Toxicological profile for

Licorice, licorice extract, root extract, powder extract

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

68916-91-6: EINECS 272-837-1; FEMA No. 2628; FEMA No. 2629; FEMA No. 2630; Gan Cao; Glycyrrhiza extract; Glycyrrhizae [Latin]; HSDB 1925; Kanzo [Japanese]; Kanzou [Chinese]; Licorice, ext; Licorice extract; Licorice extract (Glycyrrhiza spp.); Licorice extract powder (Glycyrrhiza glabra L.); Licorice root (Glycyrrhiza glabra L.); Licorice root extract; Zhi Gan Cao (ChemIDplus)

84775-66-6: CCRIS 9463; EINECS 283-895-2; Glycyrrhiza glabra; Licorice, Glycyrrhiza glabra, ext; Licorice; Liquorice; Sinu-rite; UNII-2788Z9758H (ChemIDplus)

97676-23-8: Licorice extract; Licorice oil; Oils, licorice; Secondary acid liquor; Secondary licorice extract; Tertiary acid liquor; Tertiary licorice extract (ChemIDplus)

1.3. Molecular formula

A number of substances are known to be present in licorice extract, some of which may be artifacts introduced in processing. Principal constituents consist of glycyrrhizin (20%), reducing sugars (5%), non-reducing sugars (5%), starch, dextrin and gums (30%), ash (8%) and moisture (17%). In addition licorice roots contain triterpene, flavonoids and B vitamins (Burdock, 2010); Unspecified (ChemIDplus)

1.4. Structural Formula

Licorice: not applicable, as it is a mixture.

1.5. Molecular weight (g/mol)

Licorice: not applicable, it is a mixture.

1.6. CAS registration number

68916-91-6, 84775-66-6, 97676-23-8

1.7. Properties

1.7.1. Melting point

(°C): 5 (EPISuite, 2017) (CAS RN 97676-23-8)

1.7.2. Boiling point

(°C): 193 (EPISuite, 2017) (CAS RN 97676-23-8)

1.7.3. Solubility

Soluble in water (HSDB, 2002) (CAS RN 68916-91-6); 2150 mg/L at 25ºC (EPISuite, 2017) (CAS RN 97676-23-8)

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%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

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

1.7.8. Decomposition temperature

(°C): 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

0.817 mm Hg at 25ºC (EPISuite, 2017) (CAS RN 97676-23-8)

1.7.11. log Kow

3.52 (EPISuite, 2017) (CAS RN 97676-23-8)

2. General information

2.1. Exposure

Occurrence in tobacco products

In the burnt part?	Yes
In tobacco naturally?	No evidence (Stedman 1968; Lloyd et al 1976)

“Licorice is used in cigarettes both as a flavor and as a casing material to smooth the harsh taste of certain kinds of tobacco. Most cigarette tobacco blends contain less than 1% licorice. Licorice has been used in cigarettes for decades, and is approved for cigarette use in the United Kingdom, at levels up to 4%, and in Germany. It is also used as a food and flavor in other foods and beverages.”

“Licorice (or 'liquorice') is a plant of ancient origin and steeped in history. Licorice extracts and its principle component, glycyrrhizin, have extensive use in foods, tobacco and in both traditional and herbal medicine. As a result, there is a high level of use of licorice and glycyrrhizin in the US with an estimated consumption of 0.027-3.6 mg glycyrrhizin/kg/day. ….” (Taken from Isbrucker & Burdock,

2006. Regulatory Toxicology and Pharmacology 46(3), 167-192). Available at http://www.ncbi.nlm.nih.gov/pubmed/16884839

Licorice extract (CAS RN 68916-91-6): Used in food in alcoholic beverages, baked goods, Frozen dairy, gelatin, pudding, Meat products, non alcoholic beverages and sweet sauce. The FEMA PADI (possible average daily intake) of licorice extract is 204.106 mg. Reported usage levels range from about 11 ppm in sweet sauce up to 1424 ppm in alcoholic beverages.

Reported uses for licorice extract (CAS RN 68916-91-6) (ppm): (FEMA, 1994)

Food Category	**Usual**	**Max.**	**Food Category**	**Usual**	**Max.**
Alcoholic beverages	1393.00	1424.00	Meat products	600.00	800.00
Baked Goods	570.30	630.00	Nonalcoholic beverages	169.30	196.70
Frozen dairy	468.20	553.20	Sweet sauce	10.92	15.55
Gelatins, puddings	192.90	209.80			

Reported individual intake from use as a flavouring: 0.031920 mg/kg bw/day.

Reported uses for licorice extract powder (CAS RN 977070-62-4 (ppm): (FEMA, 1994)

Food Category	**Usual**	**Max.**	**Food Category**	**Usual**	**Max.**
Alcoholic beverages	1800.00	2000.00	Hard candy	4010.00	5346.00
Baked Goods	1666.00	1908.00	Meat products	2200.00	2500.00
Chewing gum	5825.00	5825.00	Nonalcoholic beverages	24.59	35.70
Frozen dairy	200.00	400.00			

Reported individual intake from use as a flavouring: 0.03997 mg/kg bw/day.

As taken from Burdock, 2010.

Glycyrrhiza glabra leaf extract, rhizome/root extract, root, root extract, root juice, root water and root powder (all CAS RN 84775-66-6) are used as skin conditioning agents in cosmetics in the EU. In addition Glycyrrhiza glabra root extract is used as a bleaching agent, skin conditioning - emollient, perfuming, smoothing and soothing agent, Glycyrrhiza glabra root water as an antioxidant, Glycyrrhiza glabra root oil (CAS RN 84775-66-6) as a perfuming agent and Glycyrrhiza glabra rhizome/root (CAS RN 68916-91-6) as an skin conditioning - emollient, moisturising, smoothing and soothing agent in cosmetics. As taken from CosIng (Cosmetic substances and ingredients database).

Licorice root absolute and licorice root extract (both CAS RN 97676-23-8) are included on the IFRA list of fragrance ingredients (IFRA).

Licorice oils (CAS RN 97676-23-8) are listed as ingredients in personal care products by the CPID.

“Medicinal, Pharmaceutical, and Cosmetic. Licorice extracts are used extensively as ingredients in cough drops and syrups, tonics, laxatives, antismoking lozenges (see lobelia), and other preparations. They are also used as flavoring agents to mask bitter, nauseous, or other undesirable tastes in certain medicines (e.g., cascara, ammonium chloride, and quinine preparations).

Average daily doses of 5–15 g root (calculated to 200–600 mg of glycyrrhizin) or root juice (0.5–1 g for respiratory tract catarrhs, or 1.5–3 g for gastric duodenal ulcers) are used in European phytomedicine. Duration is limited to 4–6 weeks, because of potential adverse side effects (described above). Use is contraindicated for cholestatic liver disorders cirrhosis, hypertonia, pregnancy, and others. Known drug interactions include potassium loss due to thiazine diuretics, as well as increased sensitivity to digitalis glycosides.

Food. Licorice is widely used in flavouring foods.

The most well-known use of licorice and its extracts as well as ammoniated glycyrrhizin is in licorice candy where they are mixed with anise oil (see anise), with average maximum use levels of about 3.279% (32,792 ppm) and 0.151% (1512 ppm) reported for the powdered extract and ammoniated glycyrrhizin, respectively. Licorice, its extracts, and ammoniated glycyrrhizin are also used in many other food products, including alcoholic (certain kinds of beer) and nonalcoholic (e.g., root beer) beverages, frozen dairy desserts, baked goods, gelatins and puddings, and meat and meat products. Average maximum use levels reported are below 0.25% for licorice and licorice extracts; the use levels reported for ammoniated glycyrrhizin are usually below 0.01%.

Dietary Supplements/Health Foods. Root, powdered or cut and sifted, is widely used as tea ingredient; and in capsules, tablets, tinctures, and other dietary supplement formulations for flavoring and traditional indications; extracts also used in capsules, tablets, and drinks.”

“Licorice extracts are used in flavoring tobaccos.”

As taken from Khan and Abourashed, 2010.

According to Health Canada’s Natural Health Products Database, the following substances are used in non-medicinal products for the indicated purposes: Glycyrrhiza glabra (licorice) leaf extract (no CAS RN listed) as a skin conditioning agent for topical use, Glycyrrhiza glabra (licorice) rhizome/root powder (no CAS RN listed) as a flavour enhancer and fragrance ingredient for topical use, Glycyrrhiza glabra (licorice) root extract (no CAS RN listed) as a preservative antioxidant, skin-conditioning agent and humectant for topical use, and liquorice dry (no CAS RN listed) as a flavour enhancer. Glycyrrhiza glabra (no CAS RN listed) is also listed as a homeopathic substance (Health Canada, 2021).

2.2. Combustion products

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

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%

acetone	2831558	2.03	3574402	2.47
unknown	2656473	1.91	3766308	1.91
diacetyl + methyl ethyl ketone	5312732	3.82	5994452	4.14
acetic acid	65351317	46.94	60176801	41.56
acetol	10364224	7.44	10508379	7.26
furfural	4050303	2.91	4448149	3.07
furfuryl alcohol	13463052	9.67	14143421	9.77
phenol	5052284	3.63	4689736	3.24
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	7064529	5.07	7164068	4.95
2-furancarboxylic acid + unknown	10732741	7.71	11130231	7.69
p-vinylphenol	4829761	3.47	4325406	2.99
5-hydroxymethylfurfural	3813671	2.74	3610710	2.49
Total ion chromatogram	139235889	100	144833935	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2005).

Ingredient Name & CAS Number	Max. cig. Appln. Level (ppm)	Composition of pyrolysate (Compound, %)	Max. level in smoke (ug)
Licorice extract, powder 68916-91-6	20,000	Acetic acid (42.0) Acetol (11.9) Furfuryl alcohol (11.7) Diacetyl (4.1) Acetol acetate (2.0) Phenol (1.4) Cresol (0.2) Pyridine + pyrrole? (0.2) Furfural (0.2)	4,200 1,200 1,200 410 200 140 20 20 20

“The fate of glycyrrhizic acid and glycyrrhetinic acid during the smoking of cigarettes has been studied by adding these acids separately to cigarettes (Sakagami, 1973). It was found that glycyrrhizic acid decomposed to glycyrrhetinic acid and was transferred in the mainstream smoke as such. When it was glycyrrhetinic acid itself was added to cigarettes, transferred intact to the mainstream smoke. In both cases, the amount of glycyrrhetinic acid found in the smoke condensate was small, and it was concluded that the glycyrrhizic acid in licorice root used f o r tobacco flavoring was mostly decomposed on smoking.”

When licorice was heated to 900ºC at 20ºC per minute and analysed by thermogravimity/GC-MS, about 60 degraded compounds were identified, including acids, aldehydes, hydrocarbons, esters, ketones and N-containing compounds. At 37-138ºC decomposition was noted, with weight loss at

8%; 138-380ºC caused decomposition and weight loss of 44%; and 382-529ºC resulted in decomposition and weight loss of 42% (Chung & Aldridge 1999).

58 individual compounds were identified in pyrolyzed licorice extract, heated from room temperature to 900ºC in the presence of air over a period of 100 seconds (Philip Morris USA 2001).

Heating licorice in 30g portions at 700ºC until the organic matter had disappeared in an air flow of 1.5l/min resulted in the identification of 11 pyrolysis products including PAHs, quinines and phenols (Kröller 1967).

In a pyrolysis study of cigarette tobacco treated with licorice extract and heated up to 900ºC, the licorice extract (containing the non-volatile ammonium salt of glycyrrhizic acid) underwent full degradation. Glycyrrhizic acid, ammonium salt was not detect intact in the mainstream or sidestream smoke. The major pyrolysates were tentatively identified as naphthalenic compounds (Purkis et al 2011).

2.3. Ingredient(s) from which it originates

TSCA Definition 2019: Extractives and their physically modified derivatives. Glycyrrhiza, Leguminosae (CAS RN 97676-23-8).

Tinctures, concretes, absolutes, essential oils, oleoresine, terpenes. Terpene-free fractions, distillates, residues obtained from Glycyrrhiza glara, Leguminosae (CAS RN 68916-91-6).

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

Licorice extract (68916-91-6) is produced by extraction of the comminuted roots and rhizomes of Glycyrrhiza glabra [a herbaceous plant native to southern Europe], with boiling water followed by evaporation of the aqueous extract (Burdock GA, 2010).

Glycyrrhiza glabra rhizome/root (CAS RN 68916-91-6) is a plant material derived from the dried rhizomes and roots of the licorice, Glycyrrhiza glabra L., Leguminosae.

Glycyrrhiza glabra rhizome/root extract (CAS RN 84775-66-6) is the extract of the roots and rhizomes of the licorice, Glycyrrhiza glabra L., Fabaceae.

Glycyrrhiza glabra root (CAS RN 84775-66-6) is the roots of the licorice, Glycyrrhiza glabra L., Leguminosae.

Glycyrrhiza glabra root extract (CAS RN 84775-66-6) is an extract of the roots of the licorice, Glycyrrhiza glabra L., Leguminosae.

Glycyrrhiza glabra root juice (CAS RN 84775-66-6) is the juice expressed from the roots of the licorice, Glycyrrhiza glabra L., Leguminosae.

Glycyrrhiza glabra root oil (CAS RN 84775-66-6) is an essential oil obtained from the rhizomes of the licorice, Glycyrrhiza glabra L., Leguminosae.

Glycyrrhiza glabra root powder (CAS RN 84775-66-6) is the powder obtained from the roots of the licorice, Glycyrrhiza glabra L., Leguminosae.

Glycyrrhiza glabra root water (CAS RN 84775-66-6) is an aqueous solution of the steam distillate obtained from the roots of the licorice, Glycyrrhiza glabra L., Leguminosae.

Glycyrrhiza glabra leaf extract (CAS RN 84775-66-6) is an extract of the leaves of the licorice, Glycyrrhiza glabra L., Leguminosae.

As taken from CosIng (Cosmetic substances and ingredients database).

“Liquorice is a natural extract of the root of the liquorice (Glycyrrhiza glabra) plant – logically a not completely defined complex mixture of compounds.”

As taken from SCENIHR, 2016.

3. Status in legislation and other official guidance

"Licorice (or 'liquorice') is a plant of ancient origin and steeped in history. Licorice extracts and its principle component, glycyrrhizin, have extensive use in foods, tobacco and in both traditional and herbal medicine. …. Both products have been approved for use in foods by most national and supranational regulatory agencies" (Taken from Isbrucker & Burdock, 2006. Regulatory Toxicology and Pharmacology 46(3), 167-192). Available at http://www.ncbi.nlm.nih.gov/pubmed/16884839

Licorice: Decision postponed (JECFA 1978).

The EU SCF has concluded that the data are inadequate to derive an ADI for glycyrrhizinic acid and ammonium glycyrrhizinate, but felt that setting an upper limit of 100 mg/day intake for glycyrrhizinic acid provides a sufficient level of protection for the majority of the population (SCF 2003).

More recently, EFSA (2011) concluded that the novel food ingredient Glavonoid (an extract derived from the root or rootstock of Glycyrrhiza glabra L. by extraction with ethanol followed by further extraction with medium-chain triglycerides) is safe for the general adult population at up to 120 mg/day.

The Nordic Council of Ministers advocated an ADI of 1-10 mg/person/day for glycyrrhizinic acid, based on the opinion that "in most sensitive individuals, adverse effects occur at a regular daily intake of about 100 mg glycyrrhizinic acid". This was described as a provisional LOAEL (Størmer et al. 1993a,b).

The Japanese Ministry of Health, Labour and Welfare has cautioned against consumption of more than 200 mg glycyrrhizin per day (Ikegami et al. 2004).

The German National Chemists Association has warned that woman taking oral contraceptives should consume no more than 10 g licorice/day because it can cause oedema (Anon 2003).

Codex Alim.	958		
C of E no.	218 (3rd Ed.)	FEMA no.	2628;2629; 2630
TLV/OEL	Not listed		
Cosmetics (UK)	Not listed in Schedule 1		

This ingredient is a well characterized material that has been evaluated and approved as a food additive by expert bodies including USFDA, FEMA and the CoE.

Licorice extract, extract powder and root (all CAS RN 68916-91-6) have been designated as GRAS (generally recognized as safe) for use in food by FEMA (Hall & Oser, 1965).

Licorice extract (Glycyrrhiza Spp.; CAS RN 68916-91-6) is included on the FDA's inventory of "Substances Added to Food (formerly EAFUS)" as a flavoring agent or adjuvant and is covered in Title 21 of the US Code of Federal Regulations under section 184.1408 (Direct food substances affirmed as Generally Recognized As Safe, Licorice and licorice derivatives)
PART 184 -- DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE.
Subpart B - Listing of Specific Substances Affirmed as GRAS. Sec. 184.1408 Licorice and licorice derivatives.
(FDA, 2021, 2022).

Licorice extract (CAS RN 68916-91-6) is listed in the US EPA InertFinder Database (2021) as approved for food and non-food use pesticide products. For food use it is covered under 40 CFR section 180.

Pre-registered under REACH (“envisaged registration deadline 30 November 2010” for licorice, ext. (CAS RN 68916-91-6) and licorice, Glycyrrhiza glabra, ext. (CAS RN 84775-66-6); “envisaged registration deadline 31 May 2018” for oils, licorice (CAS RN 97676-23-8)). Glycyrrhiza glagra (licorice) root extract and Glycyrrhiza uralensis (licorice) root extract (no CAS RNs specified) are also pre-registered under REACH (“envisaged registration deadline 31 May 2018”).

As taken from ECHA.

Licorice, ext. (CAS RN 68916-91-6), licorice, Glycyrrhiza glabra, ext. (CAS RN 84775-66-6; EC/List no. 283-895-2), and oils, licorice (CAS RN 97676-23-8) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008. CAS RN 84775-66-6 (with EC/List no. 935-465-7 and 283-895-2) is also not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2021).

Licorice oil (CAS RN 97676-23-8) 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

Licorice extract (CAS RN 68916-91-6) “poses no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” and has been “identified as low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach”
Licorice, Glycyrrhiza glabra, extract CAS RN 84775-66-6) “poses no unreasonable risk to the environment based on Tier I assessment under the NICNAS IMAP assessment framework. Substance that is extracted from plants which is likely to be used in low quantities. The substance is a UVCB and may contain natural chemical compounds which have some toxic characteristics. However, because of degradation and low quantities of chemical compounds released to the environment from this source it is not expected to pose a concern.”

AICIS (2017).

Oils, licorice (CAS RN 97676-23-8) is listed by Australian Government Department of Health in the Australian Industrial Chemicals Introduction Scheme.

AICIS (undated)

Licorice, ext. (CAS RN 68916-91-6), licorice, Glycyrrhiza glabra, ext. (CAS RN 84775-66-6) and oils, licorice (CAS RN 97676-23-8) are included on the New Zealand Inventory of Chemicals. Licorice, ext. (CAS RN 68916-91-6) may be “used as a single component chemical under an appropriate group standard”, and licorice, Glycyrrhiza glabra, ext. (CAS RN 84775-66-6) and oils, licorice (CAS RN 97676-23-8) may be “used as a component in a product covered by a group standard but it is not approved for use as a chemical in its own right” (NZ EPA, 2006).

According to Health Canada’s Natural Health Products Ingredients Database, Glycyrrhiza glabra, Radix et Rhizoma Glycyrrhizae, Radix et Rhizoma Glycyrrhizae Preparata cum Melle and Glycyrrhiza uralensis (no CAS RNs listed) are classified as Natural Health Products (NHPs) under Schedule 1, item 1 (plant or plant material), and Extractum Glycyrrhizae and Extractum Glycyrrhizae Liquidum (no CAS RNs listed) are classified as NHPs under Schedule 1, item 2 (extract) of the Natural Health Products Regulations.

As taken from Health Canada, 2021.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

“This study was conducted to establish the multicomponent sequential metabolism (MSM) method based on comparative analysis along the digestive system following oral administration of licorice (Glycyrrhiza uralensis Fisch., leguminosae), a traditional Chinese medicine widely used for harmonizing other ingredients in a formulae. The licorice water extract (LWE) dissolved in Krebs-Ringer buffer solution (1 g/mL) was used to carry out the experiments and the comparative analysis was performed using HPLC and LC-MS/MS methods. In vitro incubation, in situ closed-loop and in vivo blood sampling were used to measure the LWE metabolic profile along the digestive system. The incubation experiment showed that the LWE was basically stable in digestive juice. A comparative analysis presented the metabolic profile of each prototype and its corresponding metabolites then. Liver was the major metabolic organ for LWE, and the metabolism by the intestinal flora and gut wall was also an important part of the process. The MSM method was practical and could be a potential method to describe the metabolic routes of multiple components before absorption into the systemic blood stream.” As taken from Zhang L et al. 2016. Biomed. Chromatogr. 30(6), 902-12. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26418123

“Traditional herbal remedies have been attracting attention as prospective alternative resources of therapy for diverse diseases across many nations. In recent decades, medicinal plants have been gaining wider acceptance due to the perception that these plants, as natural products, have fewer side effects and improved efficacy compared to their synthetic counterparts. Glycyrrhiza glabra L. (Licorice) is a small perennial herb that has been traditionally used to treat many diseases, such as respiratory disorders, hyperdipsia, epilepsy, fever, sexual debility, paralysis, stomach ulcers, rheumatism, skin diseases, hemorrhagic diseases, and jaundice. Moreover, chemical analysis of the G. glabra extracts revealed the presence of several organic acids, liquirtin, rhamnoliquirilin, liquiritigenin, prenyllicoflavone A, glucoliquiritin apioside, 1-metho-xyphaseolin, shinpterocarpin, shinflavanone, licopyranocoumarin, glisoflavone, licoarylcoumarin, glycyrrhizin, isoangustone A, semilicoisoflavone B, licoriphenone, and 1-methoxyficifolinol, kanzonol R and several volatile components. Pharmacological activities of G. glabra have been evaluated against various microorganisms and parasites, including pathogenic bacteria, viruses, and Plasmodium falciparum, and completely eradicated P. yoelii parasites. Additionally, it shows antioxidant, antifungal, anticarcinogenic, anti-inflammatory, and cytotoxic activities. The current review examined the phytochemical composition, pharmacological activities, pharmacokinetics, and toxic activities of G. glabra extracts as well as its phytoconstituents.” As taken from Batiha GE-S et al. 2020. Biomolecules 10(3), E352. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32106571/

“Liquorice [main ingredient, glycyrrhizin (GL)] is widely used as a food sweetener and herbal medicine. Occasionally, liquorice consumption causes pseudoaldosteronism as a side effect which causes oedema, hypokalaemia, and hypertension due to hyperactivity of mineral corticoid receptor. We aimed to detect GL metabolites in human blood and urine samples and to determine the pathological relationship between GL metabolites and pseudoaldosteronism. For this multi-centre, retrospective, cross-sectional study, we recruited patients who had visited Center for Kampo Medicine in Keio University Hospital, Department of Japanese Oriental (Kampo) Medicine in Chiba University Hospital, Clinic of Japanese Oriental (Kampo) Medicine in Kanazawa University Hospital, and Department of Oriental Medicine in Kameda Medical Center from November 2011 to July 2018. We collected laboratory data including concentration of serum potassium, plasma activity of renin and aldosterone, and residual blood and/or urine samples of participants who had experienced symptoms/signs of pseudoaldosteronism in the form of increase in blood pressure and occurrence or aggregation of oedema while taking liquorice-containing herbal preparations, and measured GL metabolites using a highly selective liquid chromatography tandem mass spectrometer system. We registered 97 participants (mean age 60 ± 15 years; male:female 14:83). 18β-glycyrrhetinic acid (GA) was detected in 67 serum samples (median 122 nM, range 5 nM-1.8 μM) and 18β-glycyrrhetyl-3-O-sulfate (compound 3) in 68 samples (median 239 nM, range 2 nM-4.2 μM). 3-Monoglucuronyl 18β-glycyrrhetinic acid, 22α-hydroxy-18β-glycyrrhetyl-3-O-sulfate-30-glucuronide, 22α-hydroxy-18β-glycyrrhetyl-3-O-sulfate, and GL itself were not or rarely detected. We could not find any correlation between blood pressure or peripheral oedema and serum concentration of GL metabolites.

Sulfotransferase 2A1 catalysed the metabolic reaction of GA to compound 3, a major GL metabolite in human blood. High serum concentration of compound 3 was related to lower renin, aldosterone, and potassium levels, suggesting a pathological relationship between compound 3 and liquorice-induced pseudoaldosteronism. This is the first study to identify the association between a novel metabolite, compound 3, and the incidence of pseudoaldosteronism, highlighting it as a promising biomarker." As taken from Takahashi K et al. 2019. Arch. Toxicol. 93(11), 3111-3119. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31605160/

4.2. Absorption, distribution and excretion

"The differences in gastrointestinal absorption behaviors of glycyrrhizin (GZ) between pure GZ and GZ in glycyrrhiza extract (GE) (equivalent dose as GZ) were examined in rats. Similarly to the case of pure GZ, both GZ and glycyrrhetic acid (GA) were detected in the plasma after oral administration of GE. However, the plasma concentration-time curves of GZ and GA after GE oral administration were much lower than those of pure GZ, indicating the marked reduction in bioavailability of GZ and as GA after this administration. To identify the GE components affecting the absorption of GZ, GZ was removed from GE and the effect of the remaining components on the gastrointestinal absorption process of GZ was examined. The lipophilic components of GE reduced the gastric emptying rate and the absorption of GZ from the small intestine, while these effects were not observed in the hydrophilic components. In contrast, the bioavailability of GZ as GA was increased by the hydrophilic components, but not the lipophilic ones. At least some of the factors in GE altering the bioavailability of GZ were identified."

As taken from Wang Z et al. Biol Pharm Bull. 1995 Sep; 18(9), 1238-41 available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8845813&query_hl=5&itool=pubmed_DocSum

"A study has shown that 18β-glycyrrhetinic acid crosses through the placental barrier and can be detected in the rat foetuses. Following feeding of dams with 100 mg 18β-glycyrrhetinic acid/kg/day commencing on the 13th day of gestation, on the 17th, 19th and 21st days of gestation the maternal plasma 18β-glycyrrhetinic acid concentrations were approximately 100 µg/ml, whereas the foetal concentrations were 5, 18 and 32 µ/ml, respectively" Taken from EMA 2012)

"Although various techniques have been employed to analyze drug metabolites, the metabolism of multi-component herbal medicine has seldom been fully addressed. In contrast to chemical drugs, a number of compounds in herbal medicine could get into circulation and then be metabolized. Moreover, these compounds may have metabolic interactions which make their pharmacokinetics (PK) even more complicated. The present work aims to elucidate the multi-component pharmacokinetics of a herbal medicine, and to demonstrate how PK behaviors were altered by co-existing constituents. Licorice (Glycyrrhiza uralensis Fisch.), a most commonly used herbal medicine, was chosen as a model. A strategy was proposed to compare the PK profiles of licorice extract with those of nine single compounds. These compounds were major bioactive constituents of licorice, and represented various structural types (flavanone, chalcone, isoflavone, saponin, and coumarin). We established a segmented selected reaction monitoring LC/MS/MS method to simultaneously monitor 63 licorice metabolites in rat plasma, and obtained the PK profiles of 55 metabolites. The results indicated that interactions among licorice compounds altered their PK behaviors in 4 aspects: improvement in bioavailability for aglycones (133- and 109-fold increase for liquiritigenin and isoliquiritigenin, respectively), prolongation in system circulation for glycosides (0.3h delay in T(max) for liquiritin apioside and isoliquiritin apioside), decrease of potential toxicity for saponins such as glycyrrhizic acid, and shift in plasma distribution for phase II metabolites. This is the first attempt to systematically reveal the in vivo process of licorice. Moreover, the study indicates noticeable interactions to alter pharmacokinetics among licorice compounds, which may be characteristic for

herbal medicines". As taken from Qiao X et al. 2012. J. Chromat. A 1258, 84-93. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22939378?dopt=AbstractPlus.

"To evaluate the safety and efficacy of Glycyrrhiza uralensis root produced using artificial hydroponic and artificial hydroponic-field hybrid cultivation systems, we investigated the pharmacokinetics of a major metabolite of glycyrrhizin (GL), glycyrrhetinic acid (GA). Hot water extracts obtained from the roots of the artificial hydroponic-field hybrid cultivated Glycyrrhiza uralensis were orally administered at a dose of 100 mg/kg as GL in mice and, compared with a commercial crude drug, Glycyrrhizae Radix. The temporal changes in serum GA concentration was found to depend on the GL concentration of the hot-water extracts. When hot-water extracts containing relatively high GL were administered, bimodal peaks appeared. In contrast, a broad single peak was detected when a hot-water extract containing relatively low GL content was administered. These tendencies in the serum GA concentration time course were observed for all samples, regardless of their derivation. Moreover, we compared the pharmacokinetic parameters and found that the Cmax and AUC0-48 values after oral administration of the extracts from Glycyrrhiza uralensis roots produced by the artificial cultivation system are within the range of variation for the commercial crude drugs. These results suggest the possibility that roots of Glycyrrhiza uralensis cultivated by the artificial hydroponic-field hybrid cultivation system can be used in addition to currently available commercial crude drugs produced from wild plant resources." As taken from Nose M et al. 2019. J. Nat. Med. 73(3), 661-666. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31028662/

4.3. Interactions

Effect of dietary soybean and licorice on the male F344 rat: an integrated study of some parameters relevant to cancer chemoprevention (Abstract). The individual and combined effects of dietary toasted soybean meal (3.13-25%) and dietary licorice root extract (0.38-3.0%) on selected liver and intestinal enzyme levels and on clinical chemistry and histopathological parameters were evaluated on the male F344 rats. All parameters were measured one and three months after the 50-day-old rats were started on the diets. By use of newly developed high-performance liquid chromatography-based analytic methods, measurable levels of daidzein (2.67 mug/ml) and glycyrrhetinic acid (7.87 mug/ml) were detected in the sera of rats on the 25% soybean and 3% licorice diets, respectively. Histopathological evaluations of organs and tissues yielded only nonsignificant strain-related changes. At all dosages, there were no significant soybean- or licorice-related anatomic lesions or hematologic changes. In the clinical biochemistry profile, soybean meal caused moderate but significant dose-dependent decreases in serum cholesterol and. As taken from Webb TE, Stromberg PC, Abou-Issa H, Curley RW Jr, Moeschberger M. Nutr Cancer. 1992; 18(3):215-30. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/1296195

"OBJECTIVE: To research the influence of glycyrrhiza extract on the pharmacokinetics characteristic parameters of daphnetin, which was aimed to explore the rationality of concert application of drugs. METHOD: The rats received intragastric administration of daphnetin and glycyrrhiza extract containing the same daphnetin respectively. The blood concentration of daphnetin was assayed by LC-MS. The data was processed by program DAS2.1.1. RESULT: Glycyrrhiza extract can reduce the t(1/2), tmax and Ke of daphnetin, while increased the Ka and AUC(0-infinity). CONCLUSION: Glycyrrhiza extract promoted the oral absorption of daphnetin, slowed down the elimination and increased the biological availability". As taken from Chen L et al. 2011. Zhongguo Zhong Yao Za Zhi. 36(7), 935-8. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21761739?dopt=AbstractPlus.

"Liquorice (root of Glycyrrhiza uralensis FISCH) is an ingredient of candies and used as a popular medicine in Europe and oriental countries. Cyclosporine (CsA), an immunosuppressant with narrow therapeutic window, is widely used in transplant patients. The absorption and disposition of CsA were associated with P-glycoprotein (P-gp) and cytochrome P450 3A4 (CYP3A4). This study investigated the effects of liquorice extract (LE) and its major ingredient, glycyrrhizin (GZ), on CsA

pharmacokinetics in rats. The results indicated that LE and GZ significantly decreased the peak blood concentration and the areas under the curves of CsA in rats. Mechanism studies revealed that glycyrrhetic acid (GA), the major metabolite of GZ, significantly activated the functions of P-gp and CYP3A4. In conclusion, liquorice significantly reduced the oral bioavailability of CsA through activating P-gp and CYP3A4". As taken from Hou YC et al. 2012. Food Chem. 135, 2307-2312. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22980806?dopt=AbstractPlus.

"Known drug interactions include potassium loss due to thiazide diuretics, as well as increased sensitivity to digitalis glycosides. [Leung, A.Y., Foster, S. Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. New York, NY. John Wiley & Sons, Inc. 1996., p. 348] **PEER REVIEWED**"

As taken from HSDB, 2002.

"The present study examined the effects of licorice on antioxidant defense, functional impairment, histopathology, and ultrastructural alterations in isoproterenol (ISP)-induced myocardial injury in rats. Myocardial necrosis was induced by two subcutaneous injection of ISP (85 mg/kg) at an interval of 24 h. Licorice was administered orally for 30 days in the doses of 100, 200, 400, or 800 mg/kg. ISP-treated rats showed impaired hemodynamics, left ventricular dysfunction, and caused depletion of antioxidants and marker enzymes along with lipid peroxidation from myocardium. ISP also induced histopathological and ultrastructural alterations in myocardium. Pretreatment with licorice prevented the depletion of endogenous antioxidants and myocyte injury marker enzymes, inhibited lipid peroxidation, and showed recovery of hemodynamic and ventricular functions. Licorice treatment also reduced myonecrosis, edema, and infiltration of inflammatory cells and showed preservation of subcellular and ultrastructural components. Our results demonstrate that licorice exerts cardioprotection by reducing oxidative stress, augmenting endogenous antioxidants, and restoring functional parameters as well as maintaining structural integrity." As taken from Oijha SK et al. 2015. Toxicol. Ind. Health 31(2), 140-52. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/23771872

"Inflammatory bowel disease (IBD), which includes conditions such as Crohn's disease and ulcerative colitis, is becoming more prevalent with the elderly being the fastest growing group. Parallel to this, there is an increasing interest in the use of complementary and alternative medicine (CAM). Nearly half of patients with IBD have used CAM at one time. The elderly patients, however, are burdened by comorbid conditions, polypharmacy, and altered functional status. With increasing use of complementary and alternative medicine in our elderly patients with IBD, it is vital for the provider to provide counsel on drug-herb potential interactions. CAM includes herbal products, diet, dietary supplements, acupuncture, and prayer. In this paper, we will review common CAM, specifically herbs, that are used in patients with IBD including the herb background, suggested use, evidence in IBD, and most importantly, potential interactions with IBD medications used in elderly patients. Most important evidence-based adverse events and drug-herb interactions are summarized. The herbs discussed include Triticum aestivum (wheat grass), Andrographis paniculata (chiretta), Boswellia serrata, tormentil, bilberry, curcumin (turmeric), Plantago ovata (blond psyllium), Oenothera biennis (evening primrose oil), germinated barley foodstuff, an herbal preparation of myrrh, chamomile and coffee extract, chios mastic gum, wormwood (absinthe, thujone), Cannabis sativa (marijuana, THC), tripterygium wilfordii (thunder god vine), Ulmus rubra (slippery elm bark), trigonella foenugraecum (fenugreek), Dioscorea mexicana (wild yam), Harpagophytum procumbens (devil's claw), ginger, cinnamon, licorice, and peppermint." As taken from Rahman H et al. 2017. Curr. Treat. Options Gastroenterol. 15(4), 618-636. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28918484

"The development of multi-drug resistance to existing anticancer drugs is one of the major challenges in cancer treatment. The over-expression of cytochrome P450 1B1 enzyme has been reported to cause resistance to cisplatin. With an objective to discover cisplatin-resistance reversal agents, herein, we report the evaluation of Glycyrrhiza glabra (licorice) extracts and its twelve chemical constituents for inhibition of CYP1B1 (and CYP1A1) enzyme in Sacchrosomes and live human cells.

The hydroalcoholic extract showed potent inhibition of CYP1B1 in both Sacchrosomes as well as in live cells with IC50 values of 21 and 16 µg/mL, respectively. Amongst the total of 12 constituents tested, quercetin and glabrol showed inhibition of CYP1B1 in live cell assay with IC50 values of 2.2 and 15 µM, respectively. Both these natural products were found to be selective inhibitors of CYP1B1, and does not inhibit CYP2 and CYP3 family of enzymes (IC50 > 20 µM). The hydroalcoholic extract of G. glabra and quercetin (4) showed complete reversal of cisplatin resistance in CYP1B1 overexpressing triple negative MDA-MB-468 breast cancer cells. The selective inhibition of CYP1B1 by quercetin and glabrol over CYP2 and CYP3 family of enzymes was studied by molecular modeling studies." As taken from Sharma R et al. 2017. Bioorg. Med. Chem. Lett. 27(24), 5400-5403. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29150398?dopt=AbstractPlus

"The potential of licorice dietary supplements to interact with drug metabolism was evaluated by testing extracts of three botanically identified licorice species (Glycyrrhiza glabra L., Glycyrrhiza uralensis Fish. ex DC. and Glycyrrhiza inflata Batalin) and 14 isolated licorice compounds for inhibition of 9 cytochrome P450 enzymes using a UHPLC-MS/MS cocktail assay. G. glabra showed moderate inhibitory effects against CYP2B6, CYP2C8, CYP2C9, and CYP2C19, and weak inhibition against CYP3A4 (testosterone). In contrast, G. uralensis strongly inhibited CYP2B6 and moderately inhibited CYP2C8, CYP2C9 and CYP2C19, and G. inflata strongly inhibited CYP2C enzymes and moderately inhibited CYP1A2, CYP2B6, CYP2D6, and CYP3A4 (midazolam). The licorice compounds isoliquiritigenin, licoricidin, licochalcone A, 18β-glycyrrhetinic acid, and glycycoumarin inhibited one or more members of the CYP2C family of enzymes. Glycycoumarin and licochalcone A inhibited CYP1A2, but only glycycoumarin inhibited CYP2B6. Isoliquiritigenin, glabridin and licoricidin competitively inhibited CYP3A4, while licochalcone A (specific to G. inflata roots) was a mechanism-based inhibitor. The three licorice species commonly used in botanical dietary supplements have varying potential for drug-botanical interactions as inhibitors of cytochrome P450 isoforms. Each species of licorice displays a unique profile of constituents with potential for drug interactions." As taken from Li G et al. 2017. Eur. J. Pharm. Sci. 109, 182-190. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28774812

"Glycyrrhiza glabra L. (licorice) is one of the most important medicinal plants, which is widely used throughout the world both in traditional and contemporary medical industries. This study was undertaken to investigate the potential genotoxic activity of G. glabra methanolic root extract, and its possible antigenotoxic properties against mitomycin C (MMC)-induced DNA damage in in vitro chromosome aberrations (CAs) and cytokinesis-block micronucleus (CBMN) assays in human peripheral blood lymphocytes (PBLs). Lymphocytes were treated with 25, 50, and 100 µg/ml G. glabra methanolic root extract alone as well as in combination with MMC (0.1 µg/ml) for 24 and 48 h treatment periods. It was found that there were no statistically significant differences between the negative control and the groups treated with all concentrations of G. glabra root extract of alone ($p > 0.05$), demonstrating the absence of genotoxic effects at both 24 and 48 h treatment periods. Besides, the co-treatment of G. glabra methanolic root extract and MMC significantly decreased the percentage of structural CAs and MN formation when compared with the culture treated with MMC alone ($p < 0.001$). In addition, the negative interaction factor (IF) values obtained for all combinations represent an antagonistic effect of G. glabra versus MMC. We can state that this extract acts as an antagonist and markedly decreased MMC-induced cytogenotoxicity. In conclusion, the present results demonstrate that in the tested experimental conditions, G. glabra methanolic root extract is not genotoxic in cultured human PBLs and has also antigenotoxic activity against MMC, which is widely used in chemotherapy against cancer." As taken from Yavuz Kocaman A & Güzelkokar M. 2018. Drug Chem. Toxicol. 15, 1-8. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29447011

"Ethnopharmacological relevance: Licorice (Gancao in Chinese, GC), the dried root and rhizome of Glycyrrhiza uralensis Fisch., Glycyrrhiza inflata Bat. or Glycyrrhiza glabra L., is an "essential herbal medicine" in traditional Chinese medicine (TCM). There is a classic traditional Chinese medicine theory says that "nine out of ten formulas contain licorice" and licorice is considered as one of the most important herbal medicine which can reduce toxicity and increase efficacy of certain herbal

medicine while it is combined application. In addition, it is a "medicine food homology" herbal medicine and also be widely used as a health food product and natural sweetener. However, no systematic literature review has been compiled to reveal its superiority. Herein, the aim of this work is to develop an overview of the state on phytochemicals, as well as effects of licorice in combination preparations, which can provide better understand the superiority of licorice and the special position in the application of TCM. Besides, ethnobotany, ethnopharmacological uses, quality control and toxicology of licorice have also been researched, which would provide reference for future clinical and basic research needs. Materials and methods: The information about licorice was collected from various sources including classic books about Chinese herbal medicine, and scientific databases including scientific journals, books, and pharmacopoeia. A total of 124 bibliographies, which are published from 1976 to 2019, have been searched and researched. Results: In this study, the interaction of chemical compounds between licorice and toxic herbal medicine, pharmacological effect of licorice, and the effect of licorice on pharmacokinetics of toxic compounds are considered as the main mechanisms underlying the effects of licorice in combination preparations. Besides, ethnobotany, ethnopharmacological uses and chemical constituents have been summarized. Conclusion: This work comprehensively reviews the state on ethnobotany, ethnopharmacological uses, phytochemicals, combined applications, quality control and toxicology of licorice. It will provide systematic insights into this ancient drug for further development and clinical use." As taken from Jiang M et al. 2020. J. Ethnopharmacol. 249, 112439. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31811935/

"Although compatibility is highly advocated in traditional Chinese medicine (TCM), inappropriate combination of some herbs may reduce the therapeutic action and even produce toxic effects. Kansui and licorice, one of TCM "Eighteen Incompatible Medicaments", are the most representative cases of improper herbal combination, which may still be applied simultaneously under given conditions. However, the potential mechanism of their compatibility and incompatibility is unclear. In the present study, two different ratios of kansui and licorice, representing their compatibility and incompatibility respectively, were designed to elucidate their interaction by comparative plasma/tissue metabolomics and a heatmap with relative fold change. As a result, glycocholic acid, prostaglandin F2a, dihydroceramide and sphinganine were screened out as the principal alternative biomarkers of compatibility group; sphinganine, dihydroceramide, arachidonic acid, leukotriene B4, acetoacetic acid and linoleic acid were those of incompatibility group. Based on the values of biomarkers in each tissue, the liver was identified as the compatible target organ, while the heart, liver, and kidney were the incompatible target organs. Furthermore, important pathways for compatibility and incompatibility were also constructed. These results help us to better understand and utilize the two herbs, and the study was the first to reveal some innate characters of herbs related to TCM "Eighteen Incompatible Medicaments"." As taken from Chen YY et al. 2019. J. Pharm. Anal. 9(5), 312-323. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31929940/

"The by-products of black licorice metabolism are toxic in high concentrations. Patients who consume large quantities of black licorice are at risk of developing an acquired syndrome of apparent mineralocorticoid excess. This presents clinically as hypertension, hypernatremia, and hypokalemia. Here, we present the unique case of a 74-year-old woman with a past medical history of neurogenic orthostatic hypotension, on fludrocortisone, who presented to the emergency department with asymptomatic hypokalemia (2.4 mmol/L) as detected in outpatient laboratory studies. During her hospital stay, it was discovered that the patient was consuming excessive amounts of black licorice. With this information, the synergistic interaction of fludrocortisone and black licorice was recognized as the cause of the patient's severe hypokalemia. The patient's fludrocortisone was stopped and she was treated with multiple courses of potassium repletion. Upon discharge, her fludrocortisone was discontinued, and she was prescribed midodrine to treat her neurogenic orthostatic hypertension. While small amounts of black licorice are safe, excessive licorice consumption can cause severe disease. Our case presents an opportunity to appreciate the plethora of etiologies for severe hypokalemia and the importance of taking a thorough patient history to avoid potentially fatal clinical

outcomes." As taken from Benge E et al. 2020. Cureus 12(11), e11656. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33391895/

5. Toxicity

5.1. Single dose toxicity

Species	Route	Dose data	Toxic effects	Reference
Rat	Oral	LD50: 14200 mg/kg bw	Gastrointestinal - hypermotility, diarrhea Kidney/Ureter/Bladder - other changes	OYYAA2 Oyo Yakuri. Pharmacometrics. (Oyo Yakuri Kenkyukai, CPO Box 180, Sendai 980-91, Japan) V.1- 1967- Volume(issue)/page/year: 14,535,1977
Rat	Intraperitoneal	LD50: 1420 mg/kg bw	Gastrointestinal - hypermotility, diarrhea Kidney/Ureter/Bladder - other changes	OYYAA2 Oyo Yakuri. Pharmacometrics. (Oyo Yakuri Kenkyukai, CPO Box 180, Sendai 980-91, Japan) V.1- 1967- Volume(issue)/page/year: 14,535,1977
Rat	Subcutaneous	LD50: 4200 mg/kg bw	Gastrointestinal - hypermotility, diarrhea Kidney/Ureter/Bladder - other changes	OYYAA2 Oyo Yakuri. Pharmacometrics. (Oyo Yakuri Kenkyukai, CPO Box 180, Sendai 980-91, Japan) V.1- 1967- Volume(issue)/page/year: 14,535,1977
Mouse	Oral	LD50: >- 7500 mg/kg bw	-	OYYAA2 Oyo Yakuri. Pharmacometrics. (Oyo Yakuri Kenkyukai, CPO Box 180, Sendai 980-91, Japan) V.1- 1967- Volume(issue)/page/year: 14,535,1977
Mouse	Intraperitoneal	LD50: 1500 mg/kg bw	Behavioral - convulsions or effect on seizure threshold Blood - changes in spleen	OYYAA2 Oyo Yakuri. Pharmacometrics. (Oyo Yakuri Kenkyukai, CPO Box 180, Sendai 980-91, Japan) V.1- 1967- Volume(issue)/page/year: 14,535,1977
Mouse	Subcutaneous	LD50: 4000 mg/kg bw	Behavioral - convulsions or effect on seizure threshold Blood - changes in spleen	OYYAA2 Oyo Yakuri. Pharmacometrics. (Oyo Yakuri Kenkyukai, CPO Box 180, Sendai 980-91, Japan) V.1- 1967- Volume(issue)/page/year: 14,535,1977

for CAS RN 68916-91-6 (Glycyrrhiza extract)

As taken from RTECS, 2016.

"Finney (1958), using albino mice of both sexes, reported an intraperitoneal LD of 308 mg/kg for glycyrrhetic 50 acid. Upon oral or subcutaneous administration, no deaths occurred with single doses as high as 610 mg/kg."

"In a report on the acute and subacute toxicity of Glycyrrhiza extract, it was reported that licorice powder containing 48-58% glycyrrhizin gave LD50 values of 4.0-4.4, 1.42-1.70, and 14.2-18.0 g/kg in rats and mice after 50 subcutaneous, intraperitoneal, and oral administration, respectively (Komiyama, 1977)."

"An acute toxicity assessment of licorice extract was performed by a single oral administration to Sprague-Dawley albino rats of various doses of an aqueous solution followed by a 14 day observation period. LD50 values of 3349 mg/kg (2679-4187; 95% confidence limits) for males, 2299 mg/kg (1752-3018) for females, and 2846 mg/kg (2321-3488) for combined sexes were determined."

“Tocco (1923) observed that when pigeons received subcutaneous doses of glycyrrhizin of from 450 to 500 mg/kg of body weight, they became diarrheic within an hour, and showed depression lasting about 24 hours. Guinea pigs receiving glycyrrhizin subcutaneously in doses of 1,000 mg/kg rapidly became depressed and diarrheic, showed decreased urinary volume, and died within 24 hours.In dogs, intravenous doses of glycyrrhizin of about 500 mg/kg were fatal. The same dose given subcutaneously produced only a slight depression for up to 3 hours; by the oral route, this dose produced almost no adverse reaction.”

“Probable oral lethal dose (human) 5-15 g, between 1 pint & 1 quart for 70 kg man (150 LB).”

As taken from HSDB, 2002.

“Licorice (Glycyrrhiza glabra) has been considered as an herbal drug since ancient time. Nowadays, it is a well-known spice that possesses worth pharmacological effects. However, some relevant articles have revealed negative impacts of licorice in health. By considering the great wishes in using herbal medicine, it is important to show adverse effects of herbal medicine in health. At present, there are misunderstandings toward the safety of herbal medicines. Herein, we gathered scientific research projects on the toxicity effects of licorice and glycyrrhizin to highlight their safety. In this regards, we categorized our findings about the toxicity effects of licorice and glycyrrhizin in acute, sub-acute, sub-chronic, and chronic states. Besides, we discussed on the cytotoxicity, genotoxicity, mutagenicity, and carcinogenicity of licorice and glycyrrhizin as well as their developmental toxicity. This review disclosed that G. glabra and glycyrrhizin salts are moderately toxic. They need to be used with caution during pregnancy. G. glabra and glycyrrhizin possess selective cytotoxic effects on cancerous cells. The most important side effects of licorice and glycyrrhizin are hypertension and hypokalemic-induced secondary disorders. Licorice side effects are increased by hypokalemia, prolonged gastrointestinal transient time, decreased type 2 11-beta-hydroxysteroid dehydrogenase activities, hypertension, anorexia nervosa, old age, and female sex.” As taken from Nazari S et al. 2017. Phytother. Res. 31(11), 1635-1650. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28833680

5.2. Repeated dose toxicity

Record for CAS RN 68916-91-6 (Glycyrrhiza extract):

Species	**Route**	**Dose data**	**Toxic effects**	**Reference**
Human - man	Oral	TDLo: 209 gm/kg/2Y (intermittent)	Peripheral Nerve and Sensation - spastic paralysis with or without sensory change Behavioral - muscle weakness Cardiac - EKG changes not diagnostic of specified effects	IJNSD3 Italian Journal of Neurological Sciences. (Masson Italia Periodici, Via Statuto 2/4, I-20121 Milan, Italy) V.1 n1-1979- Volume(issue)/page/year: 4,493,1983
Rat	Oral	TDLo: 37500 ug/kg/30D (continuous)	Liver - changes in liver weight Kidney/Ureter/Bladder - changes in bladder weight Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol)	OYYAA2 Oyo Yakuri. Pharmacometrics. (Oyo Yakuri Kenkyukai, CPO Box 180, Sendai 980-91, Japan) V.1- 1967- Volume(issue)/page/year: 14,535,1977
Rat	Oral	TDLo: 114 mg/kg/13W (continuous)	Liver - changes in liver weight Kidney/Ureter/Bladder - changes in bladder weight Endocrine - changes in thymus weight	OYYAA2 Oyo Yakuri. Pharmacometrics. (Oyo Yakuri Kenkyukai, CPO Box 180, Sendai 980-91, Japan) V.1- 1967- Volume(issue)/page/year: 14,535,1977

Mouse	Oral	TDLo: 2700 g/kg/90D (continuous)	Liver - changes in liver weight Nutritional and Gross Metabolic - weight loss or decreased weight gain Related to Chronic Data - death	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 31,343,1993
Human	Oral	TDLo: 79.9 mg/kg/8W (intermittent)	Kidney/Ureter/Bladder - proteinuria	TCPHP* Toxicology and Clinical Pharmacology of Herbal Products Melanie Johns Cupp ed., Humana press, 2000. Volume(issue)/page/year: - ,226,2000
Man	Oral	TDLo: 400 mg/kg/4D (intermittent)	Endocrine - androgenic Endocrine - other changes	NEJMAG New England Journal of Medicine. (Massachusetts Medical Soc., 10 Shattuck St., Boston, MA 02115) V.198- 1928- Volume(issue)/page/year: 341,1158,1999
Woman	Oral	TDLo: 2100 mg/kg/30D (intermittent)	Endocrine - other changes	STEDAM Steroids. (Holden-Day Inc., 4432 Telegraph Ave., Oakland, CA 94609) V.1- 1963- Volume(issue)/page/year: 69,763,2004

As taken from RTECS, 2016.

“Subjects who habitually eat large quantities of licorice candy (as little as 30-40 g/day) or who imbibe licorice-containing alcoholic beverages may present with some or all of the above in addn to edema, bigeminy, extrasystoles, paresis.”

“Subjects /who consume the candy as little as 30-40 g/day or licorice-containing alcoholic beverages/ may present with...quadriplegia, tetany, myoglobinuria, & convulsions.”

As taken from HSDB, 2002

According to an old report there was no evidence of adverse effects in 33 subjects who habitually consumed below 0.5 g glycyrrhizin per day (Nilon & Froment 1968). Severe metabolic disturbance is usually the result of long term ingestion of 1g daily or more of glycyrrhizic acid (Nielsen & Pedersen 1984). Myopathy with reversible myoglobinuria and muscular weakness (Cumming et al. 1977 & 1980; Lai et al. 1980) and arrhythmia leading to cardiac arrest (Bannister et al. 1977) have been documented, along with a case of extreme hypokalaemia due to licorice (Nielsen & Pedersen 1984). However, serious adverse effects including encephalopathy, hypertension and hypokalaemia, have been reported at intakes of between 50 and 400 mg glycyrrhizinic acid/day. The health of these patients recovered with treatment and cessation of licorice consumption (reviewed in SCF, 2003).

Recent studies in volunteers have established NOAELs for glycyrrhizinic acid of 130 mg/person/day (Bijlsma et al. 1996; Van Gelderen et al. 2000) and 217 mg/person/day (Bernardi et al. 1994). In the first of these studies, groups of 10 healthy women were given up to 4 mg/kg bw/day of pure glycyrrhizinic acid for 8 weeks in a placebo-controlled, double-blind design. In the latter study, Bernardi et al. gave daily “liquorice pills” containing up to 814 mg glycyrrhizinic acid to groups of 3 men and 3 women for 4 weeks. Both research groups reported that female participants were more sensitive to glycyrrhizinic acid than males (reviewed in SCF, 2003). Effects included increased sodium retention, reduction of plasma renin activity and aldosterone concentration, raised atrial natriuretic peptide, and decreased plasma potassium levels. Volume expansion, a possible precursor to the development of hypertension was also reported in the former study.

When guinea pigs and rats were administered 3 g crude licorice/kg bw/day, the treatment did not result in weight gain by either group of animals. Administration of glycyrrhetinic acid also failed to affect the weights of test animals compared to controls and the same doses did not prolong the survival time of adrenalectomized rats or guinea pigs (Card et al. 1953).

