Toxicological profile for

Cocoa, cocoa shells, extract, distillate, tincture

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

Not applicable.

1.2. Synonyms

8002-31-1: Cocao bean oil; Cocoa; Cocoa beans absolute, colourless MD; Cocoa absolute; Cocoa bean extract; Cocoa beans, methanol extract; Cacao butter; Cocoa butter; Cocoa essence, dark; Cocoa essence, white; Cocoa oil; Cocoa oil absolute; Cocoa or cocoa butter; Cocoa or cocoa butter [bean]; Cocoa shell extract; Theobroma oil; UNII-512OYT1CRR (ChemIDplus)

84649-99-0: Cacao; Cacao bean extract; Chocolate; Cocoa extract; Cocoa or cocoa butter; Cocoa or cocoa butter [bean]; EINECS 283-480-6; Theobroma cacao extract; Cacao extract; Cocoa, ext. (ChemIDplus)

1.3. Molecular formula

Unspecified (ChemIDplus)

1.4. Structural Formula

Not applicable

1.5. Molecular weight (g/mol)

Not applicable

1.6. CAS registration number

8002-31-1, 84649-99-0

1.7. Properties

1.7.1. Melting point

(°C): 349.84 (estimated) [CAS RN 8002-31-1] (EPISuite, 2017)

1.7.2. Boiling point

(°C): 798.88 (estimated) [CAS RN 8002-31-1] (EPISuite, 2017)

1.7.3. Solubility

1.099e-019 mg/L at 25ºC [CAS RN 8002-31-1] (estimated) (EPISuite, 2017)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

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

1.7.6. Flammability limits (vol/vol%)

"Inertization of Combustible Dusts": O_2 Limit Concentration 9 mol% (median fineness < 63 µm) (IGS, 2021)

1.7.7. (Auto)ignition temperature

(°C): 480 or 560 (IGS, 2021)

1.7.8. Decomposition temperature

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

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

6.84E-020mm Hg 25°C (estimated) [CAS RN 8002-31-1] (EPISuite, 2017)

1.7.11. log Kow

22.74 (estimated) [CAS RN 8002-31-1] (EPISuite, 2017)

2. General information

2.1. Exposure

No data was available on the dietary intake of cocoa products. The estimated intake of cocoa extract from use as a flavouring in the USA is 0.1680 mg/kg bw/day (Burdock GA, 2010).

The following substances are used for the indicated purposes in cosmetics in the EU:

INCI Name/Substance Name	CAS No.	EC No.	Description	Functions
HYDROLYZED THEOBROMA CACAO SEED MEAL	84649-99-0	283-480-6 (I)	Hydrolyzed Theobroma Cacao Seed Meal is the hydrolysate of the meal obtained from the seeds of Theobroma cacao (Malvaceae) derived by acid, enzyme or other method of hydrolysis.	SKIN CONDITIONING
THEOBROMA CACAO EXTRACT	84649-99-0	283-480-6	Theobroma Cacao Extract is the extract of Theobroma cacao., Sterculiaceae	SKIN CONDITIONING
THEOBROMA CACAO FLOWER EXTRACT	84649-99-0		Theobroma Cacao (Cocoa) Flower Extract is the extract of the flowers of Theobroma cacao, Malvaceae.	SKIN CONDITIONING
THEOBROMA CACAO FRUIT POWDER	84649-99-0	283-480-6	Theobroma Cacao (Cocoa) Fruit Powder is the powder obtained from the dried, ground fruit of the Cocoa, Theobroma cacao L., Sterculiaceae	SKIN CONDITIONING

THEOBROMA CACAO HUSK	84649-99-0	283-480-6	Theobroma Cacao Husk is the husk obtained from the Cocoa, Theobroma cacao L., Sterculiaceae	ABRASIVE
THEOBROMA CACAO HUSK EXTRACT	84649-99-0	283-480-6	Theobroma Cacao Husk Extract is the extract of the husk obtained from the Cocoa, Theobroma cacao L., Sterculiaceae	ANTIOXIDANT
THEOBROMA CACAO LEAF CELL EXTRACT	84649-99-0	283-480-6	Theobroma Cacao Leaf Cell Extract is the extract of a culture of the leaves cells of the Cocoa, Theobroma cacao L., Sterculiaceae	FRAGRANCE SKIN PROTECTING
THEOBROMA CACAO SEED EXTRACT	84649-99-0 / 8002-31-1	283-480-6 / -	Theobroma Cacao Seed Extract is the extract of the seeds of the Cocoa, Theobroma cacao L., Sterculiaceae	ANTIOXIDANT
THEOBROMA CACAO SEED POWDER	84649-99-0	283-480-6	Theobroma Cacao Seed Powder is the powder obtained from the dried, ground seeds of the Cocoa, Theobroma cacao L., Sterculiaceae	ABRASIVE
THEOBROMA CACAO SEED WATER	84649-99-0	283-480-6	Theobroma Cacao Seed Water is an aqueous solution of the steam distillate obtained from the seeds of the Cocoa, Theobroma cacao L., Sterculiaceae	HUMECTANT
THEOBROMA CACAO SHELL EXTRACT	84649-99-0	283-480-6	Theobroma Cacao Shell Extract is the extract of the shell of the Cocoa, Theobroma cacao L., Sterculiaceae	SKIN CONDITIONING
THEOBROMA CACAO SHELL POWDER	84649-99-0	283-480-6	Theobroma Cacao Shell Powder is a powder obtained from the dried, ground shells of the Cocoa, Theobroma cacao L., Sterculiaceae	ABRASIVE SKIN CONDITIONING
HYDROLYZED THEOBROMA CACAO SEED BUTTER	8002-31-1,110615-47-9		Hydrolyzed Theobroma Cacao (Cocoa) Seed Butter is the hydrolysate of Theobroma Cacao (Cocoa) Seed Butter derived by acid, enzyme or other method of hydrolysis, Malvaceae.	CLEANSING SKIN CONDITIONING SKIN CONDITIONING - EMOLLIENT SURFACTANT - CLEANSING SURFACTANT - FOAM BOOSTING

THEOBROMA CACAO SEED BUTTER	84649-99-0 / 8002-31-1	283-480-6 / -	Theobroma Cacao Seed Butter is a yellowish white solid material obtained from the roasted seeds of the Cocoa, Theobroma cacao L., Sterculiaceae	FRAGRANCE SKIN CONDITIONING SKIN CONDITIONING - EMOLLIENT SKIN PROTECTING

As taken from CosIng (undated).

Cocoa (CAS RN 8002-31-1) is listed as a fragrance ingredient on the US EPA InertFinder Database (2022) and cacao oil, cacao extract, cacao absolute, and cacao infusion and tincture (all CAS RN 8002-31-1) are listed by the IFRA.

Cocoa butter (CAS RN 8002-31-1) and Theobroma cacao extract (CAS RN 84649-99-0) are listed (at concentrations, where specified) as ingredients in, respectively, auto, personal care (<1%) and pet care products, and in personal care (<1%) products by the CPID.

“USES

Medicinal, Pharmaceutical, and Cosmetic. Cocoa powder (or cocoa syrup) is used in flavoring pharmaceutical preparations.

Cocoa butter is used extensively as a suppository and ointment base; also used as emollient, skin softener, and skin protectant in creams (e.g., massage), lotions, lipsticks, and soaps, among others.

Food. Beverages made from cacao flavoured with vanilla and other spices have been used by native Mexicans (Aztecs) for centuries.

Cocoa powder is used extensively as a flavor or nutrient component in non-alcoholic beverages, ice cream, cakes, biscuits, and others.

Cocoa butter is extensively used in chocolate manufacture, where it is mixed with cocoa liquor (ground cacao nibs), sugar, milk, and other ingredients such as flavors. Dark chocolate does not contain milk.

Cocoa extract is used in both alcoholic (liqueurs such as creme de cacao) and non-alcoholic beverages, frozen dairy desserts, candies, baked goods, and others.

Dietary Supplements/Health Foods. Cocoa butter is used in creams, massage oils, and other cosmetic preparations sold in health food stores (ROSE).

Traditional Medicine. Cocoa butter is used to treat neck wrinkles on neck (turkey neck), around the eyes, and at the corners of the mouth (ROSE). Reportedly used in European tradition in combination with other ingredients for infectious intestinal disease, diarrhea; bronchial expectorant in asthma, bronchitis, irritating cough, and lung congestion; to regulate function of endocrine glands, especially the thyroid.

Others. Cocoa and cocoa butter have been reported to contain fat-soluble antioxidants and could be a source of such substances.”

As taken from Khan IA and Abourshad EA, 2010.

According to Health Canada's Natural Health Products Ingredients Database, the following substances (no CAS RNs given) are used for the indicated purposes in non-medicinal health products:

Cocoa flavour is used as a flavour enhancer;

Cocoa liquor is used as a flavour enhancer;

Cocoa powder is used as a flavour enhancer, abrasive and colour additive;

Theobroma cacao (cacao) shells is used as a flavour enhancer - natural;

Theobroma cacao (cocoa) extract is used as a skin conditioning agent for topical use;

Theobroma cacao (cocoa) seed butter is used as a fragrance ingredient, skin-conditioning agent – occlusive and skin protectant for topical use;

Theobroma cacao (cocoa) seed extract is used as a colour additive for oral use, a flavour enhancer for oral use and a preservative antioxidant for topical use;

Theobroma cacao distillate is used as a flavour enhancer for oral use;

Theobroma oil (no CAS RN given) is used as a flavour enhancer, fragrance ingredient, lubricant and skin-conditioning agent – occlusive.

In addition, cacao is listed as homeopathic substances.

As taken from Health Canada, 2022.

2.2. Combustion products

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

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
acetaldehyde	3776684	1.05	trace	trace
1-pentene	trace	trace	7716447	1.61
acrolein	5054268	1.41	trace	trace
acetone	7234220	2.02	7248277	1.51
1-hexene	4609842	1.28	5678081	1.18
acetonitrile + unknown	3813959	1.06	trace	trace
1-heptene + heptane	5061014	1.41	5605807	1.17
benzene	6670236	1.86	trace	trace
dimethyl disulfide	3619870	1.01	trace	trace
acetic acid			25415439	5.30
toluene	46308425[a]	12.91[a]	9800190	2.04

pyrrole + unknown	4827431	1.35	4886236	1.02
furfural + unknown	3984264	1.11	trace	trace
furfuryl alcohol + unknown	5716516	1.59	trace	trace
nonanal	4061013	1.13	trace	trace
phenol	3927327	1.09	trace	trace
p-cresol	5011424	1.40	6274362	1.31
unknown	5094504	1.42	trace	trace
indole	4549354	1.27	trace	trace
unknown	1718678	0.60	13031386	2.72
caffeine	27409721	7.64	49284959	10.28
palmitic acid	3626269	1.01	5429577	1.13
theobromine	60301746	16.80	148506725	30.96
hexadecanamide	4235681	1.35	trace	trace
oleoamide	11809288	3.29	6730617	1.40
Total ion chromatogram	358944924	100	480758357	100

a: These compounds were not separated on total ion chromatogram under the two stage heating.

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop 2005. J. Anal. Appl. Pyrolysis 74, 145–170.

Ingredient Name & CAS Number	Max. cig. appln. level (ppm)	Composition of pyrolysate (Compound, %)	Max. level in smoke (ug)
Cocoa extract 84649-99-3 (diluted with propylene glycol)	20,000	Propylene glycol (67.2) Glycerol (26.9) Acetic acid (3.0) Methylbutanal (0.7) Isobutanal (0.4)	6700 2700 300 70 40
Cocoa powder 84649-99-0	20,000	Acetic acid (27.2) Acetol (6.6) Furfuryl alcohol (6.6) Caffeine (4.0) Pyrrole (2.8) Furfural + cyclopentanone	2,700 660 660 400 280 210 160 140

		(2.1) Phenol (1.6) Cresol + pyridenediol (1.4) 2-Butanone (0.9) Toluene (0.7) Styrene (0.2)	90 70 20
Cocoa shell extract 8002-31-2	12,000	Methyl octadecanoate (12.2) Methyl palmitate (11.9) Oleic acid (10.5) Palmitic acid (7.6) Methyl stearate (6.9) Cresol (0.2) Phenol (0.2) Furfural (0.1) Styrene (0.1)	730 710 630 460 410 12 12 6 6

At 350-750 °C, cocoa yielded phenol, cresol, xylenol, catechol, palmitic acid and stearic acid (Schlotzhauer 1978). At 900 °C, it yielded mainly theobromine, and forty-two minor products, including acetic acid, toluene, phenol and caffeine (Anonymous 2001).

“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. 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

Cocoa extract is one of three main products obtained from cocoa seeds. The other two products are cocoa powder and cocoa butter. Following curing and fermentation, the beans are dried and roasted to yield the desired flavor, color and aroma (Burdock GA, 2010).

Occurrence in tobacco products

In the burnt	Yes

part?	
In tobacco naturally?	No evidence (Stedman 1968; Lloyd et al 1976), but the majority of the volatile constituents of cocoa are identical or analogous to natural tobacco or tobacco smoke constituents (Harlee & Leffingwell, 1979).

Obtained from Theobroma cacao L. subsp. cacao (Family Sterculiaceae or Byttneriaceae).

As taken from Khan IA and Abourshad EA, 2010.

3. Status in legislation and other official guidance

No ADI or TDI identified at the current time.

This ingredient is a well characterized material that has been evaluated and approved as a food additive by expert bodies including US FDA and the CoE (C of E no: 452N). In the USA, cacao (Theobroma cacao L.) is listed in CFR section 182.20 - Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) - as GRAS for food flavouring use (FDA, 2022a). Cocoa extract (CAS RN 84649-99-0) is included on the FDA's inventory of "Substances Added to Food (formerly EAFUS)" as a flavor enhancer, flavoring agent or adjuvant (FDA, 2022b).

There are REACH dossiers for the following:

Cocoa, ext. (CAS RN 84649-99-0)

Extract obtained from the shell of Theobroma cacao (Malvaceae) by co-extraction with ethanol and propylene glycol (no CAS RN given)

Extract obtained from defatted powder of Theobroma cacao (Malvaceae) by extraction with water and ethanol (no CAS RN given)

Extract obtained from defatted powder of Theobroma cacao (Malvaceae) by extraction with ethanol (no CAS RN given)

Extract obtained from powder of Theobroma cacao (Malvaceae) by co-extraction with ethanol and propylene glycol (no CAS RN given)Cocoa-PG-extract (no CAS RN given)

Cocoa, ethanol ext. (no CAS RN given)As taken from ECHA, undated a

Cacao butter (CAS RN 8002-31-1) ("envisaged registration deadline 30 November 2010"), Cocoa, ext. (CAS RN 84649-99-0) ("envisaged registration deadline 30 November 2010"), deffated Theobroma cacao L., hydroethanolic extract (no CAS RN), theobroma cacao (no CAS RN) and Theobroma cacao L. husk, hydromethanolic extract (no CAS RN) (all "envisaged registration deadline 31 May 2018") are pre-registered under REACH (ECHA, undated b).

None of the following are classified for packaging and labelling under Regulation (EC). No. 1272/2008:

Cocoa, ext. (CAS RN 84649-99-0)

Cacao butter (CAS RN 8002-31-1)

Extract obtained from the shell of Theobroma cacao (Malvaceae) by co-extraction with ethanol and propylene glycol (no CAS RN given)

CAS RN 84649-99-0 ["No public or meaningful name is available"]Extract obtained from defatted powder of Theobroma cacao (Malvaceae) by extraction with ethanol (no CAS RN given)

Cocoa extract / ETOH (no CAS RN given)

As taken from ECHA, 2022.

Cocoa (CAS RN 8002-31-1) is listed in the US EPA InertFinder Database (2022) as approved for food, non-food and fragrance 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. Commonly consumed food commodities) and 152.25 Exemptions for pesticides of a character not requiring FIFRA regulation. (US EPA, 2022).

Cacao butter (CAS RN 8002-31-1) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule).

US EPA 2020 CDR List. US EPA TSCA inventory.

Cocoa butter (CAS RN 8002-31-1) is included on the US EPA's list of Safer Chemical Ingredients with functional use in: Emollients; Skin Conditioning Agents.

As taken from US EPA, 2022

Cacao butter (CAS RN 8002-31-1) and cocoa, ext. (CAS RN 84649-99-0) are listed on the New Zealand Inventory of Chemicals. The former donesn't have an individual approval but may be used under an appropriate group standard. The latter does not have an individual approval but may be used as a component in a product covered by a group standard. It is not approved for use as a chemical in its own right. (NZ EPA, 2006).

Cocoa (no CAS RN given) and cocoa butter (CAS RN 8002-31-1) are included on the US FDA's list of inactive ingredients for approved drug products. They are permitted for use as ingredients in product, at the following maximum potencies per unit dose:

Inactive Ingredient	Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose
COCOA BUTTER	RECTAL	SUPPOSITORY	8002311	512OYT1CRR	2280mg
COCOA	ORAL	SUSPENSION		D9108TZ9KG	246.66mg/5ml

As taken from FDA, 2022c

Cocoa butter (CAS RN 8002-31-1) has been "identified as low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach" and, together with cocoa, extract (CAS RN 84649-99-0) "poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework" (AICIS, 2017, 2019).

Theobroma cacao (no CAS RN given) is classified as a Natural Health Product (NHP) for medicinal use under Schedule 1 item 1 (plant or plant material) of the NHP Regulations. It is listed in the document: Medicated Skin Care Products Monograph with quantity in product: 50-100%.

Theobroma oil (no CAS RN given) is classified as a NHP for medicinal use under Schedule 1 item 2 (extract) of the NHP Regulations.

As taken from Health Canada, 2018, 2022.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

"Cocoa has beneficial health effects partly due to its high flavanol content. This study was aimed at assessing the absorption and metabolism of polyphenols in two soluble cocoa products: a conventional (CC) and a flavanol-rich product (CC-PP). A crossover, randomized, blind study was

performed in 13 healthy men and women. On two different days, after an overnight fast, volunteers consumed one serving of CC (15 g) or CC-PP (25 g) in 200 mL of semi-skimmed milk containing 19.80 mg and 68.25 mg of flavanols, respectively. Blood and urine samples were taken, before and after CC and CC-PP consumption, and analyzed by high-performance liquid chromatography coupled to electrospray ionisation and quadrupole time-of-flight mass spectrometry (HPLC-ESI-QToF-MS). Up to 10 and 30 metabolites were identified in plasma and urine, respectively. Phase II derivatives of epicatechin were identified with kinetics compatible with small intestine absorption, although the most abundant groups of metabolites were phase II derivatives of phenyl-γ-valerolactone and phenylvaleric acid, formed at colonic level. 5-(4'-Hydroxyphenyl)-γ-valerolactone-sulfate could be a sensitive biomarker of cocoa flavanol intake. CC and CC-PP flavanols showed a dose-dependent absorption with a recovery of 35%. In conclusion, cocoa flavanols are moderately bioavailable and extensively metabolized, mainly by the colonic microbiota." As taken from Gómez-Juaristi M et al. 2019. Nutrients 11(7), 1441. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31247980/

4.2. Absorption, distribution and excretion

“The comparative bioavailability of cocoa butter (a predominantly saturated fat) and corn oil (a predominantly unsaturated fat) was determined in male Sprague-Dawley rats by analysis of total fecal lipids following ad libitum feeding of purified diets containing 5, 10 and 20% cocoa butter or corn oil for 2 wk. Fecal lipid elimination was significantly increased (P less than 0.05) in each cocoa butter group when compared with the corresponding corn oil group, resulting in lower digestibility coefficients for cocoa butter (59-72%) than for corn oil (93-97%). Body weight gain and food intake data were similar among all treatment groups. Fecal fatty acid profiles in rats fed corn oil diets consisted primarily of 27-34% palmitic acid (16:0), 22-32% stearic acid (18:0) and 25-37% oleic acid (18:1). Palmitic, oleic and linoleic acids were also the primary fatty acids stored in epididymal fat tissue from corn oil-fed rats. In contrast, fecal fatty acids in animals fed cocoa butter diets consisted of 31-37% palmitic acid and 58-64% stearic acid; oleic acid was the major fatty acid stored in epididymal fat tissue. These results indicate that the decreased digestibility of cocoa butter is largely a result of its fatty acid composition. This reduced bioavailability of cocoa butter may be at least partially responsible for its previously described neutral effect on serum cholesterol.” As taken from Apgar JL et al. J Nutr. 1987 Apr; 117(4), 660-5. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/3585514?dopt=AbstractPlus

“The comparative absorption of cocoa butter (25.5% C16:0, 34.4% C18:0, 34.4% C18:1, 3.4% C18:2) and corn oil (11.4% C16:0, 2.0% C18:0, 26.4% C18:1, 60.0% C18:2) was assessed in six healthy male subjects. During 3-d experimental diet periods, free-living subjects consumed either cocoa butter or corn oil as virtually the sole source of dietary fat, provided at 40% of the total energy intake in the form of specially formulated cookies. Fat absorption was determined by quantifying total fecal lipid excretion over the 3-d period. Total fecal lipid and fecal fatty acids were determined. The percentage of fat excreted was significantly higher (p less than or equal to 0.001) when subjects consumed the cocoa butter (10.8 +/- 3.2%) vs the corn oil (3.5 +/- 1.0%) diet. These results indicate that the digestibility of cocoa butter is significantly less than corn oil and may explain, in part, previous reports of a neutral effect of dietary cocoa butter on plasma cholesterol concentrations.” As taken from Mitchell DC et al. Am J Clin Nutr. 1989 Nov; 50(5), 983-6. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/2816806?dopt=AbstractPlus

“To compare, in humans, the digestibility of moderate amounts of cocoa butter (30.7 g/d) consumed in the form of chocolate as part of a normal western diet with that of a well-absorbed fat (corn oil); and hence determine whether, by virtue of its apparent low absorption, cocoa butter can be considered to be a low calorie fat. Randomised, two-period crossover metabolic study, conducted under free-living conditions, but with strict control over food intake. Twelve healthy men were selected from volunteers at the Nestle Research Center and all subjects completed the study. Intervention: Two treatment periods of two weeks each: cocoa butter and control periods, with strict

dietary control separated by a two week wash out period. No differences (P>0.05) were observed in faecal weight (wet or dry), faecal fat nor in defecation frequency between treatments (cocoa butter and corn oil). Cocoa butter at a dose of 30.7 g/d in the form of black chocolate, consumed between two meals, was found to have a similar digestibility to that of corn oil (99 % of corn oil digestibility). Cocoa butter, consumed as back chocolate within a normal mixed diet, has a high digestibility, similar to that of corn oil, and a digestible energy value of 37 kJ/g in man. Thus, cocoa butter cannot be considered to be a low-calorie fat." As taken from Shahkhalili Y et al. Eur J Clin Nutr. 2000 Feb; 54(2), 120-5. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/10694782?dopt=AbstractPlus

"We investigated stearic acid (18:0) digestibility and how it affects bile acid excretion in male Sprague-Dawley rats fed diets containing (g 18:0/ 100 g fatty acids), pork lard (13); beef tallow (19); cocoa butter (35); corn oil (2) or corn oil plus cholestyramine for 25 d. Apparent lipid digestibility was reduced with increased dietary intake of 18:0 as follows: lard (90%), beef tallow (82%), cocoa butter (78%), cholestyramine (87%), and corn oil (94%); P<0.001, pooled SD = 2. Hepatic concentrations of total and esterified cholesterol were significantly less in cocoa butter-, beef tallow- and cholestyramine-fed groups compared with lard- and corn oil-fed groups. Fecal bile acid excretion was significantly greater in rats fed cocoa butter or cholestyramine compared with those fed corn oil. The half-life of intraperitoneally administered 14C-cholic acid was significantly longer in rats fed cocoa butter (1.36 +/- 0.02 d) compared with cholestyramine (0.98 +/- 0.03 d) and intermediate in those fed corn oil, lard or beef tallow (1.11-1.21 +/- 0.05 d). Fecal excretion of muricholic acids (bile acids) correlated strongly with dietary intake of 18:0 (r2 = 0.98, P<0.01), whereas excretion of bile acids derived from cholic and chenodeoxycholic acids was similar among groups. In summary, the lower digestibility of cocoa butter is associated with increased fecal bile acid excretion, reduced hepatic concentration of esterified cholesterol, decreased fractional turnover of 14C-cholic acid and increased excretion of muricholic acids in rats. The mechanism by which stearate-rich dietary fats alter bile acid and cholesterol metabolism is, however, uncertain." As taken from Monsma CC et al. J Nutr. 1996 Aug; 126(8), 2028-35. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/8759376?dopt=AbstractPlus

The use of cocoa-bean meal in the diets of horses:pharmacology and pharmacokinetics of theobromine (Abstract). The problem arising from the inclusion of cocoa-bean meal in the feed of race-horses indicated the need to investigate the pharmacodynamics aspects of theobromine when fed in this form to thoroughbred horses. The study was performed in a series of five horses (four thoroughbred and one draught type) which individually received different quatities of cocoa-bean meal. Temperature, pulse and respiratory rated and any changes in behavior were monitored daily throughout the experimental period in each case, and in most instances complementary urine and blood samples were obtained for chemical and cytological analyses and evaluation. The important findings included evidence of efficient and rapid absorption of theobromine from the gastrointestinal tract and its persistence in the body tissues for considerable periods of time, at least, as indicated by its detection in urine. Although not fully authenticated, the evidence obtained from a study of pulse rates, blood glucose, creatine phosphokinase and triiodothyronine values suggests that theobromine exerts similar pharmacokinetic effects in horses as in laboratory animal species and in man.

As taken from Kelly WR and Lambert MB. Br Vet J, 1978, 134(2), 171-180.

4.3. Interactions

Ingested cocoa was reported to prevent high-fat diet-induced obesity by modulating lipid metabolism. The authors suggested that this occurred, 'by decreasing fatty acid synthesis and transport systems, and enhancement of part of the thermogenesis mechanism in liver and white adipose tissue, (Matsui et al., 2005)

Cocoa procyanidins suppress transformation by inhibiting mitogen-activated protein kinase kinase (Abstract). Cocoa was shown to inhibit chemically induced carcinogenesis in animals and exert antioxidant activity in humans. However, the molecular mechanisms of the chemopreventive potential of cocoa and its active ingredient(s) remain unknown. Here we report that cocoa procyanidins inhibit neoplastic cell transformation by suppressing the kinase activity of mitogen-activated protein kinase kinase (MEK). A cocoa procyanidin fraction (CPF) and procyanidin B2 at 5 mug/ml and 40 mum, respectively, inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced neoplastic transformation of JB6 P+ mouse epidermal (JB6 P+) cells by 47 and 93%, respectively. The TPA-induced promoter activity and expression of cyclooxygenase-2, which is involved in tumor promotion and inflammation, were dose-dependently inhibited by CPF or procyanidin B2. The activation of activator protein-1 and nuclear factor-kappaB induced by TPA was also attenuated by CPF or procyanidin B2. The TPA-induced phosphorylation of MEK, extracellular signal-regulated kinase, and p90 ribosomal s6 kinase was suppressed by CPF or procyanidin B2. In vitro and ex vivo kinase assay data demonstrated that CPF or procyanidin B2 inhibited the kinase activity of MEK1 and directly bound with MEK1. CPF or procyanidin B2 suppressed JB6 P+ cell transformation induced by epidermal growth factor or H-Ras, both of which are known to be involved in MEK/ERK signal activation. In contrast, theobromine (up to 80 mum) had no effect on TPA-induced transformation, cyclooxygenase-2 expression, the transactivation of activator protein-1 or nuclear factor-kappaB, or MEK. Notably, procyanidin B2 exerted stronger inhibitory effects compared with PD098059 (a well known pharmacological inhibitor of MEK) on MEK1 activity and neoplastic cell transformation.

As taken from Kang N et al. J Biol Chem. 2008 Jul 25;283(30), 20664-73.

“Several concentrations of theobromine (TB) and (-)-epicatechin (EC) were coadministered to rats, and plasma EC and its metabolites were determined using ultra-high-performance liquid chromatography-tandem mass spectrometry. It has been demonstrated that TB increases the absorption of EC in a dose-dependent manner. Cocoa powder had a similar effect, and the mechanism involved is not thought to depend on tight junctions.” As taken from Yamamoto T et al. 2014. Biosci. Biotechnol. Biochem. 78(12), 2059-63. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25079983

“Cinnamon and cocoa are known to be valuable sources of bioactive phytochemicals, mainly the polyphenols. This paper investigates the potential antioxidant activity of cinnamon and cocoa extract and the interaction of their mixtures by various in vitro tests. Moreover, the combination effect of their constituents in a binary mixture was studied. Two representative active compounds of chocolate (epicatechin, catechin) were combined with seven of cinnamon (gallic acid, tannic acid, quercetin, sinapic acid, cinnamic acid, eugenol and cinnamaldehyde) in multilevel ratios. The results indicate that the addition of the cinnamon extract significantly increased the antioxidant activity of the cocoa extract. The interaction ranged from synergetic to antagonistic. The interaction was less synergetic when cinnamon extract was added in higher proportion. The interaction of their constituents substantially influenced the antioxidant activity of the mixture and was dependent on the ratio. The kinetics' study could elucidate how the polyphenols work in a mixture.” As taken from Muhammad DRA et al. 2017. Food Chem. 231, 356-364. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28450018

“The aim of the study was to examine whether a moderate zinc deficiency alters hepatic lipid composition. Male weanling rats, assigned to five groups (8 animals each), were fed low-carbohydrate high-fat diets supplemented with 7 or 50 mg Zn/kg (LZ or HZ) and 22% cocoa butter (CB) or 22% safflower oil (SF) for four weeks. One group each had free access to the LZ-CB and LZ-SF diets, one group each was restrictedly fed the HZ-CB and HZ-SF diets in matching amounts, and one group had free access to the HZ-SF diet (ad libitum control). The rats fed the LZ diets had significantly lower energy intakes and final body weights than the ad libitum control group, and lower plasma and femur Zn concentrations than the animals consuming the HZ diets. Hepatic cholesterol, triacylglycerol and phospholipid concentrations, and fatty acid composition of hepatic

triacylglycerols and phospholipids did not significantly differ between the LZ and their respective HZ groups, but were greatly affected by dietary fat source. In conclusion, the moderate Zn deficiency did not significantly alter liver lipid concentrations and fatty acid composition." As taken from Weigand E and Egenolf J. 2017. J. Nutr. Metab. 2017, 4798963. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28465837

"To contribute in the research of better drugs against dermatophytosis, we evaluated the antioxidant and antidermatophytic activities of cocoa butter, cloves essential oil, and a mixture of both extracts. The cocoa butter was obtained by boiling the cocoa paste. The essential oil extracted by hydrodistillation was chemically analysed by gas chromatography and gas chromatography coupled with mass spectrometry. The antioxidant activity was determined using the DPPH scavenging method, and the antidermatophytic activity was evaluated using the agar dilution method. The essential oil, majoritary constituated by eugenol (87.62%), β-caryophyllene (5.88%), and β-bisabolene (4.41%), had an antiradical power (4.22 x 10-2) higher than that of BHT (4.00 x 10-3), like the cocoa butter and essential oil mixture (6.06 x 10-3). The essential oil was more active than the griseofulvin: it was fungicidal at 400 ppm against Trichophyton rubrum, and at 900 ppm against Microsporum gypseumand Trichophyton tonsurans. The cocoa butter activity was low, but the mixture with the essential oil had an important activity with inhibitory percentages of 78.69 %, 88.27 %, 91.20% against T. rubrum (at 400 ppm), T. tonsurans(at 900 ppm)and M. gypseum (at 900 ppm) respectively. Cloves essential oil and the mixture with cocoa butter can be used to formulate new drugs against dermatophytes." As taken from Fankem PM et al. 2017. American Scientific Research Journal for Engineering, Technology, & Sciences 37(1), 255-272. Available at http://www.asrjetsjournal.org/index.php/American_Scientific_Journal/article/view/3449

"The consumption of food for pleasure is mainly associated with adverse health effects. This review was carried out to verify recent reports on the impact of chocolate and wine consumption on cardiovascular health, with a particular focus on atherosclerosis. On one side, these products have proven adverse effects on the cardiovascular system, but on the other hand, if consumed in optimal amounts, they have cardiovascular benefits. The submitted data suggest that the beneficial doses are 30-50 g and 130/250 mL for chocolate and wine, respectively, for women and men. The accumulated evidence indicates that the active ingredients in the products under consideration in this review are phenolic compounds, characterized by anti-inflammatory, antioxidant, and antiplatelet properties. However, there are also some reports of cardioprotective properties of other compounds such as esters, amines, biogenic amines, amino acids, fatty acids, mineral ingredients, and vitamins. Our narrative review has shown that in meta-analyses of intervention studies, consumption of chocolate and wine was positively associated with the beneficial outcomes associated with the cardiovascular system. In contrast, the assessment with the GRADE (Grading of Recommendations Assessment, Development and Evaluation) scale did not confirm this phenomenon. In addition, mechanisms of action of bioactive compounds present in chocolate and wine depend on some factors, such as age, sex, body weight, and the presence of additional medical conditions. Patients using cardiovascular drugs simultaneously with both products should be alert to the risk of pharmacologically relevant interactions during their use. Our narrative review leads to the conclusion that there is abundant evidence to prove the beneficial impact of consuming both products on cardiovascular health, however some evidence still remains controversial. Many authors of studies included in this review postulated that well-designed, longitudinal studies should be performed to determine the effects of these products and their components on atherosclerosis and other CVD (Cardiovascular Disease) disease."

Sperkowska B et al. (2021) Cardiovascular Effects of Chocolate and Wine-Narrative Review.

5. Toxicity

5.1. Single dose toxicity

“Unsweetened natural cocoa powder (UNCP) is a pulverized high-grade powder of compressed solid blocks which remains after extraction. Little scientific data is available concerning its safety despite the presence of potential toxic elements. Elemental composition in UNCP was analyzed with ED-XRF spectroscopy. Single oral high dose toxicity study was conducted on adult male Sprague-Dawley rats (150 g) by the limit test method. One group received water and the test group 2000 mg/kg UNCP. All animals were observed for 14 days and then euthanized for haematological, biochemical, and histopathological examinations. Thirty-eight (38) elements were found in UNCP. There was an increase in HDL cholesterol (P<0.05), reduction in LDL cholesterol (P>0.05), alkaline phosphatase (P<0.05), and creatinine levels, and slight increase in urea levels (P>0.05). Haematological changes were not significant. Histopathological analysis showed no toxic effect on the heart, liver, kidney, lungs, testis, and spleen. Intestinal erosion was observed in the test group. UNCP appears to be relatively safe when taken as a single oral high dose of 2000 mg/kg b.w.t. in rats. Caution should however be exercised at high doses due to the high elemental content of copper and high possibility of intestinal lining erosion.” As taken from Asiedu-Gyekye IJ et al. 2016. J. Toxicol. 2016, 4783829. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27610134

5.2. Repeated dose toxicity

“Flavonoids and related polyphenolics with antioxidant and anti-inflammatory activities may play a role in the prevention of cardiovascular disease by decreasing oxidative stress and inflammation. We wished to determine the effects of cocoa extract supplementation on markers of oxidative stress and inflammation. Healthy subjects (n = 25) were studied at baseline, after cocoa supplementation (36.9 g of dark chocolate bar and 30.95 g of cocoa powder drink) for 6 wk and after a 6-wk washout period. Fasting blood and early morning urine were collected at the three time points. Two indices of flavonoid intake, total phenols and oxygen radical absorbance capacity of plasma, were measured after an overnight fast. Neither was affected by supplementation. Measures of oxidative stress included copper-catalyzed LDL oxidation kinetics and urinary F(2) isoprostanes. LDL oxidizability was lower after chocolate supplementation as evidenced by a longer lag time (P<0.05) of conjugated diene formation (101.0 +/- 20.7 min) compared with baseline (91.3 +/- 18.0 min) and washout (96.4 +/- 7.5 min) phases. There was no effect of chocolate on urinary F(2) isoprostane levels or on markers of inflammation including the whole-blood cytokines, interleukin-1 beta, interleukin-6 and tumor necrosis factor-alpha, high sensitivity C-reactive protein and P-selectin. In conclusion, cocoa products supplementation in humans affects LDL oxidizability, but not urinary F(2) isoprostanes or markers of inflammation.” As taken from Mathur S et al. J Nutr. 2002 Dec; 132(12), 3663-7. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/12468604?dopt=AbstractPlus

Several studies have been reported on the short-term biological effects in rats of a high dietary intake of cocoa butter. None reported any gross adverse effects or toxicity, though the test animal was not examined for any tissue abnormalities beyond the immediate hypothesis being tested (Baba et al 1993; Miyasaka et al 1998a & 1998b; Aoyama et al 1995; Apgar et al 1987; Monsma et al 1996; Finley et al 1994).

Short-term repeated dose toxicity: 28 day (rat)

Cocoa extract protects against early alcohol-induced liver injury in the rat (Abstract). Oxidants have been shown to be involved in alcohol-induced liver injury. This study was designed to determine whether cocoa flavonoid extract, composed mostly of epicatechin and epicatechin oligomers, protects against early alcohol-induced liver injury in rats. Male Wistar rats were fed high-fat liquid diets with or without ethanol (10-14 g/kg per day) and cocoa extract (400 mg/kg per day) continuously for 4 weeks using an enteral feeding protocol. Mean body weight gains (approximately 4 g/day) were not significantly different between treatment groups. Cocoa extract did not affect average daily urine ethanol concentrations (approximately 200mg/dL). After 4 weeks, serum alanine amino transferase levels of the ethanol group were increased nearly fourfold (110+/-

16 IU/L) compared to control values (35+/-3 IU/L); this effect of ethanol was blocked by cocoa extract (60+/-6 IU/L). Additionally, enteral ethanol caused severe fat accumulation, mild inflammation, and necrosis in the liver; cocoa extract significantly blunted these changes. Increases in liver TNFalpha protein levels caused by ethanol were completely blocked by cocoa extract. Further, ethanol significantly increased the accumulation of protein adducts of 4-hydroxynonenal, a product of lipid peroxidation serving as an index of oxidative stress; again this was counteracted by the addition of cocoa extract. These results indicate that dietary flavanols such as those found in cocoa can prevent early alcohol-induced liver injury. As taken from McKim SE et al. Arch Biochem Biophys. 2002 Oct 1; 406(1), 40-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/12234488

The effect of Malaysian cocoa extract on glucose levels and lipid profiles in diabetic rats (Abstract). The present study aims to investigate the effect of cocoa extract on serum glucose levels and lipid profiles in streptozotocin-diabetic rats. Cocoa extract (contained 285.6 mg total polyphenol per gram extract) was prepared from fermented and roasted (140 degrees C, 20 min) beans by extracting using 80% ethanol in the ratio of 1-10. The extract of three dosages (1, 2, and 3%) was fed to normal and diabetic rats for a period of 4 weeks. In hyperglycaemic group, cocoa extract (1 and 3%) diets were found to significantly lower ($P<0.05$) the serum glucose levels compared to the control. Furthermore, supplementation of 1 and 3% cocoa extract had significantly reduced ($P<0.05$) the level of total cholesterol in diabetic rats. In addition, 1, 2, and 3% cocoa extract diets had significantly lowered ($P<0.05$) the total triglycerides. Interestingly, this study found that serum HDL-cholesterol had increased significantly ($P<0.05$) in diabetic rats fed with 2% cocoa extract, while the LDL-cholesterol had decreased significantly ($P<0.05$) in the 1% treated group. These results indicate that cocoa extract may possess potential hypoglycaemic and hypocholestrolemic effects on serum glucose levels and lipid profiles, respectively. The results also found that the effect of cocoa extract was dose-dependent. As taken from Ruzaidi A et al. Ethnopharmacol. 2005 Apr 8;98(1-2), 55-60. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/15763363

A diet rich in cocoa attenuates N-nitrosodiethylamine-induced liver injury in rats (Abstract). The effects of cocoa feeding against N-nitrosodiethylamine (DEN)-induced liver injury were studied in rats. Animals were divided into five groups. Groups 1 and 2 were fed with standard and cocoa-diet, respectively. Groups 3 and 4 were injected with DEN at 2 and 4 weeks, and fed with standard and cocoa-diet, respectively. Group 5 was treated with DEN, received the standard diet for 4 weeks and then it was replaced by the cocoa-diet. DEN-induced hepatic damage caused a significant increase in damage markers, as well as a decrease in the hepatic glutathione, diminished levels of p-ERK and enhanced protein carbonyl content, caspase-3 activity and values of p-AKT and p-JNK. The cocoa-rich diet prevented the reduction of hepatic glutathione concentration and catalase and GPx activities in DEN-injected rats, as well as diminished protein carbonyl content, caspase-3 activity, p-AKT and p-JNK levels, and increased GST activity. However, cocoa administration did not abrogate the DEN-induced body weight loss and the increased levels of hepatic-specific enzymes and LDH. These results suggested that cocoa-rich diet attenuates the DEN-induced liver injury. As taken from Granado-Serrano AB et al. Food Chem Toxicol. 2009 Oct; 47(10), 2499-506. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/19602430

Sub-chronic toxicity study: 90 day (rabbit)

Suppressive effect of cocoa powder on atherosclerosis in Kurosawa and Kusanagi-hypercholesterolemic rabbits (Abstract). We investigated the suppressive effect of cocoa powder (cacao polyphenol content: 7.8%) on atherosclerosis in a spontaneous familial hypercholesterolemic model, Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits. Six-month dietary administration of cocoa powder had no effects on body weight, hematology or blood chemistry parameters or a lipid profile in KHC rabbits. Antioxidative activity of low-density lipoprotein (LDL) was observed in the 2nd month and 3rd month of administration. Thiobarbituric acid reactive substances (TBARS), the marker of lipid peroxidation, in plasma were decreased in

the cocoa powder treated group from the 2nd month of administration during the study period compared to that in the control group. The area of atherosclerotic lesions in th aorta was significantly smaller in the cocoa powder group (30.87%) than in the control (52.39%). Tissue cholesterol content also tended to decrease. Distensibility of the aortic wall was improved significantly in the cocoa powder treated group due to decreases in fatty streaks and intimal thickening compared to that in the control group. These results suggest that cocoa powder has suppressive effect on development of atherosclerotic lesions. We consider that antioxidative activity of polyphenols rich in cocoa powder may be a key factor for the anti-atherosclerotic effect. As taken from Kurosawa T et al. J Atheroscler Thromb. 2005; 12(1), 20-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/15725692

Short-term toxicity study of irradiated cocoa beans in rats (Abstract).

Groups of rat (30 males and 30 females) were fed stock diet and diets containing 350 g/kg of cocoa beans (non-irradiated or irradiated) for 18 weeks and observed for food consumption, haematological variations, changes in growth pattern and gross pathology. Even though there was no significant difference amond the groups in haematological or gross pathological examinations, consistently less food was consumed by rats fed on diet containing either non-irradiated or irradiated cocoa beans. Furthermore, weight gain and terminal body weights of rats fed on cocoa-containing diets were less than those fed on the stock diet. Such differences, may at leat in part, be due to alkaloids in the cocoa-containing diets and not as a result of irradiation of the beans.

As taken from Takyi & Ofori-Mensa, J Sci Food Agric, 1981, 32(9), 933-944.

“OBJECTIVE: There is a substantial interest in the potential role of chocolate in the prevention of cardiovascular diseases. It has been recently reported that a higher frequency of chocolate intake is linked to lower body mass index (BMI) in adults. The aim of the present study was to determine if higher chocolate consumption also is associated with lower BMI, as well as other markers of total and central body fat, in adolescents. METHODS: This study comprised 1458 adolescents (ages 12.5-17.5 y) participating in HELENA-CSS (Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study). Dietary intake was self-registered using a computer-based tool for 24-h dietary recall on 2 non-consecutive days. Weight and height were measured, and BMI was calculated. Adiposity was estimated using skinfolds (Slaughter's equation) and bioelectrical impedance analysis (BIA). Waist circumference was measured. Sexual maturation also was recorded. Physical activity was measured by accelerometry. RESULTS: Higher chocolate consumption was associated with lower levels of total and central fatness, as estimated by BMI, body fat estimated from skinfolds and BIA, and waist circumference, regardless of potential confounders ($P \leq 0.01$). CONCLUSION: Our results demonstrate that a higher chocolate consumption was associated with lower total and central fatness in European adolescents.” As taken from Cuenca-García M et al. 2014. Nutrition 30(2), 236-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24139727

5.3. Reproduction toxicity

Hostetler et al., (1990) reported ‘The, continuous cocoa powder consumption by rats at levels as high as 5.0% of the diet was without effect on reproductive capacity under the conditions of a standard three-generation evaluation’ (Hostetler et al., 1990).

“The toxicities of theobromine and cocoa extract on the reproductive tract of male rats were compared in the present study. A cocoa powder extract containing 117 mg theobromine/g extract was prepared using 85% boiling methanol. Sprague-Dawley rats were weighed and dosed daily for 31 days with vehicle, 250 mg/kg theobromine, 2.14 g/kg cocoa extract (117 mg theobromine/g extract), or 0.43 g/kg cocoa extract by oral gavage. The animals were sacrificed on day 32. One testis and epididymis were removed and weighed. The epididymis was saved for the determination of epididymal sperm reserves. The remaining testis was fixed by whole body glutaraldehyde

perfusion and processed for morphologic examination. A decrease in body weight gain and epididymal weights were observed in theobromine and high-dose cocoa-extract-treated groups. Theobromine and high-dose cocoa extract caused vacuolation within the Sertoli cell, abnormally shaped spermatids, and failed release of late spermatids in treated animals. Most of the vacuolations were found in the earlier and middle stage seminiferous tubules (stages I to VIII). However, the frequency of some parameters of testis alterations were significantly lower in the high-dose cocoa-extract-treated group compared to the theobromine-treated group. These data demonstrate the ability of a cocoa extract containing theobromine to alter testis structure in a similar pattern but with reduced intensity compared to that observed after oral exposure to pure theobromine." As taken from Wang Y et al Reprod Toxicol. 1992; 6(4), 347-53. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/1521008?dopt=AbstractPlus

"The target cell(s) of theobromine toxicity on rat testes and reproductive toxicity induced by pure theobromine and cocoa extract are evaluated in the present studies. Theobromine (500 mg/kg x 7 days) inhibited body weight gain in treated rats. Decreased cauda epididymal sperm reserve (38%), seminiferous tubule fluid (STF) volume (33%), lactate concentration in STF (22%), inhibition of binding activity of androgen binding protein (ABP, 21%) and reduced ABP content in STF were also observed in theobromine-treated animals. Cocoa extract containing an equivalent amount of theobromine did not produce significant toxicity in treated rats. Theobromine concentrations in serum and testes from pure theobromine-treated rats were 1.8- and 1.6-fold higher, respectively, than that in rats treated with cocoa extract. The results support Sertoli cells as the primary target cells of theobromine toxicity. The lower theobromine concentrations in serum and testes of cocoa extract-treated rats could account for the lower toxicity in these animals." As taken from Wang Y and Waller DP. Toxicol Lett. 1994 Feb 1; 70(2), 155-64. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/8296320?dopt=AbstractPlus

"In a comprehensive chronic toxicity/carcinogenicity evaluation (Tarka et al., 1991) cocoa powder was fed at levels of 1.5, 3.5, and 5% for 104 weeks to the offspring (F3b generation) of rats from a multigeneration study (Hostetler et al. , 1990). At the highest level tested (5 %), which represented amounts well beyond cocoa powder consumption by humans, there was an increased incidence of bilateral diffuse testicular atrophy, and aspermatogenesis in males, non-suppurative myocarditis and interstitial fibrosis of the heart in both sexes, and some increase of both pelvic dilatation and renal pelvic microcelculi were observed. The testes effects seen in the rats after extensive doses of cocoa were interpreted by the authors to "appear to have little or no clinical significance. Whereas dietary cocoa could not be ruled out as a possible contributing factor for the renal lesions observed, an obvious cause and effect relationship was not demonstrated in the study. All these sequelae did not affect survival rates." (Tarka et al., 1991)"

"In two earlier studies, Tarka evaluated the perinatal, postnatal, and tecatogenic effects of cocoa powder and theobromine in rats and rabbits. He concluded that cocoa powder at levels up to 7.5% and theobromine at levels up to 0.1875% are not teratogenic or embryotoxic in rabbits. Further, fetal skeletal variations noted at the higher dose levels are probably related to maternal toxicity in rabbits (Tarka, 1986). In rats, the ingestion of cocoa powder at levels up to 5% of the diet was not embryotoxic or teratogenic. The ingestion of theobromine at up to approximately 97 mg /kg/day (0.135% of the diet) was not embryotoxic or teratogenic in, rats (Tarka, 1986)."

Reproductive and developmental toxicity			
Species	Test conditions	Effects	Reference
Rat (around 35 males and 35 females per	Three-generation reproduction study. Rats fed diets containing up to 5% cocoa powder [providing up to approx. 2.5 g/kg bw/day] for 12 wk (males) or 2	No effect on a wide range of reproductive endpoints in any generation No effect on tumour	Hoestetler et al., 1990a; 1990b; Tarka et al., 1991

group in each generation)	wk (females) prior to mating within treatment groups. In a subsequent study, animals from the final generation were fed the diets for a further 2 years.	incidence in F_3 animals	
Rat (20 males and 20 females per group)	In a one-generation reproduction study, rats were fed diets containing 7% cocoa butter (providing approx. 3.3 g/kg bw/day (males) and 5.7 g/kg bw/day (females) [measured]) from 12 wk prior to mating, throughout a 2-wk mating period and throughout gestation and lactation (6 wk).	No effect on a limited range of reproductive endpoints [but no data from untreated control animals available for comparison.]	Baldrick et al., 2001
Rat (4-6 males per group)	In a study of the male reproductive tract, animals were dosed with a methanol extract of cocoa powder at either 2.14 g/kg bw/day for 31 days or 4.28 g/kg bw/day for 7 days.	No effect on sperm count or testis weight; epididymis weight reduced in 31-day study but not in 7-day study.	Wang et al., 1992; Wang & Waller, 1994
Rabbit (14-15 pregnant females per group)	Developmental toxicity study with cocoa. Animals fed diets containing 2.5, 5 or 7.5% cocoa powder [equivalent to approximately 0.9, 1.8 and 2.6 g/kg bw/day [measured]] from days 6-29 of gestation. Foetuses examined for external, visceral and skeletal malformations and skeletal variations.	No foetotoxicity or teratogenicity Delayed osteogenesis [bone development] at 2.6 g/kg bw/day.	Tarka et al., 1986a
Rat (23-26 pregnant females per group)	Developmental toxicity study with cocoa. Animals fed diets containing 2.5 or 5% cocoa powder [equivalent to approximately 1.9 or 3.8 g/kg bw/day [measured]] from days 6-19 of gestation. Foetuses examined for external, visceral and skeletal malformations and skeletal variations.	No foetotoxicity or teratogenicity.	Tarka et al., 1986b
Rat (pregnant females, group size not specified in brief report)	Developmental toxicity study in which animals were fed diets containing cocoa powder throughout gestation and lactation. [No further details in brief report.]	No evidence of teratogenicity (no abnormalities observed at birth [extent of examination not clear]) Evidence of foetotoxicity (dose-dependent foetal oedema lasting 24 hr; increase in resorptions; reduction in survival; reduction in growth of foetuses)	Tarka et al., 1981
Rat (at least 28 pregnant females per group)	Peri- and post-natal toxicity study with cocoa. Animals fed diets containing 2.5, 5 or 7.5% cocoa powder [equivalent to approximately 1.9, 3.8 and 6 g/kg bw/day [measured]] throughout gestation and lactation.	No statistically significant effects on litters at birth (reductions in litter size in 7.5% group and pup survival at 5% and above).	Tarka et al., 1986b

		Small but statistically significant reduction in pup weight during lactation at 2.5% and above.	
Rat (females, [group size not specified in brief report])	Fertility and developmental toxicity study in which female rats were fed diets containing cocoa beans prior to mating and probably throughout gestation and lactation. [No further details in brief report.]	No effect on mating performance and fertility. Adverse effect on viability, growth and development of offspring prior to weaning.	Tesh et al., 1982

Reproduction studies in the rat with shea oleine and hardened shea oleine (Abstract). Shea oleine is an oil fraction derived from the nut of the tree Butyrospermum parkii, which grows in central and western Africa. There are several uses of shea oleine including its use as a frying oil and, after hardening, in margarine and toffee fat. This investigation was performed to examine the toxicity of 7 or 15% hardened shea oleine in comparison with 7 or 15% unhardened shea oleine and various commercially available materials, sheanut and palm oils, cocoa butter and toffee powder following dietary administration to rats during pre-mating, mating, pregnancy and offspring weaning in two separate investigations. Reproduction was assessed using number of litters and pups born plus survival and body weights at birth and at weaning on day 21. Skeletal evaluation using X-ray, clinical pathology and a macroscopic examination were also performed for F1 rats. Study measures for parent animals comprised evaluation of body weight, food consumption, clinical pathology, organ weights and macroscopic examination. Fatty acids and hydrocarbon levels were measured and an evaluation for lipogranulomata was made for various tissues. Results showed that shea oleine, whether unhardened or hardened, produced no evidence of reproduction toxicity and gave a similar profile to the other commercially available materials used in this study in the rat. Minor findings with shea oleine were not related to reproduction performance but comprised slightly reduced body weight gain and reduced cholesterol and raised alkaline phosphatase levels. None of the findings in this study were considered to be of toxicological significance. Thus, no evidence of reproduction toxicity was seen for both unhardened and hardened shea oleine in this investigation in the rat at levels equating to greater than 7.5 g/kg/day. As taken from Baldrick P et al. Food Chem Toxicol. 2001 Sep; 39(9), 923-30. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/11498269

Therapeutic effect of ACTICOA powder, a cocoa polyphenolic extract, on experimentally induced prostate hyperplasia in Wistar-Unilever rats (Abstract). Benign prostatic hyperplasia (BPH) is a non-malignant enlargement of the prostate that results in obstructive lower urinary tract symptoms. Plant extracts are frequently used to treat BPH rather than therapeutics that can cause severe side effects. ACTICOA() (Ba0rry Callebaut France, Louviers, France) powder (AP) is a cocoa polyphenolic extract, and we have shown in a previous study that oral treatment with AP prevented prostate hyperplasia. This study investigated whether AP could improve established prostate hyperplasia using the same testosterone propionate (TP)-induced prostate hyperplasia model in rats. Male Wistar-Unilever rats were randomly divided in four groups of 12 rats: one group injected with corn oil and orally treated with the vehicle (negative control) and three groups injected subcutaneously with TP and orally treated with the vehicle (positive control) or AP at 24 (AP24) and 48 (AP48) mg/kg/day. Treatments started 1 week after the start of the induction of prostate hyperplasia and lasted for 2 weeks. The influence of TP and AP on body weights, food and water consumptions, plasma polyphenolic concentration, and serum dihydrotestoterone (DHT) level of rats was examined. At completion of the study, rats were sacrificed, and the prostates were removed, cleaned, and weighed. The prostate size ratio (prostate weight/rat body weight) was then calculated. TP significantly influenced the body weight gain of the rats and their food and water consumptions, while AP reduced significantly these differences in a dose-dependent manner. AP

significantly reduced serum DHT level and prostate size ratio in comparison with positive controls also dose-dependently. In conclusion, AP orally administered was effective for reducing established prostate hyperplasia, especially at the dose of 48 mg/kg/day. As taken from Bisson JF et al. J Med Food. 2007 Dec; 10(4), 628-35. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/18158833

“1. Consumption of a high-fat and high-energy diet during pregnancy leads to a risk of long-term consequences on fetal development, as well as on the postnatal health of offspring. To investigate the effects of such a diet on fetal programming, we established a high-energy intake pregnant rat model using chocolate and fructose beverage as supplements to a normal chow diet. 2. Pregnant Sprague-Dawley rats were assigned to either chow (control) or a diet supplemented with chocolate and fructose beverage throughout gestation and lactation. The male F(1) pups received normal chow diet after weaning. Physiological or pathological changes in dams and pups (e.g. glucose and lipid metabolism) were evaluated. 3. The results showed that dams offered the high-fat (mainly from chocolate) and high-calorie diet during gestation consumed more energy and gained more weight than chow-fed dams. Over-consumption of chocolate reduced chow intake in dams, leading to low maternal protein supply. As a result, pups from these dams exhibited reduced birth weight that lasted until adulthood. The high-energy diet during lactation led to increased total body fat, as well as impaired liver function, in offspring; thus, the lactational diet is suggested to be a stronger determinant of offspring fat metabolism than gestational diet. 4. The results of the study suggest that over-supply of carbohydrates, such as chocolate and fructose, either during gestation or lactation has a negative impact on the well-being of offspring”. As taken from Zhang ZY et al. 2011. Clin. expt. Physiol. Pharmacol. 38, 613-622. PubMed 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/21722163?dopt=AbstractPlus.

“Epidemiological and immunological studies suggest that maternal diet during pregnancy might affect the development of allergic diseases in the offspring. The authors set out to study the effect of maternal food consumption during pregnancy on the emergence of the International Study of Asthma and Allergies in Childhood (ISAAC)-based allergic outcomes: asthma, allergic rhinitis, and wheeze by the 5 yr of age. METHODS: Data from 2441 children at 5 yr of age were analyzed within the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Nutrition Study, a population-based birth cohort study. Maternal diet was assessed with a validated food frequency questionnaire. RESULTS: In multiple regression models adjusted for known confounders, low maternal consumption of leafy vegetables (adjusted odds ratio [aOR]: 1.55; 95% CI: 1.21, 1.98), malaceous fruits (aOR: 1.45; 95% CI: 1.15, 1.84), and chocolate (aOR: 1.36; 95% CI: 1.09, 1.70) were positively associated with the risk of wheeze in children..... No associations were observed between maternal food consumption and asthma. CONCLUSIONS: Development of allergic diseases in preschool children may be influenced by intrauterine exposure to maternal diet”. As taken from Erkkola M et al. 2012. Pediatric. Allerg. Immunol. 23, 186-194. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22432883?dopt=AbstractPlus.

“AIM: According to the World Diabetes Foundation, there is an urgent need to investigate the impact of maternal health and nutrition during pregnancy to understand the background for the accelerating incidence of obesity and type 2 diabetes. In this study, we specifically concentrated on the role of overfeeding during different developmental periods. METHODS: Sprague-Dawley rats were offered chow or high-fat/high-sucrose diet (chow plus chocolate and soft drink) during gestation and lactation. At birth, offspring were randomly cross-fostered within each dietary group into small and normal litter sizes until weaning, giving four dietary groups. RESULTS: At postnatal day 1, offspring from high-fat/high-sucrose-fed dams were heavier and had increased hepatic triglycerides (TG), hepatic glycogen, blood glucose and plasma insulin compared with offspring from chow-fed dams. Hepatic genes involved in lipid oxidation, VLDL transport and insulin receptor were down-regulated, whereas FGF21 expression was up-regulated. Independent of postnatal litter size, offspring from high-fat/high-sucrose-fed dams aged 21 days had still increased hepatic TG and up-regulated FGF21 expression, while plasma insulin started to decrease. Litter size reduction in offspring from high-fat/high-sucrose-fed dams further increased body weight and adiposity, and

up-regulated genes involved in hepatic mitochondrial lipid oxidation and VLDL transport compared with all other groups. Litter size reduction did not have any impact on body weight gain and adiposity in offspring born to chow-fed dams. CONCLUSION: Our results suggest that supplementation of chocolate and soft drink during gestation and lactation contributes to early onset of hepatic steatosis associated with changes in hepatic gene expression and lipid handling." As taken from Kjaergaard M et al. 2014. Acta Physiol. (Oxf.) 210(1), 142-53. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23782871?dopt=AbstractPlus

"Unsweetened natural cocoa (UNCP) was evaluated for reproductive toxicity in rats. A preliminary genotoxic potential was evaluated by the DNA comet assay test using C57Bl/6 mice. Both therapeutic dose (TD; 900 mg/kg) and high dose (HD; 9000 mg/kg) of UNCP were used. White Wistar rats were used in two experimental groups. The females received UNCP 15 days before crossing with untreated males. The males received UNCP for 48 days before mating with untreated females. Subacute toxicity was observed during a 14-day oral administration of UNCP. Results show that a high tail DNA% was observed with methyl mesylate administration in all tissues analysed. The lowest tail DNA% value was observed in the liver (1.64 ± 0.26) and kidney (1.63 ± 0.30) during UNCP (TD) administration. UNCP did not induce observable physical congenital malformations on the pubs of treated female and male rats, lacks genotoxic potential, and did not adversely affect pregnancy index, pub weights, and survival index, but UNCP exhibited proimplantation potential ($p > 0.05$)." As taken from Asiedu-Gyekye IJ et al. 2021. J. Toxicol. 2021, 6114672. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33519930/

"Maternal consumption of polyphenol-rich foods has been associated with fetal ductus arteriosus constriction (DAC), but safety of chocolate exposure in fetal life has not been studied. This experimental study tested the hypothesis that maternal cocoa consumption in late pregnancy causes fetal DAC, with possible associated antioxidant effects. Pregnant Wistar rats, at the 21st gestational day, received by orogastric tube cocoa (720 mg/Kg) for 12 h, indomethacin (10 mg/Kg), for 8 h, or only water, before cesaren section. Immediately after withdrawal, every thorax was obtained and tissues were fixed and stained for histological analysis. The ratio of the narrowest part of the pulmonary artery to the fetal ductus inner diameter and increased ductal inner wall thickness characterized ductal constriction. Substances reactive to thiobarbituric acid were quantified. Statistical analysis used ANOVA and Tukey test. Cocoa ($n = 33$) and indomethacin ($n = 7$) reduced fetal internal ductus diameter when compared to control (water, $n = 25$) ($p < 0.001$) and cocoa alone increased ductus wall thickness ($p < 0.001$), but no change was noted in enzymes activity. This pharmacological study shows supporting evidences that there is a cause and effect relationship between maternal consumption of cocoa and fetal ductus arteriosus constriction. Habitual widespread use of chocolate during gestation could account for undetected ductus constriction and its potentially severe consequences, such as perinatal pulmonary hypertension, cardiac failure and even death. For this reason, dietary guidance in late pregnancy to avoid high chocolate intake, to prevent fetal ductal constriction, may represent the main translational aspect of this study."

Zielinsky P et al. (2021) Maternal ingestion of cocoa causes constriction of fetal ductus arteriosus in rats.

5.4. Mutagenicity

Unroasted or roasted coca powder was reported to increase the number of SCE in bone marrow cells elevated numbers of SCE of bone-marrow cells). However it was reported that Cocoa (where theobromine was removed) did not produce this effect, (Renner and Munzner, 1982)

"To further evaluate the genotoxic potential of cocoa, unroasted and roasted cocoa powder dispersed in water was administered to Chinese hamsters by stomach tube at concentrations equivalent to 5 g/kg. An increased number of sister-chromatid exchanges (SCE) in bone marrow cells was observed. Cocoa administered in the diet had no effect on bone marrow SCE. Cocoa

butter had no effect on SCE, nor did cocoa from which theobromine was extracted (Renner and Munzner, 1982).

Cocoa did not mutate Salmonella typhimurium or mouse lymphoma cells (Renner & Munzer 1982; Brusick et al 1986; Crebelli et al 1990). Cocoa pigment showed DNA damaging potential in the Bacillus subtilis rec assay (Nonaka 1989). Cocoa increased the frequency of sister chromatid exchanges in Chinese hamster and bone marrow cells (Renner & Munzer 1982), but not in Chinese hamster ovary cells or cultured human lymphocytes (Brusick et al 1986). It did not induce chromosome aberrations in Chinese hamster ovary cells, or transform mouse Balb/c-3T3 cells in vitro (Brusick et al 1986).

In vivo				
Species	**Test conditions**	**Endpoint**	**Result**	**Reference**
Chinese hamster (6 per group)	Bone marrow micronucleus test. Animals given three doses of 0.2 g roasted cocoa powder at 90-min intervals [0.6 g/animal is approximately 20 g/kg bw] and killed 27 hr after the final dose.	Chromosome damage	-ve	Renner & Münzner, 1982
Chinese hamster (6 per group)	Bone marrow chromosome aberration test. Animals given three doses of 0.2 g roasted cocoa powder at 90-min intervals [0.6 g/animal is approximately 20 g/kg bw] and killed 24 hr after the final dose.	Chromosome damage	-ve	Renner & Münzner, 1982
Chinese hamster (number undisclosed)	Bone marrow sister chromatid exchange test. Animals fed diets containing 20% cocoa. [No further details in brief report.]	Chromosome effects	-ve (small, but not statistically significant, increase in SCEs)	Renner & Münzner, 1982
Chinese hamster (4 per group)	Bone marrow sister chromatid exchange test. Animals treated by gavage with unroasted, roasted or roasted, fat-free cocoa powder in water (1:1) as a single dose of 0.1 g, or one, two or three doses, 90 min apart, of 0.2 g [0.6 g/animal is approximately 20 g/kg bw] or with 0.6 g cocoa butter.	Chromosome effects	+ve (cocoa powder, roasted, fat-free, at 7 g/kg bw and above; -ve at 3 g/kg bw; dose response) +ve (cocoa powder, roasted and unroasted, at 13 g/kg bw per day and above; -ve at 7 g/kg bw and below; dose	Renner & Münzner, 1982

			response) -ve (cocoa butter)	

In vitro					
Test system	**Test conditions**	**Endpoint**	**Activation**	**Result**	**References**
Chinese hamster ovary cells	Chromosome aberration test with cocoa at up to 1 mg/ml. Exposure time: 12 hr without S9; 2 hr with S9 and harvested 10.5 hr later.	Chromosome damage	With and without S9	-ve	Brusick et al., 1986
Chinese hamster ovary cells	Sister chromatid exchange test with cocoa at up to 0.6 mg/ml. Exposure time: 27 hr without S9; 2 hr with S9 and harvested 27 hr later.	Chromosome effects	With and without S9	weak +ve (small, but statistically significant increase at the top dose without S9; dose response)	Brusick et al., 1986
Human lymphocytes	Sister chromatid exchange test with cocoa at up to 1.25 mg/ml. Exposure time: 46 hr.	Chromosome effects	Without	-ve (limited assay, not tested with S9)	Brusick et al., 1986
Mouse lymphoma L5178Y cells	Mouse lymphoma assay with cocoa at up to 6 mg/ml. 4-hr exposure time; 2-day expression time.	Mutation	With and without S9	-ve	Brusick et al., 1986
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Ames test with cocoa at up to 5 mg/plate.	Mutation	With and without S9	-ve	Brusick et al., 1986
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Ames test with up to 0.1 ml 5% fat-free cocoa powder in water.	Mutation	With and without S9	-ve	Renner & Münzner, 1982
Salmonella	Ames test with cocoa alcoholic	Mutation	With and	-ve	Crebelli et

typhimurium TA98, TA100	extract, cocoa hydroalcoholic extract, cocoa distillate and cocoa tincture probably at up to 100 ul/plate.		without S9	limited assay, only 2 strains used	al., 1990
Salmonella typhimurium strains TA98, YG1024 and YG1029	Extracts of two instant hot cocoa products were tested, apparently at up to 2 mg/plate	Mutation	With and without S9	-ve limited assay, current protocols recommend testing in at least 4 strains	Johansson et al. 1995
[+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation]					

Radical scavenging activity, anti-bacterial and mutagenic effects of cocoa bean Maillard reaction products with degree of roasting (Abstract). Raw, pre-roasted and roasted Cocoa samples were separated into four different molecular weight fractions (> 30, 30-10, 10-5 and <5 kDa) with ultrafiltration and tested for their antibacterial, mutagenic, as well as their radical-scavenging effects. Radical-scavenging effects were tested with electro paramagnetic resonance spectroscopy, anti-mutagenicity in the Salmonella microsome assay (with and without metabolic activation), and antibacterial effects by incubating the fractions with several strains of Bifidobacteria, Enterobacter and Escherichia, and observing their growth. The radical-scavenging activity and reducing substance concentrations increased, particularly in the 5-10-kDa roasted fraction. Chromaticity testing elucidated that the 10-5-kDa fraction was one of the darkest fractions. The Salmonella microsome assay showed neither mutagenic nor anti-mutagenic effects in any of the samples at any of the different concentrations applied when using TA98, TA100 and TA102. All fractions reduced the growth of pathogenic bacteria, in particular at the highest concentration of 100 microg/mL; however, the same trends were also observed for Bifidobacteria. As taken from Summa C et al. Mol Nutr Food Res. 2008 Mar; 52(3), 342-51. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/18293302

“Nutrient excess and unbalanced diets can result in overproduction of reactive oxygen species (ROS), which are associated with oxidative stress. Cocoa extract contains antioxidants that inhibit the harmful effects of ROS. This trial analysed the effect of cocoa extract consumption integrated as a bioactive compound into ready-to-eat meals, on oxidative stress at the level of DNA in overweight/obese subjects. Fifty volunteers [57.26(5.24) years, 30.59(2.33)kg/m(2)] participated in a 4-week double-blind, randomised, placebo-controlled parallel nutritional intervention. Half of the volunteers received meals supplemented with 1.4 g/day cocoa extract, while the other half received control meals, both within a 15% energy restriction diet. Lymphocytes were isolated and endogenous strand breaks, oxidised bases and resistance to H2O2-induced damage were measured by the comet assay. The intake of ready-to-eat meals supplemented with cocoa extract did not show relevant changes in the oxidative status of DNA. However, in the cocoa group, oxidised bases negatively correlated with methyl epicatechin-O-sulphate (r = -0.76; P = -0.007) and epicatechin sulphate (r = -0.61; P = -0.046). When volunteers of both groups were analysed together, a marginal decrease (P = 0.072) in oxidised bases was observed, which attributed to weight loss. Subjects who started the intervention with higher levels of damage showed a greater

reduction in oxidised bases after 4 weeks (P = 0.040) compared to those who had lower baseline levels. In conclusion, even if 1.4 g of cocoa supplementation for 4 weeks did not show notable changes in terms of antioxidant status of DNA, the energy restriction showed a slightly decrease in oxidised bases and this was seen to a greater extent in subjects who started the intervention with higher levels of damage. On the other hand, the inverse associations found between oxidised bases and some cocoa-derived metabolites suggest that a protective effect might be seen in a longer period of time or in subjects with higher baseline DNA damage." As taken from Ibero-Baraibar I et al. 2015. Mutagenesis 30(1), 139-46. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25527736

"This study evaluated the genotoxicity of lyophilized glycolic extract of Theobroma cacao Linné seeds (TCL), using the micronucleus assay in bone marrow of mice. The interaction between TCL and doxorubicin (DXR) was also analyzed. Experimental groups were evaluated 24-48 h after treatment with N-Nitroso-N-ethylurea (NEU: 50 mg/kg), DXR (5 mg/kg), NaCl (145 mM), TCL (0.5-2 g/kg), and TCL (2 g/kg) in combination with DXR (antigenotoxic assays). Analysis of micronucleated polychromatic erythrocytes (MNPCEs) showed no significant differences between all the treatment doses of TCL and NaCl control. Mice experimentally treated with DXR and NEU significantly induced MNPCEs. However, a significant reduction of MNPCEs was also observed when TCL was administered in combination with the chemotherapeutic agent DXR. The analysis of the PCE/NCE ratio revealed no significant differences between the NaCl control, all doses of TCL, and DXR. However, there were significant differences in the PCE/NCE ratio between positive NEU control and all other treatments. The PCE/NCE ratio observed after treatment with TCL and DXR showed significant differences and intermediate values to controls (NaCl and NEU). This study suggests absence of genotoxicity and cytotoxicity of TCL, regardless of dose, sex, and time. TCL reduced genotoxic effects induced by DXR, suggesting potential antigenotoxic effects." As taken from Boriollo MFG et al. 2021. Braz. J. Biol. 81(2), 268-277. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32696851/

5.5. Cytotoxicity

Non-saponifiable fraction of cocoa shell butter: effect on rat and human skin Fibroblasts (Abstract). Synopsis Non-saponifiable lipid fraction (ICSB) extracted from cocoa shell butter was solubilized in dimethylformamide (DMF) and analysed for its biological activity on growth of rat and human fibroblasts. Non-saponifiables (10 mug ml(-1)) partially protected cells from toxicity of DMF (1%) and allowed the growth of fibroblasts cultivated in optimal conditions (10% fetal calf serum-FCS, 37 degrees C) or improved the survival of cells maintained in altered conditions (2.5% FCS, 35 degrees C). At higher concentration (ICSB 50 mug ml(-1), DMF 1%), the protective effect was suppressed. ICSB was fractionated by chromatography into four compounds: sterols, terpenic alcohols, tocopherols and hydrocarbons +/- carotenoids. We found that biological activity of ICSB was mostly due to the major fraction containing sterols.

As taken from Warocquier-Clerout R et al. Int J Cosmet Sci. 1992 Feb;14(1), 39-46.

Epicatechin and catechin in cocoa inhibit amyloid beta protein induced apoptosis (Abstract).

