake contributed to the effects observed. In addition, exposure levels are lacking. Therefore, the commission is of the opinion that these that

cannot be used for the derivation of a health-based recommended occupational exposure limit (HBROEL).

Carcinogenicity

In a case-referent study, no relationship was found between bladder cancer and exposure to sugar dust (8 cases in a group of 116 cases with urinary bladder cancer). The referent group consisted of ward personnel (n=116) and general persons (n=116). The number of cases in the referent groups was not given, but the crude Odds Ratios varied from 1.6 to 3.9, with the lower 95% confidence interval below unity, at all calculations (Hou94).

Animal data

Sucrose was not a primary skin irritant in intact and abraded skin of guinea pigs and rabbits (Rou65).

Oral LD_{50} values of 35.4 and 29.7 g/kg bw were reported for male and female rats, respectively. The initial clinical signs of toxicity were hypokinesia, prostration, cyanosis, clonic-tonic convulsions, abdominal bloating, and diarrhoea. Sublethal doses giving for 1-3 days produced anorexia, polydipsia, hypothermia, diarrhoea, and weight loss. Death was the result of respiratory failure (ACG92).

A single oral dose of 30 g/kg bw caused very severe intoxication in sheep (Del59).

A single intravenous injection of 2.0 mL of a 60% sucrose solution caused nephrosis in rats. It produced vacuolisation and loss of enzyme activity in the kidneys (Sch65).

The LD_{50} (100 days) - the daily dose that killed 50% of the animals during 100 days of administration - was 28.5 g/kg bw/day for rats. Signs of toxicity were inhibition of growth, diuresis, polydipsia, aciduria, diarrhoea, soiling, ataxia, hepatitis, nephritis, and degeneration of many organs. No mortality occurred at daily doses of 19.8 g/kg bw (Con68).

Groups of 32 male and 32 female mice (*Acomys cahirinus*, spiny mice) received a 50% sucrose diet for 18 months, which resulted in a daily sucrose intake of roughly 57 g/kg bw*. The diet was isocaloric with the regular rat chow

* Calculation based on a weekly mean food consumption of 74.1 g per pair and mean initial and final body weights of 48.4 and 47.7 g, respectively, for males and of 43.9 and 44.6 g, respectively, for females.

that was given to a control group. Both male and female mice gained significantly less weight than the control group ($p<0.01$). These changes could not be attributed to differences in the dietary intake. The greater mortality in both parents and pups was not statistically significant. According to the number of pups born and number of productive pairs, the sucrose-fed mice were also less fertile. The litter size and the number of pups born per productive pair were slightly but not significantly lower (Sha84). Due to the singular strain used, the outcome cannot be used for the risk assessment.

A group of 50 female mice (Swiss) received oral (diet) doses of 10% sucrose in polyethylene glycol for 18 months while a second group of 50 mice was pre-treated with a single oral (gavage) dose of benzo[*a*]pyrene of 50 µg in polyethylene glycol followed 7 days later by diets containing 10% sucrose. When compared to vehicle-treated controls (n=100), the mice fed 10% sucrose gained more weight. Sucrose did not show carcinogenic or tumour-promoting activity (Roe70).

Subcutaneous injection of 25% sucrose solutions into rats (Bethesda black; n=30/sex) or mice (C57BL; n=30/sex) for up to 2 years did not induce increases in tumour incidences when compared to controls (Hue65).

Mutagenicity and genotoxicity

- *In vitro* tests:
  - Gene mutation assays. Sucrose was exclusively negative when tested for forward and reverse mutations in a variety of strains, including normal, excision-repair-deficient, and plasmid-carrying strains, of *S. typhimurium* and *E. coli* under a great number of different experimental conditions in more than 15 separate laboratories (Bri81, Fal85, Ish80).
    Sucrose did not induce reversions in *S. cerevisiae* strain XV185-14C when tested with and without rat liver metabolic activation (Meh81).
    Sucrose was negative in the L5178Y mouse lymphoma cell forward mutation assay both in the absence and presence of a rat liver metabolic activation system (McG87, Mit88, Myh88).
  - Cytogenicity assays. Sucrose did not induce SCEs when tested with and without metabolic activation in CHO cells (Per81).
    Sucrose produced a dose-related increase in the frequency of chromosomal aberrations in CHO cells. However, this phenomenon was ascribed to hyperosmolarity of the solution, because other hyperosmotic solutions (NaCl, KCl) induce the same effect (Gal87). Ishidate et al. reported an increased frequency of chromosomal aberrations in a Chinese

hamster lung cell line, which can be ascribed to the osmolarity of the solution as well (Ish80).
Sucrose did not induce mitotic aneuploidy when tested in *S. cerevisiae* strain D6 with and without rat liver metabolic activation (Par81).

- Other tests. Sucrose was negative when tested with and without metabolic activation in differential killing tests in *E. coli* (Gre81, Hym80, Twe81) and in the rec-assay in *B. subtilis* (Kad81). No induction of the prophage lambda in a lysogenic strain of *E. coli* was observed (Tho81).
  Using different strains of *S. cerevisiae*, sucrose was concluded to be negative upon testing for induction of mitotic gene conversion, mitotic crossing-over, or differential inhibition (Jag81, Kas81, Sha81a, Sha81b, Zim81). Weakly positive results in tests for gene conversion and differential inhibition at relatively high dose levels (Sha81a, Sha81b) were thought to be the consequence of chemical changes following dissolving high concentrations of sucrose in DMSO at elevated temperatures (Ash81).
  Sucrose was negative in UDS tests in HeLa cells (with and without S9 mix) (Mar81) or rat hepatocytes (Nov85).
  Sucrose was positive in a degranulation test that assays for the dissociation of ribosomes or polysomes from endoplasmatic reticulum derived from rat liver. Fey et al. considered the outcome thoroughly anomalous, arisen due to chemical changes consequent upon dissolving sucrose in DMSO at high concentrations at 40ºC. In a subsequent test using water as a vehicle, sucrose produced a negative response (Fey81).

- *In vivo* tests:
  No increase in the incidence of micronuclei was found in bone marrow sampled from male B6C3F_1 mice 36, 48, or 72 hours after the final of two intraperitoneal injections, given 24 hours apart, of sucrose of 2000 mg/kg bw each (Sal81), or from CD-1 mice 6 hours after the final of two intraperitoneal injections of doses of 2000, 4000, or 8000 mg/kg bw (Tsu81).

- Other tests:
  Sucrose was negative in a cell transformation test using BHK21 C13/HRC (baby hamster kidney) cells with and without rat liver metabolic activation (Dan81, Sty81).
  It did not enhance MLV (mouse leukaemia virus) infection in contact inhibited C3H2K (mouse kidney) cells (Yos81).

Reproduction toxicity

Daily oral sucrose doses of 2000-10,000 mg/kg bw to pregnant rats on gestational days 6-15 had no detectable adverse effects on embryos or fetuses (Fri68).

A diet containing 35 parts sucrose and 65 parts Purina Chow was given to mice during gestation and lactation. The offspring was given Purina Chow after weaning. The offspring had a lower growth rate and a different disposition of glucose after a glucose-tolerance test, compared with a control group (Dav73).

7 Existing guidelines

The current occupational exposure limit (MAC) for sucrose in the Netherlands is 10 mg/m^3, 8-hour TWA.

Existing occupational exposure limits for sucrose in some European countries and in the USA are summarised in the annex.

8 Assessment of health hazard

Oral intake of sucrose causes caries. There are suggestions that a consumption level of 2.5-3.0 g/day or of 15 kg/year is a cut-off point between high-caries and low-caries incidence. Occupational exposure to sucrose may cause dental problems. However, since there were no data available on the relationship between exposure and effect or on the contribution of additional oral intake, the commission cannot use them for derivation of a HBROEL.

Sucrose was not irritating to the skin of rabbits or guinea pigs. The oral LD_{50} in rats was 30-35 g/kg bw. The compound had no mutagenic, genotoxic, carcinogenic, or tumour-promoting activity. High dietary intake caused a reduced fertility in mice (50% of the diet) and a lower growth rate in the offspring of mice (35% of the diet).

The committee considers the toxicological database on sucrose too poor to justify recommendation of a health-based occupational exposure limit.

The committee concludes there is insufficient information to comment on the level of the present MAC-value.

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Annex

Occupational exposure limits for sucrose in various countries.

country - organisation	occupational exposure limit	time-weighted average	type of exposure limit	note[a]	reference[b]

	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	-	10[c]	8 h	administrative		SZW04
Germany						
- AGS	-	-				TRG04
- DFG MAK-Kommission	-	-				DFG04
Great Britain						
- HSE	-	10	8 h	OES		HSE02
		20	15 min			
Sweden	-	-				Swe00
Denmark	-	-				Arb02
USA						
- ACGIH	-	10	8 h	TLV	A4[f]	ACG04b
- OSHA	-	15[d]	8 h	PEL		ACG04a
	-	5[e]	8 h			
- NIOSH	-	10[d]	10 h	REL		ACG04a
	-	5[e]	10 h			
European Union						
- SCOEL	-	-				EC04

[a] S = skin notation; which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] Inhalable dust.

[d] Total dust.

[e] Respirable fraction.

[f] Classified in carcinogenicity category A4, i.e., not classifiable as a human carcinogen: agents which cause concern that they could be carcinogenic for humans but which cannot be assessed conclusively because of a lack of data. *In vitro* or animal studies do not provide indications of carcinogenicity which are sufficient to classify the agent into one of the other categories.

Safety Assessment of Monosaccharides, Disaccharides, and Related Ingredients as Used in Cosmetics

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ABSTRACT

The Expert Panel assessed the safety of 25 monosaccharides, disaccharides, and related ingredients, and concluded these ingredients are safe as used in cosmetics. Many of these ingredients are common dietary sugars, dietary sugar replacements, or very closely related analogs and salts; seven of the ingredients are listed by the FDA as GRAS food substances. The most commonly reported cosmetic function is as a skin conditioning agent; other commonly-reported functions are use as a humectant or as a flavoring agent. The Panel reviewed the animal and clinical data included in this assessment, acknowledged that the oral safety of many of these ingredients has been well established, and found it appropriate to extrapolate the existing information to conclude on the safety of all the monosaccharides, disaccharides, and related ingredients.

INTRODUCTION

This report addresses the safety of the following 25 monosaccharides, disaccharides, and related ingredients as used in cosmetic formulations:

Calcium Gluconate[#]
Fructose[#]
Fucose
Galactose
Galactosyl Fructose
Galacturonic Acid
Gluconic Acid
Glucose[#]
Isomalt[##]
Kefiran
Lactitol[##]
Lactose[##]
Lactulose
Maltose
Mannose
Melibiose
Potassium Gluconate[#]
Rhamnose
Ribose
Sodium Gluconate[#]
Sucralose[#]
Sucrose[#]
Trehalose[##]
Xylobiose
Xylose

[#]generally recognized as safe (GRAS) food additive or approved direct food additive
[##]listed in the Food Chemical Codex

The monosaccharides, disaccharides, and related ingredients have a number of reported functions in cosmetics, and the most common use is as a skin conditioning agent (Table 1).[1] Other commonly-reported functions are use as a humectant or as a flavoring agent.

Most of these ingredients included in this safety assessment are common dietary sugars, dietary sugar replacements, or very closely related analogs and salts. Several are listed by the Food and Drug Administration (FDA) as GRAS food additives[2] or direct food additives, and/or are listed in the *Food Chemicals Codex*[3] as used in foods; for these ingredients, the focus of this assessment will be on dermal effects, primarily dermal irritation and sensitization. This approach is supported by the fact that some of these ingredients, namely fructose, galactose, glucose, lactose, sodium gluconate, and sucrose, are listed in Annex IV of the European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH),[4] which "sets out substances that are exempted from the registration, evaluation and downstream user provisions of REACH as sufficient information is known about these substances that they are considered to cause minimum risk because of their intrinsic properties."[5]

For those ingredients that are not identified as common dietary substances, i.e., fucose, galactosyl fructose, galacturonic acid, kefiran, lactulose, mannose, melibiose, and xylobiose, a search for oral toxicity data was performed. Very limited published data were found.

CHEMISTRY

Definition

A monosaccharide is a carbohydrate that cannot be decomposed to a simpler carbohydrate by hydrolysis, and is often called a simple sugar.[6] A disaccharide is a carbohydrate that yields two monosaccharides upon hydrolysis. Many of these ingredients exist in equilibrium between an open chain form and one or more ring forms resulting in a hemiacetal or hemiketal linkage involving the aldehyde (aldose) or ketone (ketose) moiety of the open chain form, with two possible stereochemical configurations (Figure 1). The resulting stereoisomers are called anomers and the stereocenter is referred to as the anomeric carbon.

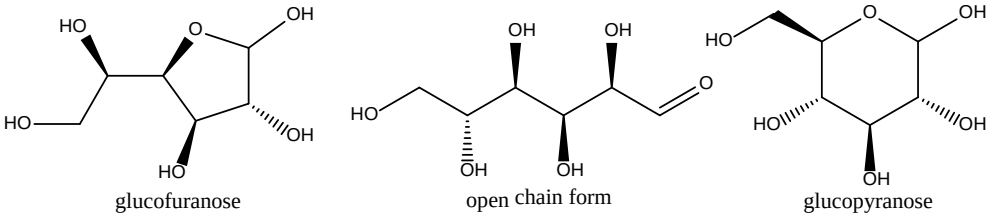


Figure 1. Structural forms of D-glucose (stereoisomer found in natural sources) that exist in equilibrium.

The definition and structure of each ingredient included in this report is provided in Table 1.

Chemical and Physical Properties

Due to the high degree of substitution with hydroxyl groups, the mono- and disaccharides are very hydrophilic and readily dissolve in aqueous solvent systems. These sugars have molecular weights ranging from 142 to 391 Daltons, and are solids at room-temperature, with many having multiple known crystalline forms (Table 2[3,7-29]).