Effects of prolonged ingestion of graded doses of licorice by healthy volunteers (Abstract). Licorice can induce a hypermineralocorticoid syndrome. Current literature usually refers to the effects of sweets containing glycyrrhizin, but little is known about the consequences of a prolonged intake of "pure licorice". We administered graded daily doses of dried, aqueous extract of licorice root, containing 108, 217, 380 and 814 mg of glycyrrhizin, to 4 groups of 6 healthy volunteers of both sexes for 4 weeks. No significant effects occurred in groups 1 and 2. After 2 weeks, side effects leading to withdrawal from the protocol occurred in a female in group 3 (headache), a male with a family history of hypertension in group 4 (arterial hypertension), and a female also taking oral contraceptives in group 4 (hypertension, hypokalaemia and peripheral edema). In group 4, transient reduction in kalaemia and increase in body weight were found after 1 and 2 weeks, respectively. A depression of plasma renin activity occurred in groups 3 and 4. In healthy subjects, only the highest doses of licorice led to untoward effects. These were favoured by subclinical disease or oral contraceptives, and were less common and pronounced than what has been reported after the intake of glycyrrhizin taken as such or as a flavouring agent in confectionery products (Bernardi M et al.,1994).

“Licorice originates from the root of Glycyrrhiza glabra, which has a herbal ingredient, glycyrrhizic acid, and has a mineralocorticoid-like effect. Chronic intake of licorice induces a syndrome similar to that found in primary hyperaldosteronism. Excessive intake of licorice may cause a hypermineralocorticoidism-like syndrome characterized by sodium and water retention, hypertension, hypokalemia, metabolic alkalosis, low-renin activity, and hypoaldosteronism. In this case report, an association of hypokalemia, edema, and thrombocytopenia that is developed due to the excessive intake of licorice is presented. There are case reports in the literature, which suggest that toxicity findings may emerge with hyperaldosteronism-like manifestations such as hypokalemia, edema, and hypertension. However, any knowledge of thrombocytopenia as a resultant was not encountered among these reported toxic effects. Our case is important because it shows that the excessive intake of licorice may cause a toxic effect in the form of thrombocytopenia. This report is the first presented case to show thrombocytopenia due to licorice syrup consumption”. As taken from Celik MM et al. 2012. Human Expt. Tox. 31, 1295-1298. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22653692?dopt=AbstractPlus.

“A 50-year-old lady on hydrochlorothiazide (HCTZ) presented to the hospital after 4 days of generalized muscle aches and dark urine. She admitted to consuming one and a half bags of black licorice bites containing 2% natural licorice during the past 3 weeks. Examination showed high blood pressure, while labs revealed elevated creatine kinase, hypokalemia, hypocalcemia and hypophosphatemia with low aldosterone and plasma renin levels and high intact PTH. The active component of licorice is glycyrrhizic acid, which inhibits an enzyme required to convert cortisol to a less active metabolite, cortisone. This causes excess cortisol, simulating syndrome of apparent mineralocorticoid excess (AME), thus resulting in hypertension, hypokalemia and metabolic alkalosis. In our patient, licorice induced hypokalemia resulted in rhabdomyolysis. The rhabdomyolysis along with the effect of licorice led to secondary hypocalcaemia, which in turn triggered secondary hyperparathyroidism. This might have had a phosphaturic effect that caused hypophosphatemia, further worsening rhabdomyolysis. Conclusion: This case illustrates the complex relationship of various electrolytes, which can lead to self perpetuation of the disease, hence demanding more vigilance”. As taken from Shah M et al. 2012. Clin. Nephrol. 77, 491-495. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22595392?dopt=AbstractPlus.

“Licorice (Glycyrrhiza glabra) has been considered as an herbal drug since ancient time. Nowadays, it is a well-known spice that possesses worth pharmacological effects. However, some relevant articles have revealed negative impacts of licorice in health. By considering the great wishes in using herbal medicine, it is important to show adverse effects of herbal medicine in health. At present, there

are misunderstandings toward the safety of herbal medicines. Herein, we gathered scientific research projects on the toxicity effects of licorice and glycyrrhizin to highlight their safety. In this regards, we categorized our findings about the toxicity effects of licorice and glycyrrhizin in acute, sub-acute, sub-chronic, and chronic states. Besides, we discussed on the cytotoxicity, genotoxicity, mutagenicity, and carcinogenicity of licorice and glycyrrhizin as well as their developmental toxicity. This review disclosed that G. glabra and glycyrrhizin salts are moderately toxic. They need to be used with caution during pregnancy. G. glabra and glycyrrhizin possess selective cytotoxic effects on cancerous cells. The most important side effects of licorice and glycyrrhizin are hypertension and hypokalemic-induced secondary disorders. Licorice side effects are increased by hypokalemia, prolonged gastrointestinal transient time, decreased type 2 11-beta-hydroxysteroid dehydrogenase activities, hypertension, anorexia nervosa, old age, and female sex." As taken from Nazari S et al. 2017. Phytother. Res. 31(11), 1635-1650. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28833680

5.3. Reproduction toxicity

When the teratogenicity of ammoniated glycyrrhizic acid was evaluated in pregnant Sprague-Dawley rats, it was concluded that the ingredient exhibited some embryotoxicity to the developing rat foetus, but not the mother, and that the foetal effects were minor (Mantovani et al. 1988).

Species	**Test conditions**	**Effects**	**Reference**
No standard reproductive toxicity studies were identified.			
Groups of 10-20 men and women	Salivary testosterone concentration was measured in subjects consuming 5.6 g liquorice (containing 0.5 g glycyrrhizic acid) daily for 4 days.	Decrease (not statistically significant) in salivary testosterone concentration	Josephs et al. 2001
7 men	Serum testosterone concentration was measured in subjects consuming liquorice supplying 0.5 g glycyrrhizic acid daily for 4 days.	Serum testosterone concentration was reduced by 35%.	Armanini et al. 1999.
Pregnant Finnish women	Epidemiological studies. Glycyrrhizinic acid intake assessed by questionnaire. Data on course of pregnancy and birth was recorded.	Investigators reported an association between high liquorice consumption and an increased risk of preterm birth. No effects on birth weight, type of delivery, or maternal blood pressure were observed.	Strandberg et al. 2001, 2002 & 2003
Groups of 14-17 pregnant Wistar rats	Developmental toxicity study on disodium glycyrrhizinate, Given at 0, 60, 290 and 1480 mg /kg bw/day by gavage on days 0-20 of gestation. At day 20, 9-12 litters per group were killed and examined for external, skeletal and visceral malformations. The remaining animals were allowed to rear the offspring to 8 wk of age.	No effects on foetuses or pups. Maternal toxicity seen at mid- and high-doses.	Itami et al. 1985

Rats and mice	Ammonium glycyrrhizinate was tested in dominant lethal tests in mice and rats and a heritable translocation test in mice. Dietary exposures were up to the maximum tolerated dose (4% for 10 wk in rats, 2.25% for 8 wk in mice)	No effects on male fertility.	Sheu et al. 1986
Groups of 16-20 pregnant Sprague-Dawley rats	Ammonium glycyrrhizinate was administered at 0, 100, 1000 or 2500 mg/litre (0, 21, 239 and 680 mg/kg bw/day) in the drinking water on days 7-18 of gestation. Rats were killed on day 20.	No effects at up to 239 mg/kg bw/day. At 680 mg/kg bw/day, minor foetal effects (increase in skeletal variants) were observed.	Mantovani et al. 1988
Rats, hamsters and rabbits.	Developmental studies on ammonium glycyrrhizinate administered orally.	No maternal or foetal effects.	Food and Drug Research Laboratories, 1972

“The effect of water extract of licorice (Glycyrrhiza uralensis), one of the most widely used medicinal plants in Oriental nations and in Europe, on male reproductive function was investigated in rats. Licorice extract was prepared as in Oriental clinics and orally administered at doses of 500, 1,000 or 2,000 mg/kg, the upper-limit dose (2,000 mg/kg) recommended in the Toxicity Test guideline of the Korea Food and Drug Administration, to 6-week-old male rats for 9 weeks. Licorice extract neither induced clinical signs, nor affected the daily feed consumption and body weight gain. There were no significant changes in testicular weights, gross and microscopic findings, and daily sperm production between vehicle- and licorice-treated animals, in spite of slight decreases in prostate weight and daily sperm production at the high dose (2,000 mg/kg). In addition, licorice did not affect the motility and morphology of sperm, although the serum testosterone level tended to decrease without significant difference, showing a 28.6% reduction in the high-dose (2,000 mg/kg) group. The results suggest that the no observed adverse-effect level of licorice extract is higher than 2,000 mg/kg, the upper-limit dose, and that long-term exposure to licorice might not cause profound adverse effects”. Taken from Shin et al. (2008).

“The developmental toxicity of water extract of licorice (Glycyrrhiza glabra) was evaluated in rats. Licorice extract (500, 1,000 or 2,000 mg/kg) was dissolved in drinking water and orally administered to male rats from 9 weeks before mating to the day of copulation, and to females from 2 weeks before mating to gestational day 19. On gestational day 20, the animals were sacrificed for Cesarian section, and maternal and fetal abnormalities were examined. Licorice extract neither induce clinical signs, nor affect the body weight gain, feed and water intake, estrous cycle, copulation and fertility rates, blood 17beta-estradiol level and organ weights of dams. Also, the implantation and development .including body weights, absorption and death of embryos and fetuses were not influenced by in utero exposure to licorice. In addition, there were no increases in external, visceral and skeletal abnormalities of fetuses. Taken together, it is suggested that no observed adverse effect level of licorice extract is higher than 2,000 mg/kg, and that long-term in utero exposure to licorice might not cause developmental toxicities of embryos and fetuses.” Taken from Shin et al (2005).

“Effects of green tea and licorice extracts on fetal abnormalities induced by cyclophosphamide were investigated in rats. Pregnant Sprague–Dawley rats were orally administered with green tea or licorice extract (100 mg/kg) for 7 days, from days 6 to 12 of gestation, and intraperitoneally exposed to cyclophosphamide (11 mg/kg) 1 h after the final treatment. On day 20 of gestation, the maternal and fetal abnormalities were examined under Caesarian section. Cyclophosphamide reduced fetal and placental weights, and induced malformations in live fetuses; 94.6%, 41.5% and 100% of

external, visceral and skeletal defects, respectively. External malformations include cranial defect and exencephaly (64.3%), micrognathia and tongue extrusion (16.1%), limb defects (74.1%), and edema and hematoma (55.4%). Major visceral malformations were cleft palate (15.1%) and dilatation of ureter (15.1%). Skeletal malformations were the most-prominent abnormalities, showing cranial loss (98.3%), vertebral defects (69.5%), costal defects (47.5%) and delayed skeletal ossification (88.1%). Green tea extract further decreased the fetal body weights and remarkably enhanced fetal defects induced by cyclophosphamide, leading to 76.3% of cranial defect and exencephaly, 29.8% of micrognathia and tongue extrusion, 13.6% of renal pelvic dilatation, 25.8% of ureteric dilatation, 75.4% of vertebral defects, 56.9% of costal defects, and 92.3% of delayed skeletal ossification. Licorice extract also further decreased the fetal body weights and markedly enhanced fetal defects, resulting in 76.4% of cranial defect and exencephaly, 22.7% of micrognathia and tongue extrusion, 85.5% of vertebral defects, 85.5% of costal defects, and 100% of delayed skeletal ossification. The results suggest that repeated pretreatment with green tea or licorice extract may aggravate body weight loss and malformations of fetuses induced by intrauterine exposure to cyclophosphamide." Taken from Jeon et al (2007).

"BACKGROUND: Since cyclophosphamide is metabolically activated to teratogenic acrolein and cytotoxic phosphoramide mustard by cytochrome P-450 type 2B (CYP2B), we assessed the effects of licorice, a CYP2B inducer, on the fetal defects induced by cyclophosphamide.

METHODS: Pregnant Sprague-Dawley rats were daily administered with licorice (100 mg/kg) by gavage for 7 days, from the 6th to 12th day of gestation, and intraperitoneally administered with cyclophosphamide (11 mg/kg) 1 hr after the final licorice treatment. On the 20th day of gestation, maternal and fetal abnormalities were determined by Cesarian section.

RESULTS: Cyclophosphamide was found to reduce fetal and placental weights without increasing resorption or death. In addition, it induced malformations in live fetuses; 93.8, 41.1, and 100% of the external (skull and limb defects), visceral (cleft palate and ureteric dilatation), and skeletal (acrania, vertebral/costal malformations, and delayed ossification) abnormalities, respectively. When pre-treated with licorice, cyclophosphamide-induced body weight loss and abnormalities of fetuses were remarkably aggravated. Moreover, repeated treatment with licorice greatly increased mRNA expression and activity of hepatic CYP2B.

CONCLUSIONS: The results indicate that repeated intake of licorice may aggravate cyclophosphamide-induced body weight loss and malformations of fetuses by upregulating CYP2B." Taken from Park et al. (2011).

"In developmental toxicity studies, glycyrrhizin (ammonium salt) exhibited some embryotoxicity to the developing rat foetus, but the foetal effects were considered as minor. These effects were shown at the dose of 100 and 250 mg/kg of ammonium glycyrrhizin from 7th to 20th day of pregnancy (soft-tissue abnormalities, mostly renal, and external haemorrhages) and at the dose of 1000 mg/kg of 18β-glycyrrhetinic acid from the 13th day of gestation (significant reduction in lamellar body content of lungs and reduced number alveolar lamellar body and surfactant clusters, but no apparent increase in malformation or foetal death rate)" (As taken from EMA 2012)

"Maternal intake of licorice from dietary sources has been associated with adverse maternal and fetal outcomes. We prospectively studied the outcome of 185 singleton pregnancies who took over-the-counter or naturopathic formulations containing licorice during their pregnancy, and 370 age-matched singleton pregnant controls that were not exposed to any potential teratogen. The indication in 56.8% of the women taking licorice was for cough and cold control, with the maximum dose of 2104 mg/day and exposure occurring between the 4th day and 25th week of gestation. The rate of stillbirths was

marginally higher among women who took licorice than those who did not (OR = 7.9; 95% CI 0.9-71.5; p = 0.048), and significantly higher when compared to the general population in the Republic of Korea (OR = 13.3; 95% CI 4.9-35.8; p < 0.001). Other fetal outcomes assessed in the study were similar between the two study groups, e.g., the OR of major malformations was 3.9 (95% CI 0.4-43.5; p = 0.27). In conclusion, the present study suggests that licorice is not a major teratogen. However, whether licorice may increase the risk of stillbirths requires careful consideration in further studies with a larger sample size". As taken from Choi JS et al. 2013. Planta Med. 79, 97-103. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23299757?dopt=AbstractPlus.

"Carbendazim is a broad spectrum carbamate fungicide used in the control of various fungal pathogens. Licorice (Glycyrrhiza glabra) is one of the widely used medicinal plants in oriental nations. The present work studied the effect of licorice aqueous extract on carbendazim-induced testicular toxicity in albino rats. Administration of carbendazim induced significant decrease in testis weight, diameter, and germinal epithelial height of the seminiferous tubules. Histological results revealed degeneration of seminiferous tubules, loss of spermatogenic cells, and apoptosis. Moreover, carbendazim caused elevation of testicular malondialdehyde (MDA), marker of lipid peroxidation, and reduced the activity of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT). Coadministration of licorice extract with carbendazim improved the histomorphological and histopathological changes observed in animals treated with carbendazim. In addition, licorice treatment leads to a significant decrease in the level of MDA and increase in the activities of SOD and CAT. According to the present results, it is concluded that licorice aqueous extract can improve the testicular toxicity of carbendazim and this effect may be attributed to antioxidant properties of one or more of its constituents." As taken from Sakr SA & Shalaby SY. 2014. Toxicol. Ind. Health 30(3), 259-67. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/22903170?dopt=AbstractPlus

"Licorice (glycyrrhiza uralensis) is known as an herb with detoxication, and it has been widely used in clinical prescription of Oriental herbal medicine. Studies on the effects of licorice in the reproductive system were very rare, especially in spermatogenesis. In order to elucidate the effects of licorice on spermatogonial proliferation and spermatocyte differentiation during neonatal mice spermatogenesis, the organ culture model of testis tissue from neonatal C57BL/6N mice (born 6 d) was established. Then, in the presence of licorice extract (LE), the proliferation activity of spermatogonia was identified with the positive rate quantitative analysis of 5-bromo-2-deoxyuridine (BrdU) and anti-proliferating cell nuclear antigen (PCNA) antibody by immunohistochemical staining. The results showed that, compared to the control group, the percentage of positive cells by BrdU staining enhanced dramatically and that the expression of PCNA protein increased significantly in the spermatogonia from the LE group and showed a concentration-dependent manner ($P < 0.05$). This indicated that the LE can significantly promote the proliferation of spermatogonia in the spermatogenesis of neonatal mice. Furthermore, proteins related to spermatocyte differentiation, synaptonemal complex protein 3 (SCP3) and meiotic recombinant protein Spo11, were detected by immunohistochemical staining. The results showed that the differentiated spermatocyte in the LE group was significantly increased compared with that of the control group and showed a concentration-dependent manner ($P < 0.05$). The above results suggested that the LE can significantly accelerate the proliferation of spermatogonia and the differentiation of spermatocytes in the testicular tissue of the neonatal mice, which may be a potential drug for male infertility." As taken from Wang C et al. 2016. In Vitro Cell. Dev. Biol. Anim. 52(2), 149-55. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26676954

"Earlier puberty, especially in girls, is associated with physical and mental disorders. Prenatal glucocorticoid exposure influences the timing of puberty in animal models, but the human relevance of those findings is unknown. We studied whether voluntary consumption of licorice, which contains glycyrrhizin (a potent inhibitor of placental 11β-hydroxysteroid dehydrogenase type 2, the "barrier" to maternal glucocorticoids), by pregnant women was associated with pubertal maturation (height, weight, body mass index for age, difference between current and expected adult height, Tanner staging, score on the Pubertal Development Scale), neuroendocrine function (diurnal salivary

cortisol, dexamethasone suppression), cognition (neuropsychological tests), and psychiatric problems (as measured by the Child Behavior Checklist) in their offspring. The children were born in 1998 in Helsinki, Finland, and examined during 2009-2011 (mean age = 12.5 (standard deviation (SD), 0.4) years; n = 378). Girls exposed to high maternal glycyrrhizin consumption (≥500 mg/week) were taller (mean difference (MD) = 0.4 SD, 95% confidence interval (CI): 0.1, 0.8), were heavier (MD = 0.6 SD, 95% CI: 0.2, 1.9), and had higher body mass index for age (MD = 0.6 SD, 95% CI: 0.2, 0.9). They were also 0.5 standard deviations (95% CI: 0.2, 0.8) closer to adult height and reported more advanced pubertal development ($P < 0.04$). Girls and boys exposed to high maternal glycyrrhizin consumption scored 7 (95% CI: 3.1, 11.2) points lower on tests of intelligence quotient, had poorer memory ($P < 0.04$), and had 3.3-fold (95% CI: 1.4, 7.7) higher odds of attention deficit/hyperactivity disorder problems compared with children whose mothers consumed little to no glycyrrhizin (≤249 mg/week). No differences in cortisol levels were found. Licorice consumption during pregnancy may be associated with harm for the developing offspring." As taken from Räikkönen K et al. 2017. Am. J. Epidemiol. 185(5), 317-328. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28158597?dopt=AbstractPlus

"The potentially deleterious effects on offspring health of excess maternal stress in pregnancy are important to understand-both whether observed associations are causal and through what mechanisms their effects may exert an influence. In this issue of the Journal, Räikkönen et al. (Am J Epidemiol. 2012;000(0):000-000) provide an ingenious test of a potential pathway through which maternal stress may influence offspring development. Licorice consumption is known to disrupt the ability of the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 to inactivate cortisol before it reaches the fetus, leading to higher levels of cortisol exposure. Higher levels of cortisol exposure are also hypothesized to underlie the mechanism through which maternal stress may disrupt fetal development. Thus licorice consumption may serve, in some ways, to mimic maternal stress. The authors report associations between heavy licorice consumption during pregnancy and a wide range of offspring outcomes, including changes in pubertal timing, intelligence quotient, and mental health. In our view, these results should be considered preliminary; more work needs to be completed to determine the relationship of prenatal licorice consumption to these outcomes. Nonetheless, these intriguing and suggestive results demonstrate that this line of work should be given high priority, and they set the stage for additional research moving forward." As taken from Keyes KM & Susser E, 2017. Am. J. Epidemiol. 185(5), 329-332. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28158433?dopt=AbstractPlus

"Licorice (Glycyrrhiza glabra) has been considered as an herbal drug since ancient time. Nowadays, it is a well-known spice that possesses worth pharmacological effects. However, some relevant articles have revealed negative impacts of licorice in health. By considering the great wishes in using herbal medicine, it is important to show adverse effects of herbal medicine in health. At present, there are misunderstandings toward the safety of herbal medicines. Herein, we gathered scientific research projects on the toxicity effects of licorice and glycyrrhizin to highlight their safety. In this regards, we categorized our findings about the toxicity effects of licorice and glycyrrhizin in acute, sub-acute, sub-chronic, and chronic states. Besides, we discussed on the cytotoxicity, genotoxicity, mutagenicity, and carcinogenicity of licorice and glycyrrhizin as well as their developmental toxicity. This review disclosed that G. glabra and glycyrrhizin salts are moderately toxic. They need to be used with caution during pregnancy. G. glabra and glycyrrhizin possess selective cytotoxic effects on cancerous cells. The most important side effects of licorice and glycyrrhizin are hypertension and hypokalemic-induced secondary disorders. Licorice side effects are increased by hypokalemia, prolonged gastrointestinal transient time, decreased type 2 11-beta-hydroxysteroid dehydrogenase activities, hypertension, anorexia nervosa, old age, and female sex." As taken from Nazari S et al. 2017. Phytother. Res. 31(11), 1635-1650. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28833680

"OBJECTIVE: To report the incidence and nature of herbal medicinal products' adverse events and herb-drug interactions used by some pregnant and postnatal women. DATA SOURCES: The Allied

and Complementary Medicine Database, the Cumulative Index to Nursing and Allied Health Literature, EMBASE, the Cochrane Library, MEDLINE, Scopus, Web of Science, and ClinicalTrials.gov were searched from inception until August 2018. METHODS OF STUDY SELECTION: Any studies reporting adverse events, herb-drug interactions or absence thereof associated with herbal medicinal products used during pregnancy or the postnatal period were included. Conference abstracts, pilot studies, and nonhuman studies were excluded. All included studies were critically appraised by two independent reviewers. TABULATION, INTEGRATION AND RESULTS: Database searches retrieved 3,487 citations. After duplicate removal and review of titles, abstracts, and full-text, 115 articles were critically appraised. After excluding irrelevant and low-quality articles, 74 articles were included for data extraction and synthesis. Adverse drug reactions, congenital malformations, fetal growth retardation or herb-drug interactions were the primary study objective reported by 19 of the 74 included studies, 16 cohort studies, one cross-sectional survey, and two randomized controlled trials. A total of 47 herbal medicinal products and 1,067,071 women were included in this review. Use of almond oil was associated with preterm birth (odds ratio 2.09, 95% CI 1.07-4.08), oral raspberry leaf was associated with cesarean delivery (adjusted odds ratio [AOR] 3.47, 95% CI 1.45-8.28); heavy licorice use was associated with early preterm birth by 3.07-fold (95% CI 1.17-8.05). African herbal medicine mwanaphepo was associated with maternal morbidity (AOR 1.28; 95% CI 1.09-1.50), and neonatal death or morbidity. Fourteen studies reported absence of adverse events. Four studies reported herb-drug interactions, but none studied adverse events arising from them. CONCLUSION: The use of herbal medicinal products during pregnancy and the postnatal period should be discouraged until robust evidence of safety is available." As taken from Muñoz Balbontín Y et al. 2019. Obstet. Gynecol. 133(5), 920-932. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30969204

"The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) has at the request of the Norwegian Food Safety Authority (Mattilsynet, NFSA) identified and characterized potential adverse effects to the fetus and long-term effects to the child that can result from maternal consumption of glycyrrhizic acid from liquorice, including at which doses these adverse effects appeared.

The human studies included in this assessment of effects on the fetus or child after their mother's intake of liquorice during pregnancy reported that high glycyrrhizin exposure versus a lower exposure did not significantly affect birth weight or maternal blood pressure (Strandberg et al., 2001). However, high glycyrrhizin exposure was significantly associated with shorter gestational duration (Strandberg et al., 2001), more than twofold increased risk of preterm (<37 weeks) delivery, and an even stronger association with early preterm (<34 weeks) delivery (Strandberg et al., 2002). When the children reached a mean age of 8.1 years, they were reported to have poorer cognitive performance, externalising symptoms and attention problems after high exposure (Räikkönen et al., 2009). They also had higher salivary cortisol awakening peak, salivary cortisol awakening slope, salivary cortisol awakening AUC and baseline TSST-C salivary cortisol levels (Räikkönen et al., 2010). At mean age 12.5 years, girls, but not boys, were taller, heavier and had higher body mass index for age, were closer to adult height and had more advanced pubertal development (Räikkönen et al., 2017). Both girls and boys scored lower on tests of intelligence quotient, had poorer memory and had higher odds of attention deficit/hyperactivity disorder problems.

In VKM's opinion, an inhibitory effect on the 11ß-hydroxysteroid dehydrogenase type 2 (11βHSD2) enzyme by glycyrrhizic acid, leading to overexposure of the fetus to cortisol, is a plausible mechanism for the adverse effects reported in the human studies in this assessment. The findings in these studies are indicative of potential adverse effects of glycyrrhizic acid on the offspring from liquorice intake during pregnancy. However, the levels of exposure of the fetus to glycyrrhizic acid are too uncertain based on the available data to be able to draw firm conclusions on cause and effects relationships. One of the uncertainties is the actual intake of glycyrrhizic acid by the mothers during pregnancy.

Based on the studies by Strandberg et al. (2001; 2002) and Räikkönen et al. (2009; 2010; 2017), the negative health effects on the mothers or their fetus or child were found with glycyrrhizin intake ≥500 mg/week, corresponding to approximately 250 g/week of liquorice, compared with lower intake (0-499 mg/week). Therefore, from these studies 500 mg/week (71.4 mg/day) of glycyrrhizin, which corresponded to average 13.7 mg/kg bw for the mothers at delivery, can be regarded as the lowest observed adverse effect level (LOAEL) (Räikkönen et al., 2017). This intake is lower than 100 mg/day suggested as a safe level in several previous risk assessments. However, this external dose level is uncertain because of inherent weaknesses in these studies as discussed in this assessment. Several toxicokinetic factors affect the internal dose of glycyrrhetic acid that eventually reach the placenta, thus determining whether the actual level of glycyrrhetic acid is sufficient to inhibit the placental 11β-HSD2 enzyme.

In these above-mentioned human studies, no recording of glycyrrhizin intake in various parts of the pregnancy was done. Thus, there is also uncertainty regarding whether the exposure to glycyrrhizin occurred in critical periods during pregnancy relevant for the effects on puberty, cortisol levels, cognitive performance, psychiatric symptoms etc. observed in the children.

VKM concludes that because of the large uncertainty associated with the relationship between the exposure dose and the observed adverse effects, a safe level cannot be established with certainty for glycyrrhizic acid or for the amount of liquorice that the pregnant mothers can consume without causing negative effects on the fetus or child."

As taken from VKM, 2018.

5.4. Mutagenicity

Genotoxicity	[+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation]			
In vivo				
NOTE: In intact mammals, glycyrrhizinic acid is hydrolysed to glycyrrhetic acid prior to absorption from the gastrointestinal tract. Therefore, both compounds have been administered in in vivo genotoxicity tests to study the genotoxicity of the former. Conversion is probably not as effective in vitro (SCF, 2003). The SCF has concluded that, based on all available data, glycyrrhizinic acid and glycyrrhetic acid are considered to be non-genotoxic (SCF, 2003).				
Species	**Test conditions**	**Endpoint**	**Result**	**Reference**
Mouse (6 males per group)	DISODIUM GLYCYRRHIZINATE Single i.p. injection of 0, 17.5, 35, 70 or 140 mg/kg bw. Examination of bone marrow for micronuclei at 24 hr. No deaths occurred.	Chromosome damage	-ve	Hayashi et al. 1988
Mouse (6 males per group)	TRISODIUM GLYCYRRHIZINATE Single i.p. injection of 0, 250, 500, 1000 or 2000 mg/kg bw. Examination of bone marrow for micronuclei at 24 hr. At the top dose, 4/6 mice died.	Chromosome damage	-ve	Hayashi et al. 1988
Mouse (6 males per group)	TRISODIUM GLYCYRRHIZINATE Daily i.p. injection of 0 or 500 mg/kg bw for 4 days, examination of bone	Chromosome damage	-ve	Hayashi et al. 1988

	marrow for micronuclei 24 hr after last dose.				
Mouse (4 males per group)	GLYCYRRHIZIN Single oral dose of 0 or 2000 mg/kg bw was given orally in a Comet assay. Animals were sacrificed at 3 and 24 hr post-treatment, and 8 organs (glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow) examined for DNA damage.	DNA damage	-ve	Sasaki et al. 2002	
Mice (males)	AMMONIUM GLYCYRRHIZINATE A review (SCF, 2003) briefly mentioned a dominant lethal assay in mice fed diets containing up to 2.25% (about 2 g/kg bw/day) for 8 wk. Presumably the males were then mated with untreated females, which were then examined for early foetal deaths	Germ cell mutation	-ve	Sheu et al. 1986	
Mice	AMMONIUM GLYCYRRHIZINATE A review (SCF, 2003) briefly mentioned a heritable translocation assay in mice fed diets containing up to 2.25% (about 2 g/kg bw/day) for 8 wk.	Germ cell mutation	-ve	Sheu et al. 1986	
Rats (males)	AMMONIUM GLYCYRRHIZINATE A review (SCF, 2003) briefly mentioned a dominant lethal assay in rats fed diets containing up to 4% (about 2 g/kg bw/day) for 10 wk. Presumably the males were then mated with untreated females, which were then examined for early foetal deaths	Germ cell mutation	+ve	Jorgenson et al. 1977; Sheu et al. 1986	
In vitro					
Test system	Test conditions	Endpoint	Activation	Result	References
Mouse lymphoma cells	Only published as an abstract, no further data given.	Somatic cell mutation	with and without S9	+ve	Heck et al. 1989
Rat hepatocytes	Unscheduled DNA synthesis	DNA damage (indicative test)	Not applicable	-ve	Heck et al. 1989
Chinese hamster lung fibroblast cells	DISODIUM and TRISODIUM GLYCYRHHIZINATES Cells incubated for 48 hr, examined for chromosome aberrations and polyploidy.	Chromosome damage and changes in chromosome numbers	Without	+ve (damage, both salts) -ve (number changes)	Ishidate et al. 1984
Salmonella typhimurium strains TA92, TA94, TA98,	DISODIUM and TRISODIUM GLYCYRHHIZINATES Ames test up to 5 (disodium) or 10 (trisodium) mg/plate. Good quality studies	Mutation	With and without S9	-ve	Ishidate et al. 1984

TA100, TA1535, TA1537					
Salmonella typhimurium strains TA97, TA98, TA100	LICORICE POWDER and AMMONIUM GLYCYRRHIZINATE Ames test, at up to 0.5 mg/ml, Only reported as an abstract, no further details given.	Mutation	With and without S9	-ve	Cooper & Berry, 1988
Salmonella typhimurium, 5 strains	Ames test on licorice. No details given, only an abstract was published	Mutation	with and without S9	-ve	Heck et al. 1989
Bacillus subtilis strains H17Rec+ and M45Rec-	Spore rec assay. Licorice extract and "pigment" were tested at ≤10mg/disk	DNA damage	with and without S9	-ve	Ishizaki et al. 1985
Bacillus subtilis strains H17 and M45	Rec assay using 100 g glycyrrhiza extract/L	DNA damage	with and without S9	+ve	Morimoto et al. 1982.
Various – see next column	GLYCYRRHIZIN Glycyrrhizin (and its di- and trisodium salts) induced chromosome aberrations in Chinese hamster lung cells (-S9). Negative results were obtained with the sodium salts in Salmonella typhimurium (Ames, +/- S9), Escherichia coli WP2 (+/-S9), hamster and human lung fibroblasts (micronuclei/SCE) and Bacillus subtilis) (the rec assay).	without	+ve -ve	Fujita & Sasaki, 1986; Ishidate, 1983; Ishidate et al. 1988; Kawachi et al. 1980 & 1981; Sasaki et al. 1980 Ishizaki et al. 1985; Prival et al. 1991.	
Antimutagenicity					
Licorice extract and several constituents and related materials have given evidence of antimutagenic potential.			Ikken et al. 1999; Reviewed in Wang et al. 2000 and Wang and Nixon, 2001.		

"Glycyrrhiza glabra Linn. (licorice) is widespread throughout the Mediterranean region and certain areas of Asia. Historically, the dried rhizome and root of the plant were used by the Chinese, Egyptian, Greek, Indian, and Roman civilizations as expectorant and carminative. In the modern medicinal system, licorice is used to treat liver ailments, dyspepsia, bronchitis, rheumatoid arthritis etc. Despite the extensive pharmacological applications, the genotoxic potential of G. glabra extract (GutGardTM) has not been evaluated. Hence, this study was conducted to investigate the genotoxic potential of GutGardTM using battery of in vitro test systems: bacterial reverse mutation test (Ames IITM), chromosome aberration (CA) and micronucleus (MN) tests. GutGard™ did not show significant increase in number of revertant colonies in Salmonella typhimurium strains (TA98 and TAMix) with/without S9 fraction. In CA and MN studies, GutGardTM did not show clastogenic effect at 4 and 18 h treatments with and without S9 fraction. Results indicated that GutGard™ is not mutagenic in a battery of genotoxicity tests". As taken from Chandrasekaran CV et al. 2011. Reg. Tox. Pharmacol. 61, 373-380. Available at http://www.sciencedirect.com/science/article/pii/S0273230011001954.

"BACKGROUND: The chemopreventive effects of certain phytoconstituents can be exploited for their use as functional foods, dietary supplements and even as drugs. The natural compounds, acting as

anti-genotoxic and free radical scavenging compounds, may serve as potent chemo-preventive agents. These can inhibit DNA modulatory activities of mutagens and help preventing pathological processes. OBJECTIVES: Present study on Glycyrrhiza glabra L., a promising medicinal plant, widely used in traditional medicine, focused on the bioassay-guided fractionation of its extracts for the isolation of certain phytochemicals with anti-genotoxic potential against oxidative mutagens. MATERIALS AND METHODS: The methanol extract of Glycyrrhiza glabra rhizomes was subjected to column chromatography, and isolated fraction was evaluated for its anti-genotoxic and antioxidant potential using SOS chromotest, Comet assay, and DPPH radical scavenging assay. RESULTS: GLG fraction, which was characterized as Glycyrrhizic acid, inhibited the genotoxicity of oxidative mutagens viz., H(2)O(2) and 4NQO quite efficiently. In SOS chromotest, using E.coli PQ37 tester strain, it inhibited induction factor induced by H(2)O(2) and 4NQO by 75.54% and 71.69% at the concentration of 121.46 µM, respectively. In Comet assay, it reduced the tail moment induced by H(2)O(2) and 4NQO by 70.21% and 69.04%, respectively, at the same concentration in human blood lymphocytes. The isolated fraction also exhibited DPPH free radical scavenging activity and was able to scavenge 85.95% radicals at a concentration of 120 µM. CONCLUSION: Glycyrrhizic acid is a potential modulator of genotoxins as well as efficient scavenger of free radicals". As taken from Kaur P et al. 2012. Pharmacognosy Res. 4, 189-195. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23225961.

"Cadmium is a modern environmental contaminant that is toxic and carcinogenic. Glycyrrhiza glabra is a traditional medicinal herb which grows in the various parts of the World. Recent studies demonstrated that G. glabra has antifungal, antimicrobial, antioxidant, and powerful antiinflammatory features. The purpose of this study was to investigate the genetic safety of extracts from G. glabra and its effects on cadmium (as CdCl(2)) induced genotoxicity. Therefore we evaluated the capability of G. glabra extract to inhibit the rate of micronucleus (MN), sister chromatid exchange (SCE) formations induced by CdCl(2). Moreover, to assess the effects of G. glabra on cell viability and oxidative status, we performed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and total antioxidant capacity (TAC) assays. Our results showed that there were significant increases ($P < 0.05$) in both SCE and MN frequencies of cultures treated with CdCl(2) (5 ppm) as compared to controls. However, co-application of G. glabra extract (5, 10 and 20 ppm) and CdCl(2) resulted in decreases of MN and SCE rates as compared to the group treated with CdCl(2) alone. Again, the results of MTT and TAC assays clearly indicated dose dependent ameliorative effects of G. glabra extracts against CdCl(2) toxicity. In conclusion, this study demonstrated for the first time that G. glabra extracts provided increased resistance of DNA against CdCl(2) induced genetic and oxidative damage in human lymphocytes. So, the risk on target tissues of CdCl(2) could be reduced and ensured early recovery from its toxicity". As taken from Dirikan E & Turkez H. 2014. Cytotechnology 66(1), 9-16. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23325115)

CAS RN 84775-66-6:

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA98
Metabolic Activation:	None
Method:	Preincubation

Dose:	0; 1.22; 2.44; 4.88; 9.77;19.5; 39.1; 78.1 ug/plate
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA1537
Metabolic Activation:	None
Method:	Preincubation
Dose:	0; 1.22; 2.44; 4.88; 9.77;19.5; 39.1; 78.1 ug/plate
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA100
Metabolic Activation:	None
Method:	Preincubation
Dose:	0; 78.1; 156; 313; 625; 1,250; 2,500; 5,000 ug/plate
Results:	Negative

Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA1535
Metabolic Activation:	None
Method:	Preincubation
Dose:	0; 78.1; 156; 313; 625; 1,250; 2,500; 5,000 ug/plate
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA1535
Metabolic Activation:	Rat, Liver, S-9, Phenobarbital and 5,6-benzoflavone
Method:	Preincubation
Dose:	0; 78.1; 156; 313; 625; 1,250; 2,500; 5,000 ug/plate
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008

Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA100
Metabolic Activation:	Rat, Liver, S-9, Phenobarbital and 5,6-benzoflavone
Method:	Preincubation
Dose:	0; 78.1; 156; 313; 625; 1,250; 2,500; 5,000 ug/plate
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	E. coli
Strain Indicator:	WP2uvrA/pKM101
Metabolic Activation:	Rat, Liver, S-9, Phenobarbital and 5,6-benzoflavone
Method:	Preincubation
Dose:	0; 78.1; 156; 313; 625; 1,250; 2,500; 5,000 ug/plate
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	E. coli

Strain Indicator:	WP2uvrA/pKM101
Metabolic Activation:	None
Method:	Preincubation
Dose:	0; 78.1; 156; 313; 625; 1,250; 2,500; 5,000 ug/plate
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA1537
Metabolic Activation:	Rat, Liver, S-9, Phenobarbital and 5,6-benzoflavone
Method:	Preincubation
Dose:	0; 19.5; 39.1; 78.1; 156; 313; 625 ug/plate
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA98

Metabolic Activation:	Rat, Liver, S-9, Phenobarbital and 5,6-benzoflavone
Method:	Preincubation
Dose:	0; 1.22; 2.44; 4.88; 9.77;19.5 ug/plate
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Chinese hamster lung (CHL/IU) cells
End Point:	In vitro chromosomal aberrations
Metabolic Activation:	None
Dose:	0; 0.025; 0.05; 0.1; 0.15; 0.2 mg/ml (Test material solvent: DMSO)
Dose Regimen:	24 hr continuous treatment; colcemid added 2 hr before harvest
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Chinese hamster lung (CHL/IU) cells
End Point:	In vitro chromosomal aberrations
Metabolic Activation:	None
Dose:	0; 0.1; 0.2; 0.3; 0.4; 0.5 mg/ml (Test material solvent: DMSO)

Dose Regimen:	6 hr treatment; 18 hr recovery, colcemid added 2 hr before harvest
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Chinese hamster lung (CHL/IU) cells
End Point:	In vitro chromosomal aberrations
Metabolic Activation:	Rat, Liver, S-9, Phenobarbital and 5,6-benzoflavone
Dose:	0; 0.2; 0.4; 0.6, 0.8 mg/ml and 0; 0.5; 0.55; 0.6; 0.65; 0.7 mg/ml (Test material solvent: DMSO)
Dose Regimen:	6 hr treatment; 18 hr recovery, colcemid added 2 hr before harvest
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Chinese hamster lung (CHL/IU) cells
End Point:	In vitro chromosomal aberrations
Metabolic Activation:	None
Dose:	0; 0.025; 0.05; 0.1; 0.15 mg/ml (Test material solvent: DMSO)
Dose Regimen:	48 hr continuous treatment; colcemid added 2 hr before harvest

Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Peripheral blood and liver polychromatic erythrocytes
End Point:	In vivo micronucleus
Species:	Rat
Strain/Sex:	F344/CuCrlCrlj/Male
Route:	Oral (gavage)
Dose:	0; 2,500; 5,000 mg/kg/day (Test material solvent: olive oil)
Dose Regimen:	Twice a day on days 1, 2, and 6 ; 24 hr apart; blood collected from tail vein on day 4; partial hepatecomy on day 5 with hepatocyte collection for micronucleus assay on day 9
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008
Test System:	Bone marrow erythrocytes
End Point:	In vivo micronucleus
Species:	Rat
Strain/Sex:	F344/CuCrlCrlj/Male
Route:	Oral (gavage)

Dose:	0; 625; 1,250; 2,500; 5,000 mg/kg/day (Test material solvent: olive oil)
Dose Regimen:	Once a day for 2 days
Results:	Negative
Reference:	[NAKAGAWA,K, HIDAKA,T, KITANO,M, ASAKURA,M, KAMIGAITO,T, NOGUCHI,T, HOSOE,K; GENOTOXICITY STUDIES ON LICORICE FLAVONOID OIL (LFO). FOOD CHEM. TOXICOL. 46(7): 2525-2532, 2008

As taken from CCRIS, 2010.

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reference
DNA repair		Bacteria - Bacillus subtilis	100 gm/L	MUREAV Mutation Research. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1964- Volume(issue)/page/year: 97,81,1982

As taken from RTECS, 2016.

“Glycyrrhiza glabra L. (licorice) is one of the most important medicinal plants, which is widely used throughout the world both in traditional and contemporary medical industries. This study was undertaken to investigate the potential genotoxic activity of G. glabra methanolic root extract, and its possible antigenotoxic properties against mitomycin C (MMC)-induced DNA damage in in vitro chromosome aberrations (CAs) and cytokinesis-block micronucleus (CBMN) assays in human peripheral blood lymphocytes (PBLs). Lymphocytes were treated with 25, 50, and 100 µg/ml G. glabra methanolic root extract alone as well as in combination with MMC (0.1 µg/ml) for 24 and 48 h treatment periods. It was found that there were no statistically significant differences between the negative control and the groups treated with all concentrations of G. glabra root extract of alone ($p > 0.05$), demonstrating the absence of genotoxic effects at both 24 and 48 h treatment periods. Besides, the co-treatment of G. glabra methanolic root extract and MMC significantly decreased the percentage of structural CAs and MN formation when compared with the culture treated with MMC alone ($p < 0.001$). In addition, the negative interaction factor (IF) values obtained for all combinations represent an antagonistic effect of G. glabra versus MMC. We can state that this extract acts as an antagonist and markedly decreased MMC-induced cytogenotoxicity. In conclusion, the present results demonstrate that in the tested experimental conditions, G. glabra methanolic root extract is not genotoxic in cultured human PBLs and has also antigenotoxic activity against MMC, which is widely used in chemotherapy against cancer.” As taken from Yavuz Kocaman A & Güzelkokar M. 2018. Drug Chem. Toxicol. 15, 1-8. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29447011

“Licorice (Glycyrrhiza glabra) has been considered as an herbal drug since ancient time. Nowadays, it is a well-known spice that possesses worth pharmacological effects. However, some relevant articles have revealed negative impacts of licorice in health. By considering the great wishes in using herbal medicine, it is important to show adverse effects of herbal medicine in health. At present, there

are misunderstandings toward the safety of herbal medicines. Herein, we gathered scientific research projects on the toxicity effects of licorice and glycyrrhizin to highlight their safety. In this regards, we categorized our findings about the toxicity effects of licorice and glycyrrhizin in acute, sub-acute, sub-chronic, and chronic states. Besides, we discussed on the cytotoxicity, genotoxicity, mutagenicity, and carcinogenicity of licorice and glycyrrhizin as well as their developmental toxicity. This review disclosed that G. glabra and glycyrrhizin salts are moderately toxic. They need to be used with caution during pregnancy. G. glabra and glycyrrhizin possess selective cytotoxic effects on cancerous cells. The most important side effects of licorice and glycyrrhizin are hypertension and hypokalemic-induced secondary disorders. Licorice side effects are increased by hypokalemia, prolonged gastrointestinal transient time, decreased type 2 11-beta-hydroxysteroid dehydrogenase activities, hypertension, anorexia nervosa, old age, and female sex." As taken from Nazari S et al. 2017. Phytother. Res. 31(11), 1635-1650. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28833680

Licorice extract (CAS RN 68916-91-6) was negative in bacterial mutagenicity (Ames) tests of Salmonella typhimurium, and Escherichia coli strain WP2 uvrA pKM101 when tested at up to 10 mg/plate in the presence or absence of activation

NTP (undated) *5.5. Cytotoxicity*

"AIM: To evaluate the antibacterial and cytotoxic effects of Liquorice as a root canal medicament and to compare its action to the commonly used root canal medicament calcium hydroxide Ca(OH)(2). METHODOLOGY: The antibacterial effect of Liquorice and Ca(OH)(2) either separately or in combination was investigated against Enterococcus faecalis. Agar-well diffusion methods, broth microdilution tests and biofilm susceptibility assays were used to determine the antibacterial activity. Human periodontal ligament fibroblast tissue culture was used to assess the cytotoxicity of the preparations under investigation. RESULTS: Liquorice extract either by itself or in combination with Ca(OH)(2) had a significant inhibitory effect against Enterococcus faecalis compared with that of Ca(OH)(2) alone. The use of Liquorice extract followed by Liquorice/Ca(OH)(2) mixture retained significantly more viable periodontal ligament cells than Ca(OH)(2) , which had a strong lethal effect on the cells. CONCLUSION: Liquorice extract either separately or as Liquorice/Ca(OH)(2) mixture had a potent bactericidal effect against Enterococcus faecalis and retained compatibility with fibroblasts in tissue culture compared to the commonly used root canal medicament Ca(OH)(2)". As taken from Badr AE et al. 2012. Int. Endod. J. 44, 51-58. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/ 20812941?dopt=AbstractPlus.

"Previously, a growth inhibiting effect of PC-Spes on head and neck carcinoma cell lines had been demonstrated. In order to determine the toxic impact of particular herbs in the mixture, we exposed the head and neck cancer cell lines FADU, HLaC79 and its Paclitaxel-resistant subline HLaC79-Clone1 as well as primary mucosal keratinocytes to increasing concentrations of the herbal mixture Prostaprotect, which has a similar formulation as PC-Spes, as well as its single herbal components Dendranthema morifolium, Ganoderma lucidium, Glycyrrhiza glabra, Isatis indigotica, Panax pseudo-ginseng, Rabdosia rubescens, Scutellaria baicalensis and Pygeum africanum. Growth inhibition was measured using the MTT assay. Expression of P-glycoprotein (P-GP), multidrug resistance protein-1 (MRP-1), multidrug resistance protein-2 (MRP-2), breast cancer resistance protein (BCRP) and androgen receptor (AR) were examined by western blot analysis. Pygeum africanum extract clearly turned out as the main cytotoxic component of the Prostaprotect prescription mixture, and initated apoptosis in sensitive cell lines. All other extracts had only minor toxic effects. Western blot analysis revealed increased expression of P-GP in HLaC79-Clone1 cells, while HLaC79 and FADU cells were negative. All three cell lines were negative for MRP-1 and BCRP but positive for MRP-2. HLaC79 and its descendant HLaC79-Clone1 both expressed AR, as verified by western blotting and immunofluorescence staining. Primary mucosal keratinocytes were negative for all multidrug resistance markers as well as for AR. Growth inhibition rates of the single herbal extracts were compared with previously published results in prostate carcinoma cell lines. The relationship between

expression levels of AR and multidrug resistance markers in relation to the measured toxicity of herbal extracts in our head and neck cancer cell system is critically discussed". As taken from Schmidt M et al. 2013. Oncol. Reps. 29, 628-636. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23165347.

"BACKGROUND: In the search for anti-viral and antitumor substances from natural resources, antiviral and antitumor activities of licorice root extract and purified ingredients were investigated. MATERIALS AND METHODS: Viability of cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. Antiviral activity was quantified by the selectivity index, defined as the ratio of the 50% cytotoxic concentration (CC50) to the 50% effective concentration against human immunodeficiency virus (HIV) or herpes simplex virus (HSV)-infected cells (EC50). The tumor specificity was calculated by the ratio of CC50 against human normal oral cells to that against human oral squamous cell carcinoma cell lines. Licorice flavonoids and lower molecular polyphenols were subjected to quantitative structure-activity relationship analysis. RESULTS: Alkaline extract of licorice root had higher anti-HIV activity than did water extracts, confirming our previous reports. On the other hand, water extract, especially the flavonoid-rich fraction, had higher anti-HSV activity than did the alkaline extract. The flavonoid-rich fraction was more cytotoxic against human oral squamous cell carcinoma cell lines compared to normal oral cells, suggesting their tumor-specific cytotoxicity. CONCLUSION: The present study suggests that water and alkaline extracts of licorice root exert different mechanisms of actions against these two viruses. Physicochemical properties, rather than the category of compounds, may be important in determining their anti-HSV activity." As taken from Fukuchi K et al. 2016. In Vivo. 30(6), 777-785. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27815461

Species	Route	Dose data	Toxic effects	Reference
Human - colon tumor	In vitro	ICLo - Inhibitor Concentration Low 35 gm/L/4H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 164,22,2015

As taken from RTECS, 2016.