To elucidate additional health benefits of cocoa phytochemicals on the neurotoxicity induced by amyloid beta protein (Abeta), PC12 cells were treated with toxic peptide (Abeta(25)(-)(35)) and the effects of epicatechin, catechin, and cocoa were studied using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction, lactate dehydrogenase (LDH) release, and trypan blue exclusion methods. Significant increase in neuronal cell death was observed on PC12 cells treated with Abeta(25)(-)(35) (25 microM), while epicatechin and catechin and their mixture prevented the Abeta-induced neuronal cell death. Abeta treatment also led to the increased membrane instability of PC12 cells. The membrane protective effects of the phenolics determined by LDH release and trypan blue exclusion assays demonstrated that epicatechin, catechin, and

their mixture protect cellular membrane from Abeta-induced cytotoxicity. In these three different cell viability assays, the mixture of epicatechin and catechin showed the highest protective effect and synergistic activity. The present results showed that the major flavonoids of cocoa, epicatechin and catechin, protect PC12 cells from Abeta-induced neurotoxicity, and suggest that cocoa may have anti-neurodegenerative effect in addition to other known chemopreventive effects. As taken from Heo HJ & Lee CY. 2005. J. Agric. Food Chem. 53(5), 1445-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/15740021

“The aims of this study were to determine the antioxidant and antiproliferative activity of the following Theobroma cacao plant part methanolic extracts: leaf, bark, husk, fermented and unfermented shell, pith, root, and cherelle. Antioxidant activity was determined using 2,2-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid-reactive substances (TBARS), and Folin-Ciocalteu assays; the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) assay was used to determine antiproliferative activity. The root extract had the highest antioxidant activity; its median effective dose (EC50) was 358.3 ± 7.0 µg/mL and total phenolic content was 22.0 ± 1.1 g GAE/100 g extract as compared to the other methanolic plant part extracts. Only the cherelle extract demonstrated 10.4% ± 1.1% inhibition activity in the lipid peroxidation assay. The MTT assay revealed that the leaf extract had the highest antiproliferative activity against MCF-7 cells [median inhibitory concentration (IC50) = 41.4 ± 3.3 µg/mL]. Given the overall high IC50 for the normal liver cell line WRL-68, this study indicates that T. cacao methanolic extracts have a cytotoxic effect in cancer cells, but not in normal cells. Planned future investigations will involve the purification, identification, determination of the mechanisms of action, and molecular assay of T. cacao plant extracts.” As taken from Baharum Z et al. 2014. Molecules 19(11), 18317-31. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25389662

“Theobroma cacao L. contains more than 500 different chemical compounds some of which have been traditionally used for their antioxidant, anti-carcinogenic, immunomodulatory, vasodilatory, analgesic, and antimicrobial activities. Spontaneous aerobic fermentation of cacao husks yields a crude husk extract (CHE) with antimicrobial activity. CHE was fractioned by solvent partition with polar solvent extraction or by silica gel chromatography and a total of 12 sub-fractions were analyzed for chemical composition and bioactivity. CHE was effective against the yeast Saccharomyces cerevisiae and the basidiomycete Moniliophthora perniciosa. Antibacterial activity was determined using 6 strains: Staphylococcus aureus, Staphylococcus epidermidis, and Bacillus subtilis (Gram-positive) and Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonella choleraesuis (Gram-negative). At doses up to 10 mg/mL, CHE was not effective against the Gram-positive bacteria tested but against medically important P. aeruginosa and S. choleraesuis with a minimum inhibitory concentration (MIC) of 5.0 mg/mL. Sub-fractions varied widely in activity and strongest antibacterial activity was seen with CHE8 against S. choleraesuis (MIC of 1.0 mg/mL) and CHE9 against S. epidermidis (MIC of 2.5 mg/mL). All bioactive CHE fractions contained phenols, steroids, or terpenes, but no saponins. Fraction CHE9 contained flavonoids, phenolics, steroids, and terpenes, amino acids, and alkaloids, while CHE12 had the same compounds but lacked flavonoids.” As taken from Santos RX et al. 2014. Genet. Mol. Res. 13(3), 7725-35. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25299086

“BACKGROUND: Over the last 400 years, cocoa and chocolate have been described as having potential medicinal value, being consumed as a beverage or eaten as food. Concentration-dependant, antiproliferation, and cytotoxic effects of some of their polyphenolic constituents have been demonstrated against various cancers. Such an effect remains to be demonstrated in ovarian cancer. OBJECTIVE: To investigate the effect of cocoa procyanidins against ovarian cancer in vitro using OAW42 and OVCAR3 cell lines. MATERIALS AND METHODS: Cocoa procyanidins were extracted and enriched from non alkalized cocoa powder. The polyphenolic content and antioxidant activity were determined. Effect on cell viability was determined after the treatment with ≤1000 µg/mL cocoa procyanidin-rich extract on OAW42 and OVCAR3 and normal human dermal fibroblasts. Similarly, chemosensitization effect was determined by pretreating cancer cell lines with

extract followed by doxorubicin hydrochloride treatment. The effect of treatment on cell cycle and P-glycoprotein (P-gp) expression was determined using flow cytometry. RESULTS: The cocoa extract showed high polyphenolic content and antioxidant activity. Treatment with extract caused cytotoxicity and chemosensitization in OAW42 and OVCAR3 cell lines. Normal dermal fibroblasts showed an increase in cell viability post treatment with extract. Treatment with extract affected the cell cycle and an increasing percentage of cells in hypodiploid sub-G1/G0 phase was observed. Treatment of OVCAR3 with the extract caused reduction of P-gp expression. CONCLUSION: Cocoa procyanidins were found to be selectively cytotoxic against epithelial ovarian cancer, interfered with the normal cell cycle and sensitized cells to subsequent chemotherapeutic treatment. Chemosensitization was found to be associated with P-gp reduction in OVCAR3 cells." As taken from Taparia S and Khanna A. 2016. Pharmacogn. Mag. 12(Suppl. 2), S109-15. PubMed, 2107 available at https://www.ncbi.nlm.nih.gov/pubmed/27279694

"Lung cancer is a common malignancy in men and the second leading cause of cancer-related mortality in men in the western world. Phenolic cocoa ingredients have a strong antioxidative activity and the potential to have a protective effect against cancer. In the present study, we have evaluated the influence of cocoa beans subjected to different processing conditions on cell viability and apoptosis of human lung cancer cells (A549). We measured the viability of lung cells treated with cocoa beans, unroasted slates (US), roasted slates (RS), unroasted well fermented (UWF) cocoa, and roasted well fermented (RWF) cocoa for 24 h. Using an MTT assay, we observed a decrease in the viability of A549 cells after treatment with cocoa bean extracts. Flow cytometer analysis revealed that cocoa beans increased the percentage of cells in sub-G1 phase and promoted up to twofold increase of apoptotic cells when compared to the control group. Taken together, the present study suggests that cocoa beans may have a protective effect against lung cancer." As taken from Bauer D et al. 2016. Oxid. Med. Cell. Longev. 2016, 7428515. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27034742

"To contribute in the research of better drugs against dermatophytosis, we evaluated the antioxidant and antidermatophytic activities of cocoa butter, cloves essential oil, and a mixture of both extracts. The cocoa butter was obtained by boiling the cocoa paste. The essential oil extracted by hydrodistillation was chemically analysed by gas chromatography and gas chromatography coupled with mass spectrometry. The antioxidant activity was determined using the DPPH scavenging method, and the antidermatophytic activity was evaluated using the agar dilution method. The essential oil, majoritary constituated by eugenol (87.62%), β-caryophyllene (5.88%), and β-bisabolene (4.41%), had an antiradical power (4.22 x 10-2) higher than that of BHT (4.00 x 10-3), like the cocoa butter and essential oil mixture (6.06 x 10-3). The essential oil was more active than the griseofulvin: it was fungicidal at 400 ppm against Trichophyton rubrum, and at 900 ppm against Microsporum gypseumand Trichophyton tonsurans. The cocoa butter activity was low, but the mixture with the essential oil had an important activity with inhibitory percentages of 78.69 %, 88.27 %, 91.20% against T. rubrum (at 400 ppm), T. tonsurans(at 900 ppm)and M. gypseum (at 900 ppm) respectively. Cloves essential oil and the mixture with cocoa butter can be used to formulate new drugs against dermatophytes." As taken from Fankem PM et al. 2017. American Scientific Research Journal for Engineering, Technology, & Sciences 37(1), 255-272. Available at http://www.asrjetsjournal.org/index.php/American_Scientific_Journal/article/view/3449

"Cocoa husk ((Theobroma cacao L.) has antioxidant and antimicrobial activity which has the potential as a natural preservative of food, but in its use the cocoa rind extract has a disadvantage because of its short shelf life and limited application to foodstuffs, therefore prevent damage and extend the shelf life, one of the efforts that can be done is to encapsulate the extract. This study aims to determine the antibacterial and antioxidant activity of encapsulated cocoa peel extract, this study begins with the treatment of extraction of cacao pods with ethanol solvent by comparison of cacao pods powder : 1: 4 solvent. Cocoa husk used are yellow harvested fruits, then chopped and dried to form flour. The sample was extracted by maceration with ethanol solvent. Antioxidant testing was carried out by DPPH method, while the antibacterial test was carried out by well diffusion method. This study used a completely randomized design method (CRD) with 5

treatments using a maltodextrin concentration of 20% (M1); 30% (M2); 40% (M3); 50% (M4) and 60% (M5) and repeated 3 times. This study concluded that encapsulant extract of cocoa husk using maltodextrin 20% had the highest antioxidant and antimicrobial activity compared to other treatments, namely 30% concentration; 40%; 50% and 60% but for treatment 20% and 30% there is no difference. Ethanol extracts of fruit peels can be made in the form of encapsulants which are very likely to be used as natural preservatives." As taken from Hasanuddin A et al. 2019. IOP Conf. Ser.: Earth Environ. Sci. 255, 012017. Available at https://iopscience.iop.org/article/10.1088/1755-1315/255/1/012017/meta

"Numerous studies have shown, rather disappointingly, that isolated bioactive phytochemicals are not as biologically effective as natural plant products. Such a discrepancy may be explained by the concept of food synergy, which was verified in this research for cocoa extract versus its major components with regard to cancer chemoprevention. The evaluation embraced the relationship between redox properties evaluated in cell-free systems with the aid of free radicals scavenging method and differential pulse voltammetry, and redox associated anticarcinogenic activities (cellular antioxidant activity, cytotoxicity, nutrigenomic activity) in human colon adenocarcinoma cell line exposed to either cocoa powder extract or artificial mixtures of cocoa bioactives at matching concentrations. In contrast to expectations, our results showed that the stepwise enrichment with antioxidants caused no gradual increase in the antioxidant activity of the model mixtures; also, these model mixtures did not reach the reducing potential of cocoa in the cell-free systems or cellular model employed. Further, the biological activities examined in colon adenocarcinoma cells did not alter in a stepwise manner that could reflect the gradual changes in composition of bioactive ingredients. In conclusion, the experiments presented here showed that the growing complexity of a mixture of phytochemicals seems to create a new redox bioactive substance rather than enrich the mixture with new activities, characteristic of the compound added. It follows that no simple, predictable relationship can be expected between the chemopreventive potential and the composition of real food items containing a complicated set of non-toxic redox active ingredients. Our observations suggest that the interactions between different bioactive compounds and food matrix components are cooperating factors determining the final bioactivity of foods." As taken from Baranowska M et al. 2020. Free Radic. Biol. Med. 154, 48-61. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32360591/

"Increasing concerns on the adverse effect of synthetic antioxidants and the emergence of antibiotic-resistant Staphylococcus aureus have become two essential problems to be addressed. To tackle them, exploration of natural resources to discover novel antioxidants and/or antibacterial agents is urgently required. The aim of this research was to investigate the correlation of phenolic and flavonoid contents of extracts to their antioxidant and antibacterial activities. Green tea, green coffee, cocoa pod husks, bee pollen, and rosella calyces were processed and subjected to 80% ethanol-based maceration procedure to obtain extracts with appropriate condition. Each extract was examined for its phenolic and flavonoid concentrations using the Folin–Ciocalteau method and the aluminum chloride colorimetric assay, respectively. Further analysis on the free-radical scavenging potential and antibacterial/antibiofilm activity against S. aureus were carried out. Samples were found to contain total phenolics (TP) and total flavonoids (TF) at different concentrations. The highest level of TP and TF was identified in green tea extract and corresponded to the lowest IC50 against DPPH and the lowest MIC against S. aureus colonies or to their respective biofilm. In contrast, low amounts of TP and TF were found in cocoa pod husks and bee pollen which were further demonstrated high IC50 and high MIC. Collectively, our results suggested the linear correlation of phenolic- and flavonoid contents to the antioxidant and antibacterial/antibiofilm activities of plant extracts. The higher the phenolics and flavonoids level, the better the antioxidant and antibacterial/antibiofilm activities obtained from the corresponding extracts." As taken from Sartini S et al. 2019. J. Phys.: Conf. Ser. 1341(7), 072009. Available at https://iopscience.iop.org/article/10.1088/1742-6596/1341/7/072009/meta

"Background: Streptococcus sanguinis is a bacterium that can cause failures in root canal treatments due its ability to penetrate the dentinal tubules to a depth of 400 μm in just two weeks.

Irrigation material is needed to stop the growth of this bacteria so that no bacteria can pass through by using chemicals, irrigation materials that are widely used such as Chlorhexidine 0,2% but still lack because it cannot be used as a single irrigation solution because its effectiveness will be reduced if it is related to protein and organic dentine matrix and low Ph saliva. Therefore, research is needed to find natural ingredient that can be an alternative such as Cocoa peel extract was chosen because it contains active compounds, in the form of saponins, tanins, alkaloids, flavonoids, and terpenoids that have been known to have antibacterial properties a concentration of 6,25% is used in accordance with the MKC of Streptococcus sanguinis. Aim: To compare the antibacterial power between cocoa peel extract (Theobroma cacao L.) 6,25% and Chlorhexidine 0,2% against Streptococcus sanguinis Method: This research is an experimental laboratory with a post-test only control group design. The diffusion method was used to determine the susceptibility of bacteria isolated from the material by planting the culture of Streptococcus sanguinis on the agar medium by swabbing the nutrient media which has been divided into 3 parts consisting of negative control, cocoa peel extract and Chlorhexidine, then each nurient media so that it is given a paper disk and 0.01ml liquid on each section. The diameter of the inhibition zone was observed after 2x24 hours using the calipers. Results: The average inhibitory zone that was formed using cocoa peel extract was 20,40 mm against Streptococcus sanguinis and Chlorhexidine was 18,36 against Streptococcus sanguinis. Conclusion: Cocoa peel extract (Theobroma cacao L.) 6,25% had higher anti-bacterial power compared to 0,2% Chlorhexidine against the growth of Streptococcus sanguinis." As taken from Wulandari NM et al. 2019. Conservative Dentistry Journal 9(1), 40-47. Available at https://e-journal.unair.ac.id/CDJ/article/view/16489/0

"Cocoa shell and cocoa husk are the waste from the processing of cocoa beans whose utilization has not been done optimally. The cocoa shell and cocoa husk contain phytochemical compounds which are potential to inhibit the growth of pathogenic bacteria in food products. The aim of this study is to find the ratio of cocoa shell extracts and cocoa husk extracts that most effectively inhibited the growth of Escherichia coli, Staphylococcus aureus and Salmonella sp. The method used experimental research followed by Randomized Block Design Factorial Pattern with 9 treatments and 3 replication. The treatment consists of two factors. The first factor was 3 types of extract (1:1, 2:3 and 3:2) and the second factor was 3 bacteria types (Escherichia coli, Staphylococcus aureus and Salmonella sp). The result showed that the extract mixture of cocoa shell and cocoa husk didn't provide interaction effects, but provided an independent effect to inhibit the various types of bacteria. The comparison of 3:2 w/w of cocoa shell and cocoa husk extract contain phenol 0.063% and flavonoid 0.0191% gave the most effective inhibitory effect on Escherichia coli with a diameter of inhibition zone 5.84 mm (resistant), Staphylococcus aureus 4.04 mm (resistant) and Salmonella sp. 21.00 mm (sensitive) and was able to reduce the total bacteria of Escherichia coli 7.67 log CFU/ml (6.19%), Staphylococcus aureus 7.06 log CFU/ml (13.65%) and Salmonella sp. 6.49 log CFU/ml (20.62%). Phytochemical tests showed that cocoa shell extract and cocoa husk extract containing phenol, flavonoid, triterpenoid, tannin, and alkaloid." As taken from Kayaputri IL et al. 2019. IOP Conference Series: Earth and Environmental Science 443, 012077. Available at https://iopscience.iop.org/article/10.1088/1755-1315/443/1/012077/pdf

"The Peruvian Amazon is considered one of the regions with the greatest diversity in flora, so the importance of its study through its hydroalcoholic extracts. The use of natural resources is an alternative for the discovery of new therapeutic agents. The active compounds derived from plants could be used as substitutes of pharmaceutical products for the control of diseases caused by microorganisms. In this study, the antibacterial potential of the hydroalcoholic extract of the fruit leaves was evaluated in gram-positive bacteria Staphylococcus aureus (ATCC 25923) and Bacillus subtilis (ATCC 6633) and gram-negative Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853). Theobroma cacao leaves were used. Cocos nucífera, Musa paradisiaca and Coffea sp. The hydroalcoholic extract was prepared by the maceration method. A phytochemical analysis was performed on the extracts to identify secondary metabolites. A total of 48 Mueller-Hinton agar plates with 1 mL of bacterial inoculum were prepared in each plate, standardized to 0.5 McFarland; the hydroalcoholic extract was added through the diffusion method,

making five holes of 5 mm each (four with concentrations and one with distilled water as a control group), the plates were incubated for 24 h at 36 °C. The halo of Inhibition was measured in mm with a Digital Vernier Caliper. The results obtained for gram-negative bacteria, antibacterial potential was observed only in Pseudomonas aeruginosa in all its concentrations, but no activity was seen in the hydroalcoholic extract of Coffea sp; for the gram positive bacteria Baccillus subtilis and Staphylococcus aureus, its antibacterial potential was demonstrated in the extracts of Cocos nucifera, Musa paradisiaca in all its concentrations, only antibacterial potential was identified in Staphylococcus aureus by Coffea sp extract; However, it should be noted that there was no reaction in E. coli." As taken from Sandoval A et al. 2020. Chemical Engineering Transactions 79, 319-324. Available at https://www.cetjournal.it/index.php/cet/article/view/CET2079054

"Cocoa pod husk (CPH) which is a waste of cocoa plantation contains phenolic compounds which can be used as antibacterial agents. Phenolic compounds in CPH include phenolic acids, flavonoids and flavones. The aim of this study was to analyze the antibacterial activity of CPH extract whis was extracted by using Microwave-Assisted Extraction (MAE) method. Extraction using 96% ethanol solvent with a ratio of 1:4, 1:6 and 1:8 (w/v) for 2, 3 and 4 minutes, respectively. The extraction results with the highest total phenolic content were tested their antibacterial activity using a disk diffusion method at an extract concentration of 5, 7.5, 10 mg/mL and 15 mg/mL with three replications, respectively. The highest total phenolic content of 453 mg GAE/g dry extract was obtained from MAE treatment with a solvent ratio of 1:4 (w/v) for 4 minutes. The results of the antibacterial activity of extracts against Escherichia coli showed that inhibitory zones had formed at a concentration of 5 mg/mL. The width of the inhibition zone increases as the concentration of extract increases." As taken from Diniardi EM et al. 2020. IOP Conference Series: Earth and Environmental Science 475, 012006. Available at https://iopscience.iop.org/article/10.1088/1755-1315/475/1/012006/pdf

"Background and Objectives. While Theobroma cacao L has long been utilized in the food, cosmetic, and pharmaceutical industries, it was also found to possess antibacterial activity. The beans comprise 10% of the fruit, while the remaining 90%, consisting of pods, is considered waste. It was reported that the pods possess antibacterial activity, and if utilized for this purpose, T. cacao pods will no longer be considered as waste. The aim of this study was to evaluate the antibacterial activity of the cream formulated from the aqueous extract of T. cacao L pods. Methods. The milled T. cacao pods were extracted using distilled water at 4°C for 24 hours. The crude extract was subjected to liquid-liquid partitioning using hexane, ethyl acetate, and n-butanol. Phytochemical screening was performed to identify the constituents present in the extract and its fractions. The extract and its fractions were tested against Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa. Determination of IC50 using 3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Reduction Assay was used to evaluate the antibacterial activity. The extract with the highest yield and the highest antibacterial activity were formulated into a cream. T. cacao cream was evaluated with quality control tests for creams and emulsions. Acute skin irritation test was performed on the T. cacao cream to assess skin irritability upon application on adult male albino rabbits. Results. T. cacao crude extract and its fractions possessed antibacterial activity. Among the fractions tested, n-butanol fraction had the highest activity against S. aureus, S. epidermidis, and P. aeruginosa. There was a significant difference between the fractions tested on the three bacterial strains ($p<0.05$). Although n-butanol fraction had the highest activity, the actual yield obtained after extraction was 0.95%. Since T. cacao aqueous extract also exhibited good antibacterial activity, it was chosen for the formulation study. There was no significant difference between the IC50 of the T. cacao crude extract and the IC50 of T. cacao cream, hence formulating it into a cream did not affect the antibacterial activity of the extract. Conclusion. T. cacao pod extract, as well as its fractions, possessed antibacterial activity against three bacterial strains. The T. cacao cream produced was a water-in-oil, non-irritant cream with antibacterial activity, and with acceptable physical attributes." As taken from Ladignon EAC and Bautista-Palacpac JS. 2020. Acta Medica Philippina 54(1), 22-30. Available at https://actamedicaphilippina.upm.edu.ph/index.php/acta/article/view/1090/964

"Theobroma cacao provides precious products such as polyphenol-rich beans that are useful for nutraceutical purposes. The geographical area may influence the chemical composition of raw cocoa beans in terms of the polyphenols and biological qualities of the products. This work aimed to investigate the biological properties and the chemical composition of two different samples of Criollo var. cocoa raw beans coming from two areas (Indonesia; Peru). Beans underwent biphasic extraction obtaining lipophilic and hydroalcoholic extracts. The extracts were tested for antiradical, antimutagenic, and antigenotoxic effects. Cell viability inhibition toward breast, gastric/esophageal colorectal adenocarcinoma, and hepatoblastoma human cell lines was evaluated. Extracts were chemically investigated through UV-Vis spectroscopy and ultra-high-pressure liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-ESI-QqTOF MS/MS). Results showed that the Indonesian bean hydroalcoholic extracts were able to scavenge 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) cation radical better than the Peruvian hydroalcoholic extracts (ECs50: 72.63 vs. 322.20 µg/mL). Extracts showed antimutagenic and antigenotoxic activity. The viability inhibitory effect on breast and hepatic cancer cells was reached only for the Indonesian hydroalcoholic extracts at hundreds of µg/mL. Phenylpropenoyl-L-amino acids, hydroxycinnamoyl aminoacids conjugates, and procyanidin compounds were found mainly in the hydroalcoholic extracts, whereas fatty acids and lyso-phospholipids were found mainly in lipophilic fractions. Fatty acid and (epi)catechins appeared to be affected by different environmental conditions of the geographical areas." As taken from Lavorgna M et al. 2021. Foods 10(3), 571. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33803449/

“Noncommunicable diseases, the leading cause of mortality around the world, are responsible for approximately 75% of premature adult deaths (ages 30-69). To tackle this issue, a healthy diet based on functional foods, including cocoa and its derivatives, has been increasingly promoted. The polyphenols present in cocoa have been of interest due to their antioxidant potential and their possible protective role in the context of noncommunicable diseases, such as diabetes and cardiovascular conditions. However, during cocoa postharvest and industrialization, the concentration of these bioactive compounds is reduced, possibly affecting their health-promoting properties. Therefore, this paper reviews in the literature in this field to find the total polyphenol content in cocoa during the postharvest and industrialization processes in order to define concentration ranges as a reference point for future research. In addition, it discusses in vitro and in vivo studies into the biological antioxidant potential of cocoa and its derivatives. This review covers publications in indexed databases from 2010 to 2020, their data were processed and presented here using box plots. As a result, we identified the concentration ranges of polyphenols depending on the type of matrix, treatment and country, as well as their relationship with the main bioactive compounds present in cocoa that are associated with their possible antioxidant biological potential and health-related benefits.”

Gil M et al. (2021) Traceability of polyphenols in cocoa during the postharvest and industrialization processes and their biological antioxidant potential.

5.6. Carcinogenicity

Species	**Test conditions**	**Evidence of carcinogenicity**	**Reference**
Human (211 cocoa consumers)	Cocoa consumption was treated as a continuous variable and the Odds Ratio for developing bladder cancer was calculated for consumption of (a) 1 serving/day; (b) total of 10 serving-years (equivalent to 1 serving/day for 10 years)	None OR for 1 serving/day: 0.77 (men), 0.92 (women) OR for 10 serving-years: 0.95 (men), 0.89 (women)	Risch et al., 1988

Human	A limited study examining trends in bladder cancer mortality and cocoa consumption in Italy between 1950-81. Breast cancer mortality rose slowly and continuously throughout this period. Cocoa intake increased between 1950-66, and thereafter decreased between 1966-81.	None This study is not very informative. Most cancers take years to develop and this study did not consider a lag pattern or any latency period.	Pannelli et al., 1989
Human (2 males and 2 females)	A study of 382 Danish patients suffering from sinonasal cancer, investigating possible occupational links. 2 men and 2 women had worked in the cocoa, chocolate and sugar confectionery manufacturing industry. In comparison, only 0.4 (men) and 02 (women) cases would have been expected statistically.	No convincing evidence. The incidence was higher in men and women (and statistically significant in the latter) but conclusions cannot be drawn due to the very small numbers, and the exposure to other chemical agents in addition to cocoa.	Olsen, 1988
Human	Breast cancer incidence figures for 23 countries were compared with estimated national average cocoa consumption (based on “food availability data”, which was not well explained). A univariate analysis was performed to calculate the correlation coefficient between age-adjusted breast cancer risk and estimated cocoa consumption.	No convincing evidence. There was some correlation between national risk and consumption ($R=0.57$; $p=0.0026$). However, no multivariate analysis was performed for cocoa. Further analysis indicated fat consumption as the major factor. Cocoa consumption might simply be a marker of the actual causative factors. Correlation studies of this type do not compare intakes of healthy subjects and cancer patients.	Kaizer et al., 1989
Groups of 90 rats/sex	Rats were fed diets containing 0, 1.5, 3.5 or 5% cocoa powder continuously for 104 weeks, and a comprehensive range of tissues and organs were examined microscopically (Treated rats were derived from the F3b phase of a three-generation reproductive toxicity study in which rats were continuously fed diets containing 0, 1.5, 3.5 or 5% cocoa powder throughout maturation, mating, pregnancy and lactation.) FDA Guideline was followed	None	Tarka et al. 1991

Rat (females, group size not specified in brief report)	Mammary tumour promotion study, only reported as an abstract. Rats were initiated with a known mammary carcinogen (7,12-dimethylbenz(a)anthracene) and, beginning 2 days later, were fed one of several high-fat diets, including one containing 20% cocoa butter, for 15 weeks. Incidences of mammary tumours were recorded.		

Flavanols and procyanidins of cocoa and chocolate inhibit growth and polyamine biosynthesis of human colonic cancer cells (Abstract). The effects of cocoa powder and extracts with different amounts of flavanols and related procyanidin oligomers were investigated on the growth of Caco-2 cells. Treatment of the cells with 50 microg/ml of procyanidin-enriched (PE) extracts caused a 70% growth inhibition with a blockade of the cell cycle at the G2/M phase. PE extracts caused a significant decrease of ornithine decarboxylase and S-adenosylmethionine decarboxylase activities, two key enzymes of polyamine biosynthesis. This led to a decrease in the intracellular pool of the polyamines. These observations indicate that polyamine metabolism might be an important target in the anti-proliferative effects of cocoa polyphenols. As taken from Carnésecchi S et al. Cancer Lett. 2002 Jan 25; 175(2), 147-55. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/11741742

“Cocoa is a rich source of bioactive compounds with potential chemopreventive ability but up to date its effectiveness in animal models of colon carcinogenesis has not been addressed. Herein, we investigated the in vivo effect of a cocoa-rich diet in the prevention of azoxymethane (AOM)-induced colon cancer and the mechanisms involved. Our results showed that cocoa feeding significantly reduced AOM-induced colonic aberrant crypt foci formation and crypt multiplicity. Oxidative imbalance in colon tissues seems to be prevented by cocoa as indicated by reduced oxidation markers levels and increased enzymatic and non-enzymatic endogenous defences. Cocoa-rich diet also exhibited antiproliferative effects by decreasing the levels of extracellular regulated kinases, protein kinase B and cyclin D1 together with pro-apoptotic effects evidenced by reduced Bcl-x(L) levels and increased Bax levels and caspase-3 activity. Our findings provide the first in vivo evidence that a cocoa-rich diet may inhibit the early stage of colon carcinogenesis probably by preventing oxidative stress and cell proliferation and by inducing apoptosis” (Rodriguez-Ramiro et al., 2011).

“Numerous lines of evidence support a relationship between intestinal inflammation and cancer. Therefore, much attention has recently been focused on the identification of natural compounds with anti-inflammatory activities as a strategy to suppress the early stages of colorectal cancer. Because cocoa is a rich source of bioactive compounds, the present study investigated its anti-inflammatory properties in a rat model of azoxymethane (AOM)-induced colon carcinogenesis and in TNF-α-stimulated Caco-2 cells. A total of forty male rats were fed with control or cocoa-enriched diets (12 %) during 8 weeks and injected with saline or AOM (20 mg/kg body weight) during the third and fourth week (n 10 rats/group). At the end of the experiment, colon samples were evaluated for markers of inflammation. The anti-inflammatory activity of a cocoa polyphenolic extract (10 μg/ml) was examined in TNF-α-stimulated Caco-2 cells, an in vitro model of experimentally induced intestinal inflammation. The signalling pathways involved, including NF-κB and the mitogen-activated protein kinase family such as c-Jun NH2-terminal kinases (JNK), extracellular signal-regulated kinases and p38, were also evaluated. The results show that the cocoa-rich diet decreases the nuclear levels of NF-κB and the expression of pro-inflammatory enzymes such as cyclo-oxygenase-2 and inducible NO synthase induced by AOM in the colon. Additionally, the experiments in Caco-2 cells confirm that cocoa polyphenols effectively down-

regulate the levels of inflammatory markers induced by TNF-α by inhibiting NF-κB translocation and JNK phosphorylation. We conclude that cocoa polyphenols suppress inflammation-related colon carcinogenesis and could be promising in the dietary prevention of intestinal inflammation and related cancer development." As taken from Rodriguez-Ramiro I et al. 2013. Br. J. Nutr. 110(2), 206-15. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23186731?dopt=AbstractPlus

"OBJECTIVE: To evaluate the chemopreventive potential of phenolic compounds - potassium apigenin, cocoa, catechins, eriocitrin and rosmarinic acid in oral carcinogenesis induced in hamsters by means of the topical application of 7,12-dimethylbenz(a)anthracene(DMBA). STUDY DESIGN: An experimental study at the University of Murcia. METHODS: 50 male Syrian hamsters (Mesocricetus auratus) were divided into five groups of ten: Group I (control group): 0.5% DMBA; Group II: 0.5% DMBA+1.1mg/15ml potassium apigenin; Group III: 05% DMBA+2.5mg/15ml cocoacatechins; Group IV: 0.5% DMBA+6mg/15ml eriocitrin; Group V: 0.5% DMBA+1.3mg/15ml rosmarinic acid. The flavonoids were administered orally. All the animals were sacrificed after 12 weeks. Macroscopic, microscopic and immunohistochemical (PCNA and p53) analyses of the lesions were performed. RESULTS: All the groups treated with phenolic compounds showed lower incidences of tumour, greater differentiation and lower scores in the tumour invasion front grading system in comparison with the control group. Potassium apigenin and rosmarinic acid achieved the best results, the former considerably reduced the carcinoma tumour volumes developed and both significantly reduced the intensity and aggression of the tumours. Immunoexpression of PCNA and p53 were significantly altered during DMBA-induced oral carcinogenesis. CONCLUSIONS: Animals treated with phenolic compounds, particularly potassium apigenin and rosmarinic acid, showed a lower incidence of tumours." As taken fromBaldasquin-Caceres B et al. 2014. Arch. Oral. Biol. 59(10), 1101-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25033381

"Recent reports on cocoa are appealing in that a food commonly consumed for pure pleasure might also bring tangible benefits for human health. Cocoa consumption is correlated with reduced health risks of cardiovascular diseases, hypertension, atherosclerosis, and cancer, and the health-promoting effects of cocoa are mediated by cocoa-driven phytochemicals. Cocoa is rich in procyanidins, theobromine, (-)-epicatechin, catechins, and caffeine. Among the phytochemicals present in consumed cocoa, theobromine is most available in human plasma, followed by caffeine, (-)-epicatechin, catechin, and procyanidins. It has been reported that cocoa phytochemicals specifically modulate or interact with specific molecular targets linked to the pathogenesis of chronic human diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, obesity, diabetes, and skin aging. This review summarizes comprehensive recent findings on the beneficial actions of cocoa-driven phytochemicals in molecular mechanisms of human health." As taken from Kim J et al. 2014. Crit. Rev. Food Sci. Nutr. 54(11), 1458-72. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24580540?dopt=AbstractPlus

"Abstract: This research aims to determine the effect of topical cocoa extract application through exspression of Bcl-2 and MDA on albino mice skin that induced by DMBA-TPA. The research was conducted at Animal Laboratory for application of DMBA, MDA-TPA and cocoa extract and Biomolecular Laboratory of Medical Faculty of Hasanuddin University Makassar as the place of ELISA examination. The method used was animal experimental with pure experimental design to know the role of topical cocoa extract towards the skin of mice which has been given DMBA-TPA three times a week in 12 weeks. The research unit was the skin of the back side of albino mice which have been excised and divided into 5 groups of treatment. The results indicate that extract of concentration cocoa 400 ppm ($p<0.05$) which is applied every day has the highest effects decreased of Bcl-2 and MDA expression after being inducted by DMBA-TPA three times a week." As taken from Djawad K et al. 2017. American Journal of Clinical and Experimental Medicine 5(3), 97-101. Available at http://article.ajcem.net/pdf/10.11648.j.ajcem.20170503.16.pdf

“Abstract: Skin cancer incidence is directly proportional to malignancies in other organs and represents a health problem. This study aims to evaluate the protective effect of topical cocoa extract application on PCNA expression in mice receiving DMBA exposure. This study was conducted in Animal Laboratory for DMBA administration and cocoa extract application, Bimolecular Laboratory of Hasanuddin University as the location of ELISA evaluation. The study used animal experimental method with pure experimental design to find out the role of topical cocoa seeds extract on the skin of mice receiving DMBA application in one week. Twenty mice were divided into 4 treatment groups: first group was control without skin protector, second group with ethanol application, third group with topical cocoa extract application 400 ppm, and fourth group was treated with 800 ppm topical cocoa with 100 ug DMBA exposure three times per week. After one week the treatment was terminated and skin biopsy was excised for PCNA expression evaluation using ELISA. Study findings indicate that 400 ppm and 800 ppm topical cocoa extract had a protective effect on PCNA expression with 400 ppm as the most effective dose.” As taken from Kirana J et al. 2017. American Journal of Clinical and Experimental Medicine 5(4), 102-107. Available at http://article.ajcem.net/pdf/10.11648.j.ajcem.20170504.11.pdf

"Obesity is associated with increased risk of many types of cancer and can be induced by various high-fat diets (HFD) from different fat sources. It remains unknown whether fatty acid composition in different HFD influences obesity-associated tumor development. Here we report that consumption of either a cocoa butter or fish oil HFD induced similar obesity in mouse models. While obesity induced by the cocoa butter HFD was associated with accelerated mammary tumor growth, consumption of the fish oil HFD uncoupled obesity from increased mammary tumor growth and exhibited a decrease in protumor macrophages. Compared with fatty acid (FA) components in both HFDs, n-3 FA rich in the fish oil HFD induced significant production of reactive oxygen species (ROS) and macrophage death. Moreover, A-FABP expression in the protumor macrophages facilitated intracellular transportation of n-3 FA and oxidation of mitochondrial FA. A-FABP deficiency diminished n-3 FA-mediated ROS production and macrophage death in vitro and in vivo. Together, our results demonstrate a novel mechanism by which n-3 FA induce ROS-mediated protumor macrophage death in an A-FABP-dependent manner. SIGNIFICANCE: This study provides mechanistic insight into dietary supplementation with fish oil for breast cancer prevention and advances a new concept that not all HFDs leading to obesity are tumorigenic." As taken from Liu L et al. 2020. Cancer Res. 80(12), 2564-2574. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32213543/

5.7. Irritation/immunotoxicity

Ramiro et al., (2005). Cocoa was reported to down-modulate T lymphocyte activation and the acquired immune response. The researchers suggested that ‘this fact could be important in some states of the immune system hyperactivity such as autoimmune or chronic inflammatory diseases’ (Ramiro et al., 2005)

“Cococa butter has been reported to have skin allergenic and comedogenic (forming blackheads) properties in animals.”

As taken from Encyclopaedia of common natural ingredients used in food, drugs, and cosmetics, 2nd edition, 2003, A. Yeung & S. Foster, pp. 181-185.

Comedogenicity in rabbit: some cosmetic ingredients/vehicles (Abstract). The rabbit external ear canal was used to define which chemicals caused comedone formation on topical application. Some of the tested ingredients are currently used in topically applied formulations. Certain raw materials have been shown to produce follicular hyperkeratosis in the rabbit ear assay. This study quantifies comedogenic potential of cosmetic materials, including: isopropyl palmitate, isopropyl myristate, butyl stearate, isopropyl isostearate, decyl oleate, isostearyl neopentanoate, isocetyl stearate, myristle myristate, cocoa butter, cetyl alcohol, paraffin, stearyl alcohol sodium lauryl sulfate (SLS), and petrolatum. The first nine were deemed positive. Factors aiding clinical

relevance are listed. As taken from Nguyen SH et al. Cutan Ocul Toxicol. 2007; 26(4), 287-92. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/18058303

Irritative effects of suppository bases on the rectal membranes in rabbits (Abstract). The irritative effects of 6 suppository bases and the interactions between the bases and aspirin (I) or indomethacin (II) on the rectal mucous membranes of rabbits were examined. Polyethylene glycol 1000 (III) and polyoxyl 40 stearate (IV) caused inflammation of the rectal membranes. However, the other bases, cocoa butter (cacao oil), Witepsol H-15, polysorbate 61 (Tween 61) and propylene glycol (V), did not cause irritation. The irritative effect induced by the solid type of III was more severe than that induced by the melting type, while the effect of IV was shown more severely through the melting type rather than the solid type. These results suggest that these bases differ qualitatively from one another in irritative effects. On the other hand, the irritative effect of I was reduced by using the above bases except for V. The irritative effect of II was enhanced by using IV as a base. It was concluded that in selecting a suppository base, the suitability for the combined use should be considered.

As taken from Satoh S et al. Arch. Pract. Pharm. (Yakuzaigaku); VOL 45 ISS Dec 20 1985, P298-303.

Sensitization:

Human - inhalation

Occupational asthma was reported in a worker exposed by inhalation to cocoa powder and confirmed by an immediate respiratory reaction to an inhalation challenge with nebulized cocoa powder extract (Malo et al., 1997).

In a group of 40 cocoa-processing workers, two had occupational asthma. Up to 30% of the group had respiratory symptoms including reduced ventilatory capacity. Decreased lung function was observed in bronchial provocation tests with cocoa dust extract. Measurement showed that the cocoa dust concentration in the workplace ranged from 2-16 mg/m3 (mean 9.1 mg/m3) and the respirable fraction from 0.9-3.5 mg/m3 (mean 2.1 mg/m3) (Zuskin et al. 1998).

Human - oral

A child was reported to have an IgE-mediated food allergy to cocoa (Crespo et al., 1995).

Of 20 patients reporting allergy to chocolate, only one gave a positive response to chocolate in a double-blind placebo-controlled food challenge and also in a skin prick test; two further patients had a positive skin prick test (Drelich et al., 1993). Five of 91 children with eczema reported it to be associated with consumption of chocolate; a double-blind food challenge performed on 4 of these individuals suggested chocolate to be the cause (Sloper et al., 1991). An oral challenge with chocolate on three separate occasions caused "flares of dermatitis" in an eczema patient (Veien et al., 1987). A review describes a number of studies reporting oral and dermal sensitization by cocoa or chocolate (Hefle et al., 1996).

“Ninety-two exclusively breast-fed Japanese infants with atopic dermatitis were studied to see whether tree nut-related foods (chocolate and coffee) and fermented foods (cheese, yogurt, bread, soy sauce, miso soup and fermented soy beans) eaten by their mothers affected their skin condition. Of the 92 infants, 67 (73%) showed improvement of skin lesions when their mothers avoided these foods and showed aggravation of skin lesions when these foods were reintroduced. The predominant offending foods were chocolate, yogurt, soy sauce and miso soup. A long-term maternal exclusion of the trigger foods brought about progressive improvement of skin lesions in the majority of the infants. These findings suggest that tree nut-related foods and fermented foods are important offending foods of atopic dermatitis in breast-fed infants” (Uenishi et al., 2011).

“Epidemiological and immunological studies suggest that maternal diet during pregnancy might affect the development of allergic diseases in the offspring. The authors set out to study the effect of maternal food consumption during pregnancy on the emergence of the International Study of

Asthma and Allergies in Childhood (ISAAC)-based allergic outcomes: asthma, allergic rhinitis, and wheeze by the 5 yr of age. METHODS: Data from 2441 children at 5 yr of age were analyzed within the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Nutrition Study, a population-based birth cohort study. Maternal diet was assessed with a validated food frequency questionnaire. RESULTS: In multiple regression models adjusted for known confounders, low maternal consumption of leafy vegetables (adjusted odds ratio [aOR]: 1.55; 95% CI: 1.21, 1.98), malaceous fruits (aOR: 1.45; 95% CI: 1.15, 1.84), and chocolate (aOR: 1.36; 95% CI: 1.09, 1.70) were positively associated with the risk of wheeze in children..... No associations were observed between maternal food consumption and asthma. CONCLUSIONS: Development of allergic diseases in preschool children may be influenced by intrauterine exposure to maternal diet". As taken from Erkkola M et al. 2012. Pediatric. Allerg. Immunol. 23, 186-194. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22432883?dopt=AbstractPlus.

Human - dermal

A positive reaction to a patch test with cocoa butter was reported in an eyelid dermatitis patient [number of patients tested is unclear in brief report; possibly up to 12] (Guin, 2004).

Patch testing with dark chocolate, cocoa liquor and drinking chocolate gave positive results in a patient who reported swelling and ulceration of the lips after eating chocolate (Taibjee et al., 2004).

Twelve of 553 atopic patients had a positive skin prick test with cocoa (Niinimaki & Hannuksela, 1981).

"We analysed the effect of (-)-epicatechin and cocoa extract on the activation of a lymphoid cell line. Particularly the expression of IL-2 receptor alpha (IL-2Ralpha or CD25) and, the secretion of IL-2 and IL-4 were established after flavonoid treatment. Two media culture conditions (1 and 10 % of fetal calf serum supplementation) and the different moments of flavonoid addition (simultaneously or 2 h before cell-activation) were compared. IL-2Ralpha (CD25) expression on activated cells was significantly reduced by epicatechin and cocoa extract in a dose-dependent manner, achieving the highest inhibition of about 50 % when flavonoids were added 2 h before stimulation. IL-2 secretion was also inhibited by the presence of both epicatechin and cocoa extract, displaying 60 and 75 % of inhibition, respectively. Cocoa flavonoids were also able to enhance 3-4.5-fold IL-4 release. In summary, cocoa extract down-modulated T lymphocyte activation and therefore the acquired immune response. This fact could be important in some states of the immune system hyperactivity such as autoimmune or chronic inflammatory diseases." As taken from Ramiro E et al. Br J Nutr. 2005 Jun; 93(6), 859-66. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/16022755?dopt=AbstractPlus

"In the present study, we report the effects of a cocoa extract on the secretion and RNA expression of various proinflammatory mediators by macrophages. Monocyte chemoattractant protein 1 and tumor necrosis factor alpha (TNFalpha) were significantly and dose-dependently diminished by cocoa extract, and this effect was higher than that produced by equivalent concentrations of epicatechin but was lower than that produced by isoquercitrin. Interestingly, cocoa extract added prior to cell activation resulted in a significantly greater inhibition of TNFalpha secretion. Both cocoa extract and epicatechin decreased TNFalpha, interleukin (IL) 1alpha, and IL-6 mRNA expression, suggesting that their inhibitory effect on cytokine secretion is produced, in part, at the transcriptional level. Cocoa extract also significantly decreased NO secretion in a dose-dependent manner and with a greater effect than that produced by epicatechin. In conclusion, our study shows that cocoa flavonoids not only inhibit NO release from macrophages but also down-regulate inflammatory cytokines and chemokines." As taken from Ramiro E et al. J Agric Food Chem. 2005 Nov 2; 53(22), 8506-11. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/16248545?dopt=AbstractPlus

"Previous studies have shown that rat intestinal immunoglobulin A (IgA) concentration and lymphocyte composition of the intestinal immune system were influenced by a highly enriched cocoa diet. The aim of this study was to dissect the mechanisms by which a long-term high cocoa

intake was capable of modifying gut secretory IgA in Wistar rats. After 7 weeks of nutritional intervention, Peyer's patches, mesenteric lymph nodes and the small intestine were excised for gene expression assessment of IgA, transforming growth factor β, C-C chemokine receptor-9 (CCR9), interleukin (IL)-6, CD40, retinoic acid receptors (RARα and RARβ), C-C chemokine ligand (CCL)-25 and CCL28 chemokines, polymeric immunoglobulin receptor and toll-like receptors (TLR) expression by real-time polymerase chain reaction. As in previous studies, secretory IgA concentration decreased in intestinal wash and fecal samples after cocoa intake. Results from the gene expression showed that cocoa intake reduced IgA and IL-6 in Peyer's patches and mesenteric lymph nodes, whereas in small intestine, cocoa decreased IgA, CCR9, CCL28, RARα and RARβ. Moreover, cocoa-fed animals presented an altered TLR expression pattern in the three compartments studied. In conclusion, a high-cocoa diet down-regulated cytokines such as IL-6, which is required for the activation of B cells to become IgA-secreting cells, chemokines and chemokine receptors, such as CCL28 and CCR9 together with RARα and RARβ, which are involved in the gut homing of IgA-secreting cells. Moreover, cocoa modified the cross-talk between microbiota and intestinal cells as was detected by an altered TLR pattern. These overall effects in the intestine may explain the intestinal IgA down-regulatory effect after the consumption of a long-term cocoa-enriched diet" (Pérez-Berezo et al., 2011).

"This study investigated the effects of cocoa butter and sunflower oil alone and in combination on performance, some biochemical parameters, immunoglobulin, and antioxidant vitamin status in Wistar rats. Forty-eight male rats were assigned to four groups, consisting of 12 rats with 3 replicates. Control received balanced rat diet without oil, cocoa butter group received 3.5% cocoa butter, sunflower oil group received 3.5% sunflower oil, the last group received 1.75% sunflower oil + 1.75% cocoa butter supplementation in the rat diet for 8 weeks. ..... The serum creatinine level was decreased in cocoa butter group compared to control. Triglyceride and VLDL cholesterol levels were decreased in only sunflower oil and only cocoa butter groups as compared to control. The level of Ig M was statistically lower in cocoa butter and cocoa butter + sunflower oil groups than in control and sunflower oil groups. There were no statistically important difference in vitamin concentrations among trial groups. It was concluded that the supplementation of cocoa butter in diet decreased Ig M level, while the supplementation of cocoa butter and sunflower oil alone decreased the triglyceride and VLDL cholesterol levels." As taken from Yildirim E et al. 2014. Biomed. Res. Int. 2014, 606575. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25136602?dopt=AbstractPlus

"Unsweetened natural cocoa powder is enriched with nutraceutical abundance of anti-asthmatic compounds theobromine and theophylline. Cocoa powder, which is prepared after removal of the cocoa butter, contains about 1.9% theobromine and 0.21% caffeine. Anecdotal reports indicate that regular consumption of unsweetened natural cocoa powder (UNCP), a common practice in Ghana, West Africa, has the potential to reduce the tendency of asthmatic episodes. In the present paper we studied the effect of regular ingestion of aqueous extract of UNCP on hematological and histopathological changes that occur in ovalbumin (OVA)-sensitized guinea pigs. OVA-sensitized guinea pigs were challenged with aerosolized OVA 1 hour after ingestion of 300 mg/kg (low dose) or 600 mg/kg (high dose) of UNCP for 35 consecutive days. Histopathological and haematological changes in the OVA-sensitized guinea pigs were evaluated. Both negative and positive controls with distilled water and prednisolone, respectively, were used. OVA-sensitized guinea pigs demonstrated concentration-independent reduction in immune response to aerosolized OVA. There were no histo-architectural changes in the bronchiolar smooth muscles of the treated groups. Unsweetened natural cocoa powder has potential anti-asthmatic properties when administered orally at the doses tested." As taken from Awortwe C et al. 2014. Int. J. Immunopathol. Pharmacol. 27(2), 203-12. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25004832?dopt=AbstractPlus

"A diet containing 10 % cocoa, a rich source of polyphenols and fibre, is able to modify intestinal immune status as well as microbiota composition. The present study was aimed at investigating whether cocoa flavonoid content is uniquely responsible for these modulatory effects of cocoa, and

to establish whether these effects depend on the rat strain. To this end, 3-week-old Wistar and Brown Norway rats were fed, for 4 weeks, either a standard diet or the following three isoenergetic diets containing increasing proportions of cocoa flavonoids from different sources: one with 0·2 % polyphenols (from conventional defatted cocoa), and two others with 0·4 and 0·8 % polyphenols (from non-fermented cocoa, very rich in polyphenols). Serum Ig concentrations, faecal IgA levels, microbiota composition and IgA-coating bacterial proportion were evaluated at the beginning and at the end of the study. After the nutritional intervention, the composition of lymphocytes in Peyer's patches and mesenteric lymph nodes was evaluated. In some respects, the Wistar strain was more sensitive to the impact of the cocoa diets than the Brown Norway strain. After 4 weeks of dietary intervention, similar modulatory effects of the diets containing 0·2 and 0·8 % polyphenols on mucosal IgA levels and microbiota composition were found, although the 0·2 % diet, with a higher proportion of theobromine and fibre, had more impact, suggesting that polyphenols are not the only components involved in such effects." As taken from Massot-Cladera M et al. 2014. Br. J. Nutr. 112(12), 1944-54. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25345541

"Cocoa powder, a rich source of polyphenols, has shown immunomodulatory properties in both the intestinal and systemic immune compartments of rats. The aim of the current study was to establish the effect of a cocoa diet in a rat oral sensitization model and also to gain insight into the mesenteric lymph nodes (MLN) activities induced by this diet. To achieve this, three-week-old Lewis rats were fed either a standard diet or a diet with 10% cocoa and were orally sensitized with ovalbumin (OVA) and with cholera toxin as a mucosal adjuvant. Specific antibodies were quantified, and lymphocyte composition, gene expression, and cytokine release were established in MLN. The development of anti-OVA antibodies was almost totally prevented in cocoa-fed rats. In addition, this diet increased the proportion of TCRγδ+ and CD103+CD8+ cells and decreased the proportion of CD62L+CD4+ and CD62L+CD8+ cells in MLN, whereas it upregulated the gene expression of OX40L, CD11c, and IL-1β and downregulated the gene expression of IL-17α. In conclusion, the cocoa diet induced tolerance in an oral sensitization model accompanied by changes in MLN that could contribute to this effect, suggesting its potential implication in the prevention of food allergies." As taken from Camps-Bossacoma M et al. 2016. Nutrients 8(4), 242. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27120615

"Previous studies have attributed to the cocoa powder the capacity to attenuate the immune response in a rat oral sensitization model. To gain a better understanding of cocoa-induced mechanisms at small intestinal level, 3-week-old female Lewis rats were fed either a standard diet or a diet containing 10% cocoa for 4 weeks with or without concomitant oral sensitization with ovalbumin (OVA). Thereafter, we evaluated the lymphocyte composition of the Peyer's patches (PPL), small intestine epithelium (IEL) and lamina propria (LPL). Likewise, gene expression of several immune molecules was quantified in the small intestine. Moreover, histological samples were used to evaluate the proportion of goblet cells, IgA+ cells and granzyme+cells as well. In cocoa-fed animals, we identified a five-time reduction in the percentage of IgA+ cells in intestinal tissue together with a decreased proportion of TLR4+ IEL. Analyzing the lymphocyte composition, almost a double proportion of TCRγδ+cells and an increase of NK cell percentage in PPL and IEL were found. In addition, a rise in CD25+, CD103+ and CD62L- cell proportions was observed in CD4+ PPL from cocoa-fed animals, along with a decrease in gene expression of CD11b, CD11c and IL-10. These results suggest that changes in PPL and IEL composition and in the gene expression induced by the cocoa diet could be involved, among other mechanisms, on its tolerogenic effect." As taken from Camps-Bossacoma M et al. 2017. J. Nutr. Biochem. 42, 182-193. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28189917

"Many flavours and fragrances are known allergens. Their selection and inclusion levels in e-liquids must therefore be guided by toxicological principles, taking into account the exposure pattern and inhalation route of exposure. For contact sensitisation, a general, agreed quantitative risk assessment approach to prevent dermal sensitisation exists. Here we propose exposure parameters and safety factors to apply this approach to e-liquid ingredients. Additionally, as a risk management approach for pre-sensitised individuals, we derive a threshold of 0.1% for indicating

the presence of a contact sensitiser in eliquid. Risk assessment for respiratory sensitisation is not well established. Occupational exposure limits that protect against respiratory allergy are generally very low. Cocoa shell extract is used as a case study to discuss the issues. A tolerable exposure level is derived and estimates of consumer exposure are presented, leading to the practical risk management approach of excluding respiratory sensitisers as eliquid ingredients. Related to this, if natural extracts are used as flavourings in e-liquids, we recommend only protein-free versions are used. Additionally, we recommend the presence of any potential food allergens should be noted on the product information." As taken from Costigan S and Lopez-Belmonte J. 2017. Regul. Toxicol. Pharmacol. 87, 1-8. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28389323

"Polyphenols-rich cocoa has many beneficial effects on human health, such as anti-inflammatory effects. Macrophages function as control switches of the immune system, maintaining the balance between pro- and anti-inflammatory activities. We investigated the hypothesis that cocoa polyphenol extract may affect macrophage proinflammatory phenotype M1 by favoring an alternative M2 anti-inflammatory state on macrophages deriving from THP-1 cells. Chemical composition, total phenolic content, and antioxidant capacity of cocoa polyphenols extracted from roasted cocoa beans were determined. THP-1 cells were activated with both lipopolysaccharides and interferon-γ for M1 or with IL-4 for M2 switch, and specific cytokines were quantified. Cellular metabolism, through mitochondrial oxygen consumption, and ATP levels were evaluated. Here, we will show that cocoa polyphenolic extract attenuated in vitro inflammation decreasing M1 macrophage response as demonstrated by a significantly lowered secretion of proinflammatory cytokines. Moreover, treatment of M1 macrophages with cocoa polyphenols influences macrophage metabolism by promoting oxidative pathways, thus leading to a significant increase in O2 consumption by mitochondrial complexes as well as a higher production of ATP through oxidative phosphorylation. In conclusion, cocoa polyphenolic extract suppresses inflammation mediated by M1 phenotype and influences macrophage metabolism by promoting oxidative pathways and M2 polarization of active macrophages." As taken from Dugo L et al. 2017. Oxid. Med. Cell Longev. 2017, 6293740. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28744339

Theobroma cacao L., stem bark, 70% ethanol extract, ethyl acetate extract of aqueous fraction (no CAS RN given):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	187.5 mg/kg/3D (intermittent)	Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 222,239,2018

As taken from RTECS, 2019a

Theobroma cacao L., stem bark, 70% ethanol extract (no CAS RN given):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published	Oral	Rodent - rat	750 mg/kg/3D (intermittent)	Biochemical - Metabolism (Intermediary) -	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub.

toxic dose				effect on inflammation or mediation of inflammation	Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 222,239,2018

As taken from RTECS, 2019b

Dermal irritation and sensitization

Ingredient and concentration	Method	Results
50.1% Theobroma cacao (cocoa) seed butter in a lip balm	HRIPT with 150 mL test material, semioccluded	Not a dermal irritant or sensitizer

Burnett CL et al. (2017), Cosmetics Ingredient Review. CIR Supplement Manuscript. Safety Assessment of Plant-Derived Fatty Acid Oils.*5.8. All other relevant types of toxicity*

5.8. All other relevant types of toxicity

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing cocoa extract (8002-31-1, 84649-99-0) 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 cocoa extract (8002-31-1, 84649-99-0) 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	286	JTI KB Study Report(s)
In vitro cytotoxicity	286	JTI KB Study Report(s)

Inhibition of inflammatory mediators by polyphenolic plant extracts in human intestinal Caco-2 cells. (Abstract). The mitogen-activated protein kinases (MAPK) and nuclear factor kappaB (NF-kappaB) are involved in transduction cascades that play a key role in inflammatory response. We tested the ability of preselected natural polyphenolic extracts (grape seed, cocoa, sugar cane, oak, mangosteen and pomegranate) to modulate intestinal inflammation using human intestinal Caco-2 cells treated for 4h with these extracts and then stimulated by cytokines for 24 or 48h. The effect of polyphenolic extracts, at 50 micromol of gallic acid equivalent/l, was investigated on inflammation-related cellular events: (i) NF-kappaB activity (cells transfected with a NF-kappaB-luciferase construct), (ii) activation of Erk1/2 and JNK (western blotting), (iii) secretion of interleukin 8 (IL-8) (ELISA), (iv) secretion of prostaglandin (PG) E(2) (ELISA), (v) production of NO (Griess method). Results show that: (i) sugar cane, oak and pomegranate extracts inhibited NF-kappaB activity (from 1.6 to 1.9-fold) (P<0.001); (ii) pomegranate slightly inhibited Erk1/2 activation (1.3-fold) (P=0.008); (iii) oak and pomegranate decreased NO synthesis by 1.5-fold (P<0.001) and that of IL-8 by 10.3 and 6.7-fold respectively; (iv) pomegranate and cocoa decreased PGE(2) synthesis by 4.6 (P<0.0001) and 2.2-fold (P=0.001), respectively. We suggest that pomegranate extract could be particularly promising in dietary prevention of intestinal inflammation.

As taken from Romier-Crouzet B et al. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/19233242

Cocoa flavonoids up-regulate antioxidant enzyme activity via the ERK1/2 pathway to protect against oxidative stress-induced apoptosis in HepG2 cells (Abstract). Oxidative stress is widely recognized as an important mediator of apoptosis in liver cells and plays a pivotal role in the pathogenesis of several diseases. Cocoa flavonoids have shown a powerful antioxidant activity providing protection against oxidation and helping prevent oxidative stress-related diseases. However, the molecular mechanisms responsible for this protection are not fully understood. Thus,

in this study we investigated the protective effect of a cocoa polyphenolic extract (CPE) against tert-butyl hydroperoxide (t-BOOH)-induced apoptosis and the molecular mechanisms involved in this process. Incubation of HepG2 cells with t-BOOH induced apoptosis as evidenced by caspase-3 activation. This effect was accompanied by increased reactive oxygen species formation and by transient activation of the extracellular regulated kinases (ERKs) as well as sustained activation of the c-Jun N-terminal kinases (JNKs). On the contrary, pretreatment of HepG2 cells with CPE prevented apoptosis through the reduction of reactive oxygen species generation and the modulation of the apoptotic pathways activated by t-BOOH. CPE treatment also activated survival signaling proteins, such as protein kinase B (AKT) and ERKs, and increased the activities of two antioxidant enzymes, glutathione peroxidase (GPx) and glutathione reductase (GR). ERK's implication on GPx and GR induction and the protective effect of CPE against t-BOOH-induced oxidative stress and apoptosis were confirmed through experiments with selective inhibitors. These findings suggest that CPE is an effective inductor of GPx and GR activities via ERK activation and that this up-regulation seems to be required to attenuate t-BOOH-induced injury. As taken from Martín MA et al. 2010. The Journal of Nutritional Biochemistry 21(3), 196-205. ScienceDirect, 2011 available at http://www.sciencedirect.com/

“In diet-induced obesity, adipose tissue (AT) is in a chronic state of inflammation predisposing the development of metabolic syndrome. Cocoa (Theobroma cacao) is a polyphenol-rich food with putative anti-inflammatory activities. Here, we examined the impact and underlying mechanisms of action of cocoa on AT inflammation in high fat-fed mice. In the present study, male C57BL/6 J mice were fed a high fat diet (HF), a HF diet with 8% (w/w) unsweetened cocoa powder (HFC), or a low-fat diet (LF) for 18 weeks. Cocoa supplementation decreased AT mRNA levels of tumor necrosis factor-α, interleukin-6, inducible nitric oxide synthase, and EGF-like module-containing mucin-like hormone receptor-like 1 by 40-60% compared to HF group, and this was accompanied by decreased nuclear protein levels of nuclear factor-κB....In conclusion, the present study has shown for the first time that long-term cocoa supplementation can reduce AT inflammation in part by modulating eicosanoid metabolism and metabolic endotoxemia.” As taken from Gu Y et al. 2014a. J. Nutr. Biochem. 25(4), 439-45. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24561154

“PURPOSE: To investigate the effect of cocoa powder supplementation on obesity-related inflammation in high fat (HF)-fed obese mice. METHODS: Male C57BL/6J (n = 126) were fed with either low-fat (LF, 10 % kcal from fat) or HF (60 % kcal from fat) diet for 18 weeks. After 8 weeks, mice from HF group were randomized to HF diet or HF diet supplemented with 8 % cocoa powder (HF-HFC group) for 10 weeks. Blood and tissue samples were collected for biochemical analyses. RESULTS: Cocoa powder supplementation significantly reduced the rate of body weight gain (15.8 %) and increased fecal lipid content (55.2 %) compared to HF-fed control mice. Further, cocoa supplementation attenuated insulin resistance, as indicated by improved HOMA-IR, and reduced the severity of obesity-related fatty liver disease (decreased plasma alanine aminotransferase and liver triglyceride) compared to HF group. Cocoasupplementation also significantly decreased plasma levels of the pro-inflammatory mediators interleukin-6 (IL-6, 30.4 %), monocyte chemoattractant protein-1 (MCP-1, 25.2 %), and increased adiponectin (33.7 %) compared to HF-fed mice. Expression of pro-inflammatory genes (Il6, Il12b, Nos2, and Emr1) in the stromal vascular fraction (SVF) of the epididymal white adipose tissue (WAT) was significantly reduced (37-56 %) in the cocoa-supplemented mice. CONCLUSIONS: Dietary supplementation with cocoa ameliorates obesity-related inflammation, insulin resistance, and fatty liver disease in HF-fed obese mice, principally through the down-regulation of pro-inflammatory gene expression in WAT. These effects appear to be mediated in part by a modulation of dietary fat absorption and inhibition of macrophage infiltration in WAT.” As taken from Gu Y et al. 2014b. Eur. J. Nutr. 53(1), 149-58. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23494741

Protective activity of Theobroma cacao L. phenolic extract on AML12 and MLP29 liver cells by preventing apoptosis and inducing autophagy (Abstract). Theobroma cacao L. is known to have potential cardiovascular and cancer chemopreventive activities because of its high content of phenolic phytochemicals and their antioxidant capacities. In this work, we show for the first time that cocoa inhibits drug-triggered liver cytotoxicity by inducing autophagy. Phenolic-rich extracts of both unroasted and roasted cocoa prevented Celecoxib-induced cell viability inhibition in MLP29 liver cells because of the accumulation of G1 cells and cell death. Death prevented by cocoa had hallmarks of apoptosis such as the sub-G1 peak at flow cytometry and activation of Bax expression, together with down-regulation of Bcl-2, released cytochrome c in the cytosol with activation of Caspase 3, indicating that components of the apoptotic pathway such as Bax or upstream are major targets of cocoa phytochemicals. The protective effect of cocoa against liver cytotoxicity by Celecoxib was probably accounted for by inducing the autophagic process, as shown by enhanced Beclin 1 expression and accumulation of monodansylcadaverine in autolysosomes. This fact suggests that apoptosis was prevented by inducing autophagy. Finally, considering all these findings, we suggest that cocoa can be added to the list of natural chemopreventive agents whose potential in hepatopathy prevention and therapy should be evaluated. As taken from Arlorio M et al. J Agric Food Chem. 2009 Nov 25;57(22), 10612-18. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/19883072

Cocoa procyanidins inhibit proliferation and angiogenic signals in human dermal microvascular endothelial cells following stimulation by low-level H2O2 (Abstract). Procyanidins extracted from cocoa play a role in the defense against oxidative stress, as well as in vascular and immune functions. We previously reported that pentameric procyanidins isolated from cocoa inhibit the expression of the tyrosine kinase ErbB2 gene, thus slowing the growth of cultured human aortic endothelial cells. We herein investigate the further consequences of such inhibition by cocoa procyanidins, particularly regarding the protein level in phosphorylation patterns and the effects on the proliferation of human dermal microvascular endothelial cells (HDMECs) following angiogenic stimulation with low-level H2O2. We report herein that both the pentameric and octameric procyanidin fractions of cocoa inhibit the proliferation of HDMECs, whereas the pentameric fraction modulates the activity of several crucial proteins in angiogenic signaling by altering their tyrosine phosphorylation. Similar to aortic endothelial cells, the pentameric procyanidin fraction down-regulates the expression of ErbB2 tyrosine kinase in HDMECs. In conclusion, we report evidence suggesting that polyphenols may influence endothelial growth signaling, thus affecting angiogenesis in vitro. If these observations are applicable in vivo, they suggest a beneficial effect for cells overexpressing ErbB2, such as in specific neoplasias. As taken from Kenny TP et al. Exp Biol Med (Maywood). 2004 Sep; 229(8), 765-71. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/15337830

Cocoa bean mulch as a cause of methylxantine toxicosis in dogs (Abstract). Background: Cocoa bean shells, a by-product of chocolate production, are sold as mulch for landscaping. Homeowners find cocoa mulch desirable because it degrades into an organic fertilizer and provides an attractive color and odor. Unprocessed beans, derived from the Theobroma cacao plant, contain 1–4% theobromine=0.07–0.36% caffeine, whereas, cocoa bean mulch contains 0.19–2.98% theobromine. Some dogs find the mulch attractive and eat small to large quantities. Case Series: In response to increasing reports of dogs eating cocoa bean mulch used in landscaping, a retrospective case study was conducted to further define this unique phenomena. Sixteen cases of cocoa mulch ingestion by dogs were managed between January 2002 and April 2003. Of these, six cases were selected for analysis because the final outcome was known, there was evidence=observation of ingestion, and the managing veterinarian assessed the causality relationship as medium or higher. In 50% of the cases vomiting was reported, 33% involved tremors, and in 17% tachycardia, hyperactivity or diarrhea was reported. In 33% of cases no clinical signs developed. In the cases in which tremors were observed, the amount ingested was described as large or significant. California accounted for 67% of cases. Conclusion: Dogs consuming cocoa bean mulch may develop methylxanthine toxicosis. Retrospective case data suggests clinical signs

following ingestion include vomiting and muscle tremors. Although oral doses could not be quantitatively determined, clinical severity increased with increasing qualitative dose descriptions. Therefore, treatment should be directed at controlling clinical signs until recovery and preventing further exposure. Pet owners should avoid use of cocoa bean mulch in landscaping around dogs with indiscriminate eating habits.

As taken from Hansen S et al. J Toxicol Clin Toxicol, 2003, 41(5), 720.

“Numerous lines of evidence support a relationship between intestinal inflammation and cancer. Therefore, much attention has recently been focused on the identification of natural compounds with anti-inflammatory activities as a strategy to suppress the early stages of colorectal cancer. Because cocoa is a rich source of bioactive compounds, the present study investigated its anti-inflammatory properties in a rat model of azoxymethane (AOM)-induced colon carcinogenesis and in TNF-α-stimulated Caco-2 cells. A total of forty male rats were fed with control or cocoa-enriched diets (12 %) during 8 weeks and injected with saline or AOM (20 mg/kg body weight) during the third and fourth week (n 10 rats/group). At the end of the experiment, colon samples were evaluated for markers of inflammation. The anti-inflammatory activity of a cocoa polyphenolic extract (10 μg/ml) was examined in TNF-α-stimulated Caco-2 cells, an in vitro model of experimentally induced intestinal inflammation. The signalling pathways involved, including NF-κB and the mitogen-activated protein kinase family such as c-Jun NH2-terminal kinases (JNK), extracellular signal-regulated kinases and p38, were also evaluated. The results show that the cocoa-rich diet decreases the nuclear levels of NF-κB and the expression of pro-inflammatory enzymes such as cyclo-oxygenase-2 and inducible NO synthase induced by AOM in the colon. Additionally, the experiments in Caco-2 cells confirm that cocoa polyphenols effectively down-regulate the levels of inflammatory markers induced by TNF-α by inhibiting NF-κB translocation and JNK phosphorylation. We conclude that cocoa polyphenols suppress inflammation-related colon carcinogenesis and could be promising in the dietary prevention of intestinal inflammation and related cancer development.” As taken from Rodriguez-Ramiro I et al. 2013. Br. J. Nutr. 110(2), 206-15. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23186731?dopt=AbstractPlus

“Background. This study sought to investigate the antidiabetic and antihypertensive mechanisms of cocoa (Theobroma cacao) bean through inhibition of α-amylase, α-glucosidase, angiotensin-1 converting enzyme, and oxidative stress. Methodology. The total phenol and flavonoid contents of the water extractable phytochemicals from the powdered cocoa bean were determined and the effects of the extract on α-amylase, α-glucosidase, and angiotensin-1 converting enzyme activities were investigated in vitro. Furthermore, the radicals [1,1-diphenyl-2 picrylhydrazyl (DPPH), 2,2..-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), hydroxyl (OH), and nitric oxide (NO)] scaenging ability and ferric reducing antioxidant property of the extract were assessed. Results. The results revealed that the extract inhibited α-amylase (1.81 ± 0.22 mg/mL), α-glucosidase (1.84 ± 0.17 mg/mL), and angiotensin-1 converting enzyme (0.674 ± 0.06 mg/mL [lungs], 1.006 ± 0.08 mg/mL [heart]) activities in a dose-dependent manner and also showed dose-dependent radicals [DPPH (16.94 ± 1.34 mg/mL), NO (6.98 ± 0.886 mg/mL), OH (3.72 ± 0.26 mg/mL), and ABTS (15.7 ± 1.06 mmol/TEAC·g] scavenging ability. Conclusion. The inhibition of α-amylase, α-glucosidase, and angiotensin-1 converting enzyme activities by thecocoa bean extract could be part of the possible mechanism by which the extract could manage and/or prevent type-2 diabetes and hypertension.” As taken from Oboh G et al. 2014. Patholog. Res. Int. 2014, 549287. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25295218

“BACKGROUND: Cocoa pod is an outer part of cocoa fruits being discarded during cocoa bean processing. Authors found out that data on its usage in literature as cosmetic materials was not recorded in vast. In this study, cocoa pod extract was investigated for its potential as a cosmetic ingredient. METHODS: Cocoa pod extract (CPE) composition was accomplished using UHPLC. The antioxidant capacity were measured using scavenging assay of 1,2-diphenyl-2-picrylhydrazyl (DPPH), β-carotene bleaching assay (BCB) and ferric reducing antioxidant power (FRAP).

Inhibiting effect on skin degradation enzymes was carried out using elastase and collagenase assays. The skin whitening effect of CPE was determined based on mushroom tyrosinase assay and sun screening effect (UV-absorbance at 200-400 nm wavelength). RESULTS: LC-MS/MS data showed the presence of carboxylic acid, phenolic acid, fatty acid, flavonoids (flavonol and flavones), stilbenoids and terpenoids in CPE. Results for antioxidant activity exhibited that CPE possessed good antioxidant activity, based on the mechanism of the assays compared with ascorbic acid (AA) and standardized pine bark extract (PBE); DPPH: AA > CPE > PBE; FRAP: PBE > CPE > AA; and BCB: BHT > CPE > PBE. Cocoa pod extract showed better action against elastase and collagenase enzymes in comparison with PBE and AA. Higher inhibition towards tyrosinase enzyme was exhibited by CPE than kojic acid and AA, although lower than PBE. CPE induced proliferation when tested on human fibroblast cell at low concentration. CPE also exhibited a potential as UVB sunscreen despite its low performance as a UVA sunscreen agent. CONCLUSIONS: Therefore, the CPE has high potential as a cosmetic ingredient due to its anti-wrinkle, skin whitening, and sunscreen effects." As taken from Karim AA et al. 2014. BMC Complement. Altern. Med. 14, 381. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25292439

AIMS: Previous epidemiological studies have suggested that ingestion of chocolate reduces the risk of cardiovascular disease. In the present study, we examined the effects of flavan-3-ols derived from cocoa on blood pressure, lipolysis, and thermogenesis in rats fed a high-fat diet and that showed early signs of metabolic syndrome. MAIN METHODS: The rats were divided into three groups, and fed either normal diet (normal), 60% fat high-fat diet (HFD), or HFD containing 0.2% flavan-3-ols (HFD-flavan) for 4 weeks. At the end of the feeding period, blood pressure was measured and animals were sacrificed under anesthesia. Lipolysis and thermogenesis-related protein levels were measured in several tissues by Western blotting, and mitochondrial DNA copy number was measured by RT-PCR. KEY FINDINGS: Mean blood pressure and epididymal adipose tissue weight of HFD-flavan were significantly lower compared with those of HFD. Uncoupling protein (UCP)1 in brown adipose tissue and UCP3 in gastrocnemius of HFD-flavan were significantly increased compared with those of HFD group. Carnitine palmitoyltransferase (CPT) 2 levels in liver and medium-chain acyl-CoA dehydrogenase (MCAD) levels in gastrocnemius and liver were significantly increased by the supplementation of flavan-3-ols. SIGNIFICANCE: In addition to having hypotensive effects, flavan-3-ols enhance thermogenesis and lipolysis and consequently reduce white adipose tissue weight gain in response to high-fat diet feeding." As taken from Osakabe N et al. 2014. Life Sci. 114(1), 51-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25132363

"Cocoa polyphenol (CP), due to their biological actions, may be supplementary treatments for adipose tissue-fat gain. However, the molecular mechanism of CPs is still ambiguous. This study investigated the hypothesis that CP treatment modulates expressing of lipid metabolism genes in mesenteric white adipose tissue (MES-WAT). Sprague-Dawley (SD) rats were fed a low-fat (LF) or high-fat (HF) diet for 12 weeks. Thereafter, HFD rats (n = 10/group) were treated at a dose of 600 mg/kg bw/day CPs (HFD + CPs) for 4 weeks. DNA microarray analysis resulted in 753 genes of the 13,008 genes expressed. Bioinformatics tools showed CP treatment significantly decreased gene expression levels for lipogenic enzymes, while increased the mRNA levels responsible for lipolysis enzymes. CP administration differentially regulates gene expression involved in lipid metabolism in MES-WAT. These data unveil a new insight into the molecular mechanisms underlying the pharmacological effect of CPs on obesity biomarkers in obese rats." As taken from Ali F et al. 2015. Genomics 105(1), 23-30. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25451742

"Obesity and related metabolic diseases (e.g., type 2 diabetes, cardiovascular diseases, and hypertension) are the most prevailing nutrition-related issues in the world. An emerging feature of obesity is their relationship with chronic inflammation that begins in white adipose tissue and eventually becomes systemic. One potential dietary strategy to reduce glucose intolerance and inflammation is consumption of polyphenol-rich cocoa-likecocoa or their by-products. In vitro as

well as in vivo data indicate that cocoa polyphenols (CPs) may exhibit antioxidant and anti-inflammatory properties. Polyphenols commonly found in cocoa have been reported to regulate lipid metabolism via inducing metabolic gene expression or activating transcription factors that regulate the expression of numerous genes, many of which play an important role in energy metabolism. Currently, several molecular targets (e.g., nuclear factor Kappa B, activated protein-1, peroxisome proliferator-activated receptors, liver X receptors, and adiponectin gene) have been identified, which may explain potential beneficial obesity-associated diseases effects of CPs. Further studies have been performed regarding the protective effects of CPs against metabolic diseases by suppressing transcription factors that antagonize lipid accumulation. Thus, polyphenols-rich cocoa products may diminish obesity-mediated metabolic diseases by multiple mechanisms, thereby attenuating chronic inflammation." As taken from Ali F et al. 2014. Mol. Nutr. Food Res. 58(1), 33-48. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24259381

"Obesity remains a major public health challenge, and its prevalence is dramatically increasing. Diet and exercise are typically recommended to prevent and manage obesity; however, the results are often conflicting. Polyphenols, a class of phytochemicals that have been shown to reduce the risk factors for diabetes type II and cardiovascular diseases, are recently suggested as complementary agents in the management of obesity through several mechanisms such as decreasing fat absorption and/or fat synthesis. Dark chocolate, a high source of polyphenols, and flavanols in particular, has lately received attention for its possible role in modulating obesity because of its potential effect on fat and carbohydrate metabolism, as well as on satiety. This outcome was investigated in animal models of obesity, cell cultures and few human observational and clinical studies. The research undertaken to date has shown promising results, with the possible implication of cocoa/dark chocolate in the modulation of obesity and body weight through several mechanisms including decreasing the expression of genes involved in fatty acid synthesis, reducing the digestion and absorption of fats and carbohydrates and increasing satiety." As taken from Farhat G et al. 2014. Phytother. Res. 28(6), 791-7. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24000103

"Chronic inflammation has been identified as a necessary step to mediate atherosclerosis and cardiovascular disease and as a relevant stage in the onset and progression of several types of cancer. Considerable attention has recently been focused on the identification of dietary bioactive compounds with anti-inflammatory activities as an alternative natural source for prevention of inflammation-associated diseases. The remarkable capacity of cocoa flavanols as antioxidants, as well as to modulate signaling pathways involved in cellular processes, such as inflammation, metabolism and proliferation, has encouraged research on this type of polyphenols as useful bioactive compounds for nutritional prevention of cardiovascular disease and cancer. Data from numerous studies suggest that cocoa and cocoa-derived flavanols can effectively modify the inflammatory process, and thus potentially provide a benefit to individuals with elevated risk factors for atherosclerosis/cardiovascular pathology and cancer. The present overview will focus on the most recent findings about the effects of cocoa, its main constituents and cocoa derivatives on selected biomarkers of the inflammatory process in cell culture, animal models and human cohorts." As taken from Goya L et al. 2016. Nutrients 8(4), 212. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27070643

"PURPOSE: Cocoa intake has been associated with health benefits, improving cardiovascular function and metabolism, as well as modulating intestinal immune function. The aim of this study was to take an in-depth look into the mechanisms affected by the cocoa intake by evaluating the colonic gene expression after nutritional intervention, and to ascertain the role of the fiber of cocoa in these effects. METHODS: To achieve this, Wistar rats were fed for 3 weeks with either a reference diet, a diet containing 10 % cocoa (C10), a diet based on cocoa fiber (CF) or a diet containing inulin (I). At the end of the study, colon was excised to obtain the RNA to evaluate the differential gene expression by microarray. Results were validated by RT-PCR. RESULTS: The C10 group was the group with most changes in colonic gene expression, most of them down-regulated

but a few in common with the CF diet. The C10 diet significantly up-regulated the expression of Scgb1a1 and Scnn1 g and down-regulated Tac4, Mcpt2, Fcer1a and Fabp1 by twofold, most of them related to lipid metabolism and immune function. The CF and I diets down-regulated the expression of Serpina10 and Apoa4 by twofold. Similar patterns of expression were found by PCR. CONCLUSION: Most of the effects attributed to cocoa consumption on genes related to the immune system (B cell and mast cell functionality) and lipid metabolism in the colon tissue were due not only to its fiber content, but also to the possible contribution of polyphenols and other compounds.".As taken from Massot-Cladera M et al. 2017. Eur. J. Nutr. 56(5), 1871-1885. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/27256297

"Prevalence of obesity worldwide has reached pandemic proportions. Despite the increasing evidence in the implication of phenolic compounds in obesity management, the real effect is not completely understood. The available in vitro and in vivo studies have demonstrated the implication of phenolic compounds in: lowering food intake, decreasing lipogenesis, increasing lipolysis, stimulating fatty acids β-oxidation, inhibiting adipocyte differentiation and growth, attenuating inflammatory responses and suppress oxidative stress. This review encompasses the most recent evidence in the anti-obesity effect of phenolic compounds from plants to different nutraceuticals and functional foods based on the in vitro, in vivo and clinical studies. For that, this review has been focused on popular plant-based products highly consumed today such as cocoa, cinnamon, and olive oil, beverages such as red wine, tea (green, white and black tea) and Hibiscus sabdariffa L. tea, among others."As taken from Rodríguez-Pérez C et al. 2019. Crit. Rev. Food Sci. Nutr. 59(8), 1212-1229.. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29156939

"Oxidative and inflammatory stress represents a major risk factor for cardiovascular disease (CVD) in overweight and obese subjects. Between the different plant foods, chocolate has been shown to decrease CVD risk due to its antioxidant and anti-inflammatory properties. However, as we recently showed in epidemiological studies, meta-analyses, and human trials, dietary antioxidants resulted more effective in subjects characterized by an ongoing oxidative stress, than in healthy people. Aim of this work was to investigate the effect of different concentrations of chocolate phenolic extract (CPE) on in vitro free radical production, stimulated by phorbol 12-myristate 13-acetate (PMA), in leukocytes extracted from blood of normo-weight and overweight/obese subjects. Neutrophils from overweight/obese group had a significantly higher free radical production compared to the normo-weight group. In neutrophils, the lowest CPE concentration significantly reduced free radical production in overweight/obese group only, and higher CPE concentrations were effective in both groups. In monocytes, the CPE concentration that was significantly effective in reducing free radical production was lower in overweight/obese subjects than in normo-weight subjects. Chocolate polyphenol extracts inhibit oxidative burst in human neutrophils and monocytes with a higher efficiency in subjects characterized by an unphysiological oxidative/inflammatory stress, such as overweight and obese. Results of this study provide further evidence about a differential role of dietary antioxidant strictly related to the "stress" condition of the subjects." As taken from Ioannone F et al. 2017. Front. Nutr. 4, 23. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28649567

"BACKGROUND: Chocolate has a reputation for contributing to weight gain due to its high fat, sugar and calorie content. However, the effect of varying concentrations of cocoa in chocolate on energy intake and appetite is not clear. OBJECTIVE: To compare the acute effect of consuming an isocaloric dose of dark, milk and white chocolate on subsequent energy intake, appetite and mood in postmenopausal women. METHODS: Fourteen healthy postmenopausal women (57.6 ± 4.8yr) attended an introductory session followed by three experimental trials performed in a counterbalanced order at a standardised time of day, each separated by one week. Ad libitum energy intake, perceived appetite, mood and appetite-related peptides were assessed in response to consumption of 80% cocoa [dark chocolate], 35% cocoa [milk chocolate] and cocoa butter [white chocolate] (2099 kJ), prepared from a single-origin cacao bean. RESULTS: Ad libitum energy intake was significantly lower following dark (1355 ± 750 kJ) compared with both milk (1693 ± 969 kJ; P = 0.008) and white (1842 ± 756 kJ; P = 0.001) chocolate consumption. Blood glucose and

insulin concentrations were transiently elevated in response to white and milk chocolate consumption compared with the dark chocolate ($P < 0.05$), while pancreatic polypeptide was elevated in response to higher cocoa content chocolate (dark and milk) compared with white chocolate ($P < 0.05$). No differences in active ghrelin or leptin were observed between conditions, nor was mood altered between conditions ($P > 0.05$). CONCLUSIONS: Dark chocolate attenuates subsequent food intake in postmenopausal women, compared to the impact of milk and white chocolate consumption." As taken from Marsh CE et al. 2017. Appetite 116, 544-551. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28572069

"Cinnamon and cocoa are known to be valuable sources of bioactive phytochemicals, mainly the polyphenols. This paper investigates the potential antioxidant activity of cinnamon and cocoa extract and the interaction of their mixtures by various in vitro tests. Moreover, the combination effect of their constituents in a binary mixture was studied. Two representative active compounds of chocolate (epicatechin, catechin) were combined with seven of cinnamon (gallic acid, tannic acid, quercetin, sinapic acid, cinnamic acid, eugenol and cinnamaldehyde) in multilevel ratios. The results indicate that the addition of the cinnamon extract significantly increased the antioxidant activity of the cocoa extract. The interaction ranged from synergetic to antagonistic. The interaction was less synergetic when cinnamon extract was added in higher proportion. The interaction of their constituents substantially influenced the antioxidant activity of the mixture and was dependent on the ratio. The kinetics' study could elucidate how the polyphenols work in a mixture." As taken from Muhammad DRA et al. 2017. Food Chem. 231, 356-364. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28450018

"Background: Root canal treatment constitutes a treatment sequence for infected pulp to eliminate the etiological factors of pulp necrosis and periapical lesion. Enterococcus faecalis (E. faecalis) is an organism commonly found in a high proportion of root canal failure because of its ability to form biofilm. Degradation of extracellular polymeric substance (EPS) by oxidizing agents such as sodium hypochlorite is the first step in removing biofilm. However, the toxicity of sodium hypochlorite constitutes the main concern and, therefore, the safest alternative irrigants possible are required. The use of fruits, herbs and plants is widespread, especially in the fields of medicine and dentistry. Food crops are known to be rich in bioactive compounds, especially polyphenols, which have antioxidant and antimicrobial properties. Cocoa pod husk extract can, therefore, represent an alternative irrigant. Purpose: This study aimed to determine the minimum inhibitory concentration of cocoa pod husk extract in relation to the thickness of E. faecalis EPS biofilm. Methods: Four groups of E. faecalis cultured biofilm samples were analysed: group one contained E. faecalis without cocoa pod husk as a positive control; group two contained E. faecalis with 1.56% cocoa pod husk extract; group 3 contained E. faecalis with 3.125% cocoa pod husk extract; and group 4 contained E. faecalis with 6.25% cocoa pod husk extract. The biofilm thickness of all groups was measured by confocal laser scanning microscopy with statistical analysis subsequently undertaken by means of a post hoc test and Tukey HSD. Results: The average values of EPS biofilm thickness were as follows: group 1: 9500 nm; group 2: 8125 nm; group 3: 8000 nm; and group 4: 6375 nm. A post hoc Tukey HSD test indicated a significant difference between group 1 and group 4, while in group 2 and group 3 compared to group 1, there were no significant differences with the values of each being $p = 0.340$ and $p = 0.267$ ($p>0.05$). Conclusion: 6.25% cocoa pod husk extract reduces E. faecalis EPS biofilm thickness." As taken from Yuanita T et al. 2019. Dental Journal 52(4), 215-218. Available at https://e-journal.unair.ac.id/MKG/article/view/16344

6. Functional effects on

6.1. Broncho/pulmonary system

"Confectionery workers are exposed to a wide variety of organic dusts and aerosols. Previous studies with workers in a confectionery plant working with cocoa and rye flour indicate that these workers are at risk of developing adverse respiratory symptoms and lung function impairment. The

effects of cocoa and rye flour extract on isolated guinea pig tracheal smooth muscle were studied using water-soluble extracts from cocoa and rye flour obtained from the studied confectionery plant. Dose-related contractions of nonsensitized guinea pig tracheal rings were demonstrated using both cocoa and rye flour extracts. Pharmacologic studies were performed by pretreating guinea pig tracheal tissue with drugs known to modulate smooth muscle contraction: atropine, indomethacin, pyrilamine, nordihydroguaiaretic acid (NDGA), acivicin, bromophenacyl bromide (BPB), 3,4,5-trimethoxybenzoate 8-(N,N-diethylamino)octyl ester (TMB8), captopril, and capsaicin. Constrictor effects of the dust extracts were inhibited by these agents, the pattern of which depended on the dust extract. Atropine consistently and significantly reduced the contractile effects of both extracts. These observations suggest a release of parasympathetic mediators by these extracts or more directly an interaction with muscarinic receptors. In addition, the constrictor effect of cocoa and rye flour extracts was significantly, but only partially, reduced by indomethacin, pyrilamine, BPB, and TMB8. Acivicin also partially decreased the constrictor effect of cocoa extract. Pretreatment of tracheal tissue with capsaicin also decreased the constrictor effects of high concentrations of cocoa and rye flour extracts. Data suggest that cocoa and rye flour extracts cause a dose-related constriction of airway smooth muscle by non immunological mechanisms involving cholinergic pathways and airway mediators such as histamine and the products of the arachadonic acid cascade. This effect is not dependent on the presensitization of guinea pigs." As taken from Schachter EN J Toxicol Environ Health A. 1999 May 28; 57(2), 137-48. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/10344228?dopt=AbstractPlus

Respiratory impairment has been reported in a group of 40 cocoa-processing workers. Up to 30% of this group showed signs of chronic respiratory symptoms, and two had occupational asthma. Acute symptoms, such as cough, occurred during work shifts and reduced ventilatory capacity was demonstrated. Decreased lung function was observed in bronchial provocation tests with cocoa dust extract. Measurement showed that the cocoa dust concentration in the workplace ranged from 2-16 mg/m3 (mean 9.1 mg/m3) and the respirable fraction from 0.9-3.5 mg/m3 (mean 2.1 mg/m3) (Zuskin et al. 1998). In common with many materials, cocoa dust seems capable of inducing the occasional case of asthma (e.g. Malo et al. 1997).