Natural Occurrence and Methods of Manufacture

The manufacture of the majority of these monosaccharides, disaccharides, and related ingredients is accomplished by extraction from plant sources (Table 3). For instance, the sugar industry processes sugar cane and sugar beets to obtain sucrose.[30] Sugar cane contains 70% water; 14% fiber; 13.3% saccharose (about 10 to 15% sucrose), and 2.7% soluble impurities. Sugar cane is extracted with water, clarified to remove mud, evaporated to prepare syrup, crystallized to separate the liquor, and centrifuged to separate molasses from the crystals. Sugar crystals are then dried and may be further refined before bagging for shipment. Sugar beet (water, 75%; sugar, 17%) processing differs in the washing, preparation, and extraction. After washing, the beet is sliced and extracted with water. Sugar refining involves removal of impurities and decolorization. The steps generally followed include affination (mingling and centrifugation), melting, clarification, decolorization (with activated carbon, ion exchange resins, etc.), evaporation, crystallization, and finishing.

Constituents/Impurities

Purity and composition specifications are available for the food and pharmaceutical uses of many of these ingredients (Table 4).

USE

Cosmetic

The ingredients included in this safety assessment have a variety of functions in cosmetics. The most common function is as a skin conditioning agent; many also are reported to function as flavoring agents (Table 1).

The FDA collects information from manufacturers on the use of individual ingredients in cosmetics as a function of cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP). VCRP data obtained from the FDA in 2014[31] and data received in response to a survey of the maximum reported use concentration by category conducted by the Personal Care Products Council (Council) in 2013[32] indicate that 22 of the 25 ingredients included in this safety assessment are used in cosmetic formulations.

According to the VCRP data, sucrose has the greatest number of reported uses, 738, followed by trehalose with 474 uses and glucose with 425 uses (Table 5).[31] A concentration of use survey conducted by the Council found that the use of these mono- and disaccharides varies widely by ingredient and use-type.[32] Glucose has the highest reported use concentration in a leave-on product; it is reported to be used at 91% in "other" non-coloring hair preparations. It is also used at 97.8% in an ingestible oral hygiene product. Sucrose has the next highest reported use concentration; it is used at up to 58% in leave-on formulations (i.e., in other skin care preparations) and 65% in rinse-off products (i.e., in other personal cleanliness products). However, most of the ingredients are used at less than 1% in leave-on products.

The three ingredients not reported to be used are galactose, galacturonic acid, and lactulose (Table 6).

VCRP data indicate that glucose, lactose, sodium gluconate, sucrose, and trehalose are used in baby products; however concentration of use data for baby products were not reported by industry. Some of the ingredients are used in products that could be incidentally, or are purposely, ingested (e.g., 97.8% glucose in an ingestible oral hygiene product), and some are used near the eye area or mucous membranes (e.g., 2% sucrose in eye lotion and 65% in personal cleanliness products, respectively). Additionally, some of these ingredients are used in cosmetic sprays and powders that could possibly be inhaled (e.g., glucose is used at 1% in a spray body and hand preparation). In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of

droplets/particles <10 µm compared with pump sprays.[33,34] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[35,36]

All of the ingredients included in this safety assessment are listed in the European Union inventory of cosmetic ingredients.[37]

Non-Cosmetic

Several of the ingredients have specific GRAS food and direct food additive uses:

- Calcium gluconate: GRAS designation; a direct food additive that meets the specifications of the *Foods Chemical Codex*; it is used as a firming agent, formulation aid, sequestrant, and texturizer at levels not to exceed current good manufacturing practices (GMP); GMP result in a maximum level, as served, of 1.75% for baked goods; 0.4% for dairy product analogs; 4.5% for gelatins and puddings; and 0.01% for sugar substitutes (21CFR184.1199)
- Fructose: a direct food additive; in high fructose corn syrup (containing approximately 42 or 55% fructose); high fructose corn syrup must conform to the identity and specifications listed in the monograph entitled "High-Fructose Corn Syrup" in the *Food Chemicals Codex*, with no limitation other than current GMP (21CFR184.1866)
- Glucose: GRAS direct food additive (D-glucose) meeting the specifications of the *Foods Chemical Codex*; it is used in foods with no limitation other than current GMP (21CFR184.1857)
- Potassium gluconate: GRAS designation; does not have a CFR citation.[2] The Select Committee on GRAS Substances (SCOGS) concluded there is no evidence in the available information on potassium gluconate that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or might reasonably be expected in the future.[38]
- Sodium gluconate: GRAS designation; as a sequestrant in food, with no limitation other than current GMP (21CFR182.6757)
- Sucralose: direct food additive as a multipurpose additive that meets the specifications of the *Foods Chemical Codex*; it is used as a sweetener in foods generally, in accordance with current GMP in an amount not to exceed that reasonably required to accomplish the intended effect (21CFR172.831)
- Sucrose: GRAS designation; a direct food additive that must be of a purity suitable for its intended use, with no limitation other than current GMP (21CFR184.1854)

Lactulose is an approved drug used to treat constipation.[39] A general list of non-cosmetic uses, including food uses that are not affirmed as GRAS or those that are inactive ingredients in approved drugs, are listed in Table 7.[3,9,10,12,26,39-41] Table 8 provides a listing of those ingredients that are nutritive and non-nutritive sweeteners.[3,14,42,43]

In Europe, the following are listed in REACH Annex IV: fructose; galactose; glucose; lactose; sodium gluconate; sucrose.[4] Substances included in Annex IV are exempted from registration (as well as downstream user requirements and evaluation) for all their possible uses irrespective of the tonnage in which they are manufactured or imported (currently or in the future).

TOXICOKINETICS

Although many of the ingredients included in this safety assessment are food ingredients, they are not all processed by the body in the same manner (see Tables 8 and 9). Some are nutrients, which are absorbed intact in the small intestines and then metabolized in the body to serve as sources of energy, and others are not (Table 8). For example, glucose[44] and potassium gluconate,[45,46] are rapidly absorbed in the small intestine (Table 9[9,12,23,27,38,44-61]). In contrast, isomalt is absorbed only to a limited extent,[12] and lactitol,[12] lactulose,[52] and sucralose,[23,55,57] are not absorbed in the gut. Trehalose can be metabolized by trehalase in the gut to produce glucose, which can then be readily absorbed. Some of these ingredients (e.g., gluconic acid, potassium gluconate, and sodium gluconate) are important intermediates in carbohydrate metabolism; gluconic acid is a normal metabolic product of glucose oxidation, and the amounts produced endogenously are much greater than what is consumed.[9] Because the absorption, distribution, metabolism, and elimination of most of the ingredients included in this safety assessment have been reviewed to evaluate their use as common dietary substances, only summary information is provided in this report.

Dermal Penetration

In Vitro

Glucose

The permeability coefficient for glucose was determined *in vitro* using full thickness mouse skin and the dermis of nude mice.[62] Unlabeled glucose, 0.01 M, was first used on both sides of the skin to saturate the sorptive capacity of the cell system. A concentration of 3.3×10^{-6} M D-[1,3-^{14}C]glucose, supplied as a sterile aq. solution containing 3% alcohol, was placed in the donor cell. After 6 h, the permeability coefficient of glucose was 9.5×10^{-5} cm/h through full-thickness skin

and 0.29 cm/h through the dermis. The permeation rate continued to increase as a function of time; the researchers stated that physical and chemical deterioration of the barrier phase seemed to be responsible for the increase in permeation.

In Vivo

Glucose

The transdermal penetration of glucose through Rhesus monkey skin was measured using optical coherence tomography (OCT).[63] The hair on the right hind leg of four anesthetized monkeys was shaved, a probe holder was taped to the shaved skin, and 0.2 ml of 20% concentrated glucose in distilled water was applied topically through the hole in the probe holder during the course of imaging. The skin was imaged using OCT for 8 min prior to application of the glucose, and then for 2 h after application. The diffusion process was monitored in a 140 µm thick region 210 µm below the dermis region. The mean permeability rate of 20% glucose was calculated to be $(4.41 \pm 0.28) \times 10^{-6}$ cm/sec.

TOXICOLOGICAL STUDIES

Most of the ingredients included in this assessment are found in foods, and the daily exposure from that food use would result in a much larger systemic dose than that resulting from use in cosmetic products. Numerous studies and reviews have been published about the safety of dietary exposure to mono- and disaccharides. Examples of these reviews include the "Evaluation of the Health Aspects of Sucrose as a Food Ingredient",[49] and "Evaluation of the Health Aspects of Sodium, Potassium Magnesium, and Zinc Gluconates as Food Ingredients".[50] Also, many of the ingredients included in this report are used as inactive ingredients in approved drugs that are administered via numerous routes. Consequently, systemic toxicity is not addressed further in this report for those ingredients that are GRAS food substances, direct food additives, or identified in the *Food Chemicals Codex* as used in foods. The focus of the safe use of those mono- and disaccharides as cosmetic ingredients is on the potential for irritation and sensitization. When available, dermal toxicity, ocular irritation, and genotoxicity studies are included.

For the ingredients that are not identified as common dietary substances, i.e., the monosaccharides fucose, galacturonic acid, and mannose and the disaccharides galactosyl fructose, kefiran, lactulose, melibiose, and xylobiose, a search for oral toxicity data was performed. However, very little published data were found.

Single Dose (Acute) Toxicity

Dermal

Lactitol

The dermal LD_{50} of lactitol in rabbits is >4500 mg/kg bw.[20]

Oral

Lactulose

The oral LD_{50} of lactulose is 48.8 ml/kg in mice and >30 ml/kg in rats.[21]

Repeated Dose Toxicity

Oral

Lactulose

Groups of eight male albino rats were fed a diet containing 0.0, 0.5, 1.0, 2.0, or 5.0% (equivalent to 0.0, 1.1, 2.2, 4.0, and 11.3 g/kg bw/day, respectively) of a 50% lactulose syrup for 21 weeks.[64] None of the animals died during the study, and no signs of general toxicity were observed. Mild diarrhea was reported for animals fed >2.2 g/kg bw/day of the test material; diarrhea subsides with 3-5 h of feeding. Feed consumption was not statistically significantly affected at any dose level. The organ weights were similar for treated and control animals. A statistically significant increase in cecal weights in the 2 and 5% groups was considered an adaptive reaction. No toxicologically-significant changes in hematology, clinical chemistry, or urinalysis parameters were reported.

Ocular Irritation

In Vitro

Gluconic Acid

The ocular irritation potential of a 50% aq. solution of gluconic acid was evaluated *in vitro* in enucleated rabbit eyes.[9] The test material was applied to four eyes and observed over a period of 4 h following application. Slight corneal swelling and slight permeability of the superficial epithelial cells were not considered to be of any toxicological significance.

Isomalt

A battery of *in vitro* tests were performed to determine the ocular irritation potential of isomalt; based on the overall results of each test included in the battery, isomalt was classified as a non-irritant. A neutral red uptake (NRU) assay was performed in human keratinocytes, and the cytotoxicity of undiluted isomalt to the cells was measured after 24-h exposure.[65] Two experiments were performed. Undiluted isomalt was classified as a non-irritant in this *in vitro* test.

A red blood cell lysis and denaturation (RBC) assay, comprised of two range-finding and denaturation assays and two lysis assays, was performed in calf red blood cells.[66] Concentrations of ≤100,000 mg/l isomalt were tested. Isomalt did not induce hemolysis or protein denaturation, and was classified as a non-irritant. Based on the lack of induction of hemolysis, the predicted *in vivo* ocular irritation potential corresponded to a modified maximum average score of 0.

The third test in the battery was the hen's egg test on the chorioallantoic membrane (HET-CAM) in which isomalt was tested undiluted according to the endpoint assessment and at concentrations of 10 and 50% (w/w) in water according to the reaction-time method.[67] Each aspect of the experiment was performed twice. According to COLIPA (now, Cosmetics Europe) classifications, undiluted isomalt was classified as a slight irritant when tested undiluted in the endpoint assessment; the 10% and 50% concentrations were classified as non-irritant using the reaction-time method.

In Vivo – Non-Human

Gluconic Acid

A 50% aq. solution of gluconic acid was not irritating to rabbit eyes.[9] A 50% solution of gluconic acid (pH 1.8; 0.1 ml) was instilled into the conjunctival sac of one eye in nine New Zealand white rabbits; the contralateral eye served as an untreated control. The eyes of three animals were rinsed after 2 sec, and of another three animals after 4 sec; the eyes of the remaining three animals were not rinsed. The eyes were examined for irritation 1, 24, 48, and 72 h and 7 days after instillation. Slight redness and conjunctival swelling were observed initially; however, no signs of irritation were observed after 72 h.

Lactitol

Lactitol was not irritating to rabbit eyes.[20] The study was performed according to the Organisation for Economic Co-operation and Development (OECD) Guideline 405.[68] No other details were provided.

In Vivo – Human

Lactose

A face and neck formulation containing 2.48% lactose did not produce irritation or hypersensitivity in a 4-wk safety-in use ophthalmological evaluation.[69] Thirty-one subjects participated in the study.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

According to the package insert for the prescription drug lactulose, in studies of mice, rats, and rabbits, doses of lactulose solution up to 6 or 12 ml/kg/day produced no deleterious effects on breeding, conception, or parturition.[21] (Details were not provided.)

GENOTOXICITY

The genotoxicity of a number of the mono- and disaccharides has been evaluated in *in vitro* and *in vivo* studies. The results of these studies are overwhelmingly negative (Table 10[9,20,27,53,70-75]).

CARCINOGENICITY

According to the package insert for the prescription drug lactulose, administration of lactulose solution in the diet of mice for 18 mos in concentrations of 3 and 10% (v/w) did not produce any evidence of carcinogenicity.[21] (Details were not provided.)

IRRITATION AND SENSITIZATION

Dermal Irritation/Sensitization

Dermal irritation and sensitization studies are summarized in Table 11. In non-human studies, a 50% aq. solution of gluconic acid was not a dermal irritant[9] and lactitol was not an irritant or sensitizer in rabbits.[20] In human repeated insult patch tests (HRIPTs), formulations containing 10% rhamnose,[76] up to 8% glucose,[77,78] 5% mannose,[79] 2.48% lactose,[69] and less than 1% isomalt,[80] kefiran,[69] lactitol,[69] sucralose,[81] and xylobiose[82] were not irritants or sensitizers. A formulation containing 10% rhamnose did induce a significant irritation reaction in one subject,[76] and irritation was observed in 16% of the subjects during induction in an HRIPT of a rinse-off hair product containing 29% sucrose (tested as a 50% dilution); no sensitization reactions were reported for this product.[83]

OCCUPATIONAL EXPOSURE LIMITS

Sucrose

The National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL) for sucrose is a time-weighted average (TWA) of 10 mg/m^3 (total exposure) and TWA of 5 mg/m^3 (respiratory exposure).[25] The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) is a TWA of 15 mg/m^3 (total) and TWA of 5 mg/m^3 (respiratory). The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) is 10 mg/m^3 as TWA; it is in category A4, not classifiable as a human carcinogen.