"Plant-derived substances (phytochemicals) are well recognized as sources of pharmacologically potent drugs in the treatment of several oxidative stress related disorders. Our study aims to evaluate the antioxidant and apoptotic effects of Glycyrrhiza glabra L. in both cell free and cell culture system. Plant fractions have been prepared with hexane, chloroform, ethyl acetate, methanol and water and their antioxidant properties are reviewed. Potent antioxidant activity has been well established in both in vitro and in silico studies which is believed to be responsible for the anticancerous nature of the plant. Results obtained indicate that methanol fraction of G. glabra L. exhibited maximum scavenging activity against DPPH and nitric oxide free radicals comparable to standard antioxidant L-AA. Administration of methanol fraction also considerably reduced the malondialdehyde produced due to lipid peroxidation in mammalian liver tissues. Moreover, the levels of antioxidant enzymes SOD, CAT, GST, GPx and GR in the oxidative stress induced tissues were refurbished significantly after treatment with plant's methanol fraction. Moreover, methanol fraction was found to be nontoxic to normal human cell line whereas it inhibited cancer cells HeLa and HepG2 considerably. Apoptosis was established by DAPI fluorescent staining and western blot analysis of pro apoptotic protein caspase-8, caspase-3 and anti-apoptotic protein Bcl-2.There is an up regulation in the levels of pro apoptotic caspase-8 and caspase-3 and down regulation of anti-apoptotic Bcl-2. Furthermore, GC-MS analysis of the methanol fraction revealed the presence of many compounds. In silico experiments using Autodock 4.2 tools showed strong affinity of plant compounds towards antioxidant

enzymes (proteins) thus validating with the conclusions of antioxidant enzyme assays and establishing a role in cancer pathogenesis." As taken from Hejazi II et al. 2017. Biomed. Pharmacother. 94, 265-279. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28763750

"Licorice (Glycyrrhiza glabra) has been considered as an herbal drug since ancient time. Nowadays, it is a well-known spice that possesses worth pharmacological effects. However, some relevant articles have revealed negative impacts of licorice in health. By considering the great wishes in using herbal medicine, it is important to show adverse effects of herbal medicine in health. At present, there are misunderstandings toward the safety of herbal medicines. Herein, we gathered scientific research projects on the toxicity effects of licorice and glycyrrhizin to highlight their safety. In this regards, we categorized our findings about the toxicity effects of licorice and glycyrrhizin in acute, sub-acute, sub-chronic, and chronic states. Besides, we discussed on the cytotoxicity, genotoxicity, mutagenicity, and carcinogenicity of licorice and glycyrrhizin as well as their developmental toxicity. This review disclosed that G. glabra and glycyrrhizin salts are moderately toxic. They need to be used with caution during pregnancy. G. glabra and glycyrrhizin possess selective cytotoxic effects on cancerous cells. The most important side effects of licorice and glycyrrhizin are hypertension and hypokalemic-induced secondary disorders. Licorice side effects are increased by hypokalemia, prolonged gastrointestinal transient time, decreased type 2 11-beta-hydroxysteroid dehydrogenase activities, hypertension, anorexia nervosa, old age, and female sex." As taken from Nazari S et al. 2017. Phytother. Res. 31(11), 1635-1650. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28833680

"OBJECTIVES: The objectives of this study were to evaluate the antimicrobial activity and total antioxidant capacity (TAC) of licorice in Saliva of HIV/AIDS patients. MATERIALS AND METHODS: Saliva specimens were collected from 20 people living with HIV infection, with CD4 count <500 cells/mm3 from people infected with HIV/AIDS in Mangalore city, India. A combination of amoxicillin-clavulanic acid and nystatin was taken as the positive control and normal saline as negative control. Results were compared using one-way analysis of variance followed by Tukey's post hoc analysis in SPSS 19. RESULTS: The TAC was evaluated spectrophotometrically at 695nm using the phosphomolybdenum method. Glycyrrhiza glabra showed a statistically significant reduction ($P < 0.05$) in total Candida count. The TAC of G. glabra was found to be 4.467 mM/L. CONCLUSIONS: G. glabra extracts showed good anticandidal activity and also high antioxidant property which reduces the oxidative stress of HIV-infected people." As taken from Aluckal E et al. 2017. J. Pharm. Bioallied Sci. 9(Suppl. 1), S237-S240. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29284971

"The prevalence of lung infection caused by Pseudomonas aeruginosa strains that are classified as multi-drug resistant has increased considerably and is mainly attributed to relative insufficiency of potent chemotherapeutic modalities. The present study was conducted to evaluate the antimicrobial activity of aquo-alcoholic extract of Glycyrrhiza glabra against the P. aeruginosa causing lung infection in Swiss albino mice. The study involves evaluation of lethal dose of P. aeruginosa in Swiss albino mice and analysis of disease manifestation that includes bacteremia, hypothermia, reduction in body weight and other parameters for 48h of infection. Physical manifestations of infected mice showed a significant decline in body temperature that is 29±0.57°C (at 48th h) from 38.81±0.33°C (0h) and 30% weight loss was observed at the end of the study. Further the efficacy of G. glabra extract against lung infection induced with the calculated lethal dose was evaluated by employing bacteremia, histopathology and radiological analysis. Bacterial burden showed that 2.30±0.02 Log10CFU/mL at day 7, a significant decline in the bacterial load as compared to day 1 when the bacterial burden was found to be 3.32±0.1 Log10CFU/mL. Histopathological results showed more diffuse and patchy accumulation of inflammatory cells within the alveolar space also the infiltrates were noted in all the lung section of infected mice. In treated animal group improved lung histology was seen with the exudates were less seen in D1 dose (20mg/kg) and disappeared in D2 dose (80mg/kg). The study clearly declares that the G. glabra extract is effective against lung infection

caused by P. aeruginosa at dose of 80mg/kg. The LCMS results revealed that the extract contains Glycyrrhizin, Stigmasterol and Ergosterol, Licochalcone and Glabridin. The current study expected to further exploit the biomedical properties of this extract in the preparation of a potent regimen against such threatening pathogen." As taken from Chakotiya AS et al. 2017. Biomed. Pharmacother. 90, 171-178. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28355591

"Background: We studied the effect of three plant extracts (Glycyrrhiza glabra, Paeonia lactiflora, Eriobotrya japonica) and six of their major secondary metabolites (glycyrrhizic acid, 18β glycyrrhetinic acid, liquiritigenin, isoliquiritigenin, paeoniflorin, ursolic acid) on the multidrug resistant human colon cancer cell line Caco-2 and human leukemia cell line CEM/ADR 5000 as compared to the corresponding sensitive cell line CCRF-CEM, and human colon cancer cells HCT-116, which do not over-express ATP-binding cassette (ABC) transporters. Methods: The cytotoxicity of single substances in sensitive and resistant cells was investigated by MTT assay. We also applied combinations of extracts or single compounds with the chemotherapeutic agent doxorubicin or doxorubicin plus the saponin digitonin. The intracellular retention of the ABC transporter substrates rhodamine 123 and calcein was examined by flow cytometry to explore the effect of the substances on the activity of ABC transporters P-glycoprotein and MRP1. Real-time PCR was applied to analyse the gene expression changes of ABCB1, ABCC1, caspase 3, caspase 8, AhR, CYP1A1, and GSTP1 in resistant cells under the treatment of the substances. Results: All the substances moderately inhibited cell growth in sensitive and resistant cells to some degree. Whereas ursolic acid showed IC50 of 14 and 22 μM in CEM/ADR 5000 and Caco-2 cells, respectively, glycyrrhizic acid and paeoniflorin were inactive with IC50 values above 400 μM. Except for liquiritigenin and isoliquiritigenin, all the other substances reversed MDR in CEM/ADR 5000 and Caco-2 cells to doxorubicin. Ue, ga, 18ga, and urs were powerful reversal agents. In CEM/ADR 5000 cells, high concentrations of all the substances, except Paeonia lactiflora extract, increased calcein or rhodamine 123 retention in a dose-dependent manner. In Caco-2 cells, all the substances, except liquiritigenin, retained rhodamine 123 in a dose-dependent manner. We also examined the effect of the plant secondary metabolite (PSM) panel on the expression of ABCB1, ABCC1, caspase 3, caspase 8, AhR, CYP1A1, and GSTP1 genes in MDR cells. Conclusions: The extracts and individual PSM could reverse MDR in CEM/ADR 5000 and Caco-2 cells, which overexpress ABC transporters, in two- and three-drug combinations. Most of the PSM also inhibited the activity of ABC transporters to some degree, albeit at high concentrations. Ue, ga, 18ga, and urs were identified as potential multidrug resistance (MDR) modulator candidates, which need to be characterized and validated in further studies." As taken from Zhou JX and Wink M. 2018. Medicines (Basel) 5(4), E123. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30428619

"Background: The phytochemical composition, antioxidant, cytotoxic, and antimicrobial activities of a methanol extract from Glycyrrhiza glabra L. (Ge), a 50% ethanol (in water) extract from Paeonia lactiflora Pall. (Pe), and a 96% ethanol extract from Eriobotrya japonica (Thunb.) Lindl. (Ue) were investigated. Methods: The phytochemical profiles of the extracts were analyzed by LC-MS/MS. Antioxidant activity was evaluated by scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals and reducing ferric complexes, and the total phenolic content was tested with the Folin-Ciocalteu method. Cytotoxicity was determined with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in murine macrophage RAW 264.7 cells. Antimicrobial activity of the three plant extracts was investigated against six bacterial strains with the broth microdilution method. Results: Only Pe showed high antioxidant activities compared to the positive controls ascorbic acid and (-)-epigallocatechin gallate (EGCG) in DPPH assay; and generally the antioxidant activity order was ascorbic acid or EGCG > Pe > Ue > Ge. The three plant extracts did not show strong cytotoxicity against RAW 264.7 cells after 24 h treatment with IC50 values above 60.53 ± 4.03 μg/mL. Ue was not toxic against the six tested bacterial strains, with minimal inhibitory concentration (MIC) values above 5 mg/mL. Ge showed medium antibacterial activity against Acinetobacter bohemicus, Kocuria kristinae, Micrococcus luteus, Staphylococcus auricularis, and Bacillus megaterium with MICs between 0.31 and 1.25 mg/mL. Pe inhibited the growth of Acinetobacter bohemicus, Micrococcus

luteus, and Bacillus megaterium at a MIC of 0.08 mg/mL. Conclusions: The three extracts were low-cytotoxic, but Pe exhibited effective DPPH radical scavenging ability and good antibacterial activity; Ue did not show antioxidant or antibacterial activity; Ge had no antioxidant potential, but medium antibacterial ability against five bacteria strains. Pe and Ge could be further studied for their potential to be developed as antioxidant or antibacterial candidates." As taken from Zhou JX et al. 2019. Medicines (Basel) 6(2), E43. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30935079

Glycyrrhiza glabra L., root, acetone extract (no CAS RN listed):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Hamster - ovary	40 mg/L/4H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	RTOPDW Regulatory Toxicology and Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1981- Volume(issue)/page/year: 61,373,2011
IC50 - Inhibitor Concentration 50	In vitro	Hamster - ovary	14.6 mg/L/18H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	RTOPDW Regulatory Toxicology and Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1981- Volume(issue)/page/year: 61,373,2011
ICLo - Inhibitor Concentration Low	In vitro	Hamster - ovary	40 mg/L/4H	In Vitro Toxicity Studies - cell proliferation: DNA incorporation, mitotic index etc.	RTOPDW Regulatory Toxicology and Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1981- Volume(issue)/page/year: 61,373,2011
ICLo - Inhibitor Concentration Low	In vitro	Hamster - ovary	14.6 mg/L/18H	In Vitro Toxicity Studies - cell proliferation: DNA incorporation, mitotic index etc.	RTOPDW Regulatory Toxicology and Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1981- Volume(issue)/page/year: 61,373,2011

As taken from RTECS, 2019

“Background: The success of an endodontic treatment depends on effective disinfection and complete sealing of root canal. The irrigants that are currently used in the field of endodontics have certain limitations, so the quest for an ideal root canal irrigant continues. Nowadays, the use of herbal extracts such as Triphala and liquorice are used for their potent antimicrobial activity and less side effects. Aim: This study aims (1) to evaluate the antimicrobial efficacy of Triphala and liquorice against Enterococcus faecalis.(2) To determine any cytotoxic effect on isolated human periodontal ligament (PDL) fibroblasts. Materials and methods: The antimicrobial efficacy of Triphala and liquorice extracts was analyzed at different concentrations (12.5, 25, 50, 75, and 100 mg/ml) using the well-diffusion method. Three percent sodium hypochlorite (NaOCl) and distilled water were taken as positive and negative controls. Minimum inhibitory concentration of the active extract was determined by the broth dilution assay. Human PDL fibroblast tissue culture was used to assess the cytotoxicity of the preparations. The data thus obtained were subjected to statistical analysis. Results: The results showed that the mean antimicrobial efficacy of Triphala and liquorice at 50 mg/ml is 20.33 and 9.33, respectively, which are statistically significant ($P < 0.0001$) as compared with a concentration 12.5 and 25 mg/ml. 50 mg/ml showed significant results ($P < 0.001$) on comparing with hypochlorite. Triphala and liquorice showed no cytotoxic effect as compared to NaOCl on human PDL fibroblasts. Conclusion: Among the three tested materials Triphala showed the highest antimicrobial efficacy followed by NaOCl and liquorice.” As taken from Satti P et al. 2019. J. Indian Soc. Pedod. Prev. Dent. 37(3), 275-281. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31584028/

“Background: We investigated the effect of root extracts from the traditional Chinese medicine (TCM) plants Glycyrrhiza glabra L., Paeonia lactiflora Pall., and the leaf extract of Eriobotrya japonica (Thunb.) Lindl., and their six major secondary metabolites, glycyrrhizic acid, 18β glycyrrhetinic acid, liquiritigenin, isoliquiritigenin, paeoniflorin, and ursolic acid, on lipopolysaccharide (LPS)-induced NF-κB expression and NF-κB-regulated pro-inflammatory factors in murine macrophage RAW 264.7 cells. Methods: The cytotoxicity of the substances was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. RAW 264.7 cells were treated with LPS (1 μg/mL) or LPS plus single substances; the gene expression levels of NF-κB subunits (RelA, RelB, c-Rel, NF-κB1, and NF-κB2), and of ICAM-1, TNF-α, iNOS, and COX-2 were measured employing real-time PCR; nitric oxide (NO) production by the cells was quantified with the Griess assay; nuclear translocation of NF-κB was visualized by immunofluorescence microscopy with NF-κB (p65) staining. Results: All the substances showed moderate cytotoxicity against RAW 264.7 cells except paeoniflorin with an IC50 above 1000 μM. Glycyrrhiza glabra extract and Eriobotrya japonica extract, as well as 18β glycyrrhetinic acid and isoliquiritigenin at low concentrations, inhibited NO production in a dose-dependent manner. LPS upregulated gene expressions of NF-κB subunits and of ICAM-1, TNF-α, iNOS, and COX-2 within 8 h, which could be decreased by 18β glycyrrhetinic acid, isoliquiritigenin and ursolic acid similarly to the anti-inflammatory drug dexamethasone. NF-κB translocation from cytoplasm to nucleus was observed after LPS stimulation for 2 h and was attenuated by extracts of Glycyrrhiza glabra and Eriobotrya japonica, as well as by 18β glycyrrhetinic acid, isoliquiritigenin, and ursolic acid. Conclusions: 18β glycyrrhetinic acid, isoliquiritigenin, and ursolic acid inhibited the gene expressions of ICAM-1, TNF-α, COX-2, and iNOS, partly through inhibiting NF-κB expression and attenuating NF-κB nuclear translocation. These substances showed anti-inflammatory activity. Further studies are needed to elucidate the exact mechanisms and to assess their usefulness in therapy.” As taken from Zhou JX and Wink M. 2019. Medicines (Basel) 6(2), 55. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31083310/

“Although a majority of nasopharyngeal carcinoma (NPC) are undifferentiated and strongly radiosensitive, many NPC patients still have troubles in recurrence. Traditional Chinese medicine (TCM) is considered as potential therapeutic drugs in NPC. However, the effect of Glycyrrhiza glabra on NPC is limited. The present study shows the decreased proliferation and high apoptosis in G. glabra root extract-treated C666-1 cells, indicating the anti-cancerous function of G. glabra in NPC. Then GC/MS-based metabolomics is employed to characterize variation of metabolomes in response to G. glabra root extract treatment. Metabolic category elaborates the higher percentage of down-regulated amino acids and lipids after G. glabra treatment. Moreover, ICA and pathway enrichment

analysis further observe that glycine, serine and threonine metabolism, fatty acid biosynthesis, alanine, aspartate and glutamate metabolism, and cysteine and methionine metabolism are four important amino acid and lipid metabolisms that likely contribute to the anti-cancer effect of G. glabra in NPC. These pathways point out the seven metabolite biomarkers, glutathione, glutamine, L-alanine, glycine, L-serine, tetradecanoic acid and stearic acid. Taken together, these findings provide potential clues that anti-cancer mechanisms of G. glabra root extract are linked to the metabolic strategies and emphasize the significance of metabolic strategies against NPC." As taken from Zheng C et al. 2019. Mol. Biol. Rep. 46(4), 3857-3864. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31066003/

"Glycyrrhiza glabra is considered as potential drug for nasopharyngeal carcinoma (NPC). However, whether the long noncoding RNAs' (lncRNAs) contributes to the anti-cancer function of this herb is unknown. In present study, we analyzed the differential expression of lncRNA between G. glabra-treated and untreated C666-1 cells. Out of those tumor-related lncRNAs, AK027294 had a strongest down-regulation upon G. glabra treatment. Knockdown of AK027294 suppresses the proliferation of C666-1 cells by inducing the apoptosis. Moreover, either G. glabra treatment or knockdown of AK027294 significantly increases the production of EZH1 (Enhancer of zeste 1 polycomb repressive complex 2 subunit). Collectively, we have identified a potential mechanism that the down-regulation of AK027294 contributes to the anti-cancer function of G. glabra and also provide the potential inter-relationship between AK027294 and EZH1." As taken from Zhang B et al. 2020. Biosci. Biotechnol. Biochem. 84(2), 314-320. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31589096/

"Herbal teas are becoming popular as functional beverages due to their various health promotional properties. This study aimed at assessing 13 hot water infusions (HWIs) from different herbs against streptococcal pharyngitis (strep throat). Licorice root exhibited the lowest minimum inhibitory concentrations (MIC) of 1.56 mg/mL, followed by barberry root, thyme, and oregano flowering shoots, with a MIC of 3.13 mg/mL. At their respective minimum bactericidal concentrations (MBC), licorice showed the bactericidal effect on S. pyogenes within 12 h after exposure while others need 24 h for a similar outcome. The HWIs exhibited inhibitory activity on biofilm formation, ranging from 1.56 to 6.25 mg/mL, which confirmed by ruptured cells or clusters of dead cell debris observed in scanning electron microscope (SEM). Overall, non-toxic concentrations of efficacious HWIs from licorice root, barberry root, thyme, and oregano flowering shoots may provide potential sources for developing herbal teas or biomedicine for the management of S. pyogenes infections." As taken from Wijesundara NM and Rupasinghe HPV. 2019. Biomedicines 7(3), 63. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31450579/

"This study aimed to compare the total phenolic (TPC), flavonoid (TFC), radical scavenging and cytotoxic activities in the aqueous methanolic extracts of Angelica sinensis, Dioscorea polystachya, Ginkgo biloba, Glycyrrhiza uralensis and Lycium barbarum with two dietary plants: Brassica oleracea and Zingiber officinale. The TPC and TFC in medicinal plant extracts were 12-93% lower than Z. officinale as follows: L. barbarum > G. uralensis > A. sinensis > G. biloba > D. polystachya. The decreasing radical scavenging activity in medicinal plant extracts shared similar trend: G. uralensis > L. barbarum > A. sinensis > G. biloba > D. polystachya. Both TPC and TFC were positively correlated with radical scavenging and cytotoxic activities. All medicinal plants were considered inactive (LC50 > 0.2 mg/ml) and safe for consumption. The TPC, TFC, radical scavenging and cytotoxic activities in the medicinal plants were plant-part dependant, in particular L. barbarum and G. uralensis." As taken from Ng ZX et al. 2020. Nat. Prod. Res. Epub ahead of print. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32290699/

5.6. Carcinogenicity

In an investigation into the potential anti-carcinogenic effects of glycyrrhizic acid, mouse skin painting with a known carcinogen and 1.2 mg glycyrrhizic acid resulted in the average number of nodules and mortality rate being significantly lower than in a non-glycyrrhizic acid control group (Nozawa 1967).

Species	Test conditions	Evidence of carcinogenicity	Reference
No carcinogenicity studies meeting modern standards were identified.			
Groups of 50-70 male and female B6C3F1 mice	Animals were administered disodium glycyrrhizinate at 0, 0.04, 0.08 and 0.15% (males) or 0, 0.08, 0.15 and 0.3% (females) in the drinking water for 96 weeks (up to 229 and 407 mg/kg bw/day in males and females, respectively. After the exposure period the animals were maintained for another 14 wk. Microscopic examination was carried out on "all visceral organs and any tumours".	None	Kobuke et al. 1985
Anticarcinogenic activity			
	Licorice extract and several constituents and related materials have given evidence of anticarcinogenic activity.		Wang et al. 2000; Reviewed in Wang & Nixon, 2001.

Species	Route	Dose data	Toxic effects	Reference
Mouse	Oral	TDLo: 7.5 mg/kg/15D (intermittent)	Tumorigenic - protects against induction of experimental tumors	BPBLEO Biological and Pharmaceutical Bulletin. (Pharmaceutical Society of Japan, 2-12-15-201 Shibuya Shibuya-ku, Tokyo 150, Japan) V.16- 1993- Volume(issue)/page/year: 30,2191,2007

As taken from RTECS, 2016

"Licorice (Gancao in Chinese) has been used worldwide as a botanical source in medicine and as a sweetening agent in food products for thousands of years. Triterpene saponins and flavonoids are its main ingredients that exhibit a variety of biological activities, including hepatoprotective, antiulcer, anti-inflammatory, antiviral and anticancer effects among others. This review attempts to summarize the current knowledge on the anticancer properties and mechanisms of the compounds isolated from licorice and obtain new insights for further research and development of licorice. A broad spectrum of in vitro and in vivo studies have recently demonstrated that the mixed extracts and purified compounds from licorice exhibit evident anticancer properties by inhibition of proliferation, induction of cell cycle arrest, apoptosis, autophagy, differentiation, suppression of metastasis, angiogenesis, and sensitization of chemotherapy or radiotherapy. A combined treatment of licorice compounds and clinical chemotherapy drugs remarkably enhances anticancer effects and reduces the side effects of chemotherapeutics. Furthermore, glycyrrhizic acid and glycyrrhetinic acid in licorice have been indicated to present obvious liver-targeting effects in targeted drug delivery systems for hepatocellular carcinoma treatment." As taken from Tang ZH et al. 2015. Planta Med. 81(18), 1670-87. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26695708

"BACKGROUND: In the search for anti-viral and antitumor substances from natural resources, antiviral and antitumor activities of licorice root extract and purified ingredients were investigated. MATERIALS AND METHODS: Viability of cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. Antiviral activity was quantified by the selectivity index, defined as the ratio of the 50% cytotoxic concentration (CC50) to the 50% effective concentration against human immunodeficiency virus (HIV) or herpes simplex virus (HSV)-infected cells (EC50). The tumor specificity was calculated by the ratio of CC50 against human normal oral cells to that against human oral squamous cell carcinoma cell lines. Licorice flavonoids and lower molecular polyphenols were subjected to quantitative structure-activity relationship analysis. RESULTS: Alkaline extract of licorice root had higher anti-HIV activity than did water extracts, confirming our previous reports. On the other

hand, water extract, especially the flavonoid-rich fraction, had higher anti-HSV activity than did the alkaline extract. The flavonoid-rich fraction was more cytotoxic against human oral squamous cell carcinoma cell lines compared to normal oral cells, suggesting their tumor-specific cytotoxicity. CONCLUSION: The present study suggests that water and alkaline extracts of licorice root exert different mechanisms of actions against these two viruses. Physicochemical properties, rather than the category of compounds, may be important in determining their anti-HSV activity." As taken from Fukuchi K et al. 2016. In Vivo. 30(6), 777-785. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27815461

"Natural products are of great surge in the identification of chemopreventive agents and biologically active molecules for the development of new promising therapeutic agents. These agents influence the cascade of biochemical and molecular signalling pathways involved in numerous physiological and pathological processes. The natural agents combat the dogma associated with the most dreaded, unconquered health concern and a multigenic disease- cancer. A category of plants known as adaptogens maintain perturbed homoeostasis, augment adaptations to noxious stimuli (exposure to cold, heat, pain, general stress, infectious organisms) and offer endurance to attenuate several disorders in human beings. The well known adaptogens and immunomodulators such as Rhodiola rosea, Withania somnifera, Tinospora cordifolia, Bacopa monnieri, Emblica officinalis, Glycyrrhiza glabra, Asparagus racemosus, Ocimum sanctum and Panax notoginseng claimed to have significant antioxidant and anticarcinogenic properties due to the presence of various biologically active chemical compounds. Their immunopotentiating activity is mediated through the modulation of T-cell immunity biochemical factors, transcription factors, some genes and factors associated with tumor development and progression. The combinatory formulation of active immunostimulating constituents from these plants may provide better homeostasis. These immunostimulant factors suggest their potential therapeutic significance in adjuvant or supportive therapy in cancer treatment." As taken from Kaur P et al. 2017. Biomed. Pharmacother. 95, 1815-1829. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28968926

"Licorice (Glycyrrhiza glabra) has been considered as an herbal drug since ancient time. Nowadays, it is a well-known spice that possesses worth pharmacological effects. However, some relevant articles have revealed negative impacts of licorice in health. By considering the great wishes in using herbal medicine, it is important to show adverse effects of herbal medicine in health. At present, there are misunderstandings toward the safety of herbal medicines. Herein, we gathered scientific research projects on the toxicity effects of licorice and glycyrrhizin to highlight their safety. In this regards, we categorized our findings about the toxicity effects of licorice and glycyrrhizin in acute, sub-acute, sub-chronic, and chronic states. Besides, we discussed on the cytotoxicity, genotoxicity, mutagenicity, and carcinogenicity of licorice and glycyrrhizin as well as their developmental toxicity. This review disclosed that G. glabra and glycyrrhizin salts are moderately toxic. They need to be used with caution during pregnancy. G. glabra and glycyrrhizin possess selective cytotoxic effects on cancerous cells. The most important side effects of licorice and glycyrrhizin are hypertension and hypokalemic-induced secondary disorders. Licorice side effects are increased by hypokalemia, prolonged gastrointestinal transient time, decreased type 2 11-beta-hydroxysteroid dehydrogenase activities, hypertension, anorexia nervosa, old age, and female sex." As taken from Nazari S et al. 2017. Phytother. Res. 31(11), 1635-1650. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28833680

"BACKGROUND: The increasing use of complementary and alternative medicine (CAM) has kindled the need for scientific evaluation of the mechanism of action of CAMs. Although, licorice, a common ingredient in many Traditional Chinese medicine (TCM) has attracted great attention for its antitumor and immunomodulatory activities, the mechanism of action of its polysaccharides is still unclear. Here we report the immunomodulatory activity of licorice polysaccharides in vivo. METHODS: The differential anticancer activities of licorice polysaccharides by tumorigenesis and immunomodulation was evaluated in vivo. Six weeks old, 120 CT-26 tumor bearing BALB/c mice, weighing 20 ± 2 g were used. They were randomly divided into six groups, three groups receiving high molecular weight

(fraction A), low molecular weight (fraction B) polysaccharides and crude extract (fraction C); positive, negative and normal groups receiving cytoxin, saline and normal diet respectively. Weight of mice and tumors was determined and tumorigenicity assay calculated to determine the anticancer effects. Immunomodulatory potential was determined by immune organ indices, immune cell population and serum cytokine levels using immune organ weight and index, flow cytometry and cytokine/chemokine bead panel kit respectively. RESULTS: Licorice polysaccharides exhibited immunomodulatory activities in CT 26 tumor bearing BALB/c mice. The polysaccharides significantly suppressed tumor growth and increased immune organ index. Furthermore, the immunomodulatory effect was evident with activation of CD4+ and CD8+ immune cells population. The polysaccharides also affected the production of various cytokines, by increasing IL 2, IL 6, IL 7 levels and a decreasing TNFα levels. CONCLUSION: In summary, licorice polysaccharide especially of low molecular weight exhibit anticancer and immunomodulatory activities by suppressing tumor growth and improving general health of mice. They also augment the thymus/spleen index and population of T lymphocytes. Furthermore, the polysaccharides enhance the levels of serum antitumor cytokines, IL 2, IL 6 and IL 7 while decreasing pro-tumor cytokine TNFα." As taken from Ayeka PA et al. 2017. BMC Complement. Altern. Med. 17(1), 536. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29246138

"Targeting altered metabolism in cancer provides a promising preventive and therapeutic approach. Natural products interplay between gene expression and metabolism either by targeting altered metabolic enzymes and/or affecting the regulating miRNAs. Licorice is a widely known product used as flavoring agent. Glycyrrhizin and other metabolites were reported to exert several metabolic benefits. Here, we investigated the effect of licorice roots extract on some metabolic pathways and their regulating miRNAs in hepatocellular carcinoma cells. Our data showed various beneficial effects of licorice roots extract including induction of apoptosis and cell cycle arrest. Second, upregulating tumor suppressor miRNAs; let7a-3p, miR-34c-5p, miR-122-5p, miR-126-3p, miR195-5p, miR-199a-5p, miR-206, and miR-326-5p. Third, inhibiting HIF1α, PI3K and C-Myc and activating AMPK and p53. Fourth, inhibiting enzymes of glycolysis; HK-2, LDH-A and PK-M2; pentose phosphate pathway; G6PD and glutaminolysis; glutaminase. However, such an extract upregulated oncogenic miRNAs; miR-21, miR-221, and miR-222. Although the present data highlights the ability of licorice roots extract to enhance apoptosis and cell cycle arrest and correct altered metabolism, it warns against its unfavorable effects, hence, its use for prevention and therapy should proceed with caution. Further experiments are required to investigate whether a specific bioactive ingredient is responsible for upregulating the oncogenic miRNAs." As taken from Abdel-Wahab AA et al. 2020. Nutr. Cancer. Epub ahead of print. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32578448/

5.7. Irritation/immunotoxicity

Various tests implicated glycyrrhizin in the rhinoconjuctivitis of a pharmaceutical laboratory worker handling this material (Martinez San Ireneo et al. 1995).

Occupational asthma and rhinitis were reported in a worker in an anis factory. Sensitization to licorice (and to other plants) was confirmed by skin prick and other tests (Gonzalez-Gutierrez et al. 2000).

A herbalist who reported occupational asthma associated with exposure to liquorice root powder reacted to a liquorice extract in skin prick tests and to liquorice root powder on inhalational challenge (Cartier et al. 2002).

Allergic dermatitis developed on the face of a Japanese woman as a result of her use of a facial cream and foundation. An oil-soluble licorice extract, a common component of skin-lightening cosmetics in Japan, was thought to be the cause; a positive reaction was seen in a patch test at 0.5% in petrolatum (Nishioka & Seguchi 1999).

A recent literature review found no reported cases of contact dermatitis to licorice root (Kiken & Cohen, 2002).

“CONTEXT: Increasing incidence and impact of inflammatory diseases have encouraged the search of new pharmacological strategies to face them. Licorice has been used to treat inflammatory diseases since ancient times in China. OBJECTIVE: To summarize the current knowledge on anti-inflammatory properties and mechanisms of compounds isolated from licorice, to introduce the traditional use, modern clinical trials and officially approved drugs, to evaluate the safety and to obtain new insights for further research of licorice. METHODS: PubMed, Web of Science, Science Direct and ResearchGate were information sources for the search terms 'licorice', 'licorice metabolites', 'anti-inflammatory', 'triterpenoids', 'flavonoids' and their combinations, mainly from year 2010 to 2016 without language restriction. Studies were selected from Science Citation Index journals, in vitro studies with Jadad score less than 2 points and in vivo and clinical studies with experimental flaws were excluded. RESULTS: Two hundred and ninety-five papers were searched and 93 papers were reviewed. Licorice extract, 3 triterpenes and 13 flavonoids exhibit evident anti-inflammatory properties mainly by decreasing TNF, MMPs, PGE2 and free radicals, which also explained its traditional applications in stimulating digestive system functions, eliminating phlegm, relieving coughing, nourishing qi and alleviating pain in TCM. Five hundred and fifty-four drugs containing licorice have been approved by CFDA. The side effect may due to the cortical hormone like action. CONCLUSION: Licorice and its natural compounds have demonstrated anti-inflammatory activities. More pharmacokinetic studies using different models with different dosages should be carried out, and the maximum tolerated dose is also critical for clinical use of licorice extract and purified compounds.” As taken from Yang R et al. 2017. Pharm. Biol. 55(1), 5-18. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27650551

“Natural products are of great surge in the identification of chemopreventive agents and biologically active molecules for the development of new promising therapeutic agents. These agents influence the cascade of biochemical and molecular signalling pathways involved in numerous physiological and pathological processes. The natural agents combat the dogma associated with the most dreaded, unconquered health concern and a multigenic disease- cancer. A category of plants known as adaptogens maintain perturbed homoeostasis, augment adaptations to noxious stimuli (exposure to cold, heat, pain, general stress, infectious organisms) and offer endurance to attenuate several disorders in human beings. The well known adaptogens and immunomodulators such as Rhodiola rosea, Withania somnifera, Tinospora cordifolia, Bacopa monnieri, Emblica officinalis, Glycyrrhiza glabra, Asparagus racemosus, Ocimum sanctum and Panax notoginseng claimed to have significant antioxidant and anticarcinogenic properties due to the presence of various biologically active chemical compounds. Their immunopotentiating activity is mediated through the modulation of T-cell immunity biochemical factors, transcription factors, some genes and factors associated with tumor development and progression. The combinatory formulation of active immunostimulating constituents from these plants may provide better homeostasis. These immunostimulant factors suggest their potential therapeutic significance in adjuvant or supportive therapy in cancer treatment.” As taken from Kaur P et al. 2017. Biomed. Pharmacother. 95, 1815-1829. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28968926

“BACKGROUND: The increasing use of complementary and alternative medicine (CAM) has kindled the need for scientific evaluation of the mechanism of action of CAMs. Although, licorice, a common ingredient in many Traditional Chinese medicine (TCM) has attracted great attention for its antitumor and immunomodulatory activities, the mechanism of action of its polysaccharides is still unclear. Here we report the immunomodulatory activity of licorice polysaccharides in vivo. METHODS: The differential anticancer activities of licorice polysaccharides by tumorigenesis and immunomodulation was evaluated in vivo. Six weeks old, 120 CT-26 tumor bearing BALB/c mice, weighing 20 ± 2 g were used. They were randomly divided into six groups, three groups receiving high molecular weight (fraction A), low molecular weight (fraction B) polysaccharides and crude extract (fraction C); positive, negative and normal groups receiving cytoxin, saline and normal diet respectively. Weight of mice and tumors was determined and tumorigenicity assay calculated to determine the anticancer effects. Immunomodulatory potential was determined by immune organ indices, immune cell population and serum cytokine levels using immune organ weight and index, flow cytometry and cytokine/chemokine

bead panel kit respectively. RESULTS: Licorice polysaccharides exhibited immunomodulatory activities in CT 26 tumor bearing BALB/c mice. The polysaccharides significantly suppressed tumor growth and increased immune organ index. Furthermore, the immunomodulatory effect was evident with activation of CD4+ and CD8+ immune cells population. The polysaccharides also affected the production of various cytokines, by increasing IL 2, IL 6, IL 7 levels and a decreasing TNFα levels. CONCLUSION: In summary, licorice polysaccharide especially of low molecular weight exhibit anticancer and immunomodulatory activities by suppressing tumor growth and improving general health of mice. They also augment the thymus/spleen index and population of T lymphocytes. Furthermore, the polysaccharides enhance the levels of serum antitumor cytokines, IL 2, IL 6 and IL 7 while decreasing pro-tumor cytokine TNFα." As taken from Ayeka PA et al. 2017. BMC Complement. Altern. Med. 17(1), 536. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29246138

"The Amino acid Derivative Reactivity Assay (ADRA) is an in chemico alternative to animal testing for skin sensitization potential, in which measurements of multi-constituent solutions were sometimes affected by co-elution with nucleophilic reagents. So, we established a means of using fluorescence detection and verified the utility of a newly developed ADRA-fluorescence detection (ADRA-FL) test method. We tested three types of plant extracts-aloe, green tea, and licorice-and although unable to quantify nucleophilic reagents using ultraviolet detection due to co-elution of multiple components, the use of fluorescence detection enabled us to detect nucleophilic reagents selectively and predict each of the extract solutions to be sensitizers. Given that plant extracts contain immunosuppressants, there is no reason to expect that positive results in ADRA-FL testing will always be concordant with in vivo results. But given its ability to predict the sensitization potential of cosmetics and other widely used multi-constituent substances that had previously been difficult to test, the newly developed ADRA-FL is expected to contribute to future assessments of sensitization risks." As taken from Fujita M et al. 2019. Toxicol. In Vitro 59, 161-178. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/31002975

"Background: Naturally derived cosmetic product ingredients of both plant and animal origin are being included increasingly in product formulations in order to cater to consumer preferences. They may be an overlooked cause of reactions to cosmetic products in some patients with dermatitis. Objectives: To identify naturally derived cosmetic product ingredients with allergenic potential (type I and type IV) and propose a cosmetic screening test series. Methods: The study was conducted in two steps. The first step was a market survey using a nonprofit application helping consumers avoid problematic substances in cosmetic products. The application contained 10 067 cosmetic products that were label checked for naturally derived cosmetic product ingredients. The second step was a literature search to examine how frequently the naturally derived ingredients were described and related to allergic reactions in cosmetics or other topically administered products. Results: We identified 121 different naturally derived cosmetic product ingredients that were included in at least 30 cosmetic products. In total, 22 ingredients were selected for a screening test series. Conclusions: We propose a supplemental patch test and a prick test screening series with naturally derived cosmetic product ingredients for patients with skin reactions to cosmetic products, aiming to identify a cause in more patients than is currently possible." As taken from Bruusgaard-Mouritsen MA et al. 2020. Contact Dermatitis 83(4), 251-270. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32248558/

"In times of health crisis, including the current COVID-19 pandemic, the potential benefit of botanical drugs and supplements emerges as a focus of attention, although controversial efficacy claims are rightly a concern. Phytotherapy has an established role in everyday self-care and health care, but, since botanical preparations contain many chemical constituents rather than single compounds, challenges arise in demonstrating efficacy and safety. However, there is ample traditional, empirical, and clinical evidence that botanicals can offer some protection and alleviation of disease symptoms as well as promoting general well-being. Newly emerging viral infections, specifically COVID-19, represent a unique challenge in their novelty and absence of established antiviral treatment or immunization. We discuss here the roles and limitations of phytotherapy in helping to prevent and

address viral infections, especially regarding their effects on immune response. Botanicals with a documented immunomodulatory, immunostimulatory, and antiinflammatory effects include adaptogens, Boswellia spp., Curcuma longa, Echinacea spp., Glycyrrhiza spp., medicinal fungi, Pelargonium sidoides, salicylate-yielding herbs, and Sambucus spp. We further provide a clinical perspective on applications and safety of these herbs in prevention, onset, progression, and convalescence from respiratory viral infections." As taken from Brendler T et al. 2020. Phytother. Res. Epub ahead of print. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33373071/

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Licorice Extract Paste (68916-91-6) 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 Licorice Extract Paste (68916-91-6) 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	91	JTI KB Study Report(s)
In vitro cytotoxicity	91	JTI KB Study Report(s)

Maser (2004) reported five enzymes were shown to initiate the detoxification of nicotine-derived nitrosamine ketone (NNK), (Maser 2004).

A report on the anti-mutagenic effects of glycyrrhizic and glycyrrhetinic acids demonstrated that both inhibited the mutagenicities of 3-amino-1-methyl-5H-pyrido[2,3-b]indol (Trp-p-2), 2-acetyl aminofluroene (AAF) and benzo(a)pyrene in the presence of S fraction hepatic enzymes (Yamaguchi & Watanabe 1984).

Induced gastric mucosal damage in rats was reduced by simultaneous administration of 100-500 mg deglycyrrhizinated licorice. Induced human faecal blood loss was less when 350 mg deglycyrrhizinated licorice was given (Rees et al. 1979).

Carbenoxolone, a glycyrrhetinic acid (a metabolic by-product of glycyrrhizic acid) analogue, did not affect sodium or potassium excretion in Sprague-Dawley rats, but promoted the antinatriuretic and kaliuretic effects of corticosterone and cortisol (Souness & Morris 1989).

Animals treated showed a reduced incidence of hepatic cells showing morphological evidence of injury (Watari 1976; Watari & Hotta 1980).

When the effects of licorice extract, ammoniated glycyrrhizic acid and deoxycorticosterone acetate (DCA) on blood pressure and subsequent tissue lesions were compared in male Sprague-Dawley rats, DCA, licorice extract or glycyrrhizic acid showed identical trends in hypertension, but the glycyrrhizic acid treatment was not polydipsic, and caused little change in body weight gain as compared to the control group. It was suggested that other constituents of licorice may affect the normal physiological metabolism of water and salts (Girerd et al. 1958).

Examination of the toxic effects of short term licorice extract administration to Wistar rats resulted in slight inhibition of body weight gain in the animals receiving the highest dose (2.5 g/kg bw/day). Haematological evaluation revealed a significant decrease in the red blood cell counts with accompanying decrease in percent hematocrit of the male, but not female rats receiving the 2 highest doses of licorice extract. Male rats also had a slightly, but significantly elevated neutrophil and decreased lymphocyte count at the highest dose. A slightly atrophic cortex and sporadic lymphofollicle formations of the thymus medulla was noted in the 2.5 g/kg bw group. The authors considered the no adverse effect level to be 0.31-0.63 g/kg bw for 90 days of treatment (Komiyama et al. 1977).

"AIM: To explore the antiangiogenic property of isoliquiritigenin (ISL) on in vivo and in vitro models. The effect of ISL on angiogenesis development was investigated using ex ovo chick chorioallantoic

membrane model. Its effect on pathological angiogenesis was examined by (1) silver nitrate cauterisation-induced corneal neovascularisation in BALB/c mice, followed by topical ISL (0.2-50 μM) and CD31 immunofluorescence of corneal blood vessels; (2) argon laser photocoagulation-induced choroidal neovascularisation in C57BL/6 mice, followed by intravitreal ISL (10-200 μM) and fundus fluorescein angiography and immunofluorescence with Griffonia simplicifolia isolectin-B4 (GSA I-B4); and (3) oxygen-induced retinopathy in C57BL/6J mice pups, followed by intravitreal ISL (1-100 μM) and GSA I-B4 immunofluorescence. The vascular area was quantified and analysed by one-way analysis of variance and Student t test. Expression of vascular endothelial growth factor (VEGF) and pigment-epithelium-derived factor in human umbilical vein endothelial cells was analysed by western blotting.

RESULTS: Ex ovo chick chorioallantoic membrane assay showed that ISL dose-dependently suppressed VEGF-induced vessel growth. In vivo experiments illustrated that topical ISL alleviated corneal neovascularisation (IC(50)=7.14 μM, day 7) and intravitreal ISL reduced vessel leakage and GSA I-B4-positive vascular area in choroidal and retinal neovascularisation. ISL was found to dose-dependently suppress VEGF and induce pigment epithelium derived factor expression in cultured endothelial cells.

CONCLUSION: Using various experimental models of ocular neovascularisation, the authors have demonstrated that ISL from licorice extract has an antiangiogenic effect. The authors' findings suggest that ISL may be a potential antiangiogenic molecule in the development of therapy for neovascularisation diseases." Taken from Jhanji et al (2011).

"BACKGROUND: Varicella-zoster virus (VZV) is the etiologic agent of two diseases, varicella (chicken pox) and zoster (shingles). Varicella is a self- limited infection, while zoster is mainly a disease of adults. The present study was conducted to isolate VZV from clinically diagnosed children using cell cultures and compare the activity of liquorice powder extract, an alternative herbal antiviral agent, with acyclovir and interferon alpha 2a (IFN-α2a) against the isolated virus. METHODS: Forty-eight VZV specimens, 26 from vesicular aspirates and 22 from vesicular swabs, from children clinically diagnosed with varicella were isolated on the Vero cell line. Isolates were propagated and identified with specific antiserum using indirect immuno-fluorescence and immunodot blotting assays. The growth kinetics of the viral isolates was studied. The antiviral activity of liquorice powder extract, acyclovir (ACV) and IFN-α2a was evaluated against the isolated virus. RESULTS: VZV was successfully isolated in 4 of the 48 specimens, all from vesicular aspirates. The growth kinetics of the viral isolates was time dependent. The inhibitory activity of liquorice powder extract (containing 125 μg/ml glycyrrhizin) when compared to ACV (250 μg/ml) and IFN-α2a is the lowest. CONCLUSIONS: VZV isolates were successfully isolated and propagated using Vero cells. Isolates were identified using indirect immunofluorescent and immunodot blotting techniques. Growth kinetics of the isolates revealed an increase in the viral infectivity titer relative to time. Glycyrrhizin in the crude form has low antiviral activity against VZV compared with acyclovir and interferon". As taken from Shebl RI et al. 2012. Chang Gung Med. J. 35, 231-239. PubMed, 2013 available at: http://www.ncbi.nlm.nih.gov/pubmed/22735054

"BACKGROUND: In the search for anti-viral and antitumor substances from natural resources, antiviral and antitumor activities of licorice root extract and purified ingredients were investigated. MATERIALS AND METHODS: Viability of cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. Antiviral activity was quantified by the selectivity index, defined as the ratio of the 50% cytotoxic concentration (CC50) to the 50% effective concentration against human immunodeficiency virus (HIV) or herpes simplex virus (HSV)-infected cells (EC50). The tumor specificity was calculated by the ratio of CC50 against human normal oral cells to that against human oral squamous cell carcinoma cell lines. Licorice flavonoids and lower molecular polyphenols were subjected to quantitative structure-activity relationship analysis. RESULTS: Alkaline extract of licorice root had higher anti-HIV activity than did water extracts, confirming our previous reports. On the other hand, water extract, especially the flavonoid-rich fraction, had higher anti-HSV activity than did the

alkaline extract. The flavonoid-rich fraction was more cytotoxic against human oral squamous cell carcinoma cell lines compared to normal oral cells, suggesting their tumor-specific cytotoxicity. CONCLUSION: The present study suggests that water and alkaline extracts of licorice root exert different mechanisms of actions against these two viruses. Physicochemical properties, rather than the category of compounds, may be important in determining their anti-HSV activity." As taken from Fukuchi K et al. 2016. In Vivo. 30(6), 777-785. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27815461

"To investigate the inhibitory effect of Glycyrrhiza uralensis (G. uralensis) and its monomeric compounds on Nav1.4 voltage-gated sodium channels (VGSCs) and analyze the relationship between the content of its marker compounds and the inhibitory rate. Based on this study, we found that 4 mg/ml ethanol extract of G. uralensis at 30%, 50%, 70% and 90% (v/v) exhibited 77.00 ± 0.03%, 34.75 ± 0.09%, 100.00 ± 0.01% and 2.00 ± 0.01% inhibitory rates on I_{Nav}1.4 respectively, and 8 mg/ml ethanol extract of G. uralensis at 30%, 50%, 70% and 90% (v/v) exhibited 99.00 ± 0.01%, 97.10 ± 0.02%, 100.00 ± 0.01% and 17.00 ± 0.04% inhibitory rates on I_{Nav}1.4 respectively. Isoliquiritigenin, echinatin, liquiritin and glycyrrhizic acid exhibited higher inhibitory rates of 39.98 ± 4.55%, 33.20 ± 1.61%, 22.62 ± 0.30% and 20.54 ± 4.82% respectively. However, liquiritigenin, formononetin, neoisoliquiritin and glycyrrhetinic acid exhibited lower inhibitory rates of less than 20%. Further, liquiritin apioside, isoliquiritin and neoliquiritin exhibited almost no effect on I_{Nav}1.4. These findings showed that glycyrrhizic acid reached a maximum concentration of 49.15 μg/ml, while echinatin had the lowest concentration. The ethanol extract of G. uralensis has significant inhibitory effects on Nav1.4 VGSCs. This may be an important mechanism in the treatment of gastrocnemius spasm and could guide further research regarding material basis and mechanism of the treatment of gastrocnemius spasm with peony and licorice decoction." As taken from Zhu G et al. 2018. J. Pharmacol. Sci. 136(2), 57-65. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29433959

"ETHNOPHARMACOLOGICAL RELEVANCE: Licorice (the roots and rhizomes of Glycyrrhiza uralensis Fisch.) is occasionally used as crude drug following processing including roasting or honey-roasting (soaking with honey before roasting) in traditional Japanese Kampo medicine and traditional Chinese medicine (TCM). AIM OF THE STUDY: We investigated the differences in the inducible effect of processed licorice products on granulocyte colony-stimulating factor (G-CSF) secretion in cultured intestinal epithelial cells and elucidated the active ingredients in both unprocessed and processed licorice products. MATERIALS AND METHODS: We prepared heat-processed licorice with or without pretreatment with honey, and fractionated the extracts by Sephadex G-100. Enterocyte-like differentiated MCE301 cells were incubated in media comprising a hot water extract of licorice products for 24h, and the concentrations of G-CSF in the media were measured using enzyme-linked immunosorbent assay (ELISA). RESULTS: Licorice extract induced G-CSF secretion in MCE301 cells, and the active ingredients of licorice were high molecular compounds. Although the roasted licorice extract exhibited the activity similar to that of the unprocessed licorice extract, honey-roasted licorice extracts exhibited a significantly higher inducible effect on G-CSF secretion in the cells than that of unprocessed or roasted licorice extracts without pretreatment with honey. This enhanced activity was dependent on the temperature and heating time. CONCLUSIONS: The enhanced inducible effect of honey-roasted licorice on G-CSF secretion might be attributed to the combined effect of licorice-derived high molecular compounds and heated-honey-derived compounds. The results of this study can scientifically explain the objective of processing via honey-roasting in TCM theory." As taken from Ota M et al. 2018. J. Ethnopharmacol. 214, 1-7. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29203272

"Women are increasingly using botanical dietary supplements (BDS) to reduce menopausal hot flashes. Although licorice (Glycyrrhiza sp.) is one of the frequently used ingredients in BDS, the exact plant species is often not identified. We previously showed that in breast epithelial cells (MCF-10A), Glycyrrhiza glabra (GG) and G. inflata (GI), and their compounds differentially modulated P450 1A1 and P450 1B1 gene expression, which are responsible for estrogen detoxification and genotoxicity, respectively. GG and isoliquiritigenin (LigC) increased CYP1A1, whereas GI and its marker

compound, licochalcone A (LicA), decreased CYP1A1 and CYP1B1 The objective of this study was to determine the distribution of the bioactive licorice compounds, the metabolism of LicA, and whether GG, GI, and/or pure LicA modulate NAD(P)H quinone oxidoreductase (NQO1) in an ACI rat model. In addition, the effect of licorice extracts and compounds on biomarkers of estrogen chemoprevention (CYP1A1) as well as carcinogenesis (CYP1B1) was studied. LicA was extensively glucuronidated and formed GSH adducts; however, free LicA as well as LigC were bioavailable in target tissues after oral intake of licorice extracts. GG, GI, and LicA caused induction of NQO1 activity in the liver. In mammary tissue, GI increased CYP1A1 and decreased CYP1B1, whereas GG only increased CYP1A1 LigC may have contributed to the upregulation of CYP1A1 after GG and GI administration. In contrast, LicA was responsible for GI-mediated downregulation of CYP1B1 These studies highlight the polypharmacologic nature of botanicals and the importance of standardization of licorice BDS to specific Glycyrrhiza species and to multiple constituents." As taken from Wang S et al. 2018. Cancer Prev. Res. (Phila.) 11(12), 819-830. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30287522

"Patients with decreased liver function or hypokalemia, women with preeclampsia or persons with apparent mineralocorticoid excess (AME), an inherited rare form of hypertension caused by mutations in the 11β-HSD2 gene, may be especially susceptible to excessive intake of liquorice."

As taken from VKM, 2018.

"Focusing on licorice, a highly used raw material in health foods, quantitative analysis of functional/medicinal components and a safety and functional evaluation was carried out for herbal medicines, health food ingredients, and so-called health foods. A functional component, glabridin, was detected in herbal medicines from Glycyrrhiza glabra and G. inflata, health food ingredients, and in commercially available health foods that contain licorice. Likewise, glycyrrhizin, a medicinal component, was detected in these sources, except in licorice oil extract. Estrogen activity in vitro was detected in some of the herbal medicines, health food ingredients, and in health foods containing licorice. In the in vivo study, liver weight in ovariectomized (OVX) mice treated with licorice oil extract was significantly higher than that in OVX and sham mice in a dose dependent manner. These results suggest that excessive intake of licorice oil extract from health foods should be avoided, even though these ingredients might be beneficial for medical use in order to maintain bone health in postmenopausal women. Measurement of hepatic cytochrome P-450 (CYP) activity, reproductive organ weight, and fat and bone mass in OVX mice was considered useful for evaluating the safety and efficacy of estrogenic health food ingredients derived from herbal medicines." As taken from Ishimi Y et al. 2019. Toxicol. Rep. 6, 904-913. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31508319/

"We present here a new selection criterion for prioritizing research on efficacious drugs for the fight against COVID-19: the relative toxicity versus safety of herbal medications, which were effective against SARS in the 2002/2003 epidemic. We rank these medicines according to their toxicity versus safety as basis for preferential rapid research on their potential in the treatment of COVID-19. The data demonstrate that from toxicological information nothing speaks against immediate investigation on, followed by rapid implementation of Lonicera japonica, Morus alba, Forsythia suspensa, and Codonopsis spec. for treatment of COVID-19 patients. Glycyrrhiza spec. and Panax ginseng are ranked in second priority and ephedrine-free Herba Ephedrae extract in third priority (followed by several drugs in lower preferences). Rapid research on their efficacy in the therapy - as well as safety under the specific circumstances of COVID-19 - followed by equally rapid implementation will provide substantial advantages to Public Health including immediate availability, enlargement of medicinal possibilities, in cases where other means are not successful (non-responders), not tolerated (sensitive individuals) or just not available (as is presently the case) and thus minimize sufferings and save lives. Moreover, their moderate costs and convenient oral application are especially advantageous for underprivileged populations in developing countries." As taken from Oesch F et al. 2021. Phytomedicine. Epub ahead of print. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33593628/

“Licorice, the root of Glycyrrhiza glabra, has been observed to possess an anti-obesity effect. Previous research has suggested that licorice acetone extract (LE) has an influence on mitotic clonal expansion (MCE) and adenosine monophosphate-activated protein kinase (AMPK), which play a key role in regulating adipogenesis. This study sought further insight into the molecular mechanism of LE's anti-obesity effect using 3T3-L1 adipocytes in vitro. LE inhibited 3T3-L1 adipogenesis, and the inhibitory effect of LE on adipogenesis was most significant in the early stage of adipogenic differentiation. LE inhibited the protein expression of cyclins and cyclin-dependent kinases in the MCE stage and arrested cells in the G1 phase of the cell cycle. Furthermore, it activated AMPK via phosphorylation. Moreover, the expression levels of lipid metabolism-related genes were regulated by LE. These findings suggest the anti-obesity effect of LE via MCE and AMPK regulation. PRACTICAL APPLICATIONS: Although the anti-obesity effects of licorice have been studied, the application of functional food-related anti-obesity effects of licorice has been less than that of other extracts. The present study increases the reliability of the anti-obesity effect of licorice by suggesting a new mechanism of action and expands the application of functional foods related to the anti-obesity effect of licorice. A new mechanistic insight will not only improve the scientific knowledge but will also help to predict the side effects of licorice's anti-obesity application.” As taken from Lee MH et al. 2020. J. Food Biochem. 44(12), e13528. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33051883/

6. Functional effects on

6.1. Broncho/pulmonary system

“Bates et al., 1999, have speculated (not peer reviewed) that licorice extract may cause bronchodilation.” As taken from Food and Chemical Toxicology, 43, 2005, pp. 1303-1322.