"Unsweetened natural cocoa powder is enriched with nutraceutical abundance of anti-asthmatic compounds theobromine and theophylline. Cocoa powder, which is prepared after removal of the cocoa butter, contains about 1.9% theobromine and 0.21% caffeine. Anecdotal reports indicate that regular consumption of unsweetened natural cocoa powder (UNCP), a common practice in Ghana, West Africa, has the potential to reduce the tendency of asthmatic episodes. In the present paper we studied the effect of regular ingestion of aqueous extract of UNCP on hematological and histopathological changes that occur in ovalbumin (OVA)-sensitized guinea pigs. OVA-sensitized guinea pigs were challenged with aerosolized OVA 1 hour after ingestion of 300 mg/kg (low dose) or 600 mg/kg (high dose) of UNCP for 35 consecutive days. Histopathological and haematological changes in the OVA-sensitized guinea pigs were evaluated. Both negative and positive controls with distilled water and prednisolone, respectively, were used. OVA-sensitized guinea pigs demonstrated concentration-independent reduction in immune response to aerosolized OVA. There were no histo-architectural changes in the bronchiolar smooth muscles of the treated groups. Unsweetened natural cocoa powder has potential anti-asthmatic properties when administered orally at the doses tested." As taken from Awortwe C et al. 2014. Int. J. Immunopathol. Pharmacol. 27(2), 203-12. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25004832?dopt=AbstractPlus

6.2. Cardiovascular system

Cocoa and cardiovascular health (Abstract). Epidemiological data demonstrate that regular dietary intake of plant-derived foods and beverages reduces the risk of coronary heart disease and stroke. Among many ingredients, cocoa might be an important mediator. Indeed, recent research demonstrates a beneficial effect of cocoa on blood pressure, insulin resistance, and vascular and

platelet function. Although still debated, a range of potential mechanisms through which cocoa might exert its benefits on cardiovascular health have been proposed, including activation of nitric oxide and antioxidant and antiinflammatory effects. This review summarizes the available data on the cardiovascular effects of cocoa, outlines potential mechanisms involved in the response to cocoa, and highlights the potential clinical implications associated with its consumption. As taken from Corti R et al. Circulation. 2009 Mar 17; 119(10), 1433-41. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/19289648

“An increasing body of epidemiologic evidence supports the concept that diets rich in fruits and vegetables can promote health and attenuate, or delay, the onset of various diseases. Epidemiologic data support the idea that these health benefits are causally linked to the consumption of certain flavonoids present in fruit and vegetables. In the context of cardiovascular health, a particular group of flavonoids, namely, the flavan-3-ols (flavanols), has received attention. Flavanol-rich, plant-derived foods and beverages include wine, tea, and various fruits and berries, as well as cocoa and cocoa products. Numerous dietary intervention studies in humans and animals indicate that flavanol-rich foods and beverages might exert cardioprotective effects with respect to vascular function and platelet reactivity. This review discusses the bioactivity of flavanols in the context of cardiovascular health, with respect to their bioavailability, their antioxidant properties, and their vascular effects.” As taken from Keen CL et al. 2005. Am. J. Clin. Nutr. 81(1 Suppl), 298S-303S. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/15640494

Cocoa, chocolate, and cardiovascular disease (Abstract). A significant body of evidence demonstrates that diets rich in fruits and vegetables promote health and attenuate, or delay, the onset of various diseases, including cardiovascular disease, diabetes, certain cancers, and several other age-related degenerative disorders. The concept that moderate chocolate consumption could be part of a healthy diet has gained acceptance in past years based on the health benefits ascribed to selected cocoa components. Specifically, cocoa as a plant and chocolate as food contain a series of chemicals that can interact with cell and tissue components, providing protection against the development and amelioration of pathological conditions. The most relevant effects of cocoa and chocolate have been related to cardiovascular disease. The mechanisms behind these effects are still under investigation. However, the maintenance or restoration of vascular NO production and bioavailability and the antioxidant effects are the mechanisms most consistently supported by experimental data. This review will summarize the most recent research on the cardiovascular effects of cocoa flavanols and related compounds. As taken from Galleano M et al. J Cardiovasc Pharmacol. 2009 Dec; 54(6), 483-90. PubMed, 201 available at http://www.ncbi.nlm.nih.gov/pubmed/19701098

Acute dark chocolate and cocoa ingestion and endothelial function: a randomized controlled crossover trial (Abstract).

BACKGROUND: Studies suggest cardioprotective benefits of dark chocolate containing cocoa.

OBJECTIVE: This study examines the acute effects of solid dark chocolate and liquid cocoa intake on endothelial function and blood pressure in overweight adults. DESIGN: Randomized, placebo-controlled, single-blind crossover trial of 45 healthy adults [mean age: 53 y; mean body mass index (in kg/m(2)), 30]. In phase 1, subjects were randomly assigned to consume a solid dark chocolate bar (containing 22 g cocoa powder) or a cocoa-free placebo bar (containing 0 g cocoa powder). In phase 2, subjects were randomly assigned to consume sugar-free cocoa (containing 22 g cocoa powder), sugared cocoa (containing 22 g cocoa powder), or a placebo (containing 0 g cocoa powder). RESULTS: Solid dark chocolate and liquid cocoa ingestion improved endothelial function (measured as flow-mediated dilatation) compared with placebo (dark chocolate: 4.3 +/- 3.4% compared with -1.8 +/- 3.3%; P<0.001; sugar-free and sugared cocoa: 5.7 +/- 2.6% and 2.0 +/- 1.8% compared with -1.5 +/- 2.8%; P<0.001). Blood pressure decreased after the ingestion of dark chocolate and sugar-free cocoa compared with placebo (dark chocolate: systolic, -3.2 +/- 5.8 mm Hg compared with 2.7 +/- 6.6 mm Hg; P<0.001; and diastolic, -1.4 +/- 3.9 mm Hg compared with

2.7 +/- 6.4 mm Hg; P = 0.01; sugar-free cocoa: systolic, -2.1 +/- 7.0 mm Hg compared with 3.2 +/- 5.6 mm Hg; P<0.001; and diastolic: -1.2 +/- 8.7 mm Hg compared with 2.8 +/- 5.6 mm Hg; P = 0.014). Endothelial function improved significantly more with sugar-free than with regular cocoa (5.7 +/- 2.6% compared with 2.0 +/- 1.8%; P<0.001). CONCLUSIONS: The acute ingestion of both solid dark chocolate and liquid cocoa improved endothelial function and lowered blood pressure in overweight adults. Sugar content may attenuate these effects, and sugar-free preparations may augment them. As taken from Faridi Z et al. Am J Clin Nutr. 2008 Jul; 88(1), 58-63. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/18614724

Effects of cocoa extracts on endothelium-dependent relaxation (Abstract). The aim of this study was to examine the effects of procyanidins derived from cocoa on vascular smooth muscle. Two hypotheses were tested: 1) extracts of cocoa, which are rich in procyanidins, cause endothelium-dependent relaxation (EDR), and 2) extracts of cocoa activate endothelial nitric oxide synthase (NOS). The experiments were carried out on aortic rings obtained from New Zealand White rabbits. The polymeric procyanidins (tetramer through decamer of catechin) caused an EDR. In addition, the Ca(2+)-dependent NOS activity, measured by the L-arginine to L-citrulline conversion assay, was significantly increased in aortic endothelial cells exposed to polymeric procyanidins, whereas monomeric compounds had no such effect. These findings demonstrate that polymeric procyanidins cause an EDR that is mediated by activation of NOS. As taken from Karim M et al. (2000). J. Nutr. 130(8S Suppl), 2105S-8S. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/10917930

“BACKGROUND: Four meta-analyses of randomized controlled trials (RCTs) based on the classical random-effects model showed that cocoa consumption can reduce systolic blood pressure (SBP) and diastolic blood pressure (DBP). Because epicatechin is suggested to be responsible for the treatment effect, changes in blood pressure should depend on the dose of ingested epicatechin, which may explain the between-study differences. OBJECTIVE: The objective was to quantify the effect of epicatechin ingested via cocoa products on changes in SBP and DBP. DESIGN: A nonlinear meta-regression model was chosen to investigate the impact of the epicatechin dose on changes in SBP and DBP. A Bayesian approach using Markov chain Monte Carlo methods was applied for an appropriate treatment of the nonlinearity. RESULTS: Data from 16 RCTs on SBP and 15 RCTs on DBP were included. The dose of epicatechin ingested via cocoa products influenced the changes in SBP and DBP. The asymptotic limit for the reduction was estimated at -4.6 mm Hg (95% CI: -5.4, -3.9 mm Hg) for SBP and at -2.1 mm Hg (95% CI: -2.7, -1.6 mm Hg) for DBP. An intake of 25 mg epicatechin/d led to a mean reduction of -4.1 mm Hg (95% CI: -4.6, -3.6 mm Hg) in SBP and of -2.0 mm Hg (95% CI: -2.4, -1.5 mm Hg) in DBP. CONCLUSIONS: Blood pressure reduction by consumption of cocoa products depends on the dose of ingested epicatechin, which explains most of the between-study differences in classical meta-analyses. Similar effects may be achieved by consumption of other foods that are also rich in epicatechin”. As taken from Ellinger et al. 2012. Am J Clin Nutr. 2012 Jun;95(6), 1365-77. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/22552030

“All along the history, many kinds of magic and aphrodisiac properties were attributed to the chocolate. Because of the presence of certain active substances, cacao and chocolate are supposed to have some potentially beneficial effects on human health, particularly on cardiovascular system. Containing flavoniods, cacao and its products have antioxidant, anti-inflammatory, anti-atherogenic, anti-thrombotic, antihypertensive and neuroprotective effects, as well as influence on insulin sensitivity, vascular endothelial function, and activation of nitric oxide. Other molecules, like methyxantin, biogenic amines and cannabinoid-like fatty acids, may have a psychoactive action. Synergic effect of all these substances could have a positive direct and indirect influence on sexual health and function. Nevertheless, randomized studies are needed to confirm these hypotheses and to elaborate recommendations about cacao consumption.” As taken

from Bianchi-Demicheli F et al. 2013. Rev. Med. Suisse 9(378), 624, 626-9. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23547364?dopt=AbstractPlus

“SCOPE: We examined whether flavan-3-ol-enriched dark chocolate, compared with standard dark and white chocolate, beneficially affects platelet function in healthy subjects, and whether this relates to flavan-3-ol bioavailability. METHODS AND RESULTS: A total of 42 healthy subjects received an acute dose of flavan-3-ol-enriched dark, standard dark or white chocolate, in random order. Blood and urine samples were obtained just before and 2 and 6 h after consumption for measurements of platelet function, and bioavailability and excretion of flavan-3-ols. Flavan-3-ol-enriched dark chocolate significantly decreased adenosine diphosphate-induced platelet aggregation and P-selectin expression in men (all $p \leq 0.020$), decreased thrombin receptor-activating peptide-induced platelet aggregation and increased thrombin receptor-activating peptide-induced fibrinogen binding in women (both $p \leq 0.041$), and increased collagen/epinephrine-induced ex vivo bleeding time in men and women ($p \leq 0.042$). White chocolate significantly decreased adenosine diphosphate-induced platelet P-selectin expression ($p = 0.002$) and increased collagen/epinephrine-induced ex vivo bleeding time ($p = 0.042$) in men only. Differences in efficacy by which flavan-3-ols affect platelet function were only partially explained by concentrations of flavan-3-ols and their metabolites in plasma or urine. CONCLUSION: Flavan-3-ols in dark chocolate, but also compounds in white chocolate, can improve platelet function, dependent on gender, and may thus beneficially affect atherogenesis.” As taken from Ostertag LM et al. 2013. Mol. Nutr. Food Res. 57(2), 191-202. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23136121?dopt=AbstractPlus

“BACKGROUND: Evidence from clinical studies has suggested that cocoa may increase high-density lipoprotein (HDL)-cholesterol concentrations. However, it is unclear whether this effect is attributable to flavonoids or theobromine, both of which are major cocoa components. OBJECTIVES: We investigated whether pure theobromine increases serum HDL cholesterol and whether there is an interaction effect between theobromine and cocoa. DESIGN: The study had a 2-center, double-blind, randomized, placebo-controlled, full factorial parallel design. After a 2-wk run-in period, 152 healthy men and women (aged 40-70 y) were randomly allocated to consume one 200-mL drink/d for 4 wk that contained 1) cocoa, which naturally provided 150 mg theobromine and 325 mg flavonoids [cocoa intervention (CC)], 2) 850 mg pure theobromine [theobromine intervention (TB)], 3) cocoa and added theobromine, which provided 1000 mg theobromine and 325 mg flavonoids [theobromine and cocoa intervention (TB+CC)], or 4) neither cocoa nor theobromine (placebo). Blood lipids and apolipoproteins were measured at the start and end of interventions. RESULTS: In a 2-factor analysis, there was a significant main effect of the TB ($P<0.0001$) but not CC ($P = 0.1288$) on HDL cholesterol but no significant interaction ($P = 0.3735$). The TB increased HDL-cholesterol concentrations by 0.16 mmol/L ($P<0.0001$). Furthermore, there was a significant main effect of the TB on increasing apolipoprotein A-I ($P<0.0001$) and decreasing apolipoprotein B and LDL-cholesterol concentrations ($P<0.02$). CONCLUSIONS: Theobromine independently increased serum HDL-cholesterol concentrations by 0.16 mmol/L. The lack of significant cocoa and interaction effects suggested that theobromine may be the main ingredient responsible for the HDL cholesterol-raising effect. This trial was registered at clinicaltrials.gov as NCT01481389.” As taken from Neufingerl N et al. 2013. Am. J. Clin. Nutr. 97(6), 1201-9. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23595874?dopt=AbstractPlus

“All along the history, many kinds of magic and aphrodisiac properties were attributed to the chocolate. Because of the presence of certain active substances, cacao and chocolate are supposed to have some potentially beneficial effects on human health, particularly on cardiovascular system. Containing flavoniods, cacao and its products have antioxidant, anti-inflammatory, anti-atherogenic, anti-thrombotic, antihypertensive and neuroprotective effects, as well as influence on insulin sensitivity, vascular endothelial function, and activation of nitric oxide. Other molecules, like methyxantin, biogenic amines and cannabinoid-like fatty acids, may have a psychoactive action. Synergic effect of all these substances could have a positive direct and

indirect influence on sexual health and function. Nevertheless, randomized studies are needed to confirm these hypotheses and to elaborate recommendations about cacao consumption." As taken from Bianchi-Demicheli F et al. 2013. Rev. Med. Suisse 9(378), 624, 626-9. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23547364?dopt=AbstractPlus

"….cocoa flavanols (mostly epicatechins) may exert an acute effect on endothelium-dependent flow-mediated dilation (ED-FMD) by enhancing nitric oxide production in the endothelium each time they are consumed….A cause and effect relationship has been established between the consumption of cocoa flavanols in the HF [high flavanol] cocoa extract (i.e. in capsules or tablets) and maintenance of normal endothelium-dependent vasodilation."

As taken from EFSA, 2014.

"Objective: This trial was undertaken to evaluate the effects of high-cocoa-content chocolate supplementation in pregnancy on several haematochemical and clinical parameters. The study had as reference population the pregnant women requesting an obstetric control at Outpatient Clinic of Obstetrics and Gynaecology of the S. Maria della Misericordia University Hospital, Perugia, Italy. Were candidated to enter in this study all Caucasian women of 18-40 year-olds, who had a single gestation pregnancy between 11th+0 and 13th+0 week gestational age. Methods: We conducted a single-center randomized controlled trial. The pregnant women selected were randomized into Group A, which received daily doses of 30 g of chocolate (70%cocoa), and Group B, which was free to increase their diet with other foods. Results: Ninety women were randomized. Significant difference was found between the two groups for diastolic blood pressure (p=0.05), systolic (P<0.0001) and levels of liver enzymes, with values lower in Group A than in Group B. Total cholesterol levels and weight gain in Group A did not increase more than in Group B. Conclusions: A modest daily intake of high-cocoa-content chocolate contributes to reduce blood pressure, glycemic and liver pattern during pregnancy without affecting the weight gain" (Di Renzo et al. 2012).

"Objective: The aim of our study was to analyze the effects of caffeine and chocolate (70% cocoa) on fetal heart rate (FHR). Study design: Fifty pregnant women with uncomplicated gestation, matched for age and parity, underwent computerized FHR recording before and after the consumption of caffeine and then, after one week, before and after 70% cocoa chocolate intake. Computerized cardiotocography (cCTG) parameters were expressed as mean and SD. The differences were tested for statistical significance using the paired t-test, with significance at $p < 0.05$. Results: The number of uterine contraction peaks, the number of small and large accelerations (10 and 15 beats per minute for 15 seconds), the duration of episodes of high variation and the short-term FHR variation were significantly higher ($p < 0.001$) after maternal coffee intake. The number of large accelerations, the duration of episodes of high variation and the short-term FHR variation were significantly higher ($p < 0.001$) after maternal consumption of chocolate, whilst no effect of cocoa was found during contractions. Conclusions: Our results suggest that maternal intake of both caffeine and 70% cocoa have a stimulating action on fetal reactivity. This finding is likely due to the pharmacological action of theobromine, a methilxanthine present in coffee and in chocolate. The correlation between maternal caffeine intake and increased uterine contraction peaks is likely due to the effect of caffeine on the uterine muscle" (Buscicchio et al. 2012).

"Introduction. The aim of this study was to assess the vascular benefits of dark chocolate in healthy and young individuals. Methods. A randomized and controlled trial was carried out involving 60 healthy volunteers, randomized into two groups: control group (CG; n = 30) and intervention group (IG; n = 30). The IG ingested a daily dosage of 10 g of dark chocolate (>75% cocoa) for a month. Blood pressure (BP), flow-mediated dilation (FMD), arterial stiffness index (ASI), aortic pulse wave velocity (PWV), and pulse wave analysis (PWA) were assessed at baseline and one week after the one-month intervention period. Results. Arterial function improved after intervention in the IG, with PWV decreasing from 6.13 ± 0.41 m/s to 5.83 ± 0.53 m/s ($P = 0.02$), with no significant differences

observed in the CG. A significant decrease in ASI (0.16 ± 0.01 to 0.13 ± 0.01; P<0.001) and AiX (-15.88 ± 10.75 to -22.57 ± 11.16; P = 0.07) was also depicted for the IG. Endothelial function improved in the IG, with the FMD increasing 9.31% after the 1-month intervention (P<0.001), with no significant variation in the CG. Conclusion. The daily ingestion of 10 g dark chocolate (>75%cocoa) during a month significantly improves vascular function in young and healthy individuals." As taken from Pereira T et al. 2014. Cardiol. Res. Pract. 2014, 945951. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24982813

"Recent reports on cocoa are appealing in that a food commonly consumed for pure pleasure might also bring tangible benefits for human health. Cocoa consumption is correlated with reduced health risks of cardiovascular diseases, hypertension, atherosclerosis, and cancer, and the health-promoting effects of cocoa are mediated by cocoa-driven phytochemicals. Cocoa is rich in procyanidins, theobromine, (-)-epicatechin, catechins, and caffeine. Among the phytochemicals present in consumed cocoa, theobromine is most available in human plasma, followed by caffeine, (-)-epicatechin, catechin, and procyanidins. It has been reported that cocoa phytochemicals specifically modulate or interact with specific molecular targets linked to the pathogenesis of chronic human diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, obesity, diabetes, and skin aging. This review summarizes comprehensive recent findings on the beneficial actions of cocoa-driven phytochemicals in molecular mechanisms of human health." As taken from Kim J et al. 2014. Crit. Rev. Food Sci. Nutr. 54(11), 1458-72. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24580540?dopt=AbstractPlus

"Previous studies in humans have shown that the cacao polyphenols, (-)-epicatechin and its oligomers, prevent in vitro and ex vivo low-density lipoprotein oxidation mediated by free radical generators and metal ions and also reduce plasma LDL-cholesterol levels. The aim of this study was to examine the effects of cacao polyphenols on the development of atherosclerosis in apolipoprotein E-deficient (-/-) mice. Mice aged 8 weeks (n = 90) were randomized into three groups, and fed either normal mouse chow (controls) or chow supplemented with 0.25 or 0.40 % cacao polyphenols for 16 weeks. The mean plaque area in cross-sections of the brachiocephalic trunk was measured and found to be lower in the 0.25 % cacao polyphenol group than in the control group (P<0.05). Pathological observations showed that accumulation of cholesterol crystals in the plaque area was greater in the control group compared with the 0.40 % cacao polyphenol group (P<0.05). Immunochemical staining in the 0.25 and 0.40 % groups showed that expression of the cell adhesion molecules (VCAM-1 and ICAM-1) and production of oxidative stress markers (4-hydroxynonenal, hexanoyl-lysine, and dityrosine) were reduced in cross-sections of the brachiocephalic trunk. These results suggest that cacao polyphenols inhibit the development of atherosclerosis in apolipoprotein E-deficient (-/-) mice by reducing oxidative stress and inflammatory responses." As taken from Natsume M & Baba S. 2014. Subcell. Biochem. 77, 189-98. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24374929

"Current evidence from experimental studies in animals and humans along with findings from prospective studies indicates beneficial effects of green and black tea as well as chocolate on cardiovascular health, and that tea and chocolate consumption may reduce the risk of stroke. The strongest evidence exists for beneficial effects of tea and cocoa on endothelial function, total and LDL cholesterol (tea only), and insulin sensitivity (cocoa only)....Awaiting the results from further long-term RCTs and prospective studies, moderate consumption of filtered coffee, tea, and dark chocolate seems prudent." As taken from Larsson SC. 2014. Stroke 45(1), 309-14. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24326448?dopt=AbstractPlus

"BACKGROUND: Cocoa flavonoids exert beneficial vascular effects and reduce the risk of cardiovascular morbidity and mortality. Nevertheless, the involved mechanisms have not been clarified and no study has yet focused on the dose-response effects. OBJECTIVES: We aimed to investigate the effects of different doses of cocoa flavonoids on flow-mediated dilation (FMD), endothelin-1 (ET-1), pulse wave velocity (PWV), and SBP and DBP. DESIGN: According to a randomized, double-blind, controlled, cross-over design, 20 healthy volunteers (1.5% improvement

in FMD in 20 individuals: 0.99 at alpha=0.05) were assigned to receive either five treatments with daily intake of 10g cocoa (0, 80, 200, 500 and 800mg cocoa flavonoids/day) in five periods lasting 1 week each. RESULTS: Cocoa dose-dependently increased FMD from 6.2% (control) to 7.3, 7.6, 8.1 and 8.2% after the different flavonoid doses, respectively (P<0.0001). Compared with the control, even 80 mg cocoa flavonoids per day increased FMD (P<0.0001). Cocoa dose-dependently decreased PWV (P<0.0001). Cocoa intake decreased office blood pressure (BP) (SBP: -4.8±1.03 mmHg, P<0.0001; DBP: -3.03±1.07mmHg, P=0.0011). With respect to control, cocoa ingestion decreased 24-h (P=0.05) and daytime (P=0.038) SBP, and 24-h (P=0.0064), daytime (P=0.0088) and night-time (P=0.0352) pulse pressure. Compared with the control, cocoa dose-dependently decreased ET-1 levels [from 17.1 (control) to 15.2, 14.5, 14.2 and 14.1pg/ml, after the different flavonoid doses, respectively (P for treatment <0.05)]. Compared with the control, significant changes were observed for all doses of flavonoids (ET-1; P<0.05). CONCLUSION: Our study showed for the first time that cocoa dose-dependently improved FMD and decreased PWV and ET-1 also by ameliorating office and monitored BP. Our findings are clinically relevant, suggesting cocoa, with very low calorie intake, might be reasonably incorporated into a dietary approach, representing a consistent tool in cardiovascular prevention." As taken from Grassi D et al. 2015. J. Hypertens. 33(2), 294-303. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25380152

"AIMS: Previous epidemiological studies have suggested that ingestion of chocolate reduces the risk of cardiovascular disease. In the present study, we examined the effects of flavan-3-ols derived from cocoa on blood pressure, lipolysis, and thermogenesis in rats fed a high-fat diet and that showed early signs of metabolic syndrome. MAIN METHODS: The rats were divided into three groups, and fed either normal diet (normal), 60% fat high-fat diet (HFD), or HFD containing 0.2% flavan-3-ols (HFD-flavan) for 4 weeks. At the end of the feeding period, blood pressure was measured and animals were sacrificed under anesthesia. Lipolysis and thermogenesis-related protein levels were measured in several tissues by Western blotting, and mitochondrial DNA copy number was measured by RT-PCR. KEY FINDINGS: Mean blood pressure and epididymal adipose tissue weight of HFD-flavan were significantly lower compared with those of HFD. Uncoupling protein (UCP)1 in brown adipose tissue and UCP3 in gastrocnemius of HFD-flavan were significantly increased compared with those of HFD group. Carnitine palmitoyltransferase (CPT) 2 levels in liver and medium-chain acyl-CoA dehydrogenase (MCAD) levels in gastrocnemius and liver were significantly increased by the supplementation of flavan-3-ols. SIGNIFICANCE: In addition to having hypotensive effects, flavan-3-ols enhance thermogenesis and lipolysis and consequently reduce white adipose tissue weight gain in response to high-fat diet feeding." As taken from Osakabe N et al. 2014. Life Sci. 114(1), 51-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25132363

"Cocoa has a high content in polyphenols, especially flavonols. Flavonols exert a favourable effect on endothelium-derived vasodilation via the stimulation of nitric oxide-synthase (NOS), the increased availability of l-arginine (NO donor) and the decreased degradation of NO. Cocoa may also have a beneficial effect via the decreased platelet aggregation, the decreased lipid oxidation and insulin resistance. These effects are associated with a modest decrease of blood pressure and a favourable trend towards a reduction in cardiovascular events and strokes." As taken from Paillard F. 2014. Presse Med. 43(7-8), 848-51. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24954290

"To assess whether antioxidant, anti-inflammatory and other cardio-protective effects attributed to cocoa are achieved when regularly consuming moderate amounts of a flavanol-rich soluble cocoa product, a non-randomized, controlled, crossover, free-living study was carried out in healthy (n = 24; 25.9 ± 5.6 years) and moderately hypercholesterolemic (200-240 mg dL(-1); n = 20; 30.0 ± 10.3 years) volunteers. Participants consumed two servings per day (7.5 g per serving) of a soluble cocoa product (providing 45.3 mg flavanols per day) in milk, which was compared with consuming only milk during a 4 week period. The effects on systolic and diastolic blood pressure and heart rate

were determined, as well as on serum lipid and lipoprotein profiles, interleukins (IL)-1β, IL-6, IL-8, IL-10, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), vascular (VCAM-1) and intercellular cell adhesion molecules (ICAM-1), serum malondialdehyde (MDA), carbonyl groups (CG), ferric reducing/antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), and free radical scavenging capacity (ABTS). During the study, the volunteers' diets and physical activity were also evaluated, as well as any changes in weight, skin folds, circumferences and related anthropometric parameters. Cocoaand certain polyphenol-rich fruits and vegetables and their derivatives were restricted. After consuming the cocoa product positive effects were observed such as an increase in serum HDL-C ($P<0.001$) and dietary fiber intake ($p = 0.050$), whereas IL-10 decreased ($p = 0.022$). Other cardiovascular-related biomarkers and anthropometric parameters were unaffected. We have therefore concluded that regular consumption of thiscocoa product in a Spanish-Mediterranean diet may protect against cardiovascular disease in healthy and hypercholesterolemic subjects without producing any weight gain or other anthropometric changes." As taken from Martínez-López S et al. 2014. Food Funct. 5(2), 364-74. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24394704

"Flavanol-enriched chocolate consumption increases endothelium-dependent vasodilation. Most research so far has focused on flow-mediated dilation (FMD) only; the effects on other factors relevant to endothelial health, such as inflammation and leukocyte adhesion, have hardly been addressed. We investigated whether consumption of regular dark chocolate also affects other markers of endothelial health, and whether chocolate enrichment with flavanols has additional benefits. In a randomized double-blind crossover study, the effects of acute and of 4 wk daily consumption of high flavanol chocolate (HFC) and normal flavanol chocolate (NFC) on FMD, augmentation index (AIX), leukocyte count, plasma cytokines, and leukocyte cell surface molecules in overweight men (age 45-70 yr) were investigated. Sensory profiles and motivation scores to eat chocolate were also collected. Findings showed that a 4 wk chocolate intake increased FMD by 1%, which was paralleled by a decreased AIX of 1%, decreased leukocyte cell count, decreased plasma sICAM1 and sICAM3, and decreased leukocyte adhesion marker expression ($P<0.05$ for time effect), with no difference between HFC and NFC consumption. Flavanol enrichment did affect taste and negatively affected motivation to consume chocolate. This study provides new insights on how chocolate affects endothelial health by demonstrating that chocolate consumption, besides improving vascular function, also lowers the adherence capacity of leukocytes in the circulation." As taken from Esser D et al. 2014. FASEB J. 28(3), 1464-73. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24302679

"The consumption of cocoa and dark chocolate is associated with a lower risk of CVD, and improvements in endothelial function may mediate this relationship. Less is known about the effects of cocoa/chocolate on the augmentation index (AI), a measure of vascular stiffness and vascular tone in the peripheral arterioles. We enrolled thirty middle-aged, overweight adults in a randomised, placebo-controlled, 4-week, cross-over study. During the active treatment (cocoa) period, the participants consumed 37 g/d of dark chocolate and a sugar-free cocoa beverage (total cocoa = 22 g/d, total flavanols (TF) = 814 mg/d). Colour-matched controls included a low-flavanol chocolate bar and a cocoa-free beverage with no added sugar (TF = 3 mg/d). Treatments were matched for total fat, saturated fat, carbohydrates and protein. The cocoa treatment significantly increased the basal diameter and peak diameter of the brachial artery by 6% (+2 mm) and basal blood flow volume by 22%. Substantial decreases in the AI, a measure of arterial stiffness, were observed in only women. Flow-mediated dilation and the reactive hyperaemia index remained unchanged. The consumption of cocoa had no effect on fasting blood measures, while the control treatment increased fasting insulin concentration and insulin resistance ($P= 0·01$). Fasting blood pressure (BP) remained unchanged, although the acute consumption of cocoa increased resting BP by 4 mmHg. In summary, the high-flavanol cocoa and dark chocolate treatment was associated with enhanced vasodilation in both conduit and resistance arteries and was accompanied by significant reductions in arterial stiffness in women." As taken from West SG et al. 2014. Br. J. Nutr. 111(4), 653-61. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24274771

“Cocoa products present great health potential due to their high content of polyphenols, mainly of flavanols. However, the antioxidant, anti-inflammatory and other health effects of regularly consuming cocoa products seem to depend on the intake and health status of the consumer, etc. and need to be further clarified. A randomised, controlled, cross-over, free-living study was carried out in healthy (n 24) and moderately hypercholesterolaemic (>2000 mg/l, n 20) subjects to assess the influence of regularly consuming (4 weeks) two servings (15 g each) of a cocoaproduct rich in fibre (containing 33·9 % of total dietary fibre (TDF) and 13·9 mg/g of soluble polyphenols) in milk v. consuming only milk (control) on (1) serum lipid and lipoprotein profile, (2) serum malondialdehyde levels, carbonyl groups, ferric reducing/antioxidant power, oxygen radical absorbance capacity and free radical-scavenging capacity, (3) IL-1β, IL-6, TNF-α, IL-10, IL-8, monocyte chemoattractant protein-1, and vascular and intracellular cell adhesion molecule levels, and (4) systolic and diastolic blood pressure and heart rate. Throughout the study, the diet and physical activity of the volunteers, as well as any possible changes in weight or other anthropometric parameters, were also evaluated. The intake of TDF increased (P<0·001) to the recommended levels. Serum HDL-cholesterol (HDL-C) levels were increased (P<0·001), whereas glucose (P= 0·029), IL-1β (P= 0·001) and IL-10 (P= 0·001) levels were decreased. The rest of the studied cardiovascular parameters, as well as the anthropometric ones, remained similar. In conclusion, regularly consuming a cocoa product with milk improves cardiovascular health by increasing HDL-C levels and inducing hypoglycaemic and anti-inflammatory effects in healthy and hypercholesterolaemic individuals without causing weight gain.” As taken from Sarriá B et al. 2014. Br. J. Nutr. 111(1), 122-34. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23823716

“Chocolate consumption has been shown to protect against various cardiovascular end points; however, little is known about the association between chocolate consumption and incident atrial fibrillation (AF). Therefore, we prospectively examined the association between chocolate consumption and incident AF in a cohort of 18,819 US male physicians. Chocolate consumption was ascertained from 1999 to 2002 through a self-administered food frequency questionnaire. Incident AF was ascertained through yearly follow-up questionnaires. Cox regression was used to estimate relative risks of AF. The average age at baseline was 66 years (±9.1). During a mean follow-up of 9.0 years (±3.0), 2,092 cases of AF occurred. Using <1 per month of chocolate consumption as the reference group, multivariable adjusted hazard ratios (95% confidence interval) for AF were 1.04 (0.93 to 1.18), 1.10 (0.96 to 1.25), 1.14 (0.99 to 1.31), and 1.05 (0.89 to 1.25) for chocolate intake of 1 to 3 per month and 1, 2 to 4, and ≥5 per week (p for trend 0.25), respectively. In a secondary analysis, there was no evidence of effect modification by adiposity (p interaction = 0.71) or age (p interaction = 0.26). In conclusion, our data did not support an association between chocolate consumption and risk of AF in US male physicians.” As taken from Khawaja O et al. 2015. Am. J. Cardiol. 116(4), 563-6. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26076989

“ OBJECTIVE: To examine the association between chocolate intake and the risk of future cardiovascular events. METHODS: We conducted a prospective study using data from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort. Habitual chocolate intake was quantified using the baseline food frequency questionnaire (1993-1997) and cardiovascular end points were ascertained up to March 2008. A systematic review was performed to evaluate chocolate consumption and cardiovascular outcomes. RESULTS: A total of 20,951 men and women were included in EPIC-Norfolk analysis (mean follow-up 11.3±2.8 years, median 11.9 years). The percentage of participants with coronary heart disease (CHD) in the highest and lowest quintile of chocolate consumption was 9.7% and 13.8%, and the respective rates for stroke were 3.1% and 5.4%. The multivariate-adjusted HR for CHD was 0.88 (95% CI 0.77 to 1.01) for those in the top quintile of chocolate consumption (16-99 g/day) versus non-consumers of chocolate intake. The corresponding HR for stroke and cardiovascular disease (cardiovascular disease defined by the sum of CHD and stroke) were 0.77 (95% CI 0.62 to 0.97) and 0.86 (95% CI 0.76 to 0.97). The propensity score matched estimates showed a similar trend. A total of nine studies with 157,809 participants were included in the meta-analysis. Higher compared to lower

chocolate consumption was associated with significantly lower CHD risk (five studies; pooled RR 0.71, 95% CI 0.56 to 0.92), stroke (five studies; pooled RR 0.79, 95% CI 0.70 to 0.87), composite cardiovascular adverse outcome (two studies; pooled RR 0.75, 95% CI 0.54 to 1.05), and cardiovascular mortality (three studies; pooled RR 0.55, 95% CI 0.36 to 0.83). CONCLUSIONS: Cumulative evidence suggests that higher chocolate intake is associated with a lower risk of future cardiovascular events, although residual confounding cannot be excluded. There does not appear to be any evidence to say that chocolate should be avoided in those who are concerned about cardiovascular risk." As taken from Kwok CS et al. 2015. Heart 101(16), 1279-87. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26076934

"BACKGROUND: We aimed to examine the association between chocolate intake and the risk of incident heart failure in a UK general population. We conducted a systematic review and meta-analysis to quantify this association. METHODS AND RESULTS: We used data from a prospective population-based study, the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort. Chocolate intake was quantified based on a food frequency questionnaire obtained at baseline (1993-1997) and incident heart failure was ascertained up to March 2009. We supplemented the primary data with a systematic review and meta-analysis of studies which evaluated risk of incident heart failure with chocolate consumption. A total of 20,922 participants (53% women; mean age 58 ± 9 years) were included of whom 1101 developed heart failure during the follow up (mean 12.5 ± 2.7 years, total person years 262,291 years). After adjusting for lifestyle and dietary factors, we found 19% relative reduction in heart failure incidence in the top (up to 100 g/d) compared to the bottom quintile of chocolate consumption (HR 0.81 95%CI 0.66-0.98) but the results were no longer significant after controlling for comorbidities (HR 0.87 95%CI 0.71-1.06). Additional adjustment for potential mediators did not attenuate the results further. We identified five relevant studies including the current study (N = 75,408). The pooled results showed non-significant 19% relative risk reduction of heart failure incidence with higher chocolate consumption (HR 0.81 95%CI 0.66-1.01). CONCLUSIONS: Our results suggest that higher chocolate intake is not associated with subsequent incident heart failure." As taken from Kwok CS et al. 2016. Nutr. Metab. Cardiovacasc. Dis. 26(8), 722-34. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27052923

"BACKGROUND: we have evaluated the antihypertensive effect of several flavonoid extracts in a rat model of arterial hypertension caused by chronic administration (6 weeks) of the nitric oxide synthesis inhibitor, L-NAME. METHODS: Sprague Dawley rats received L-NAME alone or L-NAME plus flavonoid-rich vegetal extracts (Lemon, Grapefruit + Bitter Orange, and Cocoa) or purified flavonoids (Apigenin and Diosmin) for 6 weeks. RESULTS: L-NAME treatment resulted in a marked elevation of blood pressure, and treatment with Apigenin, Lemon Extract, and Grapefruit + Bitter Orange extracts significantly reduced the elevated blood pressure of these animals. Apigenin and some of these flavonoids also ameliorated nitric oxide-dependent and -independent aortic vasodilation and elevated nitrite urinary excretion. End-organ abnormalities such as cardiac infarcts, hyaline arteriopathy and fibrinoid necrosis in coronary arteries and aorta were improved by these treatments, reducing the end-organ vascular damage. CONCLUSIONS: the flavonoids included in this study, specially apigenin, may be used as functional food ingredients with potential therapeutic benefit in arterial hypertension." As taken from Paredes MD et al. 2018. Nutrients 10(4), E484. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29652818

"Oversupply of bulk saturated fatty acids (SFA) induces metabolic disorders and myocardial dysfunction. We investigated whether, without causing metabolic disorders, the uptake of individual dietary SFA species alters lipid profiles and induces myocardial dysfunction. C57BL/6 mice were fed various customized long-chain SFA diets (40% caloric intake from SFA), including a beef tallow (HBD), cocoa butter (HCD), milk fat (HMD) and palm oil diet (HPD), for 6 months. An isocaloric fat diet, containing medium-chain triglycerides, served as a control (CHD). Long-term intake of dietary long-chain SFA differentially affected the fatty acid composition in cardiac phospholipids. All long-chain SFA diets increased the levels of arachidonic acid and total SFA in cardiac phospholipids. The preferential incorporation of individual SFA into the cardiac phospholipid fraction was dependent on the dietary SFA species. Cardiac ceramide content was elevated in all mice fed long-

chain SFA diets, while cardiac hypertrophy was only presented in mice fed HMD or HPD. We have demonstrated that the intake of long-chain SFA species differentially alters cardiac lipid profiles and induces cardiac dysfunction, without causing remarkable metabolic disorders." As taken from Chen B et al. 2018. Nutrients 10(1), E106. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29351259

"Background: Three recent meta-analyses found significant prospective inverse associations between chocolate intake and cardiovascular disease risk. Evidence from these meta-analyses suggests that such inverse associations may only apply to elderly individuals or those with pre-existing major chronic disease. Objective: We assessed the association between habitual chocolate intake and subsequent incident coronary heart disease (CHD) and stroke, and the potential effect of modification by age. Design: We conducted multivariable Cox regression analyses using data from 83,310 postmenopausal women free of baseline pre-existing major chronic disease in the prospective Women's Health Initiative cohort. Chocolate intake was assessed using a food-frequency questionnaire. Physician-adjudicated events or deaths were ascertained up to 30 September 2013. Results: After exclusions, there were 3246 CHD and 2624 stroke events or deaths, representing incidence rates of 3.9% and 3.2% during 1,098,091 and 1,101,022 person-years (13.4 y), respectively. We found no association between consumption of chocolate and risk of CHD (P for linear trend = 0.94) or stroke (P = 0.24). The results for CHD and stroke combined were similar (P = 0.30), but were significantly modified by age (P for interaction = 0.02). For women age <65 y at baseline, those who ate 1 oz (28.35 g) of chocolate <1/mo, 1 to <1.5/mo, 1.5 to <3.5/mo, 3.5/mo to <3/wk, and ≥3/wk had HRs (95% CIs) of 1.00 (referent), 1.17 (1.00, 1.36), 1.05 (0.90, 1.22), 1.09 (0.94, 1.25), and 1.27 (1.09, 1.49), respectively (P for linear trend = 0.005). No association was apparent for older women. Conclusion: We observed no association between chocolate intake and risk of CHD, stroke, or both combined in participants free of pre-existing major chronic disease. The relation for both combined was modified by age, with a significant positive linear trend and an increased risk in the highest quintile of chocolate consumption among women age <65 y. This trial was registered at clinicaltrials.gov as NCT03453073." As taken from Greenberg JA et al. 2018. Am. J. Clin. Nutr. 108(1), 41-48. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29931040

"Introduction: Since ancient times, chocolate is a food highly appreciated by people and, therefore, is present in varied eating patterns. When rich in cocoa, it has a higher concentration of flavonoids. The consumption of foods rich in flavanols has been associated with a reduction in some risk factors for cardiovascular diseases, such as hypertension. Objectives: To evaluate the effects of frequent consumption of a fixed dose of cocoa-rich chocolate on the blood pressure of healthy young individuals. Methods: Thirty healthy individuals of both sexes, aged between 18 and 35 years, were randomized, but only 28 people completed the intervention. A baseline blood pressure assessment was performed. After this the..." [No further information available.] As taken from Coutinho D et al. 2019. European Journal of Public Health 29 (S1), ckz034.091. Available at https://academic.oup.com/eurpub/article-abstract/29/Supplement_1/ckz034.091/5480823

"Abnormal lipid metabolism is a major pathogenic factor for various cardiovascular diseases. The present study investigated the lipid profile of methanol extract of unfermented Theobroma cacao (TC) in Wistar rats. 24 Male Wistar rats were divided equally into four groups. Group 1 was the control group, and was administered 0.9% normal saline. Groups 2, 3 and 4 were administered 200mg/kg, 400mg/kg and 800mg/kg methanol extract of unfermented TC. Administration was via oral gavage and lasted for 21 days. The rats were sacrificed under chloroform anaesthesia. Blood was collected through cardiac puncture, allowed to clot, and later centrifuged to get serum. Laboratory assays were done for serum concentrations of total cholesterol (Tc), triacylglycerides (TAG), high density lipoprotein (HDL-c), and low density lipoprotein (LDL-c). Administration of TC extract resulted in an increase ($p<0.05$) serum concentration of HDL-c, with a consequent reduction in the serum concentrations of Tc, TAG, and LDL-c when compared with the control. The observed results showed that consumption of unfermented Theobroma cacao within the experimental dose promotes normal lipid profile of Wistar rats. Thus, if these results are extrapolated to man,

consumption of unfermented Theobroma cacao is encouraged." As taken from Agwupuye EI et al. 2019. International Journal of Trend in Scientific Research and Development (ijtsrd) 3(3), 190-193. Available at https://bit.ly/3gky1rc

"Chocolate is well known for its fine flavor, and its history began in ancient times, when the Maya considered chocolate (a cocoa drink prepared with hot water) the "Food of the Gods". The food industry produces many different types of chocolate: in recent years, dark chocolate, in particular, has gained great popularity. Interest in chocolate has grown, owing to its physiological and potential health effects, such as regulation of blood pressure, insulin levels, vascular functions, oxidation processes, prebiotic effects, glucose homeostasis, and lipid metabolism. However, further translational and epidemiologic studies are needed to confirm available results and to evaluate other possible effects related to the consumption of cocoa and chocolate, verifying in humans the effects hitherto demonstrated only in vitro, and suggesting how best to consume (in terms of dose, mode, and time) chocolate in the daily diet." As taken from Montagna MT et al. 2019. Int. J. Environ. Res. Public Health 16(24), 4960. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31817669/

"Purpose of Review The effect of cocoa consumption on blood pressure (BP) has been investigated in previous studies; however, to date, no meta-analysis has been conducted specific to middle-aged and elderly subjects. Thus, the aim of the present study was to evaluate the effect of cocoa consumption on indices of blood pressure, in middle-aged and elderly subjects. Recent Findings Pubmed/Medline™, Cochrane Library™, Google Scholar™, and Scopus™ were searched until March 2019. The quantitative Jadad scale was used as the systematic assessment of bias in the included trials. We used a random effects model to estimate the pooled weighted mean differences (WMDs) with 95% confidence intervals (CIs). We further conducted sensitivity analysis and stratified analysis by baseline blood pressure, follow-up duration, and mean age. Thirteen studies with 758 total participants were included in the present meta-analysis. A significant reduction in SBP by 2.77 (95% CI − 5.28, − 0.27, P = 0.03, I2 = 89%) and DBP by 1.47 mm/Hg (− 95% CI − 2.40, − 0.55, P = 0.001, I2 = 45%) were observed after cocoa consumption. Stratified analyses showed BP-lowering effects of cocoa consumption in longer-term duration and hypertensive subgroups. Summary Our meta-analysis showed a significant inverse association between cocoa consumption and SBP/DBP. However, the analysis could not conclude any beneficial effect of cocoa consumption on blood pressure in normotensive/elevated blood pressure subjects. Therefore, further studies are warranted to affirm the efficacy of cocoa consumption for the improvement of blood pressure in elderly subjects." As taken from Jafarnejad S et al. 2020. Curr. Hypertens. Rep. 22(1), 1. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/31907636/

6.3. Nervous system

Smit et al., (2004), reported ‘a normal portion of chocolate exhibits psychopharmacological activity. The identical profile of effects exerted by cocoa powder and its methylxanthine constituents shows this activity to be confined to the combination of caffeine and theobromine’. The authors also stated ‘Methylxanthines may contribute to the popularity of chocolate; however, other attributes are probably much more important in determining chocolate's special appeal and in explaining related self-reports of chocolate cravings and "chocoholism’ (Smit et al., 2004),

“The aim of this work was to search for eating disorders, DSM III-R Axis I mental disorders, personality disorders, and addictive behavior, in self-labeled "chocolate addicts". Subjects were recruited through advertisements placed in a university and a hospital. Fifteen subjects were included, 3 men and 12 women aged between 18 and 49. Most of them were not overweight, although 7 thought they had a weight problem. They consumed an average of 50 g per day of pure cacao and, for 13 subjects, this consumption was lasting since childhood or adolescence. The psychological effects of chocolate, as indicated by the subjects, consisted in feelings of increased energy or increased concentration ability, and in an anxiolytic effect during stress. Seven subjects

described minor withdrawal symptoms. None of the subjects reached the thresholds for eating disorders on the EAT and BULIT scales. The structured interview (MINI) identified an important ratio of subjects with a history of major depressive episode (13/15), and one woman was currently experiencing a major depressive episode. Four people suffered, or had suffered from anxiety disorders. Although only one subject satisfied all criteria for a personality disorder on the DIP-Q, seven displayed some pathological personality features. The self-labeled "chocoholics" do not seem to suffer from eating disorders, but may represent a population of psychologically vulnerable and depression--or anxiety--prone people. They seem to use chocolate as a light psychotropic drug able to relieve some of their distress. The amount of cacao consumed, although very chronically, remains moderate, and they rarely display other addictive behaviors." As taken from Dallard I et al. Encephale. 2001 Mar-Apr; 27(2), 181-6. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/11407271?dopt=AbstractPlus

Theobromine and the pharmacology of cocoa (Abstract). The effects of theobromine in man are underresearched, possibly owing to the assumption that it is behaviourally inert. Toxicology research in animals may appear to provide alarming results, but these cannot be extrapolated to humans for a number of reasons. Domestic animals and animals used for racing competitions need to be guarded from chocolate and cocoa-containing foods, including foods containing cocoa husks. Research ought to include caffeine as a comparative agent, and underlying mechanisms need to be further explored. Of all constituents proposed to play a role in our liking for chocolate, caffeine is the most convincing, though a role for theobromine cannot be ruled out. Most other substances are unlikely to exude a psychopharmacological effect owing to extremely low concentrations or the inability to reach the blood-brain barrier, whilst chocolate craving and addiction need to be explained by means of a culturally determined ambivalence towards chocolate. As taken from Smit HJ. Handb Exp Pharmacol. 2011; 200:201-34. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/20859797

"Cocoa powder and chocolate contain numerous substances among which there is a quite large percentage of antioxidant molecules, mainly flavonoids, most abundantly found in the form of epicatechin. These substances display several beneficial actions on the brain. They enter the brain and induce widespread stimulation of brain perfusion. They also provoke angiogenesis, neurogenesis and changes in neuron morphology, mainly in regions involved in learning and memory. Epicatechin improves various aspects of cognition in animals and humans. Chocolate also induces positive effects on mood and is often consumed under emotional stress. In addition, flavonoids preserve cognitive abilities during ageing in rats, lower the risk for developing Alzheimer's disease and decrease the risk of stroke in humans. In addition to their beneficial effects on the vascular system and on cerebral blood flow, flavonoids interact with signalization cascades involving protein and lipid kinases that lead to the inhibition of neuronal death by apoptosis induced by neurotoxicants such as oxygen radicals, and promote neuronal survival and synaptic plasticity. The present review intends to review the data available on the effects of cocoa and chocolate on brain health and cognitive abilities." As taken from Nehligh A. 2013. Br. J. Clin. Pharmacol. 75(3), 716-27. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/22775434?dopt=AbstractPlus

"All along the history, many kinds of magic and aphrodisiac properties were attributed to the chocolate. …. Containing flavoniods, cacao and its products have …. neuroprotective effects, ….. Other molecules, like methyxantin, biogenic amines and cannabinoid-like fatty acids, may have a psychoactive action. ….." As taken from Bianchi-Demicheli F et al. 2013. Rev. Med. Suisse 9(378), 624, 626-9. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23547364?dopt=AbstractPlus

"All along the history, many kinds of magic and aphrodisiac properties were attributed to the chocolate. Because of the presence of certain active substances, cacao and chocolate are supposed to have some potentially beneficial effects on human health, particularly on

cardiovascular system. Containing flavoniods, cacao and its products have antioxidant, anti-inflammatory, anti-atherogenic, anti-thrombotic, antihypertensive and neuroprotective effects, as well as influence on insulin sensitivity, vascular endothelial function, and activation of nitric oxide. Other molecules, like methyxantin, biogenic amines and cannabinoid-like fatty acids, may have a psychoactive action. Synergic effect of all these substances could have a positive direct and indirect influence on sexual health and function. Nevertheless, randomized studies are needed to confirm these hypotheses and to elaborate recommendations about cacao consumption." As taken from Bianchi-Demicheli F et al. 2013. Rev. Med. Suisse 9(378), 624, 626-9. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23547364?dopt=AbstractPlus

"Recent reports on cocoa are appealing in that a food commonly consumed for pure pleasure might also bring tangible benefits for human health. Cocoa consumption is correlated with reduced health risks of cardiovascular diseases, hypertension, atherosclerosis, and cancer, and the health-promoting effects of cocoa are mediated by cocoa-driven phytochemicals. Cocoa is rich in procyanidins, theobromine, (-)-epicatechin, catechins, and caffeine. Among the phytochemicals present in consumed cocoa, theobromine is most available in human plasma, followed by caffeine, (-)-epicatechin, catechin, and procyanidins. It has been reported that cocoa phytochemicals specifically modulate or interact with specific molecular targets linked to the pathogenesis of chronic human diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, obesity, diabetes, and skin aging. This review summarizes comprehensive recent findings on the beneficial actions of cocoa-driven phytochemicals in molecular mechanisms of human health." As taken from Kim J et al. 2014. Crit. Rev. Food Sci. Nutr. 54(11), 1458-72. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24580540?dopt=AbstractPlus

"Performance in many sports is at least partially dependent on motor control, coordination, decision-making, and other cognitive tasks. This review summarizes available evidence about the ingestion of selected nutrients or isolated compounds (dietary constituents) and potential acute effects on motor skill and/or cognitive performance in athletes. Dietary constituents discussed include branched-chain amino acids, caffeine, carbohydrate,cocoa flavanols, Gingko biloba, ginseng, guarana, Rhodiola rosea, sage, L-theanine, theobromine, and tyrosine...." As taken from Baker LB et al. 2014. Nutr. Rev. 72(12), 790-802. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25400063

"BACKGROUND: Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder, characterized by pathological aggregates of amyloid peptide-β (Aβ) and tau protein. Currently available therapies mediate AD symptoms without modifying disease progression. Polyphenol-rich diets are reported to reduce the risk for AD. OBJECTIVE: In the present study, we investigated the AD disease-modifying effects of cocoa, a rich source of flavanols, which are a class of polyphenols. We hypothesized that cocoa extracts interfere with amyloid-β oligomerization to prevent synaptic deficits. METHODS: We tested the effects of three different cocoa extracts, viz. Natural, Dutched, and Lavado extracts, on Aβ42 and Aβ40 oligomerization, using photo-induced cross-linking of unmodified proteins technique. To assess the effects of cocoa extracts on synaptic function, we measured long term potentiation in mouse brain hippocampal slices exposed to oligomeric Aβ. RESULTS: Our results indicate that cocoa extracts are effective in preventing the oligomerization of Aβ, with Lavado extract being most effective. Lavado extract, but not Dutched extract, was effective in restoring the long term potentiation response reduced by oligomeric Aβ. CONCLUSION: Our findings indicate that cocoa extracts have multiple disease-modifying properties in AD and present a promising route of therapeutic and/or preventative initiatives." As taken from Wang J et al. 2014. J. Alzheimers Dis. 41(2), 643-50. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24957018

"Background: Depression is one of the most common types of neurological disorder, which is a marked pattern of disturbances in emotional behavior, memory and hedonic processing. Aim and Objectives: To investigate the role of ethanolic extract of Theobroma cacao seed on the prefrontal

cortex of female Wistar rats following reserpine-induced depression. Material and Methods: Thirty-six (36) female Wistar rats were used for this study. They were divided into six (A - F) groups (n = 6). Group A - control, Group B - 0.2 mg/kg reserpine, Group C - 10 mg/kg fluoxetine, Group D - 500 mg/kg Theobroma cacao seed extract, Group E - 0.2 mg/kg reserpine + 500mg/kg Theobroma cacao seed extract, Group F - 0.2 mg/kg reserpine + 10mg/kg fluoxetine. Animals were euthanized via cervical dislocation after the last day of administration and the prefrontal cortex and hippocampus were excised and fixed in 10% formalin solution for routine histological processing while the part used for biochemical assay were homogenized in phosphate buffer before centrifugation. Results: Morphological alteration and reduced population of prefrontal cortex and hippocampus neurons, reduced protein synthesis, poor behavioral patterns, reduced neurotransmission and induction of oxidative stress in reserpine exposed animal. Conclusion: Theobroma cacao seed extract was able to mitigate these aberrations." As taken from Adebola AO et al. 2020. Journal of Krishna Institute of Medical Sciences 9(1), 27-35. Available at https://bit.ly/3fKmbGq

"Restricted intermittent food access to palatable food (PF) induces addiction-like behaviors and plastic changes in corticolimbic brain areas. Intermittent access protocols normally schedule PF to a fixed time, enabling animals to predict the arrival of PF. Because outside the laboratory the presence of PF may occur in a random unpredictable manner, the present study explored whether random access to PF would stimulate similar addiction-like responses as observed under a fixed scheduled. Rats were randomly assigned to a control group without chocolate access, to ad libitum access to chocolate, to fixed intermittent access (CH-F), or to random unpredictable access (CH-R) to chocolate. Only the CH-F group developed behavioral and core temperature anticipation to PF access. Both groups exposed to intermittent access to PF showed binge eating, increased effort behaviors to obtain chocolate, as well as high FosB/ΔFosB in corticolimbic areas. Moreover, FosB/ΔFosB in all areas correlated with the intensity of binge eating and effort behaviors. We conclude that both conditions of intermittent access to PF stimulate addiction-like behaviors and FosB/ΔFosB accumulation in brain reward areas; while only a fixed schedule, which provides a time clue, elicited anticipatory activation, which is strongly associated with craving behaviors and may favor relapse during withdrawal." As taken from Muñoz-Escobar G et al. 2019. Sci. Rep. 9(1), 18223. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31796782/

"Migraine is a chronic disorder with episodic attacks, and patients with a migraine often report that certain factors can trigger their headache, with chocolate being the most popular type of food-based trigger. Many studies have suggested a link between chocolate and headaches; however, the underlying physiological mechanisms are unclear. As premonitory symptoms may herald migraine attacks, a question arises regarding whether eating chocolate before a headache is a consequence of a food craving or indeed a real trigger. Here, we aim to summarize the available evidence on the relationship between chocolate and migraines. All articles concerning this topic published up to January 2020 were retrieved by searching clinical databases, including EMBASE, MEDLINE, PubMed, and Google Scholar. All types of studies have been included. Here, we identify 25 studies investigating the prevalence of chocolate as a trigger factor in migraineurs. Three provocative studies have also evaluated if chocolate can trigger migraine attacks, comparing it to a placebo. Among them, in 23 studies, chocolate was found to be a migraine trigger in a small percentage of participants (ranging from 1.3 to 33), while all provocative studies have failed to find significant differences between migraine attacks induced by eating chocolate and a placebo. Overall, based on our review of the current literature, there is insufficient evidence that chocolate is a migraine trigger; thus, doctors should not make implicit recommendations to migraine patients to avoid it." As taken from Nowaczewska M et al. 2020. Nutrients 12(3), 608. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32110888/

"Cocoa has been reported to have medicinal properties. It contains a wide range of phytochemicals, including polyphenols, which have been shown to exert anti-inflammatory and antioxidant actions, and also to have a positive effect on pain. Other components of cocoa might be able to positively influence pain perception through various mechanisms. Despite encouraging

results from preclinical studies, there is a lack of evidence of antinociceptive effects of cocoa from clinical trials in humans. Further research is needed to better identify the active principles in cocoa, to understand the underlying mechanisms of action, and to establish efficacy in humans." As taken from De Feo M et al. 2020. Pain Ther. 9(1), 231-240. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32314320/

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

“We performed a functional genomic analysis to study the effect of epicatechin and polyphenolic cocoa extract in the human colon adenocarcinoma cell line Caco-2. The specific Human Hematology/Immunology cDNA arrays by Clontech, containing 406 genes in duplicate, were used. The differentially expressed genes were classified according to their level of expression, calculated as the ratio of the value obtained after each treatment relative to control cells, with a statistical significance of $P<0.05$ (upregulated: ratio > 1.5; downregulated: ratio <0.6). Treatment with epicatechin decreased the expression of 21 genes and upregulated 24 genes. Upon incubation with the cocoa polyphenolic extract, 24 genes were underexpressed and 28 were overexpressed. The changes in expression for ferritin heavy polypeptide 1 (FTH1), mitogen-activated protein kinase kinase 1 (MAPKK1), signal transducer and activator of transcription 1 (STAT1), and topoisomerase 1 upon incubation with epicatechin, and for myeloid leukemia factor 2 (MLF2), CCAAT/enhancer binding protein gamma (C/EBPG), MAPKK1, ATP-binding cassette, subfamily c member 1 (MRP1), STAT1, topoisomerase 1, and x-ray repair complementing defective repair 1 (XRCC1) upon incubation with the cocoa polyphenolic extract were validated by RT-PCR. Changes in the messenger RNA levels for MAPKK1, STAT1, MRP1, and topoisomerase 1 upon incubation with either epicatechin or cocoa extract were further confirmed at the protein level by Western blotting. The changes in the expression of STAT1, MAPKK1, MRP1, and FTH1 genes, which are involved in the cellular response to oxidative stress, are in agreement with the antioxidant properties of cocoa flavonoids. In addition, the changes in the expression of C/EBPG, topoisomerase 1, MLF2, and XRCC1 suggest novel mechanisms of action of flavonoids at the molecular level.” As taken from Noe V et al. J Nutr. 2004 Oct; 134(10), 2509-16. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/15465739?dopt=AbstractPlus

“It has been known for some time that cocoa butter, although rich in saturated fatty acids, does not raise total serum cholesterol concentrations as much as expected from its total saturated fatty acid content. Whether the effect of cocoa butter feeding on low-density-lipoprotein- (LDL)-cholesterol concentrations was also less than predicted by its total saturated fatty acid content needed to be tested. In a recent experiment cocoa butter did not raise LDL cholesterol as much as predicted by its total saturated fatty acid content. However, because of its significant palmitic acid content, cocoa butter did raise LDL-cholesterol concentrations more than do most liquid vegetable oils.” As taken from Denke MA Am J Clin Nutr. 1994 Dec; 60(6 Suppl), 1014S-1016S. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/7977142?dopt=AbstractPlus

“Cocoa products, which are rich sources of flavonoids, have been shown to reduce blood pressure and the risk of cardiovascular disease. Dark chocolate contains saturated fat and is a source of dietary calories; consequently, it is important to determine whether consumption of dark chocolate adversely affects the blood lipid profile. The objective was to examine the effects of dark chocolate/cocoa product consumption on the lipid profile using published trials. A detailed literature search was conducted via MEDLINE (from 1966 to May 2010), CENTRAL and ClinicalTrials.gov for randomized controlled clinical trials assessing the effects of flavanol-rich cocoa products or dark chocolate on lipid profile. The primary effect measure was the difference in means of the final measurements between the intervention and control groups. In all, 10 clinical trials consisting of 320 participants were included in the analysis. Treatment duration ranged from 2 to 12 weeks. Intervention with dark chocolate/cocoa products significantly reduced serum low-density lipoprotein (LDL) and total cholesterol (TC) levels (differences in means (95% CI) were -5.90 mg/dl (-10.47, -1.32 mg/dl) and -6.23 mg/dl (-11.60, -0.85 mg/dl), respectively). No statistically significant effects

were observed for high-density lipoprotein (HDL) (difference in means (95% CI), -0.76 mg/dl (-3.02 to 1.51 mg/dl)) and triglyceride (TG) (-5.06 mg/dl (-13.45 to 3.32 mg/dl)). These data are consistent with beneficial effects of dark chocolate/cocoa products on total and LDL cholesterol and no major effects on HDL and TG in short-term intervention trials" (Tokede et al., 2011).

Chemopreventive effects of cocoa polyphenols on chronic diseases (Abstract). We have explored the causes of the major chronic diseases prevailing in the world and the relevant mechanisms as a sound basis for recommendations for their prevention. Research shows that the cocoa bean, and tasty products derived from the cocoa bean such as chocolate, and the beverage cocoa, popular with many people worldwide, is rich in specific antioxidants, with the basic structure of catechins and epicatechin, and especially the polymers procyanidins, polyphenols similar to those found in vegetables and tea. Metabolic epidemiological studies indicate that regular intake of such products increases the plasma level of antioxidants, a desirable attribute as a defense against reactive oxygen species (ROS). The antioxidants in cocoa can prevent the oxidation of LDL-cholesterol, related to the mechanism of protection in heart disease. Likewise, a few studies show that ROS associated with the carcinogenic processes is also inhibited, although there have not been many studies on a possible lower risk of various types of cancer either in humans or in animal models consuming cocoa butter or chocolates. Based on the knowledge acquired thus far, it would seem reasonable to suggest inhibition of the several phases of the complex processes leading to cancer, as a function of quantitative intake of antioxidants, including those from cocoa and chocolates. Cocoa and chocolate also contain fats from cocoa butter. These are mainly stearic triglycerides (C18:0) that are less well absorbed than other fats, and are excreted in the feces. Thus, cocoa butter is less bioavailable and has minimal effect on serum cholesterol. As taken from Weisburger JH. Exp Biol Med (Maywood). 2001 Nov; 226(10), 891-7. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/11682694

"Abstract Objective: This trial was undertaken to evaluate the effects of high-cocoa-content chocolate supplementation in pregnancy on several haematochemical and clinical parameters. The study had as reference population the pregnant women requesting an obstetric control at Outpatient Clinic of Obstetrics and Gynaecology of the S. Maria della Misericordia University Hospital, Perugia, Italy. Were candidated to enter in this study all Caucasian women of 18-40 year-olds, who had a single gestation pregnancy between 11th+0 and 13th+0 week gestational age. Methods: We conducted a single-center randomized controlled trial. The pregnant women selected were randomized into Group A, which received daily doses of 30 g of chocolate (70%cocoa), and Group B, which was free to increase their diet with other foods. Results: Ninety women were randomized. Significant difference was found between the two groups for diastolic blood pressure ($p=0.05$), systolic ($P<0.0001$) and levels of liver enzymes, with values lower in Group A than in Group B. Total cholesterol levels and weight gain in Group A did not increase more than in Group B. Conclusions: A modest daily intake of high-cocoa-content chocolate contributes to reduce blood pressure, glycemic and liver pattern during pregnancy without affecting the weight gain" (Di Renzo et al., 2012).

"Objective: The aim of our study was to analyze the effects of caffeine and chocolate (70% cocoa) on fetal heart rate (FHR). Study design: Fifty pregnant women with uncomplicated gestation, matched for age and parity, underwent computerized FHR recording before and after the consumption of caffeine and then, after one week, before and after 70% cocoa chocolate intake. Computerized cardiotocography (cCTG) parameters were expressed as mean and SD. The differences were tested for statistical significance using the paired t-test, with significance at $p < 0.05$. Results: The number of uterine contraction peaks, the number of small and large accelerations (10 and 15 beats per minute for 15 seconds), the duration of episodes of high variation and the short-term FHR variation were significantly higher ($p < 0.001$) after maternal coffee intake. The number of large accelerations, the duration of episodes of high variation and the short-term FHR variation were significantly higher ($p < 0.001$) after maternal consumption of chocolate, whilst no effect of cocoa was found during contractions. Conclusions: Our results suggest that maternal intake of both caffeine and 70% cocoa have a stimulating action on fetal reactivity. This

finding is likely due to the pharmacological action of theobromine, a methilxanthine present in coffee and in chocolate. The correlation between maternal caffeine intake and increased uterine contraction peaks is likely due to the effect of caffeine on the uterine muscle" (Buscicchio et al., 2012).

Long-term ingestion of high flavanol cocoa provides photoprotection against UV-induced erythema and improves skin condition in women (Abstract). Dietary antioxidants contribute to endogenous photoprotection and are important for the maintenance of skin health. In the present study, 2 groups of women consumed either a high flavanol (326 mg/d) or low flavanol (27 mg/d) cocoa powder dissolved in 100 mL water for 12 wk. Epicatechin (61 mg/d) and catechin (20 mg/d) were the major flavanol monomers in the high flavanol drink, whereas the low flavanol drink contained 6.6 mg epicatechin and 1.6 mg catechin as the daily dose. Photoprotection and indicators of skin condition were assayed before and during the intervention. Following exposure of selected skin areas to 1.25 x minimal erythemal dose (MED) of radiation from a solar simulator, UV-induced erythema was significantly decreased in the high flavanol group, by 15 and 25%, after 6 and 12 wk of treatment, respectively, whereas no change occurred in the low flavanol group. The ingestion of high flavanol cocoa led to increases in blood flow of cutaneous and subcutaneous tissues, and to increases in skin density and skin hydration. Skin thickness was elevated from 1.11 +/- 0.11 mm at wk 0 to 1.24 +/- 0.13 mm at wk 12; transepidermal water loss was diminished from 8.7 +/- 3.7 to 6.3 +/- 2.2 g/(h x m2) within the same time frame. Neither of these variables was affected in the low flavanol cocoa group. Evaluation of the skin surface showed a significant decrease of skin roughness and scaling in the high flavanol cocoa group compared with those at wk 12. Dietary flavanols from cocoa contribute to endogenous photoprotection, improve dermal blood circulation, and affect cosmetically relevant skin surface and hydration variables. As taken from Heinrich U et al. J Nutr. 2006 Jun;136(6), 1565-9. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/16702322

Acute dark chocolate and cocoa ingestion and endothelial function: a randomized controlled crossover trial (Abstract).

BACKGROUND: Studies suggest cardioprotective benefits of dark chocolate containing cocoa.

OBJECTIVE: This study examines the acute effects of solid dark chocolate and liquid cocoa intake on endothelial function and blood pressure in overweight adults. DESIGN: Randomized, placebo-controlled, single-blind crossover trial of 45 healthy adults [mean age: 53 y; mean body mass index (in kg/m(2)), 30]. In phase 1, subjects were randomly assigned to consume a solid dark chocolate bar (containing 22 g cocoa powder) or a cocoa-free placebo bar (containing 0 g cocoa powder). In phase 2, subjects were randomly assigned to consume sugar-free cocoa (containing 22 g cocoa powder), sugared cocoa (containing 22 g cocoa powder), or a placebo (containing 0 g cocoa powder). RESULTS: Solid dark chocolate and liquid cocoa ingestion improved endothelial function (measured as flow-mediated dilatation) compared with placebo (dark chocolate: 4.3 +/- 3.4% compared with -1.8 +/- 3.3%; P<0.001; sugar-free and sugared cocoa: 5.7 +/- 2.6% and 2.0 +/- 1.8% compared with -1.5 +/- 2.8%; P<0.001). Blood pressure decreased after the ingestion of dark chocolate and sugar-free cocoa compared with placebo (dark chocolate: systolic, -3.2 +/- 5.8 mm Hg compared with 2.7 +/- 6.6 mm Hg; P<0.001; and diastolic, -1.4 +/- 3.9 mm Hg compared with 2.7 +/- 6.4 mm Hg; P = 0.01; sugar-free cocoa: systolic, -2.1 +/- 7.0 mm Hg compared with 3.2 +/- 5.6 mm Hg; P<0.001; and diastolic: -1.2 +/- 8.7 mm Hg compared with 2.8 +/- 5.6 mm Hg; P = 0.014). Endothelial function improved significantly more with sugar-free than with regular cocoa (5.7 +/- 2.6% compared with 2.0 +/- 1.8%; P<0.001). CONCLUSIONS: The acute ingestion of both solid dark chocolate and liquid cocoa improved endothelial function and lowered blood pressure in overweight adults. Sugar content may attenuate these effects, and sugar-free preparations may augment them. As taken from Faridi Z et al. Am J Clin Nutr. 2008 Jul; 88(1), 58-63. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/18614724

“Previous studies have shown that rat intestinal immunoglobulin A (IgA) concentration and lymphocyte composition of the intestinal immune system were influenced by a highly enriched cocoa diet. The aim of this study was to dissect the mechanisms by which a long-term high cocoa intake was capable of modifying gut secretory IgA in Wistar rats. After 7 weeks of nutritional intervention, Peyer's patches, mesenteric lymph nodes and the small intestine were excised for gene expression assessment of IgA, transforming growth factor β, C-C chemokine receptor-9 (CCR9), interleukin (IL)-6, CD40, retinoic acid receptors (RARα and RARβ), C-C chemokine ligand (CCL)-25 and CCL28 chemokines, polymeric immunoglobulin receptor and toll-like receptors (TLR) expression by real-time polymerase chain reaction. As in previous studies, secretory IgA concentration decreased in intestinal wash and fecal samples after cocoa intake. Results from the gene expression showed that cocoa intake reduced IgA and IL-6 in Peyer's patches and mesenteric lymph nodes, whereas in small intestine, cocoa decreased IgA, CCR9, CCL28, RARα and RARβ. Moreover, cocoa-fed animals presented an altered TLR expression pattern in the three compartments studied. In conclusion, a high-cocoa diet down-regulated cytokines such as IL-6, which is required for the activation of B cells to become IgA-secreting cells, chemokines and chemokine receptors, such as CCL28 and CCR9 together with RARα and RARβ, which are involved in the gut homing of IgA-secreting cells. Moreover, cocoa modified the cross-talk between microbiota and intestinal cells as was detected by an altered TLR pattern. These overall effects in the intestine may explain the intestinal IgA down-regulatory effect after the consumption of a long-term cocoa-enriched diet” (Pérez-Berezo et al., 2011).