SUMMARY

This report addresses the safety of 25 monosaccharides, disaccharides, and related ingredients as used in cosmetics. Many of these ingredients are GRAS food substances, direct food additives, or common dietary sugars, dietary sugar replacements, or very closely related analogs; for these ingredients, the focus of this review was on dermal irritation and sensitization. For the ingredients that are not identified as dietary substances, oral toxicity data were searched.

The monosaccharides, disaccharides, and related ingredients are reported to have a number of functions in cosmetics, and the most common function is as a skin conditioning agent; use as a humectant or flavoring agent was also common. According to VCRP data obtained from the FDA and concentration of use data obtained by the Council, 22 of the 25 ingredients reviewed in this assessment are reported to be in use. Sucrose has the greatest number of reported uses, 738, and glucose has the highest reported use concentration, 97.8% in an ingested breath freshener and 91% in "other" hair coloring products. The number of uses and maximum concentration of use varies widely by ingredient and type of use; most of the ingredients are used in leave-on products at less than 1%. Non-cosmetic uses include food use and use as inactive ingredients in approved drugs.

Although many of the ingredients included in this safety assessment are food ingredients, they are not all processed by the body in the same manner; some (e.g., glucose) are sources of energy and others (e.g., sucralose) are not. Also, absorption is not the same for each of these ingredients; some are absorbed in the intestines (e.g., glucose and potassium gluconate), while others are not absorbed in the gut (e.g., lactitol and sucralose).

In vitro, the permeability coefficient of glucose was 9.5×10^{-5} cm/h through full thickness nude mouse skin and 0.29 cm/h through the dermis (only) of nude mouse skin. *In vivo* in Rhesus monkeys, using OCT, the mean permeability rate of 20% glucose was calculated to be $(4.41 \pm 0.28) \times 10^{-6}$ cm/sec.

Lactulose fed to rats at concentrations of up to 5.0% of 50% lactulose syrup for 21 weeks did not result in toxicity. Mild diarrhea was reported for animals fed >2.2 g/kg bw/day of the test material; diarrhea subsides with 3-5 h of feeding. Doses of up to 12 ml/kg/day of lactulose solution produced no deleterious effects on breeding, conception, or parturition in mice, rats, or rabbits. No evidence of carcinogenicity was observed in mice with dosing of up to 10% lactulose solution in the diet for 18 mos.

A battery of *in vitro* tests were performed to determine the ocular irritation potential of isomalt; based on the results, isomalt was classified as a non-irritant. Gluconic acid, as a 50% aq. solution, and lactitol, concentration not specified, were not irritating to rabbit eyes. A face and neck formulation containing 2.48% lactose did not produce irritation or hypersensitivity in a 4-wk safety-in use ophthalmological evaluation

In non-human studies, a 50% aq. solution of gluconic acid was not a dermal irritant and lactitol was not an irritant or sensitizer in rabbits. In human repeated insult patch tests (HRIPTs), formulations containing 10% rhamnose, 8% glucose, 5% mannose, 2.48% lactose, and less than 1% isomalt, kefiran, lactitol, sucralose, and xylobiose were not irritants or sensitizers. A formulation containing 10% rhamnose did induce a significant irritation reaction in one subject, and irritation was observed in 16% of the subjects during induction in a HRIPT of a product containing 29% sucrose (that was a rinse-off hair product tested as a 50% dilution); no sensitization reactions were reported for this product.

Lactitol, sodium gluconate, sucralose, sucrose and trehalose were not genotoxic *in vitro*. Additionally, the genotoxic potential of sodium gluconate, sucralose, and trehalose was evaluated *in vivo*; again negative results were obtained.

DISCUSSION

The Panel reviewed this safety assessment of monosaccharides, disaccharides, and related ingredients. Most of these ingredients are common dietary sugars, dietary sugar replacements, or very closely related analogs and salts. Several are GRAS food additives, direct food additives, listed in the *Food Chemicals Codex* as used in foods, and/or listed in REACH Annex IV. Because the oral safety of these ingredients has been well-documented, systemic toxicity is not a concern of the Panel.

Some of the ingredients, however, are not GRAS food substances or direct food additives; even so, these ingredients are either listed in the *Food Chemicals Codex* as having a function in foods, listed in the Everything Added to Foods in the United States (EAFUS) inventory, and/or listed as an inactive ingredient in oral drugs. Moreover, the leave-on use concentrations of these ingredients are typically less than 1%. Therefore, the Panel stated that although oral toxicity data are very limited and reproductive toxicity data are mostly absent, the systemic toxicity of these ingredients was not a concern because of the low concentrations of use and their limited systemic exposure from dermal application.

The Panel commented that sucrose is used at high concentrations in some products that come in contact with mucous membranes (i.e., 65% in personal cleanliness products). The Panel noted that sucrose is a GRAS food substance, and therefore, the Panel was not concerned about this reported use. Additionally, the Panel observed that glucose is reported to be used at 97.8% in an ingestible oral hygiene product, but recognized that glucose is a GRAS direct food additive with no limitations other than following current good manufacturing practice. However, if an ingredient that does not have GRAS

food additive status was used at concentrations such as these with similar exposure-types, the Panel would most likely want data substantiating the safety of that use, such as metabolism after oral administration.

The Panel discussed a human repeated insult patch test of a hair product that contained 29% sucrose, diluted to 50%, that reported irritation during induction. The Panel concluded that the irritation reported was likely attributable to a surfactant effect, and was not due to sucrose itself. The Panel acknowledged that sucrose and glucose are used in cosmetics at relatively high concentrations, and that data from irritation and sensitization studies at maximum use concentrations of these ingredients are lacking; however, based on the clinical experience of the Panel, there is little concern that these ingredients are irritants or sensitizers.

Because some of the ingredients included in this safety assessment can be used in products that may be aerosolized, the Panel discussed the issue of incidental inhalation exposure. Most of the use concentrations of the ingredients used in cosmetic products that may be aerosolized are less than 1% (e.g., glucose is used at 1% in a spray body and hand preparation). In the absence of inhalation data, the Panel noted that 95% – 99% of droplets/particles produced in cosmetic aerosols would not be respirable to any appreciable amount. The Panel acknowledged that the potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs, but because of the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects.

Finally, because many of these ingredients are obtained from plant sources, the Expert Panel expressed concern regarding pesticide residues and heavy metals that may be present. They stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulation.

CONCLUSION

The CIR Expert Panel concluded that the following 25 monosaccharides, disaccharides, and related ingredients are safe in the present practices of use and concentration in cosmetics described in this safety assessment:

calcium gluconate
fructose
fucose
galactose*
galactosyl fructose
galacturonic acid*
gluconic acid
glucose
isomalt
kefiran
lactitol
lactose
lactulose*
maltose
mannose
melibiose
potassium gluconate
rhamnose
ribose
sodium gluconate
sucralose
sucrose
trehalose
xylobiose
xylose

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

TABLES

Table 1. Definitions, Structures, and Reported Functions

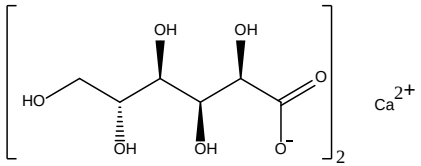
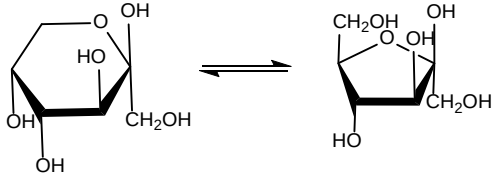
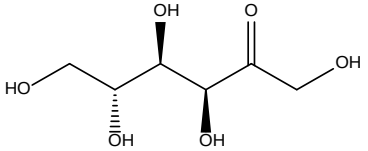
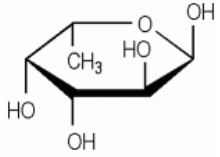
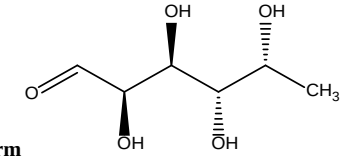
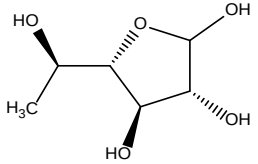
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Calcium Gluconate 299-28-5	the calcium salt of gluconic acid		chelating agent; skin-conditioning agent - miscellaneous
Fructose 30237-26-4 57-48-7 (D-)	a sugar which occurs in fruit and honey; *fructose exists in solution primarily as two cyclized forms in equilibrium, namely fructopyranose and fructofuranose.*	 ***** open chain form that exists between the furanose and pyranose forms** 	flavoring agent; humectants skin-conditioning agent - humectant
Fucose 2438-80-4 (L-) 3615-37-0 (D-)	the organic compound that conforms to the formula provided; *fucose is a deoxyhexose that is present in a wide variety of organisms; unlike most sugars, fucose occurs in nature as the L-form and lacks a hydroxyl group on the carbon at the 6-position (C-6).*	  ***** open chain form**  ***** furanose form**	skin-conditioning agent - miscellaneous

Table 1. Definitions, Structures, and Reported Functions

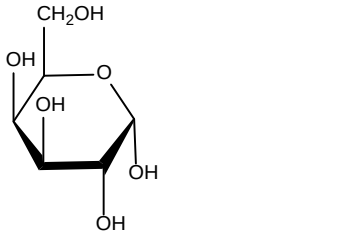
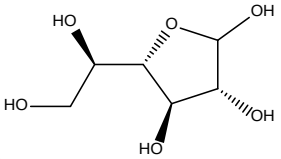
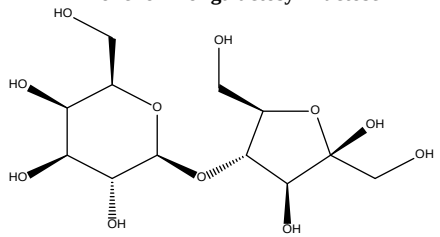
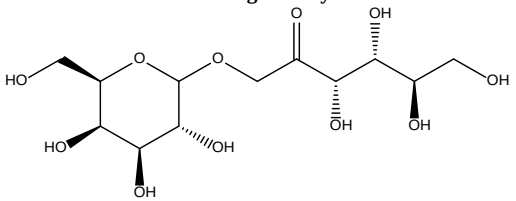
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Galactose 59-23-4	the sugar that conforms to the formula provided; g*alactose is the C4 epimer of glucose*	 *** open chain form  *** furanose form	skin-conditioning agent - miscellaneous
Galactosyl Fructose 110312-93-1	a disaccharide consisting of galactose and fructose	*** one form of galactosyl fructose  *** one form of galactosyl fructose 	skin-conditioning agent - humectant

Table 1. Definitions, Structures, and Reported Functions

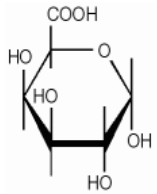
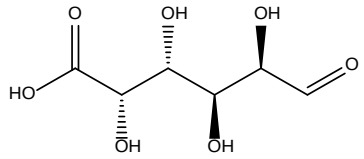
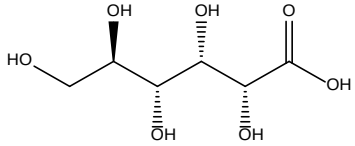
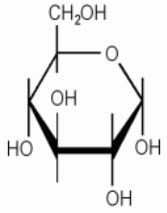
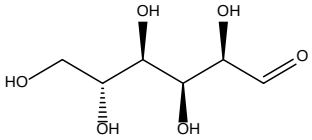
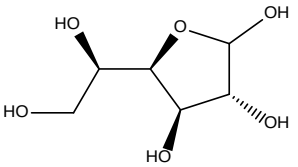
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Galacturonic Acid 14982-50-4 (DL-) 552-12-5 (D-) 685-73-4 (D-)	the organic compound that conforms to the formula provided; *galacturonic acid is the c-6 oxidation product of galactose*	  *** open chain form	chelating agent; skin-conditioning agent - humectant; pH adjuster
Gluconic Acid 133-42-6; 526-95-4	the organic compound that conforms to the formula provided; *gluconic acid is the C1 oxidation product of glucose*		chelating agent; fragrance ingredient
Glucose 50-99-7 (D-) 58367-01-4 (DL-) 5996-10-1 (DL-) 8029-43-4	a sugar that is generally obtained by the hydrolysis of starch	  *** open chain form  *** furanose form	flavoring agent; humectants; skin-conditioning agent-humectant; skin-conditioning agent – miscellaneous

Table 1. Definitions, Structures, and Reported Functions

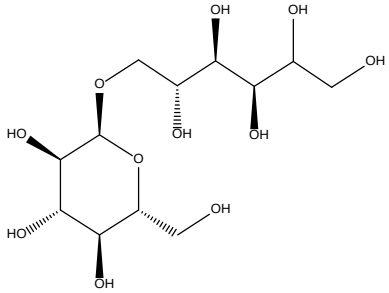
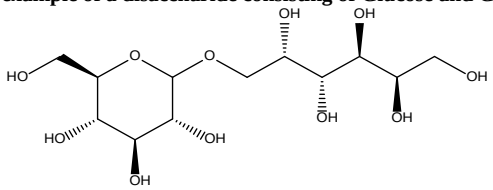
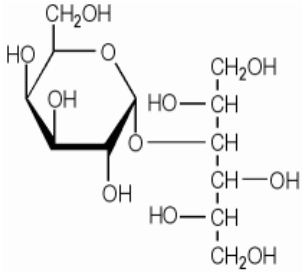
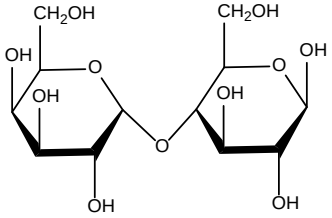
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Isomalt 64519-82-0	a mixture of polysaccharides produced by the enzymatic rearrangement of sucrose; it consists chiefly of 1-O-α-D-glucopyranosyl-D-mannitol dihydrate and 6-O-α-D-glucopyranosyl-d-sorbitol	***** one example of an isomalt form** 	anticaking agent; bulking agent; flavoring agent
Kefiran 86753-15-3	a disaccharide consisting of glucose and galactose	***** one example of a disaccharide consisting of Glucose and Galactose** 	skin-conditioning agent - humectant
Lactitol 585-86-4	a disaccharide polyol obtained by the controlled hydrogenation of lactose		flavoring agent; humectant; skin-conditioning agent - humectant
Lactose 63-42-3	the disaccharide that conforms to the formula provided; *lactose is the disaccharide (β1 →4) galactosyl-glucose*		skin-conditioning agent - humectant