“Licorice has been used as an antitussive and expectorant herbal medicine for a long history. This work evaluated the activities of 14 major compounds and crude extracts of licorice, using the classical ammonia-induced cough model and phenol red secretion model in mice. Liquiritin apioside (1), liquiritin (2), and liquiritigenin (3) at 50 mg/kg (i.g.) could significantly decrease cough frequency by 30-78% ($p < .01$). The antitussive effects could be partially antagonized by the pretreatment of methysergide or glibenclamide, but not naloxone. Moreover, compounds 1-3 showed potent expectorant activities after 3 days treatment ($p < .05$). The water and ethanol extracts of licorice, which contain abundant 1 and 2, could decrease cough frequency at 200 mg/kg by 25-59% ($p < .05$), and enhance the phenol red secretion ($p < .05$), while the ethyl acetate extract showed little effect. These results indicate liquiritin apioside and liquiritin are the major antitussive and expectorant compounds of licorice. Their antitussive effects depend on both peripheral and central mechanisms.” As taken from Kuang Y et al. 2018. Bioorg. Med. Chem. 26(1), 278-284. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29224994

“CONTEXT: The cough is a protective reflex, with 2 types, one being more sensitive to mechanical stimulation and the other to chemical stimulation, such as sulfur dioxide, ammonia, citric acid, and capsaicin. Some evidence is available that suppressant therapy is most effective when used for the short-term reduction of coughing. Today, use of herbal drugs is increasing all over the world for various ailments, including to provide antitussive activity. OBJECTIVE: The study intended to review the antitussive effects of various extracts, some fractions, and some constituents of the studied medicinal plants. DESIGN: Various databases, including the Medline, Science Direct, Scopus, and Google Scholar, were searched for studies published between 1978 and 2015, using the keywords antitussive and cough and the names of various medicinal plants and their constituents. SETTING: The study took place in the districts related to Mashhad University of Medical Sciences (Mashhad, Iran). OUTCOME MEASURES: The antitussive effects of medicinal plants and their constituents were normalized to 50 mg/kg and 1 mg/mL against various cough stimulants and compared. RESULTS: The most potent antitussive effect was observed for Nigella sativa and Linum usitatissimum on coughs induced by sulfur dioxide. Artemisia absinthium showed a higher antitussive effect on cough induced by ammonia compared with the other studied medicinal plants. The antitussive effects of Cuminum cyminum and Glycyrrhiza glabra were more potent on cough induced

by citric acid than other medicinal plants. CONCLUSIONS: These results suggest the therapeutic potential of the studied medicinal plants as antitussive therapies. However, only a few clinical studies have examined the antitussive effects of medicinal plants, and more clinical studies are needed. The underlying mechanisms of the antitussive effects of medicinal plants should be also examined in further studies." As taken from Saadat S et al. 2018. Altern. Ther. Health Med. 24(4), 36-49. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29332022

6.2. Cardiovascular system

"The roots and rhizomes of various species of the perennial herb licorice (Glycyrrhiza) are used in traditional medicine for the treatment of several diseases. In experimental and clinical studies, licorice has been shown to have several pharmacological properties including… cardioprotective effects. In recent years, several of the biochemical, molecular, and cellular mechanisms of licorice and its active components have also been demonstrated in experimental studies. In this review, we summarized the new phytochemical, pharmacological, and toxicological data from recent experimental and clinical studies of licorice and its bioactive constituents after our previous published review" As taken from Hosseinzadeh H and Nassiri-Asl M. 2015. Phytother. Res. 29(2), 1866-86. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26462981

"Medical history | We report on a 44-year-old patient with recurrent thoracic pain occurring 4 months apart. The patient complained about intense thoracic pain and acute dyspnoea in the morning. In the course of the second presentation the anamnesis revealed that the previous day the patient had consumed an entire bag of licorice (200 g). Investigations | The blood pressure was 90/65 mmHg, heart rate 68 beats / min. Neither the performed ECG nor the transthoracic echocardiography showed abnormalities. The blood tests revealed elevated troponin levels only. No coronary artery stenosis was evident on left heart catheterization. After 4 months- the symptoms reappeared- the blood pressure was 110/50 mmHg. An ECG showed infarct-typical ST elevations. The performed coronary angiography showed no stenosis or embolism. Intracoronary nitro administration resulted in significant vasodilatation. After 6 hours in the control- ECG the ST elevations were missing. We diagnosed a Prinzmetal angina. Treatment and course | The patient was given advice not to consume licorice in the future. Her medication was adjusted to 2.5 mg amlodipine per day. There has been no further presentation with similar symptoms since then. Conclusion | Case reports provide evidence of unknown potential side- effects concerning well-known medical plants or substances. It is already known that the ingredients of licorice may induce hypertension. Potential spastic reactions, such as a Prinzmetal angina, due to the possible cardiac effects caused by glycyrrhizin and glycyrrhetinic acid are rare side effects of licorice ingestion." As taken from Machalle K et al. (2015). Dtsch. Med. Wochenschr. 140(8), 590-2. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25945908

"BACKGROUND: Ethanolic extract of licorice root has been shown to reduce low-density lipoprotein (LDL) oxidation in atherosclerotic mice and in both hypercholesterolemic and normal lipidemic humans. OBJECTIVE: This study examined the effect of licorice-root extract on carotid intima-media thickness (CIMT) in individuals with hypercholesterolemia. DESIGN: Individuals with hypercholesterolemia (total cholesterol ≥6.18 mmol/L [240 mg/dL]) and without significant stenosis were randomly allocated to two groups: an experimental group that consumed 0.2 g/day of ethanolic extract of licorice root for 12 months, and a control group that received a placebo. RESULTS: Of 110 eligible participants, 94 (41-80 years old) completed the study. A significant CIMT decrease from 0.92±0.25 mm to 0.84±0.21 mm was observed in the experimental group compared with an increase from 0.85±0.17 mm to 0.88±0.19 mm in the control group. Mean plasma total cholesterol levels and LDL cholesterol decreased, at the range baseline to 1 year, from 284±32 mg/dl to 262±25 mg/dl and from 183±8.5 mg/dl to 174±9.1 mg/dl, respectively, for the experimental group ($p<0.001$) and from 291±35 to 289±31 mg/dl and from 177.6±10.7 to 179.3±9.6 ($p=0.08$), respectively, for the control group. Mean high-density lipoprotein (HDL) did not change significantly in either group. In the experimental group, systolic blood pressure decreased from 138±12 mmHg to 125±13 mmHg after

1 year (p=0.01) and increased from 136±15 mmHg to 137±13 mmHg in the control group. Diastolic blood pressure decreased from 92±9 mmHg to 84±10 mmHg (p=0.01) in the experimental group and increased from 89±11 mmHg to 90±8 mmHg in the control group. CONCLUSION: Following 1 year of licorice consumption, mean CIMT, total cholesterol, LDL levels, and blood pressure were decreased. This suggests that licorice may attenuate the development of atherosclerosis and of related cardiovascular diseases" As taken from Fogelman Y et al. 2016. Food Nutr. Res. 60, 30830. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27113136

"BACKGROUND: Licorice, also known as liquorice, refers to the root of Glycyrrhiza glabra L., a product widely available in the market in the form of licorice flavonoid oil (LFO), which is a concentrate of licorice flavonoids, being a dietary ingredient for functional foods with potential benefits for overweight subjects. PURPOSE: To summarize the results of the numerous clinical trials, and to clarify the metabolic changes after licorice consumption, through a systematic review with meta-analysis and Trial Sequential Analysis (TSA) of clinical trials. METHODS: This review was designed according to the PRISMA (Preferred Reported Items for Systematic Reviews and Meta-Analysis) recommendations. Several electronic databases were searched to identify the clinical trials. A meta-analysis approach was then developed to statistically analyze the results, followed by TSA and meta-regression analyses. RESULTS: A total 26 clinical trials were considered for the quantitative synthesis of the data, totalizing 985 patients enrolled. Overall, it was possible to verify that the licorice consumption significantly reduces the body weight (WMD: -0.433 kg; 95% CI: -0.683 to -0.183; p-value = 0.001) and consequently the body mass index (BMI) of patients (WMD: -0.150 kg/m2; 95% CI: -0.241 to -0.058; p-value = 0.001). Another result with statistical significance was the increase in the diastolic blood pressure (DBP) (1.737 mmHg; 95% CI: 0.835 to 2.621; p-value < 0.0001) observed for the group subjected to licorice consumption, which is related to the hypernatremia also caused by licorice. CONCLUSION: The present meta-analysis demonstrated the positive effects of licorice consumption on the reduction of body weight and BMI of patients. However, the results also show the increase in blood pressure of patients associated with the hypernatremia caused by licorice. Consequently, licorice consumption should be avoided by hypertensive patients." As taken from Luis A et al. 2018. Phytomedicine 39, 17-24. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29433679

"The prevalence of hypertension is likely to grow during the future years, mainly due to aging of the population and increasing prevalence of obesity, as an important risk factor for hypertension. One of the main causes of secondary hypertension, frequently ignored, is represented by certain categories of drugs, that can induce hypertension, increase the blood pressure values in previously controlled hypertension, decrease the effects of antihypertensive medication or induce a hypertensive emergency. These drugs may be over-the-counter medications, illicit drugs or prescription drugs used for the treatment of acute or chronic conditions. The most frequently incriminated drugs are steroids, nonsteroidal anti-inflammatory drugs, sympathomimetic agents, central nervous system stimulants (alcohol, amphetamine), dietary supplements (ginseng, natural liquorice etc), other therapeutic agents (sibutramine, antiemetic agents, oral physostigmine, L-dopa, leflunomide, growth hormone, thyroid hormone, recombinant human erythropoietin), antidepressants, immunosuppressants, antiangiogenic drugs, anaesthetics, heavy metals and toxins. Adding other drugs to antihypertensive treatment should be carefully evaluated by physicians, in order to avoid iatrogenic blood pressure elevations." As taken from Diaconu CC et al. 2018. Acta Cardiol. 1, 1-7. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29291681

"A 48-year-old woman presented to the Accident and Emergency department with a 4 month history of headaches, nausea and dizziness. She was found to have severe hypertension and hypokalaemia. Extensive investigations did not find any secondary cause for hypertension. The patient was discharged with oral doxazosin therapy which controlled the blood pressure. Before the follow-up appointment at the hypertension clinic, the patient and her husband identified that her headaches coincided with liquorice tea consumption of up to three cups per day. This information was not obtained in the clinical assessment. The patient is now headache and medication free after cessation of liquorice tea. Liquorice ingestion is often a forgotten reversible cause of hypertension. A good

history is key to this diagnosis." As taken from Foster S et al. 2017. BMJ Case Rep. 2017, bcr-2017-222077. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29127128

"We investigated the haemodynamic effects of two-week liquorice exposure (glycyrrhizin dose 290-370 mg/day) in 22 healthy volunteers during orthostatic challenge. Haemodynamics were recorded during passive 10-minute head-up tilt using radial pulse wave analysis, whole-body impedance cardiography, and spectral analysis of heart rate variability. Thirty age-matched healthy subjects served as controls. Liquorice ingestion elevated radial systolic ($p < 0.001$) and diastolic ($p = 0.018$) blood pressure and systemic vascular resistance ($p = 0.037$). During orthostatic challenge, heart rate increased less after the liquorice versus control diet ($p = 0.003$) and low frequency power of heart rate variability decreased within the liquorice group ($p = 0.034$). Liquorice intake increased central pulse pressure ($p < 0.001$) and augmentation index ($p = 0.002$) supine and upright, but in the upright position the elevation of augmentation index was accentuated ($p = 0.007$). Liquorice diet also increased extracellular fluid volume ($p = 0.024$) and aortic to popliteal pulse wave velocity ($p = 0.027$), and aortic characteristic impedance in the upright position ($p = 0.002$). To conclude, in addition to increased extracellular fluid volume and large arterial stiffness, two weeks of liquorice ingestion elevated systemic vascular resistance and augmentation index. Measurements performed at rest may underestimate the haemodynamic effects of liquorice ingestion, as enhanced central wave reflection and reduced chronotropic response were especially observed in the upright position." As taken from Hautaniemi EJ et al. 2017. Sci. Rep. 7(1), 10947. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28887501

"There have been numerous case reports of severe adverse events including deaths following chronic licorice ingestion. The aim of the present study was to evaluate the effect of chronic ingestion of licorice on blood pressure, plasma potassium, plasma renin activity and plasma aldosterone. A search of MEDLINE, PubMed, EMBASE, CENTRAL, DARE, CINAHL and Current Contents Connect was performed from inception through to 26 April 2017. Trials that included a treatment group ingesting a product containing at least 100 mg of glycyrrhizic acid daily were selected. Pooled mean changes from baseline with 95% confidence intervals were calculated for diastolic blood pressure, systolic blood pressure, plasma potassium, plasma renin activity and plasma aldosterone using a random effects model. An assessment of dose-response was also undertaken. A total of 18 studies (n=337) were included in the meta-analysis. There was a statistically significant increase in mean systolic blood pressure (5.45 mm Hg, 95% CI 3.51-7.39) and diastolic blood pressure (3.19 mm Hg, 95% CI 0.10-6.29) after chronic ingestion of a product containing glycyrrhizic acid. Plasma potassium (-0.33 mmol l-1, 95% CI -0.42 to 0.23), plasma renin activity (-0.82 ngml-1 per hour, 95% CI -1.27 to -0.37) and plasma aldosterone (-173.24 pmol l-1, 95% CI -231.65 to -114.83) were all significantly decreased. A significant correlation was noted between daily dose of glycyrrhizic acid and systolic blood pressure (r2=0.55) and diastolic blood pressure (r2=0.65), but not for the other outcome measures. Hence, chronic licorice ingestion is associated with an increase in blood pressure and a drop in plasma potassium, even at modest doses. This is of particular relevance for individuals with existing cardiovascular disease." As taken from Penninkilampi R et al. 2017. J. Hum. Hypertension 31(11), 699-707. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28660884

"Licorice (Glycyrrhiza glabra) has been considered as an herbal drug since ancient time. Nowadays, it is a well-known spice that possesses worth pharmacological effects. However, some relevant articles have revealed negative impacts of licorice in health. By considering the great wishes in using herbal medicine, it is important to show adverse effects of herbal medicine in health. At present, there are misunderstandings toward the safety of herbal medicines. Herein, we gathered scientific research projects on the toxicity effects of licorice and glycyrrhizin to highlight their safety. In this regards, we categorized our findings about the toxicity effects of licorice and glycyrrhizin in acute, sub-acute, sub-chronic, and chronic states. Besides, we discussed on the cytotoxicity, genotoxicity, mutagenicity, and carcinogenicity of licorice and glycyrrhizin as well as their developmental toxicity. This review disclosed that G. glabra and glycyrrhizin salts are moderately toxic. They need to be used with caution during pregnancy. G. glabra and glycyrrhizin possess selective cytotoxic effects on cancerous cells. The most important side effects of licorice and glycyrrhizin are hypertension and hypokalemic-

induced secondary disorders. Licorice side effects are increased by hypokalemia, prolonged gastrointestinal transient time, decreased type 2 11-beta-hydroxysteroid dehydrogenase activities, hypertension, anorexia nervosa, old age, and female sex." As taken from Nazari S et al. 2017. Phytother. Res. 31(11), 1635-1650. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28833680

"BACKGROUND: The purpose of this review was to create an online research summary table of heart toxicity case reports related to dietary supplements (DS; includes herbs). METHODS: Documented PubMed case reports of DS appearing to contribute to heart-related problems were used to create a "Toxic Table" that summarized the research (1966 to April, 2016, and cross-referencing). Keywords included "herb," "dietary supplement," and cardiac terms. Case reports were excluded if they were herb combinations (some exceptions), Chinese herb mixtures, teas of mixed herb contents, mushrooms, poisonous plants, self-harm (e.g. suicide), excess dose (except vitamins/minerals), drugs or illegal drugs, drug-herbal interactions, and confounders of drugs or diseases. The spectrum of heart toxicities included hypertension, hypotension, hypokalemia, bradycardia, tachycardia, arrhythmia, ventricular fibrillation, heart attack, cardiac arrest, heart failure, and death. RESULTS: Heart related problems were associated with approximately seven herbs: Four traditional Chinese medicine herbs - Don quai (Angelica sinensis), Jin bu huan (Lycopodium serratum), Thundergod vine or lei gong teng (Tripterygium wilfordii Hook F), and Ting kung teng (Erycibe henryi prain); one an Ayruvedic herb - Aswagandha, (Withania somnifera); and two North American herbs - blue cohosh (Caulophyllum thalictroides), and Yohimbe (Pausinystalia johimbe). Aconitum and Ephedra species are no longer sold in the United States. The DS included, but are not limited to five DS - bitter orange, caffeine, certain energy drinks, nitric oxide products, and a calming product. Six additional DS are no longer sold. Licorice was the food related to heart problems. CONCLUSION: The online "Toxic Table" forewarns clinicians, consumers and the DS industry by listing DS with case reports related to heart toxicity. It may also contribute to Phase IV post marketing surveillance to diminish adverse events that Government officials use to regulate DS." As taken from Brown AC. 2018. J. Diet. Suppl. 15(4), 516-555. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/28981338

"Arrhythmogenic ingredients in our diet such as mushrooms, licorice, toxic honey, liquid protein drinks, etc. have long been recognized as rare but important considerations in the differential diagnosis of arrhythmias. Anecdotal reports of torsades de pointes (TdP), arrhythmias and/or sudden death and small studies in normal subjects have suggested that simple ingredients such as grapefruit juice or ingredients in energy drinks marketed as dietary supplements could have direct arrhythmogenic actions, especially in patients with congenital long QT syndrome (cLQTS). Two recent studies that employed the industry-standard "thorough QT" trial design leave no doubt that grapefruit juice and some energy drinks can prolong the QTc interval and to exceed 500 msec. in some patients with cLQTS, a threshold known to signal imminent danger. These reports raise numerous clinically important questions such as which other patients may be at risk of arrhythmias. For example, patients with multiple clinical risk factors for TdP (hypokalemia, bradycardia, female sex, etc.) may be at risk from these and possibly other dietary ingredients ingested by millions of people each day. It is essential that further research evaluate the safety of these and similar food products and that vulnerable patients, especially those with cLQTS, be warned of this serious and emerging threat." As taken from Woosley RL. 2020. Trends Cardiovasc. Med. 30(5), 310-312. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/31477495/

"Liquorice is one of the oldest known herbs with medicinal properties and comprises up to 300 active compounds. It has been used for millennia for its digestive, anti-inflammatory and anti-infective properties. However, its possible toxic effects were described only a few years ago and there is growing interest in the side effects associated with chronic consumption. The main active component of liquorice is the prodrug glycyrrhizin and its active metabolite glycyrrhetic acid. It is a rare cause of hypokalaemia due to suppression of the renin-angiotensin-aldosterone axis, causing pseudohyperaldostenonism (PHA). We describe a rare case of secondary acute myocardial infarction in a patient with chronic consumption of liquorice." As taken from Vallejo-Garcia VE et al. 2021.

Hipertens. Riesgo Vasc. Epub ahead of print. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33715981/

“A 45-year-old man was admitted to the Emergency Department with fatigue and muscular weakness. Soon after hospital admission, he developed "torsades de pointe" and was successfully resuscitated. The admission laboratory investigations had revealed a profound hypokalemia (1.65 mmol/L). The patient had a long-term use of alcohol-free "pastis" in an attempt to reduce his chronic ethanol consumption. As the beverage likely contained a significant amount of liquorice, the diagnosis of glycyrrhizin chronic intoxication was suspected. The diagnosis of liquorice-related pseudohyperaldosteronism was assessed by normal plasma aldosterone levels and low plasma renin activity. Intravenous and oral supplementation of potassium was required for 5 days, and the patient had an uneventful follow-up.” As taken from Attou R et al. 2020. Case Rep. Emerg. Med. 2020, 3727682. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33029435/

“Rationale: Excessive ingestion of licorice can cause pseudohyperaldosteronism. A few case reports in the available literature have described significant hypokalemia secondary to licorice consumption with clinical manifestations of muscle weakness, paralysis, or severe hypertension. To our knowledge, no report has discussed severe asymptomatic hypokalemia associated with licorice consumption. Patient concerns: A 79-year-old man presented to the urology clinic with a several-month history of urinary frequency and a weak stream. Routine laboratory investigations revealed serum potassium (K) level of 1.8 mmol/L, and he was immediately admitted to the nephrology department. Diagnoses: He was in a good state of health, and systemic and neurological examinations were unremarkable. However, laboratory investigations revealed severe hypokalemia and metabolic alkalosis accompanied with renal K wasting and hypertension, suggesting a state of mineralocorticoid excess. Hormonal studies revealed low serum renin and aldosterone but normal serum cortisol levels. Detailed history taking revealed that he had used licorice tea daily during the preceding 18 months. Interventions and outcome: The patient's serum K returned to normal levels after vigorous K replacement and discontinuation of licorice intake. He was also diagnosed with benign prostatic hyperplasia during hospitalization and was treated. Lessons: Chronic licorice ingestion can precipitate severe hypokalemia, although patients may remain asymptomatic. This case report indicates that the severity of a patient's clinical presentation depends on individual susceptibility, as well as the dose and duration of licorice intake.” As taken from Kwon YE et al. 2020. Medicine (Baltimore) 99(30), e21094. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32791684/

“Long-term, excessive use of licorice can cause hypertension, hypokalemia, and disturbances of adrenal hormones, and therefore should probably be avoided during nursing.”

As taken from LactMed, 2021.

6.3. Nervous system

In the traditional system of medicine, the roots and rhizomes of Glycyrrhiza glabra (family: Leguminosae) have been employed clinically for centuries for their anti-inflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities. The present study was undertaken to investigate the effects of Glycyrrhiza glabra (popularly known as liquorice) on learning and memory in mice. Elevated plus-maze and passive avoidance paradigm were employed to test learning and memory. Three doses (75, 150 and 300 mg/kg p.o.) of aqueous extract of Glycyrrhiza glabra were administered for 7 successive days in separate groups of animals. The dose of 150 mg/kg of the aqueous extract of liquorice significantly improved learning and memory of mice. Furthermore, this dose significantly reversed the amnesia induced by diazepam (1 mg/kg i.p.) and scopolamine (0.4 mg/kg i.p.). Anti-inflammatory and antioxidant properties of liquorice may be contributing favorably to the memory enhancement effect. Since scopolamine-induced amnesia was reversed by liquorice, it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic-transmission in mouse brain. However, further studies are necessitated to identify the exact mechanism of action.

In the present investigation, Glycyrrhiza glabra has shown promise as a memory enhancing agent in all the laboratory models employed (Dhingra D et al., 2004).

Disorders of neuromuscular transmission due to natural environmental toxins (Abstract). A variety of natural toxins of animal, plant, and bacterial origin are capable of causing disorders of neuromuscular transmission. Animal toxins include venomous snakes and arthropods, venoms of certain marine creatures, skin secretions of dart-poison frogs, and poisonous fish, shellfish, and crabs. There are plant poisons such as curare, and bacterial poisons such as botulinum toxin. These act at single or multiple sites of the neuromuscular apparatus interfering with voltage-gated ion channels, acetylcholine release, depolarization of the postsynaptic membrane, or generation and spread of the muscle action potential. The specific actions of these toxins are being widely exploited in the study of neuromuscular physiology and pathology. Some toxins have proved to be valuable pharmaceutical agents. Poisoning by natural neurotoxins is an important public health hazard in many parts of the world, particularly in the tropics. Poisoning may occur by a bite or a sting of a venomous animal, or by the ingestion of poisonous fish, shellfish or other marine delicacies. Contaminated food is a vehicle for poisons such as botulinum toxin. Clinically, a cardinal feature in the symptomatology is muscle paralysis with a distribution characteristic of myasthenia gravis, affecting muscles innervated by cranial nerves, neck flexors, proximal limb muscles, and respiratory muscles. Respiratory paralysis may end fatally. This paper reviews from the clinical and pathophysiologic viewpoints, naturally occurring environmental neurotoxins acting at the neuromuscular junction. As taken from Senanayake N, Román GC. J Neurol Sci. 107(1), 1-13. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/1315843

Central hypertensinogenic effects of glycyrrhizic acid and carbenoxolone (Abstract). The apparent mineralocorticoid excess syndrome of patients ingesting large amounts of licorice or its derivatives is thought to be caused by the antagonism by these compounds of the enzyme 11 beta-hydroxysteroid dehydrogenase (11 beta-HSD). 11 beta-HSD inactivates cortisol and corticosterone, allowing the more abundantly produced glucocorticoids access to the mineralocorticoid receptor (MR) in the kidney, where they act as mineralocorticoids. We have found that the infusion of both glycyrrhizic acid, an active principle of licorice, and carbenoxolone, a synthetic analogue, into a lateral ventricle of the brain [intracerebroventricular (icv)] of a rat, at a dose less than that which has an effect when infused subcutaneously, produces hypertension. Furthermore, the hypertension produced by the oral administration of carbenoxolone or glycyrrhizic acid is blocked by the icv administration of RU 28318, an MR antagonist, at a dose below that which has an effect on blood pressure when infused subcutaneously. While the oral administration caused saline polydipsia and polyuria typical of chronic systemic mineralocorticoid excess, the icv licorice derivatives produced hypertension without affecting saline appetite. Sensitizing the rats to mineralocorticoid hypertension by renal mass reduction and increasing salt consumption was not necessary for the production of hypertension. These findings provide additional evidence for a central role in blood pressure control by mineralocorticoids that is distinct from their renal effects. They also suggest that more is involved in licorice-induced hypertension than only inhibition of 11beta-HSD. As taken from Gomez-Sanchez EP, Gomez-Sanchez CE. Am J Physiol. 1992 Dec; 263(6 Pt 1):E1125-30. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/1476186

“BACKGROUND: Posterior reversible encephalopathy syndrome is characterized by a combination of clinical-radiological findings and pathophysiologically by localized brain vasogenic edema. Many clinical illnesses may trigger the onset of posterior reversible encephalopathy syndrome and hypertension is present in about 80% of patients. METHODS: We describe a child with high consumption of licorice toffees who developed systemic hypertension followed by posterior reversible encephalopathy syndrome. RESULTS: This boy was hospitalized following a cluster of generalized tonic-clonic seizures. Monitoring his clinical parameters, we detected constant high blood pressure and a brain magnetic resonance scan showed a localized vasogenic edema; these symptoms suggested posterior reversible encephalopathy syndrome. He had been eating licorice toffees for a period of 4 months, consuming an estimated 72 mg of glycyrrhizic acid per day; this led to our

assumption of the reason for his hypertension. CONCLUSION: There are several reported examples of posterior reversible encephalopathy syndrome-induced licorice hypertension in adults, but none related to children. Our report examines a possible link between licorice consumption and hypertension/posterior reversible encephalopathy syndrome in children." As taken from Tassinari D et al. 2015. Pediatr. Neurol. 52(4), 457-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25680999

"SCOPE: Glycyrrhiza uralensis extract (GUE) has been reported to improve amyloid beta (Aβ)-induced cognitive deficits in mice. However, the mechanisms underlying this effect and the components involved have not been previously explored. Extracellular Aβ plaques are one of the major pathological hallmarks of Alzheimer's disease (AD). Therefore, decreasing Aβ levels is one strategy for preventing the etiology of AD. This study aims to test the effect of GUE and semilicoisoflavone B (SB) on Aβ secretion and investigates the mechanism underlying this effect. METHODS AND RESULTS: GUE and its bio-activated compound SB reduce Aβ secretion. We find that this effect contribute to the downregulation of the β-secretase-1 (BACE1) protein and mRNA. In a subsequent mechanism study, we find that GUE and SB regulate BACE1 transcription factors by inducing the expression of peroxisome proliferator activated receptor γ (PPARγ) and inhibiting the phosphorylation of signal transducer and activator of transcription 3. In addition, the effect of GUE and SB on BACE1 expression and Aβ secretion are attenuated by treatment with PPARγ-siRNA or its antagonist, GW9662. CONCLUSION: These findings indicate that GUE and SB may function as PPARγ agonists, thereby inhibiting BACE1 expression and ultimately reducing the secretion of Aβ." As taken from Gu MY et al. 2018. Mol. Nutr. Food Res. 62(6), e1700633. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29143445

"Licorice derived from the roots and rhizomes of Glycyrrhiza uralensis Fisch. (Fabaceae), is one of the most widely-used traditional herbal medicines in China. It has been reported to possess significant analgesic activity for treating spastic pain. The aim of this study is to investigate the spasmolytic molecular mechanism of licorice on oxytocin-induced uterine contractions and predict the relevant bioactive constituents in the aqueous extract. The aqueous extraction from licorice inhibited the amplitude and frequency of uterine contraction in a concentration-dependent manner. A morphological examination showed that myometrial smooth muscle cells of oxytocin-stimulated group were oval-shaped and arranged irregularly, while those with a single centrally located nucleus of control and licorice-treated groups were fusiform and arranged orderly. The percentage of phosphorylation of HSP27 at Ser-15 residue increased up to 50.33% at 60 min after oxytocin stimulation. Furthermore, this increase was significantly suppressed by licorice treatment at the concentration of 0.2 and 0.4 mg/mL. Colocalization between HSP27 and α-SMA was observed in the myometrial tissues, especially along the actin bundles in the oxytocin-stimulated group. On the contrary, the colocalization was no longer shown after treatment with licorice. Additionally, employing ChemGPS-NP provided support for a preliminary assignment of liquiritigenin and isoliquiritigenin as protein kinase C (PKC) inhibitors in addition to liquiritigenin, isoliquiritigenin, liquiritin and isoliquiritin as MAPK-activated protein kinase 2 (MK2) inhibitors. These assigned compounds were docked with corresponding crystal structures of respective proteins with negative and low binding energy, which indicated a high affinity and tight binding capacity for the active site of the kinases. These results suggest that licorice exerts its spasmolytic effect through inhibiting the phosphorylation of HSP27 to alter the interaction between HSP27 and actin. Furthermore, our results provide support for the prediction that potential bioactive constituents from aqueous licorice extract inhibit the relevant up-stream kinases that phosphorylate HSP27." As taken from Yang L et al. 2017. Molecules 22(9), E1392. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28850076

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

The main effect of crude licorice is a deoxycortone-like action, which may cause oedema, hypertension, myopathy and hypokalaemia (Calvert 1954; Blachley & Knochel 1980; Sundaram & Swaminathan 1981; Cugini et al. 1983). A study investigating the effects of pure glycyrrhetinic acid,

the active component of licorice, on plasma cortisol and cortisone in healthy humans found that glycyrrhetinic acid exerts mineralocortiocoid action by a direct inhibition of the conversion of cortisol to cortisone (MacKenzie et al.1990).

“In the present study we describe the mechanism by which glycyrrhizin inhibits complement. Glycyrrhizin inhibited the cytolytic activity of complement via the activation of both the classical and alternative pathways, while it had no effect on immune adherence, suggesting that it blocks C5 or a later stage of the complement cascade. Further analysis revealed that glycyrrhizin inhibits the lytic pathway in which the membrane attack complex (MAC) is formed. This mechanism suggests that glycyrrhizin may prevent tissue injury caused by MAC not only in chronic hepatitis but in many autoimmune and inflammatory diseases.” As taken from Fujisawa Y 2000. Microbiol Immunol. 44(9), 799-804. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11092245&query_hl=5&itool=pubmed_DocSum

“Licorice is known to exhibit many pharmacological activities, including estrogenic in laboratory animals, antiulcer, mineralocorticoid with sodium retention and potassium loss leading to hypertension (due to glycyrrhizin), inhibition of tumor growth (sarcoma 45 and Ehrlich ascites cells, due to a glycyrrhetinic acid salt and derivative), antitrichomonas, antiinflammatory, antiallergic, antitoxic, antitussive (comparable to codeine, due to a derivative of 18-beta-glycyrrhetinic acid), anticonvulsive, and antibacterial, among others. Liquiritigenin and isoliquiritigenin have MAO-inhibitory activities. Recent work has found that glycyrhetinic acid inhibits 11-beta-hydroxysteroid dehydrogenase in rats, and potentiates the action of hydrocortisone in humans. The flavonoids have recently been shown to have a strong antioxidant and antihepatotoxic activities.” As taken from Encyclopaedia of common natural ingredients used in food, drugs and cosmetics, 2nd edition, A. Yeung & S. Foster, 2003, pp. 346-350.

“Much of the interest on the chemopreventive properties of licorice has been focused on the plant genius Glycyrrhiza glabra. In this study the ethanol extract of Chinese licorice root, Glycyrrhiza uralensis (G. uralensis) was investigated for its estrogenic effect and the ability to inhibit cell proliferation in the MCF-7 human breast cancer cell line. The extract of the root of G. uralensis was fractionated in EtOH:H(2)O (80:20) (80% ethanol). The extract exhibited estrogenic effects similar to 17beta- estradiol (E2) and induced apoptosis at the same dose level (100 microg/ml) in MCF-7 breast cancer cells, results were associated with up-regulation of tumor suppressor gene p53 and pro-apoptotic protein Bax. G. uralensis extract caused the up-regulation of p21(waf1/cip1) and down-regulation of cdk 2 and cyclin E and most significantly, induced G1 cell cycle arrest. This is the first study to show that the ethanolic extract of the root of G. uralensis has an estrogen-like activity and anti-cancer effects against MCF-7 human breast cancer cells. Whilst the use of phytoestrogens to protect against hormone-dependent cancers or as a 'natural' alternative to hormone replacement therapy remains controversial, the data in this paper support the suggestion that extracts of root of the Chinese licorice G. uralensis might be of importance in this debate.” As taken from Jo EH et al. Cancer Lett. 230(2), 239-47. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16297710&query_hl=5&itool=pubmed_DocSum

“The extract of licorice (Glycyrrhiza uralensis FISHER, Leguminosae) showed CYP3A4 inhibitory activity with the IC50 value of 0.022 mg/ml. Bioassay-guided purification afforded nine compounds, 3-(p-hydroxyphenyl)propionic acid (1), isoliquiritigenin (2), (3R)-vestitol (3), licopyranocoumarin (4), 4-hydroxyguaiacol apioglucoside (5), liquiritin (6), liquiritigenin 7,4'-diglucoside (7), liquiritin apioside (8), and glucoliquiritin apioside (9). Among these compounds, 3, 7, and 5 showed potent CYP3A4 inhibitory activities with IC50 values of 3.6, 17, and 20 microM, respectively. Glycyrrhizin (10), a main constituent of licorice, however, was inactive for CYP3A4 inhibition.” As taken from Tsukamoto S et al. Biol Pharm Bull. 2005 Oct; 28(10), 2000-2. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16204965&query_hl=5&itool=pubmed_DocSum

“Tyrosinase is a key enzyme in the production of melanins. Phytochemical studies of a Glycyrrhiza uralensis extract were performed by measuring the tyrosinase and melanin synthesis inhibitory activity. Glycyrrhisoflavone and glyasperin C were identified as tyrosinase inhibitors for the first time. Glyasperin C showed a stronger tyrosinase inhibitory activity (IC (50) = 0.13 +/- 0.01 microg/mL) than glabridin (IC (50) = 0.25 +/- 0.01 microg/mL) and a moderate inhibition of melanin production (17.65 +/- 8.8 % at 5 microg/mL). Glycyrrhisoflavone showed a strong melanin synthesis inhibitory activity (63.73 +/- 6.8 % inhibition at 5 microg/mL). These results suggest that glyasperin C and glycyrrhisoflavone could be promising candidates in the design of skin-whitening agents.” As taken from Kim HJ et al. Planta Med. 2005 Aug; 71(8):785-7. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16142649&query_hl=5&itool=pubmed_DocSum

“(R)-4-(3,4-Dihydro-8,8-dimethyl)-2H,8H-benzo[1,2-b:3,4-b']dipyran-3yl)-1,3-benzenediol (glabridin), a flavonoid present in licorice extract, is known to have antimicrobial, anti-inflammatory, and cardiovascular protective activities. In the present study, we report the inhibitory effect of glabridin on nitric oxide (NO) production and inducible nitric oxide (iNOS) gene expression in murine macrophages. Glabridin attenuated lipopolysaccharide (LPS)-induced NO production in isolated mouse peritoneal macrophages and RAW 264.7 cells, a mouse macrophage-like cell line. Moreover, iNOS mRNA expression was also blocked by glabridin treatment in LPS-stimulated RAW 264.7 cells. Further study demonstrated that the LPS-induced nuclear factor (NF)-kappaB/Rel DNA binding activity and NF-kappaB/Rel-dependent reporter gene activity were significantly inhibited by glabridin in RAW 264.7 cells and that this effect was mediated through the inhibition of inhibitory factor-kappaB degradation and p65 nuclear translocation. Moreover, reactive oxygen species generation was also suppressed by glabridin treatment in RAW 264.7 cells. In contrast, the activity of mitogen-activated protein kinases was unaffected by glabridin treatment. In animal model, in vivo administration of glabridin increased the rate of survival of LPS-treated mice and inhibited LPS-induced increase in plasma concentrations of nitrite/nitrate and tumor necrosis factor-alpha. Collectively, these data suggest that glabridin inhibits NO production and iNOS gene expression by blocking NF-kappaB/Rel activation and that this effect was mediated, at least in part, by inhibiting reactive oxygen species generation. Furthermore, in vivo anti-inflammatory effect of glabridin suggests a possible therapeutic application of this agent in inflammatory diseases.” As taken from Kang JS et al. J Pharmacol Exp Ther. 2005 Mar; 312(3),1187-94. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15537821&query_hl=5&itool=pubmed_DocSum

“The 70% methanol soluble fraction from a licorice acetone extract was found to inhibit cell proliferation in human monoblastic leukemia U937 cells by inducing apoptosis. Separation by the methods including preparative HPLC provided us with an active compound, which was identified as licocoumarone. Several lines of evidence indicated that licocoumarone induced apoptosis in U937 cells. Thus, licocoumarone is suggested to be potentially useful as a natural anti-cancer agent.” As taken from Watanabe M et al. Biol Pharm Bull. 2002 Oct; 25(10):1388-90. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12392103&query_hl=5&itool=pubmed_DocSum

“The effect of oral administration of a water freeze-dried extract of Glycyrrhiza glabra (liquorice) has been studied at doses of 100, 250 and 500 mg/kg in rats on the plasma concentration of cortisol, adrenocorticotrophic hormone (ACTH), aldosterone, renin, sodium (Na) and potassium (K). The results indicated that treatment induced dose-dependent and mostly significant decreases in the concentration of cortisol, ACTH, aldosterone and K. There were concomitant dose-dependent increases in the concentrations of renin and Na. The results suggest a strong and dose-dependent suppression of the adrenal-pituitary axis, accompanied by stimulation of renin production from the kidney.” As taken from Al-Qarawi AA et al. Food Chem Toxicol. 2002 Oct; 40(10):1525-7. PubMed, 2010 available at

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12387318&query_hl=5&itool=pubmed_DocSum

Effect of licorice and glycyrrhizin on murine liver CYP-dependent monooxygenases (Abstract). This study is aimed to investigate the effect of the prolonged intake of conspicuous amounts of licorice (LE), or its natural constituent glycyrrhizin (G) on murine liver CYP-catalyzed drug metabolism. For this purpose the modulation of the regio- and stereo-selective hydroxylation of testosterone, together with the use of highly specific substrates as probes for different CYP isoforms such as ethoxyresorufin (CYP1A1), methoxyresorufin (1A2), pentoxyresorufin (2B1), p-nitrophenol (2E1) and aminopyrine (3A), were investigated. Daily doses of licorice root extract (3,138 or 6,276 mg/kg b.w. per os), or G (240 or 480 mg/kg b.w. per os), were administered to different groups of Swiss Albino CD1 mice of both sexes for 1, 4 or 10 consecutive days. While a single LE or G dose was unable to affect the multienzymatic CYP-system, using both schedules of repeated treatment, either LE or G were able to significantly induce hepatic CYP3A- and, to a lesser extent, 2B1- and 1A2-depend. As taken from Paolini M, Pozzetti L, Sapone A, Cantelli-Forti G. Life Sci. 1998;62(6):571-82. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/9464470

“OBJECTIVES: Various protective and therapeutic effects such as antioxidant, anti-inflammatory, anticancer, antihistaminic, and antibacterial effects have been depicted for licorice. However, its biological effects in the kidney are still not clear. Therefore, we aimed to investigate the efficiency of licorice in rats with gentamicin (GM)-induced acute tubular necrosis. DESIGN AND METHODS: Rats were randomized into the control group (only saline for 12 days), licorice group (licorice for 12 days), GM group (GM for 12 days), GM + licorice group, and licorice-treated GM group (licorice for 12 days after taking GM for 12 days). Blood urea, creatinine, and uric acid levels were measured and histopathological analyses of the kidneys were performed. The oxidative side of oxidant-antioxidant balance was evaluated by detecting lipid peroxidation (LPO) and total peroxide levels, and antioxidative side was determined by measuring total antioxidant capacity (TAC) and reduced glutathione (GSH) levels in plasma and kidney tissues. RESULTS: The oxidant-antioxidant balance seemed to be shifted to the oxidative side in the GM group when compared with the control and GM + licorice groups. In GM group, biochemical profiles showed a remarkable increase in blood uric acid, urea, and creatinine levels, and depletion of renal tissue and plasma TAC and GSH levels. In addition, histopathologic studies revealed severe acute tubular necrosis, congestion, and hyaline casts, verifying GM-induced nephrotoxicity. Licorice was effective in reduction of blood urea, creatinine, and uric acid levels, and also effective in decreasing the tubular necrosis score. Licorice treatment also significantly reduced LPO and total peroxide levels, and increased TAC and GSH levels in both renal tissue and blood. Moreover, these changes in rats subjected to the combined therapy (GM + licorice) were significantly less than those of GM group. CONCLUSIONS: Licorice ameliorates GM-induced nephrotoxicity and oxidative damage by scavenging oxygen free radicals, decreasing LPO, and improving antioxidant defense”. As taken from Aksoy L et al. 2012. J. Ren. Nutr. 22, 336-343. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22047711?dopt=AbstractPlus.

“This study was performed to investigate the effects of licorice on non-alcoholic fatty liver disease (NAFLD). In this double blind randomized clinical trial, 66 patients were divided into case and control groups. All patients had elevated liver enzymes and had increased liver echogenicity (lipid accumulation) on sonography. The case group was treated with one capsule containing 2 g aqueous licorice root extract per day for 2 months while the control group was treated in the same manner with a placebo. Weight, body mass index (BMI) and liver transaminase levels were measured for each patient before and after the study. In the case group, the mean alanine aminotransferase (ALT) level decreased from 64.09 to 51.27 IU/mL and the aspartate aminotransferase (AST) level decreased from 58.18 to 49.45 IU/mL, which were statistically significant ($p < 0.001$ and $p < 0.001$). But in the control group, a drop in the ALT and AST levels was not statistically significant. The BMI difference before and after the study was not statistically significant in both groups. Despite the significant drop in liver enzymes following administration of licorice root extract, it is recommended that further studies that include histological examination are necessary”. As taken from Hajiaghamohammadi AA et al.

2012. Phytother. Res. 26, 1381-1384. PubMed, 2013 available at: http://www.ncbi.nlm.nih.gov/pubmed/22308054.

“Licorice (Glycyrrhiza glabra Linne) is a well-known medicinal plant and glabridin is an isoflavan isolated from licorice. In this study, we investigated the anti-obesity effect of glabridin and glabridin-rich supercritical fluid extract of licorice (LSC). Glabridin effectively inhibited adipogenesis in 3T3-L1 cells. Moreover, LSC showed inhibitory effect on adipogenesis in a dose-dependent manner. The inhibitory effect of LSC resulted from inhibiting the induction of the transcriptional factors CCAAT enhancer binding protein alpha and peroxisome proliferator-activated receptor gamma. Then we fed mice with high-fat diet containing none, 0.1% and 0.25% LSC for 8weeks to explore the anti-obesity effect of LSC in vivo. LSC significantly reduced weight gain by high-fat diet in a dose-dependent manner. The reductions of the hypertrophy of white adipose tissue and of fat cell size were also observed. In the liver, LSC supplementation effectively inhibited high-fat diet-induced hepatic steatosis through downregulation of gluconeogenesis related phosphoenolpyruvate carboxykinase and glucose 6-phosphatase and upregulation of the β-oxidation related carnitine palmitoyltransferase 1. Taken together, our results suggest that glabridin and glabridin-rich licorice extract would be effective anti-obesity agents”. As taken from Ahn J et al. 2013. Fd Chem. Toxicol. 51, 439-445. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22967722.

“SCOPE: We studied the impact of dietary supplementation with licorice root components on diet-induced obesity, fat accumulation, and hepatic steatosis in ovariectomized C57BL/6 mice as a menopause model. MATERIALS AND METHODS: We evaluated the molecular and physiological effects of dietary licorice root administered to ovariectomized C57BL/6 mice as root powder (LRP), extracts (LRE), or isolated isoliquiritigenin (ILQ) on reproductive (uterus and mammary gland) and nonreproductive tissues important in regulating metabolism (liver, perigonadal, perirenal, mesenteric, and subcutaneous fat). Quantitative outcome measures including body weight, fat distribution (magnetic resonance imaging), food consumption, bone density and weight (Dual-energy X-ray absorptiometry), and gene expression were assessed by the degree of restoration to the preovariectomized health state. We characterized histological (H&E and oil red O staining) and molecular properties (expression of certain disease markers) of these tissues, and correlated these with metabolic phenotype as well as blood levels of bioactives. CONCLUSION: Although LRE and ILQ provided some benefit, LRP was the most effective in reducing body weight gain, overall fat deposition, liver steatosis, and expression of hepatic lipid synthesis genes following ovariectomy. Our data demonstrate that licorice root provided improvement of multiple metabolic parameters under conditions of low estrogen and high-fat diets without stimulating reproductive tissues.” As taken from Madak-Erdogan Z et al. 2016. Mol. Nutr. Food Res. 60(2), 369-80. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26555669

“We describe an unusual case of severe hypokalemia with electrocardiographic changes, due to licorice consumption, in a 15-year-old female student with no previous medical history. Prompt replacement of potassium and cessation of licorice ingestion resulted in a favourable outcome. We also discuss the pathophysiology and diagnosis, emphasizing the importance of a detailed anamnesis to rule out an often forgotten cause of hypokalemia as the licorice poisoning.” As taken from Caravaca-Fontan F et al. 2015. Case Rep. Nephrol. 2015, 957583. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26550501

“The roots and rhizomes of various species of the perennial herb licorice (Glycyrrhiza) are used in traditional medicine for the treatment of several diseases. In experimental and clinical studies, licorice has been shown to have several pharmacological properties including antiinflammatory, antiviral, antimicrobial, antioxidative, antidiabetic, antiasthma, and anticancer activities as well as immunomodulatory, gastroprotective, hepatoprotective, neuroprotective, and cardioprotective effects. In recent years, several of the biochemical, molecular, and cellular mechanisms of licorice and its active components have also been demonstrated in experimental studies. In this review, we summarized the new phytochemical, pharmacological, and toxicological data from recent

experimental and clinical studies of licorice and its bioactive constituents after our previous published review" As taken from Hosseinzadeh H and Nassiri-Asl M. 2015. Phytother. Res. 29(2), 1866-86. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26462981

"INTRODUCTION: The prebiotic potential of herbal medicines has been scarcely studied. METHODS: The authors therefore used anaerobic human fecal cultivation to investigate whether three herbal medicines commonly used in gastrointestinal health and disease in Ayurveda alter the growth and abundance of specific bacterial species. RESULTS: Profiling of cultures supplemented with Glycyrrhiza glabra, Ulmus rubra, or triphala formulation by 16S rDNA sequencing revealed profound changes in diverse taxa in human gut microbiota. Principal coordinate analysis highlights that each herbal medicine drives the formation of unique microbial communities. The relative abundance of approximately one-third of the 299 species profiled was altered by all 3 medicines, whereas additional species displayed herb-specific alterations. Herb supplementation increased the abundance of many bacteria known to promote human health, including Bifidobacterium spp., Lactobacillus spp., and Bacteroides spp. Herb supplementation resulted in the reduced relative abundance of many species, including potential pathogens such as Citrobacter freundii and Klebsiella pneumoniae. Herbal medicines induced blooms of butyrate- and propionate-producing species. U. rubra and triphala significantly increased the relative abundance of butyrate-producing bacteria, whereas G. glabra induced the largest increase in propionate-producing species. To achieve greater insight into the mechanisms through which herbal medicines alter microbial communities, the authors assessed the shifts in abundance of glycosyl hydrolase families induced by each herbal medicine. Herb supplementation, particularly G. glabra, significantly increased the representation and potential expression of several glycosyl hydrolase families. DISCUSSION: These studies are novel in highlighting the significant prebiotic potential of medicinal herbs and suggest that the health benefits of these herbs are due, at least in part, to their ability to modulate the gut microbiota in a manner predicted to improve colonic epithelium function, reduce inflammation, and protect from opportunistic infection. Forthcoming studies in human clinical trials will test the concordance of the results generated in vitro and the predictions made by genome analyses." As taken from Peterson CT et al. 2018. J. Altern. Complement. Med. 24(7), 656-665. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29565634

"BACKGROUND: Hyperpigmentation disorders are commonly encountered in dermatology clinics. Botanical and natural ingredients have gained popularity as alternative depigmenting products. OBJECTIVE: We sought to review clinical studies evaluating the use of different natural products in treating hyperpigmentation so clinicians are better equipped to educate their patients. Specific ingredients reviewed include azelaic acid, aloesin, mulberry, licorice extracts, lignin peroxidase, kojic acid, niacinamide, ellagic acid, arbutin, green tea, turmeric, soy, and ascorbic acid. METHODS: Systematic searches of PubMed and SCOPUS databases were performed in March 2016 using the various ingredient names, "melasma"and "hyperpigmentation." Two reviewers independently screened titles, leading to the selection of 30 clinical studies. RESULTS: Review of the literature revealed few clinical trials that evaluated the treatment of hyperpigmentation with natural ingredients. Despite the limited evidence-based research, several natural ingredients did show efficacy as depigmenting agents, including azelaic acid, soy, lignin peroxidase, ascorbic acid iontophoresis, arbutin, ellagic acid, licorice extracts, niacinamide, and mulberry. CONCLUSION: The aforementioned ingredients show promise as natural treatments for patients with hyperpigmentation disorders. These agents might also provide clinicians and researchers with a way to further characterize the pathogenesis of dyschromia. However, the paucity of clinical studies is certainly a limitation. Additionally, many of the in-vivo studies are limited by the short length of the trials, and questions remain about the long-term efficacy and safety of the ingredients used in these studies. Lastly, we suggest a standardized objective scoring system be implemented in any further comparative studies." As taken from Hollinger JC et al. 2018. J. Clin. Aesthet. Dermatol. 11(2), 28-37. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29552273

"Licorice derived from the roots and rhizomes of Glycyrrhiza uralensis Fisch. (Fabaceae), is one of the most widely-used traditional herbal medicines in China. It has been reported to possess

significant analgesic activity for treating spastic pain. The aim of this study is to investigate the spasmolytic molecular mechanism of licorice on oxytocin-induced uterine contractions and predict the relevant bioactive constituents in the aqueous extract. The aqueous extraction from licorice inhibited the amplitude and frequency of uterine contraction in a concentration-dependent manner. A morphological examination showed that myometrial smooth muscle cells of oxytocin-stimulated group were oval-shaped and arranged irregularly, while those with a single centrally located nucleus of control and licorice-treated groups were fusiform and arranged orderly. The percentage of phosphorylation of HSP27 at Ser-15 residue increased up to 50.33% at 60 min after oxytocin stimulation. Furthermore, this increase was significantly suppressed by licorice treatment at the concentration of 0.2 and 0.4 mg/mL. Colocalization between HSP27 and α-SMA was observed in the myometrial tissues, especially along the actin bundles in the oxytocin-stimulated group. On the contrary, the colocalization was no longer shown after treatment with licorice. Additionally, employing ChemGPS-NP provided support for a preliminary assignment of liquiritigenin and isoliquiritigenin as protein kinase C (PKC) inhibitors in addition to liquiritigenin, isoliquiritigenin, liquiritin and isoliquiritin as MAPK-activated protein kinase 2 (MK2) inhibitors. These assigned compounds were docked with corresponding crystal structures of respective proteins with negative and low binding energy, which indicated a high affinity and tight binding capacity for the active site of the kinases. These results suggest that licorice exerts its spasmolytic effect through inhibiting the phosphorylation of HSP27 to alter the interaction between HSP27 and actin. Furthermore, our results provide support for the prediction that potential bioactive constituents from aqueous licorice extract inhibit the relevant up-stream kinases that phosphorylate HSP27." As taken from Yang L et al. 2017. Molecules 22(9), E1392. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28850076

"Licorice (Glycyrrhiza glabra) has been considered as an herbal drug since ancient time. Nowadays, it is a well-known spice that possesses worth pharmacological effects. However, some relevant articles have revealed negative impacts of licorice in health. By considering the great wishes in using herbal medicine, it is important to show adverse effects of herbal medicine in health. At present, there are misunderstandings toward the safety of herbal medicines. Herein, we gathered scientific research projects on the toxicity effects of licorice and glycyrrhizin to highlight their safety. In this regards, we categorized our findings about the toxicity effects of licorice and glycyrrhizin in acute, sub-acute, sub-chronic, and chronic states. Besides, we discussed on the cytotoxicity, genotoxicity, mutagenicity, and carcinogenicity of licorice and glycyrrhizin as well as their developmental toxicity. This review disclosed that G. glabra and glycyrrhizin salts are moderately toxic. They need to be used with caution during pregnancy. G. glabra and glycyrrhizin possess selective cytotoxic effects on cancerous cells. The most important side effects of licorice and glycyrrhizin are hypertension and hypokalemic-induced secondary disorders. Licorice side effects are increased by hypokalemia, prolonged gastrointestinal transient time, decreased type 2 11-beta-hydroxysteroid dehydrogenase activities, hypertension, anorexia nervosa, old age, and female sex." As taken from Nazari S et al. 2017. Phytother. Res. 31(11), 1635-1650. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28833680

"Purpose: Obesity is a multi-factorial health problem which results from the interaction of environmental and genetic factors. The aim of the present study was to determine the effects of dried licorice extract with a calorie restricted diet on anthropometric indices and insulin resistance with nutrigenetic approach. Methods: For this pilot, double-blind, placebo-controlled randomized clinical trial, 72 eligible subjects were randomly allocated to Licorice or placebo group. They received a low-calorie diet either with a 1.5 g/day of Licorice extract or placebo for 8 weeks. Results: There were no significant differences in anthropometric indices and dietary intake in genotype subgroups at the baseline. Findings indicated that supplementation with Licorice extract did not change anthropometric indices and biochemical parameters significantly compared to a hypocaloric diet alone. However, from the nutrigenetic point of view, significant changes in anthropometric indices and QUICKI were observed in the Pro12Pro genotypes compared to the Pro12Ala at the end of the study ($p<0.05$ in all variables). Moreover, no interactive effect of the Licorice supplement and Pro12Ala genotype was found. Conclusion: In obese subjects, the Pro/Pro polymorphism of the PPAR-γ2 gene seems to

induce favourable effects on obesity management. Further studies are needed to clarify whether PPAR-γ2 gene polymorphisms or other obesity genes can affect responses to obesity treatment." As taken from Namazi N et al. 2017. Adv. Pharm. Bull. 7(2), 221-228. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28761824

"This case highlights the clinical course of a 54-year-old male patient presenting with hypertension and long-term refractory hypokalaemia. He reported long-term malaise, fatigue and physical discomfort. Diarrhoea, vomiting, over-the-counter drugs, dietary supplements and any kind of medical abuse were all denied. Physical examination was normal. Suppressed plasma renin activity along with a low aldosterone level and elevated urinary cortisone/cortisol metabolite excretion ratio raised the suspicion of apparent mineralocorticoid excess (AME). The patient started treatment with spironolactone, but serum potassium levels were persistently fluctuating and the patient was hospitalised for further evaluation. During hospitalisation, repeated medical history and diagnostic examinations revealed licorice-induced AME complicated by excessive use of terbutaline and massive water intake. Licorice discontinuation, reduction of terbutaline and normalisation of water intake led to fully normalised potassium levels. Despite careful clinical history and diagnostic work-up, hospitalisation may be necessary in selected patients with long-term hypokalaemia." As taken from Buhl LF et al. 2018. BMJ Case Rep., pii: bcr-2017-223918. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29674401

"Glycyrrhiza glabra (G. glabra) has been used as a flavoring and sweetener agent, in addition to its therapeutic properties. It is rich in phytoestrogen and may prevent osteoporosis caused by estrogen deficiency; however, there is no evidence for its effects on proliferation and osteogenesis in mesenchymal stem cells. So, we were encouraged to investigate whether the ethyl acetate extract of licorice root as a source of phytoestrogen can act similar to estrogen in cell culture. Furthermore, the analysis of the licorice extract (LE) based on HPLC-DAD-ESI-MS indicated that LE comprises phytoestrogen compounds, such as glabridin and glabrene. In this study, the effects of LE on proliferation of human bone-marrow mesenchymal stem cells (hBM-MSCs) were investigated using MTT assay. In addition, its effects on the osteogenesis were evaluated using alkaline phosphatase activity (ALP), calcium deposition, and bone specific gene expression such as ALP, osteocalcin, Runx2, and BMP-2. The quantitative gene expression was studied by real-time RT-PCR. Our results showed a significant increase in proliferation in presence of LE in concentration 10-50 µg/mL. The differentiation of hBM-MSCs increased in doses of LE (10-25 µg/mL) compared to the control group. The effects of LE were similar to those of 17β-estradiol (E2) (10-8 M) and were abolished by ICI 182,780 an antagonist of estrogen receptor (ER) (10-7), indicating that the stimulatory effects of LE occur through estrogen receptor-mediated mechanism. Taking these into account, LE may be a potential candidate for prevention of osteoporosis in menopausal women." As taken from Azizsoltani A et al. 2018. Iran. J. Pharm. Res. 17(3), 1057-1067. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30127828

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 licorice 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 licorice extract. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	18,802	Carmines, 2002 & Rustemeier et al., 2002
	60,000	Baker et al., 2004a
	15,000 (No CAS)	JTI KB Study Report(s)
	9610	Roemer et al, 2014
	17300 (CAS 68916-91-6)	Stabbert et al., 2019
In vitro genotoxicity	18,802	Carmines, 2002 & Roemer et al., 2002
	20,000	Baker et al., 2004c
	15,000 (No CAS)	JTI KB Study Report(s)
	30,500	fGLH Study Report (2010)
	9610	Roemer et al, 2014
	17300 (CAS 68916-91-6)	Stabbert et al., 2019
In vitro cytotoxicity	18,802	Carmines, 2002 & Roemer et al., 2002
	20,000	Baker et al., 2004c
	30,500	fGLH Study Report (2010)
	9610	Roemer et al, 2014
	17300 (CAS 68916-91-6)	Stabbert et al., 2019
Inhalation study	20,006	Gaworski et al., 1998
	18,802	Carmines, 2002 & Vanscheeuwijck et al., 2002
	20,000	Baker et al., 2004c
	9610	Schramke et al, 2014
Skin painting	18,001	Gaworski et al., 1999
In vivo genotoxicity	9610	Schramke et al, 2014

Licorice extract (block, powder or liquid) may be applied to cigarette tobacco at levels of about 1–4% to enhance and harmonize the flavor characteristics of smoke, improve moisture holding characteristics of tobacco, and act as a surface active agent for ingredient application. Neat material pyrolysis studies, and smoke chemistry and biological activity studies (bacterial mutagenicity, cytotoxicity, micronucleus, and sub-chronic inhalation) with mainstream smoke, or mainstream

smoke preparations from cigarettes containing various target levels (1.5–12%) of the licorice extracts were performed to provide data for an assessment of the use of licorice extract as a cigarette tobacco ingredient. At simulated tobacco burning temperatures up to 900 _C all forms of neat licorice extract pyrolyzed extensively, yielding small amounts of benzene, toluene, phenol and acetaldehyde with no indication that licorice extracts would transfer intact to mainstream smoke. As a single ingredient added to cigarette tobacco, block licorice extract at a target level of 12.5% increased smoke constituents including selected PAH, arsenic, lead, phenol and formaldehyde (on a TPM basis), while licorice extract powder (target level of 8% tobacco) increased select PAH, phenol and formaldehyde (on a TPM basis). Lower target application levels (including typical application levels) of block, powder or liquid licorice extract did not significantly alter the smoke chemistry profile. Biological tests indicated no relevant difference in the genotoxic or cytotoxic potential of either mainstream smoke (or smoke preparations) from cigarettes with added licorice extracts compared to control cigarettes. In subchronic 90-day rat inhalation studies, the mainstream smoke from cigarettes with 12.5% added block and 8% added powder licorice extract contained higher formaldehyde concentrations compared to control cigarette smoke. Female rats in the 12.5% block licorice extract exposure group displayed an increased incidence and severity of epithelial hyperplasia in the nose (level 2), with no relevant respiratory tract changes in the 8% powder licorice extract exposed rats. At the lower licorice extract application levels (1.25–5%), there was no indication of increased formaldehyde concentration in the smoke atmosphere and no relevant changes in respiratory tract tissues. Mineralcorticoid-like effects which have been associated with excess licorice ingestion were not found in any of the smoke inhalation studies. The results of these studies with various forms of licorice extract applied to cigarette tobacco suggest that adding licorice extract to cigarette tobacco at levels of 65% does not discernibly alter the smoke chemistry or biological effects normally associated with mainstream cigarette smoke (Carmines et al., 2005).