“The aim of this study was to compare the effects of cocoa butter and safflower oil on hepatic transcript profiles, lipid metabolism and insulin sensitivity in healthy rats. Cocoa butter-based high-fat feeding for 3 days did not affect plasma total triglyceride (TG) levels or TG-rich VLDL particles or hepatic insulin sensitivity, but changes in hepatic gene expression were induced that might lead to increased lipid synthesis, lipotoxicity, inflammation and insulin resistance if maintained. Safflower oil increased hepatic beta-oxidation, was beneficial in terms of circulating TG-rich VLDL particles, but led to reduced hepatic insulin sensitivity. The effects of safflower oil on hepatic gene expression were partly overlapping with those exerted by cocoa butter, but fewer transcripts from anabolic pathways were altered. Increased hepatic cholesterol levels and increased expression of hepatic CYP7A1 and ABCG5 mRNA, important gene products in bile acid production and cholesterol excretion, were specific effects elicited by safflower oil only. Common effects on gene expression included increased levels of p8, DIG-1 IGFBP-1 and FGF21, and reduced levels of SCD-1 and SCD-2. This indicates that a lipid-induced program for hepatic lipid disposal and cell survival was induced by 3 days of high-fat feeding, independent on the lipid source. Based on the results, we speculate that hepatic TG infiltration leads to reduced expression of SCD-1, which might mediate either neutral, beneficial or unfavorable effects on hepatic metabolism upon high-fat feeding, depending on which fatty acids were provided by the diet”. As taken from Gustavsson C et al. 2009. Lipids 44, 1011-1027. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/19806378?dopt=AbstractPlus.

“A diet containing 10 % cocoa, a rich source of polyphenols and fibre, is able to modify intestinal immune status as well as microbiota composition. The present study was aimed at investigating whether cocoa flavonoid content is uniquely responsible for these modulatory effects of cocoa, and to establish whether these effects depend on the rat strain. To this end, 3-week-old Wistar and Brown Norway rats were fed, for 4 weeks, either a standard diet or the following three isoenergetic diets containing increasing proportions of cocoa flavonoids from different sources: one with 0·2 % polyphenols (from conventional defatted cocoa), and two others with 0·4 and 0·8 % polyphenols (from non-fermented cocoa, very rich in polyphenols). Serum Ig concentrations, faecal IgA levels, microbiota composition and IgA-coating bacterial proportion were evaluated at the beginning and at the end of the study. After the nutritional intervention, the composition of lymphocytes in Peyer's patches and mesenteric lymph nodes was evaluated. In some respects, the Wistar strain was more sensitive to the impact of the cocoa diets than the Brown Norway strain. After 4 weeks of dietary intervention, similar modulatory effects of the diets containing 0·2 and 0·8 % polyphenols on

mucosal IgA levels and microbiota composition were found, although the 0·2 % diet, with a higher proportion of theobromine and fibre, had more impact, suggesting that polyphenols are not the only components involved in such effects." As taken from Massot-Cladera M et al. 2014. Br. J. Nutr. 112(12), 1944-54. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25345541

"This study investigated the effects of cocoa butter and sunflower oil alone and in combination on performance, some biochemical parameters, immunoglobulin, and antioxidant vitamin status in Wistar rats. Forty-eight male rats were assigned to four groups, consisting of 12 rats with 3 replicates. Control received balanced rat diet without oil, cocoa butter group received 3.5% cocoa butter, sunflower oil group received 3.5% sunflower oil, the last group received 1.75% sunflower oil + 1.75% cocoa butter supplementation in the rat diet for 8 weeks. ..... The serum creatinine level was decreased in cocoa butter group compared to control. Triglyceride and VLDL cholesterol levels were decreased in only sunflower oil and only cocoa butter groups as compared to control. The level of Ig M was statistically lower in cocoa butter and cocoa butter + sunflower oil groups than in control and sunflower oil groups. There were no statistically important difference in vitamin concentrations among trial groups. It was concluded that the supplementation of cocoa butter in diet decreased Ig M level, while the supplementation of cocoa butter and sunflower oil alone decreased the triglyceride and VLDL cholesterol levels." As taken from Yildirim E et al. 2014. Biomed. Res. Int. 2014, 606575. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25136602?dopt=AbstractPlus

"Although polyphenols are often merely perceived as antioxidants, their biological activities are manifold and include anti-inflammatory actions. A new area of research on polyphenols and health concerns their putative role in cholesterol metabolism, in particular, their high-density lipoprotein-cholesterol (HDL-c)-raising potential. Indeed, some human studies showed that administration of polyphenol-rich foods such as cocoa, green tea, and extra virgin olive oil modulate and increase HDL-c concentrations. This study assessed the effects of polyphenols on intestinal inflammation, using the physiologically relevant Caco-2 Transwell model and using lipopolysaccharide (LPS) to trigger inflammation. This study also investigated the mechanisms of actions behind the proposed HDL-c-increasing effects of polyphenols. The data suggest that polyphenols (at least those from red wine, cocoa, and green tea) administered at a dietary dose moderately modulate intestinal inflammation but do not increase cholesterol secretion by intestinal cells or enhance HDL functionality." As taken from Nicod N et al. 2014. J. Agric. Food Chem. 62(10), 2228-32. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24559192

"Consumption of cocoa-enriched dark chocolate (DC) has been shown to alter glucose and insulin concentration during rest and exercise compared with cocoa-depleted control (CON). However, the impact of DC consumption on exercise metabolism and performance is uncertain. Therefore, we investigated carbohydrate metabolism via stable isotope tracer techniques during exercise after subjects ingested either DC or CON. Sixteen overnight-fasted male cyclists performed a single-blinded, randomized, crossover design trial, after consuming either DC or CON at 2 h prior to 2.5 h of steady-state (SS) exercise (~45% peak oxygen uptake). This was followed by an ~15-min time-trial (TT) and 60 min of recovery. [6,6-(2)H2]Glucose and [U-(13)C]glucose were infused during SS to assess glucose rate of appearance (Ra) and disappearance (Rd). After DC consumption, plasma (-)-glucose and insulin concentrations were significantly ($P<0.001$) elevated throughout vs. CON. During SS, there was no difference in [6,6-(2)H2]glucose Ra between treatments, but towards the end of SS (last 60 min) there was a ~16% decrease in Rd in DC vs. CON ($P<0.05$). Accordingly, after DC there was an ~18% significant decrease in plasma glucose oxidation (trial effect; $p = 0.032$), and an ~15% increase in tracer-derived muscle glycogen utilization ($p = 0.045$) late during SS exercise. The higher blood glucose concentrations during exercise and recovery after DC consumption coincided with high concentrations of epicatechin and (or) theobromine. In summary, DC consumption altered muscle carbohydrate partitioning, between muscle glucose uptake and glycogen oxidation, but did not effect cycling TT performance." As taken from Stellingwerff T et al. 2014. Appl. Physiol. Nutr. Metab. 39(2), 173-82. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24476473

“Estrogen is a hormone that influences the growth of uterus. Ovariectomized rats lose their ovaries as the main source of estrogen so their uterus tends to shrink. The objective of this study was to evaluate the potency of cocoa as an estrogen substitute, which was done through an experiment that tested the influence of cocoa products on the uterus weight of ovariectomized rats. There were five treatments and in each of the treatments, six wistar rats were given the substance for three days. Four groups of treatment consisted of ovariectomized rats given cocoa extract (875.2 ppm of polyphenols, 1 g/kg of body weight, BW), cocoa powder (369.8 ppm of polyphenols, 1 g/kg BW), olive oil (10 mL/kg BW), or estradiol valerate (1 mg/kg BW). The fifth group consisted of intact (non-ovariectomized) rats given 10 mL/kg BW of water. The finding showed that the ovariectomized rats given olive oil had lower uterus weight than that of intact rats, while the ovariectomized rats given estradiol valerate had higher uterus weight compared to the intact and olive oil groups. The ovariectomized rats given cocoa powder and extract had higher uterus weight compared to those given only olive oil; although they were not significantly different. Correlation between the body weight and uterus weight varied across treatment groups. The rats given olive oil showed significant, positive correlation, while the intact rats showed moderate, positive correlation. The rats given estradiol valerate and cocoa powder showed non-significant correlation. Since the polyphenol content in cocoa powder was at lower concentration than that in the cocoa extract, it was predicted that cocoa polyphenols are more potential in the lower concentration. This study concludes that even though consumption of cocoa powder and extract did not significantly induced uterus growth, cocoa is still considered having estrogenic activity by lowering the correlation between the body weight and uterus weight in ovariectomized rats.” As taken from Sari ABT et al. 2017. Pelita 33(1), 45-50. Available at https://www.ccrjournal.com/index.php/ccrj/article/view/253

"During menopause, women undergo a series of physiological changes that include a redistribution of fat tissue. This study was designed to investigate the effect of adding 10 g of cocoa-rich chocolate to the habitual diet of postmenopausal women daily on body composition. We conducted a 6-month, two-arm randomised, controlled trial. Postmenopausal women (57·2 (sd 3·6) years, n 132) were recruited in primary care clinics. Participants in the control group (CG) did not receive any intervention. Those of the intervention group (IG) received 10 g daily of 99 % cocoa chocolate in addition to their habitual diet for 6 months. This quantity comprises 247 kJ (59 kcal) and 65·4 mg of polyphenols. The primary outcomes were the between-group differences in body composition variables, measured by impendancemetry at the end of the study. The main effect of the intervention showed a favourable reduction in the IG with respect to the CG in body fat mass (–0·63 kg (95 % CI –1·15, –0·11), P = 0·019; Cohen’s d = –0·450) and body fat percentage (–0·79 % (95 % CI –1·31, –0·26), P = 0·004; Cohen’s d = –0·539). A non-significant decrease was also observed in BMI (–0·20 kg/m2 (95 % CI –0·44, 0·03), P = 0·092; Cohen’s d = –0·345). Both the body fat mass and the body fat percentage showed a decrease in the IG for the three body segments analysed (trunk, arms and legs). Daily addition of 10 g of cocoa-rich chocolate to the habitual diet of postmenopausal women reduces their body fat mass and body fat percentage without modifying their weight." As taken from Garcia-Yu IA et al. 2020. British Journal of Nutrition 125(5), 548-556. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32746952/

7. Addiction

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

8. Burnt ingredient toxicity

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

Tobacco smoke condensates from cigarettes containing Cocoa, cocoa shells, powder and extract 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 Cocoa, cocoa shells, powder and extract. Table below provides tested level(s) and specific endpoint(s).

Endpoint	**Tested level (ppm)**	**Reference**
Smoke chemistry	9,705 (food, no CAS) 1,932 (shell extract, 8002-31-1) 772 (extract, 84649-99-0) 14,776 (Cocoa shells, no CAS)	Carmines, 2002 & Rustemeier et al., 2002
	20,000 (powder 84649-99-0) 23 ppm (cocoa oleoresin, no CAS)	Baker et al., 2004a
	10,000 (No CAS) 18,000	JTI KB Study Report(s)
	43,800	Gaworski et al., 2011 & Coggins et al., 2011c
	621 (8002-31-1) 111 (84649-99-0) 1005 (95009-22-6) 12 (977075-45-8)	Roemer et al, 2014b
	14400	Stabbert et al. ,2019
In vitro genotoxicity	9,705 (food, no CAS) 1,932 (shell extract, 8002-31-1) 772 (extract, 84649-99-0) 14,776 (Cocoa shells, no CAS)	Carmines, 2002 & Roemer et al., 2002
	37,700 (powder, 84649-99-0) 23 ppm (cocoa oleoresin, no CAS)	Baker et al., 2004c
	10,000 (No CAS)	JTI KB Study Report(s)
	25,800 (8002-31-1 and 84649-99-0)	fGLH Study Report (2010)
	43,800	Gaworski et al., 2011 & Coggins et al., 2011c
	-	Roemer et al, 2010
	621 (8002-31-1) 111 (84649-99-0) 1005 (95009-22-6) 12 (977075-45-8)	Roemer et al, 2014b

	14400	Stabbert et al. ,2019
In vitro cytotoxicity	9,705 (food, no CAS) 1,932 (shell extract, 8002-31-1) 772 (extract, 84649-99-0) 14,776 (Cocoa shells, no CAS)	Carmines, 2002 & Roemer et al., 2002
	37,700 (powder, 84649-99-0) 23 ppm (cocoa oleoresin, no CAS)	Baker et al., 2004c
	25,800 (8002-31-1 and 84649-99-0)	fGLH Study Report (2010)
	43,800	Gaworski et al., 2011 & Coggins et al., 2011c
	-	Roemer et al, 2010
	621 (8002-31-1) 111 (84649-99-0) 1005 (95009-22-6) 12 (977075-45-8)	Roemer et al, 2014b
	14400	Stabbert et al. ,2019
Inhalation study	44,000 (powder, 84649-99-0) 2 (extract, 84649-99-0)	Gaworski et al., 1998
	9,705 (food, no CAS) 1,932 (shell extract, 8002-31-1) 772 (extract, 84649-99-0) 14,776 (Cocoa shells, no CAS)	Carmines, 2002 & Vanscheeuwijck et al., 2002
	37,700 (powder, 84649-99-0) 23 ppm (cocoa oleoresin, no CAS)	Baker et al., 2004c
	10,000 (No CAS)	JTI KB Study Report(s)
	43,800	Gaworski et al., 2011 & Coggins et al., 2011c
	621 (8002-31-1) 111 (84649-99-0) 1005 (95009-22-6) 12 (977075-45-8)	Schramke et al, 2014
Skin painting	44,000 (84649-99-0)	Gaworski et al., 1999

	10,000 (No CAS)	JTI KB Study Report(s)
In vivo genotoxicity	621 (8002-31-1) 111 (84649-99-0) 1005 (95009-22-6) 12 (977075-45-8)	Schramke et al, 2014

Added at 1% (w/w), in a casing, cocoa increased cigarette smoke yields of tar, nicotine, phenol and catechol. It decreased smoke yields of isoprene, formaldehyde, nitrogen oxides, carbon monoxide and carbon dioxide (NCI 1977).

“In 1977 the Tobacco Working Group (TWG) of the National Cancer Institute (NCI) reported the results of a mouse skin painting study using condensate from cigarettes that contained 1% cocoa powder. ICR Swiss female mice (100 per group) were painted once daily, six days a week, with a condensate solution containing 12.5 mg (low dose group) or 25 mg (high dose group) of dry smoke condensate, or with positive or negative control solutions. The duration of the study was 18 months. All mice dying during the study or sacrificed at termination were necropsied. The tissues of mice visually observed to have tumors or suspected of having tumors at necropsy were histopathologically examined, and statistical analyses were based on verified tumors. The NCI studies showed a higher incidence of tumors in mice painted with condensate made from cigarettes containing cocoa at both the low and high dose levels. The report concluded that "[plowdered cocoa appears to increase the tumorigenicity of the smoke at all dose levels. 'I (NCI, 1977)”

“A second study was undertaken to clarify the results of the NCI-TWG study. Smoke condensate from cigarettes containing 0, 1%, or 3% cocoa powder were applied for 75 weeks to CD-1 female mice. Mice were painted once daily, five days a week, with condensate solution containing 12, 18, or 25 mg dry smoke condensate. All mice dying during the study or sacrificed at termination were necropsied. The tissues of mice visually observed to have tumors at necropsy were histopathologically examined, and statistical analyses were based on the verified tumors. The results of the study showed no enhancement of mouse skin tumorigenicity of smoke condensate as a result of the addition of cocoa. The authors suggest that the previous finding of the TWG-NCI study was probably a result that occurred by chance (Roemer and Hackenberg, 1990).”

When evaluated in a 75 week dermal promotion assay in CD-1 mice, there was no statistically significant effect from cigarette smoke condensate containing 3% cocoa derivatives on skin tumor incidence, when compared to a cocoa free cigarette smoke condensate. Dermal toxicity testing of cocoa was conducted. Information may be obtained from Tobacco Documents available at: http://tobaccodocuments.org/product_design/1003575547.html

“INTRODUCTION: The 2009 Family Smoking Prevention and Tobacco Control Act prohibited the use of characterizing flavors in cigarettes; however, some of these flavors are still used in cigarettes at varying levels. We reviewed tobacco industry internal documents to investigate the role of one of these flavors, cocoa, with the objective of understanding its relationship to sensory and risk perception, promotion of dependence, and enhancement of attractiveness and acceptability. METHODS: We used the Legacy Tobacco Documents Library to identify documents relevant to our research questions. Initial search terms were generated following an examination of published literature oncocoa, other cigarette additives, and sensory and risk perception. Further research questions and search terms were generated based on review of documents generated from the initial search terms. RESULTS:Cocoais widely applied to cigarettes and has been used by the tobacco industry as an additive since the early 20th century.Cocoacan alter the sensory properties of cigarette smoke, including by providing a more appealing taste and decreasing its harshness. The tobacco industry has experimented with manipulatingcocoalevels as a means of achieving sensory properties that appeal to women and youth. CONCLUSIONS: Althoughcocoais identified as a flavor on tobacco industry Web sites, it may serve other sensory purposes in cigarettes as

well. Eliminatingcocoaas an additive from tobacco products may affect tobacco product abuse liability by altering smokers' perceptions of product risk, and decreasing product appeal, especially among vulnerable populations."As taken from Sokol NA et al. 2014. Nicotine Tob. Res. 16(7), 984-91. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24610479

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing cocoa extract (8002-31-1, 84649-99-0) 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 cocoa extract (8002-31-1, 84649-99-0) 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	286	JTI KB Study Report(s)
In vitro cytotoxicity	286	JTI KB Study Report(s)

Aerosol from an electronic nicotine delivery system (ENDS) that creates a vapor by heating an e-liquid containing Cocoa extract was tested in a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic responses upon exposure. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (ppm)	Reference
Aerosol chemistry	96	Labstat International Inc. (2021)
In vitro genotoxicity	96	Labstat International Inc. (2022)
In vitro cytotoxicity	96	Labstat International Inc. (2022)

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

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	3.62	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	3.62	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	3.62	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

"Many flavours and fragrances are known allergens. Their selection and inclusion levels in e-liquids must therefore be guided by toxicological principles, taking into account the exposure pattern and inhalation route of exposure. For contact sensitisation, a general, agreed quantitative risk assessment approach to prevent dermal sensitisation exists. Here we propose exposure parameters and safety factors to apply this approach to e-liquid ingredients. Additionally, as a risk management approach for pre-sensitised individuals, we derive a threshold of 0.1% for indicating the presence of a contact sensitiser in eliquid. Risk assessment for respiratory sensitisation is not

well established. Occupational exposure limits that protect against respiratory allergy are generally very low. Cocoa shell extract is used as a case study to discuss the issues. A tolerable exposure level is derived and estimates of consumer exposure are presented, leading to the practical risk management approach of excluding respiratory sensitisers as eliquid ingredients. Related to this, if natural extracts are used as flavourings in e-liquids, we recommend only protein-free versions are used. Additionally, we recommend the presence of any potential food allergens should be noted on the product information." As taken from Costigan S and Lopez-Belmonte J. 2017. Regul. Toxicol. Pharmacol. 87, 1-8. PubMed, 2018 available at

https://www.ncbi.nlm.nih.gov/pubmed/28389323

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that cocoa butter (CAS RN 8002-31-1) is of uncertain persistence in the environment.

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

EPISuite provides the following data for CAS RN 8002-31-1: **Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:**

Bond Method :	7.05E-004 atm-m3/mole (7.14E+001 Pa-m3/mole)
Group Method:	1.07E-004 atm-m3/mole (1.08E+001 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.031E+004 atm-m3/mole (1.045E+009 Pa-m3/mole) VP: 1E-015 mm Hg (source: MPBPVP) WS: 1.1E-019 mg/L (source: WSKOWWIN)

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

Log Kow used:	22.74 (KowWin est)
Log Kaw used:	-1.540 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate),	24.280
Log Koa (experimental database),	None

Probability of Rapid Biodegradation (BIOWIN v4.10),

Biowin1 (Linear Model),	1.1853
Biowin2 (Non-Linear Model) :	0.9998
Biowin3 (Ultimate Survey Model),	2.6112 (weeks-months)
Biowin4 (Primary Survey Model) :	4.0990 (days)
Biowin5 (MITI Linear Model) :	1.4166
Biowin6 (MITI Non-Linear Model),	0.9916

Biowin7 (Anaerobic Linear Model),	1.0424
Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01),

Structure incompatible with current estimation method!

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

Vapor pressure (liquid/subcooled),	4.68E-014 Pa (3.51E-016 mm Hg)
Log Koa (Koawin est),	24.280
Kp (particle/gas partition coef. (m3/ug)), Mackay model: Octanol/air (Koa) model:	6.41E+007 4.68E+011

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 =	122.9563 E-12 cm3/molecule-sec [Cis-isomer]
OVERALL OH Rate Constant =	130.5563 E-12 cm3/molecule-sec [Trans-isomer]
Half-Life =	1.044 Hrs (12-hr day; 1.5E6 OH/cm3) [Cis-isomer]
Half-Life =	0.983 Hrs (12-hr day; 1.5E6 OH/cm3) [Trans-isomer]

Ozone Reaction:

OVERALL Ozone Rate Constant =	13.000000 E-17 cm3/molecule-sec [Cis-]
OVERALL Ozone Rate Constant =	20.000000 E-17 cm3/molecule-sec [Trans-]
Half-Life =	2.116 Hrs (at 7E11 mol/cm3) [Cis-isomer]
Half-Life =	1.375 Hrs (at 7E11 mol/cm3) [Trans-isomer]
Reaction With Nitrate Radicals May Be Important!	
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 :	1E+010 L/kg (MCI method)
Log Koc:	13.659 (MCI method)
Koc :	2.356E+013 L/kg (Kow method)
Log Koc:	13.372 (Kow method)

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

Total Kb for pH > 8 at 25 deg C:	1.455E-001 L/mol-sec
Kb Half-Life at pH 8:	55.135 days
Kb Half-Life at pH 7:	1.510 years

(Total Kb applies only to esters, carbmates, alkyl halides) **Volatilization from Water:** Henry LC: 0.000107 atm-m3/mole (estimated by Group SAR Method)

Half-Life from Model River:	19.05 hours
Half-Life from Model Lake:	454 hours (18.92 days)

Removal In Wastewater Treatment:

Total removal:	94.04 percent
Total biodegradation:	0.78 percent
Total sludge adsorption:	93.26 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	0.0438	1.05	1000
Water	17.4	900	1000
Soil	82.6	1.8e+003	1000
Sediment	3.78e-009	8.1e+003	0

Persistence Time: 1.09e+003 hr

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that cocoa butter (CAS RN 8002-31-1) is not inherently toxic to aquatic organisms and is of low ecotoxicological concern.

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

“This study demonstrated that, irrespective of hormone type or dose, administering cocoa butter implants during egg development affected the growth of female brown trout Salmo trutta and reduced the size of their offspring. Cortisol treatment also increased adult mortality. Caution is urged in the use of implants for studies of maternal hormonal influences on adult fishes and their offspring”.

As taken from Hoogenboom MO et al. 2011. J. Fish Biol. 79, 587-596. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/21884102?dopt=AbstractPlus.

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 8002-31-1:

Values used to Generate ECOSAR Profile: Log Kow: 22.739 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1.099E-019 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Esters

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Esters :	Fish	96-hr	LC50	8.91e-011 *
Esters :	Daphnid	48-hr	LC50	2.01e-011 *
Esters :	Green Algae	96-hr	EC50	3.2e-013 *
Esters :	Fish		ChV	8.61e-014 *
Esters :	Daphnid		ChV	3.53e-014 *
Esters :	Green Algae		ChV	6.9e-011 *
Esters :	Fish (SW)	96-hr	LC50	3.81e-011 *
Esters :	Mysid	96-hr	LC50	1.37e-015 *
Esters :	Fish (SW)		ChV	2.28e-010 *
Esters :	Mysid (SW)		ChV	4.43e-032
Neutral Organic SAR :	Fish	96-hr	LC50	1.68e-016 *
(Baseline Toxicity) :	Daphnid	48-hr	LC50	6.46e-016 *
	Green Algae	96-hr	EC50	1.32e-012 *
	Fish		ChV	1.57e-016 *
	Daphnid		ChV	1.3e-014 *
	Green Algae		ChV	2.46e-011 *

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.

Class Specific LogKow Cut-Offs:

If the log Kow of the chemical is greater than the endpoint specific cut-offs presented below, then no effects at saturation are expected for those endpoints.

Esters: Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50, Mysid LC50) Maximum LogKow: 6.4 (Green Algae EC50)

Maximum LogKow: 8.0 (ChV)

Baseline Toxicity SAR Limitations: Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50) Maximum LogKow: 6.4 (Green Algae EC50) Maximum LogKow: 8.0 (ChV)

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 8002-31-1:

Values used to Generate ECOSAR Profile: Log Kow: 22.739 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 1.099E-019 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations

Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters :	Earthworm	14-day	LC50	0.002 *

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.

Class Specific LogKow Cut-Offs:

If the log Kow of the chemical is greater than the endpoint specific cut-offs presented below, then no effects at saturation are expected for those endpoints.

Esters: Maximum LogKow: 6.0 (Earthworm LC50)

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that cocoa butter (CAS RN 8002-31-1) is of uncertain bioaccumulative potential.

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

EPISuite provides the following data for CAS RN 8002-31-1:

Bioaccumulation Estimates (BCFBAF v3.01),

Log BCF from regression-based method:	0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL),	2.9176 days (HL = 827.2 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:	22.74 (estimated)

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- Colorectal cancer (CRC) is the third most common malignancy in males and the second most common cancer worldwide. Chronic colonic inflammation is a known risk factor for CRC. Cocoa contains many polyphenolic compounds that have beneficial effects in humans. The objective of this study is to explore the antioxidant properties of cocoa in the mouse model of azoxymethane (AOM)/dextran sulfate sodium (DSS)-induced colitis-associated cancer, focusing on the activation of Nrf2 signaling. Mice were treated with AOM/DSS and randomized to receive either a control diet or a 5 and 10% cocoa diet during the study period. On day 62 of the experiment, the entire colon was processed for biochemical and histopathological examination and further evaluations. Increased levels of

malondialdehyde (MDA) were observed in AOM/DSS-induced mice; however, subsequent administration of cocoa decreased the MDA. Enzymatic and nonenzymatic antioxidants, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, were decreased in the AOM/DSS mice. Cocoa treatment increases the activities/levels of enzymatic and nonenzymatic antioxidants. Inflammatory mediators, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, were elevated during AOM/DSS-induction, and treatment with 5 and 10% cocoa effectively decreases the expression of iNOS and COX-2. The NF-E2-related factor 2 and its downstream targets, such as NQO1 and UDP-GT, were increased by cocoa treatment. The results of our study suggest that cocoa may merit further clinical investigation as a chemopreventive agent that helps prevent CAC.Pandurangan AK et al. (2015). Dietary cocoa protects against colitis-associated cancer by activating the Nrf2/Keap1 pathway. Biofactors. 41(1), 1-14. PubMed, 2014, available at http://www.ncbi.nlm.nih.gov/pubmed/25545372

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- Patients with inflammatory bowel disease (IBD) are at increased risk for developing ulcerative colitis-associated colorectal cancer (CRC). The interleukin-6 (IL-6)/signal transducer and activator of transcription (STAT)-3 signaling regulates survival and proliferation of intestinal epithelial cells and play an important role in the pathogenesis of IBD and CRC. Cocoa is enriched with polyphenols that known to possess antioxidant, anti-inflammatory and antitumor activities. Here, we explored the antitumor effects and mechanisms of cocoa diet on colitis-associated cancer (CAC) using the azoxymethane/dextran sulfate sodium model, with a particular focus on whether cocoa exerts its anticancer effect through the IL-6/STAT3 pathway. We found that cocoa significantly decreased the tumor incidence and size in CAC-induced mice. In addition to inhibiting proliferation of tumor epithelial cells, cocoa suppressed colonic IL-6 expression and subsequently activation of STAT3. Thus, our findings demonstrated that cocoa diet suppresses CAC tumorigenesis, and its antitumor effect is partly mediated by limiting IL-6/STAT3 activation. In addition, cocoa induces apoptosis by increased the expressions of Bax and caspase 3 and decreased Bcl-xl. Thus, we conclude that cocoa may be a potential agent in the prevention and treatment of CAC. Saadatdoust Z et al. (2015). Dietary cocoa inhibits colitis associated cancer: a crucial involvement of the IL-6/STAT3 pathway. J Nutr Biochem. 26(12), 1547-58. PubMed, 2015. Available at http://www.ncbi.nlm.nih.gov/pubmed/26355019
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13. Last audited

May 2022

SCIENTIFIC OPINION

Scientific Opinion on the substantiation of health claims related to caffeine and theobromine in cocoa *(Theobroma cacao* L.) and enhancement of mood (ID 4276) pursuant to Article 13(1) of Regulation (EC) No 1924/2006[1]

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

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 29 March 2012, replaces the earlier version published on 30 June 2011[4].

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to caffeine and theobromine in cocoa (*Theobroma cacao* L.) and enhancement of mood. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The food that is the subject of the health claim is cocoa (*Theobroma cacao* L.). The Panel considers that whereas the food, cocoa (*Theobroma cacao* L.), is not sufficiently characterised in relation to the claimed effect evaluated in this opinion, the food constituents, caffeine and theobromine in cocoa *(Theobroma cacao* L.), are sufficiently characterised.

The claimed effect is "maintenance of a normal mental health (well-being feeling, relaxation)". The target population is assumed to be the general population. In the context of the proposed wordings, the Panel assumes that the claimed effect refers to enhancement of mood. The Panel considers that enhancement of mood might be a beneficial physiological effect.

No references were provided from which conclusions could be drawn for the scientific substantiation of the claim.

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[1] On request from the European Commission, Question No EFSA-Q-2010-00229, adopted on 08 April 2011.

[2] Panel members: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Monika Neuhäuser-Berthold, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Daniel Tomé, Hendrik van Loveren and Hans Verhagen. Correspondence: nda@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank for the preparatory work on this scientific opinion: The members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Marina Heinonen, Hannu Korhonen, Martinus Løvik, Ambroise Martin, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Inge Tetens, Hendrik van Loveren and Hans Verhagen. The members of the Claims Sub-Working Group on Mental/Nervous System: Jacques Rigo, Astrid Schloerscheidt, Barbara Stewart-Knox, Sean (J.J.) Strain, and Peter Willatts.

[4] After publication of the scientific output, EFSA needed to include some editorial changes affecting pages 6 of the current opinion. Where changes have been made to the opinion, footnotes have been included to indicate the original text.

Suggested citation: EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to caffeine and theobromine in cocoa *(Theobroma cacao* L.) and enhancement of mood (ID 4276) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2011;9(6):2269. [14 pp.]. doi:10.2903/j.efsa.2011.2269. Available online: www.efsa.europa.eu/efsajournal

On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of caffeine and theobromine in cocoa *(Theobroma cacao* L.) and enhancement of mood.

KEY WORDS

Cocoa, *Theobroma cacao* L., caffeine, theobromine, mood, health claims.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

See Appendix A

EFSA DISCLAIMER

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INFORMATION AS PROVIDED IN THE CONSOLIDATED LIST

The consolidated list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006[5] submitted by Member States contains main entry claims with corresponding conditions of use and literature for similar health claims. EFSA has screened all health claims contained in the original consolidated list of Article 13 health claims which was received by EFSA in 2008 using six criteria established by the NDA Panel to identify claims for which EFSA considered sufficient information had been provided for evaluation and those for which more information or clarification was needed before evaluation could be carried out[6]. The clarifications which were received by EFSA through the screening process have been included in the consolidated list. This additional information will serve as clarification to the originally provided information. The information provided in the consolidated list for the health claims which are the subject of this opinion is tabulated in Appendix C.

ASSESSMENT

1. CHARACTERISATION OF THE FOOD/CONSTITUENT (ID 4276)

The food that is the subject of the health claim is cocoa (*Theobroma cacao* L.).

Cocoa (*Theobroma cacao* L.) contains a wide and variable range of potentially active compounds such as various amines (e.g. tyramine and phenylethylamine), methylxanthines (e.g. caffeine and theobromine), or cannabinoid-like fatty acids (Bruinsma and Taren, 1999). From the references provided, the Panel notes that the methylxanthines caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine) have been proposed as the "active" food constituents responsible for the claimed effect considered in this opinion. Caffeine and theobromine are well defined compounds which can be measured in foods by established methods.

The Panel considers that whereas the food, cocoa (*Theobroma cacao* L.), is not sufficiently characterised in relation to the claimed effect evaluated in this opinion, the food constituents, caffeine and theobromine in cocoa *(Theobroma cacao* L.), are sufficiently characterised.

2. RELEVANCE OF THE CLAIMED EFFECT TO HUMAN HEALTH (ID 4276)

The claimed effect is "maintenance of a normal mental health (well-being feeling, relaxation)". The Panel assumes that the target population is the general population.

In the context of the proposed wordings, the Panel assumes that the claimed effect refers to enhancement of mood.

The Panel considers that enhancement of mood might be a beneficial physiological effect.

3. SCIENTIFIC SUBSTANTIATION OF THE CLAIMED EFFECT (ID 4276)

Among the references provided to substantiate the claim were narrative reviews on chocolate and related substances which did not provide original data for the scientific substantiation of the claim, and a reference on the molecular mechanisms of the effects of cocoa on lipid metabolism and triacylglycerol accumulation in rats fed high-fat diets. Two abstracts did not provide sufficient details

[5] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

[6] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011. General guidance for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims. EFSA Journal, 9(4):2135, 24 pp.

for a full scientific evaluation, and for one reference, provided in Japanese, no translation into an EU language was available to the Panel. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

One reference (Smit et al., 2004) reported on two randomised, double-blind, placebo-controlled studies on the effects of methylxanthines (caffeine and theobromine) on mood. The first study tested two treatments containing identical amounts of methylxanthines (19 mg caffeine and 250 mg theobromine) in capsules, either as cocoa powder (11.5 g, treatment 1) or as pure compounds (treatment 2), against placebo (microcrystalline cellulose). Participants were required to abstain from any caffeine or cocoa/chocolate-containing drink or food from 21.00 hours the previous evening, and underwent four test sessions (two placebo sessions, treatment 1 and treatment 2). Each test session involved the completion of a pre-test (baseline) and two post-test sets of tasks at 1 and 2 h after receiving the treatment. Each set of tasks comprised a 25-item mood questionnaire, which was developed partly from the Profile of Mood States (POMS) bipolar form and from the short form of the activation-deactivation adjective list. The mood questionnaire consisted of several descriptors of alerting and energising effects, aspects of positive and negative affect, and tension related moods, a measure of "overall mood", and "hungry" and "thirsty". Then the intensity of several subjective "bodily sensations" (such as "headache", "heart pounding", "nausea", and "chills") was rated. All scales were 100 mm anchored visual-analogue scales. Of the 27 subjects recruited, 20 (17 women) completed the study[7]. In the second study by the same authors (Smit et al., 2004), visually identical 60 g portions of chocolate were prepared to contain 8 mg caffeine and 100 mg theobromine, 20 mg caffeine and 250 mg theobromine, or no methylxanthines (placebo). The two interventions and the placebo corresponded to the amounts of methylxanthines present in standard portions of milk, dark and white chocolate, respectively. Mood was assessed with the same questionnaire as in the previous study. Of the 29 participants recruited, 22 (11 women) completed the study. The Panel notes that the factors resulting from the principal component analysis of the mood questionnaire showed inconsistent outcomes (within and between factors) across the two studies[8].

The Panel concludes that a cause and effect relationship has not been established between the consumption of caffeine and theobromine in cocoa *(Theobroma cacao* L.) and enhancement of mood.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, "cocoa (*Theobroma cacao* L.)", which is the subject of the health claim, is not sufficiently characterised in relation to the claimed effect, whereas the food constituents, caffeine and theobromine in cocoa *(Theobroma cacao* L.), are sufficiently characterised.
- The claimed effect is "maintenance of a normal mental health (well-being feeling, relaxation)". The target population is assumed to be the general population. In the context of the proposed wordings, it is assumed that the claimed effect refers to enhancement of mood. Enhancement of mood might be a beneficial physiological effect.

[7] Deleted original text: The Panel notes that the factors resulting from the principal component analysis of the mood questionnaire incorporated both negative and positive mood constructs within the same factor, which cannot be interpreted with respect to enhancement of mood. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

[8] Deleted original text: The Panel notes that the factors resulting from the principal component analysis of the mood questionnaire incorporated both negative and positive mood constructs within the same factor, which cannot be interpreted with respect to enhancement of mood. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

- A cause and effect relationship has not been established between the consumption of caffeine and theobromine in cocoa *(Theobroma cacao* L.) and enhancement of mood.

DOCUMENTATION PROVIDED TO EFSA

Health claims pursuant to Article 13 of Regulation (EC) No 1924/2006 (No: EFSA-Q-2010-00229). The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The full list of supporting references as provided to EFSA is available on: http://www.efsa.europa.eu/panels/nda/claims/article13.htm.

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APPENDICES

APPENDIX A

BACKGROUND AND TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Regulation 1924/2006 on nutrition and health claims made on foods[9] (hereinafter "the Regulation") entered into force on 19th January 2007.

Article 13 of the Regulation foresees that the Commission shall adopt a Community list of permitted health claims other than those referring to the reduction of disease risk and to children's development and health. This Community list shall be adopted through the Regulatory Committee procedure and following consultation of the European Food Safety Authority (EFSA).

Health claims are defined as "any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health".

In accordance with Article 13 (1) health claims other than those referring to the reduction of disease risk and to children's development and health are health claims describing or referring to:

a) the role of a nutrient or other substance in growth, development and the functions of the body; or

b) psychological and behavioural functions; or

c) without prejudice to Directive 96/8/EC, slimming or weight-control or a reduction in the sense of hunger or an increase in the sense of satiety or to the reduction of the available energy from the diet.

To be included in the Community list of permitted health claims, the claims shall be:

(i) based on generally accepted scientific evidence; and

(ii) well understood by the average consumer.

Member States provided the Commission with lists of claims as referred to in Article 13 (1) by 31 January 2008 accompanied by the conditions applying to them and by references to the relevant scientific justification. These lists have been consolidated into the list which forms the basis for the EFSA consultation in accordance with Article 13 (3).

ISSUES THAT NEED TO BE CONSIDERED

IMPORTANCE AND PERTINENCE OF THE FOOD[10]

Foods are commonly involved in many different functions[11] of the body, and for one single food many health claims may therefore be scientifically true. Therefore, the relative importance of food e.g. nutrients in relation to other nutrients for the expressed beneficial effect should be considered: for functions affected by a large number of dietary factors it should be considered whether a reference to a single food is scientifically pertinent.

[9] OJ L12, 18/01/2007

[10] The term 'food' when used in this Terms of Reference refers to a food constituent, the food or the food category.

[11] The term 'function' when used in this Terms of Reference refers to health claims in Article 13(1)(a), (b) and (c).

It should also be considered if the information on the characteristics of the food contains aspects pertinent to the beneficial effect.

SUBSTANTIATION OF CLAIMS BY GENERALLY ACCEPTABLE SCIENTIFIC EVIDENCE

Scientific substantiation is the main aspect to be taken into account to authorise health claims. Claims should be scientifically substantiated by taking into account the totality of the available scientific data, and by weighing the evidence, and shall demonstrate the extent to which:

(a) the claimed effect of the food is beneficial for human health,

(b) a cause and effect relationship is established between consumption of the food and the claimed effect in humans (such as: the strength, consistency, specificity, dose-response, and biological plausibility of the relationship),

(c) the quantity of the food and pattern of consumption required to obtain the claimed effect could reasonably be achieved as part of a balanced diet,

(d) the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

EFSA has mentioned in its scientific and technical guidance for the preparation and presentation of the application for authorisation of health claims consistent criteria for the potential sources of scientific data. Such sources may not be available for all health claims. Nevertheless it will be relevant and important that EFSA comments on the availability and quality of such data in order to allow the regulator to judge and make a risk management decision about the acceptability of health claims included in the submitted list.

The scientific evidence about the role of a food on a nutritional or physiological function is not enough to justify the claim. The beneficial effect of the dietary intake has also to be demonstrated. Moreover, the beneficial effect should be significant i.e. satisfactorily demonstrate to beneficially affect identified functions in the body in a way which is relevant to health. Although an appreciation of the beneficial effect in relation to the nutritional status of the European population may be of interest, the presence or absence of the actual need for a nutrient or other substance with nutritional or physiological effect for that population should not, however, condition such considerations.

Different types of effects can be claimed. Claims referring to the maintenance of a function may be distinct from claims referring to the improvement of a function. EFSA may wish to comment whether such different claims comply with the criteria laid down in the Regulation.

WORDING OF HEALTH CLAIMS

Scientific substantiation of health claims is the main aspect on which EFSA's opinion is requested. However, the wording of health claims should also be commented by EFSA in its opinion.

There is potentially a plethora of expressions that may be used to convey the relationship between the food and the function. This may be due to commercial practices, consumer perception and linguistic or cultural differences across the EU. Nevertheless, the wording used to make health claims should be truthful, clear, reliable and useful to the consumer in choosing a healthy diet.

In addition to fulfilling the general principles and conditions of the Regulation laid down in Article 3 and 5, Article 13(1)(a) stipulates that health claims shall describe or refer to "the role of a nutrient or other substance in growth, development and the functions of the body". Therefore, the requirement to

describe or refer to the 'role' of a nutrient or substance in growth, development and the functions of the body should be carefully considered.

The specificity of the wording is very important. Health claims such as "Substance X supports the function of the joints" may not sufficiently do so, whereas a claim such as "Substance X helps maintain the flexibility of the joints" would. In the first example of a claim it is unclear which of the various functions of the joints is described or referred to contrary to the latter example which specifies this by using the word "flexibility".

The clarity of the wording is very important. The guiding principle should be that the description or reference to the role of the nutrient or other substance shall be clear and unambiguous and therefore be specified to the extent possible i.e. descriptive words/ terms which can have multiple meanings should be avoided. To this end, wordings like "strengthens your natural defences" or "contain antioxidants" should be considered as well as "may" or "might" as opposed to words like "contributes", "aids" or "helps".

In addition, for functions affected by a large number of dietary factors it should be considered whether wordings such as "indispensable", "necessary", "essential" and "important" reflects the strength of the scientific evidence.

Similar alternative wordings as mentioned above are used for claims relating to different relationships between the various foods and health. It is not the intention of the regulator to adopt a detailed and rigid list of claims where all possible wordings for the different claims are approved. Therefore, it is not required that EFSA comments on each individual wording for each claim unless the wording is strictly pertinent to a specific claim. It would be appreciated though that EFSA may consider and comment generally on such elements relating to wording to ensure the compliance with the criteria laid down in the Regulation.

In doing so the explanation provided for in recital 16 of the Regulation on the notion of the average consumer should be recalled. In addition, such assessment should take into account the particular perspective and/or knowledge in the target group of the claim, if such is indicated or implied.

TERMS OF REFERENCE

HEALTH CLAIMS OTHER THAN THOSE REFERRING TO THE REDUCTION OF DISEASE RISK AND TO CHILDREN'S DEVELOPMENT AND HEALTH

EFSA should in particular consider, and provide advice on the following aspects:

- Whether adequate information is provided on the characteristics of the food pertinent to the beneficial effect.

- Whether the beneficial effect of the food on the function is substantiated by generally accepted scientific evidence by taking into account the totality of the available scientific data, and by weighing the evidence. In this context EFSA is invited to comment on the nature and quality of the totality of the evidence provided according to consistent criteria.

- The specific importance of the food for the claimed effect. For functions affected by a large number of dietary factors whether a reference to a single food is scientifically pertinent.

In addition, EFSA should consider the claimed effect on the function, and provide advice on the extent to which:

- the claimed effect of the food in the identified function is beneficial.
- a cause and effect relationship has been established between consumption of the food and the claimed effect in humans and whether the magnitude of the effect is related to the quantity consumed.
- where appropriate, the effect on the function is significant in relation to the quantity of the food proposed to be consumed and if this quantity could reasonably be consumed as part of a balanced diet.
- the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.
- the wordings used to express the claimed effect reflect the scientific evidence and complies with the criteria laid down in the Regulation.

When considering these elements EFSA should also provide advice, when appropriate:

- on the appropriate application of Article 10 (2) (c) and (d) in the Regulation, which provides for additional labelling requirements addressed to persons who should avoid using the food; and/or warnings for products that are likely to present a health risk if consumed to excess.

APPENDIX B

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of the food/food constituent, a positive assessment of its safety, nor a decision on whether the food/food constituent is, or is not, classified as foodstuffs. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wordings of the claims and the conditions of use as proposed in the Consolidated List may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 13(3) of Regulation (EC) No 1924/2006.

APPENDIX C

Table 1. Main entry health claims related to caffeine and theobromine in cocoa (*Theobroma cacao* L.), including conditions of use from similar claims, as proposed in the Consolidated List.

ID	Food or Food constituent	Health Relationship	Proposed wording
4276	Cocoa (Theobroma cacao L.)	Maintenance of a normal mental health (well-being feeling, relaxation)	Improve emotional well-being. Support positive mood. Increase relaxation
	Conditions of use - Bean. At least 1g par day of bean dry extract (16:1)		

GLOSSARY AND ABBREVIATIONS

POMS	Profile of mood states

European Food Safety Authority

EFSA Journal 2014;12(5):3654

SCIENTIFIC OPINION

Scientific Opinion on the modification of the authorisation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1] following a request in accordance with Article 19 of Regulation (EC) No 1924/2006

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

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 22 July 2014, replaces the earlier version published on 05 May 2014[*]

ABSTRACT

Following an application from Barry Callebaut Belgium NV, submitted pursuant to Article 19 of Regulation (EC) No 1924/2006 via the Competent Authority of Belgium, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the modification of the authorisation of a health claim related to "cocoa flavanols help maintain the elasticity of blood vessels, which contributes to normal blood flow", pursuant to Article 13(5) of Regulation (EC) No 1924/2006. The modification concerns an extension of the authorised conditions of use of the claim to a high-flavanols (HF) cocoa extract to be consumed in capsules, tablets or added to "other foods, including beverages". Cocoa flavanols, which are the subject of the health claim, have been sufficiently characterised. Maintenance of normal endothelium-dependent vasodilation is a beneficial physiological effect. The Panel concludes that a cause and effect relationship has been established between the consumption of cocoa flavanols in the HF cocoa extract (i.e. in capsules or tablets) and maintenance of normal endothelium-dependent vasodilation. In order to obtain the claimed effect, 200 mg of cocoa flavanols should be consumed daily. This amount could be provided by less than one gram of HF cocoa extract in capsules or tablets, and can be consumed in the context of a balanced diet. The target population is the general population.

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[1] On request from the Competent Authority of Belgium following an application by Barry Callebaut Belgium NV, Question No EFSA-Q-2013-00832, adopted on 10 April 2014.

[2] Panel members: Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen. Correspondence: nda@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Marina Heinonen, Ambroise Martin, Hildegard Przyrembel, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Sean (J.J.) Strain, Inge Tetens, Hendrik Van Loveren, Hans Verhagen and Peter Willatts for the preparatory work on this scientific opinion.

[*] An editorial amendment was carried out that does not materially affect the contents or outcome of this Scientific Opinion To avoid confusion, the original version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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KEY WORDS

cocoa flavanols, extract, endothelium-dependent vasodilation, health claims

SUMMARY

Following an application from Barry Callebaut Belgium NV, submitted pursuant to Article 19 of Regulation (EC) No 1924/2006 via the Competent Authority of Belgium, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the modification of the authorisation of a health claim related to "cocoa flavanols help maintain the elasticity of blood vessels, which contributes to normal blood flow", pursuant to Article 13(5) of Regulation (EC) No 1924/2006, which was authorised by Commission Regulation No 851/2013. The authorised conditions of use of the claim are: "Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 200 mg of cocoa flavanols. The claim can be used only for cocoa beverages (with cocoa powder) or for dark chocolate which provide at least a daily intake of 200 mg of cocoa flavanols with a degree of polymerisation 1-10".

The modification concerns an extension of the authorised conditions of use of the claim to a high-flavanol (HF) cocoa extract to be consumed in capsules, tablets or added to "other foods, including beverages".

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and including a request for the protection of proprietary data.

The Panel notes that cocoa flavanols, which are the subject of the health claim, have been sufficiently characterised.

The Panel considers that maintenance of normal endothelium-dependent vasodilation is a beneficial physiological effect. The target population is the general healthy adult population.

In the partially-blinded, controlled, cross-over study, which was provided by the applicant as being pertinent to the health claim, six healthy subjects were randomised to consume cocoa flavanols from different formulations: cocoa extract in capsules, cocoa powder and dark chocolate bars. Plasma concentrations of epicatechins, which are likely to be responsible for the acute effect of cocoa flavanols on endothelium-dependent flow-mediated vasodilation, were measured at different time points after the consumption of the different formulations. The absorption of epicatechins from the HF cocoa extract in capsules when consumed with water was not lower than that observed for epicatechins in HF cocoa powder or HF dark chocolate, the food matrices for which the health claim has been authorised.

Two studies were provided by the applicant as supportive of the health claim. The Panel considers that, in the absence of a direct comparison between the food matrices investigated in these studies (milk chocolate drink and nut cream) and cocoa powder or dark chocolate, no conclusions can be drawn from these studies for the extension of the conditions of use to the food matrices investigated in the studies.

In weighing the evidence, the Panel took into account that the absorption of epicatechins from HF cocoa extract in capsules when consumed with water was not lower than that observed for epicatechins in HF cocoa powder or HF dark chocolate in a human intervention study. The Panel also took into account that, even if epicatechins are likely to be responsible for the acute effect of cocoa flavanols on endothelium-mediated vasodilation rather than for the long-term effect, it is unlikely that daily consumption of cocoa flavanols in the HF cocoa extract would have different long-term effects on endothelium-mediated vasodilation than cocoa flavanols in cocoa powder or dark chocolate. The Panel considers that the bioavailability of cocoa flavanols from HF cocoa extract in capsules and in tablets is not different from HF cocoa powder or dark chocolate.

The Panel concludes that a cause and effect relationship has been established between the consumption of cocoa flavanols in the HF cocoa extract (i.e. in capsules or tablets) and maintenance of normal endothelium-dependent vasodilation.

In order to obtain the claimed effect, 200 mg of cocoa flavanols should be consumed daily. This amount could be provided by less than one gram (0.25-0.67 g) of HF cocoa extract in capsules or tablets, and can be consumed in the context of a balanced diet. The target population is the general population.

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BACKGROUND

Regulation (EC) No 1924/2006[4] harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

The same Regulation, as referred to in Article 19, also lays down provisions for modification, suspension and revocation of authorisations. The procedures laid down in Article 15 and 18 shall apply mutatis mutandis.

According to Article 18 of that Regulation, an application for the modification, suspension or revocation of authorisations of health claims included in the Community list of permitted claims referred to in Art 13(3) shall be submitted by the applicant to the national competent authority of a Member State, who will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 17/10/2013.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.
- The scientific evaluation procedure started on 06/11/2013.
- On 22/01/2014, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application and the clock was stopped on 03/02/2014 in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- On 14/02/2014, EFSA received the requested information and the clock was restarted.
- During its meeting on 10/04/2014, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 19 of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: cocoa flavanols and maintenance of normal endothelium-dependent vasodilation.

[4] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of cocoa flavanols, a positive assessment of its safety, nor a decision on whether cocoa flavanols is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Barry Callebaut Belgium NV, Aalstersestraat 122, B-9280 Lebbeke-Wieze, Belgium.

The application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006 for one unpublished study report (ProDigest, 2012). The applicant also claimed confidentiality rights for information pertaining to the composition and the manufacturing process of the cocoa extract.

Food/constituent as stated by the applicant

According to the applicant, the food constituent that is the subject of the health claim is cocoa flavanols.

Health relationship as claimed by the applicant

The applicant indicated that a cause and effect relationship between the consumption of cocoa flavanols and endothelium-dependent vasodilation has already been established (EFSA NDA Panel, 2012; Regulation (EC) No 851/2013).

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: "Cocoa flavanols help maintain the elasticity of blood vessels, which contributes to normal blood flow".

Specific conditions of use as proposed by the applicant

According to the applicant, the target population is the general healthy adult population.

In order to obtain the claimed effect, 200 mg cocoa flavanols should be consumed daily. As an alternative to 2.5 g of high-flavanol (HF) cocoa powder or 10 g of HF dark chocolate (EFSA NDA Panel, 2012), this amount could also be provided by 0.25-0.67 g of HF cocoa extract in the form of capsules, tablets or added to food applications as such. The flavanols' content in the HF cocoa extract varies between 80% and 30% flavanols (DP1-10). The HF cocoa extract is easily dissolvable in water, and can be consumed in the context of a balanced diet. The amount of HF cocoa extracts added to food applications, including beverages, should accommodate the highest possible losses due to treatment of the food.

ASSESSMENT

The Panel has already adopted an opinion on the scientific substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation with a favourable outcome pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (EFSA NDA Panel, 2012). On 3 September 2013, the European Commission adopted Regulation No 851/2013[5], which authorised a health claim related to "cocoa flavanols help maintain the elasticity of blood vessels, which contributes to normal blood flow". The authorised conditions of use of the claim are: "Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 200 mg of

[5] Commission Regulation (EU) No 851/2013 of 3 September 2013 authorising certain health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health and amending Regulation (EU) No 432/2012. OJ L 235, 4.9.2013, p. 3–7.

cocoa flavanols. The claim can be used only for cocoa beverages (with cocoa powder) or for dark chocolate which provide at least a daily intake of 200 mg of cocoa flavanols with a degree of polymerisation 1-10".

With the present application, the applicant has requested an extension of the conditions of use to a HF cocoa extract. The amount of 200 mg of cocoa flavanols can be provided by 0.25-0.67 g of HF cocoa extract in capsules or tablets. In the HF cocoa extract, monomeric flavanols (epicatechin and catechin) account for about 23 % of total flavanols (ProDigest, 2012). Information pertaining to the manufacturing process and the nutritional composition of the HF cocoa extract has been provided. Thirty-nine months stability data of the HF cocoa extracts with different flavanols content and two months stability data of the HF cocoa extract in water-based beverages have been provided.

The applicant proposes that the HF cocoa extract providing 200 mg of cocoa flavanols could be consumed in capsules or tablets or added to "other foods, including beverages", in order to obtain the claimed effect.

Thus, the present opinion will address whether the conditions of use for this claim could be extended to the HF cocoa extract under the proposed uses (consumed in capsules, tablets or "other foods, including beverages").

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is cocoa flavanols.

The Panel notes that cocoa flavanols, which are the subject of the health claim, have been sufficiently characterised (EFSA NDA Panel, 2012).

2. Relevance of the claimed effect to human health

The Panel considers that maintenance of normal endothelium-dependent vasodilation is a beneficial physiological effect (EFSA NDA Panel, 2012). The target population proposed by the applicant is the general healthy adult population.

3. Scientific substantiation of the claimed effect

In order to apply for this extension of the conditions of use, the applicant performed a literature search in PubMed to retrieve comparative studies on the bioavailability of epicatechins, which are considered by the applicant to be responsible for the claimed effect, from different cocoa formulations. Various combinations of the following terms were used in the literature search, which was limited to "humans" and "clinical trial": "cocoa", "capsule", "tablet", "extract", "epicatechin", "flavanols", "flavan-3-ols" and "pharmacokinetics". No pertinent studies were identified by the applicant through this literature search.

The Panel noted that cocoa flavanols (mostly epicatechins) may exert an acute effect on endothelium-dependent flow-mediated dilation (ED-FMD) by enhancing nitric oxide production in the endothelium each time they are consumed. The Panel also noted that the evidence provided in support of mechanisms by which repeated consumption of cocoa flavanols may induce longer-term effects on fasting ED-FMD was weak and may have been related more to the gut metabolism of procyanidins and the fraction of epicatechin which is not absorbed in the small intestine, than to the fraction of epicatechin which is absorbed up to four to six hours after consumption (EFSA NDA Panel, 2012).

The applicant provided the unpublished study report by ProDigest (2012) as being pertinent to the health claim. This is a randomised, partially-blinded, controlled, cross-over study which compared

plasma concentrations of epicatechins at different time points after consumption of cocoa flavanols from different formulations (i.e. cocoa extract in capsules vs. cocoa powder vs. dark chocolate in bars).

Six healthy subjects (three men, mean age 26.7±10.3 years) were randomised to consume a pre-established sequence of five cocoa formulations in a single dose with a wash-out period in between of at least five days: HF cocoa extract in capsules (449 mg of flavanols per portion), low-flavanol (LF) and HF cocoa powder (27 mg and 459 mg of flavanols per portion, respectively), and LF and HF dark chocolate (60 mg and 460 mg of flavanols per portion, respectively). For the different cocoa formulations the epicatechin and catechin content per portion was provided: 96 mg and 7 mg in the HF cocoa extract in capsules, 2 mg and 4 mg in the LF cocoa powder, 95 mg and 29 mg in the HF cocoa powder, 14 mg and 6 mg in the LF dark chocolate, and 86 mg and 11 mg in the HF dark chocolate. The Panel notes that the monomeric flavanol/total flavanol ratio in the three HF formulations (cocoa powder, dark chocolate and cocoa extract) was comparable (22-27%). Although it was possible to identify the different type of cocoa formulations given to participants (i.e. capsules, powder sachet and chocolate bars), neither investigators nor participants were able to distinguish the HF and LF study products. Upon a request by EFSA for clarification on the selection of the sample size, the applicant indicated that the sample size was based on a previous study with a similar design which had investigated the rate and extent of absorption of epicatechins from five different chocolate matrices, and in which six individuals were sufficient to detect relevant differences between the interventions (Neilson et al., 2009).

After an overnight fast (except for the consumption of water which was allowed), subjects consumed the study products with 200 mL of water at the clinical facility and after two hours they consumed a standardised meal under the researchers' supervision. Subjects were requested to refrain from consuming HF-containing products for two days prior to each study day. Blood samples for the detection of plasma epicatechins were taken at baseline (t = 0) and then at 0.5, 1, 2, 4, and 6 hours after consumption of the study products. Maximum plasma concentrations achieved (C_{max}), time to maximum concentrations observed (T_{max}) and area under the curve (AUC) for plasma epicatechins were analysed using a General Linear Model. Differences between periods were determined based on the Least Significant Difference test. C_{max} and AUC values were significantly higher for HF dark chocolate and HF cocoa powder compared with their LF controls. C_{max}, T_{max} and AUC did not differ significantly between HF cocoa extract in capsules and HF cocoa powder. However, the AUC for HF cocoa extract in capsules was significantly higher than the AUC for HF dark chocolate ($p = 0.03$) and not significantly different from the AUC for HF cocoa powder. C_{max} for HF cocoa extract in capsules was not significantly different from C_{max} for HF dark chocolate and C_{max} for HF cocoa powder. The Panel notes that the amount of flavanols per portion used in this study was more than double the amount required to achieve the claimed effect, as well as the amount of epicatechins generally contained in 200 mg of cocoa flavanols. However, the Panel considers that the absorption of epicatechins from 200 mg of flavanols in the HF cocoa extract in capsules, when consumed with water, would not be lower than that observed for epicatechins from the same amount of flavanols in cocoa powder or dark chocolate, the food matrices for which the health claim has been authorised.

Through the literature search, the applicant identified two studies as supportive of the health claim. The studies investigated plasma concentrations of epicatechins or epicatechins plus catechins after consumption of a milk-containing or a milk-free chocolate drink (Keogh et al., 2007) or a nut cream (Vitaglione et al., 2013) containing HF cocoa extracts. Considering that the availability of cocoa flavanols in either cocoa powder or dark chocolate has not been investigated in these studies, the applicant was requested to provide a rationale on how these studies could provide evidence for an extension of the conditions of use of cocoa flavanols consumed as cocoa extract. The applicant acknowledged that neither study directly compared the availability of epicatechins from the tested food matrices (i.e. chocolate drinks and nut cream) against the authorised food matrices (i.e. cocoa powder and dark chocolate), and therefore did not consider that these studies were directly pertinent to the health claim but rather were only supportive. The Panel considers that, in the absence of a

direct comparison between the food matrices investigated and the authorised food matrices, no conclusions can be drawn from these two studies for the extension of the conditions of use to the food matrices investigated in the studies.

In weighing the evidence, the Panel took into account that the absorption of epicatechins from HF cocoa extract in capsules when consumed with water was not lower than that observed for epicatechins in HF cocoa powder or HF dark chocolate in a human intervention study. The Panel also took into account that, even if epicatechins are likely to be responsible for the acute effect of cocoa flavanols on endothelium-mediated vasodilation rather than for the long-term effect, it is unlikely that daily consumption of cocoa flavanols in the HF cocoa extract would have different long-term effects on endothelium-mediated vasodilation than cocoa flavanols in cocoa powder or dark chocolate. The Panel considers that the bioavailability of cocoa flavanols from HF cocoa extract in capsules and in tablets is not different from HF cocoa powder or dark chocolate.

The Panel concludes that a cause and effect relationship has been established between the consumption of cocoa flavanols in the HF cocoa extract (i.e. in capsules or tablets) and maintenance of normal endothelium-dependent vasodilation.

The Panel could not have reached its conclusions without the human intervention study claimed as proprietary by the applicant (ProDigest, 2012, unpublished).

4. Panel's comments on the proposed wording

See previous assessment of the EFSA NDA Panel (2012) and Commission Regulation[6] dated 3 September 2013.

5. Conditions and restrictions of use

In order to obtain the claimed effect, 200 mg of cocoa flavanols should be consumed daily. This amount could be provided by less than one gram (0.25-0.67 g) of HF cocoa extract in capsules or tablets. This amount of HF cocoa extract can be consumed in the context of a balanced diet. The target population is the general population.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, cocoa flavanols, which is the subject of the claim, is sufficiently characterised.
- Maintenance of normal endothelium-dependent vasodilation is a beneficial physiological effect.
- A cause and effect relationship has been established between the consumption of cocoa flavanols in the HF cocoa extract (i.e. in capsules or tablets) and maintenance of normal endothelium-dependent vasodilation.
- In order to obtain the claimed effect, 200 mg of cocoa flavanols should be consumed daily. This amount could be provided by less than one gram (0.25-0.67 g) of HF cocoa extract in

[6] Commission Regulation (EU) No 851/2013 of 3 September 2013 authorising certain health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health and amending Regulation (EU) No 432/2012. OJ L 235, 4.9.2013, p. 3–7.

capsules or tablets. This amount of HF cocoa extract can be consumed in the context of a balanced diet. The target population is the general population.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006 following a request in accordance with Article 19 of the afore-mentioned Regulation (Claim serial No: 00398_BE). October 2013. Submitted by Barry Callebaut Belgium NV.

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ABBREVIATIONS

AUC	area under the curve
C_{max}	maximum plasma concentration
ED-FMD	endothelium-dependent flow-mediated dilation
HF	high-flavanol
LF	low-flavanol
T_{max}	time to maximum concentrations observed

Danish Ministry of the Environment
Environmental Protection Agency

Bioavailability of cadmium from linseed and cocoa

A LOUS follow-up project

Environmental Project No. 1571, 2014

Title:

Bioavailability of cadmium from linseed and cocoa

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Contents

Foreword

"The List of Undesirable Substances (LOUS) was established by the Danish Environmental Protection Agency (EPA) as a guide for enterprises. It addresses chemical substances of concern, based on their hazardous properties and the volumes used in Denmark. The latest version of LOUS from 2009 includes 40 chemical substances and groups of substances [DEPA 2010].

During the period 2012-2015, all substances listed on LOUS will be surveyed and further need for risk management measures will be evaluated. In certain cases, implementation projects will be launched to achieve the goals laid down in the strategies for these substances.

The present project "Bioavailability of cadmium from linseed and cocoa" was initiated as a LOUS follow-up project by the Danish EPA. The objective of this study was to investigate the bioavailability of cadmium in selected food items. The investigation was conducted as an oral feeding study in rats in combination with *in-vitro* studies simulating the conditions in the stomach of both rats and humans. The aim of the study was to provide data which can be used to further qualify the estimated exposure of the population to cadmium via food.

The project was carried out from June2013 till February 2014 by the National Food Institute at the Technical University of Denmark (DTU Food). Participants from DTU Food were senior advisor Max Hansen, senior scientist Jens Jørgen Sloth and scientist Rie Romme Rasmussen. The quality assurance was performed by Folmer Eriksen."

Sammenfatning og konklusion

I dette projekt blev biotilgængeligheden af cadmium fra hele hørfrø, knust hørfrø, cacao og cadmiumklorid undersøgt i et fodringsforsøg med rotter og i et in vitro forsøg der skulle simulere forholdende i mavetarmkanalen hos henholdsvis rotter og mennesker.

I fodringsforsøget blev rotter inddelt i grupper, der blev doseret med cadmium fra forskellige kilder, henholdsvis hele hørfrø, knuste hørfrø, kakao og $CdCl_2$, samt en kontrolgruppe. Hørfrø eller kakao indgik med 10 % af foderets vægt og blev tilsat som erstatning for en kulhydratkilde. Dette blev gjort for at sikre koncentrationen af de øvrige nærringsstoffer i foderet forblev uændret. I cadmiumklorid gruppen blev cadmiumklorid dog iblandet foderet. Rotterne blev doseret i 3 uger og cadmium indholdet blev derefter målt i rotternes nyrer. Der blev set signifikante forskelle på indholdet af cadmium i nyrerne i de forskellige grupper. Forsøget viste at biotilgængeligheden af cadmium er nogenlunde lige stor fra de tre kilder. Der kan dog være en lidt større biotilgængelighed fra hele i forhold til knuste hørfrø og det ser ud til at biotilgængeligheden fra kakao er en smule lavere end fra hørfrø.

Der er forskel på mavetarmkanalen i rotter og mennesker. Det blev vurderet, at den væsentligste forskel i forbindelse med biotilgængeligheden af cadmium er, at pH er væsentligt lavere hos mennesker end hos rotter. Der blev derfor gennemført et in-vitro forsøg, hvor hørfrø og kakao blev ekstraheret med saltsyre i en koncentration der svarer til den der findes i maven hos rotter og mennesker. Forsøget viste, at den højere syrekoncentration i maven hos mennesker fører til en væsentlig større frigivelse af cadmium fra både hørfrø og kakao og dermed potentielt større biotilgængelighed.

Nærværende undersøgelse bekræfter den absorption af Cd fra fødevarer, der er fundet i andre studier (EFSA 2009a). Projektet kunne ikke bekræfte, at biotilgængeligheden af Cd hos rotter er lavere i hele hørfrø i forhold til knust hørfrø. En mulig forklaring kan være, at rotter, i modsætning til mennesker, tygger hørfrø og dermed øger muligheden for at Cd kan bringes i opløsning i mave-tarm kanalen. Den meget store forskel i frigivelse af Cd fra knust hørfrø i en simulation af menneskelig mavesaft sammenlignet med den rotte mavesaft indikerer, at Cd fra knust hørfrø er mere biotilgængeligt i mennesker end Cd fra hele hørfrø. Denne konklusion understøttes af resultatet i dyreforsøget, hvor det opløselige $CdCl_2$ synes at være den mest biotilgængelige. Disse resultater giver formentlig ikke Fødevarestyrelsen grund til at ændre rådgivningen af befolkningen omkring indtag af hele og knuste hørfrø. Et forsøg, hvor rotterne doseres med hele hørfrø via sonde kunne hjælpe med yderligere at afklare forskellen i biotilgængelighed mellem hele og knuste hørfrø. Biotilgængeligheden af Cd synes at være en smule lavere i kakao forhold til de andre matricer, men forskellene er ikke tilstrækkelige til, at det bør give anledning give særlige råd til befolkningen vedrørende indtag af kakao og chokolade.

Dette projekt har vist, hvordan en undersøgelse i forsøgsdyr kan bruges til at vurdere biotilgængeligheden af Cd hørfrø og kakao. Der er flere andre typer af fødevarer og fødevarekomponenter, som er væsentlige bidragydere Cd eksponering fra kosten. Det ville være yderst relevant, også at undersøge biotilgængeligheden af cadmium fra disse, på samme måde som biotilgængeligheden af cadmium i hørfrø og kakao blev undersøgt i denne undersøgelse.

Summary and conclusion

In this project, the bioavailability of cadmium from whole linseed, linseed, cocoa and cadmium chloride studied in a rat feeding study and in an in vitro assay simulating the conditions in the gastrointestinal tract in rats and humans, respectively.

In the feeding study rats were divided into groups, which were dosed with cadmium from various sources; whole linseed, milled linseed, cocoa and $CdCl_2$. Linseed or cocoa made up 10% of the feed in weight and was added as a replacement for carbohydrate source. This was done to ensure that the concentration of the other nutrients remained unchanged. In the $CdCl_2$ group cadmium chloride was mixed into the feed. The rats were dosed for 3 weeks and the cadmium content was measured in the rats' kidneys. Important differences in the level of cadmium in the kidneys were observed between the different groups. The experiment showed that the bioavailability of cadmium is much the same from the three sources. However, there may be a slightly greater bioavailability from whole linseed compared to crushed linseed and it seems that the bioavailability of cocoa is slightly lower than from linseed.

There are differences in the gastrointestinal tract in rats and humans. It was considered that the main difference in relation to the bioavailability of cadmium is that the pH is significantly lower in humans compared to rats. Therefore an in vitro study was carried out, where the linseed and cocoa was extracted with hydrochloric acid in concentrations equivalent to that found in the stomach of rats and humans. The experiment showed that the higher concentration of acid in the stomach of humans results in a substantially increased release of cadmium from both the linseed and the cocoa and thus a potentially increased bioavailability.

The present study confirms the absorption of Cd from food found in other studies reported by EFSA (EFSA 2009a). This project could not confirm that the bioavailability of Cd in rats is lower from whole linseed compared with crushed linseed. A possible explanation may be that the rats, unlike humans, are chewing linseed, thereby increasing the possibility of Cd can be brought into solution in the gastrointestinal tract. The very large difference in the release of Cd from crushed linseed in the simulation of human gastric juice compared to the rat gastric juice indicate that Cd from crushed linseed are more bioavailable in humans than Cd from whole linseed. This conclusion is supported by the results of the animal experiment, where the soluble $CdCl_2$ seems to be the most bioavailable. These results may not provide Danish Veterinary and Food Administration reason to change the advice to the public concerning the intake of whole or crushed linseed. A study in which rats are dosed with whole linseed by gavage probe could provide further information concerning the difference in bioavailability between whole or crushed linseed. The bioavailability of Cd appears to be slightly lower in cocoa compared to the other matrices, but the differences are not sufficient that it should give rise to specific advice to the public on the intake of cocoa and chocolate.

This project has shown how a study in experimental animals can be used to assess the bioavailability of Cd from linseed and cocoa. There are several other types of foods and food components that are major contributors to the Cd exposure from the diet. It would be highly relevant also to investigate the bioavailability of cadmium from these, in the same way as the bioavailability of cadmium in linseed and cocoa were investigated in this study.

1. Introduction

Cadmium (Cd) is a toxic element found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources. Food is the main source of cadmium exposure for the non-smoking general population. Besides foods, tobacco smoking and work place air have been identified as potential significant contributors to cadmium exposure.

Cadmium toxicity

Upon exposure cadmium is efficiently retained in the kidneys and liver in the human body, with a very long biological half-life ranging from 10 to 30 years. Cadmium is primarily toxic to the kidneys and may cause renal dysfunction. Cadmium can also cause bone demineralisation, either through direct bone damage or indirectly as a result of renal dysfunction. There is limited evidence for the carcinogenicity of cadmium following oral administration. In 2009 the CONTAM Panel of EFSA evaluated the dietary exposure to cadmium in the European population. Here a tolerable weekly exposure (TWI) value of 2.5 µg/kg bw/week was established, based on human studies on kidney effects (EFSA, 2009). This value was maintained in a statement from 2011 (EFSA, 2011) following a renewed evaluation due to a provisional tolerable monthly exposure (PTMI) of 25 µg/kg bw/month established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2010.

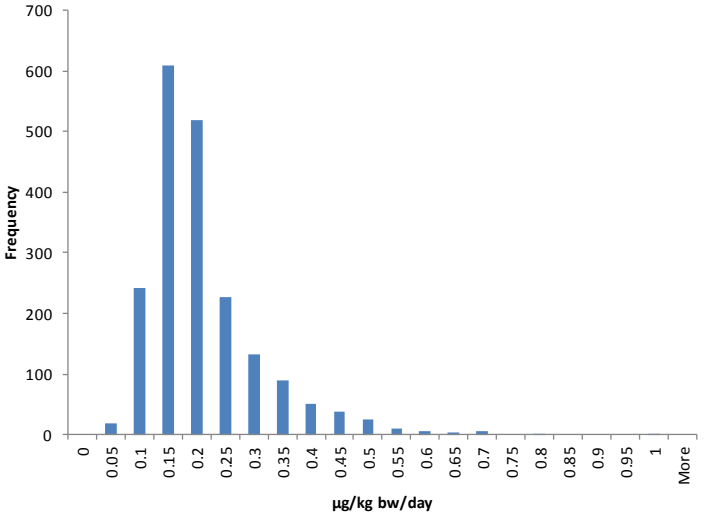
Absorption of cadmium

Absorption in rats and mice following oral administration of cadmium chloride varies from 0.2 to 3 % of the administered dose, depending on the dose and of the duration of the exposure. A refined diet high in fat and protein increases cadmium absorption, partially due to increased gastrointestinal passage time (EFSA 2009).

In humans, estimated daily intakes from the diet indicate that cadmium absorption from food is about 3-5 %. In 14 healthy adults, an average of 4.6 % of CdCl2 administered in water taken with a meal was retained (McLellan et al., 1978). The influence of chemical complexation of cadmium on absorption was evaluated in seven volunteers who ingested brown crab meat (hepatopancreas) labelled with ^{109}CdCl2 by prior feeding of the crabs; whole-body counting ranged from 1.2 to 7.6 % with a mean of 2.7% (EFSA 2009).

Dietary exposure to cadmium

Recently, DTU Food issued a report on chemical contaminants in foodstuffs and results from the analysis of various types of foodstuffs during the period 2004-2011 were compiled and the dietary exposure to cadmium in the Danish population was estimated. The most important contributors to dietary exposure to cadmium in the Danish population are the food groups: cereals and cereal products (48.8%) and vegetables and vegetable products (34.3%) (DTU Food, 2013) but cocoa or chocolate was not included in this exposure assessment.
Figure X shows the dietary exposure to cadmium in the Danish population in µg/kg bw/day. The mean exposure at 0.18 µg/kg bw/day corresponds to 50% of the established TWI value at 0.36 µg/kg bw/day (=2.5 µg/kg bw/week) by EFSA (EFSA, 2009) and about 5% of the Danish population (primarily children) has an intake, which exceeds the TWI value.



		µg/kg bw/day
Mean		0.18
sd		0.10
N		1974
Percentiles	1	0.05
	5	0.08
	25	0.12
(median)	50	0.16
	75	0.22
	95	0.38
	99	0.52
Minimum		0.02
Maximum		0.97

FIGURE 1 DISTRIBUTION OF CADMIUM EXPOSURE IN µG/KG BW/DAY IN THE DANISH POPULATION (4-75 YEARS) (DTU FOOD, 2013).

These data indicate that the intake of cadmium from food in the Danish population is at a level where a health risk for high-consuming part of the Danish population cannot be excluded. However, considerations on the possible differences in bioavailability from the different types of food have not been taken into consideration.

Data from the Danish monitoring programme show that linseeds and cocoa are food commodities, which may contain elevated levels of cadmium. Linseed samples (N=13) had cadmium concentrations in the range 0.122-0.500 mg/kg (mean=0.391 mg/kg) and cocoa powder (N=18) contained in the range 0.026-1.91 mg/kg (mean=0.248 mg/kg). These commodities are usually only consumed in low to moderate amounts by the general population, but if consumed they can be significant contributors to the total dietary exposure to cadmium. Only little is known about the bioavailability of cadmium from specific food groups, including linseeds and cocoa.

The objective of this project is to investigate the bioavailability of cadmium from selected food sources to provide the Danish Veterinary and Food Administration with a tool to refine the consumer advises. The project was intended to contain the following parts:

1. In recent years several books and articles in journals and newspapers have suggested that it would be health beneficial to supplement the food with high quantities (up to 60 g/day) of crushed linseeds. It is assumed by the authors of these papers that the potential health beneficial unsaturated fatty acids are more bioavailable when the linseeds are crushed. The Danish Veterinary and Food Administration have advised the population not to eat high quantities of crushed linseed due to the potential increased bioavailability of cadmium. On the other hand the Danish Veterinary and Food Administration find it acceptable to eat whole linseed as it is assumed that these will pass the gastro intestinal tract almost unchanged although it has not been investigated whether the cadmium in whole linseed will be released during digesting. In the present project we have investigated the difference in bioavailability of cadmium from whole linseeds and crushed linseeds in rats and compared these values with the bioavailability of cadmium from the soluble cadmium salt $CdCl_2$. We have furthermore investigated whether the differences in the pH value in the stomach in rats and humans influence the bioavailability of cadmium differently in humans and rats.