Table 1. Definitions, Structures, and Reported Functions

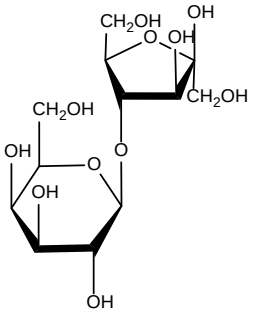
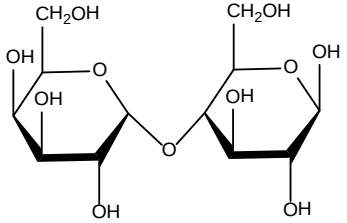
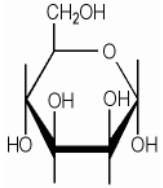
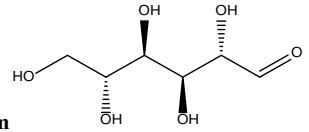
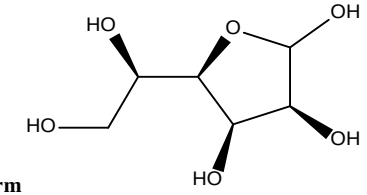
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Lactulose 4618-18-2	the disaccharide that conforms to the formula provided; *lactulose is the disaccharide (β1 →3) galactopyranosyl-fructo-furanose*		skin-conditioning agent - humectant
Maltose 16984-36-4; 69-79-4	the sugar that conforms to the formula provided; *maltose is the disaccharide α(1 →4) glucosyl-glucose*		flavoring agent; humectant; skin-conditioning agent - humectant
Mannose 3458-28-4	the sugar that conforms to the formula provided; *mannose is the C2 epimer of glucose*	 ***** open chain form**  ***** furanose form** 	humectant; skin-conditioning agent - humectant

Table 1. Definitions, Structures, and Reported Functions

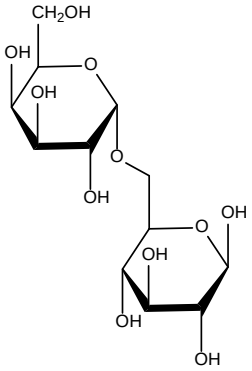
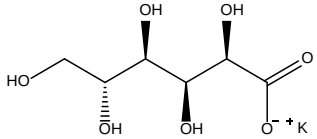
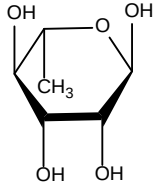
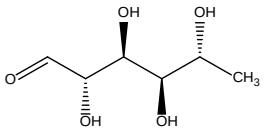
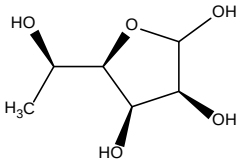
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Melibiose 5340-95-4; 585-99-9	the carbohydrate that conforms to the formula provided; *melibiose is the disaccharide α(1→6) galactosyl-glucose*		skin-conditioning agent – humectant
Potassium Gluconate 299-27-4	the potassium salt of gluconic acid		chelating agent; skin-protectant
Rhamnose 10030-85-0 3615-41-6 (L-)	the organic compound that conforms to the formula provided; *unlike most naturally abundant sugars, rhamnose occurs in nature as the L form and lacks a hydroxyl group on the carbon at the 6-position (C6)*	 ***** open chain form**   ***** furanose form**	flavoring agent; fragrance ingredient

Table 1. Definitions, Structures, and Reported Functions

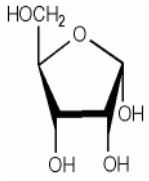
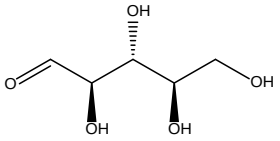
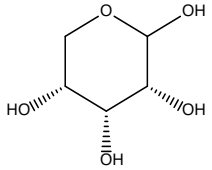
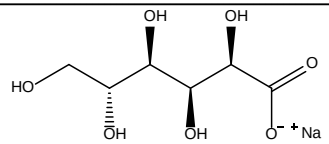
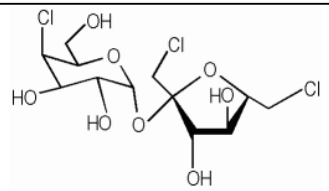
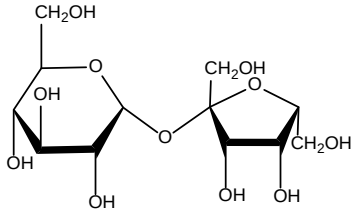
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Ribose 50-69-1	the sugar that conforms to the formula provided; *ribose is an aldopentose*	 *** open chain form   *** pyranose form	humectant; skin-conditioning agent - humectant
Sodium Gluconate 14906-97-9 527-07-1	the sodium salt of gluconic acid		chelating agent; skin-conditioning agent - miscellaneous
Sucralose 56038-13-2	the organic compound that conforms to the formula provided; *sucralose is a selectively tri-chlorinated analog of sucrose (1,6-fructo- and 4-galacto-chlorinated)*		flavoring agent
Sucrose 57-50-1	the disaccharide that conforms to the formula provided; *sucrose is the disaccharide α(1→4) glucosyl-fructose*		flavoring agent; humectant

Table 1. Definitions, Structures, and Reported Functions

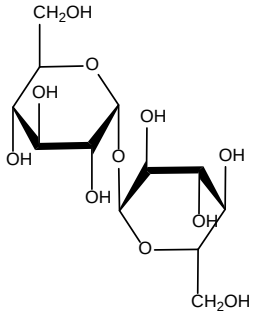
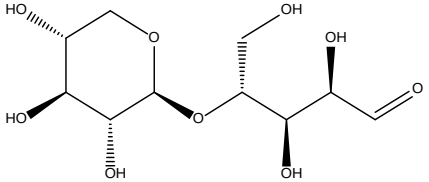
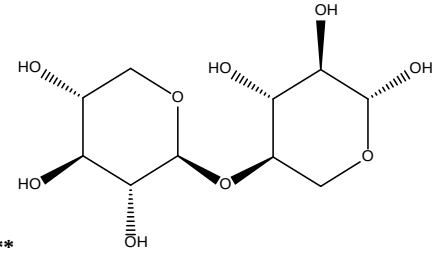
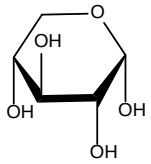
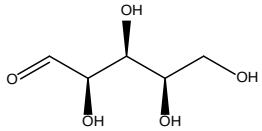
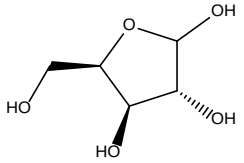
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Trehalose 99-20-7; 6138-23-4	the disaccharide that conforms to the formula provided; *trehalose is the disaccharide α(1 →1) glucosyl-glucose*		flavoring agent; humectant
Xylobiose 6860-47-5	a disaccharide consisting of two xylose units with β-1 to β-4 linkage	 ***  ***	skin-conditioning agent - humectant

Table 1. Definitions, Structures, and Reported Functions

Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Xylose 58-86-6	the sugar that conforms to the formula provided; *xylose is an aldopentose*	 ***** open chain form**  ***** furanose form** 	flavoring agent; fragrance ingredient; humectant; skin-conditioning agent - humectant

*The italicized text represents additions made by CIR staff.
Structures preceded with asterisks (***) have been added by CIR staff

Table 2. Chemical and Physical Properties

Property	Description	Reference
	Calcium Gluconate	
physical characteristics	odorless, white, crystalline granules or powder	7
molecular weight	430.4	8
melting point	120°C	9
solubility	soluble in water; insoluble in ethanol	7
density	0.30-0.65 g/cm³ (bulk density)	9
log P_{ow}	-7.51 (estimated)	9
	Fructose	
physical characteristics	D-: orthorhombic, bisphenoidal prisms from alcohol DL-: needles from methanol white crystals or powder	10 11
molecular weight	180.16	10
particle size distribution	crystalline fructose: 170-450 µm powdered fructose: 25-40 µm	12
melting point	D-: decomposes at 103-105°C DL-: 129-130°C	10
solubility	D-: freely soluble in water; slightly soluble in cold and freely soluble in hot acetone; soluble in methanol, ethanol, pyridine, ethylamine, and methylamine; insoluble in ether	3,10
specific optical rotation (α^{20}_D)	D-: shows mutarotation; -132° to -92°	10
density	1.59 kg/m^3 (20°C)	13
pK_a	D-: 12.06 (18°C)	10
specific gravity (d^{16}_4)	DL-: 1.665	10
	Fucose	
physical characteristics	D-, α-form: needles from alcohol; sweet taste L-, α-form: minute needles from absolute alcohol	10
molecular weight	164.16	10
melting point	D-, α-form: 144°C L-, α-form: 140°C	10
solubility	D-, α-form: soluble in water; moderately soluble in alcohol L-, α-form: soluble in water and alcohol	10
specific optical rotation (α^{19}_D)	D-, α-form: shows mutarotation; +127.0° (7 min) ⟶ +89.4° (31 min) ⟶ +77.2° (71 min) ⟶ +76.0° (final value 146 min)	10
specific optical rotation (α^{20}_D)	L-, α-form: shows mutarotation, -124.1° (10 min) ⟶ -108.0° (20 min) ⟶ -91.5° (36 min) ⟶ -78.6° (70 min) ⟶ -75.6° (final value, 24 hrs)	10
	Galactose	
physical characteristics	α-form: prisms from water or ethanol β-form: crystals monohydrate: prisms from water	10
molecular weight	180.16	10
melting point	α-form: 167°C β-form: 167°C monohydrate: 118-120°C	10
solubility	α-form: freely soluble in hot water; soluble in pyridine; slightly soluble in alcohol	10
specific optical rotation (α_D)	α-form: +150.7° ⟶ +80.2° (water) β-form: +52.8° ⟶ +80.2° (water) D-, α-form: (α^{20}_D): +78.0° to 81.5°	10 14
	Galactosyl Fructose	
molecular weight	342.30 (predicted)	15
boiling point	780.1 ± 60°C (at 760 Torr; predicted)	15
log P	-2.810 ± 0.846 (at 25°C; predicted)	15
	Galacturonic Acid	
physical characteristics	α-form: monohydrate, needles	10
molecular weight	194.14	10
melting point	α-form: 159°C β-form: 166°C	10
solubility	α-form: soluble in water; slightly soluble in hot alcohol; practically insoluble in ether	10
specific optical rotation	α-form, (α^{20}_D): +98.0° ⟶ +50.9° (water) β-form, (α_D): +27° ⟶ +55.6° (water)	10
	Gluconic Acid	
physical characteristics	crystals; mild acid taste white crystalline powder anhydrous: commercial form is a 50% aq. solution, which is a colorless to brownish liquid.	16 17 9,17
molecular weight	196.16	16

Table 2. Chemical and Physical Properties

Property	Description	Reference
melting point	131°C	16
solubility	freely soluble in water; slightly soluble in alcohol; insoluble in ether and most other organic solvents	16
stability	in aq. solutions, the acid is partially transformed into an equilibrium mixture with γ - and δ-gluconolactones	16
	reacts with strong oxidants	17
	on combustion, forms carbon monoxide	17
specific optical rotation (α^{20}_D)	-16.7°	16
density	1.23 g/cm^3	17
log P_{ow}	-1.87 (estimated)	17
pK_a	12.06 (18°C)	16
	Glucose	
physical characteristics	α-form monohydrate: crystals from water α-form anhydrous: crystals from hot ethanol or water β-form: crystals from hot water and ethanol, from diluted acetic acid, or from pyridine	10
	white D-glucose: powder with sweet taste	18
molecular weight	180.16	10
melting point	α-form monohydrate: 83°C α-form anhydrous: 146°C β-form: 148-155°C	10
solubility	α-form anhydrous: soluble in hot glacial acetic acid, pyridine, aniline; very sparingly soluble in absolute alcohol, ether, acetone	10
log P_{ow}	D-glucose: -3.3	18
specific optical rotation	α-form monohydrate, (α^{20}_D): +102.0° ⟶ +47.9°C (water) α-form anhydrous, (α^{20}_D): +112.2° ⟶ +52.7°C (water) β-form, (α^{20}_D): +18.7° ⟶ +52.7° (water)	10
stability	D-glucose reacts violently with strong oxidants	18
	Isomalt	
physical characteristics	white crystalline, odorless, slightly hydroscopic substance	3,19
molecular weight	380.32	3
boiling point	788.5 ± 60°C (at 760 Torr; predicted)	15
solubility	soluble in water; very slightly soluble in ethanol	3,19
log P	-2.810 ± 0.846 (at 25°C; predicted)	15
pK_a	12.89 ± 0.70 (25°C) (predicted)	15
	Lactitol	
physical characteristics	crystals from absolute ethanol; strongly hygroscopic monohydrate: white, sweet, odorless crystalline solid; non-hygroscopic dihydrate: white, sweet, odorless crystalline powder	10
molecular weight	344.31 (anhydrous); 362.37 (monohydrate)	3,10
melting point	146°C monohydrate: 94-97°C dihydrate: 75°C (food-grade)	10
partition coefficient	< -3 (20°C)	20
solubility	soluble in water, dimethyl sulfoxide, *N*,*N*-dimethylformamide; slightly soluble in ethanol, ether	10
specific optical rotation	(α^{23}_D): +14° monohydrate, (α^{22}_D): +12.3° dihydrate, (α^{25}_D): +13.5 – 15.0°	10
	Lactose	
physical characteristics	α-lactose monohydrate: monoclinic sphenoidal crystals from water; faintly sweet taste; readily absorbs odors	10
molecular weight	342.30	10
particle size distribution	varies by grade	12
melting point	α-lactose monohydrate: 201-202°C	10
solubility	α-lactose monohydrate: practically insoluble in alcohol; insoluble in chloroform, ether	10
specific optical rotation	α-lactose monohydrate, (α^{20}_D): shows mutarotation; +92.6° ⟶ +83.5° (10 min.) ⟶ +69° (50 min) ⟶ +52.3° (22 h) β-lactose, (α^{25}_D): +34° (3 min) ⟶ +39° (6 min) ⟶ +46° (1 hr) ⟶ +52.3° (22 h)	10
K_a (16.5°C)	α-lactose monohydrate: 6.0 x 10^{-13}	10
	Lactulose	
physical characteristics	hexagonal clustered plates from methanol	10
molecular weight	342.30 (anhydrous); 360.32 (monohydrate)	3,10
melting point	168-171°C	10
solubility	freely soluble in water	21
specific optical rotation (α^{22}_D)	shows mutarotation; constant value after 24 h, -51.5°	10