“OBJECTIVE: To study the in vitro effect of herb components on scavenging harmful components of cigarette smoke such as radicals, polycyclic aromatic hydrocarbons, nitrosamines in vitro, and its reducing effect on cytotoxicity of cigarette smoke. METHOD: spectrophotometry was used to examine the scavenging effect of herb components on DPPH free radicals, superoxide anion radical, and hydroxyl radical, and the results were compared with the anti-oxidation of ascorbic acid. Fluorescence spectroscopy was used to examine the scavenging effect of herb components on polycyclic aromatic hydrocarbons. UV spectrophotometry was used to examine the scavenging effect of herb components on volatile nitrosamines. MTT assay was used to examine cytotoxicity of cigarette smoke. RESULT: All the herb components showed a certain scavenging effect on DPPH free radicals, superoxide anion radical, hydroxyl radical, polycyclic aromatic hydrocarbons and volatile nitrosamines, espacially the ginkgo biloba extract (GBE), flavonoids of glycyrrhiza, procyanidine, total saponins in ophiopogonin, total saponins of astragalus and epimediun flavonoids. After these six herb components were added to cigarette, the cytotoxicity of cigarette smoke on BEP2D cells was remarkably reduced, by increasing cell survival fraction (SF, %) and mean lethal dose (DML). CONCLUSION: The herb components can scavenge harmful components of cigarette smoke such as radicals, polycyclic aromatic hydrocarbons and nitrosamines, which reduce the damage of cigarette smoke on human being”. As taken from Wu Y et al. 2011. Zhongguo Zhong Yao Za Zhi. 36(22), 3184-8. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22375404.

Transfer studies:

In a pyrolysis study of cigarette tobacco treated with licorice extract and heated up to 900ºC, the licorice extract (containing the non-volatile ammonium salt of glycyrrhizic acid) underwent full degradation. Glycyrrhizic acid, ammonium salt was not detect intact in the mainstream or sidestream smoke. The major pyrolysates were tentatively identified as naphthalenic compounds (Purkis et al 2011).

The fate of glycyrrhizic acid and glycyrrhetinic acid during the smoking of cigarettes has been studied by adding these acids separately to cigarettes with the result that glycyrrhizic acid decomposed to glycyrrhetinic acid and was transferred into mainstream smoke. When glycyrrhetinic acid itself was

added to cigarettes, it was transferred intact to the mainstream smoke. In both cases, the glycyrrhetinic acid found in the smoke condensate was small. It was concluded that the glycyrrhetinic acid in licorice root used for tobacco flavouring was mostly decomposed (Sakagami 1973).

When 5g licorice (spray dried) was applied as one of a number of ingredients including chalk (49g), tobacco dust (5g) and water (270ml); chalk (53g), tobacco extract (1g) and water (245ml); chalk (53.5g), tobacco extract synthetic analogue (0.5g) and water (250ml); and chalk (49g), tobacco dust (5g) and water (340ml), the mainstream TPMs were 14.8mg, 12.9mg, 10.4mg and 11.4mg respectively (McAdam 1997).

“This publication is part of a series of three publications and describes the non-clinical assessment performed to fulfill the regulatory requirement per Art. 6 (2) of the EU Tobacco Products Directive 2014/40/EU under which Member States shall require manufacturers and importers of cigarettes and Roll Your Own tobacco containing an additive that is included in the priority list established by Commission Implementing Decision (EU) 2016/787 to carry out comprehensive studies (European Comission, 2016). This publication contains the results of a literature search, comprehensive smoke chemistry, additive transfer, and in vitro toxicity studies for the 13 priority additives (carob bean extract, cocoa powder, fenugreek extract, fig juice concentrate, geraniol, glycerol, guaiacol, guar gum, liquorice extract powder, maltol, l-menthol (synthetic), propylene glycol, and sorbitol) commissioned by the members of the Priority Additives Tobacco Consortium to independent Contract Research Organizations. Comparisons of the 39 World Health Organisation smoke emissions in smoke from cigarettes with and without priority additives identified some differences that, with few exceptions, were minor and well within the inherent variability of the analytical method observed for the 3R4F monitor cigarette. Most differences were not statistically significant and did not show consistent additive-related increases or decreases. However, test cigarettes with guar gum showed a statistically significant, additive-related increase in formaldehyde and cadmium; test cigarettes with sorbitol showed a statistically significant, additive-related increase in formaldehyde and acrolein; test cigarettes with glycerol showed a statistically significant, additive-related decrease in phenols, benzo[a]pyrene and N-nitrosoanabasine; and test cigarettes with propylene glycol showed a statistically significant, additive-related decrease in phenol and m + p-cresols. These changes were not observed when the additives were tested as a mixture. None of the increases or decreases in smoke chemistry translated into changes in the in vitro toxicity. Comparisons of the in vitro toxicity of smoke from cigarettes with and without priority additives gave some differences that were minor, well within the inherent variability of the assays, not statistically significant, and did not show consistent additive-related increases or decreases. Thus, it can be concluded that the addition of priority additives had no effect on the in vitro toxicity of the cigarette smoke. The results obtained in our studies are consistent with those in scientific literature.” As taken from Stabbert R et al. 2019. Regul. Toxicol. Pharmacol. 104, 163-199. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30858113

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Licorice Extract Paste (68916-91-6) 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 Licorice Extract Paste (68916-91-6) 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	91	JTI KB Study Report(s)
In vitro cytotoxicity	91	JTI KB Study Report(s)

Aerosol from an electronic nicotine delivery system (ENDS) product that creates a vapor by heating an e-liquid; the vapor then passes through a capsule containing tobacco granules, containing “Licorice extract, root extract, powder” was tested in a battery of in vitro and/or in vivo 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) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed 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 and adverse effects upon exposure. The table below provides tested level(s) and specific endpoint(s):

Endpoint	Tested level	Reference
Aerosol chemistry	0.0010 mg/(tobacco portion; 310 mg)	Logic (2019)
In vitro genotoxicity	0.0010 mg/(tobacco portion; 310 mg)	Logic (2019)
In vitro cytotoxicity	0.0010 mg/(tobacco portion; 310 mg)	Logic (2019)
In vivo genotoxicity	0.0010 mg/(tobacco portion; 310 mg)	Logic (2019)
Inhalation study	0.0010 mg/(tobacco portion; 310 mg)	Logic (2019)

10. Ecotoxicity

10.1. Environmental fate

Natural Pollution Sources:

Herbaceous plant native to south europe; grows wild in eastern europe. Plant is 1-2 m (3-7 ft) high & has creeping root (secondary roots, branched), erect stalk, alternate leaves, violet flowers (from june-july), & kidney-shaped seeds. Parts of plant used: stolons & roots, at least 2 yr old. /licorice/ [Fenaroli's Handbook of Flavor Ingredients. Volume 1. Edited, translated, and revised by T.E. Furia and N. Bellanca. 2nd ed. Cleveland: The Chemical Rubber Co., 1975., p. 392] **PEER REVIEWED**

Rhizome and root of Glycyrrhiza glabra L., var. typica Regel & Herder (Spanish licorice), or of G. glabra L., var. glandulifera (Waldst. & Kit.) Regel & Herder (Russian licorice), or of other varieties of G. glabara yielding a yellow and sweet wood, Leguminosae. /Glycyrrhiza/ [The Merck Index. 10th ed. Rahway, New Jersey: Merck Co., Inc., 1983., p. 647] **PEER REVIEWED**

As taken from HSDB, 2002

EPISuite provides the following information for licorice oils (97676-23-8):

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

Bond Method :	7.00E-005 atm-m3/mole (7.09E+000 Pa-m3/mole)
Group Method:	Incomplete

Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.975E-003 atm-m3/mole (2.001E+002 Pa-m3/mole) VP: 0.721 mm Hg (source: MPBPVP) WS: 73.1 mg/L (source: WSKOWWIN)

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

Log Kow used:	3.52 (exp database)
Log Kaw used:	-2.543 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	6.063
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.3140
Biowin2 (Non-Linear Model) :	0.0451
Biowin3 (Ultimate Survey Model):	2.4160 (weeks-months)
Biowin4 (Primary Survey Model) :	3.2990 (days-weeks)
Biowin5 (MITI Linear Model) :	0.5851
Biowin6 (MITI Non-Linear Model):	0.5988
Biowin7 (Anaerobic Linear Model):	-0.7836
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):	109 Pa (0.817 mm Hg)

Log Koa (Koawin est):	6.063
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	2.75E-008 2.84E-007

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	9.95E-007
Mackay model:	2.2E-006
Octanol/air (Koa) model:	2.27E-005

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

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	11.8867 E-12 cm3/molecule-sec
Half-Life =	0.900 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	10.798 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 1.6E-006 (Junge-Pankow, Mackay avg) 2.27E-005 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	114.8 L/kg (MCI method)

Log Koc:	2.060 (MCI method)
Koc :	1169 L/kg (Kow method)
Log Koc:	3.068 (Kow method)

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

Rate constants can NOT be estimated for this structure!

Volatilization from Water:

Henry LC: 7E-005 atm-m3/mole (estimated by Bond SAR Method)

Half-Life from Model River:	11.58 hours
Half-Life from Model Lake:	229.8 hours (9.574 days)

Removal In Wastewater Treatment:

Total removal:	16.38 percent
Total biodegradation:	0.18 percent
Total sludge adsorption:	13.09 percent
Total to Air:	3.11 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.52	21.6	1000

Water	21.9	900	1000
Soil	76.5	1.8e+003	1000
Sediment	0.163	8.1e+003	0

Persistence Time: 758 hr

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that licorice oils (97676-23-8) are of uncertain persistence in the environment.

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

10.2. Aquatic toxicity

According to Canadian categorization criteria, licorice oil (97676-23-8) is not inherently toxic to aquatic organisms and is considered of low ecotoxicological concern (no further details available).

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

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 97676-23-8:

Values used to Generate ECOSAR Profile

Log Kow: 3.045 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 2150 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations

Neutral Organics

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Neutral Organics :	Fish	96-hr	LC50	14.423
Neutral Organics :	Daphnid	48-hr	LC50	9.019
Neutral Organics :	Green Algae	96-hr	EC50	10.010
Neutral Organics :	Fish		ChV	1.580
Neutral Organics :	Daphnid		ChV	1.150

Neutral Organics :	Green Algae		ChV	3.249
Neutral Organics :	Fish (SW)	96-hr	LC50	18.272
Neutral Organics :	Mysid	96-hr	LC50	6.692
Neutral Organics :	Fish (SW)		ChV	3.753
Neutral Organics :	Mysid (SW)		ChV	0.424

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.

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 97676-23-8:

Values used to Generate ECOSAR Profile

Log Kow: 3.045 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 2150 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Earthworm	14-day	LC50	206.257

“The aim of the present study was to introduce an alternative way for insects control through biodegradable plants materials. The different cold water extracts dilutions of Acacia modesta and Glycyrrhiza glabra were tested against Tribolium castaneum. The extracts dilutions of both plants caused mortality of the Tribolium castaneum. ANOVA revealed that dilutions and plants were highly significant. The interaction between plants and dilutions was also significant at $P < 0.05$. Phytotoxic activity showed that dilutions of Acacia modesta and Glycyrrhiza glabra extracts significantly inhibited the growth of Lemna minor. ANOVA showed that dilutions of both plants extracts were significant at $P < 0.05$.” As taken from Nazeefullah S et al. 2014. Pak. J. Pharm. Sci. 27(2), 217-22. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/24577905?dopt=AbstractPlus

10.5. All other relevant types of ecotoxicity

EPISuite provides the following information for licorice oils (97676-23-8)

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	1.989 (BCF = 97.61 L/kg wet-wt)
Log Biotransformation Half-life (HL):	0.0618 days (HL = 1.153 days)
Log BCF Arnot-Gobas method (upper trophic):	2.308 (BCF = 203.1)
Log BAF Arnot-Gobas method (upper trophic):	2.308 (BAF = 203.2)
log Kow used:	3.52 (expkow database)

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that licorice oils (97676-23-8) are of uncertain bioaccumulative potential in the environment.

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

11. References for conventional products

- Abdel-Wahab AA et al. (2020). A Licorice Roots Extract Induces Apoptosis and Cell Cycle Arrest and Improves Metabolism via Regulating MiRNAs in Liver Cancer Cells. Nutr. Cancer. Epub ahead of print. DOI: 10.1080/01635581.2020.1783329. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32578448/
- AICIS (2017). Australian Government Department of Health. Australian Industrial Chemicals Introduction Scheme Inventory Multi-Tiered Assessment and Prioritisation (IMAP) Tier I. Health Record for licorice, extract (CAS RN 68916-91-6). Dated 10 March 2017. Accessed March 2021. Available at: https://www.industrialchemicals.gov.au/chemical-information/search-assessments?assessmentcasnumber
- Ahn J et al. (2013). Anti-obesity effects of glabridin-rich supercritical carbon dioxide extract of licorice in high-fat-fed obese mice. Fd Chem Toxicol. 51, 439-445. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22967722
- Aksoy L et al. (2012). Protective and therapeutic effects of licorice in rats with acute tubular necrosis. J. Ren. Nutr. 22, 336-343. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22047711?dopt=AbstractPlus
- Al-Qarawi AA et al. (2002), Food Chem Toxicol. 2002 Oct; 40(10):1525-7. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12387318&query_hl=5&itool=pubmed_DocSum
- Aluckal E et al. (2017). Assessment of Total Antioxidant Capacity and Antimicrobial Activity of Glycyrrhiza glabra in Saliva of HIV-Infected Patients. J. Pharm. Bioallied Sci. 9(Suppl. 1), S237-S240. DOI: 10.4103/jpbs.JPBS_141_17. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29284971
- Anon (2003). Herbal warnings latest. Health Which? Jun 01, 33.
- Armanini D et al. (1999). Reduction of serum testosterone in men by liquorice. New England Journal of Medicine, 341, 1158.

- Attou R et al. (2020). Liquorice Intoxication Can Lead to Cardiac Arrest! Case Rep. Emerg. Med. 2020, 3727682. DOI: 10.1155/2020/3727682. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33029435/
- Ayeka PA et al. (2017). The immunomodulatory activities of licorice polysaccharides (Glycyrrhiza uralensis Fisch.) in CT 26 tumor-bearing mice. BMC Complement. Altern. Med. 17(1), 536. DOI: 10.1186/s12906-017-2030-7. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29246138
- Azizsoltani A et al. (2018). Ethyl acetate extract of licorice root (Glycyrrhiza glabra) enhances proliferation and osteogenic differentiation of human bone marrow mesenchymal stem cells. Iran. J. Pharm. Res. 17(3), 1057-1067. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30127828
- Badr AE et al. (2012). A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament. Int. Endod. J. 44, 51-58. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/20812941?dopt=AbstractPlus
- Baker R and Bishop L. (2005). The pyrolysis of non-volatile tobacco ingredients using a system that stimulates cigarette combustion conditions. J. Anal. Appl. Pyrolysis 74, 145–170.
- Baker R et al. (2004a). The effect of tobacco ingredients on smoke chemistry.Part I: Flavourings and additives. Food and Chemical Toxicology 42s, S3-S37.
- Baker R et al. (2004c). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. Food and Chemical Toxicology 42s, S53-S83.
- Bannister B et al. (1977). Cardiac arrest due to licorice induced hypokalaemia. Br. Med. J. ii: 738-739.
- Batiha GES et al. (2020). Traditional uses, bioactive chemical constituents, and pharmacological and toxicological activities of Glycyrrhiza glabra L. (Fabaceae). Biomolecules 10(3), E352. DOI: 10.3390/biom10030352. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32106571/
- Benge E et al. (2020). Trick or Treat? Licorice-Induced Hypokalemia: A Case Report. Cureus 12(11), e11656. DOI: 10.7759/cureus.11656. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33391895/
- Bernardi M et al. (1994). Effects of prolonged ingestion of graded doses of liquorice by healthy volunteers. Life Sciences, 55, 863-872. Reviewed in SCF, 2003.
- Bijlsma J A et al. (1996). [Study into effects of different dosages of glycyrrhizin in healthy female volunteers; in Dutch]. RIVM Report 348801004, RIVM, Bilthoven, The Netherlands (cited in SCF, 2003).
- Blachley J D & Knochel J P (1980). Tobacco chewer's hypokalaemia: Licorice revisited. The New England Journal of Medicine, April 3 1980, p784.
- Brendler T et al. (2020). Botanical drugs and supplements affecting the immune response in the time of COVID-19: Implications for research and clinical practice. Phytother. Res. Epub ahead of print. DOI: 10.1002/ptr.7008. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33373071/
- Brown AC. (2018). Heart toxicity related to herbs and dietary supplements: online table of case reports. Part 4 of 5. J. Diet. Suppl. 15(4), 516-555. DOI: 10.1080/19390211.2017.1356418. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/28981338

- Bruusgaard-Mouritsen MA et al. (2020). Natural ingredients in cosmetic products-A suggestion for a screening series for skin allergy. Contact Dermatitis 83(4), 251-270. DOI: 10.1111/cod.13550. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32248558/
- Buhl LF et al. (2018). Licorice-induced apparent mineralocorticoid excess compounded by excessive use of terbutaline and high water intake. BMJ Case Rep., pii: bcr-2017-223918. DOI: 10.1136/bcr-2017-223918. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29674401
- Burdock GA (2010). Fenaroli's Handbook of Flavor Ingredients. Sixth Edition. CRC Press. ISBN 978-1-4200-9077-2.
- Calvert R J (1954). Lancet 1, 805.
- Caravaca-Fontan F et al. (2015). An Unexpected Cause of Severe Hypokalemia. Case Rep. Nephrol. 2015, 957583. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26550501
- Card W I et al. (1953). Effects of licorice and its derivatives on salt and water metabolism. The Lancet. 2: 663-667.
- Carmines EL (2002), Food Chem Toxicol. 2002 Jan;40(1):77-91. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=11731038&query_hl=124&itool=pubmed_DocSum
- Carmines EL et al., (2005). Toxicologic evaluation of licorice extract as a cigarette ingredient. Food and Chemical Toxicology 43 (2005) 1303–1322.
- Cartier A et al. (2002). Occupational asthma due to liquorice roots. Allergy, 57, 863. Chemical composition of mainstream smoke. Food and Chemical Toxicology, 40, 93-104.
- CCRIS (2010). Record for licorice, Glycyrrhizia glabra ext. (CAS RN 84775-66-6). CCRIS record no. 9463. Last revision date 23 September 2010 (records no longer being updated after 2011). Available at https://www.toxinfo.io/#/chem-detail/68916-91-6
- Celik MM et al. (2012). Licorice induced hypokalemia, edema, and thrombocytopenia. Human Expt. Tox. 31, 1295-1298. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22653692?dopt=AbstractPlus
- Chakotiya AS et al. (2017). Effect of aquo-alchoholic extract of Glycyrrhiza glabra against Pseudomonas aeruginosa in Mice Lung Infection Model. Biomed. Pharmacother. 90, 171-178. DOI: 10.1016/j.biopha.2017.03.055. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28355591
- Chandrasekaran CV et al. (2011). Evaluation of the genotoxic potential of standardized extract of Glycyrrhiza glabra (GutGard™). Reg. Tox. Pharmacol. 61, 373-380. Available at http://www.sciencedirect.com/science/article/pii/S0273230011001954
- ChemIDplus. Accessed March 2021. Available at https://chem.nlm.nih.gov/chemidplus/
- Chen L et al. (2011). [Effects of glycyrrhiza extract on pharmacokinetics property of daphnetin in rats]. Zhongguo Zhong Yao Za Zhi. 36(7), 935-8. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21761739?dopt=AbstractPlus
- Chen YY et al. (2019). Elucidating the interaction of kansui and licorice by comparative plasma/tissue metabolomics and a heatmap with relative fold change. J. Pharm. Anal. 9(5), 312-323. DOI: 10.1016/j.jpha.2019.05.005. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31929940/

- Choi JS et al. (2013). Fetal and Neonatal Outcomes in Women Reporting Ingestion of Licorice (Glycyrrhiza uralensis) during Pregnancy. Planta Med. 79, 97-103. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23299757?dopt=AbstractPlus
- Chung H T & Aldridge J C (1999). Thermal study of licorice by online thermogravimetry/gas chromatography/mass spectrometry. 53rd Tobacco Science Research Conference. Sept 12-15, 1999. Montreal, Quebec, Canada.
- Cooper E P & Berry C W (1988). Mutagenic potential of glycyrrhizin. Journal of Dental Research, 67, abstract 339 (special issue, March) (cited in SCF, 2003).
- CosIng. Cosmetic substances and ingredients database. Records for Glycyrrhiza glabra (various extracts; CAS RN 84775-66-6) and Glycyrrhiza glabra rhizome/root (CAS RN 68916-91-6). Undated, accessed January 2022. Available at https://ec.europa.eu/growth/tools-databases/cosing/
- CPID (undated). Consumer Product Information Database. Record for licorice oils (CAS RN 97676-23-8). Accessed January 2022. Available at https://www.whatsinproducts.com/
- Cugini P et al. (1983). Hypertension in a licorice abuse: A case report. G. Ital. Cardiol. 13, 126-128.
- Cumming A M M (1977). Metabolic effects of licorice. Br. Med. J. 1977 (i) p906.
- Cumming A M M et al. (1980). Severe hypokalaemia with paralysis induced by small doses of licorice. Postgrad Med. J. 56:526-529.
- Dhingra D et al. (2004), J Ethnopharmacol. 2004 Apr;91(2-3), 361-5. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15120462&query_hl=5&itool=pubmed_DocSum
- Diaconu CC et al. (2018). Drug-induced arterial hypertension - a frequently ignored cause of secondary hypertension: a review. Acta Cardiol. 1, 1-7. DOI: 10.1080/00015385.2017.1421445. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29291681
- Dirikan E & Turkez H (2014). In vitro studies on protective effect of Glycyrrhiza glabra root extracts against cadmium-induced genetic and oxidative damage in human lymphocytes. Cytotechnology 66(1), 9-16. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23325115
- Doull et al. (1994). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at http://legacy.library.ucsf.edu/tid/thy03c00
- Doull et al. (1998). A safety assessment of the ingredients added to tobacco in the manufacture of cigarettes. Available at http://legacy.library.ucsf.edu/tid/wzp67e00
- ECHA (undated). European Chemicals Agency. Information on chemicals. Records for licorice, ext. (CAS RN 68916-91-6); licorice, Glycyrrhiza glabra, ext. (CAS RN 84775-66-6); licorice oils (CAS RN 97676-23-8); Glycyrrhiza Glagra (Licorice) Root Extract (no CAS RN) and Glycyrrhiza Uralensis (Licorice) Root extract (no CAS RN). Accessed January 2022. Available at: https://echa.europa.eu/information-on-chemicals/pre-registered-substances
- ECHA (2021). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 21 December2021. Available at: https://echa.europa.eu/information-on-chemicals/cl-inventory-database
- ECOSAR. Record for oils, licorice (CAS RN 97676-23-8). Accessed June 2017. (ECOSAR content has not been updated since 2012, version 1.11.) Available to download, through

EPISuite, at https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface

- EFSA (2011). European Food Safety Authority. Scientific Opinion on the safety of 'Glavonoid®', an extract derived from the roots or rootstock of Glycyrrhiza glabra L., as a Novel Food ingredient. Available at: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2287/epdf
- EMA (2012). Community herbal monograph on Glycyrrhiza glabra L. and/or Glycyrrhiza inflata Bat. and/or Glycyrrhiza uralensis Fisch., radix. European Medicines Agency. MA/HMPC/571119/2010. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_Community_herbal_monograph/2012/08/WC500131287.pdf
- EPISuite. Record for licorice oils (CAS RN 97676-23-8). Accessed June 2017. (EPISuite content has not been updated since 2012, version 4.11.) The programme is available to download via https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- EPISuite (2017). Record for licorice oils (CAS RN 97676-23-8).EPISuite version 4.11. Last updated June 2017. EPISuite is available to download at https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411
- FASEB (1974). Evaluation of the health aspects of licorice, glycyrriza and ammoniated glycyrrhizin as food ingredients. NTIS PB254529, FDABFGRAS362.
- FDA (2022). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 20 January 2022. Accessed March 2021. Available at: https://www.accessdata.fda.gov/scripts/fdcc/?set=FoodSubstances
- FDA (2021). US Food and Drug Administration. Electronic Code of Federal Regulations (eCFR). Title 21. Current as of 18 January 2022. Available at: https://www.ecfr.gov/cgi-bin/ECFR?page=browse
- fGLH Study Report (2010).
- Fogelman Y et al. (2016). Antiatherosclerotic effects of licorice extract supplementation on hypercholesterolemic patients: decreased CIMT, reduced plasma lipid levels, and decreased blood pressure. Food Nutr. Res. 60, 30830. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27113136
- Foster S et al. (2017). All sorts of tests, only one question: an unexpected cause of hypertension. BMJ Case Rep. 2017, bcr-2017-222077. DOI: 10.1136/bcr-2017-222077. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29127128
- Fujisawa Y et al., (2000), Microbiol Immunol. 2000;44(9):799-804. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11092245&query_hl=5&itool=pubmed_DocSum
- Fujita H & Sasaki M (1986). Ann. Rep. Tokyo Metr. Res. Lab. 37, 447.
- Fujita M et al. (2019). A newly developed means of HPLC-fluorescence analysis for predicting the skin sensitization potential of multi-constituent substances using ADRA. Toxicol. In Vitro 59, 161-178. DOI: 10.1016/j.tiv.2019.04.014. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/31002975
- Fukuchi K et al. (2016). Antiviral and Antitumor Activity of Licorice Root Extracts. In Vivo. 30(6), 777-785. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27815461

- Gaworski CL et al. (1998). Toxicologic evaluation of flavor ingredients added to cigarette smoke: 13-week inhalation exposures in rats. Inhalation Toxicology, 10, 357.
- Gaworski CL et al. (1999). Toxicology. 1999 Nov 29;139(1-2):1-17. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=10614684&query_hl=131&itool=pubmed_docsum
- Girerd R J et al. (1958). Production of experimental hypertension and cardiovascular-renal lesions with licorice and ammoniated glycyrrhizin. American Journal of Physiology. 194: 241-245.
- Gomez-Sanchez EP, Gomez-Sanchez CE. Am J Physiol. 1992 Dec; 263(6 Pt 1):E1125-30. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/1476186
- Gonzalez-Gutierrez M L et al. (2000). Allergy 55, 195.
- Gu MY et al. (2018). Glycyrrhiza uralensis and Semilicoisoflavone B Reduce Aβ Secretion by Increasing PPARγ Expression and Inhibiting STAT3 Phosphorylation to Inhibit BACE1 Expression. Mol. Nutr. Food Res. 62(6), e1700633. DOI: 10.1002/mnfr.201700633. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29143445
- Hajiaghamohammadi AA et al. (2012). The efficacy of licorice root extract in decreasing transaminase activities in non-alcoholic fatty liver disease: a randomized controlled clinical trial. Phytother. Res. 26, 1381-1384. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22308054
- Hall RL & Oser BL. (1965). Recent Progress in the Consideration of Flavoring Ingredients Under the Food Additives Amendment. III. GRAS Substances, Food Technology 253, 151. Available at https://www.femaflavor.org/sites/default/files/3.%20GRAS%20Substances(2001-3124)_0.pdf
- Hautaniemi EJ et al. (2017). Voluntary liquorice ingestion increases blood pressure via increased volume load, elevated peripheral arterial resistance, and decreased aortic compliance. Sci. Rep. 7(1), 10947. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28887501
- Hayashi M et al. (1988). Fd Chem. Toxic. 26, 487.
- Health Canada (2021). Drugs and Health Products. Natural Health Products Ingredients Database. Records for Glycyrrhiza glabra (various), liquorice dry, Radix et Rhizoma Glycyrrhizae, Radix et Rhizoma Glycyrrhizae Preparata cum Melle, Glycyrrhiza uralensis, Extractum Glycyrrhizae and Extractum Glycyrrhizae Liquidum (no CAS RNs listed). Last updated 07 December 2021. Accessed January 2022. Available at http://webprod.hc-sc.gc.ca/nhpid-bdipsn/search-rechercheReq.do?lang=eng
- Heck J D et al. (1989). Toxicologist 9, 257. [Abstract only]
- Hejazi II et al. (2017). New insights into the antioxidant and apoptotic potential of Glycyrrhiza glabra L. during hydrogen peroxide mediated oxidative stress: An in vitro and in silico evaluation. Biomed. Pharmacother. 94, 265-279. DOI: 10.1016/j.biopha.2017.06.108. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28763750
- Hollinger JC et al. (2018). Are Natural Ingredients Effective in the Management of Hyperpigmentation? A Systematic Review. J. Clin. Aesthet. Dermatol. 11(2), 28-37. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29552273

- Hosseinzadeh H and Nassiri-Asl M (2015). Pharmacological Effects of Glycyrrhiza spp. and Its Bioactive Constituents: Update and Review. Phytother. Res. 29(2), 1866-86. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26462981
- Hou YC et al. (2012). Liquorice reduced cyclosporine bioavailability by activating P-glycoprotein and CYP 3A. Food Chem. 135, 2307-2312. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22980806?dopt=AbstractPlus
- HSDB (2002). Record for licorice extract (CAS RN 68916-91-6). Hazardous Substances Data Bank Number 1925. Last Revision Date: 8 November 2002. Accessed March 2021. Available at: https://www.toxinfo.io/#/chem-detail/68916-91-6
- IFRA (undated). International Fragrance Association. IFRA Transparency List. Accessed January 2022. Available at https://ifrafragrance.org/priorities/ingredients/ifra-transparency-list
- Ikegami F et al. (2004). Toxicological considerations of Kampo medicines in clinical use. Toxicology, 198, 221-228.
- Ikken Y et al. (1999). J. agric. Fd Chem. 47, 3257.
- Isbrucker RA & Burdock GA (2006). Risk and safety assessment on the consumption of licorice rood (Glycyrrhiza sp.), its extract and powder as a food ingredient with emphasis on the pharmacology and toxicology of glycyrrhizin. Regulatory Toxicology and Pharmacology 46(3), 167-192. Taken from http://www.ncbi.nlm.nih.gov/pubmed/16884839
- Ishidate M (1983). Quo Vadis? Short-term Tests for Carcinogenesis. Garattini S. et al. (Eds). Centre de Recherches Clin. Midy Groupe, SANOFI. Montpellier, 4-5 Feb. 1981.
- Ishidate M et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology, 22, 623-636.
- Ishidate M et al. (1988). Mutation Res. 195, 151.
- Ishimi Y et al. (2019). Quality evaluation of health foods containing licorice in the Japanese market. Toxicol. Rep. 6, 904-913. DOI: 10.1016/j.toxrep.2019.08.013. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31508319/
- Ishizaki M et al. (1985). J. Fd Hyg. Soc., Japan 26, 523.
- Itami T et al. (1985). Effect of disodium glycyrrhizinate on pregnant rats and their offspring. J. Food Hyg. Soc. Japan. 26(5): 460-464.
- JECFA (1978). Evaluation of certain food additives. 21st JECFA report. WHO Technical Report Series 617, Geneva.
- Jeon JH et al. (2007). Effect of green tea and licorice extracts on cyclophosphamide teratogenicity. Reproductive Toxicology 24(1), 75 (abstract only).
- Jhanji V, Liu H, Law K, Lee VY, Huang S, Pang CP and Yam GH (2011). Isoliquiritigenin from licorice root suppressed neovascularization in experimental ocular angiogenesis models. British Journal of Ophthalmology 95(9), 1309-15. PubMed, available at http://www.ncbi.nlm.nih.gov/pubmed/21719569
- Jiang M et al. (2020). An "essential herbal medicine"-licorice: A review of phytochemicals and its effects in combination preparations. J. Ethnopharmacol. 249, 112439. DOI: 10.1016/j.jep.2019.112439. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31811935/
- Jo EH et al. (2005), Cancer Lett. 230(2), 239-47. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16297710&query_hl=5&itool=pubmed_DocSum

- Jorgenson et al. (1977). Study of mutagenic effects of ammoniated glycyrrhizin by dominant lethal test in rats. US NTIS PB REP PB-279650 72.
- Josephs R A et al. (2001). Liquorice consumption and salivary testosterone concentrations. The Lancet, 358, 1613-1614.
- JTI KB Study Report (s).
- JTI Study Report (s).
- Kang JS et al. (2005), J Pharmacol Exp Ther. 312(3), 1187-94. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15537821&query_hl=5&itool=pubmed_DocSum
- Kaur P et al. (2012). Modulation of genotoxicity of oxidative mutagens by glycyrrhizic acid from Glycyrrhiza glabra L. Pharmacognosy Res. 4, 189-195. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23225961
- Kaur P et al. (2017). Immunopotentiating significance of conventionally used plant adaptogens as modulators in biochemical and molecular signalling pathways in cell mediated processes. Biomed. Pharmacother. 95, 1815-1829. DOI: 10.1016/j.biopha.2017.09.081. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28968926
- Kawachi T et al. (1980). Appl. meth. Oncol. 3, 253.
- Kawachi T et al. (1981). IARC Sci. Pub. 27, 323.
- Keyes KM & Susser E (2017). Invited Commentary: An Ingenious Approach to Examining the Relationship Between Maternal Stress and Offspring Health? Am. J. Epidemiol. 185(5), 329-332. DOI: 10.1093/aje/kww173. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28158433?dopt=AbstractPlus
- Kiken D A and Cohen D E (2002). Contact dermatitis to botanical extracts. American Journal of Contact Dermatitis, 13, 148-152.
- Kim HJ et al. (2005), Planta Med. 71(8), 785-7. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16142649&query_hl=5&itool=pubmed_DocSum
- Khan IA and Abourashed EA (2010). Leung's Encyclopedia of Common Natural Ingredients used in Food, Drugs, and Cosmetics. Third Edition. John Wiley & Sons, Inc., Hoboken, New Jersey
- Kobuke T et al. (1985). Tumorigenicity study of disodium glycyrrhizinate administered orally to mice. Fd Chem. Toxic. 23(11): 979-983.
- Komiyama K et al. (1977). Acute and subacute toxicity test on the extract from Glycyrrhiza. Oyo Yakuri. 14(4):535-548.
- Kröller E (1967). Ein Beitrag zur Beurteilung von Tabakzusatzstoffen auf Grund ihrer Pyrolyseprodukte. Bundesgesundhbl 10 (1967) 277-279.
- Kuang Y et al. (2018). Antitussive and expectorant activities of licorice and its major compounds. Bioorg. Med. Chem. 26(1), 278-284. DOI: 10.1016/j.bmc.2017.11.046. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29224994
- Kwon YE et al. (2020). Severe asymptomatic hypokalemia associated with prolonged licorice ingestion: A case report. Medicine (Baltimore) 99(30), e21094. DOI: 10.1097/MD.0000000000021094. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32791684/

- LactMed (2021). Drugs and Lactation Database. Record for licorice (CAS RN 84775-66-6). Last updated 15 February 2021. Accessed March 2021. Available at https://www.ncbi.nlm.nih.gov/books/NBK501840/
- Lai F et al. (1980). Licorice, snuff and hypokalaemia. New England J. Med. 303: 463.
- Lee MH et al. (2020). Licorice extract suppresses adipogenesis through regulation of mitotic clonal expansion and adenosine monophosphate-activated protein kinase in 3T3-L1 cells. J. Food Biochem. 44(12), e13528. DOI: 10.1111/jfbc.13528. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33051883/
- Li G et al. (2017). Cytochrome P450 inhibition by three licorice species and fourteen licorice constituents. Eur. J. Pharm. Sci. 109, 182-190. DOI: 10.1016/j.ejps.2017.07.034. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28774812
- Lloyd R A et al. (1976). Flue-cured tobacco flavour. 1. Essence and essential oil components. Tobacco Science, 20, 40-48.
- Logic (2019). G.5. Nonclinical Evaluation Summary - Logic Vapeleaf (PMTA)
- Luis A et al. (2018). Metabolic changes after licorice consumption: A systematic review with meta-analysis and trial sequential analysis of clinical trials. Phytomedicine 39, 17-24. DOI: 10.1016/j.phymed.2017.12.010. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29433679
- Machalle K et al. (2015). Prinzmetal angina after licorice consumption. Dtsch. Med. Wochenschr. 140(8), 590-2. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25945908
- MacKenzie M A et al. (1990). The influence of glycyrrhetinic acid on plasma cortisol and cortisone in healthy young volunteers. Journal of Clinical Endocrinology and Metabolism. 70(6): 1637-1643.
- Madak-Erdogan Z et al. (2016). Dietary licorice root supplementation reduces diet-induced weight gain, lipid deposition, and hepatic steatosis in ovariectomized mice without stimulating reproductive tissues and mammary gland. Mol. Nutr. Food Res. 60(2), 369-80. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26555669
- Mantovani A et al. (1988). Teratogenicity study of ammonium glycyrrhizinate in the Sprague-Dawley rat. Food Cosmetic Toxicol. (1972). 26(5): 435-440.
- Martinez San Ireneo M et al. (1995). Allergy 50, 173.
- Maser E (2004). Trends Pharmacol Sci. 25(5), 235-7. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15120485&query_hl=83&itool=pubmed_docsum
- McAdam K (1997). Smokeable filler material for smoking articles; Patent (International): PCT/GB97/00589 (March 1997).
- Morimoto I et al. (1982). Mutagenicity screening of crude drugs with Bacillus subtilis rec-assay and Salmonella/microsome reversion assay. Mutation Research, 97(2), 81-102.
- Muñoz Balbontín Y et al. (2019). Herbal medicinal product use during pregnancy and the postnatal period: a systematic review. Obstet. Gynecol. 133(5), 920-932. DOI: 10.1097/AOG.0000000000003217. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30969204
- Namazi N et al. (2017). The Effect of Dried Glycyrrhiza Glabra L. Extract on Obesity Management with Regard to PPAR-γ2 (Pro12Ala) Gene Polymorphism in Obese Subjects

Following an Energy Restricted Diet. Adv. Pharm. Bull. 7(2), 221-228. DOI: 10.15171/apb.2017.027. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28761824

- Nazari S et al. (2017). Toxicological Effects of Glycyrrhiza glabra (Licorice): A Review. Phytother. Res. 31(11), 1635-1650. DOI: 10.1002/ptr.5893. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28833680
- Nazeefullah S et al. (2014). Effect of cold water extracts of Acacia modesta Wall. and Glycyrrhiza glabra Linn. on Tribolium castaneum and Lemna minor. Pak. J. Pharm. Sci. 27(2), 217-22. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/24577905?dopt=AbstractPlus
- Ng ZX et al. (2020). Comparative analyses on radical scavenging and cytotoxic activity of phenolic and flavonoid content from selected medicinal plants. Nat. Prod. Res. Epub ahead of print. DOI: 10.1080/14786419.2020.1749617. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32290699/
- Nielsen I & Pedersen R S (1984). Life-threatening hypokalaemia caused by licorice ingestion. The Lancet, June 9 1984, p1305.
- Nilon H & Froment A (1968). Rev. Lyon. Med, p99.
- Nishioka K & Seguchi T (1999). Contact allergy due to oil-soluble licorice extracts in cosmetic products. Contact Dermatitis 40, 56.
- Nose M et al. (2019). Evaluation of the safety and efficacy of Glycyrrhiza uralensis root extracts produced using artificial hydroponic-field hybrid cultivation systems II: comparison of serum concentration of glycyrrhetinic acid serum concentration in mice. J. Nat. Med. 73(3), 661-666. DOI: 10.1007/s11418-019-01312-9. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31028662/
- Nozawa J (1967). Inhibitory effect of glycyrrhizin on experimental skin carcinogenesis in mice. Jap. J. Dermatol. Ser. B. 77(4), 415.
- NTP (undated). National Toxicology Program. Chemical Effects in Biological Systems (CEBS). Record for licorice extract (CAS RN 68916-91-6). Accessed January 2022. Available at https://manticore.niehs.nih.gov/cebssearch/test_article/68916-91-6
- NZ EPA (2006). New Zealand Environmental Protection Authority. Inventory of Chemicals. Records for licorice, ext. (CAS RN 68916-91-6), licorice, Glycyrrhiza glabra, ext. (CAS RN 84775-66-6) and oils, licorice (CAS RN 97676-23-8). Date added to inventory: 1 December 2006. Accessed March 2021. Available at: https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/33691, https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/36398 and https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/38324
- OECD. Organisation for Economic Cooperation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). Oils, licorice (97676-23-8). Accessed June 2017. Available at: http://webnet.oecd.org/CCRWeb/Search.aspx.
- Oesch F et al. (2021). Toxicity as prime selection criterion among SARS-active herbal medications. Phytomedicine. Epub ahead of print. DOI: 10.1016/j.phymed.2021.153476. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33593628/
- Oijha SK et al. (2015). Licorice treatment prevents oxidative stress, restores cardiac function, and salvages myocardium in rat model of myocardial injury. Toxicol. Ind. Health 31(2), 140-52. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/23771872

- Ota M et al. (2018). Comparison of the inducible effects of licorice products with or without heat-processing and pre-treatment with honey on granulocyte colony-stimulating factor secretion in cultured enterocytes. J. Ethnopharmacol. 214, 1-7. DOI: 10.1016/j.jep.2017.11.040. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29203272
- Paolini M et al. (1998). Life Sci. 62(6), 571-82. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/9464470
- Park D et al. (2011). Licorice extract increases cyclophosphamide teratorgenicity by upregulating the expression of cytochrome P-450 2B mRNA. Birth Defects Research. Part B. Developmental and Reproductive Toxicology 92(6), 553-9. Taken from http://www.ncbi.nlm.nih.gov/pubmed/21818843
- Penninkilampi R et al. (2017). The association between consistent licorice ingestion, hypertension and hypokalaemia: a systematic review and meta-analysis. J. Hum. Hypertension 31(11), 699-707. DOI: 10.1038/jhh.2017.45. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28660884
- Peterson CT et al. (2018). Prebiotic Potential of Herbal Medicines Used in Digestive Health and Disease. J. Altern. Complement. Med. 24(7), 656-665. DOI: 10.1089/acm.2017.0422. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29565634
- Philip Morris USA (2001). Internal Report. Pyrolysis GC/MS analysis of licorice. Request 200110724. Scan P010724A.D.
- Prival M J et al. (1991). Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. Mutation Research, 321-329.
- Purkis SW, Mueller C & Intorp M (2011). The fate of ingredients in and impact on cigarette smoke. Food and Chemical Toxicology 49, 3238-3248.
- Qiao X et al. (2012). Analytical strategy to reveal the in vivo process of multi-component herbal medicine: a pharmacokinetic study of licorice using liquid chromatography coupled with triple quadrupole mass spectrometry. J. Chromat. A 1258, 84-93. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22939378?dopt=AbstractPlus
- Rahman H et al. (2017). Drug-Herb Interactions in the Elderly Patient with IBD: a Growing Concern. Curr. Treat. Options Gastroenterol. 15(4), 618-636. DOI: 10.1007/s11938-017-0154-y. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28918484
- Räikkönen K et al. (2017). Maternal Licorice Consumption During Pregnancy and Pubertal, Cognitive, and Psychiatric Outcomes in Children. Am. J. Epidemiol. 185(5), 317-328. DOI: 10.1093/aje/kww172. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28158597?dopt=AbstractPlus
- Rees W D et al. (1979). Effect of deglycyrrhizinated licorice on gastric mucosal damage by aspirin. Scand. J. Gastroenterol. 14(5), 605-607.
- Roemer E et al. (2002), Food Chem Toxicol. 40(1), 105-11. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=11731040&query_hl=124&itool=pubmed_docsum
- Roemer E et al., (2014). Toxicological assessment of kretek cigarettes Part 6: The impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity. Regulatory Toxicology and Pharmacology 70; S66-80.
- RTECS (2016). Registry of Toxic Effects of Chemical Substances. Record for Glycyrrhiza extract (68916-91-6). Last updated July 2016. Accessed March 2021.

- RTECS (2019). Registry of Toxic Effects of Chemical Substances. Record for Glycyrrhiza glabra L., root, acetone extract (no CAS RN listed). Last updated March 2019. Accessed March 2021.
- Rustemeier K et al. (2002), Food Chem Toxicol. 2002 Jan;40(1):93-104, available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=11731039&query_hl=124&itool=pubmed_docsum
- Saadat S et al. (2018). Comparative Antitussive Effects of Medicinal Plants and Their Constituents. Altern. Ther. Health Med. 24(4), 36-49. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29332022
- Sakagami H (1973). Studies on the components of licorice root used for tobacco flavouring/Part 3. The behaviour of glycyrrhizic acid and glycyrrhetinic acid added to tobacco upon smoking. Noka, 47: 623-626. English Translation.
- Sakr SA & Shalaby SY (2014). Carbendazim-induced testicular damage and oxidative stress in albino rats: ameliorative effect of licorice aqueous extract. Toxicol. Ind. Health 30(3), 259-67. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/22903170?dopt=AbstractPlus
- Sasaki M et al. (1980). Kromosomo II 20, 574.
- Sasaki Yu F et al. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutation Research, 519, 103-119.
- Satti P et al. (2019). Indigenous irrigants as potent antimicrobials in endodontic treatment: An in vitro study. J. Indian Soc. Pedod. Prev. Dent. 37(3), 275-281. DOI: 10.4103/JISPPD.JISPPD_112_19. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31584028/
- SCENIHR (2016). Scientific Committee on Emerging and Newly Identified Health Risks. Final opinion on additives used in tobacco products (opinion 1). Tobacco Additives I. Adopted on 25 January 2016. Available at: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_051.pdf
- SCF (2003). Opinion of the Scientific Committee on Food on glycyrrhizinic acid and its ammonium salt. SCF/CS/ADD/EDUL/225 Final 10 April 2003.
- Schmidt M et al. (2013). Cytotoxicity of herbal extracts used for treatment of prostatic disease on head and neck carcinoma cell lines and non-malignant primary mucosal cells. Oncol. Reps. 29, 628-636. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23165347
- Schramke H et al., (2014). Toxicological assessment of kretek cigarettes Part 7: The impact of ingredients added to kretek cigarettes on inhalation toxicity. Regulatory Toxicology and Pharmacology 70; S81-89.
- Senanayake N, Román GC. J Neurol Sci. 1992 Jan; 107(1):1-13. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/1315843
- Shah M et al. (2012). Licorice-related rhabdomyolysis: a big price for a sweet tooth. Clin. Nephrol. 77, 491-495. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22595392?dopt=AbstractPlus
- Sharma R et al. (2017). Glycyrrhiza glabra extract and quercetin reverses cisplatin resistance in triple-negative MDA-MB-468 breast cancer cells via inhibition of cytochrome P450 1B1 enzyme. Bioorg. Med. Chem. Lett. 27(24), 5400-5403. DOI: 10.1016/j.bmcl.2017.11.013. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29150398?dopt=AbstractPlus

- Shebl RI et al. (2012) Antiviral activity of liquorice powder extract against varicella zoster virus isolated from Egyptian patients. Chang Gung Med. J. 35, 231-239. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22735054
- Sheu C W et al. (1986). Tests for mutagenic effects of ammoniated glycyrrhizin, butylated hydroxytoluene and gum Arabic in rodent germ cells. Environmental Mutagenesis. 8: 357-367.
- Shin S et al (2005). Effects of licorice on embryronic and fetal development in rats. Journal of Toxcology and Public Health 21(4), 325-332.
- Shin S et al. (2008). Licorice extract does not impair the male reproductive function of rats. Experimental Animals 57(1), 11-17. http://www.ncbi.nlm.nih.gov/pubmed/18256514
- Souness G W & Morris D J (1989). The antinatriuretic and kaliuretic effects of the glucocorticoids corticosterone and cortisol following pre-treatment with carbenoxolone sodium (a licorice derivative) in the adrenalectomized rat. Endocrinology. 124 (3): 1588-1590.
- Stabbert R et al. (2019). Assessment of priority tobacco additives per the requirements in the EU Tobacco Products Directive (2014/40/EU): Part 2: Smoke chemistry and in vitro toxicology. Regul. Toxicol. Pharmacol. 104, 163-199. DOI: 10.1016/j.yrtph.2019.03.002. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30858113
- Stedman R L (1968). The Chemical composition of Tobacco and Tobacco Smoke. Chemical Reviews, 68 (2), 153-207
- Størmer F C et al. (1993a). Adverse effects of glycyrrhizinic acid in liquorice. A risk assessment. Nordiske seminar og Arbejdsrapporter 1993: 526.
- Størmer F C et al. (1993b). Glycyrrhizinic acid in liquorice - evaluation of health hazard. Food and Chemical Toxicology, 31, 303-312 (cited in SCF, 2003).
- Strandberg T E et al. (2001). Birth outcome in relation to licorice consumption during pregnancy. American Journal of Epidemiology, 153, 1085-1088.
- Strandberg T E et al. (2002). Preterm birth and licorice consumption during pregnancy. American Journal of Epidemiology, 156, 803-805.
- Strandberg T E et al. (2003). Risk factors for preterm delivery. Lancet, 361, 436.
- Sundaram M B M & Swaminathan R (1981). Total body potassium depletion and severe myopathy due to chronic licorice ingestion. Postgraduate Medical Journal, 57, 48-49.
- Takahashi K et al. (2019). Identification of glycyrrhizin metabolites in humans and of a potential biomarker of liquorice-induced pseudoaldosteronism: A multi-centre cross-sectional study. Arch. Toxicol. 93(11), 3111-3119. DOI: 10.1007/s00204-019-02588-2. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31605160/
- Tang ZH et al. (2015). A Systematic Review of the Anticancer Properties of Compounds Isolated from Licorice (Gancao). Planta Med. 81(18), 1670-87. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26695708
- Tassinari D et al. (2015). Posterior reversible encephalopathy syndrome associated with licorice consumption: a case report in a 10-year-old boy. Pediatr. Neurol. 52(4), 457-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25680999
- Tsukamoto S et al. (2005), Biol Pharm Bull. 2005 Oct;28(10):2000-2 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16204965&query_hl=5&itool=pubmed_DocSum

- US EPA (2021). Electronic Code of Federal Regulations (eCFR). Title 40. Current as of 19 January 2022. Available at: https://www.ecfr.gov/cgi-bin/ECFR?page=browse
- US EPA 2020 CDR list (Chemical Data Reporting Rule). Accessed January 2022. Available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do
- US EPA InertFinder Database (2021). Last updated 23 November 2021. Accessed January 2022. Available at https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1
- US EPA TSCA inventory. Accessed January 2022. Available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do
- Vallejo-Garcia VE et al. (2021). Acute myocardial infarction and severe hypokalaemia due to liquorice consumption during COVID-19 confinement. Hipertens. Riesgo Vasc. Epub ahead of print. [Article in Spanish]. DOI: 10.1016/j.hipert.2021.02.004. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33715981/
- Van Gelderen C E M et al. (2000). Glycyrrhizinic acid: the assessment of a no effect level. Human and Experimental Toxicology, 19, 434-439.
- Vanscheeuwijck PM et al. (2002), Food Chem Toxicol. 2002 Jan;40(1):113-31. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=11731041&query_hl=124&itool=pubmed_docsum
- VKM (2018). Norwegian Scientific Committee for Food and Environment. Hazard assessment of glycyrrhizic acid from liquorice. VKM Report 2018: 09. Available at https://www.vkm.no/download/18.948158f1633d1b9fb95930/1525764185285/Hazard%20assessment%20of%20glycyrrhizic%20acid%20from%20liquorice.pdf
- Wang Z et al. (1995), Biol Pharm Bull. 1995 Sep; 18(9):1238-41. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8845813&query_hl=5&itool=pubmed_DocSum
- Wang Z Y et al. (2000). Licorice in foods and herbal drugs: chemistry, pharmacology, toxicology and uses. Herbs, Botanicals & Teas. Mazza G. & Oomah B.D. (Eds), Functional Foods & Nutraceuticals Series. Technomic Publishing Co. Inc.
- Wang Zhi Y & Nixon D W (2001). Licorice and cancer, 39, 1-11.
- Wang C et al. (2016). Promoting effect of licorice extract on spermatogonial proliferation and spermatocytes differentiation of neonatal mice in vitro. In Vitro Cell. Dev. Biol. Anim. 52(2), 149-55. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26676954
- Wang S et al. (2018). Evidence for chemopreventive and resilience activity of licorice: Glycyrrhiza glabra and G. inflata extracts modulate estrogen metabolism in ACI rats. Cancer Prev. Res. (Phila.) 11(12), 819-830. DOI: 10.1158/1940-6207.CAPR-18-0178. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30287522
- Watanabe M et al. (2002), Biol Pharm Bull. 2002 Oct;25(10):1388-90. Pubmed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12392103&query_hl=5&itool=pubmed_DocSum
- Watari N (1976). Protective effect of glycyrrhizin in liver injury induced by a carcinogen (The First Report). J. Cell. Biol. 70(2): 1a.
- Watari N & Hotta Y (1980). Fine structural studies on the protective effect of glycyrrhizin for mouse liver injury caused by ethanol administration. Euro. J. Cell Biol. 22(1): 1677.