2. In grain and grain products cadmium is bound mainly to the bran. The concentrations of cadmium in full grain products are therefore usually higher than the concentration in refined grain products. As much of the bran will pass the gastrointestinal tract without absorption, it is likely that cadmium bound to the bran will be less available than cadmium bound to the more digestible part of the food. It was initially a purpose of the project to investigate this in the rat experiment, but it was not considered possible due to relative low concentration of cadmium found in wheat flour (both full grain and refined grain) compared with the content in the cadmium in other part of the feed used in the experiment.

3. There is little knowledge on the bioavailability of cadmium from cocoa, which may contain relatively high concentrations of cadmium. The bioavailability of cadmium from cocoa is compared to the bioavailability of cadmium from linseed and the soluble cadmium salt $CdCl_2$. The potential differences in absorption between rats and humans due to differences in pH are investigated by *in vitro* experiments.

2. Study design and methods

Introduction
In this chapter the methods used in the project will be presented, as well as the study design and changes including the scientific arguments for the modifications in the project compared to the initial plan. All results are presented in the following chapter, but references to these are given in this chapter when necessary.

Animal experiments ethics and authorization
Animal experiments were carried out at the DTU Food (Mørkhøj, Denmark) facilities. Ethical approval was given by the Danish Animal Experiments Inspectorate. The authorization number given: 2012-15-2934-00089. The experiments were overseen by the National Food Institutes in-house Animal Welfare Committee for animal care and use.

Determination of cadmium concentration
Feed and kidneys: Subsamples (homogenized feed 0.2-0.4 g and whole single kidneys 0.6-1.5 g) were digested in high-pressure quartz vessels using microwaves (Multiwave 3000, Anton Paar, Austria) with 2 ml ultrapure water and 4 ml concentrated nitric acid (PlasmaPure, SCP Science, France). Prior to analysis the digests were further diluted to a volume of 40 ml with ultrapure water from a Millipore Element apparatus (Millipore, Milford, MA, USA).
Simulated gastric juices: The simulated gastric juice suspensions were first centrifuged (4700 rpm, 10°C, 15 min) followed by filtration with single use hydrophilic syringe filters (0.45 µm, Minisart, Sartorius, Göttingen, Germany) and prior to analysis aliquots (0.4 ml) were further diluted to a volume of 5 ml with ultrapure water.

Blood samples: Whole blood rat samples (200 µL) and reference material (Seronom L-2, Trace Elements Whole Blood (SEROAS, Billingstad, Norway) were diluted 25 times with an aqueous extract containing 1.2% 2-propanol (1.2%), 0.4 g/l (NH4)2EDTA, 0.4 g/l Triton X-100, 1% TMAH (Tetramethylammonium hydroxid) and 2 µg/l internal standard (rhodium). Cadmium and rhodium (Cd111 and Rh103) were measured by ICP-MS (Thermo Scientific iCAP Q, Thermo Fisher Scientific GmbH, Bremen, Germany) in standard mode with no further sample pre-treatment. External calibration standards (0; 0.05; 0.10; 0.25; 0.60 µg/l) matching the solvent (2-propanol, (NH4)2EDTA, Triton X-100) were applied for quantification. Empty sample tubes filled with the extraction mixture were subjected to the sample preparation procedure to correct for any possible contamination of Cd (reagents blanks).

The cadmium content was subsequently determined at m/z 111 by ICP-MS using an Agilent 7500ce instrument (Agilent Technologies, Waldbronn, Germany) equipped with a glass concentric nebuliser (Agilent) and a Scott-type double-pass water-cooled spray chamber (Agilent). Typical plasma conditions were 1,500 W RF power, 15 $l min^{-1}$ plasma gas, 0.88 $l min^{-1}$ carrier gas and 0.32 $l min^{-1}$ makeup gas. Quantification was done by addition calibration with internal standardisation using ^{103}Rh as internal standard at 1 $\mu g l^{-1}$ in all blanks, standards and samples. Standard stock solutions of cadmium and rhodium were obtained from SCP Science (Courtaboeuf, France).
The method is accredited according to ISO17025 by the Danish accreditation body DANAK.

Quality assurance parameters

The limit of detection, LOD, was calculated according to the three-sigma criterion at 0.6 µg/kg (kidneys) and 3 µg/kg (feed materials) from the standard deviation of the analytical blank values (N=17). The LODs were sufficiently low to detect the cadmium content in the present study with satisfactory precision. The trueness was verified from the analysis of the certified reference material BCR186 Pig Kidney (Institute of Reference Materials and Measurements (IRMM), Geel, Belgium) 2.83±0.17 mg/kg (N=6, mean ± 2sd), which results agreed well with the certified value (2.71±0.15 mg/kg).

Pilot study with Fischer 344 SPF rats

Initial calculation based on the concentration of cadmium in the wheat flour, whole linseed, crushed linseed and cocoa and on the expected absorption of cadmium in rats (0,2 - 3 %, EFSA, 2009) indicated that it would be possible to measure the blood concentration of cadmium with sufficient precision. The hypothesis was that the blood concentration in the rats would reach a steady state after a few weeks. The purpose of the pilot study was to investigate this further and if possible confirm the hypothesis. For the pilot study 6 male Fisher 344 SPF rats at an age of 6 weeks were acclimatised for one week. After acclimatisation they were dosed with feed containing cadmium chloride at concentration similar to the concentrations expected in cocoa (2,06 µg/kg) for 2 weeks. Blood samples were taken from the tissue under the tongue after 7 days of dosage, 11 days of dosage and at sacrifice at 14 days.

As basic feed for this study a semi synthetic feed from another study at the animal facilities at DTU was used (see table 1).

TABLE 1 FEED INGREDIENTS IN 1 KG OF FEED USED IN THE PILOT RAT STUDY

Potato protein (g)	100
Corn starch (g)	700
Fish meal (g)	80
ADEK-vit/oil (g)	50
Mineral mixture(g)	28
B-vitamin (g)	12
Cellulose (g)	30

The blood concentration of cadmium after 2 weeks was 0.05 ± 0.04 µg/l (mean ± sd) . This is an extremely low value considering the relative high concentration of cadmium in the feed. As this concentration was near the detection limit of the method it was considered unlikely that the analytical method had sufficient sensitivity to determine differences in the blood concentration of cadmium due to minor changes in the cadmium concentrations in the feed. Therefore it was decided not to use blood concentration as a marker for cadmium absorption in the main study.

All parts of the feed was analysed and a high cadmium concentrations in the potato protein of 132 µg/kg and fish meal 234 µg/kg were found (see table 3 in results section).

Main study

Due to the low concentration of cadmium in wheat flour (11 mg/kg) and whole grain wheat flour (27 mg/kg) it was decided not to include this in the main study. The study therefore included animals fed with either:

- control feed
- feed containing whole linseed
- feed containing crushed linseed
- Feed containing cocoa
- feed containing the water soluble $CdCl_2$.

Due to the smaller number of dosage groups it was decided to increase the number of animals in each dosage group from 6 to 8 and to increase the timespan for dosage from 2 to 3 weeks.

Based on the results from the pilot study it was decided to change the feed composition. In order to reduce the cadmium exposure from the feed ingredients, the protein sources (fish meal and potato protein) were replaced by caseinate with a low cadmium concentration (see table 1 in results for exact values). The dosed animals were given 10 % of the feed (determined by weight) as whole linseed, crushed linseed or cocoa. To achieve this and to make as few other changes in the feed composition as possible the substances replaced a similar amount of corn starch. Thereby the concentrations of all nutritional elements were the same in the different groups although the different feeds were not isocaloric.

Since it was not possible to measure the cadmium concentration in blood with sufficient precision in the pilot study, it was decided to use the kidney concentration of cadmium. As cadmium accumulates in the kidney it was expected that the concentration in this organ was higher compared with the blood concentration. The suitability of the analytical method to analyse cadmium in rat kidney was tested on two kidneys from rats at a similar age as the animals would be at sacrifice in the feeding study. There were no methodological problems and the cadmium concentration in these kidneys was much higher than the detection limit of the method. As the kidney concentration of cadmium reflect the lifetime exposure to cadmium it was necessary to be sure that the animals did not receive significant amounts of cadmium in feed before they were delivered to DTU. Therefore the standard altromin feed used at the facilities where the rats were breed was analysed. The cadmium content of this feed was high (60 – 70 µg/kg). It was therefore decided to buy 4 weaning mother animals with 10 male pups each and let these rats go directly from breast feed to the control feed. It was only possible to buy Wistar rats on these conditions. The consequences of changing from Fisher 344 to Wistar rats was expected to be a little higher variability of the results because the Wistar strain is more genetic heterogeneous compared to the Fisher 344 rats.

TABLE 2 FEED COMPOSITION FOR 1 KG OF FEED IN EACH OF THE GROUPS IN THE MAIN STUDY:

Feed	Control	Crushed linseed	Whole Linseed	Cocoa	Cd
Caseinate (g)	180	180	180	180	180
Potato starch (g)	220	220	220	220	220
Corn starch (g)	460	360	360	360	460
Crushed linseed	0	100	0	0	0
Whole Linseed	0	0	100	0	0
Cocoa	0	0	0	100	0
ADEK-vit/oil (g)	50	50	50	50	50
Mineral mixture(g)	28	28	28	28	0
Mineral mixture(g) added Cd	0	0	0	0	28
B-vitamin (g)	12	12	12	12	12
Cellulose (g)	50	50	50	50	50

Simulation of gastric juice in humans and rats

As cadmium in food is bound as inorganic cadmium the most important differences in the conditions in the human stomach and the rat stomach was considered to be the pH value of the gastric juice, which is pH 1- -2 in humans and about pH 4 in rats. To test whether this influenced the different food matrices differently an *in-vitro* experiment was set up, which simulated the human and rat stomach, respectively. Subsamples (approx. 10 gram) of the different food items stirred for 30, 60 or 120 minutes, respectively, in 100 ml hydrochloric acid 0.1 M, with initial pH of 1.5 and addition of 0.1 M NaCl and KCl. The samples were centrifuged at 800 g for 10 minutes and the supernatants were collected. The conditions in the rat stomach were simulated by adjusting the pH value to 4 using disodiumcarbonate and the samples were treated as described in the previous part.

3. Results and discussion

Cadmium in feed and feed ingredients

Table 3 shows the results from the analysis of cadmium in feed ingredients and the complete feeds. For corn and potato starch, vitamin mixtures, cellulose powder and caseinate the cadmium concentration was in all cases very low (up to 3 µg/kg). The mineral mixture used had a concentration of Cd at 197 µg/kg and the Cd probably originated from cadmium-containing impurities in some of the minerals used in the mixture. The cocoa powder (fairtrade organic raw cacao powder from The raw chocolate company) contained a high level of Cd at almost 1400 µg/kg. The whole linseeds (organic linseed from Biogan) contained a slightly higher Cd concentration (151 µg/kg) than the crushed linseeds (crushed linseed from naturdrogeriet) (123 µg/kg).

Four different subsamples of each of the complete feed samples were analysed in order to test the homogeneity of the feed. For the control, linseed feeds and cocoa feed satisfactory homogeneity was demonstrated with RSD values ≤12%. However, for the complete feed, which was added $CdCl_2$, a high variability in the results for the four subsamples was observed (RSD=76%), indicating that the feed material was not homogeneous.

TABLE 3 CADMIUM CONCENTRATION IN FEED INGREDIENTS AND COMPLETE FEED

Feed/ingredient type	N	mean (µg/kg)	sd (µg/kg)	rsd (%)	range (µg/kg)		
Corn starch	1	< 3	-	-	-	-	-
ADEK vitamin mixture	1	< 3	-	-	-	-	-
BC Vitamin mixture	1	< 3	-	-	-	-	-
Cellulose powder	1	< 3	-	-	-	-	-
Potato protein	1	132					
Potato starch	1	< 3	-	-	-	-	-
Fish meal	1	234					
Caseinate	1	3	-	-	-	-	-
Mineral mixture	1	197	-	-	-	-	-
Cocoa powder	1	1379	-	-	-	-	-
Wheat flour	2	11	2	0.9	11	-	11
Wholemeal wheat flour	2	28	3	13	25	-	30
Linseeds, whole	2	123	2	1	122	-	124
Linseeds, crushed	2	151	1	1	150	-	152
Feed, pilot study	1	206					
Altromin whole feed	1	60-70					
Feed, control	4	6	1	12	6	-	7
Feed, crushed linseeds	4	22	1	6	21	-	24
Feed, whole linseeds	4	19	2	10	17	-	22
Feed, cocoa	4	164	3	2	161	-	169
Feed, $CdCl_2$	4	950	724	76	217	-	1795

Animals and feed intake in the main study

The feed intake was similar in all groups (see table 4). However, the weight gain was significantly higher in the groups given crushed and whole linseeds. That is probably caused by the high content of fat from the unsaturated oil in the linseed grains and The high weight gains in rats given whole linseeds indicate that the oil is bioavailable for the rats. The reason for that could be that rats, in contrast to humans, crush the whole linseeds by chewing when eating.

The high standard deviations in the feed intake are probably caused by uncertainties. The feed is a powder and some of it will be lost due to the behaviour of rat in the cage.

TABLE 4 FEED INTAKE, WEIGHT GAIN AND INTAKE OF CADMIUM IN THE MAIN STUDY.

	Control	Crushed linseed	Whole linseed	Cocoa	$CdCl_2$
Feed intake (g)	229 ± 87	195 ± 89	216 ± 83	204 ± 54	215 ± 55
Weight gain (g)	99 ± 17	136 ± 8	135 ± 11	114 ± 9	112 ± 17

Cadmium in rat kidney samples

Table 5 shows the concentration results of the analysis of cadmium in the rat kidneys. For all four groups receiving feed added linseeds, cocoa or $CdCl_2$ the concentration in the kidneys is significantly different from the control group (t-tests, 5 % level). The individual results for each rat and the mass of the kidney can be found in Annex X.

TABLE 5 CADMIUM CONCENTRATION IN RAT KIDNEYS OF EXPOSED RATS

Group	N	mean (µg/kg)	sd (µg/kg)	rsd (%)	range (µg/kg)		
Control	6	13	9	68	7	-	29
Crushed linseed	8	22	4	21	17	-	31
Whole linseed	8	28	5	19	20	-	37
Cocoa	8	156	55	35	81	-	257
$CdCl_2$	8	1379	254	18	958	-	1707

One of the results for the control group (animal no 2 at 29 µg/kg) could be identified as an outlying result by the Q-test (90% confidence level). When excluding this result the mean cadmium concentration in the control group is even lower, mean=9 µg/kg (rsd 35%).

Table 6 shows the cadmium content in the kidneys of the rats in each of the groups. These results are obtained by multiplication of the concentration with kidney weight and furthermore multiplication by 2 to get the cadmium content in both kidneys (assuming identical weight of the two kidneys).

TABLE 6 ABSOLUTE CADMIUM CONTENT IN RAT KIDNEYS OF EXPOSED RATS

	N	mean (µg)	sd (µg)	rsd (%)	range (µg)	
Control	6	24	12	49	13	- 46
Crushed linseed	8	36	7	21	25	- 49
Whole linseed	8	47	12	24	27	- 64
Cocoa	8	230	71	31	129	- 328
$CdCl_2$	8	2245	459	20	1622	- 2932

Again animal no 2 is an outlier (Q-test, 90% confidence level) and when excluded the mean content is 20 µg.

When taking the concentration of Cd in the feed into account and assuming that the amount of feed consumed by all groups is equal, a relative absorption can be estimated by division of amount of Cd in kidneys with the amount of Cd in the feed supplied throughout the feeding trial period (Table 7). The relative absorption was in the range of 0.9% to 2.0%. However, if discarding the outlying result for the control group (see text above) and using the corrected kidney mean at 9 µg/kg the factor for this group is 1.5 and similar to the factor for the other groups. The results indicate that differences in cadmium bioavailability exist from the different food items investigated in the present study. It is somewhat surprising that the bioavailability from crushed linseeds in the rat study seems to be lower than the bioavailability from the whole linseeds. This part of the study can probable not be extrapolated to humans and may be due to the crushing of the whole linseeds by chewing of the rats. In table 8 the release of Cd from the whole and crushed linseed indicate that the condition in the human stomach favour the release of cadmium from crushed linseed compared to the condition in the rat stomach and thereby increasing the bioavailability.

TABLE 7 PERCENT OF CD IN FEED DEPOSITED IN THE KIDNEY

Group	N	Percent of ingested Cd in kidneys (%)
Control	7	2,0
Crushed linseed	8	0.9
Whole linseed	8	1.5
Cocoa	8	0.7
CdCl2	8	4.6

Cadmium in simulated gastric juices

Table 8 shows the results from the analysis of cadmium in simulated gastric juices of humans and rats, respectively. The experiments were done on three different time lengths of 0.5, 1 and 2 hours, respectively. The length of the experiments did not influence the result, indicating that the bioaccessible fraction of the cadmium in the foodstuffs has been released to the liquid phase in less than half an hour and prolonging the time did not release more cadmium.

TABLE 8 CADMIUM CONCENTRATION IN SIMULATED HUMAN AND RAT GASTRIC JUICES.

		Simulated gastric juice	
Food type	Time (hours)	Human (µg/l)	Rat
Whole linseed	0.5	2,6	1,5
	1	2,5	1,7
	2	2,7	2,0
Crushed linseed	0.5	12	4,1
	1	11	4,4
	2	11	4,3
Cocoa	0.5	68	44
	1	68	45
	2	67	46

The results indicate that human gastric conditions (at pH 1.5) are more efficient to release cadmium from foodstuff compared to rat gastric conditions (at pH 4). The mean ratio between human/rat is 1.5 for both whole linseeds and cocoa, but even higher (2.6) for crushed linseeds. This factor needs to be taken into account when evaluating the data from the rat feeding trial and transferring these data to humans.
Furthermore, the data indicate that cadmium is more accessible from crushed linseeds compared to whole linseeds. When taking the concentration of cadmium in whole and crushed linseeds into account a factor describing the increased bioaccessibility of cadmium from crushed compared to whole linseeds can be calculated at 3.6 for humans and 2.0 for rats by the following approach:

$$Factor\left(\frac{crushed}{whole}\right) = \frac{C_{GJ.crushed}}{C_{GJ,whole}} \times \frac{C_{whole}}{C_{crushed}}$$, where

C_{GJ} = concentration in gastric juice
C = concentration in foodstuffs

The increased bioaccessibility in human gastric environment is most probably due to the lower pH (pH = 1.5) compared to the rat gastric environment with a higher pH value (pH = 4).
In the rat study the bioavailability was not significantly different between the rats exposed to whole linseeds and the rats exposed to crushed linseeds. This is probably because the rats crush the whole linseeds when chewing the feed and hence no difference was observed in the present study. In order to evaluate this, the linseeds should be force-fed to the rats (e.g. by gavage) in order not to let the animals chew on the linseeds prior to ingestion.

Perspective for risk managers

The present study confirms in general the expected level of absorption of cadmium from food in rats as well as humans from other studies (EFSA, 2009). The project could not confirm that the bioavailability of Cd is lower in whole linseed compared to crushed linseed in rats. The reason could be that the rats chew the linseed in contrast to humans. The very large difference in release of cadmium from crushed linseed in a simulation of human gastric juice compared with the rat gastric juice indicate that Cd from crushed linseed is more bioavailable than Cd from whole linseed. This conclusion is supported by the result in the animal experiment where the soluble $CdCl_2$ seems to be the most bioavailable. These results give no reason for the Danish Food and Veterinary Administration to change advises concerning whole and crushed linseed. A further experiment in rats where the rats are given the linseed by gavage could help clarified this issue further.

The present project has demonstrated how an animal study can be used to assess the bioavailability of Cd from two different Cd containing foodstuffs, linseeds and cocoa. There are several other types of foodstuffs that have elevated levels of Cd and also foodstuffs with lower level of Cd, but with higher consumption, which are significant contributors to dietary Cd exposure and hence it would be highly relevant to evaluate these food item similarly to the present study.
The bioavailability seems to be a little lower in cocoa compared to the other matrices but the differences are not sufficient to give special advices concerning cocoa and chocolate.

It would furthermore be very relevant to evaluate the bioavailability of other toxic elements, e.g. lead, mercury and inorganic arsenic, where there also is a lack of knowledge. Such studies would be helpful in the refinement of the risk assessment of dietary exposure to these compounds.

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Appendix 1: Results from the analysis of Cd in kidney and food

Animal no	Group	Analytical series	Kidney mass (g)	Cd result (µg/kg)	Cd in kidney (ng)
1	Control	2	1,472	7,7	22,7
2	Control	2	0,786	29,4	46,2
3	Control	3	1,130	8,4	19,0
4	Control	2	0,926	15,1	28,0
5	Control	Animal lost	-	-	-
6	Control	1	0,944	7,0	13,1
7	Control	Sample lost	-	-	-
8	Control	3	0,948	9,0	17,0
9	Crushed linseed	1	0,790	19,6	30,9
10	Crushed linseed	2	0,925	17,1	31,6
11	Crushed linseed	1	0,842	21,3	35,8
12	Crushed linseed	2	0,934	23,0	43,1
13	Crushed linseed	3	0,886	20,9	37,0
14	Crushed linseed	3	0,708	17,7	25,0
15	Crushed linseed	2	0,762	23,3	35,6
16	Crushed linseed	3	0,780	31,4	49,0
17	Whole linseed	3	0,857	27,0	46,3
18	Whole linseed	1	1,044	29,0	60,5
19	Whole linseed	1	0,768	33,2	51,0
20	Whole linseed	1	0,958	24,8	47,5
21	Whole linseed	1	0,865	36,9	63,8
22	Whole linseed	3	0,857	25,5	43,8
23	Whole linseed	1	0,792	25,2	40,0
24	Whole linseed	2	0,660	20,4	26,9
25	Cocoa	2	0,786	169	266
26	Cocoa	3	0,808	136	219
27	Cocoa	1	0,734	154	225
28*	Cocoa	1	0,399	81	129
29	Cocoa	2	0,813	202	328
30	Cocoa	2	0,769	113	174
31	Cocoa	3	0,664	135	179
32	Cocoa	3	0,626	257	322
33	$CdCl_2$	3	0,837	1436	2403
34	$CdCl_2$	1	0,731	1707	2495
35	$CdCl_2$	3	1,008	1454	2932
36	$CdCl_2$	3	0,803	1307	2099
37	$CdCl_2$	2	0,822	1605	2638
38	$CdCl_2$	2	0,707	1484	2099
39	$CdCl_2$	1	0,751	1080	1622
40	$CdCl_2$	2	0,872	958	1671

* only half of the kidney was available for analysis

Bioavailability of cadmium from linseed and cocoa

In Denmark and EU the exposure of cadmium from food is at a level that is relatively close to the Tolerable Daily Intake (TDI). This report describes an investigation of the bioavailability of cadmium in selected food items known to contain high levels of cadmium. The purpose was to provide data which can be used to further qualify the estimated exposure of the population to cadmium via food. The background for carrying out this investigation was the results from a survey of cadmium and cadmium compounds (Environmental Project no. 1471) conducted by the Danish EPA under the LOUS-review.

The investigation was conducted as a feeding study in rats in combination with in-vitro studies simulating the conditions in the stomach of both rats and humans. The results of the investigation do, however, not provide a basis for changing the current advice to the public neither regarding the intake of whole or crushed linseed nor the intake of cocoa and chocolate.

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Evaluation of certain food additives and contaminants

Seventy-seventh report of the
Joint FAO/WHO Expert Committee on
Food Additives

Summary of cadmium occurrence data and national food consumption data used in the evaluation

Table 1. Summary of individual occurrence data for cadmium in cocoa and its products as submitted (Number of ND reported) (unit: µg/kg)

Cocoa product	Czech Republic	Denmark	Estonia	France	Germany	Rumania	Singapore	Slovakia	Sweden	U.S	Ecuador	Grand total
Cocoa Beans			62	61	3(1)			2		3	320	451(1)
Cocoa beverage					122(13)					15 (1)		137(14)
Cocoa mass					85							85
Cocoa Powder	4	36		7	890 (31)		137 (120)1	206(21)	8	4		1292 (172)
Other Cocoa products	12	80 (20)	1	35	1047 (83)	39(39) 1		502(94)	22(1)	216 (28)		1954 (265)
Grand Total by country	16	116(20)	63	103	2,147 (128)	39(39)	137(120)	710(115)	30(1)	238 (29)	320	3919 (452)

Table 2. Summary of aggregated occurrence data for cadmium in cocoa and its products submitted (unit: µg/kg)

Country	Cocoa product	Number	Lower mean	Mean	Upper mean	Median
Australia	**Other cocoa products (including chocolate), nes (Milk chocolate)**	4	17	42	100	23
Ecuador	**Cocoa bean**	780	150	840	4080	-
New Zealand	**Cocoa mass**	10	8.2	25	42.4	23.5
Singapore	**Cocoa powder**	107	LOQ	LOQ	LOQ	LOQ
Singapore	**Cocoa powder**	10	410	514	740	490
Singapore	**Cocoa powder**	3	350	413.3	540	350

Table 3. Summary of Consumption data submitted by EU (number of data points)

Countries	Infant (<1 year)	Toddlers (1-<3 years)	Other children	Adolescents	Adults	Elderly	Very elderly
Austria					ASNS (2123)		
Belgium		Regional_Flanders (36)	Regional_Flanders (625)	Diet_National_ 2004 (611)	Diet_National_ 2004 (1356)	Diet_National_ 2004 (534)	Diet_National_ 2004 (744)
Bulgaria	NUTRICHILD (861)	NUTRICHILD (428)	NUTRICHILD (434)	NSFIN (161)	NSFIN(691)	NSFIN(151)	NSFIN(200)
Cyprus				Childhealth (303)			
Czch Republic			SISP04 (389)	SISP04(298)	SISP04(1666)		
Denmark			Danish_Dietary _Survey (490)	Danish_Dietary _Survey (479)	Danish_Dietary _Survey (2822)	Danish_Dietary _Survey (309)	Danish_Dietary _Survey (20)
Estonia					NDS_1997 (1866)		
Finland		DIPP(500)	DIPP (948) STRIP (250)		FINDIET_2007 (1575)	FINDIET_2007 (463)	
France			INCA2 (482)	INCA2 (973)	INCA2 (2276)	INCA2 (264)	INCA2 (84)
Germany		DONALD_2006/2007/2008 (261)	DONALD_20062007/2008 (660)	National_Nutrition_Survey_II (1011)	National_Nutrition_Survey_II (10419)	National_Nutrition_Survey_II (2006)	National_Nutrition_Survey_II (490)
Greece			Regional_Crete (847)				
Hungary					National_Repr_ Surv (1074)	National_Repr_ Surv (206)	National_Repr_ Surv (80)
Ireland					NSIFCS (958)		
Italy	INRAN_SCAI_ 2005_06 (16)	INRAN_SCAI_ 2005_06 (36)	INRAN_SCAI_ 2005_06 (193)	INRAN_SCAI_ 2005_06 (247)	INRAN_SCAI_ 2005_06 (2313)	INRAN_SCAI_ 2005_06 (290)	INRAN_SCAI_ 2005_06 (228)
Lativia			EFSA_TEST (190)	EFSA_TEST(496)	EFSA_TEST (1384)		
Netherlands		VCP_kids (322)	VCP_kids (957)		DNFCS_2003 (750)		
Poland		IZZ_FAO_2000 (79)	IZZ_FAO_2000 (409)	IZZ_FAO_2000 (666)	IZZ_FAO_2000 (2527)	IZZ_FAO_2000 (329)	IZZ_FAO_2000 (124)
Slovakia					SK_MON_2008(2761)		

Countries	Infant (<1 year)	Toddlers (1-<3 years)	Other children	Adolescents	Adults	Elderly	Very elderly
Slovenia					CRP_2008 (407)		
Spain		enKid (17)	enKid (156) NUT_INK05 (399)	AESAN_FIAB (86) enKid (209) NUT_INK05 (651)	AESAN (418) AESAN_FIAB (982)		
Sweden			NFA (1473)	NFA (1018)	Riksmaten_1997_98 (1210)		
United Kingdom					NDNS (1724)		
Total	877	1679	8902	7210	41302	4552	1970

Table 4. Summary of submitted consumption data for cocoa and its derivates **(g/kg bw/day)**

Cocoa product	Age class	country	No_of_ subjects	No _of_ consumers	Whole		Consumers_	
					Mean	97.5th percentile	Mean	97.5th percentile
Cocoa beverage	Toddlers	Bulgaria	428	4	0.028	0.000	2.943	5.856
	Other children	Bulgaria	434	11	0.038	0.188	1.489	7.143
		Finland	250	68	1.003	7.435	3.688	15.254
		France	482	85	0.593	6.211	3.365	17.731
		Germany	223	2	0.012	0.000	1.352	1.365
		Greece	847	12	0.047	0.000	3.285	8.995
		Latvia	190	38	0.827	7.167	4.132	9.942
		Sweden	1473	7	0.011	0.000	2.228	4.000
	Adolescents	Belgium	611	67	0.301	3.309	2.744	7.528
		Cyprus	303	136	1.459	6.944	3.251	8.810
		France	973	146	0.249	3.001	1.656	7.653
		Germany	1011	129	0.420	4.478	3.294	9.184
		Latvia	496	68	0.347	3.214	2.531	4.891
		Sweden	1018	13	0.021	0.000	1.658	5.000
	Adults	Belgium	1356	95	0.164	2.146	2.341	5.680
		France	2276	195	0.100	1.429	1.170	5.065
		Germany	10419	515	0.130	2.147	2.628	7.692
		Latvia	1384	41	0.045	0.880	1.523	2.875
		Netherlands	750	8	0.012	0.000	1.101	2.286
		Slovakia	2761	9	0.014	0.000	4.249	7.353
		Slovenia	407	15	0.124	2.778	3.354	5.814
		Sweden	1210	235	0.223	2.132	1.148	4.202
		United Kingdom	1724	227	0.043	0.457	0.328	1.916
	Elderly	Belgium	534	20	0.084	1.525	2.245	5.150

Cocoa product	Age class	country	No_of_ subjects	No _of_ consumers	Whole		Consumers_	
					Mean	97.5th percentile	Mean	97.5th percentile
		France	264	2	0.008	0.000	1.037	1.225
		Germany	2006	39	0.045	0.000	2.310	7.042
	Very elderly	Belgium	744	12	0.028	0.000	1.739	2.861
		France	84	6	0.028	0.300	0.395	1.313
		Germany	490	12	0.054	0.000	2.218	4.167
Cocoa powder	Infants	Bulgaria	861	6	0.001	0.000	0.161	0.329
	Toddlers	Belgium	36	1	0.014	0.513	0.513	0.513
		Bulgaria	428	32	0.009	0.133	0.124	0.517
		Finland	500	42	0.011	0.128	0.128	0.546
		Germany	261	18	0.007	0.271	0.172	0.705
		Netherlands	322	1	0.001	0.000	0.389	0.389
		Poland	79	3	0.030	0.806	0.787	1.042
		Spain	17	9	0.482	1.948	0.910	1.948
	Other children	Bulgaria	434	32	0.011	0.167	0.152	0.438
		Czech Republic	389	48	0.009	0.104	0.070	0.229
		Denmark	490	283	0.009	0.040	0.016	0.052
		Finland	1198	552	0.086	0.589	0.187	0.826
		France	482	3	0.000	0.000	0.031	0.050
		Germany	660	120	0.037	0.421	0.205	2.222
		Greece	847	36	0.013	0.068	0.302	4.610
		Italy	193	5	0.003	0.009	0.097	0.229
		Netherlands	957	4	0.000	0.000	0.053	0.061
		Poland	409	54	0.065	0.616	0.494	1.232
		Spain	555	405	0.252	1.463	0.345	1.471
		Sweden	1473	304	0.051	0.447	0.246	0.981

Cocoa product	Age class	country	No_of_ subjects	No _of_ consumers	Whole		Consumers_	
					Mean	97.5th percentile	Mean	97.5th percentile
	Adolescents	**Belgium**	611	45	0.009	0.147	0.126	0.332
		Bulgaria	162	2	0.001	0.000	0.048	0.050
		Cyprus	303	5	0.001	0.000	0.087	0.180
		Czech Republic	298	37	0.009	0.080	0.070	0.361
		Denmark	479	229	0.004	0.025	0.009	0.030
		France	973	3	0.000	0.000	0.111	0.164
		Germany	1011	28	0.004	0.040	0.147	0.439
		Italy	247	18	0.004	0.054	0.057	0.217
		Latvia	496	1	0.000	0.000	0.192	0.192
		Poland	666	70	0.027	0.313	0.261	0.570
		Spain	946	607	0.134	0.691	0.210	0.917
		Sweden	1018	236	0.048	0.405	0.209	0.667
	Adults	**Austria**	2123	55	0.001	0.000	0.040	0.215
		Belgium	1356	46	0.004	0.054	0.113	0.391
		Bulgaria	691	7	0.001	0.000	0.107	0.282
		Czech Republic	1666	156	0.002	0.030	0.024	0.069
		Denmark	2822	1278	0.003	0.015	0.007	0.027
		Estonia	1866	31	0.001	0.000	0.051	0.092
		Finland	1575	352	0.011	0.135	0.051	0.324
		France	2276	19	0.000	0.000	0.052	0.115
		Germany	10419	110	0.001	0.000	0.106	0.446
		Hungary	1074	265	0.011	0.089	0.043	0.158
		Ireland	958	111	0.001	0.012	0.010	0.029
		Italy	2313	105	0.002	0.029	0.043	0.222
		Netherlands	750	20	0.001	0.010	0.056	0.600

Cocoa product	Age class	country	No_of_ subjects	No _of_ consumers	Whole		Consumers_	
					Mean	97.5th percentile	Mean	97.5th percentile
		Poland	2527	103	0.007	0.156	0.177	0.363
		Slovakia	2761	16	0.001	0.000	0.187	0.848
		Spain	1400	31	0.003	0.000	0.131	0.250
		Sweden	1210	3	0.000	0.000	0.016	0.043
		United Kingdom	1724	8	0.000	0.000	0.036	0.176
	Elderly	**Belgium**	534	7	0.001	0.000	0.079	0.118
		Bulgaria	151	1	0.001	0.000	0.080	0.081
		Denmark	309	162	0.004	0.022	0.007	0.027
		Finland	463	59	0.005	0.078	0.041	0.234
		France	264	1	0.000	0.000	0.045	0.045
		Germany	2006	10	0.000	0.000	0.081	0.233
		Hungary	206	33	0.005	0.044	0.028	0.084
		Italy	290	7	0.001	0.000	0.022	0.081
		Poland	329	12	0.005	0.133	0.139	0.172
	Very elderly	**Belgium**	744	8	0.001	0.000	0.121	0.422
		Bulgaria	200	2	0.001	0.000	0.073	0.079
		Denmark	20	10	0.004	0.024	0.008	0.024
		France	84	1	0.009	0.000	0.783	0.783
		Germany	490	4	0.001	0.000	0.071	0.148
		Hungary	80	14	0.007	0.092	0.038	0.121
		Italy	228	4	0.001	0.000	0.033	0.089
		Poland	124	7	0.010	0.160	0.173	0.236
	General Population	**Brazil**	34003	3301	0.05	0.53	0.38	1.24
		China	65359	48	0.0005	0.0013	0.627	1.722
	Children	**China**	2784	1	0.0005	0.0005	1.397	1.397

Cocoa product	Age class	country	No_of_ subjects	No _of_ consumers	Whole		Consumers_	
					Mean	97.5th percentile	Mean	97.5th percentile
	Women of childbearing age	Brazil	10946	1293	0.05	0.52	0.34	0.97
		China	17921	17	0.0008	0.0032	0.874	3.381
Cocoa mass	Adults	Austria	2123	1	0.000	0.000	0.087	0.087
Other products	Infants	Bulgaria	861	6	0.005	0.000	0.645	1.509
	Infants	Italy	16	1	0.091	1.453	1.453	1.454
	Toddlers	Belgium	36	30	1.157	3.644	1.388	3.644
		Bulgaria	428	68	0.147	1.750	0.926	2.667
		Finland	500	58	0.055	0.673	0.474	2.462
		Germany	261	89	0.213	1.870	0.624	3.470
		Italy	36	7	0.219	2.746	1.125	2.746
		Netherlands	322	236	0.814	3.450	1.111	3.681
		Poland	79	23	0.475	3.333	1.633	3.509
		Spain	17	4	0.201	2.083	0.854	2.083
	Other children	Belgium	625	515	0.765	2.733	0.928	2.786
		Bulgaria	434	117	0.279	2.388	1.035	2.750
		Czech Republic	389	280	0.605	3.219	0.840	3.661
		Denmark	490	447	0.522	1.879	0.572	1.957
		Finland	1198	614	0.257	1.723	0.502	2.094
		France	482	441	0.783	2.857	0.856	3.355
		Germany	660	557	0.626	2.468	0.821	2.552
		Greece	847	419	0.215	1.078	0.435	1.310
		Italy	193	112	0.405	1.961	0.698	2.233
		Latvia	190	75	0.408	2.969	1.035	5.611
		Netherlands	957	760	0.822	2.951	1.035	3.333

Cocoa product	Age class	country	No_of_ subjects	No _of_ consumers	Whole		Consumers_	
					Mean	97.5th percentile	Mean	97.5th percentile
		Poland	409	156	0.636	3.578	1.668	5.389
		Spain	555	250	0.348	2.427	0.773	4.255
		Sweden	1473	494	0.212	1.583	0.632	2.469
	Adolescents	**Belgium**	611	393	0.413	1.808	0.642	2.214
		Bulgaria	162	27	0.138	1.333	0.826	4.348
		Cyprus	303	132	0.203	0.970	0.465	1.630
		Czech Republic	298	158	0.280	1.744	0.528	2.167
		Denmark	479	416	0.295	1.384	0.339	1.401
		France	973	824	0.386	1.571	0.456	1.711
		Germany	1011	373	0.177	1.234	0.480	1.764
		Italy	247	122	0.184	0.930	0.373	1.156
		Latvia	496	185	0.264	1.846	0.708	2.669
		Poland	666	205	0.299	2.105	0.970	2.674
		Spain	946	324	0.166	1.079	0.484	1.777
		Sweden	1018	395	0.191	1.215	0.492	1.875
	Adults	**Austria**	2123	535	0.150	1.521	0.594	2.353
		Belgium	1356	638	0.201	1.100	0.428	1.631
		Bulgaria	691	50	0.038	0.550	0.519	2.174
		Czech Republic	1666	300	0.056	0.676	0.312	1.250
		Denmark	2822	2035	0.133	0.682	0.184	0.773
		Estonia	1866	78	0.028	0.347	0.673	2.632
		Finland	1575	530	0.102	0.831	0.302	1.311
		France	2276	1275	0.128	0.820	0.228	0.984
		Germany	10419	3415	0.125	0.899	0.382	1.374

Cocoa product	Age class	country	No_of_ subjects	No _of_ consumers	Whole		Consumers_	
					Mean	97.5th percentile	Mean	97.5th percentile
		Hungary	1074	312	0.067	0.546	0.230	0.884
		Ireland	958	625	0.165	0.771	0.253	0.891
		Italy	2313	533	0.038	0.339	0.164	0.616
		Latvia	1384	290	0.095	0.904	0.454	1.441
		Netherlands	750	423	0.191	1.058	0.340	1.198
		Poland	2527	306	0.066	0.760	0.544	1.812
		Slovakia	2761	311	0.123	1.194	1.090	3.636
		Slovenia	407	31	0.079	1.389	1.040	1.923
		Spain	1400	539	0.108	0.828	0.280	1.200
		Sweden	1210	590	0.104	0.612	0.213	0.762
		United Kingdom	1724	942	0.128	0.738	0.234	0.874
	Elderly	Belgium	534	189	0.100	0.590	0.283	0.849
		Bulgaria	151	1	0.001	0.000	0.096	0.096
		Denmark	309	180	0.091	0.610	0.156	0.665
		Finland	463	79	0.026	0.301	0.154	0.614
		France	264	96	0.040	0.307	0.109	0.433
		Germany	2006	441	0.056	0.493	0.255	0.775
		Hungary	206	31	0.022	0.201	0.143	0.513
		Italy	290	44	0.018	0.222	0.115	0.439
		Poland	329	17	0.025	0.457	0.488	1.105
	Very elderly	Belgium	744	214	0.082	0.667	0.286	1.000
		Bulgaria	200	10	0.018	0.160	0.358	1.205
		Denmark	20	12	0.086	0.729	0.143	0.729
		France	84	27	0.059	0.513	0.182	0.766

Cocoa product	Age class	country	No_of_ subjects	No _of_ consumers	Whole		Consumers_	
					Mean	97.5th percentile	Mean	97.5th percentile
		Germany	490	134	0.060	0.392	0.218	0.746
		Hungary	80	17	0.028	0.263	0.132	0.412
		Italy	228	33	0.018	0.173	0.124	1.345
		Poland	124	6	0.018	0.241	0.362	0.686
	General Population	Brazil	34003	1530	0.05	0.48	0.88	4.33
		China	65359	120	0.001	0.003	0.513	1.79
	Children	China	2784	12	0.005	0.033	1.104	7.71
	Women of childbearing age	Brazil	10946	698	0.07	1.10	0.87	4.22
		China	17921	35	0.001	0.003	0.321	1.622

Table 4. Summary of statistical descriptors based on consumption data submitted (g/kg bw/day)

Cocoa product	Country	Age class	Whole		Consumer	
			weighted mean	97.5th percentile	weighted mean	97.5th percentile
Cocoa beverage	EU	Infants	-	-	-	-
		Toddlers*		-	(2.943)*	
		Other children	0.086	7.435	3.444	17.731
		Adolescents	0.206	6.944	2.659	9.184
		Adults	0.056	2.778	1.722	7.692
		Elderly	0.030	1.525	2.247	7.042
		Very Elderly	0.025	0.3	1.662	4.167
Cocoa powder	EU	Infants*	-	-	(0.161)*	-
		Toddlers	0.014	1.948	0.225	1.948
		Other children	0.033	1.463	0.213	4.61
		Adolescents	0.029	0.691	0.165	0.917
		Adults	0.002	0.156	0.036	0.848
		Elderly	0.002	0.133	0.027	0.234

Cocoa product	Country	Age class	Whole		Consumer	
			weighted mean	97.5th percentile	weighted mean	97.5th percentile
		Very Elderly	0.002	0.16	0.083	0.783
	China	General population	0.001	0.001	0.627	1.722
		Children**	-	-	(1.397) **	-
		Women of childbearing age	0.001	0.003	0.874	3.381
	Brazil	General population	0.05	0.53	0.38	1.24
		Women of childbearing age	0.05	0.52	0.34	0.97
Other cocoa Product	EU	Infants*	-	-	(0.761)*	-
		Toddlers	0.3	3.644	0.979	3.681
		Other children	0.458	3.578	0.787	5.611
		Adolescents	0.256	2.105	0.518	4.348
		Adults	0.109	1.521	0.329	3.636
		Elderly	0.052	0.610	0.218	1.105
		Very Elderly	0.055	0.729	0.241	1.345
	China	General population	0.001	0.003	0.513	1.79
		Children	0.005	0.033	1.104	7.71
		Women of childbearing age	0.001	0.003	0.321	1.622
	Brazil	General population	0.05	0.48	0.88	4.33
		Women of childbearing age	0.07	1.10	0.87	4.22

* Number of consumer <11, ** 1 consumer reported.

SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and *N*-methylpyridinium, and reduction of DNA damage by decreasing spontaneous DNA strand breaks pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Tchibo GmbH, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Germany, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to coffee C21 and reduction of DNA damage by decreasing spontaneous DNA strand breaks. The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. Coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and *N*-methylpyridinium (NMP), which is the subject of the health claim, is sufficiently characterised. Reduction of DNA damage by decreasing spontaneous DNA strand breaks is a beneficial physiological effect. In weighing the evidence, the Panel took into account that one human intervention study showed that daily consumption of coffee C21 (750 ml/day) for four weeks decreased spontaneous DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks, but that no other human studies in which these results have been replicated were provided, and that no evidence was provided for a mechanism by which coffee (including coffee C21) could exert the claimed effect. The Panel concludes that a cause and effect relationship has not been established between the consumption of coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, and a reduction of DNA damage by decreasing spontaneous DNA strand breaks.

KEY WORDS

coffee, C21, DNA damage, DNA strand break, health claims

[1] On request from the Competent Authority of Germany following an application by Tchibo GmbH, Question No EFSA-Q-2014-00624, adopted on 22 April 2015.

[2] Panel members: Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen. Correspondence: nda@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Marina Heinonen, Ambroise Martin, Hildegard Przyrembel, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Sean (J.J.) Strain, Inge Tetens, Hendrik Van Loveren, Hans Verhagen and Peter Willatts for the preparatory work on this scientific opinion.

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SUMMARY

Following an application from Tchibo GmbH, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Germany, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and *N*-methylpyridinium (NMP), and reduction of DNA damage by decreasing spontaneous DNA strand breaks.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.

The food that is the subject of the health claim is coffee C21. Coffee C21 is a blend of roasted coffee arabica (*Coffea arabica L.*) standardised by its content of caffeoylquinic acids, trigonelline and NMP. Caffeoylquinic acids, trigonelline and NMP can be measured in ground and brewed coffee by established methods. The Panel considers that the food, coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, is sufficiently characterised.

The claimed effect proposed by the applicant is 'reduction of the amount of spontaneous DNA strand breaks in white blood cells'. The target population proposed by the applicant is the general population. Spontaneous DNA strand breaks normally occur during the DNA repair process. DNA strand break, which may also be induced by genetic or environmental factors, is a type of DNA damage which can be measured by the comet assay (single-cell gel electrophoresis). The Panel considers that the reduction of DNA damage by decreasing spontaneous DNA strand breaks is a beneficial physiological effect.

The applicant identified six human intervention studies as being pertinent to the health claim.

These studies (except for one) were carried out with coffee types which did not comply with the specifications of coffee C21, which is standardised by its content of caffeoylquinic acids, trigonelline and NMP, were uncontrolled or did not report on spontaneous DNA strand breaks. The Panel considers that no conclusions can be drawn from these five studies for the scientific substantiation of the claim.

One placebo-controlled, randomised, single-blind, parallel study investigated the effect of consuming coffee C21 on spontaneous DNA strand breaks in healthy non-smoking males, who were accustomed to a daily coffee consumption level similar to that being investigated in this study. A total of 90 subjects were randomised (stratified by body mass index) in two groups. In the first four-week run-in period, all participants refrained from consuming coffee and instead consumed water. During the following four weeks participants consumed either coffee C21 (n=45; 45 g coffee/750 ml per day) or the same volume of water (n=45). The investigator was unaware of the treatment group allocation.

Difference in spontaneous DNA strand break changes in peripheral white blood cells between groups was the primary outcome of this study.

At baseline (i.e. after the run-in period), there was no difference in spontaneous DNA strand breaks between the groups. During the four-week intervention period spontaneous DNA strand breaks decreased in the intervention group and increased in the control group.

The Panel considers that this study shows that daily consumption of coffee C21 (750 ml/day) for four weeks decreases spontaneous DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks.

The applicant provided two animal and two *in vitro* studies in support of a mechanism by which coffee C21 could exert the claimed effect. The Panel considers that these studies do not provide

evidence for a mechanism by which coffee C21 could reduce DNA damage by decreasing spontaneous DNA strand breaks.

In weighing the evidence, the Panel took into account that one human intervention study showed that daily consumption of coffee C21 (750 ml/day) for four weeks decreased spontaneous DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks, but that no other human studies in which these results have been replicated were provided, and that no evidence was provided for a mechanism by which coffee (including coffee C21) could exert the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, and a reduction of DNA damage by decreasing spontaneous DNA strand breaks.

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BACKGROUND

Regulation (EC) No 1924/2006[4] harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 12/09/2014.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
- On 20/10/2014, during the validation process of the application, EFSA sent a request to the applicant to provide missing information.
- On 30/10/2014, EFSA received the missing information as submitted by the applicant.
- The scientific evaluation procedure started on 06/11/2014.
- On 26/11/2014, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The clock was stopped on 05/12/2014 in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- On 19/12/2014, EFSA received the applicant's reply and the clock was re-started.
- During its meeting on 22/04/2015, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and *N*-methylpyridinium, and reduction of DNA damage by decreasing spontaneous DNA strand breaks.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and *N*-methylpyridinium, and reduction of DNA damage by decreasing spontaneous DNA strand breaks.

[4] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of coffee C21, a positive assessment of its safety, nor a decision on whether coffee C21 is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address

Tchibo GmbH, Überseering 18, D-22297 Hamburg, Germany.

Food/constituent as stated by the applicant

According to the applicant, the food for which the claim is made is coffee C21, a special blend of roasted pure arabica coffee (*Coffea arabica* L.) without any non-coffee ingredients, which is standardised by its content of caffeoylquinic acids (chlorogenic acids), *N*-methylpyridinium and trigonelline.

Health relationship as claimed by the applicant

According to the applicant, consumption of coffee C21 leads to a reduction of the amount of spontaneous DNA strand breaks in white blood cells, which are measured by the comet assay.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: 'Regular consumption of coffee C21 contributes to the maintenance of DNA integrity in cells of the body'.

Specific conditions of use as proposed by the applicant

The target population proposed by the applicant is the general population.

The conditions of use proposed by the applicant are three large cups of coffee C21 (each 250 ml, prepared from two pads of 7.5 g ground coffee) per day.

ASSESSMENT

1. Characterisation of the food/constituent

The food that is the subject of the health claim is coffee C21.

Brewed coffee is a mixture of compounds, including coffee constituents, such as caffeine, caffeoylquinic acids and trigonelline, together with compounds formed during roasting, such as *N*-methylpyridinium (NMP), nicotinic acid, nicotinamide and melanoidins (Lang et al., 2008).

Coffee C21 is a blend of roasted coffee arabica (*Coffea arabica* L.). The roasting is accomplished with regular coffee manufacturing roasters which apply heat to dry beans. Roasted ground coffee (coffee C21) is standardised by its concentrations of chlorogenic acids (also called caffeoylquinic acids, polyphenols), trigonelline (alkaloid) and NMP (pyridine derivative). The concentrations of chlorogenic acids, trigonelline and the thermal degradation product NMP depend on the degree of roasting. The desired composition is obtained by blending different coffee roasts and by adjusting the roasting conditions (temperature and time). A decline of 6.6 % in caffeoylquinic acids was reported during 45 months of storage at room temperature.

The applicant indicated that ground coffee C21 contains 10.18 mg/g of caffeoylquinic acids, 3.82 mg/g of trigonelline and 1.10 mg/g of NMP. Upon a request by EFSA for clarification of the standardisation of coffee C21, the applicant indicated the minimum amount of caffeoylquinic acids, trigonelline and NMP in ground coffee C21 (9.16 mg/g, 3.44 mg/g and 0.99 mg/g, respectively).

According to the applicant, coffee C21 is prepared with a standard drip filter coffee machine, using a 20:1 (w/w) ratio of tap water to ground coffee, which results in a > 90 % extraction of caffeoylquinic acids, trigonelline and NMP.

Caffeoylquinic acids, trigonelline and NMP can be measured in ground and brewed coffee by established methods.

The Panel considers that the food, coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'reduction of the amount of spontaneous DNA strand breaks in white blood cells'. The target population proposed by the applicant is the general population.

Spontaneous DNA strand breaks normally occur during the DNA repair process. DNA strand breaks may also be induced by genetic or environmental factors (e.g. mutagenic or pro-oxidant chemicals and radiation). Such DNA strand breaks alter DNA properties, may induce anomalies during DNA replication and translation, and require repair for the maintenance of cell functioning and survival.

A DNA strand break is a type of DNA damage which can be measured by the comet assay (single-cell gel electrophoresis) (EFSA NDA Panel, 2011).

The Panel considers that the reduction of DNA damage by decreasing spontaneous DNA strand breaks is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed with the following terms: 'coffee and DNA damage', 'coffee and DNA strand break(s)', 'coffee and Comet assay', 'caffeine and DNA damage and trial', 'chlorogenic acid and DNA damage and trial', 'trigonelline and DNA damage and trial', 'methyl pyridinium and DNA damage and trial' and 'niacin and DNA damage and trial'.

The applicant identified six human intervention studies as being pertinent to the health claim.

These studies (except for one) were carried out with coffee types which did not comply with the specifications of coffee C21, which is standardised by its content of caffeoylquinic acids, trigonelline and NMP (Steinkellner et al., 2005; Bichler et al., 2007; Hoelzl et al., 2010; Misik et al., 2010), were uncontrolled (Steinkellner et al., 2005; Bichler et al., 2007; Bakuradze et al., 2011) or did not report on spontaneous DNA strand breaks (Steinkellner et al., 2005; Hoelzl et al., 2010). The Panel considers that no conclusions can be drawn from these five studies for the scientific substantiation of the claim.

The placebo-controlled, randomised, single-blind, parallel study by Bakuradze et al. (2015) investigated the effect of consuming coffee C21 on spontaneous DNA strand breaks in healthy non-smoking males (aged 19-50 years; body mass index (BMI) 19-32 kg/m^2), who were accustomed to a daily coffee consumption level similar to that being investigated in this study (i.e. 45 g/750 ml). A total of 90 subjects were randomised (stratified by BMI) in two groups. In the first four-week run-in period, all participants refrained from consuming coffee and instead consumed water (750 ml). During the following four weeks participants consumed either coffee C21 (n=45; 45 g coffee/750 ml per day) or the same volume of water (n=45). Upon a request by EFSA for clarification of the caffeine consumption, the applicant indicated that the consumption of coffee C21 led to a daily ingestion of 531 mg of caffeine in the intervention group. The investigator was unaware of the treatment group

allocation. During the run-in and the intervention periods participants were instructed to consume their usual diet and to avoid consuming coffee and caffeine-containing products and foods rich in polyphenols. Participants recorded their food intake in the final seven days of each study period. At the end of the run-in and intervention periods blood samples were taken for determination of spontaneous DNA strand breaks through the comet assay. At those visits urine samples and body weight measurements were also taken.

Difference in spontaneous DNA strand break changes in peripheral white blood cells between groups was the primary outcome of this study. The sample size (target significance level $\alpha = 5$ %, at a power of 80 %) was calculated based on a previous human intervention study, which also used the comet assay (Bakuradze et al., 2011).

Six participants withdrew from the study (n=3 in each group) owing to 'private' reasons. Compliance was assessed by monitoring trigonelline and NMP in urine. Upon a request by EFSA for clarification, the applicant indicated that these six participants were not included in the statistical analyses as they withdrew from the study in the run-in period (i.e. before the collection of baseline values).

Daily energy and macronutrient intake during the run-in and the intervention periods were not different between the groups. No changes in body weight were observed between the groups during the whole study period.

An analysis of covariance (ANCOVA) test, with baseline data as covariate, was used to assess the difference in spontaneous DNA strand break changes between groups. Additionally, the Wilcoxon rank sum test was used to cross-check the ANCOVA results.

The results of comet assays were presented as means, with standard deviations, of spontaneous DNA strand breaks, expressed as tail intensities (TI %). At baseline (i.e. after the run-in period), there was no difference in the mean of spontaneous DNA strand breaks between the groups. During the four-week intervention period the mean of spontaneous DNA strand breaks decreased in the intervention group and increased in the control group (from 0.32±0.11 to 0.27±0.09 TI % in the intervention group and from 0.31±0.12 to 0.37±0.15 TI % in the control group; ANCOVA, $p = 0.0002$; Wilcoxon rank sum test, $p = 0.0005$).

The Panel considers that this study shows that daily consumption of coffee C21 (750 ml/day) for four weeks decreases spontaneous DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks.

Mechanism by which the food could exert the claimed effect

The applicant indicated that the effect of coffee C21 on the decrease of spontaneous DNA strand breaks was mainly exerted by NMP, the major alkylpyridinium compound in roasted coffee. According to the applicant, NMP would protect against the formation of DNA strand breaks by inducing the activity of enzymes involved in the cellular defence against oxidative damage and 'other stress' by inducing the nuclear translocation of the transcription factor nuclear-factor-E2-related factor 2 (Nrf2) and thereby regulating antioxidant response element (ARE)/electrophile response element (EpRE) pathways.

The applicant provided two animal (Paur et al., 2010; Vicente et al., 2014) and two *in vitro* studies (Bakuradze et al., 2010; Boettler et al., 2011) in support of a mechanism by which coffee C21 could exert the claimed effect.

These studies investigated the effect of different coffee brews/extracts (different from coffee C21) on cytosolic and nuclear concentrations of Nrf2, and on the expression and activity of ARE/EpRE-dependent enzymes. The results on the effect of NMP on the expression of ARE-dependent enzymes in the two *in vitro* studies were inconsistent. The Panel notes that none of the

studies provided addressed whether the proposed changes in cytosolic and nuclear concentrations of Nrf2, or the expression and activity of ARE/EpRE-dependent enzymes, would affect DNA strand breaks.

The Panel considers that the animal and *in vitro* studies do not provide evidence for a mechanism by which coffee C21 could reduce DNA damage by decreasing spontaneous DNA strand breaks.

Weighing the evidence

In weighing the evidence, the Panel took into account that one human intervention study showed that daily consumption of coffee C21 (750 ml/day) for four weeks decreased spontaneous DNA strand breaks in habitual coffee drinkers after coffee withdrawal over the previous four weeks, but that no other human studies in which these results have been replicated were provided, and that no evidence was provided for a mechanism by which coffee (including coffee C21) could exert the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, and a reduction of DNA damage by decreasing spontaneous DNA strand breaks.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food, coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is 'reduction of the amount of spontaneous DNA strand breaks in white blood cells'. The target population proposed by the applicant is the general population. A reduction of DNA damage by decreasing spontaneous DNA strand breaks is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of coffee C21, a coffee standardised by its content of caffeoylquinic acids, trigonelline and NMP, and a reduction of DNA damage by decreasing spontaneous DNA strand breaks.

DOCUMENTATION PROVIDED TO EFSA

1. Health claim application on coffee C21 and reduction of DNA damage by decreasing spontaneous DNA strand breaks pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0428_DE). September 2014. Submitted by Tchibo GmbH.

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ABBREVIATIONS

ANCOVA	analysis of covariance
ARE	antioxidant response elements
BMI	body mass index
DNA	deoxyribonucleic acid
EpRE	electrophile response element
NMP	*N*-methylpyridinium
Nrf2	nuclear-factor-E2-related factor 2
TI %	tail intensity

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Safety Assessment of Plant-Derived Fatty Acid Oils

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Abstract

The Cosmetic Ingredient Review Expert Panel (Panel) assessed the safety of 244 plant-derived fatty acid oils as used in cosmetics. Oils are used in a wide variety of cosmetic products for their skin conditioning, occlusive, emollient, and moisturizing properties. Since many of these oils are edible, and their systemic toxicity potential is low, the review focused on potential dermal effects. The Panel concluded that the 244 plant-derived fatty acid oils are safe as used in cosmetics.

Keywords

oils, safety, cosmetics

Introduction

Oils derived from edible vegetables, fruits, seeds, tree, and ground nuts have been safely consumed by, and applied to the skin of, humans for thousands of years. Although nuts, fruits, and vegetables themselves may cause allergic reactions in certain individuals, the refined oils derived from these plants generally pose no significant safety concern following oral exposure, and their general biology is well characterized due to extensive use in food materials. Initially used for anointing in religious ceremonies, oils and their components have also been used on the skin for their skin conditioning, occlusive, emollient, moisturizing, and other properties.

The full list of ingredients in this report, which includes oils, hydrogenated oils, unsaponifiables, oil fatty acids, and salts of the fatty acids, is found in Table 1. Although a large number of oils derived from plants are included in this safety assessment, there is a commonality in that they all are mixtures of triglycerides that contain fatty acids and fatty acid derivatives, the safety of which in cosmetics has been established. Thus, this safety assessment focused solely on the basic chemistry, manufacturing and production methods, uses, and irritation and sensitization potential of these oils as used in cosmetic ingredients.

In preparing this report, numerous inconsistencies were noted with both taxonomic and International Nomenclature Cosmetic Ingredient (INCI) naming conventions. For example, this report includes the macadamia nut ingredients, *Macadamia integrifolia* seed oil and *Macadamia ternifolia* seed oil, which are described in the *International Cosmetic Ingredient Dictionary and Handbook*.[1] The species *M integrifolia* is currently the only species of macadamia nut which is used for oil production. The name *M ternifolia* is an old naming convention for the edible nut that is currently used to describe a noncultivated, inedible species. Both *M integrifolia* seed oil and *M ternifolia* seed oil are the same ingredient. Similar naming conflicts have been discovered with *Triticum vulgare* (wheat) germ oil and *Triticum aestivum* (wheat) germ oil, *Orbignya oleifera* seed oil and *Orbignya speciosa* kernel oil, and *Moringa pterygosperma* seed oil and *Moringa oleifera* seed oil, with these pairs being synonyms for each other. The shea plant also has 2 species names, *Butyrospermum parkii* and *Vitellaria paradoxa*. Only *B parkii* (as *B parkii*

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Table 1. Plant-Derived Fatty Acid Oils.[a]

Actinidia chinensis (kiwi) seed oil
Adansonia digitata oil (baobab)
  Adansonia digitata seed oil
  Hydrogenated *Adansonia digitata* seed oil
Aleurites moluccanus seed oil (kukui [CAS no 8015-80-3])
  Hydrogenated kukui nut oil
  Aleurites moluccanus bakoly seed oil
Amaranthus hypochondriacus seed oil (amaranth)
Anacardium occidentale (cashew) seed oil (CAS no 8007-24-7)
Arachis hypogaea (peanut) oil (CAS no 8002-03-7)
  Hydrogenated peanut oil (CAS no 68425-36-5)
  Potassium peanutate
  Sodium peanutate
  Peanut acid (CAS no 91051-35-3)
Arctium lappa seed oil (burdock)
Argania spinosa kernel oil (argan)
  Hydrogenated *Argania spinosa* kernel oil
Astrocaryum murumuru seed butter (murumuru)
  Sodium *Astrocaryum murumuruate*
Avena sativa (oat) kernel oil
Bassia butyracea seed butter
Bassia latifolia seed butter (mahwa)
Bertholletia excelsa seed oil (Brazil)
Borago officinalis seed oil (borage [CAS no 225234-12-8])
Brassica campestris (rapeseed) seed oil
  Brassica campestris (rapeseed) oil unsaponifiables
  Hydrogenated rapeseed oil
  Rapeseed acid
  Potassium rapeseedate
  Sodium rapeseedate
Brassica napus seed oil (rapeseed)
Brassica oleracea Acephala seed oil (kale)
Brassica oleracea Italica (broccoli) seed oil
Butyrospermum parkii (shea) oil
  Butyrospermum parkii (shea) butter (CAS no 68920-03-6; 194043-92-0)
  Butyrospermum parkii (shea) butter unsaponifiables (CAS no 194043-92-0; 225234-14-0)
  Hydrogenated shea butter
Camelina sativa seed oil (false flax)
  Hydrogenated *Camelina sativa* seed oil
Camellia japonica seed oil
Camellia kissi seed oil (tea)
Camellia oleifera seed oil (tea seed)
  Hydrogenated *Camellia oleifera* seed oil
Camellia sinensis seed oil
Canarium indicum seed oil (galip)
Canola oil
  Canola oil unsaponifiables
  Hydrogenated canola oil
Carica papaya seed oil (papaya)
Carthamus tinctorius (safflower) seed oil
  Hydrogenated safflower seed oil
  Potassium safflowerate
  Sodium safflowerate
  Safflower acid
Carya illinoensis (pecan) seed oil
Caryocar brasiliense fruit oil (pequi)
Chenopodium quinoa seed oil (quinoa)
Citrullus lanatus (watermelon) seed oil

(continued)

Table 1. (continued)

Citrus aurantifolia (lime) seed oil
  Citrus aurantifolia (lime) seed oil unsaponifiables
  Hydrogenated lime seed oil
  Hydrogenated lime seed oil unsaponifiables
Citrus aurantium dulcis (orange) seed oil
  Citrus aurantium dulcis (orange) seed oil unsaponifiables
  Hydrogenated orange seed oil
  Hydrogenated orange seed oil unsaponifiables
Citrus grandis (grapefruit) seed oil
  Citrus grandis (grapefruit) seed oil unsaponifiables
  Hydrogenated grapefruit seed oil
  Hydrogenated grapefruit seed oil unsaponifiables
Citrus paradisi (grapefruit) seed oil
Citrus limon (lemon) seed oil (CAS no 85085-28-5)
Cocos nucifera (coconut) oil (CAS no 8001-31-8)
  Hydrogenated coconut oil (CAS no 84836-98-6)
  Cocos nucifera (coconut) seed butter
  Magnesium cocoate
  Potassium cocoate (CAS no. 61789-30-8)
  Potassium hydrogenated cocoate
  Sodium cocoate (CAS no 61789-31-9)
  Sodium hydrogenated cocoate
  Coconut acid (CAS no 61788-47-4)
  Hydrogenated coconut acid (CAS no 68938-15-8)
Coix lacryma-jobi (Job's tears) seed oil
Corylus americana (hazel) seed oil
  Hydrogenated hazelnut oil
Corylus avellana (Hazel) seed oil
Crambe abyssinica seed oil (Abyssinian mustard)
Cucumis sativus (cucumber) seed oil (CAS no 70955-25-8)
Cucurbita pepo (pumpkin) seed oil (CAS no 8016-49-7)
  Hydrogenated pumpkin seed oil
Cynara cardunculus seed oil (artichoke [CAS no 923029-60-1])
Elaeis guineensis (palm) oil (CAS no 8002-75-3)
  Elaeis guineensis (palm) kernel oil (CAS no 8023-79-8)
  Hydrogenated palm kernel oil (CAS no 68990-82-9; 84540-04-5)
  Elaeis (palm) fruit oil
  Hydrogenated palm oil (CAS no 8033-29-2; 68514-74-9)
  Elaeis guineensis (palm) butter (CAS no 8002-75-3)
  Palm kernel acid
  Potassium palm kernelate
  Potassium palmate
  Potassium hydrogenated palmate
  Sodium palm kernelate (CAS no 61789-89-7)
  Sodium palmate (CAS no 61790-79-2)
  Sodium hydrogenated palmate
  Palm acid
  Hydrogenated palm acid
Elaeis oleifera kernel oil
Euterpe oleracea fruit oil (acai)
Fragaria ananassa (strawberry) seed oil
Fragaria chiloensis (strawberry) seed oil
Fragaria vesca (strawberry) seed oil
Fragaria virginiana (strawberry) seed oil
Garcinia indica seed butter (kokum)
Gevuina avellana oil (Chilean hazel)
  Gevuina avellana seed oil
Glycine soja (soybean) oil (CAS no 8001-22-7)
  Glycine soja (soybean) oil unsaponifiables (CAS no 91770-67 -1)
  Hydrogenated soybean oil (CAS no 8016-70-4)

(continued)

Table 1. (continued)

Soy acid (CAS no 68308-53-2)
Potassium soyate
Sodium soyate
Gossypium herbaceum (cotton) seed oil (CAS no 8001-29-4)
Hydrogenated cottonseed oil (CAS no 68334-00-9)
Cottonseed acid (CAS no 68308-51-0)
Guizotia abyssinica seed oil (ramtil/niger)
Helianthus annuus (sunflower) seed oil (CAS no 8001-21-6)
Helianthus annuus (sunflower) seed oil unsaponifiables
Hydrogenated sunflower seed oil
Sunflower seed acid (CAS no 84625-38-7)
Hippophae rhamnoides oil (sea buckthorn)
Hippophae rhamnoides fruit oil (sea buckthorn)
Hippophae rhamnoides seed oil (sea buckthorn)
Irvingia gabonensis kernel butter (dika [CAS no 192230-28-7])
Juglans regia (walnut) seed oil (CAS no 8024-09-7)
Limnanthes alba (meadowfoam) seed oil (CAS no 153065-40-8)
Hydrogenated meadowfoam seed oil
Linum usitatissimum (linseed) seed oil (CAS no 8001-26-1)
Linseed acid (CAS no 68424-45-3)
Luffa cylindrica seed oil (luffa)
Lupinus albus seed oil (white lupine)
Lupinus albus oil unsaponifiables
Lycium barbarum seed oil (goji berry)
Macadamia integrifolia seed oil
Hydrogenated macadamia seed oil
Macadamia ternifolia seed oil (CAS no 128497-20-1 or 129811-19-4)
Sodium macadamiaseedate
Mangifera indica (mango) seed oil
Mangifera indica (mango) seed butter
Sodium mangoseedate
Morinda citrifolia seed oil (noni)
Moringa oleifera seed oil (ben/moringa)
Moringa pterygosperma seed oil
Oenothera biennis (evening primrose) oil
Hydrogenated evening primrose oil
Olea europaea (olive) fruit oil (CAS no 8001-25-0)
Olea europaea (olive) oil unsaponifiables (CAS no 156798-12-8)
Hydrogenated olive oil
Hydrogenated olive oil unsaponifiables
Potassium olivate (CAS no 68154-77-8)
Sodium olivate (CAS no 64789-88-6)
Olea europaea (olive) husk oil
Olive acid (CAS no 92044-96-7)
Orbignya cohune seed oil (cohune)
Orbignya oleifera seed oil (babassu [CAS no 91078-92-1])
Potassium babassuate
Sodium babassuate
Babassu acid
Orbignya speciosa kernel oil
Oryza sativa (rice) bran oil (CAS no 68553-81-1; 84696-37-7)
Hydrogenated rice bran oil
Oryza sativa (rice) germ oil
Oryza sativa (rice) seed oil
Rice bran acid (CAS no 93165-33-4)
Passiflora edulis seed oil (passion fruit [CAS no 87676-26-1])
Hydrogenated *Passiflora edulis* seed oil
Perilla ocymoides seed oil (perilla)
Persea gratissima (avocado) oil (CAS no 8024-32-6)
Persea gratissima (avocado) oil unsaponifiables (CAS no 91770-40-0)

(continued)

Table 1. (continued)

Hydrogenated avocado oil
Persea gratissima (avocado) butter
Sodium avocadoate
Pistacia vera seed oil (pistachio [CAS no 90082-81-8; 129871-01-8])
Hydrogenated pistachio seed oil
Plukenetia volubilis seed oil (sacha inchi)
Prunus amygdalus dulcis (sweet almond) oil (CAS no 8007-69-0; 90320-37-9)
Prunus amygdalus dulcis (sweet almond) oil unsaponifiables
Hydrogenated sweet almond oil
Hydrogenated sweet almond oil unsaponifiables
Sodium sweet almondate
Prunus armeniaca (apricot) kernel oil (CAS no 72869-69-3)
Prunus armeniaca (apricot) kernel oil unsaponifiables
Hydrogenated apricot kernel oil
Hydrogenated apricot kernel oil unsaponifiables
Prunus avium (sweet cherry) seed oil
Prunus domestica seed oil (prune/plum)
Prunus persica (peach) kernel oil (CAS no 8002-78-6; 8023-98-1)
Hydrogenated peach kernel oil
Punica granatum seed oil (pomegranate)
Hydrogenated *Punica granatum* seed oil
Pyrus malus (apple) seed oil
Ribes nigrum (blackcurrant) seed oil (CAS no 97676-19-2)
Hydrogenated blackcurrant seed oil
Ribes rubrum (currant) seed oil
Rosa canina fruit oil (dog rose)
Hydrogenated *Rosa canina* fruit oil
Rubus chamaemorus seed oil (cloudberry)
Rubus idaeus (raspberry) seed oil
Hydrogenated raspberry seed oil
Schinziophyton rautanenii kernel oil (mongongo)
Sclerocarya birrea seed oil (marula)
Sesamum indicum (sesame) seed oil (CAS no 8008-74-0)
Sesamum indicum (sesame) oil unsaponifiables
Hydrogenated Sesame seed oil
Sesamum indicum (sesame) seed butter
Sodium sesame seedate
Silybum marianum seed oil (thistle)
Solanum lycopersicum (tomato) fruit oil
Solanum lycopersicum (tomato) seed oil
Theobroma cacao (cocoa) seed butter (CAS no 8002-31-1)
Sodium cocoa butterate
Theobroma grandiflorum seed butter (cupuacu [CAS no 394236-97-6])
Sodium *Theobroma grandiflorum* seedate
Torreya nucifera seed oil (Kaya)
Triticum vulgare (wheat) germ oil (CAS no 8006-95-9; 68917-73-7)
Triticum aestivum (wheat) germ oil
Triticum vulgare (wheat) germ oil unsaponifiables
Hydrogenated wheat germ oil unsaponifiables
Hydrogenated wheat germ oil
Wheat germ acid (CAS no 68938-32-9)
Vaccinium corymbosum (blueberry) seed oil
Vaccinium macrocarpon (cranberry) seed oil
Hydrogenated cranberry seed oil
Vaccinium myrtillus seed oil (bilberry [CAS no 1161921-09-0])
Vaccinium vitis-idaea seed oil (ligonberry)
Vegetable (olus) oil
Hydrogenated vegetable oil

(continued)

Table 1. (continued)

Vitis vinifera (grape) seed oil (CAS no 8024-22-4)
Hydrogenated grapeseed oil
Sodium grapeseedate
***Zea mays* (corn) oil (CAS no 8001-30-7)**
***Zea mays* (corn) oil unsaponifiables**
***Zea mays* (corn) germ oil**
***Potassium cornate* (CAS no 61789-23-9)**
***Corn acid* (CAS no 68308-50-9)**

[a]Previously reviewed ingredients are in bold and italics.

[shea] oil or butter) is the current naming convention described by the cosmetics industry.

So that all plant-derived fatty acid oils that are cosmetic ingredients are included in 1 report, several ingredients that have been reviewed previously by the Cosmetic Ingredient Review (CIR) Expert Panel (Panel) are included in this report. The ingredients, their conclusions, and citations are found in Table 2. Previously reviewed fatty acids and glyceryl triesters are also found in Table 2.

Table 2. Previously Reviewed Oil and Fatty Acid Ingredients.

Ingredients	Publication date	Conclusion
Oil ingredients		
Arachis hypogaea (peanut) oil (CAS no 8002-03-7) Hydrogenated peanut oil (CAS no 68425-36-5) Peanut acid (CAS no 91051-35-3)	*IJT*. 20(S2):65-77, 2001	Safe
Carthamus tinctorius (safflower) seed oil (CAS no 8001-23-8)	*JACT*. 4(5):171-197, 1985; rereviewed, not reopened, *IJT*. 25(2):1-89, 2006	Safe
Cocos nucifera (coconut) oil (CAS no 8001-31-8) Coconut acid (CAS no 61788-47-4) Hydrogenated coconut acid (CAS no 68938-15-8) Hydrogenated coconut oil (CAS no 84836-98-6) Magnesium cocoate Potassium cocoate (CAS no 61789-30-8) Potassium hydrogenated cocoate Sodium cocoate (CAS no 61789-31-9) Sodium hydrogenated cocoate	*JACT*. 5(3):103-121, 1986; CIR final report, 2008	Safe
Corylus americana (hazel) seed oil *Corylus avellana* (hazel) seed oil	*IJT*. 20 (S1):15-20, 2001	Insufficient data
Elaeis guineensis (palm) oil (CAS no 8002-75-3) *Elaeis guineensis* (palm) kernel oil (CAS no 8023-79-8) Hydrogenated palm oil (CAS no 8033-29-2; 68514-74-9) Hydrogenated palm kernel oil (CAS no 68990-82-9; 84540-04-5)	*IJT*. 19(S2):7-28, 2000	Safe
Gossypium herbaceum (cotton) seed oil (CAS no 8001-29-4) Cottonseed acid (CAS no 68308-51-0) Hydrogenated cottonseed oil (CAS no 68334-00-9)	*IJT*. 20(S2):21-29, 2001	Safe
Oryza sativa (rice) bran oil (CAS no 68553-81-1; 84696-37-7) *Oryza sativa* (rice) germ oil Rice bran acid (CAS no 93165-33-4)	*IJT*. 25(S2):91-120, 2006	Safe
Prunus amygdalus dulcis (sweet almond) oil (CAS no 8007-69-0)	*JACT*. 2(5):85-99, 1983; rereviewed, not reopened, *IJT*. 24(S1):1-102, 2005	Safe
Sesamum indicum (sesame) seed oil (CAS no 8008-74-0) Hydrogenated sesame seed oil *Sesamum indicum* (sesame) oil unsaponifiables Sodium sesameseedate	*JACT*. 12(3):261-277, 1993; amended final report, 2009	Safe
Zea mays (corn) oil (CAS no 8001-30-7) *Zea mays* (corn) germ oil *Zea mays* (corn) oil unsaponifiables Corn acid (CAS no 68308-50-9) Potassium cornate (CAS no 61789-23-9)	Final report, 2008	Safe
Persea gratissima (avocado) oil (CAS no 8024-32-6)	*JEPT*. 4(4):93-103, 1980; rereviewed, not reopened, *IJT*. 22(1):1-35, 2003	Safe
Triticum vulgare (wheat) germ oil (CAS no 8006-95-9; 68917-73-7)	*JEPT*. 4(4):33-45, 1980; rereviewed, not reopened, *IJT*. 22(1):1-35, 2003	Safe

(continued)

Table 2. (continued)

Ingredients	Publication date	Conclusion
Fatty acids		
Arachidonic acid (CAS no 506-32 -1)	*JACT*. 12 (5):481-559, 1993	Insufficient data
Hydroxystearic acid (CAS no 106-14-9)	*IJT*. 18(S1):1-10, 1999	Safe
Lauric acid (CAS no 143-07-7) Myristic acid (CAS no 544-63-8) Oleic acid (CAS no 112-80-1) Palmitic acid (CAS no 57-10-3) Stearic acid (CAS no 57-11-4)	*JACT*. 6(3):321-401, 1987; rereviewed, not reopened, *IJT*. 25(2):1-89, 2006	Safe
Glyceryl triesters		
Trilaurin Triarachidin Tribehenin Tricaprin Tricaprylin Trierucin Triheptanoin Triheptylundecanoin Triisononanoin Triisopalmitin Triisostearin Trilinolein Trimyristin Trioctanoin Triolein Tripalmitin Tripalmitolein Triricinolein Tristearin Triundecanoin Glyceryl triacetyl hydroxystearate Glyceryl triacetyl ricinoleate Glyceryl stearate diacetate	*IJT*. 20(S4):61-94, 2001	Safe

Abbreviations: CIR, Cosmetic Ingredient Review; IJT, International Journal of Toxicology; JACT, Journal of the American College of Toxicology; JEPT, Journal of Environmental Pathology and Toxicology.