Table 2. Chemical and Physical Properties

Property	Description	Reference
	Maltose	
physical characteristics	monohydrate: crystals from water or diluted alcohol	10
molecular weight	342.30	10
melting point	monohydrate: 102-103°C	10
solubility	α-lactose monohydrate: practically insoluble in alcohol; insoluble in chloroform, ether	10
pH	anhydrous: 3.7-4.7; monohydrate: 4.5-5.5	14
specific optical rotation (α^{20}_{D})	monohydrate: shows mutarotation; +111.7° ⟶ +130.4°	10
pK_a (21°C)	monohydrate: 12.05	10
	Mannose	
physical characteristics	α-form: crystals from methanol β-form: orthorhombic, bisphenoidal needles from alcohol or acetic acid; sweet taste with bitter aftertaste	10
molecular weight	180.16	10
melting point	α-form: 133°C β-form: decomposes at 132°C	10
specific optical rotation	α-form, (α_D): +29.3° ⟶ +14.2° (water) β-form, (α^{20}_{D}): -17.0° ⟶ +14.2° (water)	10
	Melibiose	
physical characteristics	dihydrate: monoclinic crystals from water of diluted alcohol	10
molecular weight	342.30	10
dihydrate	α-form: 84-85°C	10
specific optical rotation (α^{20}_{D})	dihydrate:: +111.7° ⟶ +129.5°	10
	Potassium Gluconate	
physical characteristics	yellowish-white crystals; mild, slightly saline, taste	16
molecular weight	234.25 (anhydrous); 252.26 (monohydrate)	3,16
melting point	decomposes at 180°C	16
solubility	freely soluble in water and glycerin; practically insoluble in alcohol, ether, benzene, and chloroform	3,16
log P_{ow}	-5.99 (estimated)	9
pH	7.5-8.5 (aq. solution)	16
stability	stable in air	16
specific optical rotation (α^{20}_{D})	-16.7°	16
density	0.80 g/cm^3 (20°C; bulk density)	9
	Rhamnose	
physical characteristics	α-form: monohydrate, holohedric rods from water; hemihedric monoclinic columns from alcohol; very sweet taste β-form: needles; hygroscopic	10
molecular weight	164.16	10
melting point	α-form: 82-92°C; sublimes at 105°C and 2 mm Hg β-form: 122-126°C	10
specific optical rotation	α-form, (α^{20}D): shows mutarotation; -7.7° ⟶ +8.9° β-form, (α^{20}_{D}): -17.0° ⟶ +31.5°	10
specific gravity (d^{20}_4)	1.4708	10
stability	α-form: loses water of crystallization upon heating, and partially changes to the β-modification β-form: changes into crystals of the α-modification upon exposure to moist air	10
	Ribose	
physical characteristics	plates from absolute alcohol	10
molecular weight	150.13	10
melting point	87°C	10
solubility	soluble in water, slightly soluble in alcohol	10
specific optical rotation (α^{20}_{D})	final, shows complex mutarotation; -25°	10
	Sodium Gluconate	
physical characteristics	white crystals white to tan, granular to fine, crystalline powder technical grade may have a pleasant odor	22 3 16
molecular weight	218.14	16
melting point	170-175°C; decomposes at 196-198°C	22
solubility	soluble in water; sparingly soluble in alcohol; insoluble in ether	16
log P_{ow}	-5.99 (estimated)	22
density	1.8 g/cm^3	22
	Sucralose	
physical characteristics	anhydrous crystalline form: orthorhombic needle-like crystals; intensely sweet taste	10
molecular weight	397.63	10

Table 2. Chemical and Physical Properties

Property	Description	Reference
particle size distribution	90% <12 µm	12
solubility	soluble in water	23
octanol/water partition coefficient	-0.51 ($\log_{10}$ P)	12
specific optical rotation (α_D)	+68.2°	10
	(α^{20}_D): +84.0° to +87.5°, calculated on the anhydrous basis	3
	Sucrose	
physical characteristics	monoclinic sphenoidal crystals, crystalline masses, blocks, or powder; sweet taste	10
	hard, white, odorless crystals, lumps, or powder; may have a characteristic caramel odor when heated	24
molecular weight	342.30	10
melting point	decomposes at 160-186°C	10
solubility	moderately soluble in glycerol, pyridine; practically insoluble in dehydrated alcohol	10
log P_{ow}	-3.67	25
specific optical rotation	(α^{20}_D): +65.9° to +66.7°	3
	(α^{25}_D): +66.47 to +66.49°	10
pK_a	12.62	10
specific gravity (d^{25}_4)	1.587	10
stability	stable in air	10
	hydrolyzed to glucose and fructose by diluted acids and by invertase	
	Trehalose	
physical characteristics	orthorhombic, bisphenoidal crystals for diluted alcohol; sweet taste	10
	typically found in the dihydrate form; characterized by low hygroscopicity	26,27
molecular weight	342.30	10
melting point	the dihydrate melts at 97°C; additional heat drives off the water of crystallization until it resolidifies at 130°C; the anhydrous then melts at 210°C	27
solubility	very soluble in water, formamide, and dimethyl sulfoxide; soluble hot alcohol; slightly soluble to insoluble in ether	3,10
stability	very stable and chemically unreactive; does not dissociate into two reducing monosaccharidic constituents unless exposed to extreme hydrolytic conditions or to the actions of trehalase	28
specific optical rotation (α^{20}_D)	+178°	10
	Xylobiose	
molecular weight	282.24 (predicted)	15
boiling point	604 ± 55°C (at 760 Torr; predicted)	15
log P	-2.900 ± 0.852 (at 25°C; predicted)	15
pK_a	12.40 ± 0.20 (25°C) (predicted)	15
	Xylose	
physical characteristics	monoclinic needles or prisms; very sweet taste	10
	white, odorless, crystal or crystalline powder with a sweet taste	29
molecular weight	150.13	10
melting point	144-145°C	10
solubility	soluble in glycerol, pyridine, hot alcohol	10
specific optical rotation (α^{20}_D)	shows mutarotation; +92° → +18.6° (16 hrs)	10
pK_a (18°C)	12.14	10
specific gravity (d^{20}_4)	1.525	10

Table 3. Natural Occurrence and /or Methods of Preparation

Ingredient	Natural Occurrence and/or Method of Preparation	Reference
Fructose	- occurs in many fruits and in honey	10
	- prepared by adding absolute alcohol to the syrup obtained from the acid hydrolysis of inulin; prepared from dextrose; prepared from sucrose by enzymatic conversion	
	- obtained from glucose in corn syrup by the use of glucose isomerase	3,13
Fucose	D-: obtained from glucosides found in various species of Convolvulaceae	10
	L-: occurs in seaweed - *Ascophyllum nodosum, (Fucus nodosus*), *Fucus vesiculosu., F. serratus, F. virsoides, Fucaceae* - and in gum tragacanth	
	L-: a common component of many N- and O-linked glycans and glycolipids produced by mammals	48
Galactose	- constituent of many oligo- and polysaccharides in pectins, gums, and mucilages; isolation in the processing of the red algae, *Porphyra umbilicalis*	10
	- a product of lactose metabolism	14
Galacturonic Acid	obtained by hydrolysis of pectin where it is present as polygalacturonic acid	10
Gluconic Acid	- prepared by oxidation of glucose; produced commercially using *Aspergillus niger, A. fumaricus, Aerobacter aceti, Penicillium chrysogenum,* or other *Penicillia*	16,50

Table 3. Natural Occurrence and /or Methods of Preparation

Ingredient	Natural Occurrence and/or Method of Preparation	Reference
Glucose	- produced by the complete hydrolysis of corn starch with safe and suitable acids or enzymes, followed by refinement and crystallization from the resulting hydrolysate - occurs naturally and in the free state in fruits and other parts of plants; combined in glucosides, in disaccharides and oligosaccharides, in the cellulose and starch of polysaccharides, and in glycogen; manufactured on a large scale from starch; below 50°C, α-D-glucose hydrate is the stable crystalline form, above 50°C, the anhydrous form is obtained, and at higher temperatures, β-D-glucose is formed - normal human blood contains 0.08-0.1%	21CFR184.1857 10
Isomalt	produced from food-grade sucrose in a two-stage process: beet sugar is converted by enzymatic transglucosidation into isomaltulose, which undergoes catalytical hydrogenation to produce isomalt	12
Lactitol	prepared by the hydrogenation of lactose	10
Lactose	- present in the milk of mammals: human, 6.7%: cow, 4.5% - by-product of the cheese industry, produced from whey - β-lactose: obtained by crystallizing concentrated solutions of α-lactose above 93.5°C	10
Lactulose	- synthetic disaccharide composed of galactose and fructose - can be produced from agricultural by-products and from lactose	10 64
Maltose	obtained in 80% yield by enzymatic (diastase) degradation of starch	10
Mannose	α-form prepared by treating ivory nut shavings with H_2SO_4	10
Melibiose	prepared from raffinose by fermentation with top yeast, which removes the fructose	10
Potassium Gluconate	prepared by the reaction of potassium hydroxide or carbonate with gluconic acid	45
Rhamnose	- occurs free in poison sumac; combined in the form of glycosides of many plants; isolated from the walls of gram-negative bacteria α-form: obtained by crystallization from water or ethyl alcohol β-form: prepared by heating α-rhamnose monohydrate on a steam bath	10
Ribose	prepared by hydrolysis of yeast-nucleic acid; obtained from glucose, nucleosides, D-erythrose, and L-glutamic acid; obtained by the reduction of D-ribonic acid	10
Sucralose	- chlorinated derivative of sucrose - synthesized by selective chlorination of sucrose at three of the primary hydroxyl groups - can be synthetized by the reaction of sucrose (or an acetate) with thionyl chloride	10 55 12
Sucrose	- obtained from sugar cane and sugar beet: sugar cane (*Saccharum officinarum*) contains 10-15% sucrose, sugar beet (*Beta vulgaris*) contains 10-17% sucrose - sucrose is obtained by crystallization from sugar cane or sugar beet juice that has been extracted by pressing or diffusion, then clarified and evaporated - most abundant carbohydrate in the sap of land plants	10 21CFR184.1854 49
Trehalose	- found in fungi, bacteria, yeasts, and insects; isolated from the ergot of rye; isolated from yeast - produced from starch using the enzymes maltooligosyl-trehalose synthase and maltooligosyl-trehalose trehalohydrolase	10 27
Xylose	- widely distributed in plant materials, especially wood (maple, cherry), in straw, and in hulls; not found in the free state – is found in the form of xylan, a polysaccharide consisting of D-xylose units occurring in association with cellulose; also occurs as part of glycosides; can be isolated from corn cobs - produced industrially by hydrolysis of extracts from cotton seed shells, press residue of sugarcane and beech tree chips	10 29

Table 4. Purity specifications

Ingredient	Purity Specifications
Fructose	food use: NMT 0.018% chloride; NMT 0.1 mg/kg lead; NMT 0.5% glucose; NMT 0.1% hydroxymethylfurfural, calculated on the dried ash and free-ash basis; NMT 0.5% loss on drying; NMT 0.5% residue on ignition (sulfated ash)[3] USP: NMT 1 ppm arsenic;; NMT 5 ppm heavy metals: NMT 0.5% loss on drying; NMT 0.5% residue on ignition[14]
Galactose	USP: NMT 1.0% water; NMT 0.1% residue on ignition[14]
Isomalt	food use: NMT 7% water; NMT 0.05% sulfated ash; NMT 3% D-mannitol; NMT 6% D-sorbitol; NMT 0.3% reducing sugars; NMT 2 mg/kg nickel; NMT 1 mg/kg lead[19]; - NMT 1 mg/kg lead; NMT 2 mg/kg nickel; NMT 3% mannitol and NMT 6% sorbitol; NMT 0.3% cuprous oxide (as glucose); NMT 7.0% water; NMT 0.05% residue on ignition (sulfated ash)[3] USP: NMT 7% water; NMT 1 µg/g nickel; NMT 10 µg/g heavy metals; NMT 0.3% reducing sugars[14]
Lactitol	food use: NMT 1 mg/kg lead; NMT 1 mg/kg nickel; NMT 4.0% other hydrogenated saccharides (polyols); NMT 5% water; NMT 0.3% cuprous oxide residue; NMT 0.1% residue on ignition (sulfated ash)[3] USP: 4.5-5.5% water, monohydrate, or 10.5%, dihydrate; NMT 0.5% water, anhydrate; NMT 0.5% residue on ignition[14]
Lactose	food use: NMT 0.5 mg/kg arsenic; NMT 0.5 mg/kg lead; NMT 0.3% residue on ignition (sulfated ash)[3]; loss on drying: not less than 4.5% and NMT 5.5%, monohydrate and spray-dried mixture; NMT 1.0%, anhydrous[3] USP: water: NMT 1.0%, anhydrous, 4.5-6.5%, monohydrate; heavy metals: 5 µg/g, anhydrous and monohydrate; loss on drying: NMT 0.5%, anhydrous and monohydrate; residue on ignition: NMT 0.1%, anhydrous and monohydrate[14]
Maltose	USP: water: NMT 1.5%, anhydrous, 4.5-5.5%, monohydrate; NMT 5 µg/g heavy metals; NMT 0.05% residue on ignition[14]