- Webb TE et al. (1992). Nutr Cancer. 1992; 18(3):215-30. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/1296195
- Wijesundara NM and Rupasinghe HPV (2019). Herbal tea for the management of pharyngitis: inhibition of Streptococcus pyogenes growth and biofilm formation by herbal infusions. Biomedicines 7(3), 63. DOI: 10.3390/biomedicines7030063. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31450579/
- Woosley RL. (2020). Arrhythmogenic foods - A growing medical problem. Trends Cardiovasc. Med. 30(5), 310-321. DOI: 10.1016/j.tcm.2019.08.007. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/31477495/
- Wu Y et al. (2011). [Research of herb components on scavenging harmful components and reducing cytotoxicity of cigarette smoke]. Zhongguo Zhong Yao Za Zhi.; 36(22):3184-8. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22375404
- Yamaguchi T & Watanabe T (1984). Effects of triterpenes on the mutagenicities of various mutagens toward Salmonella. Agric. Biol. Chem. 49(12): 3137-3139.
- Yang L et al. (2017). Spasmolytic Mechanism of Aqueous Licorice Extract on Oxytocin-Induced Uterine Contraction through Inhibiting the Phosphorylation of Heat Shock Protein 27. Molecules 22(9), E1392. DOI: 10.3390/molecules22091392. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28850076
- Yang R et al. (2017). The anti-inflammatory activity of licorice, a widely used Chinese herb. Pharm. Biol. 55(1), 5-18. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27650551
- Yavuz Kocaman A & Güzelkokar M. (2018). The genotoxic and antigenotoxic potential of the methanolic root extract of Glycyrrhiza glabra L. on human peripheral blood lymphocytes. Drug Chem. Toxicol. 15, 1-8. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29447011
- Yeung A. & Foster S., Encyclopaedia of common natural ingredients used in food, drugs and cosmetics, 2nd edition, 2003, pp. 346-350
- Zani F et al. (1993). Inhibition of mutagenicity in Salmonella typhimurium by Glycyrrhiza glabra extract, glycyrrhizic acid 18a- and 18b-glycyrrhetinic acids. Planta Med. 59(6): 502-507.
- Zhang L et al. (2016). Metabolic routes along digestive system of licorice: multicomponent sequential metabolism (MSM) method in rat. Biomed. Chromatogr. 30(6), 902-12. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26418123
- Zhang B et al. (2020). Glycyrrhiza glabra suppresses nasopharyngeal carcinoma cell proliferation through inhibiting the expression of lncRNA, AK027294. Biosci. Biotechnol. Biochem. 84(2), 314-320. DOI: 10.1080/09168451.2019.1673695. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31589096/
- Zheng C et al. (2019). A metabolic investigation of anticancer effect of G. glabra root extract on nasopharyngeal carcinoma cell line, C666-1. Mol. Biol. Rep. 46(4), 3857-3864. DOI: 10.1007/s11033-019-04828-1. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31066003/
- Zhou JX and Wink M. (2018). Reversal of multidrug resistance in human colon cancer and human leukemia cells by three plant extracts and their major secondary metabolites. Medicines (Basel) 5(4), E123. DOI: 10.3390/medicines5040123. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30428619

- Zhou JX and Wink M (2019). Evidence for anti-inflammatory activity of isoliquiritigenin, 18β glycyrrhetinic acid, ursolic acid, and the traditional Chinese medicine plants Glycyrrhiza glabra and Eriobotrya japonica, at the molecular level. Medicines (Basel) 6(2), 55. DOI: 10.3390/medicines6020055. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31083310/
- Zhou JX et al. (2019). Antioxidant, cytotoxic, and antimicrobial activities of Glycyrrhiza glabra L., Paeonia lactiflora Pall., and Eriobotrya japonica (Thunb.) Lindl. extracts. Medicines (Basel) 6(2), E43. DOI: 10.3390/medicines6020043. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30935079
- Zhu G et al. (2018). The effect of ethanol extract of Glycyrrhiza uralensis on the voltage-gated sodium channel subtype 1.4. J. Pharmacol. Sci. 136(2), 57-65. DOI: 10.1016/j.jphs.2017.11.008. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29433959

12. References for reduced-risk products

- Abdel-Wahab AA et al. (2020). A Licorice Roots Extract Induces Apoptosis and Cell Cycle Arrest and Improves Metabolism via Regulating MiRNAs in Liver Cancer Cells. Nutr. Cancer. Epub ahead of print. DOI: 10.1080/01635581.2020.1783329. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32578448/
- AICIS (undated). Australian Government Department of Health. Australian Industrial Chemicals Introduction Scheme. Record for 97676-23-8. Accessed January 2022. https://www.industrialchemicals.gov.au/search-inventory
- AICIS (2017). Australian Government Department of Health. Australian Industrial Chemicals Introduction Scheme Inventory Multi-Tiered Assessment and Prioritisation (IMAP) Tier I. Health Record for licorice, extract (CAS RN 68916-91-6) and Licorice, Glycyrrhiza glabra, extract (CAS RN 84775-66-6). Dated 10 March 2017 and 30 June 2017 respectively. Accessed January 2022. Available at: https://www.industrialchemicals.gov.au/chemical-information/search-assessments?assessmentcasnumber
- Ahn J et al. (2013). Anti-obesity effects of glabridin-rich supercritical carbon dioxide extract of licorice in high-fat-fed obese mice. Fd Chem Toxicol. 51, 439-445. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22967722
- Aksoy L et al. (2012). Protective and therapeutic effects of licorice in rats with acute tubular necrosis. J. Ren. Nutr. 22, 336-343. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22047711?dopt=AbstractPlus
- Al-Qarawi AA et al. (2002), Food Chem Toxicol. 2002 Oct; 40(10):1525-7. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12387318&query_hl=5&itool=pubmed_DocSum
- Aluckal E et al. (2017). Assessment of Total Antioxidant Capacity and Antimicrobial Activity of Glycyrrhiza glabra in Saliva of HIV-Infected Patients. J. Pharm. Bioallied Sci. 9(Suppl. 1), S237-S240. DOI: 10.4103/jpbs.JPBS_141_17. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29284971
- Anon (2003). Herbal warnings latest. Health Which? Jun 01, 33.
- Armanini D et al. (1999). Reduction of serum testosterone in men by liquorice. New England Journal of Medicine, 341, 1158.

- Attou R et al. (2020). Liquorice Intoxication Can Lead to Cardiac Arrest! Case Rep. Emerg. Med. 2020, 3727682. DOI: 10.1155/2020/3727682. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33029435/
- Ayeka PA et al. (2017). The immunomodulatory activities of licorice polysaccharides (Glycyrrhiza uralensis Fisch.) in CT 26 tumor-bearing mice. BMC Complement. Altern. Med. 17(1), 536. DOI: 10.1186/s12906-017-2030-7. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29246138
- Azizsoltani A et al. (2018). Ethyl acetate extract of licorice root (Glycyrrhiza glabra) enhances proliferation and osteogenic differentiation of human bone marrow mesenchymal stem cells. Iran. J. Pharm. Res. 17(3), 1057-1067. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30127828
- Badr AE et al. (2012). A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament. Int. Endod. J. 44, 51-58. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/20812941?dopt=AbstractPlus
- Bannister B et al. (1977). Cardiac arrest due to licorice induced hypokalaemia. Br. Med. J. ii: 738-739.
- Batiha GES et al. (2020). Traditional uses, bioactive chemical constituents, and pharmacological and toxicological activities of Glycyrrhiza glabra L. (Fabaceae). Biomolecules 10(3), E352. DOI: 10.3390/biom10030352. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32106571/
- Benge E et al. (2020). Trick or Treat? Licorice-Induced Hypokalemia: A Case Report. Cureus 12(11), e11656. DOI: 10.7759/cureus.11656. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33391895/
- Bernardi M et al. (1994). Effects of prolonged ingestion of graded doses of liquorice by healthy volunteers. Life Sciences, 55, 863-872. Reviewed in SCF, 2003.
- Bijlsma J A et al. (1996). [Study into effects of different dosages of glycyrrhizin in healthy female volunteers; in Dutch]. RIVM Report 348801004, RIVM, Bilthoven, The Netherlands (cited in SCF, 2003).
- Blachley J D & Knochel J P (1980). Tobacco chewer's hypokalaemia: Licorice revisited. The New England Journal of Medicine, April 3 1980, p784.
- Brendler T et al. (2020). Botanical drugs and supplements affecting the immune response in the time of COVID-19: Implications for research and clinical practice. Phytother. Res. Epub ahead of print. DOI: 10.1002/ptr.7008. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33373071/
- Brown AC. (2018). Heart toxicity related to herbs and dietary supplements: online table of case reports. Part 4 of 5. J. Diet. Suppl. 15(4), 516-555. DOI: 10.1080/19390211.2017.1356418. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/28981338
- Bruusgaard-Mouritsen MA et al. (2020). Natural ingredients in cosmetic products-A suggestion for a screening series for skin allergy. Contact Dermatitis 83(4), 251-270. DOI: 10.1111/cod.13550. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32248558/
- Buhl LF et al. (2018). Licorice-induced apparent mineralocorticoid excess compounded by excessive use of terbutaline and high water intake. BMJ Case Rep., pii: bcr-2017-223918. DOI: 10.1136/bcr-2017-223918. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29674401

- Burdock GA (2010). Fenaroli's Handbook of Flavor Ingredients. Sixth Edition. CRC Press. ISBN 978-1-4200-9077-2.
- Calvert R J (1954). Lancet 1, 805.
- Caravaca-Fontan F et al. (2015). An Unexpected Cause of Severe Hypokalemia. Case Rep. Nephrol. 2015, 957583. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26550501
- Card W I et al. (1953). Effects of licorice and its derivatives on salt and water metabolism. The Lancet. 2: 663-667.
- Cartier A et al. (2002). Occupational asthma due to liquorice roots. Allergy, 57, 863. Chemical composition of mainstream smoke. Food and Chemical Toxicology, 40, 93-104.
- CCRIS (2010). Record for licorice, Glycyrrhizia glabra ext. (CAS RN 84775-66-6). CCRIS record no. 9463. Last revision date 23 September 2010 (records no longer being updated after 2011). Available at https://www.toxinfo.io/#/chem-detail/68916-91-6
- Celik MM et al. (2012). Licorice induced hypokalemia, edema, and thrombocytopenia. Human Expt. Tox. 31, 1295-1298. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22653692?dopt=AbstractPlus
- Chakotiya AS et al. (2017). Effect of aquo-alchoholic extract of Glycyrrhiza glabra against Pseudomonas aeruginosa in Mice Lung Infection Model. Biomed. Pharmacother. 90, 171-178. DOI: 10.1016/j.biopha.2017.03.055. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28355591
- Chandrasekaran CV et al. (2011). Evaluation of the genotoxic potential of standardized extract of Glycyrrhiza glabra (GutGard™). Reg. Tox. Pharmacol. 61, 373-380. Available at http://www.sciencedirect.com/science/article/pii/S0273230011001954
- ChemIDplus. Accessed March 2021. Available at https://chem.nlm.nih.gov/chemidplus/
- Chen L et al. (2011). [Effects of glycyrrhiza extract on pharmacokinetics property of daphnetin in rats]. Zhongguo Zhong Yao Za Zhi. 36(7), 935-8. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21761739?dopt=AbstractPlus
- Chen YY et al. (2019). Elucidating the interaction of kansui and licorice by comparative plasma/tissue metabolomics and a heatmap with relative fold change. J. Pharm. Anal. 9(5), 312-323. DOI: 10.1016/j.jpha.2019.05.005. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31929940/
- Choi JS et al. (2013). Fetal and Neonatal Outcomes in Women Reporting Ingestion of Licorice (Glycyrrhiza uralensis) during Pregnancy. Planta Med. 79, 97-103. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23299757?dopt=AbstractPlus
- Cooper E P & Berry C W (1988). Mutagenic potential of glycyrrhizin. Journal of Dental Research, 67, abstract 339 (special issue, March) (cited in SCF, 2003).
- CosIng. Cosmetic substances and ingredients database. Records for Glycyrrhiza glabra (various extracts; CAS RN 84775-66-6) and Glycyrrhiza glabra rhizome/root (CAS RN 68916-91-6). Undated, accessed January 2022. Available at https://ec.europa.eu/growth/tools-databases/cosing/
- CPID (undated). Consumer Product Information Database. Record for licorice oils (CAS RN 97676-23-8). Accessed January 2022. Available at https://www.whatsinproducts.com/
- Cugini P et al. (1983). Hypertension in a licorice abuse: A case report. G. Ital. Cardiol. 13, 126-128.

- Cumming A M M (1977). Metabolic effects of licorice. Br. Med. J. 1977 (i) p906.
- Cumming A M M et al. (1980). Severe hypokalaemia with paralysis induced by small doses of licorice. Postgrad Med. J. 56:526-529.
- Dhingra D et al. (2004), J Ethnopharmacol. 2004 Apr;91(2-3), 361-5. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15120462&query_hl=5&itool=pubmed_DocSum
- Diaconu CC et al. (2018). Drug-induced arterial hypertension - a frequently ignored cause of secondary hypertension: a review. Acta Cardiol. 1, 1-7. DOI: 10.1080/00015385.2017.1421445. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29291681
- Dirikan E & Turkez H (2014). In vitro studies on protective effect of Glycyrrhiza glabra root extracts against cadmium-induced genetic and oxidative damage in human lymphocytes. Cytotechnology 66(1), 9-16. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/23325115
- ECHA (undated). European Chemicals Agency. Information on chemicals. Records for licorice, ext. (CAS RN 68916-91-6); licorice, Glycyrrhiza glabra, ext. (CAS RN 84775-66-6); licorice oils (CAS RN 97676-23-8); Glycyrrhiza Glagra (Licorice) Root Extract (no CAS RN) and Glycyrrhiza Uralensis (Licorice) Root extract (no CAS RN). Accessed January 2022. Available at: https://echa.europa.eu/information-on-chemicals/pre-registered-substances
- ECHA (2021). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 21 December 2021. Available at: https://echa.europa.eu/information-on-chemicals/cl-inventory-database
- ECOSAR. Record for oils, licorice (CAS RN 97676-23-8). Accessed June 2017. (ECOSAR content has not been updated since 2012, version 1.11.) Available to download, through EPISuite, at https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- EFSA (2011). European Food Safety Authority. Scientific Opinion on the safety of 'Glavonoid®', an extract derived from the roots or rootstock of Glycyrrhiza glabra L., as a Novel Food ingredient. Available at: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2287/epdf
- EMA (2012). Community herbal monograph on Glycyrrhiza glabra L. and/or Glycyrrhiza inflata Bat. and/or Glycyrrhiza uralensis Fisch., radix. European Medicines Agency. MA/HMPC/571119/2010. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_Community_herbal_monograph/2012/08/WC500131287.pdf
- EPISuite. Record for licorice oils (CAS RN 97676-23-8). Accessed June 2017. (EPISuite content has not been updated since 2012, version 4.11.) The programme is available to download via https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
- EPISuite (2017). Record for licorice oils (CAS RN 97676-23-8). EPISuite version 4.11. Last updated June 2017. EPISuite is available to download at https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411
- FASEB (1974). Evaluation of the health aspects of licorice, glycyrriza and ammoniated glycyrrhizin as food ingredients. NTIS PB254529, FDABFGRAS362.

- FDA (2022). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 20 January 2022. Accessed January 2022. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances
- FDA (2021). US Food and Drug Administration. Electronic Code of Federal Regulations (eCFR). Title 21. Current as of 18 January 2022. Available at: https://www.ecfr.gov/cgi-bin/ECFR?page=browse
- Fogelman Y et al. (2016). Antiatherosclerotic effects of licorice extract supplementation on hypercholesterolemic patients: decreased CIMT, reduced plasma lipid levels, and decreased blood pressure. Food Nutr. Res. 60, 30830. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27113136
- Foster S et al. (2017). All sorts of tests, only one question: an unexpected cause of hypertension. BMJ Case Rep. 2017, bcr-2017-222077. DOI: 10.1136/bcr-2017-222077. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29127128
- Fujisawa Y et al., (2000), Microbiol Immunol. 2000;44(9):799-804. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11092245&query_hl=5&itool=pubmed_DocSum
- Fujita H & Sasaki M (1986). Ann. Rep. Tokyo Metr. Res. Lab. 37, 447.
- Fujita M et al. (2019). A newly developed means of HPLC-fluorescence analysis for predicting the skin sensitization potential of multi-constituent substances using ADRA. Toxicol. In Vitro 59, 161-178. DOI: 10.1016/j.tiv.2019.04.014. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/31002975
- Fukuchi K et al. (2016). Antiviral and Antitumor Activity of Licorice Root Extracts. In Vivo. 30(6), 777-785. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27815461
- Girerd R J et al. (1958). Production of experimental hypertension and cardiovascular-renal lesions with licorice and ammoniated glycyrrhizin. American Journal of Physiology. 194: 241-245.
- Gomez-Sanchez EP, Gomez-Sanchez CE. Am J Physiol. 1992 Dec; 263(6 Pt 1):E1125-30. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/1476186
- Gonzalez-Gutierrez M L et al. (2000). Allergy 55, 195.
- Gu MY et al. (2018). Glycyrrhiza uralensis and Semilicoisoflavone B Reduce Aβ Secretion by Increasing PPARγ Expression and Inhibiting STAT3 Phosphorylation to Inhibit BACE1 Expression. Mol. Nutr. Food Res. 62(6), e1700633. DOI: 10.1002/mnfr.201700633. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29143445
- Hajiaghamohammadi AA et al. (2012). The efficacy of licorice root extract in decreasing transaminase activities in non-alcoholic fatty liver disease: a randomized controlled clinical trial. Phytother. Res. 26, 1381-1384. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22308054
- Hall RL & Oser BL. (1965). Recent Progress in the Consideration of Flavoring Ingredients Under the Food Additives Amendment. III. GRAS Substances, Food Technology 253, 151. Available at https://www.femaflavor.org/sites/default/files/3.%20GRAS%20Substances(2001-3124)_0.pdf
- Hautaniemi EJ et al. (2017). Voluntary liquorice ingestion increases blood pressure via increased volume load, elevated peripheral arterial resistance, and decreased aortic

compliance. Sci. Rep. 7(1), 10947. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28887501

- Hayashi M et al. (1988). Fd Chem. Toxic. 26, 487.
- Health Canada (2021). Drugs and Health Products. Natural Health Products Ingredients Database. Records for Glycyrrhiza glabra (various), liquorice dry, Radix et Rhizoma Glycyrrhizae, Radix et Rhizoma Glycyrrhizae Preparata cum Melle, Glycyrrhiza uralensis, Extractum Glycyrrhizae and Extractum Glycyrrhizae Liquidum (no CAS RNs listed). Last updated 07 December 2021. Accessed January 2022. Available at http://webprod.hc-sc.gc.ca/nhpid-bdipsn/search-rechercheReq.do?lang=eng
- Heck J D et al. (1989). Toxicologist 9, 257. [Abstract only]
- Hejazi II et al. (2017). New insights into the antioxidant and apoptotic potential of Glycyrrhiza glabra L. during hydrogen peroxide mediated oxidative stress: An in vitro and in silico evaluation. Biomed. Pharmacother. 94, 265-279. DOI: 10.1016/j.biopha.2017.06.108. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28763750
- Hollinger JC et al. (2018). Are Natural Ingredients Effective in the Management of Hyperpigmentation? A Systematic Review. J. Clin. Aesthet. Dermatol. 11(2), 28-37. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29552273
- Hosseinzadeh H and Nassiri-Asl M (2015). Pharmacological Effects of Glycyrrhiza spp. and Its Bioactive Constituents: Update and Review. Phytother. Res. 29(2), 1866-86. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26462981
- Hou YC et al. (2012). Liquorice reduced cyclosporine bioavailability by activating P-glycoprotein and CYP 3A. Food Chem. 135, 2307-2312. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22980806?dopt=AbstractPlus
- HSDB (2002). Record for licorice extract (CAS RN 68916-91-6). Hazardous Substances Data Bank Number 1925. Last Revision Date: 8 November 2002. Accessed March 2021. Available at: https://www.toxinfo.io/#/chem-detail/68916-91-6
- IFRA (undated). International Fragrance Association. IFRA Transparency List. Accessed January 2022. Available at https://ifrafragrance.org/priorities/ingredients/ifra-transparency-list
-
- Ikegami F et al. (2004). Toxicological considerations of Kampo medicines in clinical use. Toxicology, 198, 221-228.
- Ikken Y et al. (1999). J. agric. Fd Chem. 47, 3257.
- Isbrucker RA & Burdock GA (2006). Risk and safety assessment on the consumption of licorice rood (Glycyrrhiza sp.), its extract and powder as a food ingredient with emphasis on the pharmacology and toxicology of glycyrrhizin. Regulatory Toxicology and Pharmacology 46(3), 167-192. Taken from http://www.ncbi.nlm.nih.gov/pubmed/16884839
- Ishidate M (1983). Quo Vadis? Short-term Tests for Carcinogenesis. Garattini S. et al. (Eds). Centre de Recherches Clin. Midy Groupe, SANOFI. Montpellier, 4-5 Feb. 1981.
- Ishidate M et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology, 22, 623-636.
- Ishidate M et al. (1988). Mutation Res. 195, 151.
- Ishimi Y et al. (2019). Quality evaluation of health foods containing licorice in the Japanese market. Toxicol. Rep. 6, 904-913. DOI: 10.1016/j.toxrep.2019.08.013. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31508319/

- Ishizaki M et al. (1985). J. Fd Hyg. Soc., Japan 26, 523.
- Itami T et al. (1985). Effect of disodium glycyrrhizinate on pregnant rats and their offspring. J. Food Hyg. Soc. Japan. 26(5): 460-464.
- JECFA (1978). Evaluation of certain food additives. 21st JECFA report. WHO Technical Report Series 617, Geneva.
- Jeon JH et al. (2007). Effect of green tea and licorice extracts on cyclophosphamide teratogenicity. Reproductive Toxicology 24(1), 75 (abstract only).
- Jhanji V, Liu H, Law K, Lee VY, Huang S, Pang CP and Yam GH (2011). Isoliquiritigenin from licorice root suppressed neovascularization in experimental ocular angiogenesis models. British Journal of Ophthalmology 95(9), 1309-15. PubMed, available at http://www.ncbi.nlm.nih.gov/pubmed/21719569
- Jiang M et al. (2020). An "essential herbal medicine"-licorice: A review of phytochemicals and its effects in combination preparations. J. Ethnopharmacol. 249, 112439. DOI: 10.1016/j.jep.2019.112439. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31811935/
- Jo EH et al. (2005), Cancer Lett. 230(2), 239-47. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16297710&query_hl=5&itool=pubmed_DocSum
- Jorgenson et al. (1977). Study of mutagenic effects of ammoniated glycyrrhizin by dominant lethal test in rats. US NTIS PB REP PB-279650 72.
- Josephs R A et al. (2001). Liquorice consumption and salivary testosterone concentrations. The Lancet, 358, 1613-1614.
- JTI KB Study Report (s).
- Kang JS et al. (2005), J Pharmacol Exp Ther. 312(3), 1187-94. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15537821&query_hl=5&itool=pubmed_DocSum
- Kaur P et al. (2012). Modulation of genotoxicity of oxidative mutagens by glycyrrhizic acid from Glycyrrhiza glabra L. Pharmacognosy Res. 4, 189-195. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23225961
- Kaur P et al. (2017). Immunopotentiating significance of conventionally used plant adaptogens as modulators in biochemical and molecular signalling pathways in cell mediated processes. Biomed. Pharmacother. 95, 1815-1829. DOI: 10.1016/j.biopha.2017.09.081. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28968926
- Kawachi T et al. (1980). Appl. meth. Oncol. 3, 253.
- Kawachi T et al. (1981). IARC Sci. Pub. 27, 323.
- Keyes KM & Susser E (2017). Invited Commentary: An Ingenious Approach to Examining the Relationship Between Maternal Stress and Offspring Health? Am. J. Epidemiol. 185(5), 329-332. DOI: 10.1093/aje/kww173. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28158433?dopt=AbstractPlus
- Kiken D A and Cohen D E (2002). Contact dermatitis to botanical extracts. American Journal of Contact Dermatitis, 13, 148-152.
- Kim HJ et al. (2005), Planta Med. 71(8), 785-7. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16142649&query_hl=5&itool=pubmed_DocSum

- Khan IA and Abourashed EA (2010). Leung's Encyclopedia of Common Natural Ingredients used in Food, Drugs, and Cosmetics. Third Edition. John Wiley & Sons, Inc., Hoboken, New Jersey
- Kobuke T et al. (1985). Tumorigenicity study of disodium glycyrrhizinate administered orally to mice. Fd Chem. Toxic. 23(11): 979-983.
- Komiyama K et al. (1977). Acute and subacute toxicity test on the extract from Glycyrrhiza. Oyo Yakuri. 14(4):535-548.
- Kuang Y et al. (2018). Antitussive and expectorant activities of licorice and its major compounds. Bioorg. Med. Chem. 26(1), 278-284. DOI: 10.1016/j.bmc.2017.11.046. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29224994
- Kwon YE et al. (2020). Severe asymptomatic hypokalemia associated with prolonged licorice ingestion: A case report. Medicine (Baltimore) 99(30), e21094. DOI: 10.1097/MD.0000000000021094. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32791684/
- LactMed (2021). Drugs and Lactation Database. Record for licorice (CAS RN 84775-66-6). Last updated 15 February 2021. Accessed March 2021. Available at https://www.ncbi.nlm.nih.gov/books/NBK501840/
- Lee MH et al. (2020). Licorice extract suppresses adipogenesis through regulation of mitotic clonal expansion and adenosine monophosphate-activated protein kinase in 3T3-L1 cells. J. Food Biochem. 44(12), e13528. DOI: 10.1111/jfbc.13528. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33051883/
- Lai F et al. (1980). Licorice, snuff and hypokalaemia. New England J. Med. 303: 463.
- Li G et al. (2017). Cytochrome P450 inhibition by three licorice species and fourteen licorice constituents. Eur. J. Pharm. Sci. 109, 182-190. DOI: 10.1016/j.ejps.2017.07.034. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28774812
- Lloyd R A et al. (1976). Flue-cured tobacco flavour. 1. Essence and essential oil components. Tobacco Science, 20, 40-48.
- Logic (2019). G.5. Nonclinical Evaluation Summary - Logic Vapeleaf (PMTA)
- Luis A et al. (2018). Metabolic changes after licorice consumption: A systematic review with meta-analysis and trial sequential analysis of clinical trials. Phytomedicine 39, 17-24. DOI: 10.1016/j.phymed.2017.12.010. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29433679
- Machalle K et al. (2015). Prinzmetal angina after licorice consumption. Dtsch. Med. Wochenschr. 140(8), 590-2. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25945908
- MacKenzie M A et al. (1990). The influence of glycyrrhetinic acid on plasma cortisol and cortisone in healthy young volunteers. Journal of Clinical Endocrinology and Metabolism. 70(6): 1637-1643.
- Madak-Erdogan Z et al. (2016). Dietary licorice root supplementation reduces diet-induced weight gain, lipid deposition, and hepatic steatosis in ovariectomized mice without stimulating reproductive tissues and mammary gland. Mol. Nutr. Food Res. 60(2), 369-80. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26555669
- Mantovani A et al. (1988). Teratogenicity study of ammonium glycyrrhizinate in the Sprague-Dawley rat. Food Cosmetic Toxicol. (1972). 26(5): 435-440.

- Martinez San Ireneo M et al. (1995). Allergy 50, 173.
- Maser E (2004). Trends Pharmacol Sci. 25(5), 235-7. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15120485&query_hl=83&itool=pubmed_docsum
- Morimoto I et al. (1982). Mutagenicity screening of crude drugs with Bacillus subtilis rec-assay and Salmonella/microsome reversion assay. Mutation Research, 97(2), 81-102.
- Muñoz Balbontín Y et al. (2019). Herbal medicinal product use during pregnancy and the postnatal period: a systematic review. Obstet. Gynecol. 133(5), 920-932. DOI: 10.1097/AOG.0000000000003217. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30969204
- Namazi N et al. (2017). The Effect of Dried Glycyrrhiza Glabra L. Extract on Obesity Management with Regard to PPAR-γ2 (Pro12Ala) Gene Polymorphism in Obese Subjects Following an Energy Restricted Diet. Adv. Pharm. Bull. 7(2), 221-228. DOI: 10.15171/apb.2017.027. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28761824
- Nazari S et al. (2017). Toxicological Effects of Glycyrrhiza glabra (Licorice): A Review. Phytother. Res. 31(11), 1635-1650. DOI: 10.1002/ptr.5893. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28833680
- Nazeefullah S et al. (2014). Effect of cold water extracts of Acacia modesta Wall. and Glycyrrhiza glabra Linn. on Tribolium castaneum and Lemna minor. Pak. J. Pharm. Sci. 27(2), 217-22. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/24577905?dopt=AbstractPlus
- Ng ZX et al. (2020). Comparative analyses on radical scavenging and cytotoxic activity of phenolic and flavonoid content from selected medicinal plants. Nat. Prod. Res. Epub ahead of print. DOI: 10.1080/14786419.2020.1749617. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/32290699/
- Nielsen I & Pedersen R S (1984). Life-threatening hypokalaemia caused by licorice ingestion. The Lancet, June 9 1984, p1305.
- Nilon H & Froment A (1968). Rev. Lyon. Med, p99.
- Nishioka K & Seguchi T (1999). Contact allergy due to oil-soluble licorice extracts in cosmetic products. Contact Dermatitis 40, 56.
- Nose M et al. (2019). Evaluation of the safety and efficacy of Glycyrrhiza uralensis root extracts produced using artificial hydroponic-field hybrid cultivation systems II: comparison of serum concentration of glycyrrhetinic acid serum concentration in mice. J. Nat. Med. 73(3), 661-666. DOI: 10.1007/s11418-019-01312-9. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31028662/
- Nozawa J (1967). Inhibitory effect of glycyrrhizin on experimental skin carcinogenesis in mice. Jap. J. Dermatol. Ser. B. 77(4), 415.
- NTP (undated). National Toxicology Program. Chemical Effects in Biological Systems (CEBS). Record for licorice extract (CAS RN 68916-91-6). Accessed January 2022. Available at https://manticore.niehs.nih.gov/cebssearch/test_article/68916-91-6
- NZ EPA (2006). New Zealand Environmental Protection Authority. Inventory of Chemicals. Records for licorice, ext. (CAS RN 68916-91-6), licorice, Glycyrrhiza glabra, ext. (CAS RN 84775-66-6) and oils, licorice (CAS RN 97676-23-8). Date added to inventory: 1 December 2006. Accessed March 2021. Available at: https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/33691, https://www.epa.govt.nz/database-

search/new-zealand-inventory-of-chemicals-nzioc/view/36398 and https://www.epa.govt.nz/database-search/new-zealand-inventory-of-chemicals-nzioc/view/38324

- OECD. Organisation for Economic Cooperation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). Oils, licorice (97676-23-8). Accessed June 2017. Available at: http://webnet.oecd.org/CCRWeb/Search.aspx.
- Oesch F et al. (2021). Toxicity as prime selection criterion among SARS-active herbal medications. Phytomedicine. Epub ahead of print. DOI: 10.1016/j.phymed.2021.153476. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33593628/
- Oijha SK et al. (2015). Licorice treatment prevents oxidative stress, restores cardiac function, and salvages myocardium in rat model of myocardial injury. Toxicol. Ind. Health 31(2), 140-52. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/23771872
- Ota M et al. (2018). Comparison of the inducible effects of licorice products with or without heat-processing and pre-treatment with honey on granulocyte colony-stimulating factor secretion in cultured enterocytes. J. Ethnopharmacol. 214, 1-7. DOI: 10.1016/j.jep.2017.11.040. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29203272
- Paolini M et al. (1998). Life Sci. 62(6), 571-82. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/9464470
- Park D et al. (2011). Licorice extract increases cyclophosphamide teratorgenicity by upregulating the expression of cytochrome P-450 2B mRNA. Birth Defects Research. Part B. Developmental and Reproductive Toxicology 92(6), 553-9. Taken from http://www.ncbi.nlm.nih.gov/pubmed/21818843
- Penninkilampi R et al. (2017). The association between consistent licorice ingestion, hypertension and hypokalaemia: a systematic review and meta-analysis. J. Hum. Hypertension 31(11), 699-707. DOI: 10.1038/jhh.2017.45. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28660884
- Peterson CT et al. (2018). Prebiotic Potential of Herbal Medicines Used in Digestive Health and Disease. J. Altern. Complement. Med. 24(7), 656-665. DOI: 10.1089/acm.2017.0422. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29565634
- Prival M J et al. (1991). Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. Mutation Research, 321-329.
- Qiao X et al. (2012). Analytical strategy to reveal the in vivo process of multi-component herbal medicine: a pharmacokinetic study of licorice using liquid chromatography coupled with triple quadrupole mass spectrometry. J. Chromat. A 1258, 84-93. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22939378?dopt=AbstractPlus
- Rahman H et al. (2017). Drug-Herb Interactions in the Elderly Patient with IBD: a Growing Concern. Curr. Treat. Options Gastroenterol. 15(4), 618-636. DOI: 10.1007/s11938-017-0154-y. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28918484
- Räikkönen K et al. (2017). Maternal Licorice Consumption During Pregnancy and Pubertal, Cognitive, and Psychiatric Outcomes in Children. Am. J. Epidemiol. 185(5), 317-328. DOI: 10.1093/aje/kww172. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28158597?dopt=AbstractPlus
- Rees W D et al. (1979). Effect of deglycyrrhizinated licorice on gastric mucosal damage by aspirin. Scand. J. Gastroenterol. 14(5), 605-607.

- RTECS (2016). Registry of Toxic Effects of Chemical Substances. Record for Glycyrrhiza extract (68916-91-6). Last updated July 2016. Accessed March 2021.
- RTECS (2019). Registry of Toxic Effects of Chemical Substances. Record for Glycyrrhiza glabra L., root, acetone extract (no CAS RN listed). Last updated March 2019. Accessed March 2021.
- Saadat S et al. (2018). Comparative Antitussive Effects of Medicinal Plants and Their Constituents. Altern. Ther. Health Med. 24(4), 36-49. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29332022
- Sakr SA & Shalaby SY (2014). Carbendazim-induced testicular damage and oxidative stress in albino rats: ameliorative effect of licorice aqueous extract. Toxicol. Ind. Health 30(3), 259-67. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/22903170?dopt=AbstractPlus
- Sasaki M et al. (1980). Kromosomo II 20, 574.
- Sasaki Yu F et al. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutation Research, 519, 103-119.
- Satti P et al. (2019). Indigenous irrigants as potent antimicrobials in endodontic treatment: An in vitro study. J. Indian Soc. Pedod. Prev. Dent. 37(3), 275-281. DOI: 10.4103/JISPPD.JISPPD_112_19. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31584028/
- SCENIHR (2016). Scientific Committee on Emerging and Newly Identified Health Risks. Final opinion on additives used in tobacco products (opinion 1). Tobacco Additives I. Adopted on 25 January 2016. Available at: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_051.pdf
- SCF (2003). Opinion of the Scientific Committee on Food on glycyrrhizinic acid and its ammonium salt. SCF/CS/ADD/EDUL/225 Final 10 April 2003.
- Schmidt M et al. (2013). Cytotoxicity of herbal extracts used for treatment of prostatic disease on head and neck carcinoma cell lines and non-malignant primary mucosal cells. Oncol. Reps. 29, 628-636. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23165347
- Senanayake N, Román GC. J Neurol Sci. 1992 Jan; 107(1):1-13. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/1315843
- Shah M et al. (2012). Licorice-related rhabdomyolysis: a big price for a sweet tooth. Clin. Nephrol. 77, 491-495. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22595392?dopt=AbstractPlus
- Sharma R et al. (2017). Glycyrrhiza glabra extract and quercetin reverses cisplatin resistance in triple-negative MDA-MB-468 breast cancer cells via inhibition of cytochrome P450 1B1 enzyme. Bioorg. Med. Chem. Lett. 27(24), 5400-5403. DOI: 10.1016/j.bmcl.2017.11.013. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29150398?dopt=AbstractPlus
- Shebl RI et al. (2012) Antiviral activity of liquorice powder extract against varicella zoster virus isolated from Egyptian patients. Chang Gung Med. J. 35, 231-239. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22735054
- Sheu C W et al. (1986). Tests for mutagenic effects of ammoniated glycyrrhizin, butylated hydroxytoluene and gum Arabic in rodent germ cells. Environmental Mutagenesis. 8: 357-367.

- Shin S et al (2005). Effects of licorice on embryronic and fetal development in rats. Journal of Toxcology and Public Health 21(4), 325-332.
- Shin S et al. (2008). Licorice extract does not impair the male reproductive function of rats. Experimental Animals 57(1), 11-17. http://www.ncbi.nlm.nih.gov/pubmed/18256514
- Souness G W & Morris D J (1989). The antinatriuretic and kaliuretic effects of the glucocorticoids corticosterone and cortisol following pre-treatment with carbenoxolone sodium (a licorice derivative) in the adrenalectomized rat. Endocrinology. 124 (3): 1588-1590.
- Stedman R L (1968). The Chemical composition of Tobacco and Tobacco Smoke. Chemical Reviews, 68 (2), 153-207
- Størmer F C et al. (1993a). Adverse effects of glycyrrhizinic acid in liquorice. A risk assessment. Nordiske seminar og Arbejdsrapporter 1993: 526.
- Størmer F C et al. (1993b). Glycyrrhizinic acid in liquorice - evaluation of health hazard. Food and Chemical Toxicology, 31, 303-312 (cited in SCF, 2003).
- Strandberg T E et al. (2001). Birth outcome in relation to licorice consumption during pregnancy. American Journal of Epidemiology, 153, 1085-1088.
- Strandberg T E et al. (2002). Preterm birth and licorice consumption during pregnancy. American Journal of Epidemiology, 156, 803-805.
- Strandberg T E et al. (2003). Risk factors for preterm delivery. Lancet, 361, 436.
- Sundaram M B M & Swaminathan R (1981). Total body potassium depletion and severe myopathy due to chronic licorice ingestion. Postgraduate Medical Journal, 57, 48-49.
- Takahashi K et al. (2019). Identification of glycyrrhizin metabolites in humans and of a potential biomarker of liquorice-induced pseudoaldosteronism: A multi-centre cross-sectional study. Arch. Toxicol. 93(11), 3111-3119. DOI: 10.1007/s00204-019-02588-2. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31605160/
- Tang ZH et al. (2015). A Systematic Review of the Anticancer Properties of Compounds Isolated from Licorice (Gancao). Planta Med. 81(18), 1670-87. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26695708
- Tassinari D et al. (2015). Posterior reversible encephalopathy syndrome associated with licorice consumption: a case report in a 10-year-old boy. Pediatr. Neurol. 52(4), 457-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25680999
- Tsukamoto S et al. (2005), Biol Pharm Bull. 2005 Oct;28(10):2000-2 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16204965&query_hl=5&itool=pubmed_DocSum
-
- US EPA (2021). Electronic Code of Federal Regulations (eCFR). Title 40. Current as of 19 January 2022. Available at: https://www.ecfr.gov/cgi-bin/ECFR?page=browse
- US EPA 2020 CDR list (Chemical Data Reporting Rule). Accessed January 2022. Available at https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.do
- US EPA InertFinder Database (2021). Last updated 23 November 2021. Accessed January 2022. Available at https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1
- US EPA TSCA inventory. Accessed January 2022. Available at

- https://sor.epa.gov/sor_internet/registry/substreg/LandingPage.doVallejo-Garcia VE et al. (2021). Acute myocardial infarction and severe hypokalaemia due to liquorice consumption during COVID-19 confinement. Hipertens. Riesgo Vasc. Epub ahead of print. [Article in Spanish]. DOI: 10.1016/j.hipert.2021.02.004. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/33715981/
- Van Gelderen C E M et al. (2000). Glycyrrhizinic acid: the assessment of a no effect level. Human and Experimental Toxicology, 19, 434-439.
- VKM (2018). Norwegian Scientific Committee for Food and Environment. Hazard assessment of glycyrrhizic acid from liquorice. VKM Report 2018: 09. Available at https://www.vkm.no/download/18.948158f1633d1b9fb95930/1525764185285/Hazard%20assessment%20of%20glycyrrhizic%20acid%20from%20liquorice.pdf
- Wang Z et al. (1995), Biol Pharm Bull. 1995 Sep; 18(9):1238-41. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8845813&query_hl=5&itool=pubmed_DocSum
- Wang Z Y et al. (2000). Licorice in foods and herbal drugs: chemistry, pharmacology, toxicology and uses. Herbs, Botanicals & Teas. Mazza G. & Oomah B.D. (Eds), Functional Foods & Nutraceuticals Series. Technomic Publishing Co. Inc.
- Wang Zhi Y & Nixon D W (2001). Licorice and cancer, 39, 1-11.
- Wang C et al. (2016). Promoting effect of licorice extract on spermatogonial proliferation and spermatocytes differentiation of neonatal mice in vitro. In Vitro Cell. Dev. Biol. Anim. 52(2), 149-55. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26676954
- Wang S et al. (2018). Evidence for chemopreventive and resilience activity of licorice: Glycyrrhiza glabra and G. inflata extracts modulate estrogen metabolism in ACI rats. Cancer Prev. Res. (Phila.) 11(12), 819-830. DOI: 10.1158/1940-6207.CAPR-18-0178. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30287522
- Watanabe M et al. (2002), Biol Pharm Bull. 2002 Oct;25(10):1388-90. Pubmed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=12392103&query_hl=5&itool=pubmed_DocSum
- Watari N (1976). Protective effect of glycyrrhizin in liver injury induced by a carcinogen (The First Report). J. Cell. Biol. 70(2): 1a.
- Watari N & Hotta Y (1980). Fine structural studies on the protective effect of glycyrrhizin for mouse liver injury caused by ethanol administration. Euro. J. Cell Biol. 22(1): 1677.
- Webb TE et al. (1992). Nutr Cancer. 1992; 18(3):215-30. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/1296195
- Wijesundara NM and Rupasinghe HPV (2019). Herbal tea for the management of pharyngitis: inhibition of Streptococcus pyogenes growth and biofilm formation by herbal infusions. Biomedicines 7(3), 63. DOI: 10.3390/biomedicines7030063. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31450579/
- Woosley RL. (2020). Arrhythmogenic foods - A growing medical problem. Trends Cardiovasc. Med. 30(5), 310-312. DOI: 10.1016/j.tcm.2019.08.007. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/31477495/
- Yamaguchi T & Watanabe T (1984). Effects of triterpenes on the mutagenicities of various mutagens toward Salmonella. Agric. Biol. Chem. 49(12): 3137-3139.

- Yang L et al. (2017). Spasmolytic Mechanism of Aqueous Licorice Extract on Oxytocin-Induced Uterine Contraction through Inhibiting the Phosphorylation of Heat Shock Protein 27. Molecules 22(9), E1392. DOI: 10.3390/molecules22091392. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28850076
- Yang R et al. (2017). The anti-inflammatory activity of licorice, a widely used Chinese herb. Pharm. Biol. 55(1), 5-18. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27650551
- Yavuz Kocaman A & Güzelkokar M. (2018). The genotoxic and antigenotoxic potential of the methanolic root extract of Glycyrrhiza glabra L. on human peripheral blood lymphocytes. Drug Chem. Toxicol. 15, 1-8. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29447011
- Yeung A. & Foster S., Encyclopaedia of common natural ingredients used in food, drugs and cosmetics, 2nd edition, 2003, pp. 346-350
- Zani F et al. (1993). Inhibition of mutagenicity in Salmonella typhimurium by Glycyrrhiza glabra extract, glycyrrhizic acid 18a- and 18b-glycyrrhetinic acids. Planta Med. 59(6): 502-507.
- Zhang L et al. (2016). Metabolic routes along digestive system of licorice: multicomponent sequential metabolism (MSM) method in rat. Biomed. Chromatogr. 30(6), 902-12. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26418123
- Zhang B et al. (2020). Glycyrrhiza glabra suppresses nasopharyngeal carcinoma cell proliferation through inhibiting the expression of lncRNA, AK027294. Biosci. Biotechnol. Biochem. 84(2), 314-320. DOI: 10.1080/09168451.2019.1673695. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31589096/
- Zheng C et al. (2019). A metabolic investigation of anticancer effect of G. glabra root extract on nasopharyngeal carcinoma cell line, C666-1. Mol. Biol. Rep. 46(4), 3857-3864. DOI: 10.1007/s11033-019-04828-1. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31066003/
- Zhou JX and Wink M. (2018). Reversal of multidrug resistance in human colon cancer and human leukemia cells by three plant extracts and their major secondary metabolites. Medicines (Basel) 5(4), E123. DOI: 10.3390/medicines5040123. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30428619
- Zhou JX and Wink M (2019). Evidence for anti-inflammatory activity of isoliquiritigenin, 18β glycyrrhetinic acid, ursolic acid, and the traditional Chinese medicine plants Glycyrrhiza glabra and Eriobotrya japonica, at the molecular level. Medicines (Basel) 6(2), 55. DOI: 10.3390/medicines6020055. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31083310/
- Zhou JX et al. (2019). Antioxidant, cytotoxic, and antimicrobial activities of Glycyrrhiza glabra L., Paeonia lactiflora Pall., and Eriobotrya japonica (Thunb.) Lindl. extracts. Medicines (Basel) 6(2), E43. DOI: 10.3390/medicines6020043. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30935079
- Zhu G et al. (2018). The effect of ethanol extract of Glycyrrhiza uralensis on the voltage-gated sodium channel subtype 1.4. J. Pharmacol. Sci. 136(2), 57-65. DOI: 10.1016/j.jphs.2017.11.008. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29433959

13. Other information

- Klus H et al. (2012). Influence of Additives on Cigarette Related Health Risks. Beiträge zur Tabakforschung 25(3), 412–493. Available at: http://www.degruyter.com/view/j/cttr.2012.25.issue-3/cttr-2013-0921/cttr-2013-0921.xml?rskey=wBFXae&result=2
- Paschke T et al. (2002). Effects of Ingredients on Cigarette Smoke Composition and Biological Activity: A Literature Overview. Beiträge zur Tabakforschung 20(3), 107–247. Available at: http://www.degruyter.com/view/j/cttr.2002.20.issue-3/cttr-2013-0736/cttr-2013-0736.xml?rskey=wBFXae&result=4
- Rodgman A (2002). Some Studies of the Effects of Additives on Cigarette Mainstream Smoke Properties. II. Casing Materials and Humectants. Beiträge zur Tabakforschung 20(4), 279–299. Available at: http://www.degruyter.com/view/j/cttr.2002.20.issue-4/cttr-2013-0742/cttr-2013-0742.xml?rskey=wBFXae&result=1
- Rodgman A (2004). Some Studies of the Effects of Additives on Cigarette Mainstream Smoke Properties. III. Ingredients Reportedly Used in Various Commercial Cigarette Products in the USA and Elsewhere. Beiträge zur Tabakforschung 21(2), 47-104. Available at http://www.degruyter.com/view/j/cttr.2004.21.issue-2/cttr-2013-0771/cttr-2013-0771.xml?rskey=wBFXae&result=6
- Harding V & Stebbing J. (2017). Liquorice: a treatment for all sorts? Lancet Oncol. 18(9), 1155. DOI: 10.1016/S1470-2045(17)30628-9. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28884688
- Liew ZH & Lee KG. (2017). Liquorice-Induced Severe Hypokalaemic Rhabdomyolysis with Acute Kidney Injury. Ann. Acad. Med. Singapore 46(9), 354-355. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29022037
- Sayiner ZA et al. (2017). A rare cause of thyrotoxic periodic paralysis: liquorice consumption. Postgrad. Med. J. 93(1099), 295-296. DOI: 10.1136/postgradmedj-2016-134716. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28258118
- Ali M et al. (2018). Selected hepatoprotective herbal medicines: Evidence from ethnomedicinal applications, animal models, and possible mechanism of actions. Phytother. Res. 32(2), 199-215. DOI: 10.1002/ptr.5957. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29047177
- Cao LJ et al. (2017). Evid. Based Complement. Alternat. Med. 2017, 2752389. DOI: 10.1155/2017/2752389. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29234377
- Ju SM et al. (2017). Licorice and its active compound glycyrrhizic acid ameliorates cisplatin-induced nephrotoxicity through inactivation of p53 by scavenging ROS and overexpression of p21 in human renal proximal tubular epithelial cells. Eur. Rev. Med. Pharmacol. Sci. 21(4), 890-899. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28272690?dopt=AbstractPlus
- Kargozar R et al. (2017). A review of effective herbal medicines in controlling menopausal symptoms. Electron Physician 9(11), 5826-5833. DOI: 10.19082/5826. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29403626
- Lim C et al. (2018). Licorice Pretreatment Protects Against Brain Damage Induced by Middle Cerebral Artery Occlusion in Mice. J. Med. Food. Epub ahead of print. DOI: 10.1089/jmf.2017.4044. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29341803

- Mostafa HE et al. (2017). Alleviative effect of licorice on copper chloride-induced oxidative stress in the brain: biochemical, histopathological, immunohistochemical, and genotoxic study. Environ. Sci. Pollut. Res. Int. 24(22), 18585-18595. DOI: 10.1007/s11356-017-9503-6. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28647874
- Najafi S et al. (2017). Preventive Effect of Glycyrrhiza Glabra Extract on Oral Mucositis in Patients Under Head and Neck Radiotherapy: A Randomized Clinical Trial. J. Dent. (Tehran) 14(5), 267-274. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29296112
- Pastorino G et al. 2018. Liquorice (Glycyrrhiza glabra): A phytochemical and pharmacological review. Phytother. Res. 32(12), 2323-2339. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30117204
- Kokoska L et al. 2018. Plant-Derived Products as Antibacterial and Antifungal Agents in Human Health Care. Curr. Med. Chem. Epub ahead of print. PubMed, 2019 Available at https://www.ncbi.nlm.nih.gov/pubmed/30182844
- Kimyon RS et al. (2019). Allergic contact dermatitis to licorice root extract. Dermatitis 30(3), 227-228. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31045935/

14. Last audited

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EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

22 May 2012
EMA/HMPC/571119/2010
Committee on Herbal Medicinal Products (HMPC)

Community herbal monograph on *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch., radix

Final

Discussion in Working Party on Community monographs and Community list (MLWP)	September 2010 January 2011 March 2011 May 2011
Adoption by Committee on Herbal Medicinal Products (HMPC) for release for consultation	July 2011
End of consultation	15 November 2011
Rediscussion in Working Party on Community monographs and Community list (MLWP)	November 2011 January 2012 March 2012
Adoption by Committee on Herbal Medicinal Products (HMPC)	22 May 2012

Keywords	Herbal medicinal products; HMPC; Community herbal monographs; traditional use; *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch., radix; liquorice root

BG (bălgarski): Сладник	LT (lietuvių kalba):
CS (čeština): Lékořicový kořen	LV (latviešu valoda): Lakricu saknes
DA (dansk): Lakridsrod, uskrællet	MT (malti): Għerq ta' Għud is-Sus
DE (Deutsch): Süßholzwurzel	NL (nederlands): Zoethoutwortel
EL (elliniká): Γλυκύρριζα	PL (polski): Korzeń lukrecji
EN (English): Liquorice root	PT (português): Alcaçuz, raiz
ES (espanol): Regaliz, raíz de	RO (română): Rădăcină de lemn dulce
ET (eesti keel): Magusjuurejuur	SK (slovenčina): Sladkovkový koreň
FI (suomi): lakritsi, juuri	SL (slovenščina): Korenina golostebelnega sladkega korena
FR (français): Réglisse (racine de)	SV (svenska): Lakritsrot
HU (magyar): Édesgyökér	*IS (íslenska)*:
IT (italiano): Liquirizia, radice	*NO (norsk):* Lakrisrot, uskrelt

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Community herbal monograph on *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch., radix

1. Name of the medicinal product

To be specified for the individual finished product.

2. Qualitative and quantitative composition[1,2]

Well-established use	Traditional use
	With regard to the registration application of Article 16d(1) of Directive 2001/83/EC as amended *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch., radix (liquorice root) i) Herbal substance Not applicable. ii) Herbal preparations a) Comminuted herbal substance b) Soft extract (DER 1:0.4-0.5), extraction solvent water c) Soft extract (DER 3:1), extraction solvent water d) Dry extracts that correspond to preparations mentioned under b) and c)

3. Pharmaceutical form

Well-established use	Traditional use
	Comminuted herbal substance as a herbal tea for oral use. Herbal preparations in solid or liquid dosage forms for oral use. The pharmaceutical form should be described by the European Pharmacopoeia full standard term.

1 The declaration of the active substance(s) for an individual finished product should be in accordance with relevant herbal quality guidance.
2. The material complies with the Ph. Eur. monograph (ref.: 01/2010: 0277).

4. Clinical particulars

4.1. Therapeutic indications

Well-established use	Traditional use
	Indication 1 Traditional herbal medicinal product for the relief of digestive symptoms including burning sensation and dyspepsia. Indication 2 Traditional herbal medicinal product used as an expectorant in cough associated with cold. The product is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.

4.2. Posology and method of administration[3]

Well-established use	Traditional use
	Posology Indication 1 *Adults and elderly* Single dose a) Comminuted herbal substance Herbal tea: 1.5 - 2 g of comminuted herbal substance in 150 ml of boiling water as a herbal infusion 2 to 4 times daily or 1.5 - 2 g of comminuted herbal substance in 150 ml of water as a decoction 2 to 4 times daily. Take one cup after meals. b) Soft extract (DER 1:0.4-0.5) 32 mg 2-3 times daily for oral use. Not more than 160 mg (32 mg 5 times) daily. d) doses of dry extracts corresponding to b)

[3] For guidance on herbal substance/herbal preparation administered as herbal tea or as infusion/decoction/macerate preparation, please refer to the HMPC 'Glossary on herbal teas' (EMA/HMPC/5829/2010 Rev.1).