Chemistry

The group of ingredients characterized as fats and oils are the glyceryl esters of fatty acids (triglycerides) normally found in plants, including those that have been hydrogenated to reduce or eliminate unsaturation.[1] Figure 1 represents the general structure of fats and oils. The raw oil may include diglycerides, monoglycerides, free fatty acids, plant sterols, pigments, glucosides, proteins, natural antioxidants, vitamins, and impurities.[2,3] The extent to which these components are removed during processing varies. The available information on chemical properties of oils in this report, including Food Chemicals Codex specifications when provided, is found in Table 3.[4] The available fatty acid compositions for the oils in this report are found in Table 4.

The percentage of chemical constituents in individual oil types is dependent on the region where the oilseed plant is grown, individual cultivars, and plant genetics.[3] This is especially true with rapeseed, where the erucic acid content varies from 1% to 58.6%. Low erucic acid rapeseed oil is also known as canola oil.

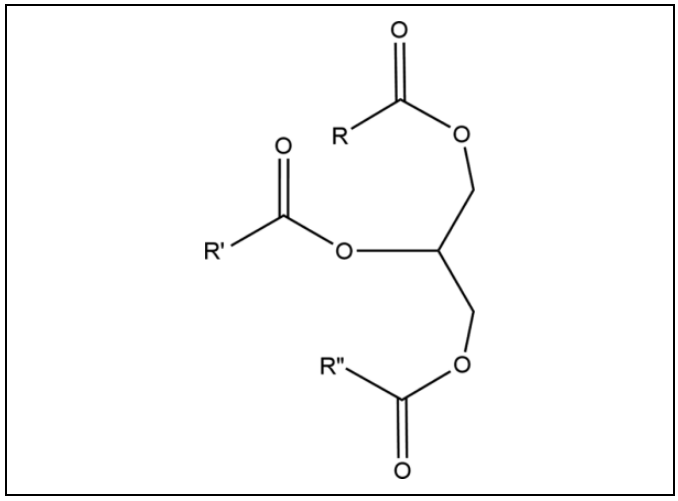

Figure 1. General structure of fats and oils, wherein RC(O)−, R′C(O)− and R″C(O)− may be the same or different fatty acid radicals.[1]

Table 3. Chemical Properties for Plant-Derived Fatty Acid Oils.

Properties and constituents	*Actinidia chinensis* (kiwi) seed oil[58]	*Adansonia digitata* oil [59,60]	Aleurites moluccana seed oil (kukui)[61–64]	*Anacardium occidentale* (cashew) seed oil[65]	*Arachis hypogaea* (peanut) oil[3,63,66–69]	*Argania spinosa* kernel oil[70,71]	*Astrocaryum murumuru* seed butter[3,72]
Appearance		Pale yellow	Clear yellow liquid		Light yellow	Yellow	Pale brown waxy solid at room temperature
Specific gravity			0.920-0.930 (20°C)		0.912-0.920 (20°C)	0.908-0.918 (20°C)	0.890-0.910 (25°C)
Refractive index			1.470-1.480 (20°C)		1.46-1.475 (20°C)		
Iodine value		65-95	130-175		74-107	95	15 max
Saponification value		190-210	185-210		180-208		270-350
Peroxide value, mEq/kg	44.37	5.0-10	5.0 max	0.22	0.39-5.0 max	10.0 max	20.0 max
Melting point (°C)							25-37
Unsaponifiable matter (%)			0.3-1		≤1.0		
Free fatty acids (%)	1.2	2.0 max as oleic acid	0.1-4		0.2-2.08		12.56 as oleic acid
Titer (°C)					26-32		
Acid value					0.5	3-4	

Properties and constituents	*Avena sativa* (oat) kernel oil[73]	*Bertholletia excelsa* seed oil[65,74]	*Borago officinalis* seed oil[75,76]	*Brassica campestris* (rapeseed) seed oil[3]	Hydrogenated rapeseed oil[4]	Rapeseed acid[77]	Canola oil[4]
Appearance	Yellow		Clear, pale yellow-golden		White waxy solid		Light yellow oil
Specific gravity	0.914-0.932 (25°C)	1.473	0.918-0.928 (20°C)				
Refractive index	1.469-1.471 (25°C)	0.914 (20°C)	1.474-1.479 (20°C)				1.465-1.467 (40°C)
Iodine value		74.2	130-155	81-112	4 max	119-120 g/100 g	110-126
Saponification value	176-186	192.4	184-194	168-192			
Peroxide value, mEq/kg	0.6-1.1	0.16	10.0 max		2.0 max		10 max
Melting point (°C)							
Unsaponifiable matter (%)	3.7-4.3			0.5-2			1.5 max
Free fatty acids (%)	0.1-0.3			1	2.0 max as oleic acid		0.1% max as oleic acid
Titer (°C)							
Acid value			1.0 max			197-200 mg KOH/g	

Properties and constituents	*Brassica oleracea* acephala seed oil[78]	*Brassica oleracea* Italica (broccoli) seed oil[79]	*Butyrospermum parkii* (shea) butter[3,63,80–83]	*Butyrospermum parkii* (shea) oil[4]	*Camellia oleifera* seed oil[84,85]	*Canarium indicum* oil[86,87]	Carica papaya seed oil[88,89]
Appearance	Yellow	Golden	Grey, tallow-like	Pale yellow	Clear, pale yellow or "water white"	Cream to golden	Pale yellow
Specific gravity	0.9010 (20°C)	0.910-0.918 (20°C)	0.918 (15°C)				
Refractive index	1.4741 (23°C)	1.465-1.475 (20°C)	1.468 (25°C)			1.45-1.47	
Iodine value	61.2	90-120	45-77	28-43	80-94		65-100
Saponification value	123.06		165-190	185-195	188-196		
Peroxide value (mEq/kg)			5.0 max	≤10	10.0 max	≤20	10.0 max
Melting point (°C)			32-46; 28-42 (slip)				
Unsaponifiable matter (%)	1.6		3-13	≤1.5	1.5 max	≤1	
Free fatty acids (%)			1.0 max as oleic acid	≤0.1 as oleic acid		0.2	0.8-3
Titer (°C)			49-54				
Acid value	2.1	1.5	1.5		1.0 max	≤10	

(continued)

Table 3. (continued)

Properties and constituents	*Carthamus tinctorius* (safflower) seed oil[4]	*Carya illinoensis* (pecan) seed oil[63,65,74]	*Caryocar brasiliense* fruit oil (pequi)[77,90]	*Citrullus lanatus* (watermelon) seed oil[3,91]	*Citrus aurantifolia* (lime) seed oil[92,93]	*Citrus aurantium* dulcis (orange) seed oil[94,95]	*Citrus paradisi* (grapefruit) seed oil[96,97]
Appearance	Light yellow oil		Yellow[90]	Pale to golden yellow liquid	Clear yellow	Clear, light yellow	Clear yellow
Specific gravity		0.924 (25°C)		0.8930-0.9166		0.910-0.920 (20°C)	
Refractive index		1.472		1.4668		1.466 -1.475 (20°C)	
Iodine value	135-150	100-105	48.65-74.80[90]; 50-70 g/100 g[77]	113-123		90-110	80-125
Saponification value		190	160.15-202[90]; 190-210 mg KOH/g	193-195		185-200	
Peroxide value (mEq/kg)	10 max	0.15	0.99-5.22[90]; ≤20[77]	≤5.0	5.0 max	5-10	5-10
Melting point (°C)							
Unsaponifiable matter (%)	1.5 max	0.35-40					
Free fatty acids (%)	0.1 max as oleic acid		0.98-2.85 (mg KOH/g)[90]	<5.0 as oleic acid		0.5 as oleic acid	
Titer (°C)							
Acid value			10 mg KOH/g max[77]		1.0 max	0.8 max	1.0 max

Properties and constituents	*Cocos nucifera* (coconut) oil[3,4,63]	*Cucurbita pepo* (pumpkin) seed oil[98,99]	*Elaeis guineensis* (palm) oil[3,4]	*Elaeis guineensis* (palm) kernel oil[3,4]	*Fragaria ananassa* (strawberry) seed oil[3,100,101]	*Fragaria chiloensis* (strawberry) seed oil[102,103]	*Garcinia indica* seed butter (kokum)[104–106]
Appearance	White to light yellow-tan	Dark green	Pale yellow to deep orange in color	Nearly colorless	Light golden/yellow to yellow	Light yellow with some green	
Specific gravity	0.917-0.919 (25°C/15.5°C)		0.921-0.925 (40°C)		0.93-0.95	0.912-0.930	
Refractive index	1.448-1.450 (40°C)		1.453-1.458 (40°C)			1.465-1.485	1.4565-1.4575 (40°C)
Iodine value	6-11	110-330	44-58	14-33		170-190	30-50
Saponification value	248-265	174-197	195-205	245-255		180-195	185-195
Peroxide value, mEq/kg	≤10	5.0 max	10 max	10 max	<15	10 max	
Melting point (°C)	22-26		25-50	25-30			37-43; 27 (slip)
Unsaponifiable matter (%)	≤0.5	1.5	0.2-0.8	1.5 max			1.5 max; 18-20; 32-40
Free fatty acids (%)	≤0.1% as oleic acid; ≤0.07% as lauric acid	1.5 as oleic acid	0.1 max as oleic acid; 0.09 as palmitic acid	0.1 max as oleic acid; 0.07 max as lauric acid		3	0.1-1
Titer (°C)	20-24						
Acid value					18 max		

Properties and constituents	*Glycine soja* (soybean) oil[3,4]	*Gossypium herbaceum* (cotton) seed oil[3,4]	*Guizotia abyssinica* seed oil[3]	Hazel seed oil[a,66,107–109]	*Helianthus annuus* (sunflower) seed oil[3,4]	Sunflower seed acid[77]	*Hippophae rhamnoides* fruit oil[110]
Appearance	Light amber oil	Dark red-brown oil	Pale yellow with a bluish tint		Light amber oil		Orange-red
Specific gravity			0.910-0.928	0.912-0.917 (15.5°C); 0.905-0.925 (20°C)	0.894-0.899 (60°C)		0.90
Refractive index			1.467-1.471	1.467-1.474 (20°C)			

(continued)

Table 3. (continued)

Properties and constituents	*Glycine soja* (soybean) oil[3,4]	*Gossypium herbaceum* (cotton) seed oil[3,4]	*Guizotia abyssinica* seed oil[3]	Hazel seed oil[a,66,107–109]	*Helianthus annuus* (sunflower) seed oil[3,4]	Sunflower seed acid[77]	*Hippophae rhamnoides* fruit oil[110]
					1.4597-1.4745 (25°C)		
Iodine value	120.9-151.4	90-113	126-139	83-100	128-144	125-140 g/100 g	
Saponification value		180-198	180-195	180-200	188-194		
Peroxide value, mEq/kg	10 max	10 max		0.43; 10.0 max	10 max		10 max
Melting point (°C)					0		
Unsaponifiable matter (%)	0.3-0.6	1.5 max	0.5-1	≤1.0	0.3-0.5		
Free fatty acids (%)	0.05-0.7	0.1 max as oleic acid	0.4-3	0.2 max as oleic acid	0.1 max as oleic acid		
Titer (°C)							
Acid value				≤0.5		125-140 mg KOH/g	18 max

Properties and constituents	*Hippophae rhamnoides* seed oil[111–113]	*Irvingia gabonenesis* kernel butter[114]	*Juglans regia* (walnut) seed oil[63,66,74]	*Linum usitatissimum* (linseed) seed oil[3]	Macadamia nut oil[66,74,115–117]	*Mangifera indica* (mango) seed oil[3]	*Moringa oleifera* seed oil[118–120]
Appearance	Orange				Pale to golden yellow	Pale yellow to ivory cream color	
Specific gravity	0.890-0.955 (20°C)		0.917 (25°C)	0.927-0.931 (20°C)	0.911-0.918 (20°C)	0.91	0.908 (20°C); 0.8933 (24°C)
Refractive index	1.4650-1.4825 (20°C)		1.475 (25°C)	1.4786-1.4815	1.466 -1.470 (20°C)	1.456	1.4566 (40°C)
Iodine value	130-200		150-162	170-204	62-82	32-93	66.47
Saponification value	184-210		190-197	189-196	190-200	190-195	164.27; 192
Peroxide value, mEq/kg	5-10 max		0.37		0.36; 10.0 max		0.45; 10.0
Melting point (°C)				0		34-43	18.93
Unsaponifiable matter (%)	1.0	0.13	0.5	0.5-1.5	1.5	0.8-2.9	0.58
Free fatty acids (%)	2.0 max; 18 max	0.30	0.2-2.5	5	0.5 max; 1.0 max as oleic acid		2.55 as oleic acid
Titer (°C)							
Acid value	15				1		

Properties and constituents	*Oenothera biennis* (evening primrose) oil[121,122]	*Olea europaea* (olive) fruit oil[3]	*Olea europaea* (olive) husk oil[123]	Olive acid[77]	*Oryza sativa* (rice) bran oil[124,125]	*Oryza sativa* (rice) bran oil[124,125]	*Passiflora edulis* seed oil (passion fruit)
Appearance	Light yellow	Almost colorless to yellow, greenish, or brown in color			Light golden yellow	Light golden yellow	Golden-orange
Specific gravity	0.920-0.930 (20°C)	0.914-0.918			0.916-0.922 (15.5°C)	0.916-0.922 (15.5°C)	0.917 (20°C)
Refractive index	1.475-1.480 (20°C)	1.469-1.484			1.470-1.473 (20°C)	1.470-1.473 (20°C)	1.468-1.473 (20°C)
Iodine value	145-165	64-88; refined 75-94		85-91 g/100 g	92-115	92-115	119.9-129.29[126]
Saponification value	180-195	185-212; refined 184-186			180-195	180-195	176-187.4
Peroxide value, mEq/kg	10.0 max	20 max (refined)	14.33		10.0 max	10.0 max	1.37-2.23
Melting point (°C)							
Unsaponifiable matter (%)		0.6-1.2; 1.5 max refined					0.9-2.86

(continued)

Table 3. (continued)

Properties and constituents	*Oenothera biennis* (evening primrose) oil[121,122]	*Olea europaea* (olive) fruit oil[3]	*Olea europaea* (olive) husk oil[123]	Olive acid[77]	*Oryza sativa* (rice) bran oil[124,125]	*Oryza sativa* (rice) bran oil[124,125]	*Passiflora edulis* seed oil (passion fruit)
Free fatty acids (%)		0.6-1.4; 0.3 max refined			1.0 as oleic acid	1.0 as oleic acid	
Titer (°C)							
Acid value	1-2			190-201 mg KOH/g			2.11-2.36

Properties and constituents	*Persea gratissima* (avocado) oil[3]	*Pistacia vera* seed oil[65]	*Plukenetia volubilis* seed oil[127]	*Prunus amygdalus* (sweet almond) oil[3,57,63,66,128–130]	*Prunus armeniaca* (apricot) kernel oil	*Prunus avium* (sweet cherry) seed oil[131,132]
Appearance			Yellow-amber	Colorless to pale yellow liquid		Clear light yellow
Specific gravity	0.910-0.916		0.90-0.93 (20°C)	0.911-0.920 (20°C)	0.923[3]	0.905-0.925 (20°C)
Refractive index	1.461-1.465		1.478-1.481 (20°C)	1.467-1.473 (20°C)	1.4672-1.4722[3]	1.463 -1.480 (20°C)
Iodine value	71-95		180-200	93-106	81-123[3]	90-115
Saponification value	177-198		180-210	183-197	191[3]	105-135
Peroxide value, mEq/kg		0.22	0-15	0.19		10.0 max
Melting point (°C)						
Unsaponifiable matter (%)				0.4-1.0	0.4-1.4	
Free fatty acids (%)				1.0 max		0.5% max
Titer (°C)					0-6[133]	
Acid value			0-2	0.5		1.0 max

Properties and constituents	*Prunus domestica* seed oil[134,135]	*Prunus persica* (peach) kernel oil[3,136]	*Punica granatum* seed oil[137,138]	*Pyrus malus* (apple) seed oil[139]	*Ribes nigrum* (blackcurrant) seed oil[140–142]	*Ribes rubrum* (currant) seed oil[143]
Appearance		Pale yellow (refined)	Golden to dark yellow		Pale yellow or slightly greenish	Pale yellow or slightly greenish
Specific gravity		0.910-0.920 (20°C) refined	0.935 (15.5°C)	0.902-0.903 (25°C)	0.92	0.92
Refractive index				1.465-1.466 (40°C)		
Iodine value	90-108	90-115 (refined)	190-230	94.14-101.15	145-185	
Saponification value				179.01-197.25		
Peroxide value, mEq/kg	10.0 max	5.0 max (refined)	10.0 max	2.43-2.52	1-10	10 max
Melting point (°C)						
Unsaponifiable matter (%)						
Free fatty acids (%)	2.0 max as oleic acid		1.4; 5.0 max as oleic acid		0.2	
Titer (°C)						
Acid value				4.036-4.323	3; 18 max	18 max

(continued)

Table 3. (continued)

Properties and constituents	*Rubus chamaemorus* seed oil[144]	*Rubus idaeus* (raspberry) seed oil[145–147]	*Schinziophyton rautanenii* kernel oil[148]	*Sclerocarya birrea* seed oil (marula)[149]	*Solanum lycopersicum* (tomato) seed oil[150]	*Theobroma cacao* (cocoa) seed butter[3]
Appearance	Yellow-red	Yellow or yellow-red	Light yellow		Clear golden yellow to darker red	
Specific gravity	0.92	0.92			0.9135-0.9357	0.950-0.998
Refractive index			1.4830	1.46	1.4577-1.4771	1.453-1.458
Iodine value		175-195		100.25	105-130.5	35-40
Saponification value		180-200		162.70	156-194.9	190-200
Peroxide value, mEq/kg	10 max	5.0 max; 10 max	10 mg/kg	4.58		
Melting point (°C)				26-28		33.5
Unsaponifiable matter (%)				3.06		
Free fatty acids (%)		1.5 max as oleic acid				
Titer (°C)						
Acid value	18 max	18 max		33.70		

Properties and constituents	*Vaccinium corymbosum* (blueberry) seed oil[58,151,152]	*Vaccinium macrocarpon* (cranberry) seed oil[3,58,153–156]	*Vaccinium myrtillus* seed oil[157]	*Vaccinium vitis-idaea* seed oil[158]	*Vitis vinifera* (grape) seed oil[3]	*Zea mays* (corn) oil[159,160]
Appearance	Green with yellow tint or dark green/ brown	Pale yellow to greenish; light green	Pale yellow to greenish	Pale yellow		Clear, bright golden yellow
Specific gravity		0.923	0.93	0.92	0.91-0.93	0.920-0.928 (15.5°C)
Refractive index					1.470-1.476	1.472-1.476 (20°C)
Iodine value	155-175	140-180			125-143	103-128
Saponification value		170-200			176-206	185-195
Peroxide value, mEq/kg	20-24.62	<15; 10 max	10 max	10 max		10.0 max
Melting point (°C)						
Unsaponifiable matter (%)						
Free fatty acids (%)	0.67; 2.0 as oleic acid	0.7; 1.0 as oleic acid				
Titer (°C)						
Acid value		2.0 max; 18 max	18 max	18 max		0.2 max

Abbreviation: max, maximum.
[a]Information mainly on *Corylus avellana*.

Table 4. Total Fatty Acid Composition of Plant-derived Fatty Acid Oils (%).

Fatty acids	*Actinidia chinensis* (kiwi) seed oil[58]	*Adansonia digitata* oil (baobab) [59,60]	*Aleurites moluccana* seed oil (kukui)[61–63]	*Amaranthus hypochondriacus* seed oil (amaranth)[161]	*Anacardium occidentale* (cashew) seed oil[65]	*Arachis hypogaea* (peanut) oil[3,67,68]	*Arctium lappa* seed oil[162]	*Argania spinosa* kernel oil (argan)[70,71]	*Astrocaryum murumuru* seed butter (murumuru)[72]	*Avena sativa* (oat) kernel oil[73,163]
Caproic (C6)										
Caprylic (C8)									1.85	
Capric (C10)									1.85	
Lauric (C12)	0.02								47.46	
Myristic (C14)	0.03				0.07		0.01		26	0.2-0.3
Myristoleic (C14:1)										
Palmitic (C16)	5.96	18-30	5-8	19-20	9.9	5-16	7.27	10-15	6.28	13.9-18.82
Palmitoleic (C16:1)		1	0.5		0.4		0.01			0.1-0.4
Heptadecanoic (C17:0)					0.1					
Stearic (C18)	3.09	2-8	0.1-6.7	3	8.7	1-6.5	32.56	5-6.5	2.65	0.8-2.79
Oleic (C18:1)	14.6	30-40	10-35	22-26	57.2	33.3-76	50.21	45-55	12.56	31.4-51.26
Linoleic (C18:2)	17.55	24-34	35-50	46-50	20.8	8-47.5	3.18	28-36	2.87	22.8-43.1
Linolenic (C18:3)	57.4	1-3	24-40		0.2	0-0.6				0.64-2.1
Arachidic (C20)	0.34		1.5		1	0.17-3	0.22			
Eicosenoic (C20:1)			1		0.3	0.33-3	0.33			0.5-1
Eicosadienoic (C20:2)										
Arachidonic (C20:4)										
Behenic (C22)					0.4	1-5				
Erucic (C22:1)					0.3	0.5				
Docosadienoic (C22:2)										
Docosahexaenoic (C22:6)										
Lignoceric (C24)						0.2-3	0.49			
Others						<C16:0-0.4	heptadecenoic = 0.02; nonadecadienoic acid = 2.99; heneicosanoic acid = 1.07; dicosanoic acid = 0.43			arachidic (C20) + eicosadienoic (C20:2) = 0.1-0.3; C18:1, n-11 = 0.9-1.3

(continued)

Table 4. (continued)

Fatty acids	*Bassia butyracea* seed butter[a,104]	*Bassia latifolia* seed butter (Mahwa)[b,104]	*Bertholletia excelsa* seed oil (Brazil)[65]	*Borago officinalis* seed oil (borage)[75,76]	*Brassica campestris* (rapeseed) seed oil[3]	Rape seed acid[77]	*Brassica napus* seed oil (rapeseed)[164]	Hydrogenated rapeseed oil[4]	Canola oil[4]
Caproic (C6)									
Caprylic (C8)									
Capric (C10)									
Lauric (C12)									
Myristic (C14)			0.06			≤0.5		<1.0	<0.2
Myristoleic (C14:1)									
Palmitic (C16)	60.8	23.7-24.7	13.5	9-13	1.5-3	≤8	2	3-5.0	<6.0
Palmitoleic (C16:1)			0.3			≤2			<1.0
Heptadecanoic (C17:0)			0.2						
Stearic (C18)	3.2	19.3-29.9	11.8	3-5	0.7-1.3	≤3	1	38-42	<2.5
Oleic (C18:1)	30.9	36.3-43.3	29.1	10-22	12.1-57.4	54-70	21	1	>50
Linoleic (C18:2)	4.9	11.6-15.8	42.8	33-46	11.4-22.1	18-24	20	< 1.0	<40.0
Linolenic (C18:3)			0.2	18-25	8.3-12.5	5-10	2		<14
Arachidic (C20)			0.5			≤6	1	8-10.0	<1.0
Eicosenoic (C20:1)			0.2	2-6	5.6-3.1			< 1.0	<2.0
Eicosadienoic (C20:2)									
Arachidonic (C20:4)									
Behenic (C22)			0.1					42-50	<0.5
Erucic (C22:1)			0.3	1-3.5	1-58.6		53	< 1.0	<2.0
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)							2	1-2.0	<0.2
Others				α-linolenic (C18:3) = 0.4%; γ-linolenic = 1%-3.5%		<C14 = ≤0.5; >C18:3 = ≤5; >C20 = ≤6			<C14 = <0.1; C24:1 = <0.2

(continued)

Table 4. (continued)

Fatty acids	*Brassica oleracea* Acephala seed oil (kale)[78]	*Brassica oleracea* Italica (broccoli) seed oil[79]	*Butyrospermum parkii* (shea) oil[4]	*Butyrospermum parkii* (shea) butter[3,80–82]	*Camelina sativa* seed oil (false flax)[165]	*Camellia japonica* seed oil[166]	*Camellia kissi* seed oil[166]	*Camellia oleifera* seed oil (tea seed)[84,85]	*Camellia sinensis* seed oil[166]
Caproic (C6)									
Caprylic (C8)									
Capric (C10)									
Lauric (C12)									
Myristic (C14)				0.5					
Myristoleic (C14:1)									
Palmitic (C16)	4.4	0-5	3.8-4.1	3-9	7.8	7.9		6.1-15	8-10
Palmitoleic (C16:1)						0.16			
Heptadecanoic (C17:0)									
Stearic (C18)	0.7	0-5	41.2-56.8	30-50	2.96	2.46		0.8-2	1.5-3.5
Oleic (C18:1)	11.3	10-20	34.0-46.9	38-50	16.77	84.99	80	72-87	78-86
Linoleic (C18:2)	12.6	10-20	3.7-6.5	3-8	23.08	3.76		5.3-14.3	7-10
Linolenic (C18:3)	10.2	5-10		0.5 max	31.2				0.2-0.8
Arachidic (C20)	8.2		1-2	2.5-3		0.49			
Eicosenoic (C20:1)	0.4	5-10			11.99				
Eicosadienoic (C20:2)									
Arachidonic (C20:4)									
Behenic (C22)									
Erucic (C22:1)	51.8	40-50			2.8				
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)									
Others					3.4				

(continued)

Table 4. (continued)

Fatty acids	*Canarium indicum* oil (galip)[86,87]	*Carica papaya* seed oil (papaya)[88,89]	*Carthamus tinctorius* (safflower) seed oil[28,167]	*Carya illinoensis* (pecan) seed oil[63,65]	*Caryocar brasiliense* fruit oil (pequi)[c,77,90]	*Chenopodium quinoa* seed oil (quinoa)[168]	*Citrullus lanatus* (watermelon) seed oil[91]	*Citrus aurantifolia* (lime) seed oil[92,93]	*Citrus aurantium dulcis* (orange) seed oil[94,95]
Caproic (C6)									
Caprylic (C8)									
Capric (C10)									
Lauric (C12)	≤ 2								
Myristic (C14)	≤ 2			Trace	0.5	0.2		1	
Myristoleic (C14:1)									
Palmitic (C16)	28-38	8-18	2	3-4.3	34.4-44.3	9.9-11	8.0-13.0	20-30	14-22
Palmitoleic (C16:1)	≤2	2		0.1	1.3	0.1	<1.0		
Heptadecanoic (C17:0)	≤2			0.1					
Stearic (C18)	10-20	2-6		1.8-2	0.66-1.8	0.7-0.8	8.0-12.0	3-8	2-6
Oleic (C18:1)	30-40	60-77	26	40.6-79	54.55-57.4	22-50.2	15.0-30.0	20-38	26-35
Linoleic (C18:2)	12-22	3-25	68	16-50.3	0.84-2.8	1.2-56	55.0-65.0	30-45	35-45
Linolenic (C18:3)		0.8	Trace	0.7	0.18 -1.0	0.7-7	<1.0	5-15	2-6
Arachidic (C20)			Trace	Trace		0.7	<1.0	2	0.5
Eicosenoic (C20:1)		2		1.2			<1.0		
Eicosadienoic (C20:2)									
Arachidonic (C20:4)									
Behenic (C22)				0.2			<1.0		
Erucic (C22:1)				0.3					
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)							<2.0		
Lignoceric (C24)									
Others	Others ≤ 2	α-linolenic (C18:3) 2%					<1.0		

(continued)

Table 4. (continued)

Fatty acids	*Citrus grandis* (grapefruit) seed oil[96,97]	*Citrus limon* (lemon) seed oil[169]	*Citrus paradisi* (seed) oil[170]	*Cocos nucifera* (coconut) oil[29]	*Coix lacryma-jobi* (job's tears) seed oil[171]	*Corylus americana* (hazel) seed oil[164]	*Corylus avellana* (hazel) seed oil[9,107–109]	*Crambe abyssinica* seed oil (Abyssinian mustard)[164,172]	*Cucumis sativus* (cucumber) seed oil[173]	*Cucurbita pepo* (pumpkin) seed oil[98,99]
Caproic (C6)				0-1						
Caprylic (C8)				5-9						
Capric (C10)				6-10				<0.01-0.11		
Lauric (C12)	1.5		2.95	44-52				<0.01-0.14		
Myristic (C14)	1		1.01	13-19			≤0.2	<0.01-0.43		
Myristoleic (C14:1)								<0.01-0.09		
Palmitic (C16)	18-30	18.8	36.25	8-11	16.0	6	4-9	0.81-5.55	9-13	10-16
Palmitoleic (C16:1)				0-1			0.2-1	<0.01-0.77		
Heptadecanoic (C17:0)		0.08					≤0.1			
Stearic (C18)	2-8	3.5	5.95	1-3	trace	3	1-6	0.6-10.42	6-9	3-7
Oleic (C18:1)	20-38	30.1	18.34	5-8	53	76	66-85	12.8-23.13	14-20	18-38
Linoleic (C18:2)	30-48	33.4	29.26	Trace-2.5	30.5	15	7-25	9.08-15.86	60-68	40-62
Linolenic (C18:3)	2-6	13.5	3.58		trace		≤0.6	3.27-9.43	<1	1
Arachidic (C20)		0.3	0.38				≤0.5	<0.01 -1.19		
Eicosenoic (C20:1)		0.03	0.84				≤0.5	<0.01-6		
Eicosadienoic (C20:2)								<0.01-0.21		
Arachidonic (C20:4)								<0.01		
Behenic (C22)		0.08					≤0.3	<0.01-2.59		
Erucic (C22:1)							Trace-0.01	48.86-60		
Docosadienoic (C22:2)										
Docosahexaenoic (C22:6)								<0.01-1.34		
Lignoceric (C24)		0.2					0.01	<0.01-1.85		
Others		C23:0 = <0.01; C26:0 = 0.01	C12:1=1.44				C17:1 = ≤0.1	C20:3 = <0.01-0.19; C20:5 = <0.01-1.91		

(continued)

Table 4. (continued)

Fatty acids	*Cynara cardunculus* seed oil (artichoke)[174]	*Elaeis guineensis* (palm) oil[23]	*Elaeis guineensis* (palm) kernel oil[23]	*Elaeis oleifera* kernel oil[175]	*Euterpe oleracea* fruit oil (acai)[176]	*Fragaria ananassa* (strawberry) seed oil[58,100,101]	*Fragaria chiloensis* (strawberry) seed oil[103]	*Garcinia indica* seed butter (kokum)[d,114,177]	*Gevuina avellana* oil (Chilean hazel)[178]
Caproic (C6)			0.3	0.1					
Caprylic (C8)			4.4	0.9					
Capric (C10)			3.7	0.8					
Lauric (C12)		0.2	48.3	29.3					
Myristic (C14)		1.1	15.6	25.7		0.05			
Myristoleic (C14:1)									
Palmitic (C16)	12	44	7.8	10.1	22	4.32	3-5	2-8	1.9
Palmitoleic (C16:1)		0.1			2		0-0.2		22.7
Heptadecanoic (C17:0)									
Stearic (C18)	3	4.5	2	1.8	2	1.68	1-2	50-67.4	0.5
Oleic (C18:1)	25	39.2	15.1	26.4	60	10-20	15-18	27-42	39.4
Linoleic (C18:2)	60	10.1	2.7	4.5	12	28.5-50	40-46	0.5-2	5.6
Linolenic (C18:3)		0.4			Trace	25-40	30-36		0.1
Arachidic (C20)		0.4			2.5	0.71	0-0.2	0.7	1.4
Eicosenoic (C20:1)							0-0.2		3.1
Eicosadienoic (C20:2)									
Arachidonic (C20:4)									
Behenic (C22)									2.2
Erucic (C22:1)									
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)									0.5
Others			0.2	0.4		5.5-8.5	C18:3 w6 = 0-0.1		C18:1Δ12 = 6.2; C20:1Δ15 = 6.6; C22:1Δ17 = 7.9; C22:1Δ19 = 1.6

(continued)

Table 4. (continued)

Fatty acids	*Glycine soja* (soybean) oil[3]	*Gossypium herbaceum* (cotton) seed oil[24]	*Guizotia abyssinica* seed oil (Ramtil/Niger)[3]	*Helianthus annuus* (sunflower) seed oil[3]	Sunflower seed acid[77]	*Hippophae rhamnoides* fruit oil[f,110,179]	*Hippophae rhamnoides* seed oil[112,113,179]	*Irvingia gabonenesis* kernel butter[114,114]	*Juglans regia* (walnut) seed oil[180]
Caproic (C6)									
Caprylic (C8)									
Capric (C10)									
Lauric (C12)								35-51.1	
Myristic (C14)		2			≤2	0.4-0.6		36.8-58	
Myristoleic (C14:1)						0.2			
Palmitic (C16)		21	5.0-13	5.0-7.2	6-11	24-42	5-11.3	3.9-5	3-7
Palmitoleic (C16:1)						24-42	4.4		
Heptadecanoic (C17:0)									
Stearic (C18)		Trace	2.0-11	2.0-6.5	3-7	0.9-2.1	2-5	0.4-0.7	0.5-3
Oleic (C18:1)	11.5-60.0	30	6.0-40	14.7-37.2	19-31	3-30	11-30	0.6-2.7	9-30
Linoleic (C18:2)	0000	45	45-77	51.5-73.5	57-66		28-45	0.60	57-76
Linolenic (C18:3)	2.9 - 12.1			Trace-0.3	≤1	1.7-6.8	24.9-38	1.3	2-16
Arachidic (C20)		Trace		0.3-1	≤3				
Eicosenoic (C20:1)									
Eicosadienoic (C20:2)									
Arachidonic (C20:4)									
Behenic (C22)									
Erucic (C22:1)									
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)			2 max						
Others					>C20 = ≤3	vakccenic C18:1 (n-7) = 7.3-7.5; α-linoleic C18:2 = 4.1-5.5	vakccenic C18:1 (n-7) = 3.2; α-linoleic C18:2 = 34.1; others = 3 max		

(continued)

Table 4. (continued)

Fatty acids	*Limnanthes alba* (meadowfoam) seed oil[3]	*Linum usitatissimum* (linseed) seed oil[3]	*Luffa cylindrica* seed oil[181]	*Lupinus albus* seed oil[182]	*Lycium barbarum* seed oil[183]	*Macadamia integrifolia* seed oil[f,115–117,184]	*Mangifera indica* (mango) seed oil[g,3]	*Morinda citrifolia* seed oil[185]	*Moringa oleifera* seed oil (Ben/ Moringa)[118,119,186]	*Oenothera biennis* (evening primrose) oil[121,122]
Caproic (C6)										
Caprylic (C8)								1.44		
Capric (C10)										
Lauric (C12)						0.1-1.4				
Myristic (C14)			0.1			0.7-1.5			Trace	
Myristoleic (C14:1)										
Palmitic (C16)		5.5	12.2	14.44-21.57		6-12	5-8	9.0	5-9.3	4-10
Palmitoleic (C16:1)			0.1	0.36-1.03		12-25		0.12	1.5-3	
Heptadecanoic (C17:0)								0.13		
Stearic (C18)		3.5	0.1	1.37-3.91	3	0.5-8	33-48	4.07	3-8	2-4
Oleic (C18:1)		19.1	19.6	42.78-52.87	19.1	50-67	35-50	17.45	65-80	5-12
Linoleic (C18:2)		15.3	59.7	9.20-17.23	68.3	1.5-5	4.0-8	59.45	1.5-5	60-85
Linolenic (C18:3)		57		4.81-9.02	2.8	0.5 -1.9		0.27	1 -1.5	
Arachidic (C20)				1.61-2.30		1.5-5	1-7	0.51	2-5	
Eicosenoic (C20:1)	52 - 77			3.86-5.30		1.5-3.1		0.2	2.5-4	
Eicosadienoic (C20:2)										
Arachidonic (C20:4)					0.68					
Behenic (C22)				4.75-5.99		0.3-1			8-8.6	
Erucic (C22:1)	8.0-29			0.51-1.47		1			3	
Docosadienoic (C22:2)	7.0-20									
Docosahexaenoic (C22:6)										
Lignoceric (C24)									Trace	
Others										α-linolenic (C18:3) = 1%; γ-linolenic = 7%-12%

(continued)

Table 4. (continued)

Fatty acids	*Olea europaea* (olive) oil[3]	*Olea europaea* (olive) husk oil[123]	Olive acid[77]	*Orbignya cohune* seed oil (Cohune)[3]	*Orbignya oleifera* seed oil (babassu)[3]	*Orbignya speciosa* kernel oil[187]	*Oryza sativa* (rice) bran oil[125]	*Oryza sativa* (rice) germ oil[25]	*Passiflora edulis* seed oil (passion fruit)[126]
Caproic (C6)									
Caprylic (C8)				7.5	4-8	2-10			
Capric (C10)				6.5	4-8	2-12			
Lauric (C12)				46.5	44-47	35-50			
Myristic (C14)	Trace		≤1.0	16	15-20	12-25		6.92[25]	0.03
Myristoleic (C14:1)									
Palmitic (C16)	7.5-20	14.96	9-15	9.5	6-9	4-15	14	9.28	8.57
Palmitoleic (C16:1)	0.3-3.5	2.18	≤2					4.41[25]	0.23
Heptadecanoic (C17:0)			≤0.5						
Stearic (C18)	0.5-3.5	1	2-5	3	3-5	1-7	2	7.91[25]	1.66
Oleic (C18:1)	53-86	64.08	69-78	10	10-12	5-20	45	17.81[25]	16.25
Linoleic (C18:2)	3.5-20	16.09	8-14	1	1 to 3	<3	34	16.22[25]	72.69
Linolenic (C18:3)	0-1.5	0.71	≤3.5				1	15.56[25]	0.26
Arachidic (C20)	Trace							3.08[25]	
Eicosenoic (C20:1)									
Eicosadienoic (C20:2)									
Arachidonic (C20:4)								5.48[25]	
Behenic (C22)	Trace								
Erucic (C22:1)									
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)	Trace								
Others								arachidontrienoic = 5.21[25]	Unspecified other fatty acids = 0.31

(continued)

Table 4. (continued)

Fatty acids	*Perilla ocymoides* seed oil (perilla)[3]	*Persea gratissima* (avocado) oil[3]	*Pistacia vera* seed oil (pistachio)[65]	*Plukenetia volubilis* seed oil (sacha inchi)[188]	*Prunus amygdalus* (sweet almond) oil[3,63,128–130,189]	*Prunus armeniaca* (apricot) kernel oil[33]	*Prunus avium* (sweet) cherry seed oil[h,131,132]	*Prunus domestica* seed oil (prune/ plum)[134,135]
Caproic (C6)								
Caprylic (C8)								
Capric (C10)								
Lauric (C12)								
Myristic (C14)			0.09	0.02	1			
Myristoleic (C14:1)								
Palmitic (C16)		13-17	7.4	4.72	4-9	4.6-6	4-10	4-9
Palmitoleic (C16:1)		3-5.1	0.7	0.04	0.8	1-2		1
Heptadecanoic (C17:0)				0.12	0.2			
Stearic (C18)			0.9	3.33	2-3	0.5-1.2	1-4	3
Oleic (C18:1)	14-23	67-72	58.2	10.46	62-86	58-65.7 (total 18:1)	23-55	60-80
Linoleic (C18:2)	16	10 to 12	30.3	37.64	20-30	29-33; 28.5 (undef 18:2)	30-55	15-25
Linolenic (C18:3)	63-70		0.4	48.96	0.4	05-1.0 (undef 18:3)	13	1
Arachidic (C20)			0.6	0.09	0.2	0.2	2	
Eicosenoic (C20:1)			0.6	0.3	0.3			
Eicosadienoic (C20:2)								
Arachidonic (C20:4)								
Behenic (C22)			0.3		0.2			
Erucic (C22:1)			0.6		0.1			
Docosadienoic (C22:2)								
Docosahexaenoic (C22:6)								
Lignoceric (C24)								
Others				C17:1 = 0.06; gamma C18:3 = 0.24; others = 0.02	<C16:0 = 0.1	Oleic/Linoleic = 90%-93%	Eleostearic (C18:3 conj) = 10%	

(continued)

Table 4. (continued)

Fatty acids	*Prunus persica* (peach) kernel oil[136]	*Punica granatum* seed oil (pome granate)[137,138]	*Pyrus malus* (apple) seed oil[139]	*Ribes nigrum* (black currant) seed oil[140–142]	*Ribes rubrum* (currant) seed oil[143,190]	*Rosa canina* seed oil (dog rose)[169,191]	*Rubus chamaemorus* seed oil[144]	*Rubus idaeus* (raspberry) seed oil[58,145–147]
Caproic (C6)								
Caprylic (C8)								
Capric (C10)								
Lauric (C12)								
Myristic (C14)						0.11-0.21		0.07
Myristoleic (C14:1)								
Palmitic (C16)	2.0-7	1-10	6.51-6.60	6-10	4.6-4.8	1.71-4.6		2-2.43
Palmitoleic (C16:1)			0-0.05			0.24-1.01		
Heptadecanoic (C17:0)						0.04		
Stearic (C18)	0.5-3.5	1-5	1.75-1.96	1-4	2-3	1.69-2.47		0.9-1
Oleic (C18:1)	55-70	3-12	37.49-38.55	9-16	17.1-17.8	14.71-21.7	13-19	8-13
Linoleic (C18:2)	22-33	2-12	50.70-51.40	40-54	36-48	47.9-54.41	40-52	47-63
Linolenic (C18:3)	$\leq$1		0.19-0.30	11-18	15-30	16.42-21.8	27-38	25-40
Arachidic (C20)			1.49-1.54	1		1.0-2.61		0.37
Eicosenoic (C20:1)			0.51-0.56	3		0.3		
Eicosadienoic (C20:2)						0.07		
Arachidonic (C20:4)								
Behenic (C22)			0-0.40	1		0.1-0.64		
Erucic (C22:1)				1				
Docosadienoic (C22:2)								
Docosahexaenoic (C22:6)								
Lignoceric (C24)						0.04		
Others		punicic (C18:3 conj) = 60-80; other C18:3 conj = 18%		C18:3 (n-6) = 11-18; C18:4 (n-3) = 2-5	C18:1n-7 = 0.5-0.6; C18:3n-6 = 5.6-12; C18:4n-3 = 2-5; others = 0-0.3	C17:1 = 0.01; C21:0 = 0.01; C23:0 = 0.03		

(continued)

Table 4. (continued)

Fatty acids	*Schinziophyton rautanenii* kernel oil[148]	*Sclerocarya birrea* seed oil (marula)[149,192]	*Sesamum indicum* (sesame) seed oil[22,48]	*Silybum marianum* seed oil (thistle)[193]	*Solanum lycopersicum* (tomato) seed oil[50]	*Solanum lycopersicum* (tomato) fruit oil[i,194]	*Theobroma cacao* (cocoa) seed butter[3]	*Theobroma grandiflorum* seed butter (cupuacu)[195]
Caproic (C6)		1.41						
Caprylic (C8)								
Capric (C10)								
Lauric (C12)					Trace-0.3			
Myristic (C14)		2.12	<0.5		1.5-2.3			Trace
Myristoleic (C14:1)					Trace			
Palmitic (C16)	8	9-12; 22.56	7.0-12.0	9.4	16.9-23.4	47	24-29	7.2
Palmitoleic (C16:1)		0.05-0.15	<0.5		3.3-6.8			0.1
Heptadecanoic (C17:0)								0.2
Stearic (C18)	9	5-8; 50.76	3.5-6.0	6.6	4.0-9.5	3	34-36	30.8
Oleic (C18:1)	15	4.13; 70 - 78	35-50	21.3	18.3-29.7	30	30-40	43.9
Linoleic (C18:2)	37	4.0-7.0	35-50	53.3	37.6-42.8	12	2.4	4.6
Linolenic (C18:3)	25	0.1-0.6	<1.0	trace	Trace-0.7			Trace
Arachidic (C20)		0.3-0.7	<1.0	3.8	0.8-1.3			11
Eicosenoic (C20:1)		0.1-0.5	<0.5	0.5				
Eicosadienoic (C20:2)								
Arachidonic (C20:4)		8.46						
Behenic (C22)		5.14	<0.5	2.4	Trace-0.7			
Erucic (C22:1)		0.1-0.5						
Docosadienoic (C22:2)								
Docosahexaenoic (C22:6)								
Lignoceric (C24)		4.13		0.7				
Others		butyric = 0.35%	Trace C14			Other (C14 + C20) = 8		

(continued)

Table 4. (continued)

Fatty acids	*Torreya nucifera* seed oil (kaya)[196]	*Triticum vulgare* (wheat) germ oil[26,46]	*Vaccinium corymbosum* (blueberry) seed oil[58,151,152]	*Vaccinium macrocarpon* (cranberry) seed oil[58,153–156]	*Vaccinium myrtillus* seed oil (bilberry)[157,197]	*Vaccinium vitis-idaea* seed oil (lingonberry)[158,197]	*Vitis vinifera* (grape) seed oil[3]	*Zea mays* (corn) oil[47,159,160]	*Zea mays* (corn) oil[47,159,160]
Caproic (C6)									
Caprylic (C8)									
Capric (C10)									
Lauric (C12)			0.02	0.14					
Myristic (C14)	Trace		0.09	0.08	2.2-2.5	1.6-2.6		0.1-1.7	0.1-1.7
Myristoleic (C14:1)									
Palmitic (C16)	6.03	11.0-16	3-8	4-6	4.8-7.4	4.4-6.7	7-9.5	8-16.5	8-16.5
Palmitoleic (C16:1)	Trace							0.2-1.6	0.2-1.6
Heptadecanoic (C17:0)	Trace								
Stearic (C18)	2.51	1.0-6	0.5-3.5	1-1.25	2.2-2.5	1.2-1.9	3.5-5.5	0-4.5	0-4.5
Oleic (C18:1)	30.35	8.0-30	15-25	15-25.3	17.4-23	10-25	14-44	19-49	19-49
Linoleic (C18:2)	51.26	44-65	35-45	32-42	35-47.5	30-46.8	46-74	34-66	34-66
Linolenic (C18:3)	0.23	4.0-10	22-38	30-40	23.1-40	25.2-55		0-2	0-2
Arachidic (C20)			0.25	0.07				1	1
Eicosenoic (C20:1)	0.28							1	1
Eicosadienoic (C20:2)	0.98								
Arachidonic (C20:4)									
Behenic (C22)									
Erucic (C22:1)									
Docosadienoic (C22:2)									
Docosahexaenoic (C22:6)									
Lignoceric (C24)									
Others	C18:1 Δ11 = 0.57; C18:3 Δ5,9,12 = 0.08; C20:2 Δ5,11 = 0.79; C20:3 Δ5,11, 14 = 6.68; others = 0.24	0-1.2 C20-22 saturated acids		α-linolenic (C18:3) = 34%-35%					

Abbreviations: max, maximum; undef, undefined; conj, conjugated alkene.
[a]As Bassia butyracea seed fat.
[b]As Bassia latifolia seed fat or Madhuca indica seed fat.
[c]As Caryocar brasiliense pulp oil.
[d]As *Garcinia indica* seed fat.
[e]As Hippophae pulp oil.
[f]*Macadamia integrifolia* and *Macadamia ternifolia* are synonyms; information is being reported under the more common name.
[g]As mango kernel fat.
[h]As cherry kernel oil.
[i]With palm oil.

The nutritional content of these oils varies with oil type. For example, sunflower oil contains high levels of vitamins A, D, and K, whereas palm oil is a rich source of vitamins A and E. Crude sunflower oil also has the highest content of vitamin E in the form of α-tocopherol among vegetable oils.[3]

Vegetable oil and hydrogenated vegetable oil are cosmetic labeling names for blends of plant-derived oils.[5] The composition of a blend is determined by the desired physical properties. Vegetable oil and hydrogenated vegetable oil may include, but are not limited to, canola oil, *Brassica campestris* (rapeseed) oil, *Carthamus tinctorius* (safflower) seed oil, *Helianthus annuus* (sunflower) seed oil, *Sesamum indicum* (sesame) seed oil, *Elaeis guineensis* (palm) oil, *E guineensis* (palm kernel) oil, *Cocos nucifera* (coconut) oil, *Gossypium herbaceum* (cottonseed) oil, *Glycine soja* (soybean) oil, *Zea mays* (corn) oil, *Olea europaea* (olive) oil, *Prunus amygdalus dulcis* (sweet almond) oil, and hydrogenated products of these oils.

Method of Manufacturing

The oil may be directly expressed from the source (seed or pulp) followed by solvent extraction. *Bailey Industrial Oil and Fat Products* states that the removal of pigments and polar materials is mandatory for most cosmetic applications.[6] The process used for oil refining for foods may be adequate for this purpose, or additional steps may be required. Special refining methods to yield colorless and odorless oils are used by the cosmetic industry and include proprietary adsorption chromatography and supercritical fluid extractions.

The majority of the oils presented in this report are produced either from mechanical extraction or solvent extraction or a hybrid of both methods, known as prepress solvent extraction.[3] In solvent extraction, hexane is the most commonly used solvent, as it is economical and easily removed from the extracted oil. Seeds that are rich in oil can be cold pressed to extract oil without the use of solvents.[7]

After the initial extraction by methods such as solvent extraction, the crude (degummed) oil is often refined.[3] The first step is treating the oil with caustic soda to neutralize free fatty acids, hydrolyze phosphatides, and remove some colored pigments and unsaponifiable materials. Soap stock is usually a byproduct of this step. The next step involves treating the neutralized oil with activated earth to further adsorb pigments. The last major step in refining oil is deodorizing, usually by a type of steam distillation, which is intended to remove all oxidative cleavage products that impart odor or flavor to the oil. Deodorization also removes tocopherols, sterols, and other minor constituents of free fatty acids and undesirable foreign materials. Figure 2 is a flowchart of the basic refinement process.

After deodorization, oils can be further processed by hydrogenation, which makes oil more resistant to oxidative and thermal damage, and by winterization, where oil is slowly cooled to promote formation of crystals that cause cloudiness, and then filtered to remove the crystals.

Cosmetic grade fatty acid plant oils may include a physical refining step that involves heating crude oil under vacuum.[7] This step allows for the removal of volatile components such as color compounds, odor compounds, and free fatty acids, which gives the refined oil a lighter color, less odor, and lower acid values.

Analytical Methods

Near-infrared spectroscopy and gas chromatography have been used, respectively, to phenotype and analyze fatty acid profiles in shea fat (described as *V paradoxa*, not *B parkii*).[8] The fatty acid composition of hazel seed oil (*Corylus avellana*, in crude form) has also been analyzed by gas chromatography.[9] The triacylglycerol and diacylglycerol composition oils from hazelnut, pistachio, almond, Brazil nut, and macadamia nuts have been characterized using high-performance liquid chromatography with atmospheric pressure chemical ionization and UV detection.[10] The triacylglycerol profile of Brazil nut oil has also been quantified using dry matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.[11]

Impurities

Proteins. Many edible fatty acid oils are derived from foods that are recognized as potent food allergens. It has been shown that an individual who is allergic to a food will generally not react to the refined oil, especially if the oil has been "hot pressed" or has undergone more processing.[12,13] A prime example is *Arachis hypogaea* (peanut) oil. Peanuts are extremely allergenic to a large population, but reaction to the oil is rare. In its safety assessment on *A hypogaea* (peanut) oil, the Panel noted that the major concern associated with allergic reactions to peanuts is the protein.[14] The protein does not partition into the refined oil, and therefore, the oil is safe for use in cosmetics. However, researchers have reported protein levels in processed oils. Halsey et al reported that Lowry protein determinations of cold-pressed and refined sunflower oil were found to be 2 to 8 μg/mL protein,[15] whereas Zitouni et al reported trace amounts of protein in the refined oil.[16] Olszewski et al found 0.1 to 0.2 μg protein per gram of peanut oil,[17] whereas Ramazzotti et al reported finding immunoglobulin E (IgE)-responsive residual proteins in peanut oil extracts.[18] Porras et al found soy protein in some samples of soy oil, but not others.[19] Awazuhara et al reported 1.4 to 4.0 μg protein per 100 g of soy oil.[20] Although Paschke et al found approximately 35 μg/L protein content in refined soybean oil, no IgE-binding activity was detectable.[21]

Although the Panel has found a general lack of clinical effects for fatty acid oils already reviewed,[14,22–30] other groups have raised concerns. The European Medicines Agency (EMEA) Working Party on Herbal Medicinal Products concluded that soy and peanut products "should be treated as allergenic unless they have an analytically monitored non-allergenic specification and a safe maximum daily dose."[31] The EMEA found that threshold concentrations for induction of a protein contact dermatitis were not available and recommended, "all medications for topical use containing soya or peanut products should be treated as allergenic."

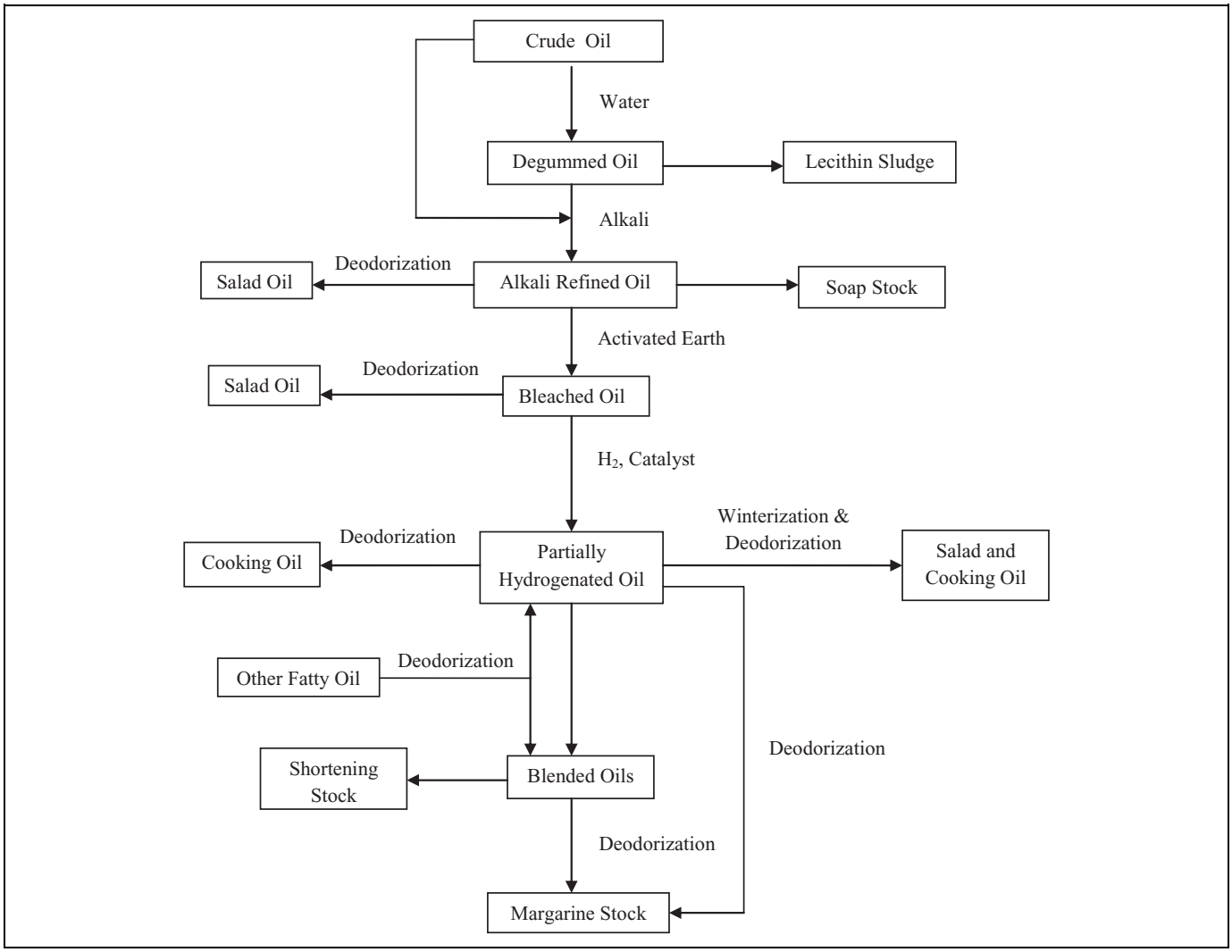

Figure 2. Basic oil refinement flowchart.[3]

Aflatoxin. Aflatoxins are metabolic products of the molds *Aspergillus flavus* and *Aspergillus parasiticus*. They are most often produced in stored agricultural crops (such as peanuts and other nut crops) when growth conditions and genetic requirements are favorable.[32–34] The International Agency for Research on Cancer (IARC) categorized aflatoxins as group 1 agents, "carcinogenic to humans."[35,36]

The US government places the following limitations on peanuts to be considered "negative" for aflatoxin: $\leq$15 ppb for "peanuts which have been certified as meeting edible quality grade requirements" and $\leq$25 ppb for "nonedible quality categories" (7 Code of Federal Regulations (CFR) sections 997.30 and 998.200).[37] Aflatoxin contamination was not a concern in the previous CIR safety assessments of peanut oil,[17] hazelnut oil,[41] or coconut oil.[29]

Glycidol. Glycidol and glycidol fatty acid esters have been detected in refined fatty acid oils.[38–41]

Gossypol. Gossypol reportedly is present in refined cottonseed oil at a concentration of $\leq$0.01%.[24] The concentration of gossypol in modified cottonseed products intended for human consumption is limited by federal regulation (21CFR 112.894).

Use

Cosmetic

The safety of the cosmetic ingredients addressed in this safety assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council, of maximum reported use concentrations by product category.

There are 244 oil ingredients included in this safety assessment, 146 of which are reported to be used; 118 of the in-use ingredients have never been reviewed by CIR, while 28 have

been reviewed previously. For the ingredients being reviewed for the first time, the frequency of use[42] and/or concentration of use[43–45] can be found in Table 5. (Also included in Table 5 are 3 ingredients, *Citrullus vulgaris* (watermelon) seed oil, macadamia nut oil, and *Vaccinium oxycoccos* (cranberry) seed oil, that do not have identifiable INCI names; these ingredients are not part of this assessment, but they are very similar to the oils that are part of this assessment, and information on them is included in this report for completeness.) For the ingredients that have been reviewed previously, the current and historical[23–26,28,46–48] frequency and concentration of use is given in Table 6. The 97 ingredients not currently reported to be used are listed in Table 7.[42–45,49,50]

Of the oils included in this report, *B parkii* (shea) butter has the most reported uses in cosmetic and personal care products, with a total of 1,950; 1,680 of those uses are in leave-on formulations. A recent survey of use concentrations for *B parkii* (shea) butter reports a maximum use concentration of 60% in leave-on products as a cuticle softener, a manicuring application.[51] *Helianthus annuus* (sunflower) seed oil has the second greatest number of overall uses reported, with a total of 1,414; 1,054 of those uses are in leave-on formulations, having use concentrations up to 96%. Many other ingredients are used in an extensive number of formulations. For example, prunus amygdalus dulcis (sweet almond) oil, *O europaea* (olive) fruit oil, and *G soja* (soybean) oil have 1,127, 915, and 912 uses, respectively. Most of the in-use ingredients have uses in both leave-on and rinse-off product types, many are used in products that are applied around the eye and some are used in a way they can possibly be ingested. Some are used in products that involve mucous membrane exposure, and a few are used in underarm deodorant formulations. Many of the products are used in formulations at relatively high concentrations. *Olea europaea* (olive) fruit oil is used at up to 100%, *Persea gratissima* (avocado) oil is used at up to 98%, *H annuus* (sunflower) seed oil at up to 96%, and *G soja* (soybean) oil at 95%.

Oils are used in a wide variety of cosmetic products for their skin conditioning, occlusive, emollient, moisturizing, and other properties. Some of the oils included in this report are used in products that can be inhaled, and effects on the lungs that may be induced by aerosolized products containing these ingredients are of concern. The particle size of aerosol hair sprays and of pump hair sprays is 38 and >80 μm, respectively, and is relatively large compared to respirable particle sizes (≤10 μm). Therefore, because of their size, most aerosol particles are deposited in the nasopharyngeal region and are not respirable.

None of the oils, hydrogenated oils, unsaponifiables, oil fatty acids, and salts of the fatty acids described in this report were restricted from use in any way under the rules governing cosmetic products in the European Union.[52]

Noncosmetic

The primary use of plant-derived fatty acid oils is for cooking. Palm oil is the world's most widely consumed edible oil (41.7 million metric tons), followed by soybean oil, rapeseed oil, sunflower seed oil, cottonseed oil, peanut oil, palm kernel oil, coconut oil, and olive oil.[3,53] Nonfood, noncosmetic uses for edible fatty acid oils are found in Table 8.

Toxicological Studies

Many of the fatty acid oils in this assessment are edible, and exposure to the oils from food use would result in a much larger systemic dose than that resulting from use in cosmetic products. Consequently, their systemic toxicity potential, except as discussed below relating to carcinogenicity, is not addressed in this report. The safety focus of use of these oils as cosmetic ingredients is the potential for irritation and sensitization.

Carcinogenicity

The safety of glycidol fatty acid esters in refined vegetable oils was assessed by IARC. Glycidol was determined to be a group 2A (probably carcinogenic to humans) chemical, while glycidol fatty acid esters were determined to be a group 3 (not classifiable as to carcinogenicity to humans) chemical.[40,41]

The Federal Institute for Risk Assessment in Germany released a summary of their initial evaluation of the assessment of levels of glycidol fatty acid esters detected in refined vegetable fats.[39] Although acknowledging that the levels of glycidol that may be released from glycidol fatty acid esters are not known, the evaluation noted that glycidol is classified as probably carcinogenic to humans. The evaluation was based on findings of the German Chemical and Veterinary Test Agency that noted that glycidol is converted to 3-chloropropanediol and it appeared to be the 3-chloropropanediol that was detected in the vegetable fat.[38] The levels of 3-chloropropanediol were negligible at the crude oil, degummed, neutralized, and bleached stages, but levels were significant at the deodorized stage.

Anacardium occidentale *(Cashew) Seed Oil*

The modulatory effect of *A occidentale* (cashew) seed oil on antioxidant potential was investigated in female Swiss albino mice in a 120-day skin papillomagenesis study.[54] The mice were divided into 4 groups of 15 and 1 group of 10 (vehicle control). Test groups were as follows: group I was the vehicle control, receiving 0.1 mL acetone; group II was the positive control, receiving a single dose of 7,12-dimethylbenz(*a*)anthracene (DMBA; 0.005 mg/0.05 mL acetone) followed by applications of 2% croton oil 3 times a week until study termination; group III received a single dose of DMBA followed by applications of 2.5% cashew nut kernel oil 3 times a week until study termination; group IV received a single dose of DMBA followed by applications of 5% cashew nut kernel oil 3 times a week until study termination; and group V received 5% cashew nut kernel oil applied until study termination. The oil was applied to the clipped dorsal scapular region that was 2 cm in diameter. Body weights were recorded at regular intervals.

Table 5. Frequency and Concentration of Use According to Duration and Exposure.[a]

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)
	Actinidia chinensis (kiwi) seed oil		*Adansonia digitata* oil		*Aleurites moluccanus* seed oil		*Anacardium occidentale* (cashew) seed oil		*Argania spinosa* kernel oil		*Astrocaryum murumuru* seed butter	
Totals[b]	7	0.1	6	0.01	141	0.00001-5	10	0.002-1	100	0.001-10	192	0.001-7
Duration of use												
Leave-on	5	NR	4	0.01	87	0.00002-5	9	0.04-1	87	0.001-10	171	0.001-7
Rinse-off	2	0.1	2	NR	54	0.00001-3	1	0.002	13	0.001-2	21	0.001-0.2
Exposure type												
Eye area	NR	NR	NR	NR	6	0.0001-0.005	NR	NR	11	0.1-1	21	0.06-0.5
Possible ingestion	1	NR	NR	0.01	1	0.01	NR	NR	9	0.1-1	22	1-7
Inhalation	1	NR	NR	NR	15	0.1	NR	NR	NR	0.01	NR	NR
Dermal contact	5	NR	5	0.01	76	0.00001-5	9	0.002-1	88	0.001-10	178	0.001-7
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.001	NR	NR
Hair—noncoloring	2	0.1	1	NR	58	0.00002-0.1	1	NR	8	0.01-1	11	0.001-0.2
Hair—coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.07-0.1	3	NR
Nail	NR	NR	NR	NR	4	NR	NR	NR	2	0.001-0.1	NR	NR
Mucous membrane	NR	NR	NR	NR	5	0.00001-0.4	NR	NR	2	0.001-2	3	NR
Bath products	NR	NR	NR	NR	6	0.01-0.3	NR	NR	1	0.05	NR	NR
Baby products	NR	NR	1	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Sodium *Astrocaryum murumuruate*		*Avena sativa* (oat) kernel oil		*Bassia latifolia* seed butter		*Bertholletia excelsa* seed oil		*Borago officinalis* seed oil		*Brassica campestris* (rapeseed) seed oil	
Totals	NR	0.002-0.005	43	0.01-3	22	0.001-2	55	0.0003-0.5	180	0.001-1	27	0.007-17
Duration of use												
Leave-on	NR	0.002	37	0.1-3	17	0.001-0.05	18	0.0003-0.5	160	0.001-1	23	0.007-17
Rinse-off	NR	0.002-0.005	6	0.001-0.1	5	0.001-2	37	0.01-0.2	20	0.001-0.01	4	0.1-1
Exposure type												
Eye area	NR	NR	NR	0.2	4	0.01	1	NR	7	0.001-0.5	2	NR
Possible ingestion	NR	NR	NR	2	NR	NR	NR	NR	NR	0.01	1	9
Inhalation	NR	NR	NR	NR	NR	NR	1	NR	3	0.1	NR	NR
Dermal contact	NR	0.002-0.005	41	0.001-3	22	00.01-2	29	0.0003-0.5	168	0.001-1	27	0.007-17
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	2	0.1	NR	0.001-0.5	12	0.03-0.2	10	NR	NR	0.1
Hair—coloring	NR	NR	NR	NR	NR	NR	14	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	0.002	2	0.01-0.1	5	NR	7	0.01	4	0.001-0.01	1	NR
Bath products	NR	NR	1	NR	NR	NR	3	NR	1	NR	NR	NR
Baby products	NR	NR	6	0.1	NR	NR	NR	NR	3	NR	NR	NR

(continued)

Table 5. (continued)

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)
	Hydrogenated rapeseed oil		*Brassica oleracea* Italica (broccoli) seed oil		*Butyrospermum parkii* (shea) oil		*Butyrospermum parkii* (shea) butter		*Butyrospermum parkii* (shea) butter unsaponifiables		Hydrogenated shea butter	
Totals	1	0.3-4	NR	0.001-3	22	0.01-15	1950	0.0005-60	38	0.06-3	4	1
Duration of use												1
Leave-on	NR	0.3-4	NR	3	16	0.01-15	1680	0.001-60	35	0.06-3	2	1
Rinse-off	1	NR	NR	0.001-0.5	22	0.6-1	270	0.0005-30	3	NR	2	1
Exposure type												
Eye area	NR	2	NR	NR	1	NR	108	0.1-8	7	0.2-0.7	NR	NR
Possible ingestion	NR	NR	NR	NR	NR	15	128	0.5-26	2	3-Jan	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR	17	0.001-3	NR	NR	NR	NR
Dermal contact	1	0.3-4	NR	NR	22	0.6-15	1724	0.001-45	33	0.06-3	4	1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	2	1	NR	NR	NR	NR
Hair—noncoloring	NR	NR	NR	NR	NR	NR	210	0.0005-3	5	2	NR	NR
Hair—coloring	NR	NR	NR	0.001-3	NR	NR	4	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	0.01-1	7	0.01-60	NR	NR	NR	NR
Mucous membrane	NR	NR	NR	NR	3	0.6	101	0.003-5	NR	NR	NR	NR
Bath products	NR	NR	NR	NR	3	1	13	1	NR	NR	2	NR
Baby products	NR	NR	NR	NR	NR	NR	24	0.01-5	NR	NR	NR	NR
	Camelina sativa seed oil		*Camellia japonica* seed oil		*Camellia kissi* seed oil		*Camellia oleifera* seed oil		Hydrogenated *Camellia oleifera* seed oil		*Camellia sinensis* seed oil	
Totals	76	0.002-1	NR	0.01-0.2	47	0.1-10	25	0.003-3	1	NR	12	0.1
Duration of use												
Leave-on	61	0.002-1	NR	0.01-0.2	34	0.1-10	23	0.003-3	1	NR	8	0.1
Rinse-off	15	1	NR	0.1	13	0.1-3	2	0.01-0.1	NR	NR	4	0.1
Exposure type												
Eye area	NR	0.05	NR	0.01	4	0.1	NR	2	NR	NR	NR	NR
Possible ingestion	34	0.05-0.5	NR	0.1	1	0.1	3	3	NR	NR	1	0.1
Inhalation	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Dermal contact	47	0.002-1	NR	0.01-0.2	36	0.1-10	23	0.003-3	1	NR	10	0.1
Deodorant (underarm)	NR	NR	NR	0.01	NR	NR	NR	NR	NR	NR	NR	0.1
Hair—noncoloring	29	1	NR	0.1	11	0.1-1	2	2	NR	NR	2	0.1
Hair—coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	NR	0.1	1	0.1	NR	0.01-0.1	NR	NR	2	0.1
Bath products	NR	NR	NR	NR	1	0.3	NR	0.05	NR	NR	NR	NR
Baby products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

(continued)

Table 5. (continued)

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)
	Canola oil		Canola oil unsaponifiables		Hydrogenated canola oil		*Carica papaya* seed oil		*Caryocar brasiliense* fruit oil		*Chenopodium quinoa* seed oil	
Totals	132	0.0002-73	NR	0.001	3	NR	NR	0.1	31	0.0005-0.2	1	0.3
Duration of use												
Leave-on	112	0.002-73	NR	NR	2	NR	NR	0.1	29	0.0005-2	1	NR
Rinse-off	20	0.02-33	NR	0.0001	1	NR	NR	NR	2	NR	NR	0.3
Exposure type												
Eye area	3	0.002-0.03	NR	NR	NR	NR	NR	NR	12	NR	NR	NR
Possible ingestion	62	0.3-70	NR	NR	NR	NR	NR	NR	12	0.2	NR	NR
Inhalation	1	0.0002-17	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Dermal contact	113	0.0002-73	NR	NR	3	NR	NR	0.1	30	0.0005-0.2	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair—noncoloring	19	0.006-24	NR	0.001	NR	NR	NR	NR	1	NR	1	NR
Hair—coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.3
Nail	NR	5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	2	0.02-1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Bath products	1	1-33	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Citrullus lanatus (watermelon) seed oil		*Citrullus vulgaris* (watermelon) seed oil[c]		*Citrus limon* (lemon) seed oil		*Citrus paradisi* (grapefruit) seed oil		*Crambe abyssinica* seed oil		*Cucumis sativus* (cucumber) seed oil	
Totals	1	2	5	NR	6	6	NR	0.01-20	6	NR	6	NR
Duration of use												
Leave-on	1	2	3	NR	5	5	NR	0.08-20	5	NR	5	NR
Rinse-off	NR	NR	2	NR	1	1	NR	0.01-1	1	NR	1	NR
Exposure type												
Eye area	NR	NR	NR	NR	NR	1	NR	NR	NR	NR	1	NR
Possible ingestion	NR	NR	NR	NR	NR	NR	NR	5	NR	NR	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR	NR	2	NR	NR	NR	NR
Dermal contact	1	2	5	NR	6	5	NR	2-5	6	NR	5	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	NR	NR	NR	1	NR	0.01-20	NR	NR	1	NR
Hair—coloring	NR	NR	NR	NR	NR	NR	NR	9	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	1	NR	1	NR	NR	NR	1	NR	NR	NR
Bath products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

(continued)

Table 5. (continued)

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)
	Cucurbita pepo (pumpkin) seed oil		Palm kernel acid		Potassium palm kernelate		Potassium palmate		Sodium palm kernelate		Sodium palmate	
Totals	18	0.003-0.1	72	0.2-12	7	0.3-30	5	0.3-3	194	12-44	212	3-68
Duration of use												
Leave-on	17	0.003-0.1	3	NR	NR	NR	NR	NR	10	NR	7	NR
Rinse-off	1	NR	69	0.2-12	7	0.3-30	5	0.3-3	184	12-44	205	3-68
Exposure type												
Eye area	1	0.003	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Possible ingestion	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Inhalation	1	NR	NR	NR	NR	NR	NR	NR	1	NR	1	NR
Dermal contact	18	0.003-0.1	71	0.2-12	7	0.3-30	5	0.3-3	194	12-44	212	3-68
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair—noncoloring	1	NR	1	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair—coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	64	0.2-3	1	0.3-30	2	0.3-3	173	16-44	189	3-68
Bath products	NR	NR	NR	NR	NR	NR	NR	NR	3	NR	1	NR
Baby products	NR	NR	NR	NR	NR	NR	NR	NR	4	NR	3	NR
	Palm acid		*Elaeis oleifera* kernel oil		*Euterpe oleracea* fruit oil		*Garcinia indica* seed butter		*Gevuina avellana* oil		*Glycine soja* (soybean) oil	
Totals	33	1-17	5	NR	29	0.00001-0.5	30	0.1-2	5	0.002-0.2	912	0.0002-95
Duration of use												
Leave-on	1	NR	NR	NR	19	0.00001-0.5	27	0.1-2	5	0.04-0.2	718	0.0005-95
Rinse-off	32	1-17	5	NR	10	0.05	3	NR	NR	0.002-0.01	194	0.0002-95
Exposure type												
Eye area	NR	NR	NR	NR	2	0.5	1	NR	NR	NR	53	0.04-2
Possible ingestion	NR	NR	NR	NR	1	0.002	3	0.1-2	NR	NR	103	0.6-4
Inhalation	1	NR	NR	NR	1	NR	NR	NR	NR	NR	6	0.03-0.5
Dermal contact	33	1-17	NR	NR	14	0.00001-0.5	30	0.1-2	4	0.002-0.2	800	0.0005-93
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.01-0.5
Hair—noncoloring	NR	NR	2	NR	15	NR	NR	NR	NR	NR	97	0.0002-95
Hair—coloring	NR	NR	3	NR	NR	NR	NR	NR	NR	NR	5	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	6	0.02-95
Mucous membrane	31	1-4	NR	NR	3	NR	1	NR	NR	NR	70	0.01-52
Bath products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	19	0.1-78
Baby products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	21	2

(continued)

Table 5. (continued)

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)
	Glycine soja (soybean) oil unsaponifiables		Hydrogenated soybean oil		*Helianthus annuus* (sunflower) seed oil		*Helianthus annuus* (sunflower) seed oil unsaponifiables		Hydrogenated sunflower oil		*Hippophae rhamnoides* oil	
Totals	12	0.0001-0.2	36	0.001-42	1414	0.000007-96	10	0.005-2	NR	6-35	15	0.2-0.7
Duration of use												
Leave-on	12	0.0001-0.2	33	0.001-39	1054	0.0002-96	10	0.005-2	NR	6-35	10	0.2-0.7
Rinse-off	NR	NR	3	0.05-42	360	0.000007-92	NR	0.002	NR	15-35	5	0.2
Exposure type												
Eye area	NR	NR	4	0.03-7	64	0.0005-19	2	0.02	NR	7	NR	NR
Possible ingestion	NR	NR	3	0.1-39	260	0.08-41	NR	NR	NR	6	NR	NR
Inhalation	NR	NR	NR	NR	3	0.0002-85	NR	NR	NR	NR	NR	NR
Dermal contact	12	0.0001-0.2	34	0.01-39	707	0.0002-96	10	0.005-2	NR	6-35	1	0.2-0.7
Deodorant (underarm)	NR	NR	NR	NR	1	0.0003-4	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	1	0.1	179	0.000007-92	NR	NR	NR	NR	6	NR
Hair—coloring	NR	NR	NR	NR	85	0.03-35	NR	NR	NR	15-35	NR	NR
Nail	NR	NR	NR	0.001-25	8	0.05-30	NR	NR	NR	NR	8	NR
Mucous membrane	NR	NR	NR	0.05-6	52	0.0003-4	NR	0.002	NR	NR	1	0.2
Bath products	NR	NR	NR	5-42	11	0.005-75	NR	NR	NR	NR	NR	NR
Baby products	NR	NR	NR	NR	18	0.2	NR	NR	NR	NR	NR	NR
	Hippophae rhamnoides fruit oil		*Irvingia gabonensis* kernel butter		*Juglans regia* (walnut) seed oil		*Limnanthes alba* (meadowfoam) seed oil		*Linum usitatissimum* (linseed) seed oil		Linseed acid	
Totals	7	0.004-2	109	0.003-0.4	15	0.00003-0.2	316	0.002-74	102	0.001-10	3	NR
Duration of use												
Leave-on	7	0.004-2	109	0.003-0.4	12	0.01-0.2	225	0.002-74	52	0.002-10	3	NR
Rinse-off	NR	NR	NR	NR	3	0.00003-0.1	91	0.01-2	50	0.001-0.4	NR	NR
Exposure type												
Eye area	1	NR	2	NR	1	NR	30	0.1-20	3	0.01	NR	NR
Possible ingestion	NR	NR	64	0.003-0.3	NR	NR	67	0.6-26	NR	0.01	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR	1	0.1-3	3	NR	NR	NR
Dermal contact	6	2	108	0.003-0.4	15	0.003-0.2	211	0.002-74	58	0.003-4	3	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.05-0.1	NR	NR
Hair—noncoloring	NR	NR	1	NR	NR	0.00003-0.1	47	0.1-1	42	0.001-0.1	NR	NR
Hair—coloring	NR	NR	NR	NR	NR	NR	46	0.2-2	NR	NR	NR	NR
Nail	1	0.004	NR	NR	NR	NR	NR	0.5	2	0.002-0.05	NR	NR
Mucous membrane	NR	NR	NR	NR	NR	NR	4	0.001-0.6	5	0.003-0.4	NR	NR
Bath products	NR	NR	NR	NR	2	NR	2	0.5-0.9	1	0.02-0.2	NR	NR
Baby products	NR	NR	NR	NR	NR	NR	1	NR	2	NR	1	NR