Table 4. Purity specifications

Ingredient	Purity Specifications
Potassium Gluconate	food use: NMT 1% calculated as D-glucose;[84] NMT 2 mg/kg lead;[3,84] NMT 1.0% reducing substances; NMT 3.0% (anhydrous) and 6.0-7.5% (monohydrate) loss on drying[3] USP: NMT 0.002% heavy metals; NMT 1.0% reducing substances; loss on drying: NMT 3.0%, anhydrous, and 6.0-7.5%, monohydrate [14]
Sodium Gluconate	food use: NMT 2 mg/kg lead; NMT 0.5% reducing substances, calculated as D-glucose [3] USP: NMT 0.001% lead; NMT 0.002% heavy metals; NMT 0.5% reducing substances[14]
Sucralose	food use: NMT 1 mg/kg lead; NMT 2.0% water; NMT 0.1% methanol; NMT 0.7% residue on ignition (sulfated ash)[3] USP: NMT 2.0% water; NMT 0.001% heavy metals; NMT 0.7% residue on ignition[14]
Sucrose	food use: NMT 1 mg/kg arsenic; NMT 0.1 mg/kg lead; NMT 0.1% invert sugars; NMT 0.15% residue on ignition (sulfated ash); NMT 0.1% loss on drying[3] USP: NMT 5 ppm heavy metals; NMT 0.05% residue on ignition[14]
Trehalose	food use: NMT 0.1 mg/kg lead; NMT 11.0% water; NMT 0.05% residue on ignition (sulfated ash)[3]
Xylose	USP: NMT 5 ppm iron; NMT 0.001% heavy metals; NMT 0.1% loss on drying; NMT 0.5% residue on ignition[14]

Abbreviation: NMT – not more than

Table 5. Frequency and concentration of use according to duration and type of exposure

	# of Uses[31]	*Max. Conc. of Use (%)*[32]	*# of Uses*[31]	*Max. Conc. of Use (%)*[32]	*# of Uses*[31]	*Max. Conc. of Use (%)*[32]
	Calcium Gluconate		**Fructose**		**Fucose**	
Totals*	**68**	**0.0000075-1**	**172**	**0.0001-20**	**3**	**NR**
Duration of Use						
Leave-On	*50*	*0.0000075-1*	*144*	*0.0002-2*	*3*	*NR*
Rinse Off	*18*	*0.0000075-0.1*	*28*	*0.0001-20*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	4	0.0000075-.05	10	0.002-0.075	1	NR
Incidental Ingestion	NR	0.00006-0.5	1	NR	NR	NR
Incidental Inhalation-Spray	21[a]; 13[b]	spray: 0.0006-0.1 0.0000075-0.01[a]	1; 62[a]; 50[b]	0.23; aerosol: 0.0002 0.08-2[a]	2[b]	NR
Incidental Inhalation-Powder	2; 13[b]	0.2	50[b]	0.002	2[b]	NR
Dermal Contact	61	0.0000075-1	153	0.0003-20	3	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	7	0.008-0.1	18	0.0001-0.1	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	4	0.00006-0.5	4	0.0015-0.002	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

	Galactosyl Fructose		**Gluconic Acid**		**Glucose**	
Totals*	**1**	**NR**	**2**	**0.0001-0.18**	**425**	**0.00003-97.8**
Duration of Use						
Leave-On	*1*	*NR*	*2*	*0.0001-0.18*	*276*	*0.0001-91*
Rinse Off	*NR*	*NR*	*NR*	*NR*	*140*	*0.00003-97.8*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*9*	*19*
Exposure Type						
Eye Area	NR	NR	NR	NR	28	0.0001-0.48
Incidental Ingestion	NR	NR	NR	NR	1	0.059-97.8 (97.8 is an ingested breath freshener)
Incidental Inhalation-Spray	1[b]	NR	2[a]	NR	1; 38[a]; 101[b]	0.24; spray: 1 0.0045-2.9[a]
Incidental Inhalation-Powder	1[b]	NR	NR	NR	3[c]; 101[b]	NR
Dermal Contact	1	NR	2	0.001-0.18	319	0.0001-84
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	36	0.00003-91
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	1	0.0004
Mucous Membrane	NR	NR	4	NR	29	0.00063-97.8 (97.8 is an ingested breath freshener)
Baby Products	NR	NR	NR	NR	4	NR

Table 5. Frequency and concentration of use according to duration and type of exposure

	# of Uses[31]	*Max. Conc. of Use (%)*[32]	*# of Uses*[31]	*Max. Conc. of Use (%)*[32]	*# of Uses*[31]	*Max. Conc. of Use (%)*[32]
	Isomalt		**Kefiran**		**Lactitol**	
Totals*	**12**	**0.19-7.77**	**2**	**0.1**	**9**	**0.15-0.2**
Duration of Use						
Leave-On	*11*	*0.19*	*2*	*0.1*	*NR*	*NR*
Rinse Off	*1*	*7.77*	*NR*	*NR*	*9*	*0.15-0.2*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	2	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	2[a]; 5[b]	NR	2[b]	NR	NR	NR
Incidental Inhalation-Powder	5[b]	NR	2[b]	NR	NR	NR
Dermal Contact	10	0.19	2	0.1	4	0.15-0.2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	5	NR
Hair-Coloring	NR	7.77	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	1	0.2
Baby Products	NR	NR	NR	NR	NR	NR

	Lactose		**Maltose**		**Mannose**	
Totals*	**77**	**0.0005-9.4**	**3**	**0.3-0.5**	**5**	**5**
Duration of Use						
Leave-On	*28*	*0.0005-6*	*3*	*0.3-0.5*	*5*	*5*
Rinse Off	*48*	*0.038-9.4*	*NR*	*0.5*	*NR*	*NR*
Diluted for (Bath) Use	*1*	*8*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	8	NR	1	NR	1	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	4[a]; 10[b]	0.0005[a]; 6[b]	NR	NR	4[a]	NR
Incidental Inhalation-Powder	10[b]	6[b]	NR	NR	NR	NR
Dermal Contact	70	0.001-6	1	0.3-0.5	5	5
Deodorant (underarm)	NR	aerosol: 0.038 not spray: 0.075-0.25	NR	NR	NR	NR
Hair - Non-Coloring	3	0.0005-9.4	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	3	0.3	1	NR	NR	NR
Mucous Membrane	33	0.038-8 diluted use product:	NR	NR	NR	NR
Baby Products	1	NR	NR	NR	NR	NR

	Melibiose		**Potassium Gluconate**		**Rhamnose**	
Totals*	**2**	**0.1-0.25**	**8**	**0.002-0.1**	**7**	**5-10**
Duration of Use						
Leave-On	*2*	*0.1-0.25*	*7*	*0.002-0.1*	*7*	*5-10*
Rinse Off	*NR*	*NR*	*1*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	1	0.1	1	NR	1	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	1[a]; 3[b]	0.05[a]	4[a]	NR
Incidental Inhalation-Powder	NR	NR	3[b]	NR	NR	NR
Dermal Contact	2	0.1-0.25	7	0.002-0.1	7	5-10
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	1	0.05	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

Table 5. Frequency and concentration of use according to duration and type of exposure

	# of Uses[31]	Max. Conc. of Use (%)[32]	# of Uses[31]	Max. Conc. of Use (%)[32]	# of Uses[31]	Max. Conc. of Use (%)[32]
	Ribose		**Sodium Gluconate**		**Sucralose**	
Totals*	**13**	**0.05**	**168**	**0.0000075-12**	**84**	**0.012-1.2**
Duration of Use						
Leave-On	*11*	*0.05*	*78*	*0.0000075-12*	*39*	*0.2-0.6*
Rinse Off	*2*	*NR*	*90*	*0.0000075-0.8*	*45*	*0.012-1.2*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	5	0.0000075-0.2	1	NR
Incidental Ingestion	NR	NR	NR	0.00006-0.75	68	0.012-1.2
Incidental Inhalation-Spray	7[a]; 2[b]	NR	29[a]; 27[b]	spray: 0.0006 0.0000075-0.6[a]	3[a]	0.012-0.95[a]
Incidental Inhalation-Powder	2[b]	NR	27[b]	NR	NR	NR
Dermal Contact	13	0.05	104	0.0000075-5	16	0.5-0.6
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	61	0.2-12	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	19	0.00006-0.8	68	0.012-1.2
Baby Products	NR	NR	1	NR	NR	NR

	Sucrose		**Trehalose**		**Xylobiose**	
Totals*	**738**	**0.001-65**	**474**	**0.0001-2**	**2**	**0.0075-0.15**
Duration of Use						
Leave-On	*423*	*0.001-58*	*356*	*0.00055-2*	*2*	*0.075-0.15*
Rinse Off	*303*	*0.001-65*	*118*	*0.0001-1*	*NR*	*0.0075-0.05*
Diluted for (Bath) Use	*12*	*1-52*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	57	0.0035-2	47	0.02-1.1	NR	NR
Incidental Ingestion	4	9-45	3	0.005-0.1	NR	NR
Incidental Inhalation-Spray	4; 157[a]; 84[b]	0.002; spray: 1; 0.002-2[a]	4; 163[a]; 88[b]	0.002-1[a]	1[a]; 1[b]	0.091[a]
Incidental Inhalation-Powder	4; 84[b]	NR	1; 88[b]; 1[c]	0.12	1[b]	NR
Dermal Contact	672	0.001-65	376	0.00055-2	2	0.0075-0.15
Deodorant (underarm)	NR	aerosol: 0.004 not spray: 0.005-0.009	NR	NR	NR	NR
Hair - Non-Coloring	53	0.001-10.5	91	0.0001-1	NR	0.091
Hair-Coloring	5	NR	NR	NR	NR	NR
Nail	2	13.6	1	1	NR	NR
Mucous Membrane	205	0.001-65	11	0.005-0.1	NR	0.0075
Baby Products	1	NR	1	NR	NR	NR

	Xylose			
Totals*	**75**	**0.1-1**		
Duration of Use				
Leave-On	*68*	*0.1-0.11*		
Rinse Off	*7*	*0.1-1*		
Diluted for (Bath) Use	*NR*	*NR*		
Exposure Type				
Eye Area	1	NR		
Incidental Ingestion	NR	NR		
Incidental Inhalation-Spray	4; 10[a]; 13[b]	pump spray: 0.11		
Incidental Inhalation-Powder	13[b]	NR		
Dermal Contact	28	NR		
Deodorant (underarm)	NR	NR		
Hair - Non-Coloring	47	0.1-0.11		
Hair-Coloring	NR	1		
Nail	NR	NR		
Mucous Membrane	NR	NR		
Baby Products	NR	NR		

* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types my not equal the sum of total uses

a Includes products that can be sprays, but it is not known whether the reported uses are sprays

b Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

c Includes products that can be powders, but it is not known whether the reported uses are powders

NR – not reported

Table 6. Ingredients Not Reported to be Used

Galactose
Galacturonic Acid
Lactulose

Table 7. Examples of non-cosmetic uses

Ingredient	Use	Reference
Calcium Gluconate	- a direct food additive used as a firming agent, formulation aid, sequestrant, and texturizer	21CFR184.1199
	- used as mineral supplements in pharmaceutical injection solutions	[9]
	- GRAS in animal feed	21CFR582.1199; 21CFR582.6199
Fructose	- listed in the *United States Pharmacopeia* (USP)*/National Formulary* (NF)	[3]
	- inactive ingredient for approved drugs; used in oral, intravenous, and rectal drugs	[14]
	- can function as a dissolution enhancer, flavoring agent, sweetening agent, and tablet diluent in pharmaceuticals, is used tablets, syrups, and solutions as a flavoring and sweetening agent	[40] [12]
Galactose	- listed in the USP/NF	[14]
	- inactive ingredient for approved drugs; used in oral and rectal products	[40]
Gluconic Acid	industrial cleaning; metal surface treatment; textile bleach stabilizer; aluminum processing ; chelating agent in dispersive cements, cleaning products, pharmaceuticals, and food stuff; sequestering agent in dispersive building materials	[9]
Glucose	- in sweeteners and table syrups, with specifications defined in the CFR	21CFR168.110, 111, 120, 121
	- in a glucose/glycine/electrolyte in animal drugs, feeds, and related products	21CFR520.550
	- listed in the USP/NF as a liquid	[14]
	- approved as an inactive ingredient for approved drugs; used in oral products	[40]
Isomalt	- listed in the *Foods Chemicals Codex* as a texturizer, formulation aid, surface finishing agent, stabilizer, thickener	[3]
	- listed in the USP/NF	[14]
	- inactive ingredient for approved drugs; used in oral products	[40]
	- can function as a coating agent, granulation aid, medicated confectionary base, sweetening agent, or tablet and capsule diluent in pharmaceuticals; a non-cariogenic excipient used in tablets or capsules, coatings, sachets, and effervescent tablets; often used in buccal applications	[12]
Lactitol	- listed in the *Foods Chemicals Codex* as a humectant, stabilizer	[3]
	- listed in the USP/NF	[14]
	- inactive ingredient for approved drugs; used in oral products (the monohydrate)	[40]
	- can function as a sweetening agent, tablet and capsule diluent, and therapeutic agent in pharmaceuticals; used as a non-cariogenic replacement for sucrose, a diluent in solid dosage forms, and therapeutically in the treatment of encephalopathy and as a laxative	[12]
Lactose	- in sweeteners and table syrups, with specifications defined in the CFR	21CFR168.122
	- used as a nutrient in the preparation of modified milk and food for infants and convalescents (predominantly the α-form, but also the β-form)	[10]
	- listed in the *Foods Chemicals Codex* as a processing aid, humectant (anhydrous form), texturizer	[3]
	- inactive ingredient for approved drugs; used in transdermal, oral, sublingual, buccal, inhalation, subcutaneous, vaginal, intravenous, intramuscular, and rectal drugs	[40]
	- in pharmaceuticals, lactose, anhydrous can function as a directly compressible tablet excipient, dry powder inhaler carrier, lyophilization aid, tablet and capsule diluent, tablet and capsule filler; widely used in direct compression tableting applications and as a tablet and capsule filler and binder, and it can be used in i.v. injections	[12]
	- lactose, monohydrate can function as a dry powder inhaler carrier, lyophilization aid, tablet binder, tablet and capsule diluent, tablet and capsule filler; is widely used as a filler and diluent in tablets and capsules	
	- lactose, inhalation can function as a diluent and as a dry powder inhaler carrier; it is widely used as a carrier, diluent, and flow aid in dry powder formulations, and when of suitable particle size, it can be used to prepare soft pellets of dry powder inhaler formulations	
	- lactose, spray-dried can function as a directly compressible tablet excipient, tablet and capsule diluent, tablet and capsule filler; widely used as a binder, filler-binder, and flow aid in direct compression tableting	
Lactulose	- listed in the USP/NF as a concentrate	[14]
	- an approved drug used to treat constipation; used in oral and rectal products	[39]
Maltose	-listed in the Everything Added to Food in the United States (EAFUS) inventory	[41]
	- listed in the USP/NF	[14]
	- inactive ingredient for approved drugs; used in oral drugs (the anhydrous form)	[40]
	- can function as a sweetening agent and tablet excipient in pharmaceuticals	[12]
Mannose	inactive ingredient for approved drugs; used in oral drugs (D-mannose)	[40]
Potassium Gluconate	- listed in the *Foods Chemicals Codex* as a sequestrant	[3]
	- listed in the USP/NF	[14]
Rhamnose	listed in the EAFUS inventory	[41]
Ribose	listed in the EAFUS inventory	[41]
Sodium Gluconate	- GRAS as a sequestrant in animal drugs, feeds, and related products, with no limitation other than current GMP	21CFR582.6757
	- listed in the *Foods Chemicals Codex* as sequestrant	[3]
	- listed in the USP/NF	[14]
	- inactive ingredient for approved drugs; used in oral products	[40]
Sucralose	- listed in the *Foods Chemicals Codex* as a flavor enhancer	[3]
	- listed in the USP/NF	[14]
	- inactive ingredient for approved drugs; used in oral, sublingual, and buccal drugs	[40]
	- can function as a sweetening agent in pharmaceuticals	[12]