	Indication 2 *Adults and elderly* Single dose a) Comminuted herbal substance 1.5 g of comminuted herbal substance in 150 ml of boiling water as a herbal infusion 2 times daily or 1.5 g of comminuted herbal substance in 150 ml of water as a decoction 2 times daily. c) Soft extract (DER 3:1) 1.2-1.5 g 3-4 times daily. d) doses of dry extracts corresponding to c) The use in children and adolescents under 18 years of age is not recommended (see section 4.4 'Special warnings and precautions for use'). **Duration of use** Indication 1 Not to be used for more than 4 weeks. If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted. Indication 2 If the symptoms persist longer than 1 week during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted. **Method of administration** Oral use.

4.3. Contraindications

Well-established use	Traditional use
	Hypersensitivity to the active substance(s).

4.4. Special warnings and precautions for use

Well-established use	Traditional use
	Indication 1 and 2

Well-established use	Traditional use
	The use in children and adolescents under 18 years of age has not been established due to lack of adequate data. If the symptoms worsen during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted. Patients taking liquorice medication should not take other liquorice containing products as serious adverse events may occur such as water retention, hypokalemia, hypertension, cardiac rhythm disorders. Liquorice medication is not recommended to be used in patients affected by hypertension, kidney diseases, liver or cardiovascular disorders or hypokalemia, as they are more sensitive to the adverse effects of liquorice. Concomitant use with diuretics, cardiac glycosides, corticosteroids, stimulant laxatives or other medications which may aggravate electrolyte imbalance is not recommended (see section 4.5). Indication 2 If dyspnoea, fever or purulent sputum occurs, a doctor or a qualified health care practitioner should be consulted.

4.5. Interactions with other medicinal products and other forms of interaction

Well-established use	Traditional use
	Liquorice root may counteract antihypertensive action of prescribed medications. Not to be used concomitantly with diuretics, cardiac glycosides, corticosteroids, stimulant laxatives or other medications which may aggravate electrolyte imbalance (see section with 4.4).

4.6. Fertility, pregnancy and lactation

Well-established use	Traditional use
	Studies in animals have shown reproductive

	toxicity (see section 5.3 'Preclinical safety data'). Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended. No fertility data available.

4.7. Effects on ability to drive and use machines

Well-established use	Traditional use
	No studies on the effect on the ability to drive and use machines have been performed.

4.8. Undesirable effects

Well-established use	Traditional use
	If adverse reactions occur, a doctor or a qualified health care practitioner should be consulted.

4.9. Overdose

Well-established use	Traditional use
	Cases of overdose have been reported with prolonged use (more than 4 weeks) and/or intake of high amount of liquorice, with symptoms such as water retention, hypokalaemia, hypertension, cardiac rhythm disorders, hypertensive encephalopathy.

5. Pharmacological properties

5.1. Pharmacodynamic properties

Well-established use	Traditional use
	Not required as per Article 16c (1)(a)(iii) of Directive 2001/83/EC as amended.

5.2. Pharmacokinetic properties

Well-established use	Traditional use
	Not required as per Article 16c (1)(a)(iii) of Directive 2001/83/EC as amended.

5.3. Preclinical safety data

Well-established use	Traditional use
	Not required as per Article 16c (1)(a)(iii) of Directive 2001/83/EC as amended, unless necessary for the safe use of the product. Adequate tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed. A study has shown that 18β-glycyrrhetinic acid[4] crosses through the placental barrier and can be detected in the rat foetuses. Following feeding of dams with 100 mg 18β-glycyrrhetinic acid/kg/day commencing on the 13th day of gestation, on the 17th, 19th and 21st days of gestation the maternal plasma 18β-glycyrrhetinic acid concentrations were approximately 100 µg/ml, whereas the foetal concentrations were 5, 18 and 32 µ/ml, respectively. In developmental toxicity studies, glycyrrhizin (ammonium salt) exhibited some embryotoxicity to the developing rat foetus, but the foetal effects were considered as minor. These effects were shown at the dose of 100 and 250 mg/kg of ammonium glycyrrhizin from 7th to 20th day of pregnancy (soft-tissue abnormalities, mostly renal, and external haemorrhages) and at the dose of 1000 mg/kg of 18β-glycyrrhetinic acid from the 13th day of gestation (significant reduction in lamellar body content of lungs and reduced number alveolar lamellar body and surfactant clusters, but no apparent increase in malformation or foetal death rate). Another study suggested that 100 mg/kg of liquorice extract repeated for 7 days may also aggravate body weight loss and malformations of foetuses, induced by intrauterine exposure to cyclophosphamide.

[4] Where herbal preparations from Liquiritiae radix are used, the total exposure to 18β-glycyrrhizic acid should be considered from a safety standpoint.

6. Pharmaceutical particulars

Well-established use	Traditional use
	Not applicable.

7. Date of compilation / last revision

22 May 2012

European Food Safety Authority

EFSA Journal 2011;9(7):2287

SCIENTIFIC OPINION

Scientific Opinion on the safety of 'Glavonoid®', an extract derived from the roots or rootstock of Glycyrrhiza glabra L., as a Novel Food ingredient[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of 'Glavonoid' as a food ingredient in the context of Regulation (EC) No 258/97 taking into account the comments and objections of a scientific nature raised by Member States. Glavonoid is an extract derived from the root or rootstock of *Glycyrrhiza glabra* L. by extraction with ethanol followed by further extraction with medium-chain triglycerides. The applicant provided sufficient information on the specification, production, composition and the stability of Glavonoid. The applicant intends to market Glavonoid as food supplements and as an ingredient for fruit juices, yoghurts and yoghurt drinks up to a dose of 300 mg per day to the general adult population. A 90-day rat study showed a lowest observed adverse effect level (LOAEL) at a dose of 400 mg/kg bw per day. Prothrombin-time and activated partial thromboplastin time (APTT) were analysed using a Benchmark Dose (BMD) modelling approach. The BMD lower confidence limit ($BMDL_{05}$) for this study derived from APTT data is 167 mg/kg bw per day. There are no concerns related to genotoxicity. Studies on reproductive and developmental toxicity were not provided. Extrapolation of the $BMDL_{05}$ from the rat study to a maximum intake for a 70 kg person results in 117 mg Glavonoid/day. The human studies provided do not raise safety concerns. The Panel considers that the human studies are consistent with the maximum level derived from the BMD approach. The Panel considers that there are no concerns related to genotoxicity. The safety of Glavonoid for pregnant and breast-feeding women has not been established. The Panel concludes that the novel food ingredient Glavonoid is safe for the general adult population up to 120 mg/day.

KEY WORDS

Glycyrrhiza glabra, liquorice, ethanolic extract.

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[1] On request from the European Commission, Question EFSA-Q-2009-00749. Adopted on 30 June 2011.

[2] Panel members: Carlo V. 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

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of 'Glavonoid' as a food ingredient in the context of Regulation (EC) No 258/97 taking into account the comments and objections of a scientific nature raised by Member States.

Glavonoid is an extract rich in polyphenolic type substances, derived from the root or rootstock of *Glycyrrhiza glabra* L. (liquorice root) by extraction with ethanol followed by further extraction of this ethanolic extract with medium-chain triglycerides (MCT). According to the specification proposed by the applicant Glavonoid is a dark-brown coloured liquid, standardised using MCT to a content of approximately 3.0 % of the prenylated flavonoid glabridin, which is the most abundant constituent of the polyphenol fraction contained in the product. Other identified prenylated flavonoids are glabrene, glabrol and 4'-O-methylglabridin. The forty-five identified polyphenolic type substances were classified as chalcones, isoflavans, isoflavenes, 3-aryl coumarins, pterocarpans, 2-aryl benzofurans, flavones, isoflavones, flavanones and flavanols. Batch testing confirmed that the product complies with the given specifications. The applicant provided sufficient information regarding the specification, manufacture, composition and stability of Glavonoid.

Glycyrrhiza glabra L., the source of the novel food ingredient is a member of the Fabaceae family, and has a history of human consumption. The roots are chewed as a mouth freshener. In the EU extracts produced by boiling the root of *Glycyrrhiza glabra* L. and subsequently evaporating most of the water, are widely used in candies and confectionary. Liquorice products are also used in soft drinks, herbal teas and chewing gum. According to the information provided, Glavonoid is on the market in Japan and the USA.

The applicant intends to market Glavonoid as food supplements and as an ingredient for fruit juices, yoghurts and yoghurt drinks up to a dose of 300 mg per day. The target population is the general adult population.

In a subchronic (90-day) oral toxicity study a "Liquorice Flavonoid Oil (LFO) - concentrated form" containing 3 % glabridin, which can be considered representative for Glavonoid, induced an effect on blood coagulation parameters, evidenced in a prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT), which caused haemorrhage in several organs and tissues and the death of several animals in the highest dose group (1600 mg/kg bw). Additional studies showed that the anticoagulant effect was caused by inhibition of the synthesis of vitamin K-dependent coagulation factors, though the identity of the responsible substance(s) remains unknown.

A prolonged PT was statistically significant in male animals receiving the lowest dose level of 400 mg/kg bw per day. Therefore this dose is considered as the lowest observed adverse effect level (LOAEL). There are no further studies e.g. a long-term exposure studies or reproductive and developmental toxicity studies that might have shed light on the NOAEL. Prothrombin-time and APTT were also analysed using a Benchmark Dose (BMD) modelling approach, following the recommendations in the guidance document of the Scientific Committee of EFSA. The values for the BMD and BMD lower confidence limit ($BMDL_{05}$) derived from the APTT data of male rats provided lower values than the PT data. The $BMDL_{05}$ for this study derived from APTT data is 167 mg/kg bw. The extrapolation of this study $BMDL_{05}$ to a maximum intake for a 70 kg person results in 117 mg Glavonoid/day, rounded up to 120 mg.

The Panel considers that there are no concerns related to genotoxicity.

The reported six human studies examining a dose range of 100 to 600 mg Glavonoid per day did not show relevant changes in haematology, coagulation, clinical-chemistry and urinalysis parameters. Because of the short treatment times, the dose levels used, and in particular the low number of study participants receiving higher doses, the Panel considers that these studies are not adequate to derive a

safe level of intake, especially for longer-term consumption. However, since the human studies do not raise safety concerns, the Panel considers that the human studies are consistent with the maximum level derived from the BMD approach.

Regarding the uncertainties concerning a possible impact on anticoagulant therapy, the Panel notes that no studies have been conducted to evaluate a possible interaction between Glavonoid and drugs on blood coagulation. The safety of Glavonoid for pregnant and breast-feeding women has not been established.

The Panel concludes that the novel food ingredient Glavonoid is safe for the general adult population up to 120 mg/day.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 1 November 2007, KANEKA Pharma Europe N.V. submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to the competent authorities of Belgium for placing on the market Glavonoid as a novel food ingredient and food supplement.

On 23 January 2009, the competent authorities of Belgium forwarded to the Commission their initial assessment report, which came to the conclusion that health risks related to the placing on the market of Glavonoid as food supplement are extremely low.

On 19 February 2009, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted comments or raised objections.

In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) No 258/97.

The concerns of a scientific nature raised by the Member States can be summarized as follows:

- Specification of the source and composition of the novel food ingredient is not sufficient. In particular, clarification is needed regarding the composition of the lipid fraction including the polyphenol-type substances as well as the fraction of uncharacterised constituents. The use of minimum and maximum values is recommended. Furthermore, the documents provided do not show whether the testing laboratories were accredited under an internationally recognised system, which is a general requirement in the case of approval applications.

- The Folin-Ciocalteu test does not allow determination of the polyphenol content but determination of the total reducing capacity. Determination by HPLC is regarded as a suitable method to determine the polyphenol content.

- The use of medium-chain triglycerides (MCT) is questionable. In the scientific literature gastrointestinal problems after ingestion of MCTs were reported. In addition, MCTs may alter the blood lipid concentrations (increase of cholesterol and triglyceride levels).

- The manufacturing process of Glavonoid is described in sufficient detail except for the preliminary treatment of the raw material. There is no mention of an HACCP plan.

- Information on the stability of Glavonoid in the different food matrices is lacking.

- The applicant is of the opinion that Glavonoid is substantially equivalent to liquorice extracts currently on the market except for the glycyrrhizinic acid content. However, there is no information on possible selective isolation and concentration of constituents relative to existing liquorice products (i.e. the root and aqueous root extracts) that are used as the basis for arguments for a history of apparent safe use. Therefore toxicological studies with the novel food ingredient are needed.

- With regards to the use of Glavonoid in foodstuffs no information on the intended use levels was provided and no assessment of the anticipated intake levels on the basis of representative consumption data of the respective foodstuffs in the EU has been carried out.

- The maximum daily intake of Glavonoid as recommended by the applicant (300 mg/day) is very close to the maximum doses tested in humans. The doses used in the human studies are considered relatively low. No long-term studies have been conducted in humans.

- The equivalence between Liquorice Flavonoid Oil (LFO) used as test material in the clinical trials and Glavonoid needs to be demonstrated. It is not clear whether or not LFO, which is described as

a diluted form of Glavonoid, has also undergone extraction with MCT under the same conditions and therefore what is the content of glycyrrhizinic acid and other constituents in LFO.

- There is a concern regarding the addition of Glavonoid to general foodstuffs that may cause an uncontrolled and excess intake in the elderly as well as in children. No data on the likely intake in teenagers and younger children were provided but many of the food categories that might contain Glavonoid are potentially attractive to young age groups.

- Full reports of the toxicological studies should be provided. In the subchronic oral toxicity study in rats an anti-coagulant effect was observed and it was considered questionable to derive a no-observable adverse effect level (NOAEL). A higher safety factor should be applied to derive an acceptable intake level for long-term human consumption. Detailed results of the examinations in humans were not provided. Thus there are concerns over the potential for effects on blood coagulation, particularly in sensitive individuals including patients undergoing anti-coagulant therapy. The potential for an interaction with other factors (e.g. aspirin intake) should also be examined. In a scientific publication (Aoki et al., 2007) changes in haematology and related parameters were reported to have occurred in humans.

- Specific constituents of the extract have structural similarities with oestrogens and there is therefore a potential for interaction with oestrogen receptors. Furthermore, since a high proportion of the components in the extract have not been characterised the potential of Glavonoid to induce endocrine effects, including effects in infants and young children, should be examined. Questions also arise over potential effects on the efficacy of drugs (e.g. Tamoxifen). Additional animal experiments are necessary to rule out undesirable effects on embryonic or foetal development.

- The applicant should further clarify the record of the *in vivo* micronucleus test in rat liver cells. A test on unscheduled DNA synthesis (UDS) on mammalian cells *in vivo* should also be conducted to confirm the negative results. The effects of chronic exposure in experimental animals have not been examined and there is no proof that Glavonoid is not carcinogenic.

- Long-term clinical studies on nutritional safety and effectiveness are missing and there are concerns in relation to usefulness and necessity. The effects of Glavonoid on the availability and metabolism of nutrients in the diet cannot be evaluated.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for Glavonoid as food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of scientific nature in the comments raised by the other Member States.

ASSESSMENT

In accordance with the Commission Recommendation 97/618/EC (EC, 1997) Glavonoid derived from the root or rootstock of *Glycyrrhiza glabra* L. is allocated to Class 2.1 'a complex (non-GM derived) novel food ingredient the source of the novel food having a history of food use in the community'. The assessment of the safety of this novel food ingredient is based on data supplied in the original application, the initial assessment by the Belgian competent authority, the concerns and objections of the other Member States and the responses of the applicant to these questions and those of Belgium. The data are required to comply with the information required for novel foods of Class 2.1 i.e. structured schemes I, II, III, IX, X, XI, XII and XIII.

In its initial assessment report, the competent authority of Belgium came to the conclusion that health risks related to the placing on the market of Glavonoid as food supplement are extremely low. Regarding the use of Glavonoid in foods it was considered that health risks are extremely low at doses of 300 mg/day with proper labelling and clear information for consumers on the maximum tolerated doses. However, particular attention should be paid to potential risks linked to a multiplicity of applications for the placing on the market of products containing polyphenols. It is noted that the novel food ingredient is intended by the applicant to be marketed to overweight subjects in order to reduce body fat and body weight as well as total and LDL blood cholesterol. This assessment concerns only risk that might be associated with consumption and is not an assessment of the efficacy of Glavonoid with regard to any claimed benefit.

1. Specification of the Novel Food (NF)

Glavonoid is an extract rich in polyphenolic type substances, derived from the root or rootstock of *Glycyrrhiza glabra* L. (liquorice root) by extraction with ethanol followed by further extraction of this ethanolic extract with medium-chain triglycerides (MCT). According to the specification proposed by the applicant, Glavonoid is a dark-brown coloured liquid, standardised using MCT to a content of 3.0 % +/- 0.5 % glabridin, which is the most abundant constituent of the polyphenol fraction contained in the product (Table 1).

According to the information in the application dossier, Glavonoid comprises 30 % ethanolic extract from liquorice roots and 70 % MCT. The content of polyphenolic type substances was determined using a colourimetric method (Folin-Ciocalteu) and a single value of 24 % was reported. In addition, single values determined by HPLC were reported for the contents of glabridin (3%), glabrene (0.3 %), glabrol (0.6 %) and 4'-O-methylglabridin (0.6 %).

Table 1: Specification for Glavonoid proposed by the applicant

Parameter	Specification	Method of analysis
Appearance	Dark brown coloured liquid Distinct smell and taste	Visual check and organoleptic examination
Identification	Correspond to the standard UV-VIS chart Correspond to the standard HPLC chart	UV-VIS HPLC
Glabridin	3.0 % +/- 0.5 %	HPLC
Glycyrrhizinic acid	< 0.005 % (w/w) *	HPLC
Peroxide Value	≤ 0.5 meq/kg	Conform to standard method for the analysis of fats, oil and related materials (Japanese Oil Chemists' Society)
Aerobic Plate Count	≤ 1000 cfu/g	Standard agar plating method

Coliforms	Negative /2.22 g	Brilliant green lactose bile (BGLB) method

* quantification limit 0.005 % (w/w); detection limit 0.001 % (w/w)

These specifications were verified for each production batch. Additional analyses were performed on some production batches to assure quality control. Pesticide residues were determined for every new batch of the starting material *Glycyrrhiza glabra* L. roots (Table 2).

Table 2: Additional quality criteria for Glavonoid suggested by the applicant

Parameter	In-house criterion	Method of analysis
Residual ethanol	≤ 0.5 % (w/w)	Gas chromatography
Moisture	≤ 0.5 % (w/w)	Karl Fisher's method
Residue on ignition	≤ 0.5 %	Conform to Japanese standard of food additives
Arsenic	≤ 2 ppm	
Lead	≤ 0.3 ppm	
Pesticide residues (benzene hexachlorides (BHC), di-chlorodiphenyltrichloroethane (DDT), aldrin, endrin, dieldrin, parathion, malathion, fenitrothion)	Not detected	GC-MS
E. coli / *Salmonella*	Negative [a]	Conform to USP 2021
Yeasts and moulds	Negative [b]	Standard agar plating method [c]

[a] negative/10 g Glavonoid capsules (containing approximately 2 g Glavonoid). Criterion for Salmonella does not comply with requirements of EU Regulation

[b] negative/1 g Glavonoid capsules (containing approximately 0.2 g Glavonoid)

[c] According to the additional information provided by the applicant the method complies with 'the standard methods of analysis in food safety regulation' issued by the Health Labour and Welfare Ministry of Japan

Analysis of three representative batches of Glavonoid confirmed that the product complies with the proposed specification (Table 1) and the additional in-house criteria listed in Table 2. However, the NDA Panel notes that the analysis of microbial contaminants does not comply with the EU standard.

At the request of the Panel the applicant provided additional information concerning the composition of Glavonoid. The results are presented in Tables 3 and 4.

Table 3: Nutrient composition of Glavonoid (based on the analysis of three batches)

Parameter	Test results	Method of analysis
Water (g/100 g)	0.2 - 0.5	Atmospheric heated-air drying method
Protein (g/100 g)	0.1	Kjeldahl method
Fat (g/100 g)	99.4 – 99.7 *	Ether extraction method
Ash (g/100 g)	< 0.1	Direct ashing method
Carbohydrate (g/100 g)	0	Enzymatic-gravimetric method
Energy (kcal/100 g)	895 – 897	Calculation
Sodium (mg/100 g)	0.6 – 1.1	Atomic absorption method

* including polyphenol-type substances

Table 4: Lipid composition of Glavonoid (based on the analysis of one batch)

Lipid fraction		Method of analysis
MCT	67.3 g/100 g	Gas chromatography
Fatty acid composition of MCT-esterified fatty acids		Gas chromatography
8 : 0	99.7 %	
10 : 0	0.3 %	
Free fatty acids (total)	0.27 g/100 g	Gas chromatography
Octanoic acid	0.04 g/100 g	
Palmitic acid	0.05 g/100 g	
Stearic acid	0.02 g/100 g	
Oleic acid	0.04 g/100 g	
Linoleic acid	0.05 g/100 g	
Linolenic acid	0.01 g/100 g	
Behenic acid	0.05 g/100 g	
Lignoceric acid	0.01 g/100 g	
Total sterol	0.070 g/100 g	Gas chromatography
Cholesterol	0.001 g/100 g	
Brassicasterol	n.d.	
Campesterol	0.007 g/100 g	
Stigmasterol	0.021 g/100 g	
7-ergosterol	n.d.	
Beta-sitosterol	0.038 g/100 g	
Isofucosterol	0.003 g/100 g	
7-stigmasterol	n.d.	
Apenasterol	n.d.	
Phospholipids	0.031 g/100 g	Standard method for the analysis of oil and fat (Japanese Oil Chemists' Society)

n.d. – not detected; detection limit 1 mg/100 g

Considering that Glavonoid had a total fat content determined by extraction with ether (including polyphenolic type substances) of more than 99 % (Table 3) and that the total content of MCT, free fatty acids, phospholipids and sterols was approximately 68 % (Table 4), the applicant anticipated that the remaining constituents in the ether extract of Glavonoid, i.e. approximately 30 %, are hydrophobic polyphenolic-type substances.

In addition, the applicant provided a HPLC chromatogram (UV detector at 282 nm) of an ethanolic extract of *Glycyrrhiza glabra* L. roots. The roots were extracted with 95 % ethanol. Separation and refinement of the extract were performed with reverse-phase and normal-phase silica gel column chromatography and preparative HPLC with a reverse-phase column. All peaks, whose area was larger than 0.2 % of the total peak area, were further analysed by mass spectrometry and structures for 45 compounds were tentatively assigned. The most prominent peak corresponded to the prenylated flavonoid glabridin. Other prominent peaks were identified as the prenylated flavonoids glabrene, glabrol and 4'-O-methylglabridin. The 45 polyphenolic-type substances, for which structures were tentatively assigned, were classified as chalcone, isoflavan, isoflavene, 3-arylcoumarin, pterocarpan, 2-arylbenzofuran, flavone, isoflavone, flavanone and flavanol. In addition, comparisons of HPLC chromatograms (UV detector at 282 nm and 254 nm) of five batches of ethanolic extracts of *Glycyrrhiza glabra* L. roots showed that the patterns of peaks were qualitatively and quantitatively relatively consistent.

The applicant claimed that there was no difference in polyphenolic type compounds between the ethanolic extract of *Glycyrrhiza glabra* L. root and the final product Glavonoid, which is obtained by further extraction of the ethanolic extract using MCT. The total glabridin content in the end product

Glavonoid (one batch) corresponded to 87 % of the amount present at the first stage of the production process, i.e. after ethanol extraction of *Glycyrrhiza glabra* L. root. According to the applicant, the recovery rate is also applicable to the total amount of hydrophobic polyphenols. Determination of the relative peak areas corresponding to 28 polyphenolic type substances in relation to the glabridin peak area revealed that the relative abundance of these 28 substances at the first of the three stages of the production process, i.e. after ethanol extraction, was in the same range as in the end product Glavonoid.

The Panel concludes that the product is sufficiently characterised.

2. Effect of the production process applied to the NF

Root or rootstock of *Glycyrrhiza glabra* L. (liquorice root) is extracted with ethanol and food grade MCT under proprietary process conditions, which are fully disclosed to the Member States, the European Commission and EFSA.

According to the applicant, extraction of the ethanolic extract from liquorice root with MCT reduces the amount of glycyrrhizinic acid from approximately 0.2 % in the concentrated ethanol solution to below the detection limit of 0.001 % in Glavonoid (see section 1). The applicant anticipates that the levels of other constituents, e.g. glycyrrhetinic acid, liquiritin and liquiritigenin, are also reduced although this was not substantiated by analytical data.

The food grade MCT used in the production process is produced by esterification of glycerol and fatty acids derived from coconut and/or palm oil followed by refining and deodorisation processes. It contains mixed tocopherols obtained from soybean oil diluted with rice oil as a natural preservative. A product specification for MCT was provided.

The NDA Panel asked for information on whether a HACCP or an equivalent system is applied to ensure the consistency of the production process and a constant quality and composition of the novel food ingredient. The applicant confirmed that manufacture of Glavonoid is carried out in compliance with Good Manufacturing Practice (GMP) provisions. As for HACCP, hazard analysis is based on the procedure described in "Recommended International Code of Practice General Principle of Food Hygiene (CAC/RCP 1-1969, Rev.4-2003)".

The stability of Glavonoid under different storage conditions (-4, +4, 15 and 40° C) was analysed by determination of the levels of the prenylated flavonoids glabridin, glabrene, glabrol and 4'O-methylglabridin using HPLC analysis. Under all conditions applied, the levels of glabrene, glabrol and 4'O-methylglabridin after 6 months were practically identical to the levels at the start of the experiment. The levels of glabridin were slightly reduced after storage at 25 and 40° C but still 98.0 and 92.7 %, respectively, of the initial levels after 6 months.

In a photostability study (test apparatus EYELA, LST-300D, 25° C, 60 % relative humidity, 5000 Lux) the levels of glabridin, glabrene, glabrol and 4'-O-methylglabridin after 8 weeks were 91.5, 77.9, 93.3 and 104.7 %, respectively, of the initial levels as determined by HPLC analysis.

In response to the Member States' comments, the applicant provided information on the stability of water-soluble formulations in powder form containing 10 % and 30 % Glavonoid. The amount of glabridin was determined (method not indicated) after storage at 5, 25 and 40° C. After storage for 12 months at 25° C the level of glabridin in the 10 % and 30 % formulation was 94.7 % and 95.1 %, respectively, of the initial level. After storage of both formulations at 40° C the amount of glabridin was 90.6 % and 92.7 %, respectively, of the initial level after 6 months, which was the last time point.

3. History of the organism used as the source of the NF

Glycyrrhiza glabra L., the plant used as the source for the production of Glavonoid, is a member of the Fabaceae family and has a history of human consumption. The roots are chewed as a mouth freshener. In the EU extracts produced by boiling the root of *Glycyrrhiza glabra* L. and subsequently evaporating most of the water, are widely used in candies and confectionary. These extracts contain glycyrrhizinic acid, a sweetener more than 50 times as sweet as sucrose, which also has pharmacological effects. Liquorice root extracts are also used in soft drinks, herbal teas and chewing gum. Liquorice extract is also added to tobacco products.

In the USA liquorice (glycyrrhiza) root, liquorice root extract (extracted by boiling water) and ammoniated glycyrrhizinic acid are direct food substances generally recognised as safe (GRAS) in accordance with 21 CFR 184.1408. These regulations allow the use of these ingredients as a flavour enhancer and flavouring agent in various food categories at specified maximum levels.

According to the applicant ethanolic extracts from the roots of *Glycyrrhiza glabra* L. are used in Japan and the USA as food additives and as health-ingredients in food supplements.

4. Anticipated intake/extent of the use of the NF

As a reaction to the Member States' concerns regarding potential intake levels of Glavonoid for specific population groups, the applicant provided more detailed information regarding the intended uses. Glavonoid is intended to be marketed first as food supplement in the form of capsules and tablets containing 100 to 300 mg of the novel food ingredient. Daily dose, directions for use and specific warnings would be included in the product label. The applicant proposes that pregnant or breast-feeding women as well as individuals taking prescription drugs should consult their healthcare practitioner prior to use.

The applicant had originally proposed to incorporate Glavonoid also into a relatively large variety of food products. However, as a reaction to the Member States' comments, the number of food products was reduced to fruit juices, yoghurts and yoghurt drinks (Table 5).

Table 5: Proposed food uses and use-levels for Glavonoid

Food Category	Proposed food use	Serving size	Use-level (mg/serving)	Use-level (g/100 g)*
Fruit and vegetable juices, soft drinks and bottled water	Fruit juices	250 mL	100 – 150	0.04 – 0.06
Milk and dairy-based products	Yoghurt	150 g	100 - 150	0.07 – 0.1
	Yoghurt drinks	200 mL	100 – 150	0.05 – 0.07

* Serving sizes in mL converted to g using the specific gravities provided by the Food Standards Agency

The applicant proposes to advise consumers by product labelling to limit their daily intake to a maximum of two products containing the novel ingredient, equivalent to a maximum total daily intake of 300 mg Glavonoid. The specific warnings for particular population groups (as indicated above for supplements) would also be included in the label.

Based on data from the Concise European Food Consumption Database for the United Kingdom (UK) the applicant has estimated the potential intake of Glavonoid resulting from the proposed food uses for the UK population (16-64 years) (UKDA, 2002). The results are summarised in Table 6. The mean (consumer only) combined intake from consumption of fruit juices, yoghurts and yoghurt drinks was estimated to be 273 mg Glavonoid/day. This is still below the intake of 300 mg/day, which should not be exceeded according to the recommendation made by the applicant. High level consumption (95th

percentile) would result in a combined Glavonoid intake of 675 mg/day. It is noted that this type of intake assessment methodology is generally considered to be "worst case" as a result of several conservative assumptions made in the consumption estimates, assuming that all food items within a food category contain the ingredient at the maximum specified level of use.

Table 6: Summary of the estimated daily intake of Glavonoid by the UK population (16 – 64 years) from all proposed food uses

Food Group	Consumer only intake (mg/day)	
	Mean	95th percentile
Fruit juices	60	169
Yoghurts	50	138
Yoghurt drinks	163	368
Total	273	675

The NDA Panel noted that in the intake assessment only the population group from 16 to 64 years was considered. On request of the Panel to provide an intake assessment for children (12-16 years), the applicant indicated that he does not conduct additional intake estimation for children between 12 and 16 years and that products containing Glavonoid would specifically be targeted to the general adult population.

5. Information from previous exposure to the NF or its source

A diluted form of Glavonoid, i.e. Liquorice Flavonoid Oil (LFO), is marketed by Kaneka Corporation in the USA. The company has notified the use of LFO to the Food and Drug Administration (FDA) as a new dietary ingredient to be used in dietary supplements (capsules) at doses up to 900 mg/day, which is equivalent to 300 mg Glavonoid/day (Docket No.1995S-0316, RPT348).

According to the applicant there were no reports of undesirable events from more than 66,000 "bottles" sold in the Japanese market and from more than 56,400 products sold in the US market from 2008 onwards.

One "bottle" refers to a food supplement product that contains capsules for one month with a daily dose of 300 mg Glavonoid for the USA and 200 mg for Japan (until February 2009, 3000 bottles were sold in Japan with a daily dose of only 100 mg). Kaneka indicates that 3,600 and 5,700 bottles were sold to its employees which provided capsules with a daily dose of 100 and 300 mg, respectively and which contain a safety survey in the form of a questionnaire.

6. Nutritional information on the NF

Glavonoid contains approximately 70 g medium-chain triglycerides (MCT) per 100 g. With regard to the fatty acid content, 99.7 % is octanoic acid (C8:0, common name caprylic acid) and 0.3 % is decanoic acid (C10:0, common name capric acid). The energy content of MCT is 8.4 kcal/g. Considering a daily intake of 300 mg Glavonoid/person/day, which should not be exceeded as suggested by the applicant, the nutritional value contributed by the additional MCT intake is marginal in relation to the total daily lipid intake.

MCT are normal constituents of the human diet; they occur for example in coconut oil, palm kernel oil and in the milk fat of cows, goats and sheep. In the human gastrointestinal tract MCT are hydrolysed, and the fatty acids are absorbed and further metabolised using the normal pathways of

fatty acid metabolism. Considering the occurrence of MCT in the human diet and the very low anticipated intake level resulting from consumption of Glavonoid, the NDA Panel sees no concern regarding MCT.

7. Microbiological information on the NF

The microbiological analysis of Glavonoid showed numbers for aerobic plate counts < 1000 cfu/g, and absence of coliforms/2.22 g. *E. coli* and *Salmonella* were absent in 2 g and fungi were absent in 0.2 g (see section 2 of the opinion). These data do not raise concern but the NDA Panel notes that the microbiological analysis was not carried out according to the EU requirements.

Analyses of two batches of Glavonoid for the presence of mycotoxins showed that the levels of aflatoxins (B1, B2, G1, G2) and ochratoxin A were below the minimum limit of detection of the methods applied (5 ppb and 0.05 ppm, respectively).

8. Toxicological information on the NF

The applicant has conducted a study on subchronic oral toxicity using rats as well as several genotoxicity studies using a concentrated form of liquorice flavonoid oil (LFO) as test material. A summary of these toxicological studies was published by Nakagawa et al. (2008a and 2008b). On request of EFSA, the applicant provided the full study reports and confirmed that the production process of LFO, which is a commercial product containing 1 % glabridin, is identical with that of Glavonoid except that the latter product is further diluted with MCT in order to obtain a glabridin concentration of 1 % in LFO. Thus, the test material containing 3 % glabridin, which is used in these studies (named "LFO - concentrated form") can be considered representative for Glavonoid.

In addition, the Panel requested additional information on the bioavailability of Glavonoid in rats and humans and a comparison and estimate to which extent the bioavailability may differ between these species. As a response, the applicant provided data on the bioavailability of glabridin determined by administration of LFO containing 1 % glabridin in rats (Ito et al., 2007). The respective information for humans is described under "Clinical trials".

8.1. Kinetics

Male Sprague-Dawley rats (n = 12) were administered by stomach tube a single dose of LFO containing 1 % glabridin, equivalent to a dose of approximately 10 mg glabridin/kg bodyweight (bw) (Ito et al., 2007). Blood samples were obtained 0, 0.5, 1, 2, 4, 6, 16 and 24 hrs after the administration and the concentration of glabridin was determined. Glabridin showed a maximum concentration in blood of 145 nmol/L (46.9 µg/L) at 1 hr after the administration which decreased gradually over 24 hr after dosing with elimination $T_{½}$ of 8.5 hr. The bioavailability $AUC_{(0-\infty)}$ was calculated to be 1.3 µmol/L x h (0.42 µg/mL x h). Further examinations showed that glabridin was also detectable in the liver, kidneys, mesenteric fat and kidney leaf fat 2 hr after the administration.

8.2. Subchronic oral toxicity

"LFO - concentrated form" was administered by gastric intubation to groups of 10 male and 10 female Sprague-Dawley rats at doses of 400, 600, 800 or 1600 mg/kg bw per day for 90 days (Kawabe, 2004; Nakagawa et al., 2008b). The vehicle control group received MCT, and an additional control group received corn oil.

The animals were observed daily for clinical signs of toxicity and mortality. During the treatment period several rats died (one female in the MCT control group, one female each in the groups receiving 400 and 800 mg/kg bw per day, and two females receiving 1600 mg/kg bw per day; two

males receiving 800 mg/kg bw per day and eight males receiving 1600 mg/kg bw per day). Considering the results of the haematology, macroscopic and histopathological examinations (haemorrhage in several organs and tissues, see below) the death of eight males as well as one female receiving 1600 mg/kg bw per day is related to the test material. Due to the number of only two surviving animals, the data for males of the highest dose group were not included in the statistical analysis. There were no relevant differences in food and water intake between the groups.

Haematology examinations carried out at the end of the treatment period showed a number of differences in groups receiving LFO compared with the MCT control group. In particular, in male rats, prothrombin time (PT) was statistically significantly prolonged at 400 mg/kg bw (12.5 sec versus 11.1 sec in the MCT control group) and 800 mg/kg bw (21.9 sec), and a trend was evident at 600 mg/kg bw (15.2 sec) and 1600 mg/kg bw (24.1 sec). Activated partial thromboplastin time (APTT) was statistically significantly prolonged at 800 mg/kg bw (81.8 sec versus 32.9 sec in the MCT control group), and trends were evident at 400 mg/kg bw (37.5 sec), 600 mg/kg bw (48.9 sec) and 1600 mg/kg bw (97.1 sec). These effects were dose-related. PT and APTT were also prolonged in female animals of the highest dose group. In addition, male animals showed a dose-related decrease in red blood cell counts at 600 mg/kg bw and higher doses and haematocrit at 400 mg/kg bw and higher doses, and there was an increase of mean corpuscular haemoglobin (MCH) (800 mg/kg bw and 1600 mg/kg bw) and in mean corpuscular haemoglobin concentration MCHC (600 mg/kg bw and higher doses). White blood cell counts in male animals administered LFO were consistently higher reaching statistical significance only in the group receiving 600 mg/kg bw. An increase in the reticulocyte counts was identified in males of the highest dose group.

In clinical-chemistry analyses males of the groups receiving 400 mg/kg bw and higher doses and females receiving 600 mg/kg bw and higher doses showed a dose-related increase in alanine amino transferase (ALT) activity compared with the MCT control group (regarding the male animals in relation to the corn oil control group, a relevant increase in ALT activity was only seen in the highest dose group). The activity of aspartate amino transferase (AST) was lower in males (800 mg/kg bw and 1600 mg/kg bw) and females (1600 mg/kg bw), which is not considered toxicologically relevant. Females of the highest dose group showed a higher alkaline phosphatase (ALP) activity, and in males the values were consistently higher (600 mg/kg bw and higher doses) although not statistically significant. Males and females of the highest dose group showed a higher total bilirubin level. In males (800 mg/kg bw and 1600 mg/kg bw) creatinine levels were higher. Female animals showed a reduced glucose level at 600 mg/kg bw and higher doses compared with the MCT control group and at 800 and 1600 mg/kg bw also in relation to the corn oil control group. In males the levels of total cholesterol and phospholipids were higher (400 mg/kg bw and higher doses) compared with the MCT control group but comparable to the levels in the corn oil control croup. These differences in glucose, cholesterol and phospholipid levels are not considered toxicologically relevant. Male animals (600 mg/kg bw and higher doses) showed a dose-dependently reduced blood potassium level compared with the MCT control group.

Determinations of the weights of selected organs and tissues at necropsy showed higher absolute but not relative pituitary weights in male animals (600 mg/kg bw and higher doses). The differences were not dose-related and not accompanied by histopathological changes in this organ. In females of the highest dose group, absolute spleen weights were lower and relative kidney weights were higher. Also in these organs no histopathological changes were identified.

Macroscopic examination of the animals in the high dose group (1600 mg/kg bw), whose deaths were due to treatment with LFO showed signs of haemorrhage, such as dark red discoloration in several organs. In one of the surviving male rats dark red discoloration of the musculature was found, which is also probably related to the test material. Histopathological examination of these animals showed signs of haemorrhage in several organs and tissues, including lymph nodes, thymus, nasal cavity, stomach, pancreas, testes, epididymides, prostate, skeletal musculature, brain and skin. Haematopoesis in the bone marrow was also noted. Furthermore, inflammatory lesions, apoptosis,

necrosis and atrophy, were observed in several organs and considered to be changes accompanying the haemorrhage. In the salivary glands, minimal or slight hypertrophy of acinar cells was frequently found. Several other findings, which appeared with equal frequency and severity in the high-dose group and the control groups, were considered unrelated to the test material.

In the 90-day study a variety of changes were observed in animals administered LFO. The decisive effect is an impact on blood coagulation, i.e. prolongation of PT and APTT, which caused haemorrhage and the death of one female and eight male animals at the highest dose level. Some of the observed other changes in haematology and clinical-chemistry parameters, e.g. reticulocyte counts, red blood cell counts and haematocrit are probably related to the blood coagulation effect and the resulting haemorrhage. Since the difference in PT in male animals was statistically significant even at the lowest dose level, the lowest dose administered in this study, i.e. 400 mg/kg bw per day, should be regarded as the lowest observed adverse effect level (LOAEL).

Prothrombin-time and APTT were also analysed using a Benchmark Dose (BMD) modelling approach, following the recommendations in the guidance document of the Scientific Committee of EFSA (EFSA, 2009). The values for the BMD and BMD lower confidence limit ($BMDL_{05}$) derived from the APTT data of male rats provided lower values than the PT data. The $BMDL_{05}$ for this study derived from APTT data is 167 mg/kg bw (see Appendix for the details).

The publication of Nakagawa (2008b) contains a summary of an additional study, which was conducted in order to determine the mechanism of the anticoagulant effect induced by LFO in the 90-day rat study. Three groups of male Sprague-Dawley rats (n = 5 or 3) received a diet containing 5 % "LFO – concentrated form" for 20 days. One group (n = 5) received a diet with 5 % LFO for 13 days followed by a normal rodent diet for the remaining 7 days. Two control groups (n = 5 or 3) received a standard rodent diet for 20 days. One of the groups (n = 5) fed with the LFO-containing diet received doses of 70 mg/kg bw by i.p. injection on days 13 and 14. As in the 90-day study administration of LFO induced prolongation of PT and APTT compared with the control group. The values returned to normal levels within 2 days after vitamin K injection. Furthermore, PT and APTT values returned to normal levels within 2 days after cessation of feeding the LFO-containing diet. At day 20 the activities of the vitamin K-dependent coagulation factors II, VII, IX and X were decreased in LFO-treated animals, while there was no decrease in the group receiving the additional treatment with vitamin K. The PT and APTT remained prolonged on day 20 in animals treated with LFO, but animals that had received vitamin K on days 13 and 14 showed normal levels at day 20 indicating that the effect of vitamin K was maintained for at least 7 days. In animals receiving only LFO the concentration of fibrinogen was increased compared with untreated animals.

Based on these results the authors concluded that the blood anticoagulant effect was caused by an inhibition of the synthesis of vitamin K-dependent coagulation factors II, VII, IX and X. The mechanism of action of LFO was considered to be the same, in terms of inhibition of the synthesis of vitamin K-dependent coagulation factors, as in the case of warfarin, which is used as a rodenticide and anticoagulant drug. The similarly is also reflected in observed higher sensitivity of male rats in comparison with female rats. The authors assume that flavonoids contained in LFO, in particular compounds with structures similar to that of coumarin, are responsible for the anticoagulant effect. The NDA Panel is aware that specific coumarin derivatives, i.e. 4-hydroxycoumarins like warfarin, are therapeutically used as anticoagulants. All 4-hydroxycoumarin anticoagulants share an enolic benzopyran structure, which is considered essential to their common pharmacological activity as vitamin K antagonists (Au and Rettie, 2008). However, none of the polyphenolic type substances identified in Glavonoid shows this specific chemical structure or that of the structurally related indane-1,3-diones, which also acts as vitamin K antagonist. The substance(s) responsible for the anticoagulant effect of Glavonoid and also the exact mechanism of action remains unknown.

The Panel notes by *in vitro* data that glabridin inhibits the activity of cytochrome P450 (CYP) enzymes 3A4, 2B6 and 2C9 in a reconstituted system. Glabridin as well as liquorice root extract

inhibit the activity of the major human drug metabolising isozyme CYP3A4 in a time, concentration- and NADPH-dependent manner. The concentration required for half-maximal inactivation by glabridin was 7 µM for CYP3A4 and 12 µM for CYP2B6. The Panel notes that the observed steady state levels of plasma glabridin reached in the human studies after oral administration of LFO are low in relation to the inactivation constant (K_I) (Aoki et al. 2007). However, the inhibition of CYP 3A4 and 2B6 by the flavan glabridin is a mechanism-based irreversible inactivation and correlates with a loss of the intact haem moiety of the enzyme (Kent et al., 2002). If this effect occurs *in vivo* it can be anticipated that the inactivated isozymes have to be replaced by newly synthesised CYP proteins, which would cause effects on substrate/drug pharmacokinetics. The impact of inactivation of CYP3A4 moreover depends on the relative contribution of intestinal and hepatic metabolism to the first–pass metabolism of a given drug (Zhou et al., 2005a). No information has been provided relating to this kinetic context. Moreover, information on whether an irreversible inhibition of CYP3A4 and 2B6 by glabridin and liquorice root extract also occurs *in vivo* is not available.

The Panel notes that despite the knowledge of *in vitro* data of a mechanism-based inhibition, the clinical importance of CYP3A4 inactivation cannot be assessed without *in vivo* studies, i.e. the risk of a potential food-drug interaction and subsequent toxicities of concomitant drugs that are CYP3A4 substrates, is neither qualitatively nor quantitatively predictable exclusively by *in vitro* data.

The Panel concludes that in the absence of *in vivo* data (e.g. the altered clearance of coadministered drugs) the relevance of the mentioned findings with regard to a possible impact on concomitant drug metabolism or therapy cannot be assessed.

According to the applicant Glavonoid does not only contain the most abundant polyphenolic flavonoid among all liquorice flavanoids, the glabridin, but also polyphenols such as glabrene, glabrol and 4'-O-methylglabridin. No data have been provided that could exclude further inactivations of human cytochrome P450s by these structure-analogous components.

The phase I - metabolism of the prodrug tamoxifen is catalysed by the cytochromes CYP3A4 /5 to N-Desmethyl-Tamoxifen and additionally by CYP 2C9 to 4-hydroxytamoxifen in humans; the CYP2D6 and CYP3A5 isoforms are predominantly involved in the ensuing formation of Endoxifen, which is the pharmacologically active substance (Goetz et al., 2008; Jin et al., 2005). Although it is known that the anticancer agent tamoxifen and its main metabolites are potent inhibitors of oxidases of the cytochrome P-450 system, clinical data on tamoxifen-food interactions are scant (Zhou et al. 2005b). The Panel notes that due to a possible prothrombin time prolongation in humans the tamoxifen therapy is not recommended in combination with anticoagulant drugs of coumarin-type. As such, S-Warfarin is mainly metabolised through CYP2C9. Glabridin competitively inhibits this isozyme *in vitro*. The Panel notes that no human intervention data have been provided from which the relevance of a potential pharmacokinetic interaction of glabridin-tamoxifen could have been assessed *in vivo*.

8.3. Genotoxicity

Tests on gene mutations in bacteria using "LFO - concentrated form" were conducted in accordance with OECD Guideline 471 using 4 strains of *Salmonella enterica* var. Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* strain WP2 uvrA (pKM101) and the pre-incubation method (Kamigaito, 2003). There was no increase in the number of revertant colonies in any of the five tested strains up to the highest concentration of 5000 µg LFO/plate in the presence and absence of metabolic activation (S9 mix).

"LFO-concentrated form" was tested for induction of chromosomal aberrations in mammalian cells in accordance with OECD Guideline 473 using Chinese hamster lung (CHL/IU) cells (Asakura, 2003). There was no increase in the number of cells with structural chromosomal aberrations or polyploidy after continuous treatment of cells (for 24 hours and 48 hours) and after short-time treatment (6 hrs) in the absence of S9 mix. However, there was an increase in the number of cells with structural

chromosome aberrations (chromatid breaks and chromatid exchanges) at the highest concentration that could be evaluated, after short-time treatment in the presence of S9 Mix. In a confirmation test (short-time treatment in the presence of S9 mix, only) a dose-dependent increase in the number of cells with structural chromosome aberrations was observed. Although the purpose of the test is to detect structural chromosome aberrations, the NDA Panel notes that in both the main (short-time treatment in the presence of S9 mix, only) and the confirmation test the number of cells showing polyploidy was also increased, indicating that the test material may have the potential to induce numerical chromosome aberrations. This issue was not specifically addressed in the study report.

A bone marrow micronucleus test with "LFO - concentrated form" was carried out in male Fisher (F344) rats, the protocol being largely in accordance with OECD Guideline 474 (Noguchi, 2003a). The animals received LFO twice at 24 hour intervals by gavage at doses of 625, 1250, 2500 and 5000 mg/kg bw per day (n = 5). A negative control group as well as a solvent control group were included in the study. A positive control group received mitomycin C (MMC) by i.p. injection. Twenty-four hours after the second treatment bone marrow cells were isolated and analysed. The frequencies of micronucleated polychromatic erythrocytes and the ratio of polychromatic erythrocytes (PCE) to total erythrocytes were counted. At the highest dose LFO induced bone marrow toxicity (decreased ratio of PCE to total erythrocytes) showing that the test material has reached the target organ. The test material did not increase the frequency of micronucleated polychromatic erythrocytes. The NDA Panel agrees with the conclusion of the study report that LFO was not mutagenic in this assay.

The applicant also provided a micronucleus test, in which peripheral blood erythrocytes were analysed (Noguchi, 2003b). Groups of male F344 rats (n = 4) were administered "LFO - concentrated form" by gavage at doses of 2500 or 5000 mg/kg bw/day on day 1 and 2 of the study. Both doses are higher than the limit dose recommended in OECD Guideline 474 (2000 mg/kg bw). The negative control group received olive oil on the same days. A positive control group was administered a single dose of MMC by i.p. injection on day 2. On day 4 peripheral blood was collected and smear preparations were made and stained. There was no increase in the frequency of micronucleated polychromatic erythrocytes in blood obtained from rats treated with LFO and from the negative control group, whereas a statistically significant increase was observed in the positive control group. The Panel notes that exposure to the target tissue can be concluded on the basis of the study above (Noguchi, 2003a).

In the same study also the frequency of micronucleated hepatocytes in the liver was determined. For this purpose, two-thirds of the livers of the same animals were excised (partial hepatectomy) on day 5, and the animals received an additional treatment with the test material or olive oil on day 6. On day 9 hepatocytes were obtained from the liver by collagenase treatment, fixed and stained. There was no increase in the frequency of micronucleated hepatocytes obtained from rats treated with LFO and from the negative control group, whereas a statistically significant increase was observed in the positive control group.

The NDA Panel considers that the endpoint chromosome mutations has been adequately analysed. Although the *in vitro* chromosomal aberration test showed a positive result after short-time treatment with LFO in the presence of metabolic activation, the NDA Panel sees no concern since there were no indications for chromosomal mutations in the two *in vivo* studies showing that the positive *in vitro* effect is not expressed *in vivo*. Regarding the endpoint gene mutations the Panel notes that a study on gene mutations in mammalian cells has not been provided.

8.4. Other toxicological studies

Studies on reproductive and developmental toxicity have not been carried out.

A study on chronic toxicity/carcinogenicity was not provided.

The applicant has studied the tumour promoting potential of LFO (concentrated form) in male F344 rats after initiation of hepatocarcinogenesis by a single i.p. injection of diethylnitrosamine (DEN) (Yoshino, 2004). Starting two weeks later, the animals received LFO at dose levels of 0 (MCT control), 150, 300 or 600 mg/kg bw per day for six weeks. A positive control group received sodium phenobarbital (SPB). Additional control groups not treated with DEN received MCT or 600 mg LFO/kg bw per day. The animals were subjected to partial hepatectomy at the end of week 3 and sacrificed at the end of week 8. There was a statistically significant increase in liver weights in all groups receiving LFO. According to the study report, the changes were slight and microscopic analysis of liver sections did not reveal changes. Microscopic analysis showed that in the high-dose group with DEN initiation the numbers of GST-P (glutathione-S-transferase P) positive foci per liver section as well as the areas of GST-P positive foci per liver section were statistically significantly decreased compared with the control group, whereas the positive control group showed the expected increases. According to the study report, the results demonstrate that LFO lacks promoting potential for liver carcinogenesis at the tested dose levels, and at a dose of 600 mg/kg bw per day inhibition was evident. In the opinion of the NDA Panel the study, which is normally not part of the standard toxicological testing programme, does not add much information to the safety evaluation.

8.5. Oestrogenic activity

According to scientific publications, glabridin, glabrene and other constituents present in liquorice root showed oestrogen-like activity *in vitro* and/or *in vivo* (e.g. Somjen, et al., 2004; Tamir et al., 2000 and 2001). With regard to potential oestrogenic activity of Glavonoid, the applicant argues that 45 phenolic compounds have been identified in Glavonoid. Five of these are isoflavones, the total amount of these being approximately 0.6 % corresponding to 1.8 mg/300 mg of Glavonoid. According to the Food Safety Commission of the Japanese authorities, the average daily intake of soy isoflavone aglycone from foods is estimated to be 16-22 mg/day, and the upper limit of safe daily intake was set at 70-75 mg/day. The French food safety authority AFSSA has assessed the safety (and health benefits) of phytoestrogens and came to the conclusion that an intake of 1 mg/kg bw per day of aglycone isoflavones, i.e. 60 mg for a person weighing 60 kg, presents no risk for the general population. Some consumers, however, need to take special precautions, i.e. people with breast cancer or a personal or family history of breast cancer as well as infants and young children taking soy protein-based formula. Considering a maximum daily intake of 300 mg Glavonoid/day, the NDA Panel concludes that the resulting intake of 1.8 mg isoflavones/day does not raise concern in terms of oestrogenic activity.

In addition, the applicant makes reference to a scientific publication describing characteristic phenolic compounds present in *Glycyrrhiza* species and biological activities of some of these (Nomura et al., 2002). According to this publication, about 100 phenolic compounds from medicinal plants and their derivatives were evaluated for potential oestrogenic activity using an oestradiol receptor binding assay. Altogether 13 compounds, of which six compounds were isolated from *Glycyrrhiza* species, exhibited weak binding affinities (IC50 < 1 µg/mL). The IC50 value of 17ß-estradiol was 0.47-2.0 nM (128 – 544 pg/mL according to the applicant). In comparison, the IC50 of phenolic compounds present in *Glycyrrhiza glabra* was higher than 0.2 µg/mL, which is 370 – 1560 fold higher than that of 17ß-estradiol. According to the applicant, the relative binding affinities (in relation to 17ß-estradiol) of typical polyphenols present in Glavonoid, e.g. glabrene (0.0022), glabridin (<0.0016) and glabrol (<0.0016), are similar to the binding affinities of isoflavones present in soybean, i.e. genistein (0.004) and daidzein (0.00035).

The NDA Panel considers that the information provided on the potential oestrogenic activity of these specific constituents does not indicate a safety concern. However, the Panel noted that the information only relates to a limited number of substances, whereas experimental studies using Glavonoid, which contains a complex mixture of polyphenolic type compounds with largely unknown biological activities have not been carried out. Therefore, the Panel has asked for experimental data, e.g. a rat

uterotrophic bioassay using Glavonoid as test material. However, the applicant was unable to submit such a study. The Panel agrees with the applicant in that there were no indications for effects on reproductive organs in the subchronic rat study. However, this study provides only limited information on other relevant parameters related to reproduction.

8.6. Clinical trials

The results of several human studies using LFO (diluted form containing 1 % glabridin) as test material were presented. On request of the NDA Panel the applicant provided the full study reports. The data from the first three studies described below were also published by Aoki et al. (2007).