(continued)

Table 5. (continued)

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)
	Luffa cylindrica seed sil		*Lupinus albus* seed oil		*Lycium barbarum* seed oil		*Macadamia integrifolia* seed oil		*Macadamia ternifolia* seed oil		Macadamia nut oil[c]	
Totals	21	0.01	1	NR	2	NR	41	0.00006-5	533	0.0003-30	208	NS
Duration of use												
Leave-on	21	NR	1	NR	2	NR	25	0.00006-5	482	0.001-30	191	NS
Rinse-off	NR	0.01	NR	NR	NR	NR	16	0.006-3	51	0.0003-10	17	NS
Exposure type												
Eye area	1	NR	NR	NR	1	NR	3	0.1	16	0.1-15	22	NS
Possible ingestion	9	NR	NR	NR	1	NR	4	1	33	0.1-30	11	NS
Inhalation	NR	NR	NR	NR	NR	NR	NR	0.5	12	0.007-16	2	NS
Dermal contact	21	0.01	1	NR	2	NR	36	0.00006-5	493	0.001-30	170	NS
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NS
Hair—noncoloring	NR	NR	NR	NR	NR	NR	12	0.01-0.03	33	0.0003-16	9	NS
Hair—coloring	NR	NR	NR	NR	NR	NR	NR	NR	3	0.02	NR	NS
Nail	NR	NR	NR	NR	NR	NR	NR	3	1	0.001-0.5	NR	NS
Mucous membrane	NR	0.01	NR	NR	NR	NR	10	2	12	0.02-10	NR	NS
Bath products	NR	NR	NR	NR	NR	NR	1	0.5	2	1-10	1	NS
Baby products	NR	NR	NR	RN	NR	NR	NR	NR	4	NR	NR	NS
	Mangifera indica (Mango) seed oil		*Mangifera indica* (mango) seed butter		Sodium mango seedate		*Moringa oleifera* seed oil		*Moringa pterygosperma* seed oil		*Oenothera biennis* (evening primrose) oil	
Totals	72	0.003-6	175	0.0005-3	1	NR	NR	0.001	15	0.003-3	150	0.00002-58
Duration of use												
Leave-on	64	0.003-6	134	0.01-5	NR	NR	NR	0.001	13	0.004-3	113	0.00002-58
Rinse-off	8	0.05-0.2	41	0.0005-0.5	1	NR	NR	NR	2	0.003	37	0.002-0.2
Exposure type												
Eye area	13	5	6	0.02	NR	NR	NR	NR	4	3	4	0.00002-0.5
Possible ingestion	7	0.03-6	25	1-5	NR	NR	NR	NR	1	NR	14	0.1-15
Inhalation	1	NR	2	0.02	NR	NR	NR	NR	NR	NR	2	NR
Dermal contact	60	0.003-6	147	0.0005-5	1	NR	NR	0.001	11	0.003-3	109	0.00002-58
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.2
Hair—noncoloring	12	0.05-0.2	12	0.02-0.5	NR	NR	NR	NR	1	0.02	37	0.05-0.1
Hair—coloring	NR	0.05	16	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	0.5	NR	NR	NR	NR	NR	NR	4	0.001-3
Mucous membrane	2	0.1	10	0.0005-0.5	1	NR	NR	NR	NR	0.003	4	0.1-0.2
Bath products	NR	NR	1	NR	NR	NR	NR	NR	NR	NR	2	0.2
Baby products	NR	NR	3	NR	NR	NR	NR	NR	NR	NR	3	NR

(continued)

Table 5. (continued)

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)
	Hydrogenated evening primrose oil		*Olea europaea* (olive) fruit oil		*Olea europaea* (olive) oil unsaponifiables		Hydrogenated olive oil		Hydrogenated olive oil unsaponifiables		Potassium olivate	
Totals	14	NR	915	0.0005-100	77	0.0001-3	50	0.0005-12	2	0.05-5	3	NR
Duration of use												
Leave-on	14	NR	617	0.001-100	68	0.0001-3	36	0.1-12	2	0.05-5	NR	NR
Rinse-off	NR	NR	298	0.0005-94	9	0.04-0.3	14	0.0005-0.1	NR	NR	3	NR
Exposure type												
Eye area	1	NR	26	0.004-17	12	0.02-0.4	13	0.1-3	NR	0.3-2	NR	NR
Possible ingestion	NR	NR	26	0.7-26	1	0.08	7	0.1-12	NR	NR	NR	NR
Inhalation	NR	NR	6	0.2-5	NR	3	NR	NR	NR	NR	NR	NR
Dermal contact	14	NR	711	0.0005-100	67	0.0001-3	34	0.0005-12	2	0.05-5	3	NR
Deodorant (underarm)	NR	NR	3	0.02-0.1	NR	NR	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	190	0.006-94	6	0.02-0.3	11	0.01-0.1	NR	NR	NR	NR
Hair—coloring	NR	NR	NR	0.2-0.5	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	5	1-40	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	121	0.0005-3	4	NR	1	0.0005	NR	NR	1	NR
Bath products	NR	NR	14	0.9-17	NR	NR	NR	NR	NR	NR	NR	NR
Baby products	NR	NR	9	0.2	NR	0.04	NR	0.4	NR	NR	NR	NR
	Sodium olivate		*Orbignya cohune* seed oil		*Orbignya oleifera* seed oil		Sodium babassuate		*Orbignya speciosa* kernel oil		*Passiflora edulis* seed oil	
Totals	16	4-18	1	NR	161	0.0009-27	NR	8	8	0.5-0.9	62	0.0007-3
Duration of use												
Leave-on	5	NR	NR	NR	118	0.0009-4	NR	NR	1	0.9	53	0.003-5
Rinse-off	11	4-18	1	NR	43	0.01-27	NR	8	7	0.5	9	0.0007-0.005
Exposure type												
Eye area	NR	NR	NR	NR	7	0.5-0.6	NR	NR	NR	NR	3	0.8
Possible ingestion	NR	NR	NR	NR	57	0.001-2	NR	NR	NR	NR	14	0.6-3
Inhalation	NR	NR	NR	NR	5	0.02-2	NR	NR	NR	NR	3	NR
Dermal contact	16	4-18	NR	NR	110	0.0009-27	NR	8	NR	NR	49	0.003-3
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.003
Hair—noncoloring	NR	NR	1	NR	43	0.02-2	NR	NR	5	0.5-0.9	10	0.007-0.5
Hair—coloring	NR	NR	NR	NR	8	NR	NR	NR	3	NR	3	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	9	4-18	NR	NR	5	27	NR	8	NR	NR	1	NR
Bath products	NR	NR	NR	NR	2	0.01-0.1	NR	NR	NR	NR	NR	0.01-0.05
Baby products	1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

(continued)

Table 5. (continued)

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)
	Perilla ocymoides seed oil		*Persea gratissima* (avocado) oil unsaponifiables		Hydrogenated avocado oil		*Persea gratissima* (avocado) butter		Sodium avocadoate		*Pistacia vera* seed oil	
Totals	7	NR	63	0.2-6	11	0.5	15	NR	1	NR	158	0.003-1
Duration of use												
Leave-on	5	NR	57	0.5-6	9	NR	15	NR	NR	NR	107	0.08-0.2
Rinse-off	2	NR	6	0.2	2	0.5	NR	NR	1	NR	51	0.003-1
Exposure type												
Eye area	2	NR	9	0.5	NR	NR	NR	NR	NR	NR	7	NR
Possible ingestion	NR	NR	2	3	2	NR	11	NR	NR	NR	6	NR
Inhalation	NR	NR	4	NR	NR	NR	NR	NR	NR	NR	NR	NR
Dermal contact	5	NR	56	0.2-3	8	NR	15	NR	1	NR	133	0.003-0.2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair—noncoloring	2	NR	2	6	3	0.5	NR	NR	NR	NR	16	0.05-1
Hair—coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	3	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	NR	NR	NR	NR	NR	NR	1	NR	19	NR
Bath products	NR	NR	4	NR	NR	NR	NR	NR	NR	NR	8	NR
Baby products	NR	NR	1	NR	NR	NR	NR	NR	NR	NR	3	NR
	Plukenetia volubilis seed oil		Hydrogenated sweet almond oil		Sodium sweet almondate		*Prunus armeniaca* (apricot) kernel oil		Hydrogenated apricot kernel oil		*Prunus avium* (sweet cherry) seed oil	
Totals	13	0.05-0.6	21	0.5	4	15	588	0.00001-89	2	NR	2	0.01-0.02
Duration of use												
Leave-on	12	0.05-0.6	13	0.5	4	NR	449	0.0001-40	2	NR	NR	NR
Rinse-off	1	NR	8	0.5	NR	15	139	0.00001-89	NR	NR	2	0.01-0.02
Exposure type												
Eye area	1	NR	NR	NR	NR	NR	25	0.002-18	NR	NR	NR	NR
Possible ingestion	3	0.6	1	NR	NR	NR	38	0.001-5	NR	NR	NR	NR
Inhalation	NR	NR	NR	NR	NR	NR	5	0.0009-1	NR	NR	NR	NR
Dermal contact	13	0.6	15	0.5	4	15	486	0.00001-18	2	NR	2	0.01-0.02
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	1	0.003-0.1	NR	NR	NR	NR
Hair—noncoloring	NR	NR	6	0.5	NR	NR	78	0.0001-89	NR	NR	NR	NR
Hair—coloring	NR	NR	NR	NR	NR	NR	10	0.1	NR	NR	NR	NR
Nail	NR	0.05	NR	NR	NR	NR	10	0.002-40	NR	NR	NR	NR
Mucous membrane	NR	NR	1	NR	NR	15	24	0.01-9	NR	NR	2	0.01-0.02
Bath products	NR	NR	1	NR	NR	NR	8	4	NR	NR	NR	NR
Baby products	NR	NR	NR	NR	NR	NR	7	NR	NR	NR	NR	NR

(continued)

Table 5. (continued)

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)
	Prunus domestica seed oil		*Prunus persica* (peach) kernel oil		*Punica granatum* seed oil		*Pyrus malus* (apple) seed oil		*Ribes nigrum* (black currant) seed oil		*Rosa canina* fruit oil	
Totals	NR	0.04	22	0.003-22	46	0.001-1	8	NR	53	0.000001-0.3	121	0.001-19
Duration of use												
Leave-on	NR	NR	16	0.05-22	44	0.001-1	8	NR	45	0.000001-0.3	106	0.001-19
Rinse-off	NR	0.04	6	0.003-6	2	0.001-0.1	NR	NR	8	0.05	15	0.001-0.5
Exposure type												
Eye area	NR	NR	NR	NR	2	NR	NR	NR	2	0.08	17	0.1-0.5
Possible ingestion	NR	NR	NR	0.04-22	30	1	1	NR	7	0.03-0.1	7	0.001-2
Inhalation	NR	NR	NR	2	NR	NR	NR	NR	NR	NR	1	NR
Dermal contact	NR	0.04	18	0.003-22	46	0.001-1	8	NR	43	0.000001-0.3	109	0.008-19
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	4	NR	NR	NR	NR	NR	5	NR	9	0.001-0.5
Hair—coloring	NR	NR	NR	0.1	NR	0.1	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	0.001	NR	NR	5	0.2	1	0.1-2
Mucous membrane	NR	NR	1	NR	2	0.001	NR	NR	2	NR	3	0.001
Bath products	NR	NR	1	0.1-1	NR	NR	NR	NR	NR	NR	1	0.5
Baby products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Rubus chamaemorus seed oil		*Rubus idaeus* (raspberry) seed oil		*Schinziophyton rautanenii* kernel oil		*Sclerocarya birrea* seed oil		*Silybum marianum* seed oil		*Solanum lycopersicum* (tomato) fruit oil	
Totals	3	0.1	10	0.1-5	6	NR	29	1	NR	0.5	NR	0.01-1
Duration of use												
Leave-on	*3*	*0.1*	*8*	*0.1-5*	*4*	*NR*	*23*	*1*	*NR*	*0.5*	*NR*	*0.001-1*
Rinse-off	*NR*	*NR*	*2*	*NR*	*2*	*NR*	*6*	*1*	*NR*	*NR*	*NR*	*NR*
Exposure type												
Eye area	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.01
Possible ingestion	NR	NR	1	NR	NR	NR	6	NR	NR	NR	NR	0.001
Inhalation	NR	NR	NR	NR	NR	NR	2	NR	NR	NR	NR	NR
Dermal contact	3	0.1	8	0.1-5	3	NR	23	1	NR	0.5	NR	0.001-1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	NR	NR	3	NR	6	1	NR	NR	NR	NR
Hair—coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	2	NR	NR	NR	2	NR	NR	NR	NR	NR
Bath products	NR	NR	1	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

(continued)

Table 5. (continued)

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)	No. of uses[42]	Conc. of use (%)
	Solanum lycopersicum (tomato) seed oil		*Theobroma cacao* (cocoa) seed butter		*Theobroma grandiflorum* seed butter		*Triticum vulgare* (wheat) germ oil unsaponifiables		Wheat germ acid		*Vaccinium macrocarpon* (cranberry) seed oil	
Totals	1	NR	442	0.000002-37	153	0.00005-7	17	0.2	16	NR	21	0.002-2
Duration of use												
Leave-on	*1*	*NR*	*367*	*0.000002-37*	*119*	*0.00005-7*	*17*	*0.2*	*3*	*NR*	*18*	*0.002-2*
Rinse-off	*NR*	*NR*	*75*	*0.0001-2*	*34*	*0.001-1*	*NR*	*NR*	*13*	*NR*	*3*	*0.003-0.1*
Exposure type												
Eye area	NR	NR	11	0.0002-9	21	0.1-2	1	NR	NR	NR	2	NR
Possible ingestion	NR	NR	33	37	49	7	NR	NR	NR	NR	NR	0.3
Inhalation	NR	NR	2	0.4	NR	NR	NR	NR	NR	NR	NR	NR
Dermal contact	1	NR	417	0.000002-37	141	0.00005-7	17	0.2	NR	NR	17	0.002-2
Deodorant (underarm)	NR	NR	NR	0.001-1	NR	0.1	NR	NR	NR	NR	NR	NR
Hair—noncoloring	NR	NR	24	0.01-2	9	0.001-1	NR	NR	16	NR	4	0.01-0.1
Hair—coloring	NR	NR	NR	0.1	3	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	0.1-1	NR	NR	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	35	0.02-2	19	0.05-0.1	NR	NR	NR	NR	1	0.003-0.1
Bath products	NR	NR	4	0.1-1	4	NR	NR	NR	NR	NR	NR	NR
Baby products	NR	NR	8	0.01	NR	NR	NR	NR	NR	NR	NR	NR
	Vaccinium myrtillus seed oil		*Vaccinium oxycoccos* (cranberry) seed oil[c]		*Vaccinium vitis-idaea* seed oil		Vegetable (olus) oil		Hydrogenated vegetable oil		*Vitis vinifera* (grape) seed oil	
Totals	33	0.01-0.1	4	NS	9	NR	165	0.0005-31	457	0.0004-60	465	0.001-43
Duration of use												
Leave-on	*32*	*0.01-0.12*	*3*	*NS*	*9*	*NR*	*135*	*0.0005-11*	*439*	*0.0005-60*	*368*	*0.001-41*
Rinse-off	*1*	*NR*	*1*	*NS*	*NR*	*NR*	*30*	*0.002-31*	*18*	*0.0004-8*	*97*	*0.001-43*
Exposure type												
Eye area	NR	NR	NR	NS	NR	NR	11	0.01-11	102	0.008-49	14	0.01-5
Possible ingestion	29	0.01	NR	NS	NR	NR	74	0.03-11	216	0.8-60	34	0.03-7
Inhalation	NR	NR	NR	NS	NR	NR	1	0.0005-0.02	1	3	6	0.001-7
Dermal contact	33	0.01-0.1	4	NS	1	NR	143	0.0005-31	450	0.005-60	401	0.001-41
Deodorant (underarm)	NR	NR	NR	NS	NR	NR	NR	–	NR	NR	NR	0.001-0.2
Hair—noncoloring	NR	NR	NR	NS	NR	NR	2	0.02-2	2	0.0005-0.09	46	0.01-0.3
Hair—coloring	NR	NR	NR	NS	NR	NR	18	–	NR	0.0004-1	10	43
Nail	NR	NR	NR	NS	8	NR	1	2	1	0.2	8	0.001-35
Mucous membrane	NR	NR	NR	NS	NR	NR	1	0.03-2	2	2-4	21	0.001-7
Bath products	NR	NR	NR	NS	NR	NR	2	0.002-0.02	NR	0.5	8	0.01-2
Baby products	NR	NR	NR	NS	NR	NR	1	–	NR	NR	5	NR

(continued)

Table 5. (continued)

	No. of uses	Conc. of use (%)	No. of uses	Conc. of use (%)
	Hydrogenated grapeseed oil		Sodium grapeseedate	
Totals	7	0.3-0.5	4	NR
Duration of use				
Leave-on	4	0.3-0.5	4	NR
Rinse-off	3	0.5	NR	NR
Exposure type				
Eye area	NR	NR	NR	NR
Possible ingestion	1	0.5	NR	NR
Inhalation	NR	NR	NR	NR
Dermal contact	5	0.5	NR	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair—noncoloring	1	NR	4	NR
Hair—coloring	NR	NR	NR	NR
Nail	1	0.3	NR	NR
Mucous membrane	1	NR	NR	NR
Bath products	NR	NR	NR	NR
Baby products	NR	NR	NR	NR

Abbreviations: NR, not reported to the Voluntary Cosmetic Registration Program (VCRP) or Personal Care Products Council; NS, not surveyed.
[a]Ingredients not previously reviewed by the Cosmetic Ingredient Review (CIR).
[b]Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[c]Not listed as an International Nomenclature Cosmetic Ingredient (INCI) name; included because of similarity.

Table 6. Current and Historical Frequency and Concentration of Use According to Duration and Type of Exposure—Previously Reviewed Ingredients.[a]

	No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)	
	Arachis hypogaea (peanut) oil				Hydrogenated peanut oil				*Carthamus tinctorius* (safflower) seed oil				*Cocos nucifera* (coconut) oil			
Data year	1998	2010	1984	2010	1998	2010	1998	2010	2002	2010	2003	2010	2007	2010	2008	2010
Totals[b]	22	74	Mostly ≤25; >50 (1 use)	0.0001-30	19	12	[c]	2-5	142	508	0.00005-84	NS	626	798	0.0001-80	NS
Duration of use																
Leave-on	14	59	[c]	0.0001-1	19	12	[c]	2-5	114	402	0.00005-84	NS	243	409	0.005-80	NS
Rinse-off	8	15	[c]	0.0002-30	NR	NR	[c]	NR	28	106	0.001-72	NS	383	389	0.0001-16	NS
Exposure type																NS
Eye area	NR	4	[c]	NR	NR	NR	[c]	NR	5	15	1-6	NS	7	25	0.01-80	NS
Possible ingestion	3	NR	[c]	NR	NR	NR	[c]	2	18	83	0.1-60	NS	19	44	0.2-51	NS
Inhalation	NR	2	[c]	NR	NR	NR	[c]	NR	3	5	5	NS	7	10	0.01-26	NS
Dermal contact	19	53	[c]	0.0001-1	19	12	[c]	2-5	113	395	0.001-72	NS	380	548	0.005-80	NS
Deodorant (underarm)	NR	NR	[c]	NR	NR	NR	[c]	NR	NR	NR	NR	NS	NR	NR	0.1-16	NS
Hair—noncoloring	3	21	[c]	25-30	NR	NR	[c]	NR	28	79	0.00005-27	NS	97	176	0.0001-13	NS
Hair—coloring	NR	NR	[c]	NR	NR	NR	[c]	NR	NR	20	1	NS	145	69	NR	NS
Nail	NR	NR	[c]	NR	NR	NR	[c]	NR	1	32	84	NS	2	5	0.005-2	NS
Mucous membrane	4	2	[c]	NR	NR	NR	[c]	NR	NR	31	NR	NS	12	161	0.0005-16	NS
Bath products	NR	NR	[c]	NR	NR	NR	[c]	NR	NR	3	7	NS	141	15	0.004-23	NS
Baby products	NR	NR	[c]	NR	NR	NR	[c]	NR	NR	6	10	NS	12	15	0.010-0.3	NS
	Hydrogenated coconut oil				Magnesium cocoate				Potassium cocoate				Sodium cocoate			
Data year	2007	2010	2008	2010	2007	2010	2008	2010	2007	2010	2008	2010	2007	2010	2008	2010
Totals[b]	62	105	0.001-50	NS	11	9	NR	NS	24	40	0.003-40	NS	230	340	1-52	NS
Duration of use																
Leave-on	55	79	0.001-50	NS	NR	NR	NR	NS	4	NR	28	NS	12	16	NR	NS
Rinse-off	7	26	0.001-38	NS	11	9	NR	NS	20	40	0.03-40	NS	218	324	1-52	NS
Exposure type																
Eye area	9	7	0.2-22	NS	NR	NR	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS
Possible ingestion	6	10	0.7-29	NS	NR	NR	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS
Inhalation	NR	NR	0.3	NS	NR	NR	NR	NS	NR	NR	NR	NS	1	NR	NR	NS
Dermal contact	3	102	0.001-25	NS	11	9	NR	NS	22	38	0.3-40	NS	175	269	1-52	NS
Deodorant (underarm)	NR	NR	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS
Hair—noncoloring	3	3	0.001-2	NS	NR	NR	NR	NS	2	2	15	NS	55	71	2	NS
Hair—coloring	NR	NR	0.5-0.6	NS	NR	NR	NR	NS	NR	NR	0.003	NS	NR	NR	NR	NS
Nail	NR	NR	0.8-25	NS	NR	NR	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS
Mucous membrane	NR	18	1-17	NS	NR	NR	NR	NS	NR	8	0.3	NS	1	238	1-2	NS
Bath products	1	NR	0.5-39	NS	NR	NR	NR	NS	11	NR	0.3-40	NS	149	3	1-52	NS
Baby products	1	1	2-50	NS	NR	NR	NR	NS	NR	NR	NR	NS	2	5	NR	NS

(continued)

Table 6. (continued)

	No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)	
	Coconut acid				Hydrogenated coconut acid				*Corylus americana* (hazel) seed oil				*Corylus avellana* (hazel) seed oil			
Data year	2007	2010	2008	2010	2007	2010	2008	2010	1998[d]	2010	1998	2010	1998[d]	2010	1997	2010
Totals[b]	142	141	0.03-14	NS	NR	NR	6-10	NS	[d]	10	[c]	NR	85	150	≤100	0.005-98
Duration of use																
Leave-on	18	17	NR	NS	NR	NR	6	NS	[d]	9	[c]	NR	74	131	[c]	0.005-98
Rinse-off	124	124	0.03-14	NS	NR	NR	10	NS	[d]	1	[c]	NR	11	19	[c]	0.005-5
Exposure type				NS				NS								
Eye area	1	1	NR	NS	NR	NR	NR	NS	[d]	NR	[c]	NR	2	9	[c]	0.1
Possible ingestion	NR	NR	NR	NS	NR	NR	NR	NS	[d]	NR	[c]	NR	NR	NR	[c]	14
Inhalation	NR	NR	NR	NS	NR	NR	NR	NS	[d]	NR	[c]	NR	NR	2	[c]	NR
Dermal contact	140	140	0.04-14	NS	NR	NR	6-10	NS	[d]	10	[c]	NR	83	147	[c]	0.005-98
Deodorant (underarm)	NR	NR	NR	NS	NR	NR	NR	NS	[d]	NR	[c]	NR	NR	NR	[c]	NR
Hair—noncoloring	2	1	0.03-0.3	NS	NR	NR	NR	NS	[d]	NR	[c]	NR	1	2	[c]	NR
Hair—coloring	NR	NR	NR	NS	NR	NR	NR	NS	[d]	NR	[c]	NR	NR	NR	[c]	NR
Nail	NR	NR	NR	NS	NR	NR	NR	NS	[d]	NR	[c]	NR	1	1	[c]	NR
Mucous membrane	1	101	0.04-2	NS	NR	NR	NR	NS	[d]	1	[c]	NR	4	1	[c]	NR
Bath products	93	NR	0.04-14	NS	NR	NR	NR	NS	[d]	NR	[c]	NR	2	2	[c]	NR
Baby products	1	1	NR	NS	NR	NR	NR	NS	[d]	NR	[c]	NR	NR	1	[c]	NR
	Elaeis guineensis (palm) oil				*Elaeis guineensis* (palm) kernel oil				Hydrogenated palm kernel oil				Hydrogenated palm oil			
Data year	1997	2010	1997	2010	1997	2010	1997	2010	1997	2010	1997	2010	1997	2010	1997	2010
Totals[b]	36	272	[c]	0.002-48	11	77	[c]	0.05-23	29	47	[c]	0.4-13	13	152	[c]	0.2-30
Duration of use																
Leave-on	28	171	[c]	0.008-13	9	60	[c]	0.8-3	27	45	[c]	0.4-13	13	134	[c]	0.2-30
Rinse-off	8	101	[c]	0.002-48	2	17	[c]	0.05-23	2	2	[c]	0.6-2	NR	18	[c]	2
Exposure type																
Eye area	NR	12	[c]	0.04-2	NR	10	[c]	0.8	5	2	[c]	2-10	1	61	[c]	0.2-30
Possible ingestion	NR	11	[c]	2	NR	6	[c]	NR	2	5	[c]	3-13	3	12	[c]	2-30
Inhalation	1	3	[c]	NR	NR	NR	[c]	NR	NR	1	[c]	NR	NR	NR	[c]	1
Dermal contact	36	229	[c]	0.002-48	11	71	[c]	0.05-2	24	47	[c]	0.4-13	12	123	[c]	0.4-30
Deodorant (underarm)	NR	NR	[c]	NR	NR	NR	[c]	NR	NR	NR	[c]	NR	NR	NR	[c]	NR
Hair—noncoloring	NR	43	[c]	2-34	NR	6	[c]	0.9-23	NR	NR	[c]	NR	NR	NR	[c]	NR
Hair—coloring	NR	NR	[c]	NR	NR	NR	[c]	NR	NR	NR	[c]	NR	NR	NR	[c]	NR
Nail	NR	NR	[c]	NR	NR	NR	[c]	3	NR	NR	[c]	NR	NR	NR	[c]	NR
Mucous membrane	7	68	[c]	0.002-48	NR	10	[c]	0.05	2	2	[c]	0.9-2	NR	17	[c]	2
Bath products	NR	NR	[c]	NR	NR	1	[c]	NR	NR	NR	[c]	NR	NR	NR	[c]	NR
Baby products	1	2	[c]	NR	NR	NR	[c]	NR	NR	NR	[c]	NR	NR	NR	[c]	NR

(continued)

Table 6. (continued)

	No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)	
	Gossypium herbaceum (cotton) seed oil				Hydrogenated cottonseed oil				*Oryza sativa* (rice) bran oil				*Oryza sativa* (rice) germ oil			
Data year	1998	2010	1998	2010	1998	2010	1998	2010	2002	2010	2000-2003	2010	2002	2010	2000-2003	2010
Totals[b]	4	83		0.004-32	272	362	[c]	0.001-24	39	371	0.1-39	0.0003-78	6	34	0.1	0.003-3
Duration of use																
Leave-on	1	68		0.08-32	272	358	[c]	0.001-24	32	267	0.1-8	0.0003-78	5	29	0.1	0.003-3
Rinse-off	3	15	[c]	0.004-29	NR	4	[c]	0.01-0.1	7	104	0.2-39	0.005-6	1	5	NR	0.003-3
Exposure type																
Eye area	NR	4		0.1-11	116	155	[c]	0.5-24	NR	5	0.1-1	0.5-0.8	NR	2	NR	0.01-1
Possible ingestion	NR	9	[c]	0.2-1	151	NR	[c]	8-12	NR	17	0.1-1	0.1-8	NR	4	NR	0.1-3
Inhalation	NR	12	[c]	0.2	NR	NR	[c]	NR	NR	11	NR	0.1	NR	NR	NR	NR
Dermal contact	4	78	[b]	0.004-29	156	356	[c]	0.001-24	36	321	0.1-39	0.0003-27	6	32	0.1	0.003-3
Deodorant (underarm)	NR	1	[c]	0.2	NR	NR	[c]	NR	NR	NR	NR	0.5	NR	NR	NR	0.003
Hair—noncoloring	NR	2	[c]	NR	NR	4	[c]	0.01-0.1	3	42	0.3	0.005-0.5	NR	NR	NR	NR
Hair—coloring	NR	NR	[c]	NR	NR	NR	[c]	NR	NR	NR	NR	0.3	NR	NR	NR	NR
Nail	NR	1	[c]	0.5-32	NR	NR	[c]	NR	2	5	NR	0.02-78	NR	NR	NR	NR
Mucous membrane	NR	7	[c]	0.004-0.01	NR	NR	[c]	NR	NR	48	1	0.0006-6	NR	1	NR	0.003-0.005
Bath products	NR	NR	[c]	NR	NR	NR	[c]	NR	1	17	1-39	0.2	NR	1	NR	0.5
Baby products	NR	NR	[c]	NR	NR	8	[c]	NR	NR	1	NR	NR	NR	NR	NR	NR
	Persea gratissima (avocado) oil				Prunus amygdalus dulcis (sweet almond) oil				*Sesamum indicum* (sesame) seed oil				*Sesamum indicum* (sesame) oil unsaponifiables			
Data year	2001	2010	2001	2010	2002	2010	2002	2010	2009	2010	2008	2010	2009	2010	2008	2010
Totals[b]	188	883	0.001-23	0.0001-98	375	1127	0.004-76	0.0001-77	402	480	0.0001-73	NS	6	17	0.01-0.03	NS
Duration of use																
Leave-on	40	657	0.001-23	0.0005-98	302	791	0.004-76	0.001-77	313	374	0.0001-73	NS	NR	17	0.01-0.03	NS
Rinse-off	148	226	0.1-5	0.0001-15	73	336	0.01-2	0.0001-43	89	106	0.001-68	NS	NR	NR	NR	NS
Exposure type																
Eye area	8	24	0.1-3	0.05-2	6	28	0.4	0.1-22	11	14	0.0008-10	NS	NR	NR	0.01	NS
Possible ingestion	29	60	0.7-21	0.05-26	3	55	0.5	0.1-19	57	52	0.1-16	NS	NR	11	0.03	NS
Inhalation	2	11	0.02-3	0.01-8	3	18	1-3	0.5-39	5	5	2	NS	NR	NR	NR	NS
Dermal contact	165	685	0.001-23	0.0005-98	323	986	0.04-11	0.001-46	346	414	0.0008-73	NS	6	17	0.01-0.03	NS
Deodorant (underarm)	NR	NR	NR	0.1	NR	2	0.004	0.02-1	NR	NR	NR	NS	NR	NR	NR	NS
Hair—noncoloring	11	189	0.002-3	0.0001-41	46	116	0.3-3	0.001-19	50	59	0.0001-30[e]	NS	NR	NR	NR	NS
Hair—coloring	8	NR	NR	0.3	2	2	0.1	0.02	NR	NR	0.03-0.8[f]	NS	NR	NR	NR	NS
Nail	4	7	0.4-19	0.001-34	4	13	1-76	0.001-77	6	7	≤1-10	NS	NR	NR	NR	NS
Mucous membrane	NR	43	0.1-5	0.002-3	19	93	0.5	<0.1-23	4	28	NR	NS	NR	NR	NR	NS
Bath products	5	25	0.1-5	0.6-6	10	41	0.01-0.1	0.1-43	27	5	0.09-68	NS	NR	NR	NR	NS
Baby products	NR	9	NR	NR	7	14	NR	2-3	1	3	6	NS	NR	NR	NR	NS

(continued)

Table 6. (continued)

	No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)		No. of uses		Conc. of use (%)	
	Triticum vulgare (wheat) germ oil				*Zea mays* (corn) oil				*Zea mays* (corn) oil unsaponifiables				*Zea mays* (corn) germ oil			
Data year	2001	2010	2001	2010	2007	2010	2006	2010	2007	2010	2006	2010	2007	2010	2006	2010
Totals[b]	303	527	0.00002-18	0.0001-28	498	598	0.00003-14	NS	7	1	NR	NS	37	53	0.2-25	NS
Duration of use																
Leave-on	80	373	0.00002-18	0.0001-28	241	361	0.00003-14	NS	6	1	NR	NS	25	34	3-25	NS
Rinse-off	223	154	0.00002-5	0.001-2	257	237	0.001-0.07	NS	1	NR	NR	NS	12	19	0.2-3	NS
Exposure type																
Eye area	9	12	0.00004-3	0.0001-0.5	39	35	0.0008-0.2	NS	NR	NR	NR	NS	NR	NR	NR	NS
Possible ingestion	33	29	0.1-3	0.3-5	29	30	0.003-10	NS	NR	NR	NR	NS	NR	NR	NR	NS
Inhalation	2	7	0.0002-0.01	0.0001-0.0005	1	1	0.001-0.1	NS	NR	NR	NR	NS	NR	NR	NR	NS
Dermal contact	220	360	0.00002-18	0.0005-23	276	371	0.00003-14	NS	7	1	NR	NS	31	50	3-25	NS
Deodorant (underarm)	NR	NR	0.02	NR	1	4	NR	NS	NR	NR	NR	NS	NR	NR	NR	NS
Hair—noncoloring	63	142	0.0001-2	0.0001-<1	38	40	0.0001-0.02	NS	NR	NR	NR	NS	4	3	0.2	NS
Hair—coloring	12	20	0.1	0.01-0.2	182	183	0.004-0.007	NS	NR	NR	NR	NS	NR	NR	NR	NS
Nail	4	2	0.1-4	0.1-28	1	3	0.001-5	NS	NR	NR	NR	NS	NR	NR	NR	NS
Mucous membrane	3	22	0.02-1	0.01-0.5	2	2	0.004-0.01	NS	NR	NR	NR	NS	4	3	3	NS
Bath products	1	2	0.001-2	0.5	NR	NR	0.001-0.01	NS	NR	NR	NR	NS	3	4	NR	NS
Baby products	1	9	0.5	NR	8	8	0.004	NS	NR	NR	NR	NS	2	4	NR	NS

Abbreviations: NR, not reported to the Voluntary Cosmetic Registration Program (VCRP) or the Council; NS, not surveyed.
[a]Ingredients that were recently reviewed were not resurveyed for concentration of use.
[b]Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[c]Concentration of use data were not given in the original report.
[d]Was not distinguished whether *C americana* or *C avellana* was reported; arbitrarily reported under *C Avellana* (hazel) seed oil for this table.
[e]15% after dilution.
[f]0.4 after dilution.

Table 7. Ingredients With No Reported Use Concentrations or Uses.

Adansonia digitata seed oil
Aleurites moluccanus bakoly seed oil
Amaranthus hypochondriacus seed oil
Arctium lappa seed oil
Babassu acid
Bassia butyracea seed butter
Brassica campestris (rapeseed) oil unsaponifiables
Brassica napus seed oil
Brassica oleracea acephala seed oil
Canarium indicum seed oil
Carya illinoensis (pecan) seed oil
Citrus aurantifolia (lime) seed oil
Citrus aurantifolia (lime) seed oil unsaponifiables
Citrus aurantium dulcis (orange) seed oil
Citrus aurantium dulcis (orange) seed oil insaponifiables
Citrus grandis (grapefruit) seed oil
Citrus grandis (grapefruit) seed oil unsaponifiables
Cocos nucifera (coconut) seed butter
Coix lacryma-jobi (Job's tears) seed oil
Corn acid
Cottonseed acid
Cynara cardunculus seed oil
Elaeis (palm) fruit oil
Elaeis guineensis (palm) butter
Fragaria ananassa (strawberry) seed oil
Fragaria chiloensis (strawberry) seed oil
Fragaria vesca (strawberry) seed oil
Fragaria virginiana (strawberry) seed oil
Guizotia abyssinica seed oil
Hippophae rhamnoides seed oil
Hydrogenated *Adansonia digitata* seed oil
Hydrogenated apricot kernel oil unsaponifiables
Hydrogenated *Argania spinosa* kernel oil
Hydrogenated blackcurrant seed oil
Hydrogenated *Camelina sativa* seed oil
Hydrogenated cranberry seed oil
Hydrogenated grapefruit seed oil
Hydrogenated grapefruit seed oil unsaponifiables
Hydrogenated hazelnut oil
Hydrogenated kukui nut oil
Hydrogenated lime seed oil
Hydrogenated lime seed oil unsaponifiables
Hydrogenated macadamia seed oil
Hydrogenated meadowfoam seed oil
Hydrogenated orange seed oil
Hydrogenated orange seed oil unsaponifiables
Hydrogenated palm acid
Hydrogenated *Passiflora edulis* seed oil
Hydrogenated peach kernel oil
Hydrogenated pistachio seed oil
Hydrogenated pumpkin seed oil
Hydrogenated *Punica granatum* seed oil
Hydrogenated raspberry seed oil
Hydrogenated rice bran oil
Hydrogenated *Rosa canina* fruit oil
Hydrogenated safflower seed oil
Hydrogenated sesame seed oil
Hydrogenated sweet almond oil unsaponifiables
Hydrogenated wheat germ oil
Hydrogenated wheat germ oil unsaponifiables
Lupinus albus oil unsaponifiables
Morinda citrifolia seed oil
Olea europaea (olive) husk oil
Olive acid
Oryza sativa (rice) seed oil
Peanut acid
Potassium babassuate
Potassium cornate
Potassium hydrogenated cocoate
Potassium hydrogenated palmate
Potassium peanutate
Potassium rapeseedate
Potassium safflowerate
Potassium soyate
Prunus amygdalus dulcis (sweet almond) oil unsaponifiables
Prunus armeniaca (apricot) kernel oil unsaponifiables
Rapeseed acid
Ribes rubrum (currant) seed oil
Rice bran acid
Safflower acid
Sesamum indicum (sesame) seed butter
Sodium cocoa butterate
Sodium hydrogenated cocoate
Sodium hydrogenated palmate
Sodium macadamiaseedate
Sodium peanutate
Sodium rapeseedate
Sodium safflowerate
Sodium sesameseedate
Sodium soyate
Sodium *Theobroma grandiflorum* seedate
Soy acid
Sunflower seed acid
Torreya nucifera seed oil
Triticum aestivum (wheat) germ oil
Triticum vulgare (wheat) germ oil unsaponifiables
Vaccinium corymbosum (blueberry) seed oil

Skin papillomas greater than 1 mm in diameter at the application sites were recorded weekly and included in the data analysis if they persisted for more than 2 weeks. The positive control group yielded expected results (86% tumor incidence). No tumors were observed in the vehicle control or the other test groups. The authors concluded that cashew nut kernel oil did not exhibit any solitary carcinogenic activity.

Dermal Irritation and Sensitization Studies

Nonhuman

Dermal irritation and sensitization nonhuman studies, including photosensitization and comedogenicity studies, are summarized in Tables 9 and 10. Undiluted, technical grade, *A hypogaea* (peanut) oil was moderately irritating to rabbits and

Table 8. Examples of Noncosmetic Uses of Oils.

Oil	Use[3,64,105,180,189,198–200]
Aleurites moluccanus seed oil (kukui)	Wood preservative, varnishes, paint oil, illumination, soap making, waterproofing paper, rubber substitute, insulating material
Arachis hypogaea (peanut) oil	Pharmaceutical, soap making, lubricants, emulsions for insect control, diesel engine fuel
Brassica napus seed oil (rapeseed)/canola oil	Rubber additive, lubricants, fat liquoring of leather, varnishes and lacquers, textile chemicals, detergent additives, plasticizers, weed control
Butyrospermum parkii (shea) oil	Illumination
Camelina sativa seed oil (false flax)	Drying oil, manufacturing of varnishes and paints
Citrullus lanatus (watermelon) seed oil	Illumination
Cocos nucifera (coconut) oil	Lubricants, hydraulic fluid, paints, synthetic rubber, plastics, illumination
Elaeis guineensis (palm) oil	Crayon and candle manufacturing, tin plate industry
Elaeis guineensis (palm) kernel oil	Detergent production, pharmaceutical, crayon and candle manufacturing, tin plate industry
Garcinia indica seed butter (kokum)	Candle and soap making, sizing of cotton yarn, pharmaceutical
Guizotia abyssinica seed oil (Niger/Ramtil)	Paint, lubricant, pharmaceutical
Helianthus annuus (sunflower) seed oil	Manufacturing of lacquers, copolymers, polyester films, modified resins, plasticizers, alkyl resins, other similar products
Juglans regia (walnut) seed oil	Paints, soap making
Linum usitatissimum (linseed) seed oil	Manufacturing of linoleum, cloth oil, printing and lithographic inks, core oils, linings, packings, oil-modified alkyd resins, caulking compounds, putties, leather-finishing compounds, lubricants, greases, polishes, pyrotechnic compositions, pigment binder in petrochemicals, concrete protector, stabilizer/plasticizer for vinyl plastics, industrial stains, jute textiles, drying oil in paints and varnishes
Mangifera indica (mango) seed butter	Substitute for cocoa butter
Olea europaea (olive) fruit oil	Textile industry, pharmaceutical
Orbignya cohune seed oil	Manufacturing of soaps, candles, nightlights, cotton dyeing, ointment base, substitute for cocoa butter in food
Perilla ocymoides seed oil (perilla)	Substitute for linseed oil in the manufacture of paints, varnishes, linoleum, oilclothes, and printing inks
Prunus amygdalus dulcis (sweet almond) oil	Pharmaceutical, energy source
Prunus armeniaca (apricot) kernel oil	Pharmaceutical
Theobroma cacao (cocoa) seed butter	Pharmaceutical
Vitis vinifera (grape) seed oil	Substitute for linseed oil in the manufacture of paints, and varnishes

Table 9. Dermal Effects—Nonhuman Studies.

Ingredient	Concentration	Animals	Procedure	Results	Reference
Dermal irritation and sensitization					
Adansonia digitata seed oil					
Adansonia digitata (baobab) oil	100%		MatTek EpiDerm MTT viability assay; 100 μL of test material for 1-24 hours	Classified as nonirritating	[201]
Arachis hypogaea (peanut) oil					
Arachis hypogaea (peanut) oil		Hartley and/or Himalayan guinea pigs	Single drops of a store-bought peanut oil were applied to clipped skin on the backs of 4 guinea pigs. Applications were made at 2- to 6-week intervals, for a total of 7 applications over a 5-month period. It appears that the test sites were not covered. The test sites were scored 24 hours after application. Well-defined erythema was considered a positive reaction	None of the animals had a positive reaction following the initial application. Two animals had positive reactions following application at weeks 6 and 12, while 1 animal had a positive reaction following dosing at week 12 only	[14]

(continued)

Table 9. (continued)

Ingredient	Concentration	Animals	Procedure	Results	Reference
			Butyrospermum parkii (shea) butter		
Butyrospermum parkii (shea) butter	Not specified	3 male New Zealand white (NZW) rabbits	0.5 mL applied to the shaved dorso-lumbar region under an occlusive patch for 4 hours	Very slight erythema with or without edema was observed in 2 rabbits; resolved by day 3 or 4	202
Butyrospermum parkii (shea) butter	Induction: 75%; challenge: 20% and 50%	10 female albino Hartley/Dunkin guinea pigs	Maximization study with Freund's complete adjuvant (FCA) during induction	No evidence of delayed hypersensitivity	203
			Crambe abyssinica seed oil		
Crambe abyssinica seed oil	Undiluted		Dermal irritation study; details not provided	Not a dermal irritant	204
			Hippophae rhamnoides seed oil		
Hippophae rhamnoides seed oil		Albino rabbits, number not specified	0.5 mL applied under an occlusive patch for 4 hours	No irritation	205
			Olea europaea (olive) fruit oil		
Olea europaea (olive) fruit oil		12 Harley and/or Himalayan guinea pigs	Single drops of a USP-grade olive oil that had been stored in its original metal container for 10 years were applied to a clipped area on the backs of 12 guinea pigs. (The composition of the oil was not determined.) Applications were made at 2- to 6-week intervals over a period of 5 months. Four guinea pigs were treated similarly using store-bought virgin olive oil	None of the animals had a positive reaction following the initial application of either oil. With 10-year-old olive oil, 11 of 12 of the animals had a positive reaction at some point. Some, but not all, of these guinea pigs reacted consistently following the first positive reaction; 2 animals had only 1 positive reaction; 2 guinea pigs in this group died by week 16. In the group dosed with virgin olive oil, 1 animal had a positive reaction at week 2 and 1 animal had a positive reaction at weeks 4 and 6	206
		22 guinea pigs sensitive to the 10-year-old USP olive oil	Cross-reactivity to store-bought olive oil, another store-bought olive oil (not specified as virgin olive oil), corn oil, and peanut oil was determined. The 5 oils were applied simultaneously to the backs of the guinea pigs	18 of the animals reacted to the virgin olive oil, and 18 reacted to the other store-bought olive oil. (Overlap of these animals was not complete.) Cross-reactivity to corn or peanut oil was not observed	
		8 sensitized and 4 nonsensitized guinea pigs	Single drops of the unsaponifiable fraction of the 10-year-old oil were applied	All of the sensitized animals reacted to the unsaponifiable fraction, while the nonsensitized animals did not	
			Zea mays (corn) oil		
Corn oil, store-bought		6 Hartley and/or Himalayan guinea pigs	Sensitization study, details not specified	0 of the animals had a positive reaction following the initial application; 2 animals had positive reactions following application at weeks 4 and 6, while 1 animal had a positive reaction following application at week 12	206
			Phototoxicity		
			Butyrospermum parkii (shea) butter		
Butyrospermum parkii (shea) butter	10% and 20% in acetone	10 Pirbright white guinea pigs	Animals were treated with test compound, then irradiated with UV-B light for 80 seconds followed by UV-A light for 80 minutes	Not phototoxic	207

Table 10. Dermal Effects—Nonhuman Studies—Summarized From Previous CIR reports.

Procedure and results	Reference
Dermal irritation and sensitization	
Arachis hypogaea (peanut) oil	
Undiluted technical grade *Arachis hypogaea* (peanut) oil was moderately irritating to rabbits and guinea pig skin and mildly irritating to rat skin following exposure; there was no indication that the test site was occluded. However, in a 48-hour occlusive patch test using miniature swine, technical grade *Arachis hypogaea* (peanut) oil was not irritating	14
Carthamus tinctorius (safflower) oil	
Undiluted *Carthamus tinctorius* (safflower) seed oil was minimally irritating in a repeat open patch test using rabbits and was not a primary irritant or sensitizer in a maximization study using guinea pigs	28
Cocos nucifera (coconut) oil	
Undiluted *Cocos nucifera* (coconut) oil was nonirritating to rabbit skin. In guinea pigs, undiluted *Cocos nucifera* (coconut) oil was not a sensitizer in a Magnusson-Kligman maximization study	29
Hydrogenated coconut oil	
Undiluted hydrogenated coconut oil was nonirritating to rabbit skin. In guinea pigs, undiluted hydrogenated coconut oil was not a sensitizer in a Buehler test	29
Coconut acid	
Undiluted coconut acid was minimally irritating to rabbit skin	29
Sodium cocoate	
In single-insult occlusive patch tests of a 5% aqueous solution of a bar soap containing 13% sodium cocoate, scores of 1.6 to 4.0/8.0 were reported	29
Elaeis guineensis (palm) oil	
Undiluted *Elaeis guineensis* (palm) oil was practically nonirritating to minimally irritating to rabbit skin. *Elaeis guineensis* (palm) oil, 5%, was nonallergenic in a maximization study	23
Gossypium herbaceum (cotton) seed oil	
Cosmetic formulations containing 3.4% to 8.97% hydrogenated cottonseed oil were not irritating to rabbit skin	24
Oryza sativa (rice) bran oil	
Undiluted *Oryza sativa* (rice) bran oil was not irritating to rabbits, and in a guinea pig maximization study, no reactions were observed when 5% was used at induction and 25% and 50% *Oryza sativa* (rice) bran oil were used at challenge. An *Oryza sativa* (rice) bran oil/ *Oryza sativa* (rice) germ oil mixture, concentrations not stated, did not cause a contact allergy response. Undiluted hydrolyzed rice protein was also not irritating or sensitizing	25
Oryza sativa (rice) germ oil	
Oryza sativa (rice) germ oil was not a primary dermal irritant	25
Prunus amygdalus dulcis (sweet almond) oil	
Undiluted prunus amygdalus dulcis (sweet almond) oil and 2 moisturizer formulations, each containing 25% prunus amygdalus dulcis (sweet almond) oil, were tested for skin irritancy in rabbits using occlusive patches. Undiluted prunus amygdalus dulcis (sweet almond) oil was nonirritating (primary irritation index, PII = 0/4). The formulations containing 25% prunus amygdalus dulcis (sweet almond) oil were minimally irritating (PIIs = 0.28 and 0.72, respectively). In a 60-day cumulative irritation test, 10% and 100% prunus amygdalus dulcis (sweet almond) oil was applied to rabbits. When tested in 7 separate trials, 100% prunus amygdalus dulcis (sweet almond) oil produced mean maximum irritation indices (MMIIs) ranging from 0.34 to 1.34 (maximum score = 8). At a concentration of 10%, MMIIs for this ingredient ranged from 0 to 0.66. Results indicated that, when applied to the skin over a long period of time, prunus amygdalus dulcis (sweet almond) oil is slightly irritating; whereas at 10%, it is practically nonirritating A maximization assay was used to determine the sensitizing potential of prunus amygdalus dulcis (sweet almond) oil using guinea pigs. Intradermal induction used concentrations of 5% amygdalus dulcis (sweet almond) oil, the dose-range phase of the experiment used a single dermal application of 5%, 10%, or 100% Prunus amygdalus dulcis (sweet almond) oil, a booster induction injection of 100% Prunus amygdalus dulcis (sweet almond) oil was applied occlusively for 48 hours 1 week later, challenge was with 5% prunus amygdalus dulcis (sweet almond) oil in petrolatum applied topically under occlusion for 24 hours. Prunus amygdalus dulcis (sweet almond) oil was nonsensitizing Undiluted prunus amygdalus dulcis (sweet almond) oil was tested for irritancy in groups of 6 male albino rabbits. The test material was applied under occlusion to the clipped intact and abraded dorsal skin of each animal. Twenty-three hours later, patches were removed; sites were scored at 24 and 48 hours. The primary irritation indices (PIIs) for 7 test samples of prunus amygdalus dulcis (sweet almond) oil ranged from 0 to 0.18 (maximum score = 8), indicating that this ingredient is practically nonirritating to skin	208

(continued)

Table 10. (continued)

Procedure and results	Reference
Dermal irritation and sensitization	
Sesamum indicum (sesame) seed oil	
Undiluted *Sesamum indicum* (sesame) seed oil was nonirritating or minimally irritating to rabbit skin	48
Triticum vulgare (wheat) germ oil	
Triticum vulgare (wheat) germ oil, undiluted and at 2% in formulation, was nonirritating to mildly irritating, and undiluted *Triticum vulgare* (wheat) germ oil was not sensitizing to guinea pigs	26
Phototoxicity	
Elaeis guineensis (palm) oil	
A facial lotion containing 1.5% *Elaeis guineensis* (palm) oil was not phototoxic in the phototoxicity yeast assay	23
Oryza sativa (rice) bran oil	
Oryza sativa (rice) bran oil, tested undiluted during induction at 10% at challenge, was not a photosensitizer in guinea pigs	25
Oryza sativa (rice) germ oil	
Oryza sativa (rice) germ oil, $\leq$75%, was not phototoxic or photosensitizing	25
Comedogenicity	
Corylus avellana (hazel) seed oil	
A comedogenicity study was conducted in which 0.1 mL of *Corylus avellana* (hazel) seed oil (pH 6) was applied to the pinna of the ear of albino rabbits. No local irritation was noted at the application site. A "slight difference in the number and size of the pilosebaceous follicles" was noted via magnifying glass. A "slight excess of sebum and a dilation of the follicles" was noted upon microscopic examination of the treated areas	30

Abbreviation: CIR, Cosmetic Ingredient Review.

guinea pig skin, and 5% aqueous solutions of a bar soap containing 13% sodium cocoate had irritation scores of 1.6 to 4.0 of 8 in animal studies. However, the majority of the remaining animal irritation and/or sensitization studies conducted on a large number of the oils included in this report, primarily in formulation, did not report any significant irritation or sensitization reactions, indicating that refined oils derived from plants are not dermal irritants or sensitizers. None of the tested oils, including *B parkii* (shea) butter (up to 20%) and *Oryza sativa* (rice) germ oil ($\leq$ 75%), were phototoxic in animal studies. The comedogenicity of *C avellana* (hazel) seed oil was evaluated using rabbits, and a slight difference in the number and size of the pilosebaceous follicles and a slight excess of sebum and a dilation of the follicles were observed.

Human

Plant-derived fatty acid oils are commonly believed to be safe for use on the skin.[6] de Groot notes that no documentation exists to show that high-quality edible lipids cause adverse reactions in normal individuals (except for potential comedogenicity).[55] Very few reports of adverse reactions to cosmetic use of edible fatty acid oils have been reported.

Many plant-derived fatty acid oils are derived from foods that are recognized as potent food allergens. The allergic reactions are thought to be caused by the proteins present in the food. It has been shown that an individual who is allergic to a food will generally not react to the refined oil, especially if the oil has been "hot pressed" or has undergone more processing.[12,13] In its safety assessment on *A hypogaea* (peanut) oil, the Panel noted that while peanuts are extremely allergenic to a large population, reaction to the oil is rare. The major concern associated with allergic reactions to peanuts is the protein,[14] which does not partition into the refined oil; therefore, the oil is safe for use in cosmetics. Crevel et al also concluded that chemically refined peanut oil is safe for the majority of peanut allergic individuals.[13] They stated that "as peanut is acknowledged to be one of the most potent food allergens, it is reasonable to extrapolate the conclusions drawn up for peanut oil to other edible oils." However, they concede that validated analytical methodology for establishing the protein content of oil is needed.

In support of the conclusions stated earlier, Crevel et al also examined the allergenicity of some other oils. Very few instances of allergic reactions to other major edible fatty acid oils have been reported. Even sesame oil, which differs from the other oils, is used as a flavorant and, therefore, is not refined, is expected to contain significantly more protein than the other edible fatty acid oils, and has had very few reports of allergic reaction. Additional studies demonstrating safety are summarized later in this section.[15,56]

A large number of clinical irritation and sensitization studies were made available on many of the oils, primarily in formulation, and these studies are summarized in Table 11. All of the data indicated that the oils were not irritants or sensitizers. Summary statements of human dermal studies, including

Table 11. Dermal Effects—Human Studies.

Ingredient and concentration	Participants completed	Method	Results	Reference
Dermal irritation and sensitization				
		Adansonia digitata seed oil		
0.01% *Adansonia digitata* seed oil in a lip product	106	Human repeat insult patch test (HRIPT) with 0.2 g test material, semi-occluded	Not a dermal irritant or sensitizer	209
100% *Adansonia digitata* seed oil	107	HRIPT with 0.02-0.05 ml test material, semi-occluded	Not a dermal irritant or sensitizer	210
		Aleurites moluccana seed oil		
0.005% aleurites moluccana seed oil in scalp conditioner/hair wax	104	HRIPT; occlusive; applied neat	Not a dermal irritant or sensitizer	211
~3% in a skin cleanser	110	Modified HRIPT; semi-occlusive; 10% dilution in distilled water	Not a dermal irritant or sensitizer	212
		Arachis hypogaea (peanut) oil		
Dermatologic product containing 0.01% fluocinolone and refined *Arachis hypogaea* (peanut) oil	Peanut-sensitive participants; 8 children, 6 adults	Skin prick test with peanut extracts, a solution of 50% glycerin (negative control), a solution of 1.8 mg/mL histamine phosphate in 50% glycerin (positive control), the complete test product, vehicle only (without fluocinolone), and refined *Arachis hypogaea* (peanut) oil	1 child had a trace positive reaction	213
		Patch test with product, vehicle only, and refined *Arachis hypogaea* (peanut) oil	No reactions	
		Argania spinosa kernel oil		
5% *Argania spinosa* kernel oil in a face serum	108	Primary cutaneous irritation	No primary irritation	214
5% *Argania spinosa* kernel oil in a face serum	108	HRIPT; occlusive; applied neat	Not an irritant or a sensitizer	214
10% *Argania spinosa* kernel oil in a skin salve	209	HRIPT; occlusive; applied neat	Not a sensitizer	215
10% *Argania spinosa* kernel oil in a skin salve	51	4-week use test; applied to lips, hands/nails, elbows, knees, feet/heels	Did not elicit significant dermal irritation or dryness; 2 participants had level 1(mild, very slight erythema) on the lips, and 5 had level 1 erythema on the elbows, lips, or knees; 15 participants reported subjective irritation	216
		Astrocaryum murumuru		
1% *Astrocaryum murumuru* seed butter in a lipstick	97	HRIPT with 150 mg test material, semi-occluded	Not a dermal irritant or sensitizer	217
4% *Astrocaryum murumuru* seed butter in a lipstick	108	HRIPT, occluded	Not a dermal irritant or sensitizer	218
4% *Astrocaryum murumuru* seed butter in a lipstick	108	HRIPT, occluded	Not a dermal irritant or sensitizer	219
4% *Astrocaryum murumuru* seed butter in a lipstick	108	HRIPT, occluded	Not a dermal irritant or sensitizer	220
4% *Astrocaryum murumuru* seed butter in a lipstick	106	HRIPT, occluded	Not a dermal irritant or sensitizer	221
4% *Astrocaryum murumuru* seed butter in a lipstick	106	HRIPT, occluded	Not a dermal irritant or sensitizer	222
4% *Astrocaryum murumuru* seed butter in a lipstick	108	HRIPT, occluded	Not a dermal irritant or sensitizer	223

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
		Dermal irritation and sensitization		
		Avena sativa (oat) kernel oil		
3% *Avena sativa* (oat) kernel oil in a body and hand formulation	100	HRIPT with 0.2 mL, occluded	Not a dermal irritant or sensitizer	224
		Bassia latifolia seed butter		
2% *Bassia latifolia* seed butter in a body scrub	110	HRIPT with 1% aqueous solution of the formulation, semi-occluded	Not a dermal irritant or sensitizer	225
		Borago officinalis seed oil		
1% *Borago officinalis* seed oil in a body and hand formulation	213	HRIPT with 0.2 g, occluded	Not a dermal irritant or sensitizer	226
2% *Borago officinalis* seed oil in a face serum	108	primary cutaneous irritation	No primary irritation	214
2% *Borago officinalis* seed oil in a face serum	108	HRIPT; occlusive; applied neat	Not an irritant or a sensitizer	214
		Brassica campestris (rapeseed) oil		
5% Hydrogenated rapeseed oil in a baby oil	105	HRIPT with 0.2 mL, semi-occluded	Not a dermal irritant or sensitizer	227
		Brassica oleracea Italica (broccoli) seed oil		
0.5% *Brassica oleracea* Italica (broccoli) seed oil in a hair conditioner	102	HRIPT with 150 μL of test material, 10% dilution, semi-occluded	Not a dermal irritant or sensitizer	228
		Butyrospermum parkii (shea) butter		
Butyrospermum parkii (shea) butter and fractions of unsaponifiable lipids from *Butyrospermum parkii* (shea) butter; the "liquid" sample was obtained from a supplier; the unsaponifiable fraction was obtained through low temperature crystallization of the supplied sample	21	Single applications to normal skin and sodium lauryl sulfate (SLS)-irritated skin; right volar forearm was treated with 50 μL of each test material in 12-mm Finn chambers for 48 hours; the left volar forearm was treated with 50 μL of 14% aqueous SLS for 7 hours, rinsed, dried, and then treated with 50 μL of each test material for 17 hours; cutaneous blood flow (CBF) and transepidermal water loss (TEWL) were measured	Normal skin: barely perceptible erythema observed in a "small" number of participants at 24 hours after treatment with shea butter; no irritation to the shea unsaponifiable fraction; no significant difference in CBF or TEWL SLS-treated skin: 2 participants had a slight- and moderate reaction to the unsaponifiable fraction; no significant difference in CBF or TEWL	229
0.1% *Butyrospermum parkii* (shea) butter in a scalp conditioner	114	Primary cutaneous irritation; formulation diluted to 1%	No primary irritation	230
2% *Butyrospermum parkii* (shea) butter in a cream	119	Primary cutaneous irritation	No primary irritation	231
0.1% *Butyrospermum parkii* (shea) butter in a scalp conditioner	110	HRIPT; occlusive; formulation diluted to 1%	Not a dermal irritant or sensitizer	230
2% *Butyrospermum parkii* (shea) butter in a cream	118 (irritation)/ 116 (sensitization)	HRIPT; occlusive	Not a dermal irritant or sensitizer	231
4% *Butyrospermum parkii* (shea) butter in a face cream	51	HRIPT with 20 μL test material, occluded	Not a dermal irritant or sensitizer	232
4% *Butyrospermum parkii* (shea) butter in an eye cream	108	HRIPT with 20 μL test material, occluded	Not a dermal irritant or sensitizer	233
23.5% *Butyrospermum parkii* (shea) butter in a lip gloss	104	HRIPT	Not a dermal irritant or sensitizer	234

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
		Dermal irritation and sensitization		
23.7% *Butyrospermum parkii* (shea) butter in a lip gloss	104	HRIPT	Irritation on induction days 5 to 9 in 1 participant; no sensitization	235
24.1% *Butyrospermum parkii* (shea) butter in a lip wax	113	HRIPT	Not a dermal irritant or sensitizer	236
24.1% *Butyrospermum parkii* (shea) butter in a lip wax	2 runs	Episkin	Average viability 67.3%—no irritation potential	237
24.7% *Butyrospermum parkii* (shea) butter in a lip gloss	40	28-day use study, 2-6 times/day	1 participant with desquamation	238
45% *Butyrospermum parkii* (shea) butter in a body/hand massage	109[a]	HRIPT	Not a dermal irritant or sensitizer	239
45% *Butyrospermum parkii* (shea) butter in a body/hand massage	109[a]	HRIPT	Not a dermal irritant or sensitizer	240
45% *Butyrospermum parkii* (shea) butter in a body/hand massage	109[a]	HRIPT	Not a dermal irritant or sensitizer	241
45% *Butyrospermum parkii* (shea) butter in a body/hand massage	109[a]	HRIPT	Not a dermal irritant or sensitizer	242
45% *Butyrospermum parkii* (shea) butter in a body/hand massage	31	2-week use study, 2 times per day	No erythema, edema, or dryness	243
60% *Butyrospermum parkii* (shea) butter in a cuticle cream	111	HRIPT	Not a dermal irritant or sensitizer	244
		Camelina sativa seed oil		
0.25% *Camelina sativa* seed oil in a body powder	204	HRIPT with 0.1 g, semi-occluded	Not a dermal sensitizer	245
7% *Camelina sativa* seed oil in an oil treatment	103	HRIPT with 200 μL test material, semi-occluded	Grade 1 (mild erythema) reactions in 4 participants for 1 or 2 patches in the induction phase, grade 1 (mild erythema) in different participants at the 48-hour challenge reading. Study concluded test material was not a dermal irritant or sensitizer	246
		Camellia sinensis seed oil		
0.0985% *Camellia sinensis* seed oil in a lipstick	108	HRIPT with 0.2 g, occluded	Not a dermal irritant or sensitizer	247
0.0985% *Camellia sinensis* seed oil in a lipstick	108	HRIPT with 0.2 g, occluded	Not a dermal irritant or sensitizer	248
		Canola oil		
74.7% canola oil in a body oil	101	HRIPT with 150 μL test material, semi-occluded	Not a dermal irritant or sensitizer	249
		Carthamus tinctorius (safflower) oil		
5% *Carthamus tinctorius* (safflower) seed oil in a cleansing oil rinse-off	214	HRIPT with 0.2 mL of a 10% vol/vol aqueous solution, semi-occluded	3 participants had a "?" reaction following a patch during the induction and 1 participant had definite erythema with no edema or damage to the epidermis (+D) following the seventh patch. No reactions were observed at a new test site. No other reactions were observed. Study concluded test material was not a dermal sensitizer	250

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
		Dermal irritation and sensitization		
30% *Carthamus tinctorius* (safflower) seed oil in a massage oil	107	HRIPT with 0.2 mL test material, semi-occluded	1 participant had slight erythema following the seventh patch that did not reoccur, no other reactions observed. Not a dermal irritant or sensitizer	[251]
		Caryocar brasiliense fruit oil		
0.1% *Caryocar brasiliense* fruit oil in a lipstick	100	HRIPT with 200 mg test material, semi-occluded	Not a dermal irritant or sensitizer	[252]
		Chenopodium quinoa seed oil		
1% *Chenopodium quinoa* seed oil in a UV SPF cream	105	HRIPT with 0.02 mL test material, occluded	"An acceptable level of irritation" was observed in the induction phase consisting of grade 1 (mild erythema) in 39 participants, with 1 additional subject exhibiting a grade 2 (moderate erythema) reaction. No evidence of skin sensitization was observed	[253]
1% *Chenopodium quinoa* seed oil in a UV SPF cream	102	HRIPT with 0.02 mL test material, occluded	"An acceptable level of irritation" was observed in the induction phase, with 54% of the participants exhibiting a grade 1 (mild erythema) reaction and 3% of the participants exhibiting a grade 2 (moderate erythema) reaction. One participant had a strong reaction to the third induction patch and discontinued the induction phase after the sixth application. At challenge, the participant had only papules at 96 hours. Due to reactions to other materials tested at the same time, it could not be determined if the test material was the causative agent. No evidence of skin sensitization was observed in the remaining participants	[254]
		Citrullus lanatus (watermelon) seed oil		
2% *Citrullus lanatus* (watermelon) seed oil in a facial oil	105	HRIPT, semi-occluded	Not a dermal irritant or sensitizer	[255]
		Cocos nucifera (coconut) fruit oil		
0.15% *Cocos nucifera* (coconut) oil in a scalp conditioner/hair wax	104	HRIPT; occlusive; applied neat	Not a dermal irritant or sensitizer	[211]
31% *Cocos nucifera* (coconut) oil in a lip balm	222	HRIPT with 0.2 g test material, occluded	2 participants had low-level, transient (±) reactions during the induction, no other reactions were observed. Study concluded that test material was not a dermal sensitizer	[256]
		Corylus avellana (hazel seed) oil		
1% *Corylus avellana* (hazel) seed oil in a moisturizing cream	25	Amended Draize patch test, 10% standard concentration	Nonirritating	[257]
1% *Corylus avellana* (hazel) seed oil in a moisturizing cream	32	60 day clinical study	"Fairly good acceptability"	[258]

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
		Dermal irritation and sensitization		
5% *Corylus avellana* (hazel) seed oil in a massage oil	107	HRIPT with 0.2 mL test material, semi-occluded	1 participant had slight erythema following the seventh patch that did not reoccur, no other reactions observed. Not a dermal irritant or sensitizer	251
		Crambe abyssinica seed oil		
5% *Crambe abyssinica* seed oil in a face and neck product	54	HRIPT; semi-occluded, undiluted	Not a dermal irritant or sensitizer	259
100% *Crambe abyssinica* seed oil in an unspecified product	107	HRIPT; undiluted	Not a dermal irritant or sensitizer	204
		Elaeis guineensis (palm) oil		
15.7% sodum palm kernelate in a soap	42	28-day use test	Good acceptability for use	260
61.6% sodium palmate in a soap	42	28-day use test	Good acceptability for use	260
		Euterpe oleracea fruit oil		
0.5% *Euterpe oleracea* fruit oil in an eye treatment	104	HRIPT with 150 μL test material, semi-occluded	Not a dermal irritant or sensitizer	261
		Glycine soja (soybean) oil		
0.19% *Glycine soja* (soybean) unsaponifiables in a face and neck product	50	HRIPT, occluded	Not a dermal irritant or sensitizer	262
39% Hydrogenated soybean oil in a lipstick	108	HRIPT, occluded	Not a dermal irritant or sensitizer	263
		Garcinia indica seed butter		
0.3869% *Garcinia indica* seed butter in a body and hand product	101	HRIPT, 0.2 g applied, occlusive	Not a sensitizer; irritation was observed in 1 subject	264
		Gossypium herbaceum (cotton) seed oil		
3.6% Hydrogenated cottonseed oil in a lip balm	222	HRIPT with 0.2 g test material, occluded	2 participants had low-level, transient (±) reactions during the induction, no other reactions were observed. Study concluded that test material was not a dermal sensitizer	256
		Helianthus annuus (sunflower) seed oil		
6% *Helianthus annuus* (sunflower) seed oil in a skin cream	108	Primary cutaneous irritation	No primary irritation	265
20% *Helianthus annuus* (sunflower) seed oil in a face serum	108	Primary cutaneous irritation	No primary irritation	214
0.264% *Helianthus annuus* (sunflower) seed oil in a cream	57	HRIPT; Finn chambers, applied neat	Not a dermal irritant or sensitizer	266
6% *Helianthus annuus* (sunflower) seed oil in a skin cream	106	HRIPT, occlusive	Not a dermal irritant or sensitizer	265
20% *Helianthus annuus* (sunflower) seed oil in a face serum	108	HRIPT; occlusive; applied neat	Not an irritant or a sensitizer	214
1% *Helianthus annuus* (sunflower) seed oil in a soap	42	28-day use test	Good acceptability for use	260
39.8% *Helianthus annuus* (sunflower) seed oil in a massage oil	107	HRIPT with 0.2 mL test material, semi-occluded	1 participant had slight erythema following the seventh patch that did not reoccur, no other reactions observed. Not a dermal irritant or sensitizer	251

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
Dermal irritation and sensitization				
Helianthus annuus (sunflower) seed oil unsaponifiables				
2% *Helianthus annuus* (sunflower) seed oil unsaponifiables in a night product	100	HRIPT, semi-occluded	Not a dermal irritant or sensitizer	[262]
2% *Helianthus annuus* (sunflower) seed oil unsaponifiables in a face and neck product	100	HRIPT, semi-occluded	Not a dermal irritant or sensitizer	[262]
Hippophae rhamnoides seed oil				
5% *Hippophae rhamnoides* seed oil	10	Cutaneous local tolerance test, 0.02 mL single 48 hours occlusive application	Not an irritant; average irritation score of 0	[267]
Irvingia gabonensis kernel butter				
0.31% *Irvingia gabonensis* kernel butter in a face and neck product	52	HRIPT, occluded	Not a dermal irritant or sensitizer	[262]
Limnanthes alba (meadowfoam) seed oil				
71.3% *Limnanthes alba* (meadowfoam) seed oil in a facial repair product	109	HRIPT, semi-occluded	7 participants had $\pm$ on the first day of the induction only, no other reactions. Not a dermal irritant or sensitizer	[268]
Linum usitatissimum (linseed) seed oil				
9.4% *Linum usitatissimum* (linseed) seed oil in mascara	105	HRIPT with 0.2 g test material, semi-occluded	Not a dermal irritant or sensitizer	[269]
Luffa cylindrica seed oil				
0.01% *Luffa cylindrica* seed oil in a body wash	102	HRIPT; 0.2 mL of a 1% dilution using distilled water was applied to a $1'' \times 1''$ pad applied with a semi-occlusive patch	Not a dermal irritant or sensitizer	[270]
Macadamia ternifolia seed oil				
0.5% *Macadamia ternifolia* seed oil in a cleansing oil rinse-off	214	HRIPT with 0.2 mL of a 10% vol/vol aqueous solution, semi-occluded	3 participants had a "?" reaction following a patch during the induction and 1 participant had definite erythema with no edema or damage to the epidermis (+D) following the seventh patch. No reactions were observed at a new test site. No other reactions were observed. Study concluded test material was not a dermal sensitizer	[250]
30% *Macadamia ternifolia* seed oil in a body and hand product	55	HRIPT; semi-occluded, undiluted	Not a dermal irritant or sensitizer	[259]
Mangifera indica (mango) seed oil				
2% *Mangifera indica* (mango) seed oil in a lipstick	100	HRIPT with 150 μL test material, semi-occluded	Not a dermal irritant or sensitizer	[271]
3.87% *Mangifera indica* (mango) seed oil in an eyeliner	102	HRIPT with 0.2 g of test material, semi-occluded	Not a dermal irritant or sensitizer	[272]
Mangifera indica (mango) seed butter				
1% *Mangifera indica* (mango) seed butter in a facial lotion	100	HRIPT with 200 μL test material, semi-occluded	Not a dermal irritant or sensitizer	[273]
9% *Mangifera indica* (mango) seed butter in a body product	102	HRIPT with 0.2 g, semi-occluded	Not a sensitizer	[274]

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
Dermal irritation and sensitization				
		Moringa oleifera seed oil		
0.01% *Moringa oleifera* seed oil in a cleansing oil rinse-off	214	HRIPT with 0.2 mL of a 10% vol/vol aqueous solution, semi-occluded	3 participants had a "?" reaction following a patch during the induction and 1 participant had definite erythema with no edema or damage to the epidermis (+D) following the seventh patch. No reactions were observed at a new test site. No other reactions were observed. Study concluded test material was not a dermal sensitizer	250
		Moringa pterygosperma seed oil		
3% *Moringa pterygosperma* seed oil in an eye treatment	104	HRIPT with 150 μL test material, semi-occluded	Not a dermal irritant or sensitizer	275
		Oenothera biennis (evening primrose) oil		
1.99% *Oenothera biennis* (evening primrose) oil in a foundation	600	HRIPT, occluded	Not a dermal irritant or sensitizer	276
		Olea europaea (olive) fruit oil		
0.7% *Olea europaea* (olive) fruit oil in a scalp conditioner	114	Primary cutaneous irritation; formulation diluted to 1%	No primary irritation	230
0.1595% *Olea europaea* (olive) fruit oil in a scalp conditioner/hair wax	104	HRIPT; occlusive; applied neat	Not a dermal irritant or sensitizer	211
0.7% *Olea europaea* (olive) fruit oil in a scalp conditioner	110	HRIPT; occlusive; formulation diluted to 1%	Not a dermal irritant or sensitizer	230
1.6% *Olea europaea* (olive) fruit oil in a body lotion	110	HRIPT with 0.02 mL test material, occluded	1 participant had slight erythema following the seventh patch that did not reoccur, no other reactions observed. Not a dermal irritant or sensitizer	277
10% *Olea europaea* (olive) fruit oil in a skin salve	209	HRIPT; occlusive applied neat	Not a sensitizer	215
22% *Olea europaea* (olive) fruit oil in a body moisturizer	105	HRIPT, semi-occluded	Not a dermal irritant or sensitizer	278
58.7% *Olea europaea* (olive) fruit oil in a conditioning hair oil	102	HRIPT with 0.2 mL, semi-occluded	Not a dermal irritant or sensitizer	279
69.6% *Olea europaea* (olive) fruit oil in a foundation	209	HRIPT with 200 μL test material, occluded	Not a dermal irritant or sensitizer	280
10% *Olea europaea* (olive) oil in a skin salve	51	4-week use test; applied to lips, hands/nails, elbows, knees, feet/heels	Did not elicit significant dermal irritation or dryness; 2 participants had level 1 (mild, very slight erythema on the lips) and 5 had level 1 erythema on the elbows, lips, or knees; 15 participants reported subjective irritation	216
		Olea europaea (olive) oil unsaponfiables		
2.5% *Olea europaea* (olive) oil unsaponfiables in a bath body mist	107	HRIPT with 150 μL test material, semi-occluded	Not a dermal irritant or sensitizer	281
		Hydrogenated olive oil		
12% hydrogenated olive oil in a lipstick	108	HRIPT, occluded	Not a dermal irritant or sensitizer	263

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
Dermal irritation and sensitization				
		Hydrogenated olive oil unsaponifiables		
2% Hydrogenated olive oil unsaponifiables in a face and neck product	50	HRIPT, occluded	Not a dermal irritant or sensitizer	262
5% hydrogenated olive oil unsaponifiables in a skin cleansing product	57	HRIPT, semi-occluded, 10% dilution of product	Not a dermal irritant or sensitizer	262
		Sodium olivate		
17.64% sodium olivate in a body bar soap	107	HRIPT, semi-occluded	Not a dermal irritant or sensitizer	282
		Orbignya oleifera seed oil		
3.79% *Orbignya oleifera* seed oil in a cream cleanser	104	HRIPT with 0.2 mL of a 10% dilution of formulation, semi-occluded	Not a dermal irritant or sensitizer	283
		Orbignya speciosa kernel oil		
0.4125% *Orbignya speciosa* kernel oil in a hair conditioner	104	Modified HRIPT; semi-occlusive; 10% dilution in distilled water	Not a dermal irritant or sensitizer	284
		Persea gratissima (avocado) oil		
0.2% *Persea gratissima* (avocado) oil in a scalp conditioner	114	Primary cutaneous irritation; formulation diluted to 1%	No primary irritation	230
0.2% *Persea gratissima* (avocado) oil in a scalp conditioner	110	HRIPT; occlusive; formulation diluted to 1%	Not a dermal irritant or sensitizer	230
10% *Persea gratissima* (avocado) oil in a skin salve	51	4-week use test; applied to lips, hands/nails, elbows, knees, feet/heels	Did not elicit significant dermal irritation or dryness; 2 participants had level 1(mild, very slight erythema on the lips) and 5 had level 1 erythema on the elbows, lips, or knees; 15 participants reported subjective irritation	216
		Plukenetia volubilis seed oil		
0.51% *Plukenetia volubilis* seed oil in a lipstick	0.51% *Plukenetia volubilis* seed oil in a lipstick	0.51% *Plukenetia volubilis* seed oil in a lipstick	0.51% *Plukenetia volubilis* seed oil in a lipstick	0.51% *Plukenetia volubilis* seed oil in a lipstick
		Prunus amygdalus dulcis (sweet almond) oil		
7% *Prunus amygdalus dulcis* (sweet almond) oil in an oil treatment	103	HRIPT with 200 μL test material, semi-occluded	Grade 1 (mild erythema) reactions in 4 participants for 1 or 2 patches in the induction phase, grade 1 (mild erythema) in different participants at the 48-hour challenge reading. Study concluded test material was not a dermal irritant or sensitizer	246
10% *Prunus amygdalus dulcis* (sweet almond) oil in a face serum	108	Primary cutaneous irritation	No primary irritation	214
10% *Prunus amygdalus dulcis* (sweet almond) oil in a face serum	108	HRIPT; occlusive; applied neat	Not an irritant or a sensitizer	214
10% *Prunus amygdalus dulcis* (sweet almond) oil in a skin salve	209	HRIPT; occlusive applied neat	Not a sensitizer	215

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
		Dermal irritation and sensitization		
10% *Prunus amygdalus dulcis* (sweet almond) oil in a skin salve	51	4-week use test; applied to lips, hands/nails, elbows, knees, feet/heels	Did not elicit significant dermal irritation or dryness; 2 participants had level 1 (mild, very slight erythema on the lips) and 5 had level 1 erythema on the elbows, lips, or knees; 15 participants reported subjective irritation	216
15% *Prunus amygdalus dulcis* (sweet almond) oil in a massage oil	107	HRIPT with 0.2 mL test material, semi-occluded	1 participant had slight erythema following the seventh patch that did not reoccur, no other reactions observed. Not a dermal irritant or sensitizer	251
25% *Prunus amygdalus dulcis* (sweet almond) oil in a lip balm	222	HRIPT with 0.2 g test material, occluded	2 participants had low-level, transient (±) reactions during the induction, no other reactions were observed. Study concluded that test material was not a dermal sensitizer	256
~31% *Prunus amygdalus dulcis* (sweet almond) oil in a facial oil	108	Modified HRIPT; semi-occlusive; applied neat	Not a dermal irritant or sensitizer	285
45.25% *Prunus amygdalus dulcis* (sweet almond) oil in a facial oil	109	HRIPT; semi-occlusive; applied neat	Not a dermal irritant or sensitizer	286
46% *Prunus amygdalus dulcis* (Sweet Almond) Oil in a cuticle softener	106	Modified Draize assay with an induction phase (3×/week for 10 applications) and a challenge phase, applied neat, occlusive	Not a dermal irritant or sensitizer	287
		Prunus armeniaca (apricot) kernel oil		
2% *Prunus armeniaca* (apricot) kernel oil in a face cream	51	HRIPT with 20 μL test material, occluded	Not a dermal irritant or sensitizer	232
2% *Prunus armeniaca* (apricot) kernel oil in an eye cream	108	HRIPT with 20 μL test material, occluded	Not a dermal irritant or sensitizer	233
2.5% *Prunus armeniaca* (apricot) kernel oil in a cream	119	Primary cutaneous irritation	No primary irritation	231
19.749% *Prunus armeniaca* (apricot) kernel oil in a face serum	108	Primary cutaneous irritation	No primary irritation	214
0.005% *Prunus armeniaca* (apricot) kernel oil in a scalp conditioner/hair wax	104	HRIPT; occlusive; applied neat	Not a dermal irritant or sensitizer	211
1% *Prunus armeniaca* (apricot) kernel oil in a cream	57	HRIPT; Finn chambers, applied neat	Not a dermal irritant or sensitizer	266
2.5% *Prunus armeniaca* (apricot) kernel oil in a cream	118 (irritation)/ 116 (sensitization)	HRIPT; occlusive	Not a dermal irritant or a sensitizer	231
19.749% *Prunus armeniaca* (apricot) kernel oil in a face serum	108	HRIPT; occlusive; applied neat	Not an irritant or a sensitizer	214
		Prunus domestica seed oil		
0.04% *Prunus domestica* seed oil in a preshave lotion	105	HRIPT with 0.2 mL, occluded	Not a sensitizer	288

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
		Dermal irritation and sensitization		
		Prunus persica (peach) kernel oil		
24% *Prunus persica* (peach) kernel oil in a lip balm	222	HRIPT with 0.2 g test material, occluded	2 participants had low-level, transient (±) reactions during the induction, no other reactions were observed. Study concluded that test material was not a dermal sensitizer	256
		Ribes nigrum (blackcurrant) seed oil		
0.1% *Ribes nigrum* (blackcurrant) oil in a scalp conditioner	114	Primary cutaneous irritation; diluted to 1%	No primary irritation	230
0.25% *Ribes nigrum* (blackcurrant) oil in a cream	119	Primary cutaneous irritation	No primary irritation	231
0.1% *Ribes nigrum* (blackcurrant) Oil in a scalp conditioner	110	HRIPT; occlusive; diluted to 1%	Not a dermal irritant or sensitizer	230
0.2% *Ribes nigrum* (blackcurrant) seed oil is an eye mask	228	HRIPT, occluded	4 participants had "?" or "+" reaction during induction that were not considered clinically relevant, no other reactions observed. Not sensitizing	289
0.2% *Ribes nigrum* (blackcurrant) oil in a skin cream	106	HRIPT, occlusive	Not a dermal irritant or sensitizer	265
0.25% *Ribes nigrum* (blackcurrant) oil in a cream	118 (irritation)/ 116 (sensitization)	HRIPT; occlusive	Not a dermal irritant or a sensitizer	231
0.2% *Ribes nigrum* (blackcurrant) seed oil is an eye mask	195	4-week safety in-use study	No adverse reactions reported. Product considered suitable for sensitive skin	290
		Rosa canina fruit oil		
0.39% *Rosa canina* fruit oil in a skin cream	108	Primary cutaneous irritation	No primary irritation	265
0.39% *Rosa canina* fruit oil in a skin cream	106	HRIPT, occlusive	Not a dermal irritant or sensitizer	265
		Rubus chamaemorus seed oil		
2.5% *Rubus chamaemorus* seed oil in product	10	Single occlusive patch test for 48 hours with 25 μL	Not an irritant	291
		Rubus idaeus (raspberry) seed oil		
5% *Rubus idaeus* (raspberry) seed oil in a face and neck product	102	HRIPT, occluded	Not a dermal irritant or sensitizer	262
		Sesamum indicum (sesame) seed oil		
25% *Sesamum indicum* (sesame) seed oil in a face serum	108	Primary cutaneous irritation	No primary irritation	214
8% *Sesamum indicum* (sesame) seed oil in a skin salve	209	HRIPT; occlusive applied neat	Not a sensitizer	215
25% *Sesamum indicum* (sesame) seed oil in a face serum	108	HRIPT; occlusive; applied neat	Not an irritant or a sensitizer	214

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
		Dermal irritation and sensitization		
8% *Sesamum indicum* (Sesame) seed oil in a skin salve	51	4-week use test; applied to lips, hands/nails, elbows, knees, feet/heels	Did not elicit significant dermal irritation or dryness; 2 participants had level 1 (mild, very slight erythema on the lips), and 5 had level 1 erythema on the elbows, lips, or knees; 15 participants reported subjective irritation	216
		Solanum lycopersicum (tomato) seed oil		
0.0023% *Solanum lycopersicum* (tomato) seed oil in a cream cleanser	104	HRIPT with 0.2 mL of a 10% dilution of the formulation, semi-occluded	Not a dermal irritant or sensitizer	292
		Theobroma cacao (cocoa) seed butter		
50.1% *Theobroma cacao* (cocoa) seed butter in a lip balm	106	HRIPT with 150 μL test material, semi-occluded	Not a dermal irritant or sensitizer	293
		Theobroma grandiflorum seed butter[294]		
5% *Theobroma grandiflorum* seed butter in a lip balm	106	HRIPT with 150 μL test material, semi-occluded	Not a dermal irritant or sensitizer	295
		Triticum vulgare (wheat) germ oil		
0.005% *Triticum vulgare* (wheat) germ oil in a scalp conditioner/hair wax	104	HRIPT; occlusive; applied neat	Not a dermal irritant or sensitizer	211
		Vaccinium macrocarpon (cranberry) seed oil		
0.04% *Vaccinium macrocarpon* (cranberry) seed oil in a face and neck product	53	HRIPT, occluded	Not a dermal irritant or sensitizer	262
		Vaccinium myrtillus seed oil		
~1% *Vaccinium myrtillus* seed oil in a facial oil	116	Modified HRIPT; semi-occlusive; volatilized	Not a dermal irritant or sensitizer	294
		Vaccinium vitis-idaea seed oil		
5% *Vaccinium vitis-idaea* seed oil in product	10	Single occlusive patch test of 48 hours with 0.02 mL	Not an irritant	296
		Vegetable oil		
4% vegetable oil in a foundation	115	HRIPT, semi-occluded	1 participant had ± on the first day of the induction only, no other reactions. Not a dermal irritant or sensitizer	297
4% vegetable oil in a lipstick	106	HRIPT with 0.2 g, occluded	Not a dermal irritant or sensitizer	298
11% vegetable oil in an eye shadow	106	HRIPT, semi-occluded	Not a dermal irritant or sensitizer	299
		Vitis vinifera (grape) seed oil		
39% *Vitis vinifera* (Grape) seed oil in a preshave lotion	105	HRIPT with 0.2 mL, occluded	Not a sensitizer	288
90% *Vitis vinifera* (grape) seed oil in a fragranced oil	105	HRIPT; semi-occluded; applied neat	Not a dermal irritant or sensitizer	300
0.5% Hydrogenated grapeseed oil in a lip product	53	HRIPT; semi-occluded	Not a dermal irritant or sensitizer	301

(continued)

Table 11. (continued)

Ingredient and concentration	Participants completed	Method	Results	Reference
Dermal irritation and sensitization				
		Zea mays (corn) germ oil		
20% *Zea mays* (Corn) germ oil in a cleansing oil rinse-off	214	HRIPT with 0.2 mL of a 10% vol/vol aqueous solution, semi-occluded	3 participants had a "?" reaction following a patch during the induction and 1 participant had definite erythema with no edema or damage to the epidermis (+D) following the seventh patch. No reactions were observed at a new test site. No other reactions were observed. Study concluded test material was not a dermal sensitizer.	250
Comedogenicity				
		Ribes nigrum (blackcurrant) seed oil		
0.2% *Ribes nigrum* (blackcurrant) seed oil in an eye mask formulation	6	Applied undiluted; occlusive	Average score of 0.00 comedones/cm^2; noncomedogenic	302

Abbreviations: HRIPT, human repeat insult patch test; SPF, sun protection factor; UV ultraviolet light. [a]The same 109 panelists tested these 4 formulations that differed only in color and fragrance.

phototoxicity/photosensitization data, from previous CIR reports on oils are provided in Table 12.