Table 7. Examples of non-cosmetic uses

Ingredient	Use	Reference
Sucrose	- as the starting material in the fermentative production of ethanol, butanol, glycerol, citric acid, and levulinic acid	10
	- listed in the *Foods Chemicals Codex* as a formulation and texturizing aid	3
	- inactive ingredient for approved drugs; used in topical, oral, sublingual, buccal, subcutaneous, intravenous, and rectal drugs	40
	- functions as a confectionary base, coating agent, granulation aid, suspending agent, sweetening agent, tablet binder, tablet and capsule diluent, tablet filler, therapeutic agent, and viscosity increasing agent in pharmaceuticals; widely used in oral formulations	12
Trehalose	- listed in the *Foods Chemicals Codex* as a humectant, stabilizer, thickener, texturizer	3
	- used as an excipient in a few monoclonal antibody products	26
	- can function as a color adjuvant, flavor enhancer, freeze-drying agent, humectant, stabilizing agent, sweetening agent, table diluent, and thickening agent in pharmaceuticals; used for the lyoprotection of therapeutic proteins	12
Xylose	- listed in the EAFUS inventory	41
	- listed in the USP/NF	14

Table 8. Nutritive and non-nutritive sweeteners and food additives

Nutritive[3,14,42,85]	Non-Nutritive[3,43]
fructose	lactitol
galactose	sucralose
glucose	xylose
isomalt	
lactose	
maltose	
potassium gluconate	
sodium gluconate	
sucrose	
trehalose	

Table 9. Summary metabolism data

Ingredient (GRAS foods are noted)	Metabolism Data	Reference
	Absorbed and Metabolized (Nutritive)	
Calcium Gluconate (GRAS)	calcium and the gluconate anion are common constituents of food and are metabolized by the normal metabolic processes in man	47
Fucose	L-fucose is a common component of many *N*- and *O*-linked glycans and glycolipids produced by mammalian cells	48
Fructose (GRAS)	- metabolism of fructose occurs mainly in the liver; it is converted partially to dextrose and to lactic and pyruvic acid; further metabolism occurs by a variety of metabolic pathways	12
	- serum fructose levels were higher in adult humans fed sucrose than when fed a mixture of glucose and fructose; release of fructose by hydrolysis of sucrose within the brush border may facilitate absorption of fructose; also the furanose ring structure of fructose as released may be more readily absorbed than the equilibrium mixture of pyranose and furanose forms attained after being in solution for some time	49
Galactose	actively absorbed from the gut; converted in the liver through the Leloir pathway to yield glucose-6-phosphate	85-87
Gluconic Acid	a normal metabolic product of glucose oxidation, is an important intermediate in carbohydrate metabolism in mammals; contributes to the synthesis of nicotinamide-adenine dinucleotide phosphate (NADPH), and it leads to the formation of ribose-5-phosphate; the amount produced endogenously is many times greater than the largest amounts likely to be consumed from food; the daily production of gluconate from endogenous sources is about 450 mg/kg for a 60 kg person	9,38,50
Glucose (GRAS)	rapidly absorbed from the small intestine, principally by an active mechanism	44
Potassium Gluconate (GRAS)	- important intermediate in carbohydrate metabolism	9
	- readily absorbed in the intestine, the potassium ion ionize almost immediately to potassium and gluconic acid; with parental administration, a significant portion (60-85%) is excreted unchanged in the urine	45,46
Ribose	rapidly and extensively metabolized; converted to glucose via the pentose phosphate pathway in the liver and other tissues	88
Sodium Gluconate (GRAS)	important intermediate in carbohydrate metabolism	9

Table 9. Summary metabolism data

Ingredient (GRAS foods are noted)	Metabolism Data	Reference
Sucrose	- known to be a relatively efficient source of energy; rapidly metabolizable for utilization and storage - hydrolyzed in the small intestine by sucrose to yield dextrose and fructose, which are then absorbed - there is evidence that sucrose can be absorbed unchanged to a small extent, particularly at high dietary level; nearly all ingested sucrose is absorbed as glucose and fructose, its metabolism is essentially that of these two monosaccharides - excreted unchanged in the urine when administered intravenously	51 12 49 12
	Metabolized in the small intestines	
Lactose	broken down in the gut by lactase to produce glucose and galactose	85
Maltose	broken down in the gut by maltase to yield two glucose molecules	85
Trehalose	- rapidly metabolized in the gut to glucose by trehalase - metabolism is essentially identical to that of other disaccharides that are consumed as part of the human diet	12 27,53
	Not Absorbed (or Limited Absorption)	
Isomalt	hydrolysis and absorption in the small intestine is limited because the glycoside linkage between the mannitol or sorbitol moiety and the glucose moiety is very stable; the majority of isomalt is fermented in the large intestine (nutritive)	12
Lactitol	not absorbed in the small intestine; broken down by microflora in the large intestine (non-nutritive)	12
Lactulose	-not readily absorbed from the intestine in humans; not hydrolyzed by intestinal disaccharidases; <1% of a 5 g dose given orally was recovered in the urine - reaches the large intestine essentially unchanged, where it is metabolized by bacteria with the formation of low molecular weight acids	52 21
Mannose	little disposition of glycogen in the liver following oral ingestion; transport across the liver is approximately 1/10 that of glucose, suggesting diffusion; significant amounts excreted in the urine following oral administration; no significant reabsorption by the kidney	54
Sucralose (GRAS)	- highly water-soluble, not lipophilic, and does not bioaccumulate; the major portion of an oral dose of sucralose is unabsorbed and excreted unchanged in the feces of rats, mice, rabbits, dog, and man; only two minor metabolites were detected following oral dosing in the mouse, rat, and man, and only one urinary metabolite was found in the rabbit and the dog - not metabolized or used for energy in mammalian systems	23,55-59,89 60
	Limited Absorption/Not Metabolized	
Xylose	- D-xylose is passively absorbed in rats; in rats and man, oral absorption was incomplete (about 70% absorbed) and xylose was eliminated primarily unchanged in the urine	61

Table 10. Genotoxicity studies

Test Article	Concentration/Vehicle	Procedure	Test System	Results	Reference
		IN VITRO			
Calcium Gluconate	12.5, 25 and 50 µg/ml	Ames test; with and without metabolic activation	*Salmonella typhimurium* strains TA1535, TA1537, TA1538	negative	[70]
Calcium Gluconate	7.5, 15 and 30 µg/ml	with and without metabolic activation	*Saccharomyces cerevisiae* strain D4	negative	[70]
Lactitol	not provided	reverse mutation assay; details not provided	*S. typhimurium* (stains not specified)	negative	[20]
Lactitol	not provided	mammalian gene mutation assay; details not provided	human lymphocytes	negative	[20]
Sodium Gluconate	0.006, 0.012, and 0.024 µg/ml	Ames test, with and without metabolic activation; appropriate positive and negative controls were used	*S. typhimurium* strains TA1535, TA1537, TA1538	negative	[71]
Sodium Gluconate	12.5, 25, and 50 µg/ml	Ames test, with and without metabolic activation; appropriate positive and negative controls were used	*Saccharomyces cerevisiae* strain D4	negative	[71]
Sucralose	0.16-10 mg/plate; distilled water was the vehicle	Ames test, with and without metabolic activation; appropriate positive and negative controls were used	*S. typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100	negative	[72]
Sucralose	0.16-10 mg/plate; distilled water was the vehicle	DNA damage test; appropriate positive and negative controls were used	*Escherichia coli* strains W3110 and P3478	negative	[72]
Sucralose	≤10 mg/ml; distilled water was the vehicle	mouse lymphoma assay, with and without metabolic activation; appropriate positive and negative controls were used	L5178Y TK +/- mouse lymphoma cells	originally classified as equivocal results; redefined as negative using revised criteria	[72]
Sucralose	8, 40, and 200 µg/ml; distilled water was the vehicle	human peripheral lymphocyte assay, without metabolic activation; appropriate positive and negative controls were used	human lymphocytes	negative	[72]
Sucrose	156-5000 µg/ml	mouse lymphoma assay, with and without metabolic activation; appropriate controls were used	L5178Y mouse lymphoma cells	negative	[73]
Sucrose	156-5000 µg/ml	mouse lymphoma assay, with and without metabolic activation; appropriate controls were used	L5178Y mouse lymphoma cells	negative	[74]
Sucrose	1311-5000 µg/ml	mouse lymphoma assay, with and without metabolic activation; appropriate controls were used	L5178Y mouse lymphoma cells	negative	[75]
Trehalose	312.5-5000 µg/plate	Ames test, with and without metabolic activation; appropriate controls were used	*S. typhimurium* strains TA1535, TA1537, TA98, and TA100; *E. coli* strain WP2 uvrA	negative	[27]
Trehalose	to 312, 1250, or 5000 µg/ml	chromosomal aberration assay, with and without metabolic activation; appropriate controls were used	Chinese hamster ovary cells	negative	[27]
		IN VIVO			
Sodium Gluconate	0, 2.5, 5, or 10 g/kg in physiological saline	chromosomal aberration assay; mice were given a single oral 1 ml dose	mouse bone marrow cells; C57BL male mice, 3/group	not clastogenic; all animals of the 5 and 10 g/kg groups died	[9]
Sodium Gluconate	0, 1.25,or 2.5 g/kg in physiological saline	chromosomal aberration assay; mice were dosed orally with 1 ml, 1x/day for 4 days	mouse bone marrow cells; C57BL male mice, 2 (control and low dose) or 3 (high dose)/group	not clastogenic; 1 animal of each test group died	[9]
Sucralose	0.5, 1, and 2 g/kg bw in distilled water	chromosomal aberration assay; rats were dosed by gavage daily for 5 days; aberrations were evaluated 6 h after the final dose	rat bone marrow cells; male and female Sprague-Dawley rats, 5/group	negative; no mortality	[72]
Sucralose	2 or 10 g/kg bw in distilled water	micronucleus test; 5 male and 5 female CD-1 COBS Swiss mice were dosed twice by gavage in 24 h; micronuclei were evaluated after 6 h, the study was preliminary and was not Good Laboratory Practices (GLP)-compliant	male and female CD-1 COBS Swiss mice; 5/sex/group	negative	[72]
Sucralose	1 or 5 g/kg bw in distilled water	micronucleus test; mice were given a single dose by gavage, and micronuclei were evaluated 24, 48,or 72 h after dosing	female CD-1 Swiss mice; 5/sex/group	negative	[72]
Trehalose	1250, 2500, or 5000 mg/kg	micronucleus test; mice were dosed intraperitoneally and then killed 1 or 2 days after dosing; cyclophosphamide was used as the positive control.	male and female mice; 5/group	negative	[27]

Table 10. Genotoxicity studies

Test Article	Concentration/Vehicle	Procedure	Test System	Results	Reference
Trehalose	1.25, 2.5, and 5 g/kg in distilled water	micronucleus test; mice were dosed by gavage for 3 days and killed on day 4	male mice; 10/group	negative; no mortality	[53]

Table 11. Irritation and Sensitization Studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			NON-HUMAN		
Gluconic Acid	50% aq. solution 0.5 ml	6 rabbits/group	- 1 sq. in. occlusive patch was applied for 4 h - test sites of one group was abraded - test sites were scored after 24, 48, and 72 h	- slight erythema observed during the initial observation; it is not clear if this is only for abraded skin - no signs of irritation at 72 h	[9]
Lactitol	not specified	rabbits; no./group not specified	- study was performed according to the OECD Guidelines 404 and 406, respectively.[90,91] (No other details were provided)	- not an irritant or sensitizer	[20]
			HUMAN		
hair styling cream containing **0.08% glucose**	applied neat	100 subjects	HRIPT induction: the test material was applied neat under semi-occlusive patches; 9 applications were made over a 3-wk period; the first patch was applied for 48 h, and the remainder for 24 h challenge: the patch was applied after a 2-wk non-treatment period to a previously untreated site; the test sites were scored 48 and 96 h after application.	not an irritant or a sensitizer	.[78]
a leave-in hair product containing **8% glucose**	applied neat 0.2 ml	208 subjects	HRIPT; 24-h, 2 cm^2, semi-occlusive patches were used	not a sensitizer 1% of subjects had a "+" reaction during induction	[77]
mixture containing **isomalt**	final applied concentration of isomalt is 0.94% 20 µl	49 subjects	- single insult patch test; test material was applied to the ventral forearm using Finn Chambers, and the test site was scored 15 min, 24 h, and 48 h after patch removal - SDS (not defined) was used as a positive control - water was the negative control	not an irritant; no reactions to the test formulation were observed	[80]
face and neck product containing **0.1% kefiran**	applied neat	100 subjects	HRIPT using semi-occlusive patches	not an irritant or sensitizer	[69]
paste mask and mud pack containing **0.15% lactitol**	applied neat	28 subjects	4-wk in-use dermal study with open applications	not an irritant	[69]
paste mask and mud pack containing **0.15% lactitol**	applied neat	110 subjects	HRIPT using semi-occlusive patches	not an irritant or sensitizer	[69]
face and neck product containing **2.48% lactose**	applied neat	114 subjects	HRIPT using occlusive patches	not an irritant or sensitizer	[69]