Ocular Irritation Studies

Nonhuman

Ocular irritation studies were performed using animals and alternative assays on a number of plant-derived fatty acid oils. Although the majority of the oils were nonirritating to mildly irritating, a few studies indicated some significant irritation. For example, a lotion containing 1.5% *E guineensis* (palm) oil was moderately irritating to rabbit eyes, and a mascara containing 9.4% *Linum usitatissimum* (linseed) seed oil was predicted to be moderately irritating in an alternative assay. Available ocular irritation studies are summarized in Table 13. Summary statements of ocular irritation studies from previous CIR reports on oils are provided in Table 14.

Human

In clinical ocular irritation studies, formulations containing 9.4% *L usitatissimum* (linseed) oil and 0.2% *Ribes nigrum* (blackcurrant) seed oil did not produce adverse reactions and were considered safe for contact lens wearers. These studies are also summarized in Table 13.

Clinical Studies

Clinical Trials/Case Studies

Plant-derived fatty acid oils have been used as vehicles for delivery of therapeutic agents or used alone in treating skin disorders. Adverse reactions to the oils were notably absent in such clinical tests. Case studies have reported isolated allergic reactions. The available data are summarized in Table 15.

Summary

The report addresses the safety of plant-derived fatty acid oils. These oils, which are derived from vegetable and fruit plants, are composed of monoglycerides, diglycerides, and primarily triglycerides, free fatty acids, and other minor components, including natural antioxidants and fat-soluble vitamins. The percentage of chemical constituents and nutritional content of individual oil types is dependent on region where the oil plant is grown, individual cultivars, and plant genetics. Oils used in cosmetics are likely produced in the same manner as those used in the food industry. Oils may be expressed through mechanical or solvent extraction. The oils may undergo further refining, such as neutralizing, bleaching, and deodorizing, to remove pigments, odors, unsaponifiable materials, and other undesirables.

So that all plant-derived fatty acid oils that are cosmetic ingredients are included in 1 report, several ingredients that have been reviewed previously by the Panel are included in this report. The ingredients, their conclusions, and citations are found in Table 2. Some study results utilized in those previous safety assessments were also utilized in this safety assessment and cited below where appropriate.

Individuals who have food allergies to a plant protein rarely exhibit allergic reactions when exposed to refined oils of the same plant. Data evaluation by the Panel regarding the method of manufacture indicates that protein constituents do not partition into the refined oils. The Panel has also found a general lack of clinical effects for fatty acid oils that they have already reviewed; however, other researchers have raised concerns about the presence of residual proteins in oils, such as peanut and soy.

Table 12. Dermal Effects—Human Studies—Summarized From Previous CIR Reports.

Procedure and results	Reference
Dermal irritation and sensitization	
Carthamus tinctorius (safflower) oil	
Cosmetic formulations containing 3% to 5% *Carthamus tinctorius* (safflower) seed oil were not irritating to humans in occlusive patch tests and were not primary irritants or sensitizers in repeated insult patch tests.	28
Cocos nucifera (coconut) fruit oil	
An HRIPT was performed using 103 participants with a tanning butter containing 2.5% *Cocos nucifera* (coconut) oil; no erythematous reactions were seen at challenge; A bar soap containing 13% *Cocos nucifera* (coconut) oil produced very mild irritation when tested as a 1% aqueous solution on 106 participants, and it was minimally to mildly irritating in a soap chamber test with a 8% aqueous solution; the soap produced no unusual irritation response in a 2-week normal use test; undiluted *Cocos nucifera* (coconut) oil was not an allergen in 12 participants.	29
Hydrogenated coconut oil	
Four lipstick formulations containing 10% hydrogenated coconut oil were tested with a single 48-hour application on 204 females; there was no evidence of primary irritation and no indication of sensitization on retests performed 14 days later.	29
Potassium cocoate	
In a test using 40 healthy participants and 480 patients with active skin disease, 5% aqueous potassium cocoate produced 5 positive responses.	29
Corylus avellana (hazel seed) oil	
A patch testing reference book by de Groot noted that the published literature does not contain recommended test concentrations concerning hazel seed oil. To serve as a guide to the reader, de Groot reported that an unpublished (and at the time, ongoing) study found no irritant reaction in 1 to 20 patients having or suspected to have cosmetic product contact allergy who had been patch tested with 30% hazel seed oil in petrolatum.	30
Elaeis guineensis (palm) oil	
Elaeis guineensis (palm) oil, 15% in petrolatum or cosmetic formulations containing 1.0% to 2.0%, was not an irritant or sensitizer in clinical studies. Bar soap flakes, tested at dilutions that contained $\leq$2.13% palm kernel oil, were not irritating or sensitizing.	23
Gossypium herbaceum (cotton) seed oil	
Patients who were hypersensitive to cottonseed proteins were not sensitive to cottonseed oil in a skin prick test	24
Hydrogenated cottonseed oil	
In a clinical patch test, the irritation potential of a cosmetic formulation containing 3.4% hydrogenated cottonseed oil was mildly low, and the severity of reaction to 10.4% hydrogenated cottonseed oil was acceptably low in a use study. Cosmetic formulations containing 10.6% to 20.86% hydrogenated cottonseed oil were not irritating or sensitizing.	24
Oryza sativa (rice) bran oil	
Rice is generally regarded as hypoallergenic, although some case studies of allergic reactions to raw rice have been reported. In clinical testing, formulations containing 1.04% to 8.0% *Oryza sativa* (rice) bran oil were not irritating or sensitizing. Hydrolyzed rice protein was not irritating to human participants.	25
Persea gratissima (avocado) oil	
Persea gratissima (avocado) oil was not an irritant or sensitizer when human participants were patch tested with cosmetic formulations containing up to 10.7% *Persea gratissima* (avocado) oil or in patch tests using 100% *Persea gratissima* (avocado) oil.	27
Prunus amygdalus dulcis (sweet almond) oil	
Undiluted *Prunus amygdalus dulcis* (sweet almond) oil was nonirritating in a single insult patch test with 101 participants, and it was nonirritating and nonsensitizing in an HRIPT using 52 participants. Cosmetic formulations containing 0.1% to -5% were practically nonirritating and nonsensitizing in HRIPTs performed with 6,906 participants. In the Lanman-Maibach 21-day cumulative irritancy assay, a moisturizer containing 25% *Prunus amygdalus dulcis* (sweet almond) oil had a total irritancy score of 14 of 630.	208
Sesamum indicum (sesame) seed oil	
In clinical testing, undiluted *Sesamum indicum* (sesame) seed oil was not irritating. Cosmetic formulations containing 8% to 14.3% *Sesamum indicum* (sesame) seed oil were nonirritating to essentially nonirritating. Prophetic patch testing with formulations containing 10% to 11% *Sesamum indicum* (sesame) seed oil were not irritating with or without UV light. Patients with contact allergy to *Sesamum indicum* (sesame) seed oil were patch tested, and most had positive reactions to sesamol, sesamin, and sesamolin.	48

(continued)

Table 12. (continued)

Procedure and results	Reference
Dermal irritation and sensitization	
Triticum vulgare (wheat) germ oil	
In clinical testing, *Triticum vulgare* (wheat) germ oil was not an irritant or a sensitizer.	26
Phototoxicity/photosensitization	
Cocos nucifera (coconut) oil	
Bar soaps made with 13% *Cocos nucifera* (coconut) oil, tested as a 3% aqueous solution, tested using 10 participants, and a similar soap, prepared as 1% or 3% aqueous solutions, tested on 52 panelists, did not produce any evidence of photosensitization.	29
Sodium cocoate	
Bar soaps 13% sodium cocoate, prepared as a 3% aqueous solution, tested using 10 participants did not produce any evidence of photosensitization.	29
Prunus amygdalus dulcis (sweet almond) oil	
Formulations containing 0.1% to 2.0% *Prunus amygdalus dulcis* (sweet almond) oil, tested for photosensitization in a total of 764 participants, did not manifest photosensitivity in any of the test participants.	208
Oryza sativa (rice) bran oil	
Formulations containing 1.04% *Oryza sativa* (rice) bran oil were not photosensitizing.	25

Abbreviation: CIR, Cosmetic Ingredient Review; HRIPT, human repeat insult patch test.

Table 13. Ocular Irritation—Nonhuman and Human.

Ingredient	Concentration	Test group	Procedure	Results	Reference
Nonhuman studies					
Adansonia digitata seed oil					
Baobab oil	100%		MatTek EpiOcular MTT viability assay; 100 μL of test material for 16 to 256 minutes	Nonirritating	201
Aleurites moluccana seed oil					
Aleurites moluccana oil			Draize test	Not an ocular irritant	303
Aleurites moluccana oil			In vitro conjunctival cell assay	Not cytotoxic	303
Aleurites moluccana oil			Ocular burn treatment efficacy test	No adverse effects	304
Butyrospermum parkii (shea) butter					
Butyrospermum parkii (shea) butter	Undiluted	3 male Kleinrussen Chbb: HM rabbits	0.1 mL instilled into the conjunctival sac of 1 eye for 24 hour	Not irritating; mild conjunctival reactions	305
Crambe abyssinica seed oil					
Crambe abyssinica seed oil	Undiluted		Details not provided	An ocular irritant, but not corrosive	204
Fragaria ananassa (strawberry) seed oil					
Fragaria ananassa (strawberry) seed oil	5% to 50% in a lipophilic solvent		Neutral red release test	IC_{50} > 50%; negligible cytotoxicity	306
Hippophae rhamnoides seed oil					
Hippophae rhamnoides seed oil	5% to 50% in a lipophilic solvent		Neutral red release test	IC_{50} > 50%; negligible cytotoxicity	307

(continued)

Table 13. (continued)

Ingredient	Concentration	Test group	Procedure	Results	Reference
Nonhuman studies					
Linum usitatissimum (linseed) seed oil					
Mascara containing 9.4% *Linum usitatissimum* (linseed) oil	Diluted at 0% ot 50% in mineral oil		Neutral red release test	NR_{50} > 50%; slightly cytotoxic	308
Mascara containing 9.4% *Linum usitatissimum* (linseed) oil	67.1% solution in mineral oil		Hen egg test-chorioallantoic membrane assay (HET-CAM)	Moderately irritating	308
Mascara containing 9.4% *Linum usitatissimum* (linseed) oil	66.9% solution in mineral oil		Reconstituted epithelial culture assay	Slightly cytotoxic	308
Olea europaea (olive) fruit oil					
Olea europaea (olive) fruit oil, "high purity"	Undiluted	Rabbits; number not specified	Draize test	Not irritating	303
Olea europaea (olive) fruit oil, "high purity"			In vitro study using human conjunctival epithelial cells	Did not induce cellular necrosis or apoptosis	303
Ribes nigrum **(**blackcurrant**)** seed oil					
Eye mask containing 0.2% black Ribes (blackcurrant) seed oil	50% dilution		HET-CAM assay	Practically no irritation	309
Rubus chamaemorus seed oil					
Product containing 2.5% *Rubus chamaemorus* seed oil			Neutral red release assay	Negligible cytotoxicity; product was considered well tolerated	310
Vaccinium vitis-idaea seed oil					
Vaccinium vitis-idaea seed oil	5% to 50% in a lipophilic solvent		Neutral red release test	IC_{50} > 50%; negligible cytotoxicity	311
Zea mays (corn) oil					
Zea mays (corn) oil, "high purity"	Undiluted	Rabbits, number not specified	Draize test	Not irritating	303
Zea mays (corn) oil, "high purity"			In vitro study using human conjunctival epithelial cells	Did not induce necrosis or apoptosis	303
Human studies					
Linum usitatissimum (linseed) seed oil					
9.4% *Linum usitatissimum* (linseed) seed oil in a mascara		33 female participants	4 week study; 16 wore contact lenses, 17 had "sensitive" eyes	No subjective irritation and no adverse reports; clinically safe for use by contact lens-wearers	312
Ribes nigrum (blackcurrant) seed oil					
0.2% *Ribes nigrum* (blackcurrant) seed oil in an eye mask	Undiluted	52 participants	4 week in-use study	No adverse reactions; safe for contact-lens wearers	313

Abbreviations: IC_{50}, half maximal inhibitory concentration; NR_{50}, midpoint cytotoxicity

Glycidol fatty acid esters are possible impurities in refined vegetable oils. Although the amount of glycidol that may be present with glycidol fatty acid esters is not known, the IARC has noted that glycidol is probably carcinogenic to humans and that glycidol fatty acid esters are not classifiable as to carcinogenicity in humans. Peanuts and soy may contain aflatoxins, metabolic products of certain molds that are carcinogenic to humans.

Of the oils described in this report, *B parkii* (shea) butter has the most reported uses in cosmetic and personal care products with a total of 1,950 and is used at a maximum concentration of 60%. Oils are used in a wide variety of cosmetic products,

Table 14. Ocular Irritation—Nonhuman—Summarized From Previous CIR Reports.

Procedure and results	Reference
Cocos nucifera (coconut) oil	
Undiluted *Cocos nucifera* (coconut) oil, instilled into rabbit eyes without rinsing, produced minimal eye irritation.	29
Hydrogenated coconut oil	
Undiluted hydrogenated coconut oil produced mild irritation in 1 study, minimal irritation in another, negligible, or minimal irritation in 8 additional tests. Two lipstick formulations containing 10% hydrogenated coconut oil both produced slight conjunctivitis.	29
Coconut acid	
Undiluted coconut acid produced mild irritation in rabbit eyes in 2 studies and minimal irritation in a third.	29
Elaeis guineensis (palm) oil	
Undiluted *Elaeis guineensis* (palm) oil and cosmetic lotions and creams containing 1.5% to 2.0% *Elaeis guineensis* palm) oil were minimally irritating to the eyes of rabbits, whereas 1 lotion containing 1.5% *Elaeis guineensis* (palm) oil was moderately irritating.	23
Hydrogenated palm oil	
Hydrogenated palm oil suppositories were mildly irritating to rabbit eyes.	23
Hydrogenated cottonseed oil	
Cosmetic formulations containing 3.4% to 12.3% hydrogenated cottonseed oil were mildly irritating to the eyes of rabbits.	24
Oryza sativa (rice) bran oil	
A mixture of *Oryza sativa* (rice) bran oil and *Oryza sativa* (rice) germ oil, concentrations not stated, were not irritating to rabbit eyes. Undiluted *Oryza sativa* (rice) bran oil was considered minimally irritating.	25
Oryza sativa (rice) germ oil	
Oryza sativa (rice) germ oil, concentration not stated, was not a primary irritant.	25
Prunus amygdalus dulcis (sweet almond) oil	
The ocular irritation potential of undiluted *Prunus amygdalus dulcis* (sweet almond) oil and cosmetic formulations containing up to 25% *Prunus amygdalus dulcis* (sweet almond) oil were evaluated using rabbits. Undiluted *Prunus amygdalus dulcis* (sweet almond) oil was practically nonirritating or minimally irritating, and formulations containing up to 25% *Prunus amygdalus dulcis* (sweet almond) oil were nonirritating to minimally irritating. In most instances, reactions that occurred were limited to conjunctival irritation, which cleared by the third day of observation.	208
Sesamum indicum (sesame) seed oil	
Undiluted *Sesamum indicum* (sesame) seed oil was nonirritating to minimally irritating to rabbit eyes, and a lipstick containing 10% to 11% *Sesamum indicum* (sesame) seed oil was not an ocular irritant.	48
Triticum vulgare (wheat) germ oil	
Undiluted *Triticum vulgare* (wheat) germ oil was, at most, a minimal ocular irritant, and 2% in a water emulsion was not irritating.	26

Abbreviation: CIR, Cosmetic Ingredient Review.

including use in hair spray and other aerosolized products. None of the oils, or the related counterparts, described in this report is restricted from use in the European Union.

Anacardium occidentale (cashew) seed oil was not a tumor promoter in a DMBA skin test system. The safety focus of use of these oils as cosmetic ingredients is on the potential for irritation and sensitization. Undiluted, technical grade, *A hypogaea* (peanut) oil was moderately irritating to rabbits and guinea pig skin, and 5% aqueous solutions of a bar soap containing 13% sodium cocoate had irritation scores of 1.6 to 4.0 of 8 in animal studies. However, the majority of the remaining animal irritation and/or sensitization studies conducted on a large number of the oils included in this report, primarily in formulation, did not report any significant irritation or sensitization reactions, indicating that refined oils derived from plants are not dermal irritants or sensitizers. None of the tested oils, including *B parkii* (shea) butter (up to 20%) and *O sativa* (rice) germ oil ($\leq$ 75%), were phototoxic in animal studies. The comedogenicity of *C avellana* (hazel) seed oil was evaluated using rabbits, and a slight difference in the number and size of the pilosebaceous follicles and a slight excess of sebum and a dilation of the follicles were observed.

The results of a large number of clinical irritation, sensitization, and photoxicity/photosensitziation studies indicated that plant-derived fatt acid oils were not irritants or sensitizers in humans. In clinical testing with an eye mask containing 0.2% *Ribes nigrum* (blackcurrant) seed oil (undiluted), the formulation was noncomedogenic.

Table 15. Clinical Trials/Case Studies.

Ingredient	Patients/condition	Effect/observation	Reference
		Aleurites moluccana seed oil	
Aleurites moluccana oil	15; mild, stable plaque psoriasis	Efficacy study "just enough (oil) to moisten the plaque" was applied 3× daily for 12 weeks; no side effects or adverse events were reported.	314
		Anacardium occidentale (cashew) seed oil	
Anacardium occidentale (cashew) seed oil	37-year-old male resin researcher	Presentation of bullae on his right leg after dropping pure oil from a bottle on his right thigh; skin was thoroughly washed immediately; erythema developed 10 days after exposure.	315
		Patch testing was performed with cashew nut oil 3% alcohol, cashew nut oil 0.3% alcohol, cashew nut oil 0.03% alcohol, and urushiol 0.01% petrolatum; a "+" reaction was reported on day 2 and "++" reactions on days 3 and 4 to the 3% dilution; a "+" reactions to the 0.3% dilution and urushiol was reported on days 2 to 4; a "+" reaction was observed on days 2 and 3 and a "+" reaction was observed on day 4 to the 0.03% dilution.	
		Cocos nucifera (coconut) oil	
Cocos nucifera (coconut) oil		Did not produce adverse effects in several therapeutic studies.	29
		Glycine soja (soybean) oil	
Glycine soja (soybean) oil	7; history of immediate hypersensitivity reaction after the ingestion of soybeans	A double-blind crossover study; the patients were first skin tested by the puncture method with a crude whole soybean extract, a partially hydrogenated oil, a nonhydrogenated oil, and a cold-pressed soybean oil; olive oil from a retailer was used as a negative control.	56
		Since all 7 patients had negative skin tests to the oils and positive reactions to the crude soybean extract, they were challenged orally with capsules of each of the oils in random order on 4 separate days. None of the patients reacted to the oral challenges; the researchers remarked that although a reaction to the cold-pressed soybean oil did not occur in this study, cold-pressed oils may contain soybean protein and should be avoided.	
Soy oil proteins	4; known allergy to soybean	Sera was used to examine the allergenicity; neither the IgE nor the IgG_4 in the sera reacted to protein in the soy oil.	20
		Helianthus annuus (sunflower) oil	
Refined and cold-pressed sunflower oils	Patients had anaphylactic reactions following ingestion of sunflower seeds	No reactions were seen upon oral or open challenge with refined or cold-pressed sunflower oils, both of which were shown to contain detectable amounts of protein.	15
	1 woman; desensitized to mugwort (of the Compositae family) pollen for a year, and then had an anaphylactic reaction to sunflower (also of the Compositae family) seeds.	A delayed positive reaction to sunflower oil in a skin prick test was discovered; prick test results with 10 control participants were negative. In an oral challenge test, a delayed reaction was again observed, with symptoms occurring 2.25 to 8 hours after administration.	316
		Macadamia seed oil	
Macadamia seed oil in a lipstick	28-year-old woman; chelitis	Chelitis case reported after lipstick use; patient was patch tested with ingredients contained in the lipstick; positive reactions to diisostearyl malate and macadamia seed oil were reported; the condition improved after discontinuing the use of lipsticks containing these 2 ingredients.	317

(continued)

Table 15. (continued)

Ingredient	Patients/condition	Effect/observation	Reference
		Olea europaea (olive) fruit oil	
Olea europaea (olive) fruit oil		Throughout the literature, it is stated that sensitization to *Olea europaea* (olive) fruit oil is considered rare. Case reports have been described, however, and generally involved patients with venous eczema, some type of dermatitis or lesion, or an occupational exposure. Patch testing with *Olea europaea* (olive) fruit oil produced positive reactions in most of these cases, and these results were usually regarded as allergenic. The concentrations of *Olea europaea* (olive) fruit oil tested were not always given, but, when stated, test concentrations giving positive results, ranged from 30% to100%. When the constituents of olive oil were tested as well, the results of that testing were negative.	318–325
Olea europaea (olive) fruit oil		Whether the reactions to olive oil were contact sensitization or irritation were investigated using open and occlusive testing. It was concluded that olive oil presented as a weak irritant rather than a contact sensitizer in the few case studies was observed.	326
		Persea gratissima (avocado) oil	
Persea gratissima (avocado) oil	1 female; dermatitis around the eyes and earlobes	Patch testing with her sunscreen resulted in positive results. In subsequent patch testing of the individual ingredients, a positive reaction to undiluted oil, but not to the active ingredient, was observed; 20 control participants were involved, and reactions to *Persea gratissima* (avocado) oil were not seen.	327
		Sesamum indicum (sesame) seed oil	
Sesamum indicum (sesame) seed oil in an ointment	Female	Pruritic erythema, papules, and vesicles appeared after application of the ointment; patch testing was performed with the ointment and with the individual ingredients, including undiluted *Sesamum indicum* (sesame) seed oil. Both the ointment and *Sesamum indicum* (sesame) seed oil produced positive reactions on days 2, 3, 4, and 1; the other components did not cause a reaction. Results were negative in patch testing of *Sesamum indicum* (sesame) seed oil using 20 healthy participants.	328

Abbreviations: IgE, immunoglubulin E; IgG4, immunoglobulin G4.

The ocular irritation potential of a number of the oils, mostly in formulation, was evaluated by testing using animals or alternative assays. The majority of the test results did not report significant ocular irritation; however, a lotion containing 1.5% *E guineensis* (palm) oil was moderately irritating to rabbit eyes and a mascara containing 9.4% *L usitatissimum* (linseed) seed oil was moderately irritating in an alternative assay.

In human testing, a mascara containing 9.4% *L usitatissimum* (linseed) seed oil did not produce ocular irritation or adverse effects in contact lens wearers or participants with sensitive eyes. An eye mask containing 0.2% *R nigrum* (blackcurrant) seed oil (undiluted) was tested and considered safe for contact lens wearers.

Plant-derived fatty acid oils have been used as vehicles for delivery of therapeutic agents or used alone in treating skin disorders. Adverse reactions to the oils were notably absent in such clinical tests. Case studies have reported isolated allergic reactions.

Discussion

Plant-derived fatty acid oils, oils which have been hydrogenated to reduce or eliminate unsaturation, fatty acid salts, and oil unsaponifiables were reviewed by the Panel. Most of these ingredients in this report are mixtures of triglycerides containing fatty acids and fatty acid derivatives, the safety of which in cosmetics has been established. Upon review of these ingredients, the Panel expressed concern regarding gossypol (for cotton-derived ingredients), pesticide residues, and heavy metals that may be present in botanical ingredients. The Panel stressed that the cosmetics industry should continue to use the

necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulations.

Additionally, the Panel considered the safety of glycidol and glycidol fatty acid esters in refined vegetable oils. Although the Panel recognizes that these impurities may be carcinogenic, absorption through the skin would be very low and likely does not pose a significant hazard. Nonetheless, suppliers should take steps to eliminate or reduce the presence of glycidol and glycidol fatty acid esters in plant-based fatty acid oils that are used in cosmetic products. Aflatoxins, which are potent carcinogens, may be present in moldy nuts and coconut copra but are not found in oils expressed from these nuts and copra. The Panel adopted the US Department of Agriculture designation of $\leq$15 ppb as corresponding to "negative" aflatoxin content.

Certain plant-derived oils are used in cosmetic products that may be inhaled during their use. In practice, however, the particle sizes produced by the cosmetic aerosols are not respirable.

The Panel discussed the relationship between food allergies and exposure to refined oils. Individuals who have food allergies to a plant protein rarely exhibit allergic reactions when exposed to refined oils of the same plant. The Panel has found a general lack of clinical effects for plant-derived fatty acid oils already reviewed.

Fatty acid composition data were available for the majority of the oils included in this review and the Panel agreed that the composition data, in combination with the available data on method of manufacture, impurities, safety test data, a long history of safe use in foods, and an absence of adverse reactions in clinical experience, were a sufficient basis for determining safety. The Panel did note that vegetable oil is a blend of a number of different oils and that a specific composition of vegetable oil was not available. The Panel determined that the safety of vegetable oil as used in cosmetic formulations has been established, providing that the blend contains oils for which the fatty acid composition is known.

Additionally, although data on the fatty acid composition of *Fragaria vesca* (strawberry) seed oil and *Fragaria virginiana* (strawberry) seed oil were not available, data were available for *Fragaria ananassa* (strawberry) seed oil and *Fragaria chiloensis* (strawberry) seed oil. In that the fatty acid compositions of *F ananassa* and *F chiloensis* (strawberry) seed oil were similar to each other and it was assumed that *F vesca* and *F virginiana* (strawberry) seed oils would also have similar fatty acid compositions.

The Panel also noted that arachidonic acid is a fatty acid constituent of *Lycium barbarum* seed oil, *O sativa* (rice) germ oil, and *Sclerocarya birrea* seed oil. Although a previously published CIR evaluation concluded that insufficient data exist to support the safety of arachidonic acid in cosmetic products, the Panel was of the opinion that the concentration of use of these ingredients was sufficiently low that the amount of free arachidonic acid from these oils would not warrant concern.

Finally, the conclusion reached by the Panel on the safety of the plant-derived fatty acid oils supersedes the 2001 conclusion of insufficient data for *Corylus americana* and *C avellana* (hazel) seed oil.

Conclusion

The Panel concluded that the 244 plant-derived fatty acid oils included in this review are safe in the present practices of use and concentration described in this safety assessment. Were ingredients not in current use (as indicated by *) to be used in the future, the expectation is that they would be used in product categories and concentrations comparable to others in this group. The ingredients found safe are:

Actinidia chinensis (kiwi) seed oil
Adansonia digitata oil
Adansonia digitata seed oil*
Aleurites moluccanus bakoly seed oil*
Aleurites moluccanus seed oil
Amaranthus hypochondriacus seed oil*
Anacardium occidentale (cashew) seed oil
Arachis hypogaea (peanut) oil
Arctium lappa seed oil*
Argania spinosa kernel oil
Astrocaryum murumuru seed butter
Avena sativa (oat) kernel oil
Babassu acid*
Bassia butyracea seed butter*
Bassia latifolia seed butter
Bertholletia excelsa seed oil
Borago officinalis seed oil
Brassica campestris (rapeseed) oil unsaponifiables*
Brassica campestris (rapeseed) seed oil
Brassica napus seed oil*
Brassica oleracea Acephala seed oil*
Brassica oleracea Italica (broccoli) seed oil
Butyrospermum parkii (shea) butter
Butyrospermum parkii (shea) butter unsaponifiables
Butyrospermum parkii (shea) oil
Camelina sativa seed oil
Camellia japonica seed oil
Camellia kissi seed oil
Camellia oleifera seed oil
Camellia sinensis seed oil
Canarium indicum seed oil*
Canola oil
Canola oil unsaponifiables
Carica papaya seed oil
Carthamus tinctorius (safflower) seed oil
Carya illinoensis (pecan) seed oil*
Caryocar brasiliense fruit oil
Chenopodium quinoa seed oil
Citrullus lanatus (watermelon) seed oil
Citrus aurantifolia (lime) seed oil*
Citrus aurantifolia (lime) seed oil unsaponifiables*
Citrus aurantium dulcis (orange) seed oil*
Citrus aurantium dulcis (range) seed oil unsaponifiables*
Citrus grandis (grapefruit) seed oil*
Citrus grandis (grapefruit) seed oil unsaponifiables*
Citrus limon (lemon) seed oil*

Citrus paradisi (grapefruit) seed oil
Coconut acid
Cocos nucifera (coconut) oil
Cocos nucifera (coconut) seed butter*
Coix lacryma-jobi (Job's tears) seed oil*
Corn acid*
Corylus americana (hazel) seed oil
Corylus avellana (hazel) seed oil
Cottonseed acid*
Crambe abyssinica seed oil
Cucumis sativus (cucumber) seed oil
Cucurbita pepo (pumpkin) seed oil
Cynara cardunculus seed oil*
Elaeis (palm) fruit oil*
Elaeis guineensis (palm) butter*
Elaeis guineensis (palm) kernel oil
Elaeis guineensis (palm) oil
Elaeis oleifera kernel oil
Euterpe oleracea fruit oil
Fragaria ananassa (strawberry) seed oil*
Fragaria chiloensis (strawberry) seed oil*
Fragaria vesca (strawberry) seed oil*
Fragaria virginiana (strawberry) seed oil*
Garcinia indica seed butter
Gevuina avellana seed oil
Gevuina avellana oil
Glycine soja (soybean) oil
Glycine soja (soybean) oil unsaponifiables
Gossypium herbaceum (cotton) seed oil
Guizotia abyssinica seed oil*
Helianthus annuus (sunflower) seed oil
Helianthus annuus (sunflower) seed oil unsaponifiables
Hippophae rhamnoides fruit oil
Hippophae rhamnoides oil
Hippophae rhamnoides seed oil*
Hydrogenated *Adansonia digitata* seed oil*
Hydrogenated apricot kernel oil
Hydrogenated apricot kernel oil unsaponifiables*
Hydrogenated *Argania spinosa* kernel oil*
Hydrogenated avocado oil
Hydrogenated blackcurrant seed oil*
Hydrogenated *Camelina sativa* seed oil*
Hydrogenated *Camellia oleifera* seed oil
Hydrogenated canola oil
Hydrogenated coconut acid
Hydrogenated coconut oil
Hydrogenated cotton seed oil
Hydrogenated cranberry seed oil*
Hydrogenated evening primrose oil
Hydrogenated grapefruit seed oil*
Hydrogenated grapefruit seed oil unsaponifiables*
Hydrogenated grape seed oil
Hydrogenated hazelnut oil*
Hydrogenated kukui nut oil*
Hydrogenated lime seed oil*
Hydrogenated lime seed oil unsaponifiables*
Hydrogenated macadamia seed oil*
Hydrogenated meadowfoam seed oil*
Hydrogenated olive oil
Hydrogenated olive oil unsaponifiables
Hydrogenated orange seed oil*
Hydrogenated orange seed oil unsaponifiables*
Hydrogenated palm acid*
Hydrogenated palm kernel oil
Hydrogenated palm oil
Hydrogenated *Passiflora edulis* seed oil*
Hydrogenated peach kernel oil*
Hydrogenated peanut oil
Hydrogenated pistachio seed oil*
Hydrogenated pumpkin seed oil*
Hydrogenated *Punica granatum* seed oil*
Hydrogenated rape seed oil*
Hydrogenated raspberry seed oil
Hydrogenated rice bran oil*
Hydrogenated *Rosa canina* fruit oil*
Hydrogenated safflower seed oil*
Hydrogenated sesame seed oil*
Hydrogenated shea butter
Hydrogenated soybean oil
Hydrogenated sunflower seed oil
Hydrogenated sweet almond oil
Hydrogenated sweet almond oil unsaponifiables*
Hydrogenated vegetable oil
Hydrogenated wheat germ oil*
Hydrogenated wheat germ oil Unsaponifiables*
Irvingia gabonensis kernel butter
Juglans regia (walnut) seed oil
Limnanthes alba (meadowfoam) seed oil
Linseed acid
Linum usitatissimum (linseed) seed oil
Luffa cylindrica seed oil
Lupinus albus oil unsaponifiables*
Lupinus albus seed oil
Lycium barbarum seed oil
Macadamia integrifolia seed oil
Macadamia ternifolia seed oil
Magnesium cocoate
Mangifera indica (mango) seed butter
Mangifera indica (mango) seed oil
Morinda citrifolia seed oil*
Moringa oleifera seed oil
Moringa pterygosperma seed oil
Oenothera biennis (evening primrose) oil
Olea europaea (olive) husk oil*
Olea europaea (olive) oil unsaponifiables
Olea europaea (olive) fruit oil
Olive acid*
Orbignya cohune seed oil
Orbignya oleifera seed oil
Orbignya speciosa kernel oil
Oryza sativa (rice) bran oil
Oryza sativa (rice) germ oil

Oryza sativa (rice) seed oil*
Palm acid
Palm kernel acid
Passiflora edulis seed oil
Peanut acid*
Perilla ocymoides seed oil
Persea gratissima (avocado) butter
Persea gratissima (avocado) oil
Persea gratissima (avocado) oil unsaponifiables
Pistacia vera seed oil
Plukenetia volubilis seed oil
Potassium babassuate*
Potassium cocoate
Potassium cornate*
Potassium hydrogenated cocoate*
Potassium hydrogenated palmate*
Potassium olivate
Potassium palm kernelate
Potassium palmate
Potassium peanutate
Potassium rapeseedate*
Potassium safflowerate*
Potassium soyate*
Prunus amygdalus dulcis (sweet almond) oil
Prunus amygdalus dulcis (sweet almond) oil unsaponifiables*
Prunus armeniaca (apricot) kernel oil
Prunus armeniaca (apricot) kernel oil unsaponifiables*
Prunus avium (sweet cherry) seed oil
Prunus domestica seed oil
Prunus persica (peach) kernel oil
Punica granatum seed oil
Pyrus malus (apple) seed oil
Rapeseed acid*
Ribes nigrum (blackcurrant) seed oil
Ribes rubrum (currant) seed oil*
Rice bran acid*
Rosa canina fruit oil
Rubus chamaemorus seed oil
Rubus idaeus (raspberry) seed oil
Safflower acid*
Schinziophyton rautanenii kernel oil
Sclerocarya birrea seed oil
Sesamum indicum (sesame) oil unsaponifiables
Sesamum indicum (sesame) seed butter*
Sesamum indicum (sesame) seed oil
Silybum marianum seed oil (thistle)
Sodium *Astrocaryum murumuruate*
Sodium avocadoate
Sodium babassuate
Sodium cocoa butterate*
Sodium cocoate
Sodium grapeseedate
Sodium hydrogenated cocoate*
Sodium hydrogenated palmate*
Sodium macadamiaseedate*
Sodium mangoseedate
Sodium olivate
Sodium palm kernelate
Sodium palmate
Sodium peanutate*
Sodium rapeseedate*
Sodium safflowerate*
Sodium sesameseedate
Sodium soyate*
Sodium sweet almondate
Sodium *Theobroma grandiflorum* seedate*
Solanum lycopersicum (tomato) fruit oil
Solanum lycopersicum (tomato) seed oil
Soy acid*
Sunflower seed acid*
Theobroma cacao (cocoa) seed butter
Theobroma grandiflorum seed butter
Torreya nucifera seed oil*
Triticum aestivum (wheat) germ oil*
Triticum vulgare (wheat) germ oil
Triticum vulgare (wheat) germ oil unsaponifiables*
Vaccinium corymbosum (blueberry) seed oil*
Vaccinium macrocarpon (cranberry) seed oil
Vaccinium myrtillus seed oil
Vaccinium vitis-idaea seed oil
Vegetable (olus) oil
Vitis vinifera (grape) seed oil
Wheat germ acid
Zea mays (corn) germ oil
Zea mays (corn) oil
Zea mays (corn) oil unsaponifiables

Authors' Note

Unpublished sources cited in this report are available from the Executive Director, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.

Author Contributions

Christina L. Burnett contributed to conception and design; contributed to acquisition, analysis, and interpretation; and drafted the manuscript. Bart Heldreth contributed to conception and design; contributed to analysis and interpretation; and critically revised the manuscript. Monice M. Fiume contributed to conception and design; contributed to analysis and interpretation; drafted the manuscript; and critically revised the manuscript. Wilma F. Bergfeld, Donald V. Belsito, Ronald A. Hill, Curtis D. Klaassen, Daniel Liebler, James G. Marks, Ronald C. Shank, Thomas J. Slaga, and Paul W. Snyder contributed to conception and design; contributed to analysis and interpretation; and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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TECHNICAL REPORT

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Technical Report on the notification of pulp from *Theobroma cacao* L. as a traditional food from a third country pursuant to Article 14 of Regulation (EU) 2015/2283

European Food Safety Authority (EFSA),

Abstract

Following a notification from CABOSSE Naturals NV (Belgium), submitted to the European Commission under Article 14 of Regulation (EU) 2015/2283 to place on the market the pulp from *Theobroma cacao* L. (subjected to pasteurization and freezing) as a traditional food (TF) from a third country, and in line with Article 15(2) of that Regulation, EFSA was asked by the European Commission whether there are duly reasoned safety objections to the placing on the market of the TF within the European Union (EU). The approach of EFSA for the evaluation of TF notifications is based on the EFSA guidance for stakeholders on notifications for authorisation of TF and on the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. EFSA considers that the available data on composition and history of use of the TF do not raise safety concerns. Considering the available data, EFSA does not raise safety objections to the placing on the market of the requested TF (i.e. pulp from *Theobroma cacao* L. - subjected to pasteurization and freezing) within the EU.

Key words: Theobroma cacao, pulp, cacao, traditional food, third country

Requestor: European Commission following a notification from CABOSSE Naturals NV (Belgium)

Question number: EFSA-Q-2019-00395

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 28 March 2019, CABOSSE Naturals NV (Belgium) submitted a notification under Article 14 of the Novel Food Regulation (EU) 2015/2283[1] to place on the market pulp from *Theobroma cacao* L. as a traditional food (TF) from a third country.

On 20 June 2019, the European Commission forwarded to EFSA the notification on pulp from *Theobroma cacao* L. as a TF in accordance with Article 15(1) of Regulation (EU) 2015/2283.

In accordance with Article 15(2) of Regulation (EU) 2015/2283, EFSA may submit to the European Commission duly reasoned safety objections to placing on the market within the EU pulp from *Theobroma cacao* L. as TF.

2. Data and Methodologies

2.1. Data

The data provided to EFSA concern a notification for pulp from *Theobroma cacao* L. as a TF pursuant to Regulation (EU) 2015/2283 and Commission Implementing Regulation (EU) 2017/2468.[2]

Additional data, which were not included in the notification, were retrieved and considered by EFSA.

A common and structured format on the presentation of TF notifications is described in the EFSA guidance on the preparation and presentation of TF notifications.[3]

2.2. Methodologies

The approach of EFSA for the evaluation of TF notifications is based on the EFSA guidance for stakeholders on notifications for authorisations of TF[3] and on the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. As indicated in the EFSA guidance on TF, it is the duty of applicants to provide all the available data that are pertinent to the safety of the TF. EFSA may retrieve additional publicly available information in order to perform comprehensive hazard identification and evaluate whether identified hazards may pose a risk to human health.

Based on that evaluation, EFSA may submit to the European Commission duly reasoned safety objections in accordance with Article 15(2) of Regulation (EU) 2015/2283.

3. Assessment

3.1. Introduction

The TF consists of the pulp (subjected to pasteurization and freezing) from *Theobroma cacao* L. that, according to the applicant, has been consumed for more than 25 years in Brazil.

The pulp is obtained by splitting cocoa pods and separating the pulp from husks and beans; the pulp is subjected to freezing and pasteurization.

The TF is proposed to be marketed in the EU as either fruit pulp (intended for final consumers) or as a food ingredient (e.g. for fruit preparations, confectionary, bakery products, edible ices, yoghurts).

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 concerning novel foods. OJ L 327, 11.12.2015, pp. 1–22.

[2] Commission Implementing Regulation (EU) 2017/2468 of 20 December 2017 laying down administrative and scientific requirements concerning traditional foods from third countries in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 55–63.

[3] EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle H, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tome D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Schlatter J, Gelbmann W, de Sesmaisons-Lecarré A, Verhagen H and van Loveren H, 2016. Guidance on the preparation and presentation of the notification and application for authorisation of traditional foods from third countries in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4590, 16 pp. doi:10.2903/j.efsa.2016.4590

A summary of the notification on pulp from *Theobroma cacao* L. has been published by the European Commission on its website.[4]

3.2. Identity of the TF

The TF consists of the pulp from *Theobroma cacao* L. (genus: *Theobroma*; family: *Malvaceae*). According to The Plant List[5] *Theobroma cacao* L. is an accepted scientific name. Other synonyms are: *Cacao minar* Gaertn.; *Cacao minus* Gaertn.; *Cacao sativa* Aubl.; *Cacao theobroma* Tussac; *Theobroma cacao f. leiocarpum* (Bernoulli) Ducke; *Theobroma cacao subsp. leiocarpum* (Bernoulli) Cuatrec.; *Theobroma cacao var. leiocarpum* (Bernoulli) Cif.; *Theobroma cacao* subsp. pentagona (Bernoulli) León; *Theobroma cacao subsp. sativa* (Aubl.) León; *Theobroma integerrima* Stokes; *Theobroma kalagua* De Wild.; *Theobroma leiocarpum* Bernoulli; *Theobroma pentagonum* Bernoulli; *Theobroma saltzmanniana* Bernoulli; *Theobroma sapidum* Pittier; *Theobroma sativa* (Aubl.) Lign. & Le Bey; *Theobroma sativa var. leucosperma* A. Chev.; *Theobroma sativa* var. *melanosperma* A. Chev.; *Theobroma sativum* (Aubl.) Lign. & Bey.

Either cocoa or cacao are the common names used by the applicant.

Cocoa plants originate from South and Central America.

The part of the cocoa plant used for the TF is the pulp, which is the 'aqueous, mucilaginous and acidic substance in which the seeds [of cocoa] are embedded' (Codex Alimentarius, 2013). Pulp and beans are included in pods.

3.3. Production process

Cocoa plants used for obtaining the pulp were grown in Ecuador, near the production facility.

Once harvested, cocoa fruits (i.e. pods), which include pulp and beans, are transported to production facilities where they are cleaned and opened (either manually or mechanically). The pulp is removed from the beans through a sequence of three drums. The pulp is cooled down, stored in frozen conditions and subjected to pasteurization. The pulp can be subjected to further processing (e.g. enzymatic treatment, pasteurization, filtration, concentration) to obtain either juice or concentrated juice, which are finally frozen.

The applicant indicated that biological hazards are controlled via freezing and pasteurisation.

EFSA notes that according to the description provided in the notification, harvesting and production processes of the TF follow conventional agricultural and technological practices.

3.4. Compositional data

The applicant provided publications on the composition of cocoa pulp which contains approximately 80% water and 10-15% sugars (e.g. glucose, fructose, sucrose), and has a low pH (3.3 – 4.0) owing to the presence of citric acid (Adams et al., 1982; Schwan and Wheals, 2004; Henderson et al., 2007; Aprotosoaie et al., 2016). According to Codex Alimentarius (2013), the pulp contains up to 10% pectin.

The applicant provided results on the analyses of five batches of cacao pulp, either juice and juice concentrate from cacao pulp in terms of macronutrients, minerals, vitamins and phytochemicals (Table 1).

No inherent substances of possible concern to human health have been identified by the applicant.

No publications on substances of possible concern (including known allergens) for human health have been retrieved by EFSA, following a literature search on cocoa pulp.

[4] https://ec.europa.eu/food/safety/novel_food/authorisations/summary-applications-and-notifications_en
[5] http://www.theplantlist.org/

Table 1: Compositional analyses of batches of cacao pulp, juice and juice concentrate from cacao pulp.

		Juice Average ± SD (n=5)	**Juice concentrate Average ± SD (n=5)**	**Pulp Average ± SD (n=5)**
Parameter	**Unit**			
Physico-chemical				
Ash 500-550 °C	% (m/m)	0.5 ± 0.2	1.2 ± 0.2	0.5 ± 0.1
Moisture, vacuum	% (m/m)	78.7 ± 1.1	38.4 ± 0.4	79.4 ± 1.5
Energy				
Energy value (kcal)	kcal/100 g	83 ± 5	242 ± 4	79 ± 5
Energy kJ (calculated)	kJ/100 g	348 ± 19	1000 ± 0	332 ± 22
Macronutrients				
Protein (Nx6,25)	% (m/m)	0.6 ± 0.2	1.4 ± 0.2	0.7 ± 0.1
Carbohydrates, calculated	% (m/m)	19.5 ± 1.2	57.9 ± 0.7	18.1 ± 1.4
Total sugar, as glucose	% (m/m)	18.0 ± 1.1	51.4 ± 1.1	16.5 ± 1.5
Total fat, Soxhlet	% (m/m)	<0.2	<0.2	<0.2
Insoluble High Molar Weight Dietary Fiber	% (m/m)	0.6 (*)	0.4 ± 0.2	0.7 ± 0.2
Soluble High Molar Weight Dietary Fiber	% (m/m)	0.6 ± 0.2	0.9 ± 0.3	0.7 ± 0.5
Total dietary fibre	% (m/m)	0.8 ± 0.5	1.2 ± 0.3	1.1 ± 0.9
Minerals				
Calcium	mg/kg	73 ± 3	208 ± 19	54 (*)
Phosphorus (P)	mg/kg	123 ± 6	352 ± 8	140 (*)
Iron (Fe)	mg/kg	2.6 ± 0.3	7.1 ± 0.3	2.9 ± 0.5
Potassium (K)	mg/kg	2020 ± 164	5860 ± 152	1700 (*)
Copper (Cu)	mg/kg	0.7 ± 0.1	1.8 ± 0.1	0.7 ± 0.1
Magnesium (Mg)	mg/kg	220 ± 12	632 ± 11	200 (*)
Sodium	mg/kg	11 ± 3	24 ± 11	6 ± 1
Zinc (Zn)	mg/kg	2.0 ± 0.1	5.7 ± 0.0	1.9 (*)
Manganese (Mn)	mg/kg	0.6 ± 0.0	1.6 ± 0.0	0.5 ± 0.0
Vitamins				
beta-Carotene	µg/100 g	<5 LOQ	<5 LOQ	<5 LOQ
Vitamin B12 (cyanocobalamin)	µg/100 g	<5 LOQ	<5 LOQ	0.02 (*)
Riboflavin (vitamin B2)	mg/100 g	Not provided	Not provided	<0.01 LOQ
Niacin (vitamin B3)	mg/100 g	0.51 ± 0.07	1.63 ±0.11	0.61 (*)
Pantothenic acid (vitamin B5)	mg/100 g	0.04 ± 0.00	0.14 ± 0.01	0.04 (*)
Pyridoxine (vitamin B6)	mg/100 g	0.04 ± 0.00	0.11 ± 0.11	0.05 (*)
Biotin (vitamin B8)	mg/kg	0.01 (*)	0.51 ± 0.87	<0.01 LOQ
Ascorbic acid (vitamin C)	mg/100 g	<0.5 LOQ	7.02 ± 4.21	16.10 (*)
Ergocalciferol (vitamin D2)	µg/100 g	<0.25 LOQ	<0.25 LOQ	<0.25 LOQ
alpha-Tocopherol (vitamin E)	mg/100 g	<0.08 LOQ	<0.08 LOQ	<0.08 LOQ
beta-Tocopherol (vitamin E)	mg/100 g	<0.5 LOQ	<0.5 LOQ	<0.5 LOQ
delta-Tocopherol (vitamin E)	mg/100 g	<0.5 LOQ	<0.5 LOQ	<0.5 LOQ
gamma-Tocopherol (vitamin E)	mg/100 g	<0.5 LOQ	<0.5 LOQ	<0.5 LOQ
Sum of tocopherols	mg/100 g	<0.5 LOQ	<0.5 LOQ	0.12(*)
Phytochemicals				
Theobromine	% (w/w)	0.00055 ± 0.00013	0.00173 ± 0.00044	0.00065 ± 0.0003
Theophylline	% (w/w)	ND	ND	ND
Caffeine	% (w/w)	ND	ND	ND
Polyphenols calculated as gallic acid equivalent	mg/kg	619.4 ± 39.0	1604 ± 68	1065 ± 442

ND: not detected; LOQ: limit of quantification; (*): value calculated from one or two samples.

3.5. Specifications

The specifications of the TF proposed by the applicant are presented in Table 2 (typical nutritional composition), Table 3 (analytical specifications) and Table 4 (microbiological parameters).

Table 2: Typical nutritional composition of the TF as reported by the applicant

	Cacao fruit juice Cacao fruit pulp	**Cacao fruit juice concentrate**
Protein (g/100g)	0-1.0	1.0-2.0
Total fat (g/100g)	0-0.2	0-0.2
Available carbohydrates (g/100g)	18.5-22.0	57.5-59.5
Total sugars (g/100g)	17.0-20,0	51.0-53.0

Table 3: Analytical specifications of the TF as reported by the applicant

	Cacao fruit juice Cacao fruit pulp	**Cacao fruit juice concentrate**	**Method**
Ash (%)	<1.0	<1.5	AOAC 940.26
Moisture (g/100g)	77.0-81.0	37.0-40.0	(vacuum) 70° C (6h)
BRIX	16.0-20.0	58.0-62.0	ISO 2173 - Refractometer

Table 4: Microbiological parameters for the TF as reported by the applicant

	Cacao fruit juice Cacao fruit pulp	**Cacao fruit juice concentrate**	**Method**
Total Plate Count (aerobic) (cfu/g)	<10000	<10000	ISO 4833-1
Moulds (cfu/g)	<50	<50	ISO 21527-2
Yeasts (cfu/g)	<50	<50	ISO 21527-2
Enterobacteriaceae (cfu/g)	<10	<10	ISO 21528-2
Coliforms (cfu/g)	<10	<10	ISO 4832
Salmonella spp. (/25g)	absent	absent	ISO 6579-1
TAB (/50g)	absent	absent	IFU Method No. 6
ACB (/50g)	absent	absent	IFU Method No. 12

ACB: Alicyclobacillus; cfu: colony forming unit; TAB: Thermophilic Acidophilic Bacteria

3.6. Experience of continued food use in a third country

Extent of use of the TF

The applicant provided publications to document the use of cocoa pulp for producing juice, jelly, alcohol, soft drinks and vinegar in Brazil (CEPLAC, 1982; Figueira et al., 1993; Henderson et al., 2007).

The Report by CEPLAC (Comissão Executiva do Plano da Lavoura Cacaueira, 1982) describes the development in the production of juice from cocoa pulp (which is called 'cacao sweating') which is a by-product obtained from the fermentation of cocoa beans, as part of the cocoa production. Evidence on the production of juice from cocoa pulp traced back to the 1970s.

CEPLAC (1982) refers to a publication dated 1975 which describes the production of 'cacao sweating' from the pulp of cacao pods which consists in breaking cacao pods, pressing, and collecting the juice with banana leaves or cactus or wood. Producers of cocoa have developed 'press' techniques to collect cacao 'sweating'. 'Cacao sweating' was used to prepare other foods (e.g. sweets, jellies, liquors, alcoholic beverages, vinegar). In 1975-1977 five producers of cacao juices on industrial scales were

identified (i.e. back to 1979, approximately 20,000 liters of cacao fruit juice were produced and commercially marketed).

CEPLAC (1982) also refers to the production of 'cacao jelly' (i.e. ready to use jam-type product which is made with 'cacao sweetings' by addition of sugars and concentrating until jellification), which took place in three industrial facilities and increased from an average of 27.3 tons in 1976 to 38.6 tons in 1978.

Henderson et al. (2007) reported the use of the fruit pulp surrounding the cacao seeds as a drink or to be fermented to produce 'Chica', an alcoholic beverage.

Figueira et al. (1993) reported on local small industry in Bahia (Brazil) for production of fresh juice and cocoa pulp concentrates; cacao pulp was consumed fresh in the form of juices and "shakes." Figueira et al. (1993) also described the production of juice from cocoa pulp, which can be preserved by freezing and used for food production of ice-cream, yogurt flavoring, or juice concentrates.

Codex Alimentarius (2013) reports the use of cocoa pulp for making jams and jellies as well as alcoholic beverages and vinegar.

The applicant provided a list of products from cacao pulp and cacao pulp juice which have been commercialized in Brazil in the last decades.

The applicant also provided references on the use of cacao pulp, as a by-product from cacao production, to produce alcoholic beverages, jams, marmalades and syrup in other countries such as Africa (Buamah et al., 1997, Adomako et al., 2006; Vasquez et al. 2019).

Characteristics of the population group(s) of consumers

The applicant indicated that the TF and products thereof such as juice have been consumed by the general population.

Role in the diet

The applicant indicated that the TF has been consumed as other fruit pulp, in the form of beverages or as a food ingredient.

Information on the handling and preparation of the food

No information on handling and preparation of the TF has been indicated by the applicant.

Precautions for the preparation and restrictions of use

No precautions for the preparation or restrictions of use of the TF have been indicated by the applicant.

Human data

No publications on human data have been retrieved either by the applicant or by EFSA on cocoa pulp.

3.7. Other information on the TF

No publications on toxicity have been retrieved either by the applicant or by EFSA on cocoa pulp.

3.8. Proposed conditions of use for the EU market

Target population

The target population proposed by the applicant is the general population.

Proposed uses and use levels

The TF is intended to be marketed in the EU as such or as a juice (for final consumers) or as a food ingredient (e.g. for fruit preparations, confectionary, bakery products, edible ices, yoghurts).

The applicant indicated that the TF is expected to be consumed as any fruit pulp.

Taking into account the compositional data on cocoa pulp (section 3.4) and the intended uses, EFSA does not consider the consumption of TF at the intended uses to be of concern.

Intended role in the diet

The TF is expected to be consumed as any fruit pulp.

Precautions and restrictions of use

No precautions or restrictions of use have been indicated by the applicant.

4. Conclusions

EFSA considers that the available data on composition and history of use of the TF do not raise safety concerns.

Considering the available data, EFSA does not raise safety objections to the placing on the market of the requested TF (i.e. pulp from *Theobroma cacao* L. - subjected to pasteurization and freezing) within the EU.

5. Documentation as provided to EFSA

On 20 June 2019, a valid notification on fruit pulp from *Theobroma cacao* L. as a TF, which was submitted by CABOSSE Naturals NV (BE), was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1014).

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Abbreviations

EU	European Union
TF	traditional food

NI Cocoa Powder

Safety Data Sheet

According To Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules And Regulations

Revision Date: 09/06/2019 Date of Issue: 09/06/2016 Version: 2.0

SECTION 1: IDENTIFICATION

1.1. Product Identifier

Product Form: Substance

Product Name: NI Cocoa Powder

Synonyms: Cocoa

1.2. Intended Use of the Product

Use of the Substance/Mixture: Ingredient in the confectionery and pharmaceutical industry.

1.3. Name, Address, and Telephone of the Responsible Party

Company/Manufacturer:

Blommer Chocolate Company
1101 Blommer Drive
East Greenville, PA 18041
215-679-4472

1.4. Emergency Telephone Number

Emergency Number : 215-541-2430

SECTION 2: HAZARDS IDENTIFICATION

2.1. Classification of the Substance or Mixture

GHS-US Classification

Comb. Dust

Full text of hazard classes and H-statements : see section 16

2.2. Label Elements

GHS-US Labeling

Signal Word (GHS-US) : Warning

Hazard Statements (GHS-US) : May form combustible dust concentrations in air.

2.3. Other Hazards

Dust may cause mechanical irritation to eyes, nose, throat, and lungs. Exposure may aggravate pre-existing eye, skin, or respiratory conditions.

2.4. Unknown Acute Toxicity (GHS-US)

No data available

SECTION 3: COMPOSITION/INFORMATION ON INGREDIENTS

3.1. Substance

Name : NI Cocoa Powder

Name	Product Identifier	%	GHS-US classification
Cocoa, extract	(CAS No) 84649-99-0	100	Not classified

3.2. Mixture

Not applicable

SECTION 4: FIRST AID MEASURES

4.1. Description of First-aid Measures

First-aid Measures General: Never give anything by mouth to an unconscious person. If you feel unwell, seek medical advice (show the label where possible).

First-aid Measures After Inhalation: Using proper respiratory protection, move the exposed person to fresh air at once. Encourage exposed person to cough, spit out, and blow nose to remove dust. Immediately call a poison center, physician, or emergency medical service.

First-aid Measures After Skin Contact: Remove contaminated clothing. Drench affected area with water for at least 15 minutes. Obtain medical attention if irritation develops or persists.

First-aid Measures After Eye Contact: Rinse cautiously with water for at least 15 minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Obtain medical attention.

First-aid Measures After Ingestion: Rinse mouth. Do NOT induce vomiting. Obtain medical attention.

4.2. Most Important Symptoms and Effects Both Acute and Delayed

Symptoms/Injuries: Dust may cause mechanical irritation to eyes, nose, throat, and lungs.

Symptoms/Injuries After Inhalation: Dust may be harmful or cause irritation.

Symptoms/Injuries After Skin Contact: Prolonged exposure may cause skin irritation.

Symptoms/Injuries After Eye Contact: May cause slight irritation to eyes.

Symptoms/Injuries After Ingestion: Adverse effects not expected from this product.

Chronic Symptoms: None known.

4.3. Indication of Any Immediate Medical Attention and Special Treatment Needed

If exposed or concerned, get medical advice and attention. If medical advice is needed, have product container or label at hand.

SECTION 5: FIRE-FIGHTING MEASURES

5.1. Extinguishing Media

Suitable Extinguishing Media: Use extinguishing media appropriate for surrounding fire.

Unsuitable Extinguishing Media: Do not use a heavy water stream. Use of heavy stream of water may spread fire.

5.2. Special Hazards Arising From the Substance or Mixture

Fire Hazard: Combustible Dust.

Explosion Hazard: Dust explosion hazard in air.

Reactivity: Hazardous reactions will not occur under normal conditions.

5.3. Advice for Firefighters

Precautionary Measures Fire: Exercise caution when fighting any chemical fire.

Firefighting Instructions: Use water spray or fog for cooling exposed containers.

Protection During Firefighting: Do not enter fire area without proper protective equipment, including respiratory protection.

Hazardous Combustion Products: Carbon oxides (CO, CO_2). Nitrogen oxides.

Other Information: Risk of dust explosion.

SECTION 6: ACCIDENTAL RELEASE MEASURES

6.1. Personal Precautions, Protective Equipment and Emergency Procedures

General Measures: Avoid prolonged contact with eyes, skin and clothing. Avoid breathing dust. Avoid generating dust. Remove ignition sources. Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking.

6.1.1. For Non-Emergency Personnel

Protective Equipment: Use appropriate personal protection equipment (PPE).

Emergency Procedures: Evacuate unnecessary personnel.

6.1.2. For Emergency Personnel

Protective Equipment: Equip cleanup crew with proper protection.

Emergency Procedures: Upon arrival at the scene, a first responder is expected to recognize the presence of dangerous goods, protect oneself and the public, secure the area, and call for the assistance of trained personnel as soon as conditions permit.

6.2. Environmental Precautions

Prevent entry to sewers and public waters.

6.3. Methods and Materials for Containment and Cleaning Up

For Containment: Contain solid spills with appropriate barriers and prevent migration and entry into sewers or streams. Avoid generation of dust during clean-up of spills.

Methods for Cleaning Up: Clean up spills immediately and dispose of waste safely. Use explosion proof vacuum during cleanup, with appropriate filter. Do not mix with other materials. Vacuum clean-up is preferred. If sweeping is required use a dust suppressant. Use only non-sparking tools. Contact competent authorities after a spill.

6.4. Reference to Other Sections

See Section 8 for exposure controls and personal protection and Section 13 for disposal considerations.

SECTION 7: HANDLING AND STORAGE

7.1. Precautions for Safe Handling

Additional Hazards When Processed: Accumulation and dispersion of dust with an ignition source can cause a combustible dust explosion. Keep dust levels to a minimum and follow applicable regulations.

Precautions for Safe Handling: Wash hands and other exposed areas with mild soap and water before eating, drinking or smoking and when leaving work. Avoid prolonged contact with eyes, skin and clothing. Avoid breathing dust. Avoid creating or spreading dust. Keep away from heat, sparks, open flames, hot surfaces. – No smoking.

Hygiene Measures: Handle in accordance with good industrial hygiene and safety procedures.

7.2. Conditions for Safe Storage, Including Any Incompatibilities

Technical Measures: Comply with applicable regulations. Avoid creating or spreading dust. Use explosion-proof electrical, ventilating, lighting equipment. Proper grounding procedures to avoid static electricity should be followed.

Storage Conditions: Keep container closed when not in use. Store in a dry, cool place. Keep/Store away from direct sunlight, extremely high or low temperatures and incompatible materials.

Incompatible Products: Strong acids, strong bases, strong oxidizers.

Maximum Storage Period: 24 months in optimal storage conditions

Storage Temperature: 15.6 - 21.1 °C (60 - 70 °F) and < 60 % relative humidity

7.3. Specific End Use(s)

Ingredient in the confectionery and pharmaceutical industry.

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1. Control Parameters

For substances listed in section 3 that are not listed here, there are no established exposure limits from the manufacturer, supplier, importer, or the appropriate advisory agency including: ACGIH (TLV), AIHA (WEEL), NIOSH (REL), or OSHA (PEL).

8.2. Exposure Controls

Appropriate Engineering Controls : Ensure adequate ventilation, especially in confined areas. Ensure all national/local regulations are observed. Proper grounding procedures to avoid static electricity should be followed. Use explosion-proof equipment. Use local exhaust or general dilution ventilation or other suppression methods to maintain dust levels below exposure limits. Power equipment should be equipped with proper dust collection devices. It is recommended that all dust control equipment such as local exhaust ventilation and material transport systems involved in handling of this product contain explosion relief vents or an explosion suppression system or an oxygen-deficient environment.

Personal Protective Equipment : Dust formation: dust mask. In case of dust production: protective goggles.

Hand Protection : Wear protective gloves.

Eye Protection : Chemical safety goggles.

Skin and Body Protection : Wear suitable protective clothing.

Respiratory Protection : If exposure limits are exceeded or irritation is experienced, approved respiratory protection should be worn.

Other Information : When using, do not eat, drink or smoke.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

9.1. Information on Basic Physical and Chemical Properties

Physical State	:	Solid
Appearance	:	Light Brown Powder
Odor	:	Chocolate
Odor Threshold	:	No data available
pH	:	5.1 - 5.9
Evaporation Rate	:	No data available
Melting Point	:	No data available
Freezing Point	:	No data available
Boiling Point	:	No data available
Flash Point	:	No data available
Auto-ignition Temperature	:	No data available
Decomposition Temperature	:	No data available
Flammability (solid, gas)	:	No data available
Vapor Pressure	:	No data available
Relative Vapor Density at 20°C	:	No data available
Relative Density	:	No data available
Solubility	:	No data available
Partition Coefficient: N-Octanol/Water	:	No data available
Viscosity	:	No data available
Water Activity (Aw)	:	< 0.86
Viscosity	:	No data available
Moisture	:	5 % Max

9.2. Other Information

No additional information available

SECTION 10: STABILITY AND REACTIVITY

10.1. Reactivity: Hazardous reactions will not occur under normal conditions.

10.2. Chemical Stability: Stable under recommended handling and storage conditions (see section 7).

10.3. Possibility of Hazardous Reactions: Hazardous polymerization will not occur.

10.4. Conditions to Avoid: Any activities that could increase the water activity level. Direct sunlight, extremely high or low temperatures, and incompatible materials. Sparks, heat, open flame and other sources of ignition. Dust accumulation (to minimize explosion hazard).

10.5. Incompatible Materials: Strong acids, strong bases, strong oxidizers.

10.6. Hazardous Decomposition Products: None known.

SECTION 11: TOXICOLOGICAL INFORMATION

11.1. Information on Toxicological Effects

Acute Toxicity: Not classified

Skin Corrosion/Irritation: Not classified (**pH:** 5.1 - 5.9)

Serious Eye Damage/Irritation: Not classified (**pH:** 5.1 - 5.9)

Respiratory or Skin Sensitization: Not classified

Germ Cell Mutagenicity: Not classified

Carcinogenicity: Not classified

Reproductive Toxicity: Not classified

Specific Target Organ Toxicity (Single Exposure): Not classified

Specific Target Organ Toxicity (Repeated Exposure): Not classified

Aspiration Hazard: Not classified

Symptoms/Injuries After Inhalation: Dust may be harmful or cause irritation.

Symptoms/Injuries After Skin Contact: Prolonged exposure may cause skin irritation.

Symptoms/Injuries After Eye Contact: May cause slight irritation to eyes.

Symptoms/Injuries After Ingestion: Adverse effects not expected from this product.

Chronic Symptoms: None known.

SECTION 12: ECOLOGICAL INFORMATION

12.1. Toxicity

Ecology - General : Not classified.

12.2. Persistence and Degradability

NI Cocoa Powder	
Persistence and Degradability	Not established.

12.3. Bioaccumulative Potential

NI Cocoa Powder	
Bioaccumulative Potential	Not established.

12.4. Mobility in Soil No additional information available

12.5. Other Adverse Effects

Other Information : Avoid release to the environment.

SECTION 13: DISPOSAL CONSIDERATIONS

13.1. Waste Treatment Methods

Waste Disposal Recommendations: Dispose of contents/container in accordance with local, regional, national, and international regulations.

Ecology - Waste Materials: Avoid release to the environment.

SECTION 14: TRANSPORT INFORMATION

The shipping description(s) stated herein were prepared in accordance with certain assumptions at the time the SDS was authored, and can vary based on a number of variables that may or may not have been known at the time the SDS was issued.

14.1. In Accordance with DOT Not regulated for transport

14.2. In Accordance with IMDG Not regulated for transport

14.3. In Accordance with IATA Not regulated for transport

SECTION 15: REGULATORY INFORMATION

15.1. US Federal Regulations

NI Cocoa Powder	
SARA Section 311/312 Hazard Classes	Fire hazard Sudden release of pressure hazard

15.2. US State Regulations Neither this product nor its chemical components appear on any US state lists.

SECTION 16: OTHER INFORMATION, INCLUDING DATE OF PREPARATION OR LAST REVISION

Revision Date : 09/06/2019

Other Information : This document has been prepared in accordance with the SDS requirements of the OSHA Hazard Communication Standard 29 CFR 1910.1200

GHS Full Text Phrases:

Comb. Dust	Combustible Dust

This information is based on our current knowledge and is intended to describe the product for the purposes of health, safety and environmental requirements only. It should not therefore be construed as guaranteeing any specific property of the product.

SDS US (GHS HazCom)