Table 11. Irritation and Sensitization Studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
leave-on facial product containing **5% mannose**	applied neat	103 subjects	HRIPT with 48-72 h occlusive induction patches and a 48-h challenge patch - distilled water was used as a negative control.	not an irritant or a sensitizer	[79]
leave-on formulation containing **10% rhamnose**	applied neat	106 subjects	HRIPT using 48-72 h occlusive patches - distilled water was used as a negative control.	- not a sensitizer - irritation reaction consisting of severe to mild erythema, bulla, coloration, fissuring, and scabbing was observed in one subject	[76]
lip balm formulation containing **0.6% sucralose**	applied neat	50 subjects	modified Draize HRIPT; similar to that described previously, with the exceptions that all the induction patches were applied for 24 h, the challenge patch was applied for 24 h, and the challenge sites scored 24 and 48 h after application	not an irritant or sensitizer	[81]
rinse-off hair product containing **29% sucrose**	diluted to 50% in distilled water 0.02 ml over 50 mm^2	102 subjects	HRIPT using 48-72 h occlusive patches for induction, and a 48-h patch at challenge	- not an irritant or sensitizer - mean irritation index of <0.25; 16% of the subjects presented with score ≥2 reactions during induction	[83]
eye cream formulation containing **0.1% xylobiose**	applied neat	56 subjects	HRIPT using 24-h occlusive patches	not an irritant or sensitizer	[82]

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SAFETY DATA SHEET

1. Identification

Product identifier: Sucrose

Other means of identification
Product No.: 6320, L376, 37727

Recommended use and restriction on use
Recommended use: Not determined.
Restrictions on use: Not determined.

Details of the supplier of the safety data sheet

Avantor Performance Materials, LLC.
3477 Corporate Parkway
Center Valley, PA 18034

Telephone:
Customer Service: 855-282-6867
Fax: 610-573-2610
Contact Person: Environmental Health & Safety
E-mail: info@avantormaterials.com

Emergency telephone number:
CHEMTREC: 1-800-424-9300 within US and Canada

2. Hazard(s) identification

Hazard Classification

Label Elements

Hazard Symbol:	No symbol
Signal Word:	No signal word.
Hazard Statement:	May form combustible dust concentrations in air.
Precautionary Statements	
Prevention:	Prevent dust accumulation to minimize explosion hazard.

Other hazards which do not result in GHS classification: None.

3. Composition/information on ingredients

Substances

Chemical name	Common name and synonyms	CAS number	Content in percent (%)*
SUCROSE		57-50-1	100%

* All concentrations are percent by weight unless ingredient is a gas. Gas concentrations are in percent by volume.

4. First-aid measures

General information: Get medical advice/attention if you feel unwell. Show this safety data sheet to the doctor in attendance.

Ingestion: Rinse mouth thoroughly. Call a POISON CENTER/doctor if you feel unwell.

Inhalation: Move to fresh air. Get medical attention if symptoms persist.

Skin Contact: Wash skin thoroughly with soap and water. Get medical attention if irritation persists after washing. Wash contaminated clothing before reuse.

Eye contact: Flush thoroughly with water. If irritation occurs, get medical assistance.

Most important symptoms/effects, acute and delayed

Symptoms: May cause irritation to skin, eyes, and respiratory tract.

Hazards: None known.

Indication of immediate medical attention and special treatment needed

Treatment: Treat symptomatically. Symptoms may be delayed.

5. Fire-fighting measures

General Fire Hazards: Dust clouds may be explosive under certain conditions.

Suitable (and unsuitable) extinguishing media

Suitable extinguishing media: Water spray, dry powder or carbon dioxide.

Unsuitable extinguishing media: None known.

Specific hazards arising from the chemical: Dust may form explosive mixture with air.

Special protective equipment and precautions for firefighters

Special fire fighting procedures: Move containers from fire area if you can do so without risk. Use water spray to keep fire-exposed containers cool. Cool containers exposed to flames with water until well after the fire is out.

Special protective equipment for fire-fighters: Firefighters must use standard protective equipment including flame retardant coat, helmet with face shield, gloves, rubber boots, and in enclosed spaces, SCBA.

6. Accidental release measures

Personal precautions, protective equipment and emergency procedures: Keep unauthorized personnel away. Use personal protective equipment. Keep upwind. Ventilate closed spaces before entering them. Avoid inhalation of dust. See Section 8 of the SDS for Personal Protective Equipment.

Methods and material for containment and cleaning up: Avoid dust formation. Sweep up and place in a clearly labeled container for chemical waste. Clean surface thoroughly to remove residual contamination.

Notification Procedures: Prevent runoff from entering drains, sewers, or streams. Inform authorities if large amounts are involved.

Environmental Precautions: Prevent further leakage or spillage if safe to do so. Avoid discharge into drains, water courses or onto the ground.

7. Handling and storage

Precautions for safe handling: Use personal protective equipment as required. Avoid contact with eyes, skin, and clothing. Avoid inhalation of dust. Avoid generation and spreading of dust. DO NOT handle, store or open near an open flame, sources of heat or sources of ignition. Protect material from direct sunlight. Wash thoroughly after handling.

Conditions for safe storage, including any incompatibilities: Keep container tightly closed. Store in a well-ventilated place. Store in a dry place.

8. Exposure controls/personal protection

Control Parameters

Occupational Exposure Limits

Chemical Identity	Type	Exposure Limit Values	Source
SUCROSE	TWA	10 mg/m3	Canada. Alberta OELs (Occupational Health & Safety Code, Schedule 1, Table 2) (07 2009)
SUCROSE - Total dust.	TWA	10 mg/m3	Canada. British Columbia OELs. (Occupational Exposure Limits for Chemical Substances, Occupational Health and Safety Regulation 296/97, as amended) (07 2007)
SUCROSE - Respirable fraction.	TWA	3 mg/m3	Canada. British Columbia OELs. (Occupational Exposure Limits for Chemical Substances, Occupational Health and Safety Regulation 296/97, as amended) (07 2007)
SUCROSE	TWA	10 mg/m3	Canada. Manitoba OELs (Reg. 217/2006, The Workplace Safety And Health Act) (03 2011)
SUCROSE	TWA	10 mg/m3	Canada. Ontario OELs. (Control of Exposure to Biological or Chemical Agents) (11 2010)
SUCROSE	8 HR ACL	10 mg/m3	Canada. Saskatchewan OELs (Occupational Health and Safety Regulations, 1996, Table 21) (05 2009)
	15 MIN ACL	20 mg/m3	Canada. Saskatchewan OELs (Occupational Health and Safety Regulations, 1996, Table 21) (05 2009)
SUCROSE	TWA	10 mg/m3	Canada. Quebec OELs. (Ministry of Labor - Regulation Respecting the Quality of the Work Environment) (12 2008)
SUCROSE	TWA	10 mg/m3	US. ACGIH Threshold Limit Values (2011)

Appropriate Engineering Controls No data available.

Individual protection measures, such as personal protective equipment

General information: Good general ventilation (typically 10 air changes per hour) should be used. Ventilation rates should be matched to conditions. If applicable, use process enclosures, local exhaust ventilation, or other engineering controls to maintain airborne levels below recommended exposure limits. If exposure limits have not been established, maintain airborne levels to an acceptable level. An eye wash and safety shower must be available in the immediate work area.

Eye/face protection: Use tight fitting goggles if dust is generated.

Skin Protection

Hand Protection: Wear protective gloves.

Other: Wear suitable protective clothing.

Respiratory Protection: In case of inadequate ventilation use suitable respirator.

Hygiene measures: Provide eyewash station and safety shower. Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing and protective equipment to remove contaminants.

9. Physical and chemical properties

Appearance

Physical state:	Solid
Form:	Crystals or powder.
Color:	White
Odor:	Odorless
Odor threshold:	No data available.
pH:	No data available.
Melting point/freezing point:	185,5 °C
Initial boiling point and boiling range:	No data available.
Flash Point:	Not applicable
Evaporation rate:	No data available.
Flammability (solid, gas):	No data available.
Upper/lower limit on flammability or explosive limits	
Flammability limit - upper (%):	No data available.
Flammability limit - lower (%):	No data available.
Explosive limit - upper (%):	No data available.
Explosive limit - lower (%):	No data available.
Vapor pressure:	No data available.
Vapor density:	No data available.
Density:	1,58 g/cm3
Relative density:	No data available.
Solubility(ies)	
Solubility in water:	2.000 g/l
Solubility (other):	No data available.
Partition coefficient (n-octanol/water):	-3,70
Auto-ignition temperature:	No data available.
Decomposition temperature:	No data available.

Viscosity: No data available.

10. Stability and reactivity

Reactivity: No dangerous reaction known under conditions of normal use.

Chemical Stability: Material is stable under normal conditions.

Possibility of hazardous reactions: Hazardous polymerization does not occur.

Conditions to avoid: Heat, sparks, flames. Dust clouds may be explosive under certain conditions. Contact with incompatible materials.

Incompatible Materials: Strong oxidizing agents. Inorganic peroxides.

Hazardous Decomposition Products: Thermal decomposition may release oxides of carbon.

11. Toxicological information

Information on likely routes of exposure

Inhalation: Dust may irritate respiratory system.

Skin Contact: May cause irritation.

Eye contact: May irritate eyes.

Ingestion: Expected to be a low ingestion hazard.

Information on toxicological effects

Acute toxicity (list all possible routes of exposure)

Oral
Product: No data available.

Dermal
Product: No data available.

Inhalation
Product: No data available.

Repeated dose toxicity
Product: No data available.

Skin Corrosion/Irritation
Product: May cause skin irritation.

Serious Eye Damage/Eye Irritation
Product: May irritate eyes.

Respiratory or Skin Sensitization
Product: Not a skin sensitizer.

Carcinogenicity

Product: This substance has no evidence of carcinogenic properties.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans:
No carcinogenic components identified

US. National Toxicology Program (NTP) Report on Carcinogens:
No carcinogenic components identified

ACGIH Carcinogen List:
No carcinogenic components identified

Germ Cell Mutagenicity

In vitro
Product: No mutagenic components identified

In vivo
Product: No mutagenic components identified

Reproductive toxicity
Product: No components toxic to reproduction

Specific Target Organ Toxicity - Single Exposure
Product: None known.

Specific Target Organ Toxicity - Repeated Exposure
Product: None known.

Aspiration Hazard
Product: Not classified

Other effects: None known.

12. Ecological information

Ecotoxicity:

Acute hazards to the aquatic environment:

Fish
Product: No data available.

Aquatic Invertebrates
Product: No data available.

Chronic hazards to the aquatic environment:

Fish
Product: No data available.

Aquatic Invertebrates
Product: No data available.

Toxicity to Aquatic Plants
Product: No data available.

Persistence and Degradability

Biodegradation
Product: Expected to be readily biodegradable.

BOD/COD Ratio
Product: No data available.

Bioaccumulative potential
Bioconcentration Factor (BCF)
Product: No data available on bioaccumulation.

Partition Coefficient n-octanol / water (log Kow)
Product: Log Kow: -3,70

Mobility in soil: The product is water soluble and may spread in water systems.

Other adverse effects: The product components are not classified as environmentally hazardous. However, this does not exclude the possibility that large or frequent spills can have a harmful or damaging effect on the environment.

13. Disposal considerations

Disposal instructions: Discharge, treatment, or disposal may be subject to national, state, or local laws.

Contaminated Packaging: Since emptied containers retain product residue, follow label warnings even after container is emptied.

14. Transport information

TDG
Not regulated.

IMDG
Not regulated.

IATA
Not regulated.

Transport in bulk according to Annex II of MARPOL and the IBC Code: Not applicable

15. Regulatory information

Canada Federal Regulations
List of Toxic Substances (CEPA, Schedule 1)
Not Regulated

Export Control List (CEPA 1999, Schedule 3)
Not Regulated

National Pollutant Release Inventory (NPRI)
Canada. National Pollutant Release Inventory (NPRI) Substances, Part 5, VOCs with Additional Reporting Requirements
NPRI PT5 Not Regulated

Canada. National Pollutant Release Inventory (NPRI) (Schedule 1, Parts 1-4)
NPRI Not Regulated

Greenhouse Gases
Not Regulated

Controlled Drugs and Substances Act

CA CDSI	Not Regulated
CA CDSII	Not Regulated
CA CDSIII	Not Regulated
CA CDSIV	Not Regulated
CA CDSV	Not Regulated
CA CDSVII	Not Regulated
CA CDSVIII	Not Regulated

Precursor Control Regulations
Not Regulated

International regulations

Montreal protocol
Not applicable

Stockholm convention
Not applicable

Rotterdam convention
Not applicable

Kyoto protocol
Not applicable

Inventory Status:

Australia AICS:	On or in compliance with the inventory
Canada DSL Inventory List:	On or in compliance with the inventory
EINECS, ELINCS or NLP:	On or in compliance with the inventory
Japan (ENCS) List:	Not in compliance with the inventory.
China Inv. Existing Chemical Substances:	Not in compliance with the inventory.
Korea Existing Chemicals Inv. (KECI):	On or in compliance with the inventory
Canada NDSL Inventory:	Not in compliance with the inventory.
Philippines PICCS:	On or in compliance with the inventory
US TSCA Inventory:	On or in compliance with the inventory
New Zealand Inventory of Chemicals:	On or in compliance with the inventory
Japan ISHL Listing:	On or in compliance with the inventory
Japan Pharmacopoeia Listing:	Not in compliance with the inventory.

16.Other information, including date of preparation or last revision

Revision Date:	18.05.2018
Version #:	1.1
Further Information:	No data available.

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