LFO was administered orally (after breakfast) to groups of healthy male subjects (n = 5) at single doses of 300, 600 or 1200 mg/day, corresponding to doses of 100, 200 or 400 mg Glavonoid/day (Ikematsu, 2004a). Blood samples to determine plasma glabridin concentration were collected at 0 (pre-dose), 2, 4, 6, 8 and 24 hours after dosing. Glabridin was absorbed and plasma levels reached the maximum concentration after approximately 4 hours. The maximum concentration and area under the curve (AUC) increased almost linearly with dose. Glabridin was eliminated relatively slowly with a $T_{½}$ of approximately 10 h at all doses. Considering the respective data in rats (see "Kinetics"), the bioavailability of glabridin in humans was estimated by the applicant to be about 5 times higher than in rats. The Panel notes that the evidence provided does not support accepting glabridin as a marker for the bioavailability of the bulk of other substances in Glavonoid since even small differences in chemical structure may have a profound impact on absorption, distribution, metabolism and elimination of a substance. The Panel considers that no conclusions regarding the bioavailability of the unidentified substance(s), which is responsible for the anticoagulant effect in rats, can be drawn based on data for glabridin.

In a repeated-dose study groups of male (n = 5) and female (n = 5) subjects received once daily LFO at doses of 0 (placebo), 300, 600 or 1200 mg for 7 days, corresponding to doses of 0, 100, 200 or 400 mg Glavonoid/day (Ikematsu, 2004b). Blood samples for measurement of plasma glabridin levels were collected at pre-dose, 4 and 24 h after dosing on the first and on the last day of the treatment period. The plasma glabridin levels increased almost linearly with the doses of LFO administered and were higher on day 7 than on day 1. In the high-dose group (corresponding to a dose of 400 mg Glavonoid) mean plasma glabridin levels were 2.88 and 0.66 ng/mL after 4 and 24 h, respectively, on day 1 and increased to 4.47 and 0.87 ng/mL after 4 and 24 h, respectively, on day 7.

In a single-blind study healthy males (n = 7) and females (n = 7) consumed LFO at doses of 300, 600 or 1200 mg/day, corresponding to 100, 200 or 400 mg Glavonoid/day for 4 weeks (Ikematsu, 2005). Additional groups of males (n = 9) and females (n = 7) received a placebo. Prior to the start of the treatment, after two and 4 weeks as well as two weeks after the end of the treatment period, a number of parameters were evaluated, including body weight, blood pressure, pulse rate, haematology and clinical-chemistry parameters (including WBC count, differential WBC count, RBC count, haemoglobin, haematocrit, platelet count, PT, APTT, blood lipids, blood glucose, insulin, AST, ALT, gamma-GT, LDH, ALP, total bilirubin, protein and albumin, urea nitrogen, uric acid, creatinine, Na, K, Cl,) and urinalysis parameters. Comparison of the mean values after treatment with Glavonoid with the values prior to the treatment, showed no relevant differences. Mean glabridin plasma levels in the high-dose group were 1.82 ng/mL after 2 weeks and 1.75 ng/mL after 4 weeks suggesting that a steady-state was reached.

In a double-blind study using a similar design mildly obese but otherwise healthy male (n = 10) and female (n = 10) subjects consumed LFO at a dose of 1800 mg/day, corresponding to 600 mg Glavonoid/day for four weeks (Ikematsu and Nakamura, 2005; Tominaga et al., 2006). Groups of mildly obese but otherwise healthy male (n = 10) and female (n = 10) subjects received a placebo. One male receiving Glavonoid showed minor increases in AST and ALT at week 2 of the treatment, which, according to the author of the report, was not related to the treatment. Apart from this, there

were no relevant changes in values after treatment with Glavonoid compared with the values prior to the start of the treatment.

In a double-blind study groups of healthy male (n = 13 or 14) and female (n = 7) subjects received LFO at doses of 300, 600 or 900 mg/day, corresponding to 100, 200 or 300 mg Glavonoid/day for 8 weeks (Arai, 2004; Tominaga et al., 2006). Healthy males (n = 12) and females (n = 7) received a placebo. Analysis of haematology and clinical-chemistry parameters prior to treatment and after 4 and 8 weeks did not show relevant changes after treatment with Glavonoid.

The publication of Tominaga et al. (2006) also reports a randomised, double-blind, placebo-controlled study with overweight subjects. Men (n = 32) and women (n = 19) were administered LFO at a dose of 300 mg/day, corresponding to 100 mg Glavonoid/day, for 12 weeks. The control group (31 men and 21 women) received a placebo. At the start of the treatment, after 4, 8 and 12 weeks as well as 4 weeks after the end of the treatment period, a number of parameters were evaluated, including body weight, body fat ratio, blood pressure, pulse rate, haematology and clinical-chemistry parameters (i.e. RBC count, haemoglobin level, WBC and platelet counts, PT, APTT, total protein, albumin, albumin/globulin ratio, blood lipid, blood glucose and insulin levels, AST, ALT, gamma-GT, ALP, LDH activities, urea nitrogen, total bilirubin, creatinine, Na, K and Cl levels). There were no relevant differences between the test and control group. Compared with the baseline situation, several statistically significant differences were observed in both groups. However, the changes were within physiological ranges, showed no clear time dependency and were thus not considered clinically relevant.

In sum, the six human studies examining a dose range of 100 to 600 mg Glavonoid per day did not show changes in haematology, coagulation, clinical-chemistry and urinalysis parameters after administration of Glavonoid. The highest dose repeatedly (4 weeks) administered to humans in these studies was 600 mg/day (approximately 8.6 mg/kg bw per day for a 70 kg adult).

8.7. Studies using different liquorice extracts

The results of a number of animal and human studies using different extracts of *Glycyrrhiza glabra* roots were provided by the applicant. However, the method of production and composition of these extracts differed considerably from that of Glavonoid. Therefore these studies were not considered relevant for the safety evaluation of Glavonoid.

8.8. Allergenicity

According to the applicant no reports of allergic reactions or sensitivity following consumption of liquorice or its components were reported in the published scientific literature.

The protein content of LFO, the three-fold dilution of Glavonoid with MCT, was determined to be less than 0.1 g/100 g (Kjeldahl method). Using a different analytical method (Bradford method) the protein content of Glavonoid was determined to be less than 0.2 mg/mL.

Case reports of contact allergy in Japan associated with ethanolic liquorice root extracts contained in cosmetic products were published in the scientific literature (Matsunaga and Fujisawa, 1995; Nishioka and Seguchi, 1999). A case of contact allergy in the United Kingdom was also associated with the use of a cosmetic product. When the patient was subsequently patch tested, she reacted positive to liquorice root extract, one of the ingredients of the cream that had induced the previous reaction (O'Connell et al, 2008).

In addition, a case of occupational rhinitis and asthma in an anis liqueur factory worker was reported. The worker developed IgE-mediated sensitisation to five of the eight plants, which he handled habitually, among them *Glycyrrhiza glabra*. The symptoms appeared mainly while he was handling

Glycyrrhiza glabra, and skin prick test and RAST values for *Glycyrrhiza glabra* were higher than for the other species. Therefore the authors considered that this species was the major source of the respiratory symptoms of the patient who showed oral tolerance to liquorice (González-Gutièrrez et al., 2000). A case of a herbalist who developed occupational asthma was described. Skin prick test with a liquorice extract gave a positive reaction and inhalation challenges using liquorice root powder induced an immediate fall in forced expiratory volume (FEV) without a significant late reaction. The authors concluded that liquorice roots could cause occupational allergy through an apparently IgE-mediated mechanism as seen by immediate skin reactivity reaction to the powder of liquorice root (Cartier et al., 2002).

The described cases relate to inhalation exposure to the plant *Glycyrrhiza glabra* at the workplace and dermal exposure to root extracts contained in cosmetic products. Allergic reactions after consumption of *Glycyrrhiza glabra* extracts have not been reported in the scientific literature. Regarding the low protein content of Glavonoid and the anticipated intake levels, the NDA Panel is of the opinion that allergic reactions induced by consumption of Glavonoid are unlikely.

DISCUSSION

The applicant has provided sufficient information regarding the composition and specification of Glavonoid and on the manufacturing process. Glavonoid consists of an extract derived from *Glycyrrhiza glabra* roots (approximately 30 %) and MCT (approximately 70 %). The plant-derived material is rich in polyphenolic type substances of which 45 substances have been identified. In contrast to traditional products derived from the roots of *Glycyrrhiza glabra*, Glavonoid does not contain detectable levels of glycyrrhizinic acid. Analyses of several batches using recognised methods have confirmed that the manufacturing process is well controlled and the product meets specification. There are no anticipated problems with heavy metals, microbial or pesticide contaminants. The stability of Glavonoid was analysed under relevant storage conditions and considered sufficient.

Glavonoid is intended to be marketed as food supplement in the form of capsules and tablets containing up to 300 mg and as an ingredient in fruit juices, yoghurts and yoghurt drinks. The applicant proposes to advise consumers by product labelling to limit their daily intake to a maximum of 300 mg Glavonoid. The target population are adults. Based on intake data from the Concise European Food Consumption Database for the UK population aged 16 - 64 years, it was estimated that the mean and 95th percentile (consumer only) intakes from combined consumption of fruit juices, yoghurts and yoghurt drinks would be 273 mg/day and 675 mg/day, respectively. These would be "worst case" scenarios based on all food categories consumed having the maximum level of the novel ingredient added.

Tests on the induction of gene mutations in bacteria produced negative results. Studies on gene mutations in mammalian cells, which would have added further relevant information for the genotoxicity assessment, were not provided. Tests on chromosomal aberrations in mammalian cells showed an increase in the number of cells with structural chromosome aberrations after short-time treatment in the presence of metabolic activation. However, the NDA Panel sees no concern since there were no indications for chromosomal mutations in two *in vivo* studies showing that the positive *in vitro* effect is not expressed *in vivo*.

In a subchronic (90-day) oral toxicity study "LFO - concentrated form" containing 3 % glabridin, which can be considered representative for Glavonoid, induced an effect on blood coagulation parameters, evidenced in a prolongation of PT and APTT, which caused haemorrhage in several organs and tissues and the death of several animals in the highest dose group (1600 mg/kg bw per day). Additional examinations showed that the anticoagulant effect was caused by inhibition of the synthesis of vitamin K-dependent coagulation factors. A substance or substances having a structure similar to that of specific coumarin derivatives are considered likely to be responsible for the anticoagulant effect, but the identity of the substance(s) and also the exact mechanism of action

remains unknown. A prolonged PT was statistically significant in male animals receiving the lowest dose level of 400 mg/kg bw per day. Therefore this dose is considered as the lowest observed adverse effect level (LOAEL). There are no further studies e.g. a long-term exposure studies or reproductive and developmental toxicity studies that might have shed light on the NOAEL.

Prothrombin-time and APTT were also analysed using a BMD modelling approach. The values for the BMD and BMD lower confidence limit ($BMDL_{05}$) derived from the APTT data of male rats provided lower values than the PT data. The $BMDL_{05}$ for this study derived from APTT data is 167 mg/kg bw. The extrapolation of this study $BMDL_{05}$ to a maximum intake for a 70 kg person results in 117 mg (rounded up to 120 mg) Glavonoid/day. The application of an uncertainty factor of three on the LOAEL of 400 mg/kg of this study in addition to the application of the default factor of 100 would provide a similar, albeit slightly lower value (93 mg).

The reported six human studies examining a dose range of 100 to 600 mg Glavonoid per day did not show relevant changes in haematology, coagulation, clinical-chemistry and urinalysis parameters. Because of the short treatment time, the dose levels used and in particular the low number of study participants receiving higher doses, the Panel considers that these studies are not adequate to derive a safe level of intake, especially in the case of longer-term consumption. However, since the human studies do not raise safety concerns, the Panel considers that the human studies are consistent with the maximum level derived from the BMD approach.

Regarding the uncertainties concerning a possible impact on anticoagulant therapy, the Panel notes that no studies have been conducted to evaluate interaction between Glavonoid and drugs with an effect on blood coagulation. The safety of Glavonoid for pregnant and breast-feeding women has not been established.

CONCLUSIONS

The Panel concludes that the novel food ingredient Glavonoid is safe for the general adult population up to 120 mg/day.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on Glavonoid. September 2009. Submitted by Kaneka Pharma Europe N.V. Additional information was submitted on 11 and 26 March, 14 and 20 April, 25 May, 24 June and 16 December 2010.

2. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety of the safety of 'Glavonoid (Flavonoids and Polyphenols, in particular Glabridin from *Glycyrrhiza glabra*)'. SANCO E4/AK/bs (2009) D/540491.

3. Initial assessment report carried out by Belgium: Advisory Report of the Superior Health Council on a marketing authorization application for Glavonoid as a novel food ingredient and food supplement under Regulation EC No 258/97

4. Member States' comments and objections

5. Response by the applicant to the initial assessment report and the Member States' comments and objections

REFERENCES

Aoki F, Nakagawa K, Kitano M, Ikematsu H, Nakamura K, Yokota S, Tominaga Y, Arai N, Mae T, 2007. Clinical Safety of Liquorice Flavonoid Oil (LFO) and Pharmacokinetics of Glabridin in Healthy Humans. Journal of the American College of Nutrition 26(3):209-218.

Arai, 2004. Study protocol. Confirmation of the body fat reducing effect of LFO. Effective dose finding study. Protocol No. 16139.

Asakura M, 2003. Chromosomal Aberration Test of Liquorice Flavonoid Oil in Cultured Mammalian Cells. Study No. 7352. Study Report.

Au N and Rettie E, 2008. Pharmacogenomics of 4-hydroxycoumarin anticoagulants. Drug Metabolism Reviews 40, 355-375.

Cartier A, Malo J-L, Labrecque M, 2002. Occupational asthma due to liquorice roots. Allergy 57, 863.

EC (European Commission), 1997. Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. Official Journal of the European Communities L 253, 16.09.1997, p. 1-36.

EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal (2009) 1150, 1-72 2 External expert of the EFSA Scientific Committee.

Gonzales-Gutierrez ML, Sanchez-Fernandez C, Esteban-Lopez MI, Sempere-Ortells JM, Diaz-Alperi P, 2000. Allergy to anis. Allergy 55, 195-204

Goetz MP, Kamal A, Ames MM., 2008. Tamoxifen pharmacogenomics: the role of CYP2D6 as a predictor of drug response. Clin Pharmacol Ther. 2008 Jan;83(1):160-6. Review.

Hikino H, 1985, Recent research on oriental medicinal plants.

Ikematsu H, 2004a. Study Report: Safety Study of LFO in Single-dose Ingestion. Protocol No. H16-KA0212. Total Technological Consultant, Co., Ltd.

Ikematsu H, 2004b. Study Report. Safety Study of LFO in I-week Repeated-dose Ingestion.Protocol No. 161 12. Total Technological Consultant, Co., Ltd.

Ikematsu H, 2005. Study Report. Safety Study of LFO in 4-week Repeated-dose Ingestion. Protocol No. 16113. Total Technological Consultant, Co., Ltd.

Ikematsu H and Nakamura H, 2005. Study Report. Safety Study of LFO (1800 mg) in 4-week Repeated-ose Ingestion. Protocol No. 17218. Total Technological Consultant, Co., Ltd.

Ito C, Oi N, Hashimoto T, Nakabayashi H, Aoki F, Tominaga Y, Yokota S, Hosoe K and Kanazawa K, 2007. Absorption of dietary liquorice isoflavan glabridin to blood circulation in rats. Journal of Nutritional Science and Vitaminology 53, 358 – 365.

Jin Y, Desta Z, Stearns V, Ward B, Ho H, Lee KH, Skaar T, Storniolo AM, Li L, Araba A, Blanchard R, Nguyen A, Ullmer L, Hayden J, Lemler S, Weinshilboum RM, Rae JM, Hayes DF, Flockhart DA, 2005. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst. 97(1):30-9.

Kamigaito T, 2003. Reverse Mutation Test of Liquorice Flavonoid Oil in Bacteria. Study No 6208. Final Report.

Kawabe M, 2004. 90-day repeated dose toxicity study of liquorice flavonoid oil in rats. Study No. 0326. Final Report. Daiyu-kai Institute of Medical Science. Ichinomiya, Japan.

Kent U, Aviram M, Rosenblat M and Hollenberg PF, 2002. The liquorice root derived isoflavan glabridin inhibits the activities of human cytochrome P450s 3A4, 2B6 and 2C9. Drug Metabolism and Disposition 30, 709 – 715

Matsunaga K and Fujisawa Y, 1995. 2 cases with allergic contact dermatitis due to whitening agents. Aesthetic Dermatology 5: 81–86.

Nakagawa K, Hidaka T, Kitano M, Asakura M, Kamigaito T, Noguchi T, Hosoe K, 2008a. Genotoxicity studies on liquorice flavonoid oil (LFO). Food and Chemical Toxicology 46(7):2525-32.

Nakagawa K, Kitano M, Kishida H, Hidaka T, Nabae K, Kawabe M, Hosoe K, 2008b. 90-Day repeated-dose toxicity study of liquorice flavonoid oil (LFO) in rats. Food and Chemical Toxicology 46(7):2349-57.

Nishioka K and Seguchi T, 1999. Contact allergy due to oil-soluble liquorice extracts in cosmetic products. Contact Dermatitis 40: 56.

Noguchi T, 2003a. Bone Marrow Micronucleus test of Liquorice Flavonoid Oil with Rats. Study No. 7038, Study Report.

Noguchi T, 2003b. Liver and Peripheral Blood Micronucleus Test of Liquorice Flavonoid Oil with Rats. Study No. 7035, Study Report.

Nomura T, Fukai T, Akiyama T, 2002. Chemistry of phenolic compounds of liquorice (Glycyrrhiza species) and their estrogenic and cytotoxic activities. Pure and Applied Chemistry 74 (7):1199-1206

O'Connell RL, White IR, White JM, McFadden JP, 2008. Liquorice extract in a cosmetic product causing contact allergy. Contact Dermatitis 59(1):52.

Somjen D, Knoll E, Vaya J, Stern N, Tamir S, 2004. Estrogen-like activity of liquorice root constituents; glabridin and glabrene, in vascular tissues in vitro and in vivo. Journal of Steroid Biochemistry and Molecular Biology 91(3):147-155.

Tamir S, Eizenberg M, Somjen D, Stern N, Shelach R, Kaye A, Vaya J. 2000. Estrogenic and antiproliferative properties of glabridin from liquorice in human breast cancer cells. Cancer Research 60(20):5704-9.

Tamir S, Eizenberg M, Somjen D, Izrael S, Vaya J, 2001. Estrogen-like activity of glabrene and other constituents isolated from liquorice root. Journal of Steroid Biochemistry and Molecular Biology 78(3):291-8.

Tominaga Y, Mae T, Kitano M, Sakamoto Y, Ikematsu H, Nakagawa K, 2006. Liquorice flavonoid oil effects body weight loss by reduction of body fat mass in overweight subjects. Journal of Health Science 52(6):672-683.

UKDA, Henderson L, Irving K, Gregory J, 2002. National Diet and Nutrition Survey: Adult aged 19 to 64 Years. A survey carried in Great Britain on behalf of the Food Standards Agency, Office for National Statistics and Medical Research, Council Human Nutrition Research.

Yoshino H, 2004. Rat Medium-term Liver Carcinogenesis Bioassay of Liquorice Flavonoid Oil. Study No. 0406. DIMS Institute of Medical Science Inc. English translation dated April 19, 2005.

Zhou, S, Chan, E, Li, X, Huang, M, 2005a. Clinical outcomes and management of mechanism-based inhibition of cytochrome P4503A4. Therap Clin Risk Manag. 1(1): 3–13.

Zhou S, Yung Chan S, Cher Goh B, Chan E, Duan W, Huang M, McLeod HL, 2005b. Mechanism-based inhibition of cytochrome P450 3A4 by therapeutic drugs Clin Pharmacokinet. 2005;44(3):279-304.

APPENDIX - BENCHMARK ANALYSIS

A. The data

Prothrombin time and APTT results from the 90 day rat study using "LFO- concentrated solution" (Kawabe, 2004; Nakagawa et al., 2008b).

Sex	Dose (mg/kg BW)	No. of samples	PT	APTT
Female	0 (MCT)	9	10.60 ± 0.63	29.31 ± 11.33
	400	9	10.30 ± 0.27	31.57 ± 9.12
	600	10	10.16 ± 0.63	29.75 ± 7.60
	800	9	10.03 ± 0.46	32.87 ± 9.07
	1600	8	12.99 ± 2.59*	52.70 ± 18.79**
Male	0 (MCT)	9	11.09 ± 0.65	32.88 ± 9.88
	400	10	12.45 ± 1.28*	37.52 ± 9.15
	600	10	15.23 ± 5.40	48.91 ± 23.24
	800	8	21.89 ± 4.54**	81.83 ± 37.03**
	1600	2	24.10 ± 0.85	97.05 ± 1.20

* Significantly different from vehicle control (MCT) group at P < 0.05.
** Significantly different from vehicle control (MCT) group at P < 0.01.

B. BMR: Default value (percent change = 5 %)

C. Software used: PROAST version 28.1
http://www.rivm.nl/en/foodnutritionandwater/foodsafety/proast.jsp

D. Additional assumptions: None

E. Table of BMD results from analysis of APTT data

Model	No of parameters	Log-likelihood		BMD_{05}* (mg/kg BW)		$BMDL_{05}$* (mg/kg BW)	
		Exponential	Hill	Exponential	Hill	Exponential	Hill
M1	2	-52.37		-	-	-	-
I__M2-	3	- 42.42	-44.47	-	-	-	-
I__M2-a	4	-29.94	-35.26	-	-	-	-
I__M2-b	4	-27.16	- 35.96	-	-	-	-
I__M2-ab	5	-26.32	-34.47	-	-	-	-
I__M3-a	5	n. r.	-28.67	-	-	-	-
I__M3-b	5	-27.09	-28.22*	-	*	-	*
I__M3-ab	6	n. r.	-23.63	-	-	-	-
I__M4-a	5	n. r.	-30.69	-	-	-	-
I__M4-b	5	-28.39	n. r.	-	-	-	-
I__M5-a	6	n. r.	-28.56	-	-	-	-
I__M5-b	6	-22.19	n. r.	313	-	**167.4**	-
I__M5	5	-41.40	-41.41	-	-	-	-
Full model	11	-20.94		-	-	-	-

* In the Hill family models, M3-b was the selected model. However, all models of the Hill family were significantly worse that the full model. Hence no BMD_{05} and $BMDL_{05}$ values were derived from Hill family models.

n. r. = the application of the model does not provide a result (data not suitable for this model).

F. Figures

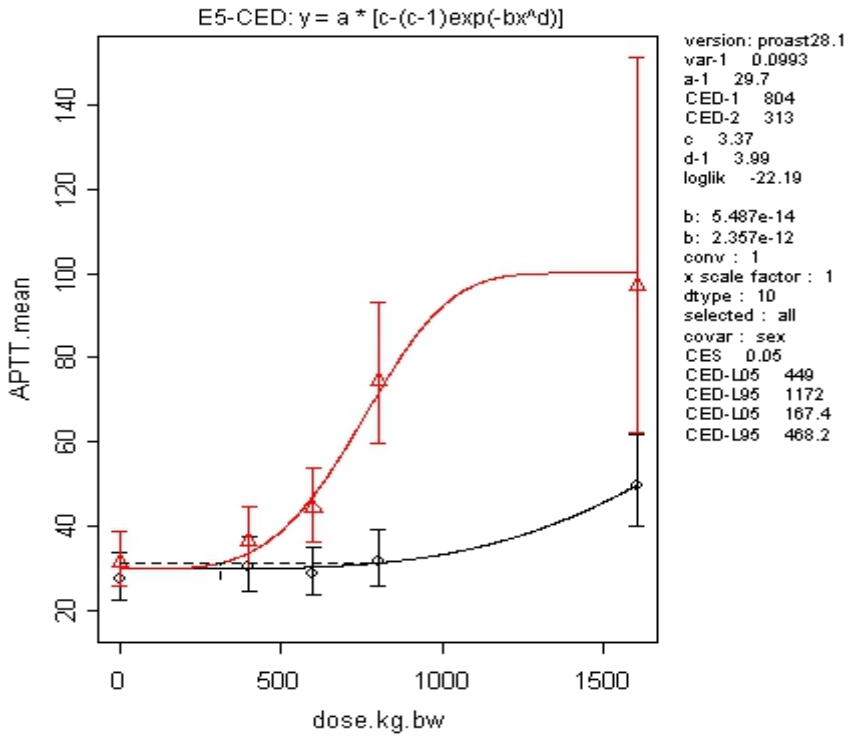

Figure: Fitted curves for male (red) and female (black) APTT data

GLOSSARY AND ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alanine Amino Transferase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Amino Transferase
AUC	Area Under the Curve
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower confidence limit
DEN	Diethylnitrosamine
GMP	Good Manufacturing Practice
GST-P	Glutathione-S-Transferase P-type
LDL	Low Density Lipoproteins
LFO	Liquorice Flavonoid Oil
LOAEL	Lowest observed adverse effect level
MCT	Medium-Chain Triglycerides
NOAEL	No observed adverse effect level
PT	Prothrombin time
RBC	Red Blood Cells
UDS	Unscheduled DNA synthesis
WBC	White Blood Cells

PRIVILEGED AND CONFIDENTIAL

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

FINAL REPORT: 2005
For 2004 list of ingredients

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

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33	2-,5-, or 6-Methoxy-3-methylpyrazine	02847-30-5	24
34	2-Methylheptanoic acid	01188-02-9	25
35	2-Methylpyrazine	00109-08-0	26
36	gamma-Octalactone	00104-50-7	29
37	2,3-Pentanedione	00600-14-6	29
38	2-Phenethyl acetate	00103-45-7	29
39	Phenylacetaldehyde	00122-78-1	29
40	Sodium bicarbonate	00144-55-8	29
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189	2, 5-Dimethyl-4-hydroxy-3(2h)-furanone (4-Hydroxy-2,5-dimethyl-3(2h)furanone)	03658-77-3	95
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INTRODUCTION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

INGREDIENTS REVIEW

CATEGORY: NEW INGREDIENTS

PARA-TOLUALDEHYDE
CAS: 104-87-0

Number of relevant papers: 7

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

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

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

COMMENTS: Comments are provided above.

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

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

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

COMMENTS: Comments are provided above.

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

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

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

COMMENTS: Comments are provided above.

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

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

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

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

COMMENTS: Comments are provided above.

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

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

COMMENTS: Comments are provided above.

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

Source: EPA/OTS; Doc #86-920000590

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

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

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

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

COMMENTS: Additional comments not necessary, abstract satisfactory.

CITRONELLOL
CAS: 106-22-9

Number of relevant papers: 3

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

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

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

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

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

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

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

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

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

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

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

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

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

ETHYL HEPTANOATE
CAS: 106-30-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOAMYL FORMATE
CAS: 110-45-2

Number of relevant papers: 2

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

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

ABSTRACT: N/A

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

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

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

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

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

HEXYL ACETATE
CAS: 142-92-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PECTIN
CAS: 9000-69-5

Number of relevant papers: 3

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

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

ABSTRACT: N/A

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

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

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

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

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

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

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

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

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

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

CORN STARCH
9005-25-8

Number of relevant papers: 2

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

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

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

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

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

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

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

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

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

L-MENTHONE
14073-97-3

Number of relevant papers: 1

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

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

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

COMMENTS: Abstract summary of the paper is adequate.

CATEGORY: HIGH MUL'S INGREDIENTS

ACETIC ACID
CAS: 64-19-7

Number of relevant papers: 2

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

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

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

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

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

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

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

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

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

BENZALDEHYDE
CAS: 100-52-7

Number of relevant papers: 2

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

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

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

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

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

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

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

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

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

BUTYRIC ACID
CAS: 107-92-6

Number of relevant papers: 1

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

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

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

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

CAPRYLIC/CAPRIC TRIGLYCERIDE
CAS: 65381-09-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

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

2,5-DIMETHYLPYRAZINE
123-32-0

Number of relevant papers: 3

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

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

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

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

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

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

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

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

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

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

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

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

COMMENTS: POSTER PRESENTATION -- Paper N/A

ETHYL BUTYRATE
CAS: 105-54-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL DECANOATE
CAS: 110-38-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Number of relevant papers: 1

1. Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning

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

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

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

2-METHYLHEPTANOIC ACID
CAS: 1188-02-9

Number of relevant papers: 1

1. Evaluation of certain food additives and contaminants -

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

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

COMMENTS: The abstract describes this report well.

2-METHYLPYRAZINE
CAS: 109-08-0

Number of relevant papers: 3

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

SUCROSE OCTAACETATE
CAS: 126-14-7

Number of relevant papers: 1

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

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

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

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

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

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

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

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

CATEGORY: MAJOR INGREDIENTS

GLYCEROL
CAS: 56-81-5

Number of relevant papers: 1

1. Glycerol transfer in cigarette mainstream smoke

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

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

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

CARBON
CAS: 7440-44-0

Number of relevant papers: 4

GENERAL COMMENTS ON CARBON AND GRAPHITE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4. Ultrafine particle deposition in subjects with asthma -

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

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

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

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

GRAPHITE
CAS: 7782-42-5

Number of relevant papers: 2

1. Translocation of inhaled ultrafine particles to the brain -

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

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

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

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

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

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

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

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

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

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

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

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

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

SUCROSE
CAS: 57-50-1

Number of relevant papers: 3

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

PROPYLENE GLYCOL
57-55-6

Number of relevant papers: 2

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

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

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

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

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

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

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

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

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

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

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

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

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

Number of relevant papers: 6

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

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

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

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

2. Mentholated cigarette smoking inhibits nicotine metabolism

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

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

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

3. Epidemiology of menthol cigarette use -

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

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

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

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

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

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

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

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

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

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

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

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

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

6. Percutaneous penetration enhancers in cigarette mainstream smoke -

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

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

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

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

POTASSIUM CARBONATE
CAS: 584-08-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

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

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

Number of relevant papers: 7

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5. Percutaneous penetration enhancers in cigarette mainstream smoke.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

Number of relevant papers: 1

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

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

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

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

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

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

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

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

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

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

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

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

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

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

UREA
CAS: 57-13-6

Number of relevant papers: 1

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

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

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

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

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

SODIUM CARBONATE
CAS: 497-19-8

Number of relevant papers: 1

1. Cancer incidence in textile manufacturing workers in Australia

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

ABSTRACT: N/A

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

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

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

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

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

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

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

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

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

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

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

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

VANILLIN
CAS: 121-33-5

Number of relevant papers: 1

1. Mutagens and Sensitizers-An Unequal Relationship? -

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

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

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

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CATEGORY: STANDARD INGREDIENTS

1. Percutaneous penetration enhancers in cigarette mainstream smoke -

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

This paper examines a number of standard ingredients including:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Number of relevant papers: 1

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

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

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

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

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

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ANISE STAR OIL
CAS: 8007-70-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ANISYL ACETATE
CAS: 104-21-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

L-ARGININE
CAS: 74-79-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ASCORBIC ACID
CAS: 50-81-7

Number of relevant papers: 1

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

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

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

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

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

L-ASPARTIC ACID
CAS: 56-84-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BALSAM PERU AND OIL
CAS: 8007-00-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BEET JUICE CONCENTRATE
CAS: 89957-90-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZALDEHYDE
CAS: 100-52-7

SEE HIGH MUL'S INGREDIENTS

BENZALDEHYDE GLYCERYL ACETAL
CAS: 1319-88-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZOIC ACID
CAS: 65-85-0

Number of relevant papers: 1

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

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

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

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

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

BENZYL ALCOHOL
CAS: 100-51-6

Number of relevant papers: 3

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

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

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

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

2. Neurologic issues with solvents

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

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

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

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

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

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

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

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

BENZYL BENZOATE
CAS: 120-51-4

Number of relevant papers: 2

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

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

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

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

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

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

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

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

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

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

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

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

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

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

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

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

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

Number of relevant papers: 1

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

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

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

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

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

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

N-BUTYL ISOVALERATE
CAS: 109-19-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3-BUTYLIDENEPHTHALIDE
CAS: 551-08-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

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

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

CARBON
CAS: 7440-44-0
SEE MAJOR INGREDIENTS

CARBON DIOXIDE
CAS: 124-38-9

Number of relevant papers: 3

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

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

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

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

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

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

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

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

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

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

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

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

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

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CAROTENE
CAS: 7235-40-7

Number of relevant papers: 8

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

CHICORY EXTRACT
CAS: 68650-43-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHOCOLATE AND CHOCOLATE LIQUOR

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMALDEHYDE
CAS: 104-55-2

Number of relevant papers: 2

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

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

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

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

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

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

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

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

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

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMYL ACETATE
CAS: 103-54-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMYL ALCOHOL
CAS: 104-54-1

Number of relevant papers: 2

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

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

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

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

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

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

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

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

CINNAMYL CINNAMATE
CAS: 122-69-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CITRAL
CAS: 5392-40-5

Number of relevant papers: 5

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4. Analysis of thresholds for carcinogenicity. -

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

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

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

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

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

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

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

COMMENTS: Abstract sufficient, no additional comments needed.

CITRIC ACID
CAS: 77-92-9

Number of relevant papers: 1

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

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

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

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

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

CITRONELLOL
CAS: 106-22-9
SEE NEW INGREDIENTS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Number of relevant papers: 1

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

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

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

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

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

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

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

DELTA-DODECALACTONE
CAS: 713-95-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GAMMA-DODECALACTONE
CAS: 2305-05-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ACETATE
CAS: 141-78-6

Number of relevant papers: 1

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

INGREDIENTS USED IN MIXTURE STUDIES TOBACCO SMOKE STUDIES

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

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

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

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

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

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

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

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

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

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

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

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

7. The pyrolysis of tobacco ingredients -

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

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

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

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

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

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

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

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

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

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

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

Baker RR, Massey ED, Smith G.
Food Chem Toxicol. 2004;42 Suppl:S53-83.

ABSTRACT: This paper presents an overview of a series of studies designed to assess the influence of 482 tobacco ingredients on cigarette smoke chemistry and toxicity. The studies are: pyrolysis of the ingredients; influence of the ingredients on smoke constituents believed by regulatory authorities to be relevant to smoking-related diseases ("Hoffmann analytes"); influence of the ingredients on in vitro genotoxicity and cytotoxicity of smoke partiulate matter; and influence of the ingredients on the inhalation toxicity of smoke. The present paper brings the salient features of these studies together. A pyrolysis technique has been developed which, as far as practicably possible, mimics the combustion conditions inside a burning cigarette. The results from 291 single-substance ingredients indicate that almost a third would transfer out of the cigarette burning zone at least 99% intact (i.e. less than 1% pyrolysis), and almost two thirds would transfer at least 95% intact. Of the ingredients that underwent some degree of pyrolysis, a few "Hoffmann analytes" were detected amongst the pyrolysis products of 19 ingredients. Taking into account maximum use levels, their maximum pyrolysis levels were generally small and often insignificant compared to the levels typically present in smoke. Possible exceptions were acetaldehyde and benzene from the pyrolysis of malic acid. However, subsequent smoke chemistry studies indicated that the maximum levels predicted from pyrolysis of this involatile substance were overestimated, suggesting that malic acid does not undergo complete pyrolysis in the burning cigarette and/or generates acetaldehyde and benzene at similar rates to that of tobacco on a per weight basis. When added to tobacco, many of the ingredient mixtures produced no significant effect on the levels of many of the "Hoffmann analytes" in smoke, while some produced increases or decreases relative to the relevant control cigarettes. The study has concentrated on the increases. Many of the differences were found to be not significant when the long-term variability of the analytical methodology was taken into account. However, even taking this into account, the smoke formaldehyde levels in two of the test cigarettes were significantly increased relative to their controls, by up to 26 microg (73%). These increases are likely to be due to the pyrolysis of sugars, cellulose and other polysaccharide materials. The activity of smoke particulate matter from cigarettes containing tobacco ingredients has been determined with three in vitro bioassays, two for genotoxicity and one for cytotoxicity. These were the Ames test, the mammalian cell micronucleus assay, and the neutral red uptake cytotoxicity assay. Within the sensitivity and specificity of these bioassays, the specific activity of the cigarette smoke particulate matter was not changed by the addition of ingredients to the cigarette. Three 90-day sub-chronic inhalation studies have been undertaken and histopathological and histomorphometric assessments made within the respiratory tracts of animals exposed to smoke from cigarettes containing the various ingredient mixtures and their control

cigarettes. The response due to tobacco smoke exposure was not distinguishable between the test and control cigarettes, indicating that the presence of the ingredients had made no discernable differences to the type and severity of the treatment-related changes.

RELEVANT REVIEWS & INTERESTING PAPERS

1. Evaluation of certain food additives and contaminants -

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

GENERAL COMMENT: In this document, examples of additives that were reviewed include citric acid, 2 methylheptanoic, citral, citronellol and much more.

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

COMMENTS: This is a massive report that one needs to be aware of since this Committee had access to documents called Technical Data Sheets, which were prepared using new or existing food additives and which had not been published because the detailed information on manufacturing processes described therein could be commercially sensitive. These documents, however, also contain valuable information, which was not made public, on chemical and technological approaches.

The Committee recognized the need for a working definition of the term "flavouring agent" and recommended that such a definition be agreed at a future meeting. At its present meeting, the Committee noted that a range of regulatory definitions of "flavouring" and similar terms exist in different countries and concluded that any definition would need to be elaborated in an international forum, such as the Codex Alimentarius Commission. The Committee reiterated the criteria that need to be met for an individual flavouring agent to be evaluated by the existing Procedure for the Safety Evaluation of Flavouring Agents: • The substance should be chemically defined, such that at least 95% of the commercially used material consists either of the named chemical, or of the named chemical and identified secondary constituents. The substance is added to food for flavouring purposes, including the generation of active flavouring substances during storage or processing of the food. • There is a valid estimate of current exposure to the named substance and, if appropriate, its breakdown or reaction products. Some substances that have a use as flavouring agents may have been evaluated previously by the Committee in relation to other food additive functions. The use of such a substance, or its breakdown or reaction products, as a flavouring agent is included in the relevant, previously-established ADI.

2. Human functional neuroimaging in nicotine and tobacco research: Basics, background, and beyond - 2004 –

F. Joseph McClernon and David G. Gilbert
Nicotine & Tobacco Research Volume 6, Number 6 : 941 - 959

ABSTRACT: Modern functional neuroimaging techniques allow nicotine and tobacco researchers to investigate the neurobiological basis of addiction in humans. We introduce the methods and measures of the following neuroimaging techniques: Electroencephalography and event-related cortical potentials, positron emission tomography, and functional magnetic resonance imaging. We outline strengths and limitations across modalities and describe new and emerging technologies. We provide summaries of recent neuroimaging findings in the field of nicotine and tobacco research for neurochemistry, smoking and nicotine administration, craving and cue-reactivity, cognitive and affective information processing, and tobacco withdrawal. We address limitations of studies to date and identify opportunities for future research.

3. **Effect of exposure to secondhand smoke on markers of inflammation: the ATTICA study –**

Demosthenes B. Panagiotakos PhDa, , , Christos Pitsavos MD, PhDa, Christina Chrysohoou MD, PhDa, John Skoumas MDa, Constadina Masoura MDa, Pavlos Toutouzas MD, PhDa and Christodoulos Stefanadis MD, PhDa
The American Journal of Medicine Volume 116, Issue 3 , 1 February 2004, Pages 145-150

ABSTRACT: We sought to investigate the effect of secondhand smoke exposure on inflammatory markers related to cardiovascular disease. Methods. During 2001 to 2002,

we randomly selected a stratified (age-sex) sample of adults without clinical evidence of cardiovascular disease. Exposure to secondhand smoke (>30 minutes per day and ≥1 day per week) was recorded. Multivariate regression analysis was used to evaluate the effects of exposure to secondhand smoke on levels of C-reactive protein, fibrinogen, homocysteine, and oxidized low-density lipoprotein (LDL) cholesterol, and on white blood cell count. Results. One hundred and thirty-seven (38%) of the 357 men who had never smoked and 211 (33%) of the 638 never-smoking women reported current exposure to secondhand smoke. Compared with those who were not exposed to secondhand smoke, those exposed more than 3 days per week had higher white blood cell counts (by 600 cells per μL; P = 0.02), as well as higher levels of C-reactive protein (by 0.08 mg/dL; P = 0.03), homocysteine (by 0.4 μmol/L; P = 0.002), fibrinogen (by 5.2 mg/dL; P = 0.4), and oxidized LDL cholesterol (by 3.3 mg/dL; P = 0.03), after adjusting for several potential confounders. Conclusion: Our findings suggest another pathophysiological mechanism by which exposure to secondhand smoke is associated with the development of atherosclerosis.

4. **Influence of smoking and sinus on the prevalence and incidence of type 2 diabetes amongst men: the northern Sweden MONICA study**

M. Eliasson1,2, K. Asplund2, S. Nasic2 & B. Rodu3
Journal of Internal Medicine Volume 256 Issue 2 Page 101 - August 2004

ABSTRACT: To explore the effect of smoking and smokeless tobacco, 'snus', on the risk of type 2 diabetes. Design. Population-based cross-sectional and prospective follow-up study in northern Sweden. Subjects. A total of 3384 men, aged 25–74 years, who participated in the MONICA study in 1986, 1990, 1994 or 1999, 1170 of whom had an oral glucose tolerance test. In 1999, 1757 men from previous cohorts returned for re-examination. Main outcome measures. We compared the prevalence of type 2 diabetes or pathological glucose tolerance (PGT) amongst tobacco users to that of nonusers at entry into the study and at follow-up, using odds ratios. Results. Compared with never users, the ageadjusted risk of prevalent clinically diagnosed diabetes for ever smokers was 1.88 (CI 1.17–3.0) and for smokers 1.74 (0.94–3.2). Corresponding odds ratios for snus users were 1.34 (0.65–2.7) and 1.18 (0.48–2.9). We found no increased risk of prevalent PGT in snus users or smokers. Former smokers and snus users had an insignificantly increased risk for PGT. Compared with nonusers, the age-adjusted risk of developing clinically diagnosed diabetes during follow-up was 4.63 (1.37–16) in consistent exclusive smokers, 3.20 (1.16–8.8) in ex-smokers and no cases in consistent snus users. The risk of PGT during follow-up was not increased in consistent tobacco users but evident, although not statistically significant, in those who quit snus during the follow-up period, 1.85 (0.60–5.7). Adjustment for physical activity and alcohol consumption did not change the major findings. Conclusions. The risk of diabetes for snus users was not significantly increased. Smoking was associated with prevalent and incident cases of diabetes. Ex-tobacco users tended towards more PGT.

COMMENTS: This paper describes an epidemiological study comparing the effects of smoking and smokeless tobacco use on type 2 diabetes. The study confirmed previous

findings that smoking is a risk factor for type 2 diabetes, but did not find a similar association with the use of smokeless tobacco.

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

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

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

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

COMMENTS: This paper provides a review of 69 chemicals tested in the National Cancer Institute / National Toxicology Program (NCI/NTP) carcinogenicity bioassay database. The selected chemicals are those that have shown an association with renal tubule tumors in rat and/or mouse, and was focused on oral exposures.

6. Cigarette smoking exacerbates chronic alcohol-induced brain damage: A preliminary metabolite imaging study -

Durazzo TC, Gazdzinski S, Banys P, Meyerhoff DJ.
Alcohol Clin Exp Res. 2004 Dec;28(12):1849-60.

ABSTRACT: Cigarette smoking is common among alcohol-dependent individuals. Nevertheless, previous research has typically not accounted for the potential independent or compounding effects of cigarette smoking on alcohol-induced brain injury and neurocognition. METHODS: Twenty-four 1-week-abstinent recovering alcoholics (RAs; 14 smokers and 10 nonsmokers) in treatment and 26 light-drinking controls (7 smokers and 19 nonsmokers) were compared on measures of common brain metabolites in gray matter and white matter of the major lobes, basal ganglia, midbrain, and cerebellar vermis, obtained via multislice short-echo time proton magnetic resonance spectroscopic imaging. Smoking and nonsmoking RAs were also contrasted on measures of neurocognitive functioning, as well as laboratory markers of drinking severity and nutritional status. RESULTS: Chronic alcohol dependence, independent of smoking, was associated with lower concentrations of frontal N-acetylaspartate (NAA) and frontal choline-containing compounds, as well as lower parietal and thalamic choline. Smoking RAs had lower NAA concentrations in frontal white matter and midbrain and lower midbrain choline than nonsmoking RAs. A four-group analysis of covariance also demonstrated that chronic cigarette smoking was associated with lower midbrain NAA and choline and with lower vermian choline. In smoking RAs, heavier drinking was associated with heavier smoking, which correlated with numerous subcortical metabolite abnormalities. The 1-week-abstinent smoking and nonsmoking RAs did not differ

significantly on a brief neurocognitive battery. In smoking RAs, lower cerebellar vermis NAA was associated with poorer visuomotor scanning speed and incidental learning, and in nonsmoking RAs lower vermis NAA was related to poorer visuospatial learning and memory. CONCLUSIONS: These human in vivo proton magnetic resonance spectroscopic imaging findings indicate that chronic cigarette smoking exacerbates chronic alcohol-induced neuronal injury and cell membrane damage in the frontal lobes of RAs and has independent adverse effects on neuronal viability and cell membranes in the midbrain and on cell membranes of the cerebellar vermis. Higher smoking levels are associated with metabolite concentrations in select subcortical structures. Greater consideration of the potential effects of comorbid cigarette smoking on alcohol-induced brain damage and other diseases affecting the central nervous system is warranted.

7**. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: A review of agents and causative mechanisms**

Urmila Nair, Helmut Bartsch and Jagadeesan Nair
Mutagenesis vol. 19 no. 4 pp. 251-262, July 2004

ABSTRACT: In south-east Asia, Taiwan and Papua New Guinea, smoking, alcohol consumption and chewing of betel quid with or without tobacco or areca nut with or without tobacco are the predominant causes of oral cancer. In most areas, betel quid consists of a mixture of areca nut, slaked lime, catechu and several condiments according to taste, wrapped in a betel leaf. Almost all habitual chewers use tobacco with or without the betel quid. In the last few decades, small, attractive and inexpensive sachets of betel quid substitutes have become widely available. Aggressively advertised and marketed, often claimed to be safer products, they are consumed by the very young and old alike, particularly in India, but also among migrant populations from these areas world wide. The product is basically a ¯avoured and sweetened dry mixture of areca nut, catechu and slaked lime with tobacco (gutkha) or without tobacco (pan masala). These products have been strongly implicated in the recent increase in the incidence of oral submucous ®brosis, especially in the very young, even after a short period of use. This precancerous lesion, which has a high rate of malignant transformation, is extremely debilitating and has no known cure. The use of tobacco with lime, betel quid with tobacco, betel quid without tobacco and areca nut have been classi®ed as carcinogenic to humans. As gutkha and pan masala are mixtures of several of these ingredients, their carcinogenic affect can be surmised. We review evidence that strongly supports causative mechanisms for genotoxicity and carcinogenicity of these substitute products. Although some recent curbs have been put on the manufacture and sale of these products, urgent action is needed to permanently ban gutkha and pan masala, together with the other established oral cancer-causing tobacco products. Further, education to reduce or eliminate home-made preparations needs to be accelerated.

COMMENTS: Well-marketed and conveniently packaged commercial preparations containing chewing tobacco with various combinations of lime, betel quid and areca nut have popularized the use of these products in Asia. The authors summarize available

evidence of the carcinogenic potential of these mixtures, and suggest a ban on products such as gutkha and pan masala.

8. **Alcohol, acetaldehyde, and digestive tract cancer**

SALASPURO, M. Alcohol, acetaldehyde, and digestive tract cancer. In: Nutrition and alcohol, pp. 393-411. Boca Raton, CRC Press, 2004.
Book Chapter

ABSTRACT N/A

COMMENTS: This monograph reviews the health issues associated with use of alcohol and states that cancer risk is dose-dependent and alcohol and smoking is synergistic, producing a greater effect together than either alone. Moderate smoking without drinking and moderate drinking without smoking had a slight or negative effect on esophageal cancer risk. But simultaneous exposure to the same moderate amounts increased risk 12 to 19-fold in men and women respectively.

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees II; scientific co-operation and networks

Scientific Committee on Food **SCF/CS/ADD/EDUL/225 Final**
10 April 2003

OPINION OF THE SCIENTIFIC COMMITTEE ON FOOD

ON

GLYCYRRHIZINIC ACID AND ITS AMMONIUM SALT

(opinion expressed on 4 April 2003)

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Opinion of the scientific committee on food on glycyrrhizinic acid and its ammonium salt

[Please note that more details on the use levels in food and on individual studies together with their reference citations can be found on the Annexes of this document]

Terms of Reference

The Committee is asked to consider if the opinion of the Committee expressed in 1991 on glycyrrhizin is still valid in the light of additional information resulting from toxicological and clinical studies published since then on both glycyrrhizinic acid and its salts. The Committee is asked to take into account dietary exposure from all known sources, including contributions due to its natural occurrence in liquorice and through the ingestion of food products to which it is added as a flavouring substance.

The Committee is also asked to evaluate ammonium glycyrrhizinate as a chemically defined flavouring substance for the possible acceptability of its inclusion in the Community Register.

Previous evaluations

Glycyrrhizinic acid has been given Generally Recognized as Safe (GRAS) status in the USA in 1985, however with upper limits for use levels in foods (Anonymous, 1985).

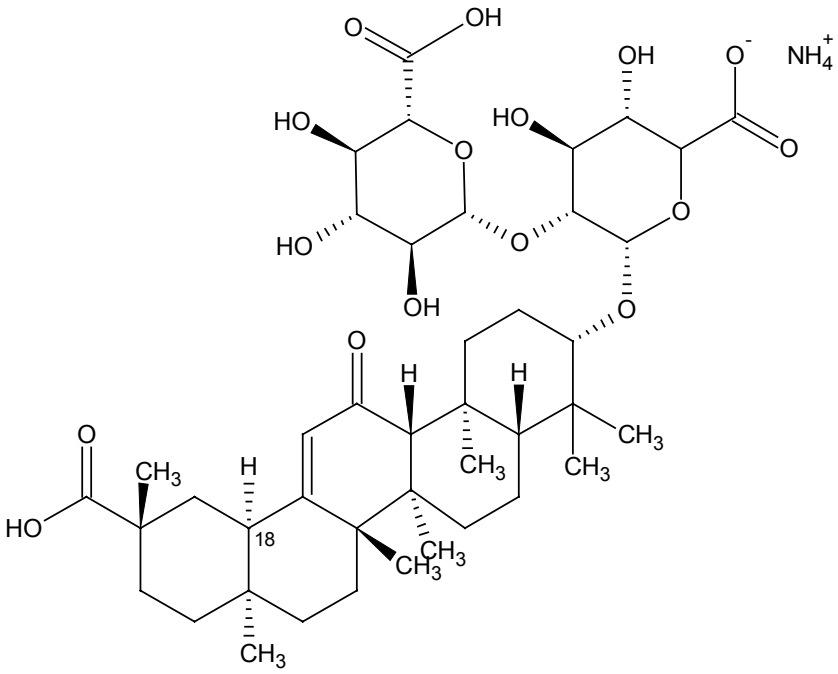
The Committee has considered the use of glycyrrhizinic acid as a sweetener in 1985. At that time the Committee was unable to endorse the use of the substance as a sweetener. In 1991 the Committee has evaluated the toxicological information obtained in human volunteer studies and the clinical information on glycyrrhizinic acid and concluded that the data were inadequate to derive an ADI. The data did indicate a concern for some sectors of the population, especially those suffering from hypertension. The Committee considered it prudent that regular ingestion should not exceed 100 mg/day, while it was explicitly mentioned that this was a provisional figure, which should be updated when new data

would become available. It was recognised that studies in human volunteers were in progress, the results of which were expected to be highly relevant (SCF, 1991).

In 1993 the Nordic Council of Ministers published an evaluation on glycyrrhizinic acid (Størmer *et al.*, 1993a). These authors concluded that: *"it appears that in the most sensitive individuals adverse effects occur at a regular daily intake of about 100 mg glycyrrhizinic acid. A regular daily intake of 100 mg/day was, established as a provisional LOAEL for adults."* In a subsequent publication, these authors advocated an ADI for glycyrrhizinic acid of 1-10 mg/person/day by applying an uncertainty factor of 10 to the above mentioned lowest-observed-adverse-effect level (LOAEL) (Størmer *et al.*, 1993b)

Chemical Identity

This document gives an evaluation of glycyrrhizinic acid and its monoammonium salt, which has the following chemical structure:



Substance Name	Glycyrrhizinic acid
Chemical name (IUPAC name not identified)	(3-beta, 20-beta)-20-carboxy-11-oxo-30-norolean-12-en-3-yl 2-O-beta-D-glucopyranuronosyl-alpha-D-glucopyranosiduronic acid
synonyms	Glycyrrhizin
CAS nr	1405-86-3
EINECS nr	215-785-7
FL nr	16.012
Molecular formula	$C_{42}H_{62}O_{16}$
Molecular weight	822.94 D*

* no information is available whether glycyrrhizinic acid may contain crystal water.

Substance Name	Ammonium glycyrrhizinate
Chemical name (IUPAC name not identified)	(3-beta, 20-beta)-20-carboxy-11-oxo-30-norolean-12-en-3-yl 2-O-beta-D-glucopyranuronosyl-alpha-D-glucopyranosiduronic acid, monoammonium salt
synonyms	Glycyrrhizinic acid, ammonium salt
CAS nr	53956-04-0
EINECS nr	258-887-7
FL nr	16.060
Molecular formula	$C_{42}H_{61}O_{16}\cdot NH_4$ (anhydrous)
Molecular weight	839.96 D (anhydrous)

Glycyrrhizinic acid is a naturally occurring triterpenoid saponin, which can be found in extracts of roots and rhizomes of the Liquorice plant *Glycyrrhiza glabra*, together with a number of other substances including other triterpenoids, polyphenols, polysaccharides essential oils and flavonoids. The crude dried aqueous extracts (also known as "block liquorice") may contain 4-25% glycyrrhizinic acid in the form of calcium, magnesium and potassium salts (Wang *et al.*, 2000; Størmer *et al.*, 1993a; EFFA, 2001). The ammoniated salt is manufactured by acid treatment of the aqueous extracts, followed by neutralisation

of the precipitated material with diluted ammonia. The monoammonium salt is then further purified by solvent extraction and other separation techniques (EFFA, 2001). Glycyrrhizinic acid can be considered as the di-glucuronic acid conjugate of glycyrrhetic acid. This aglycone part of the molecule may occur in two forms, (18-alpha- and 18-beta-). Although the 18-alpha form has been found in amounts up to 13% of the total glycyrrhetic acid present, it is not clear whether this form occurs naturally or whether it is only formed during processing due to isomerisation (Størmer *et al.*, 1993a).

Both glycyrrhizinic acid and ammonium glycyrrhizinate are in the Community register of chemically defined flavouring substances, adopted in Commission Decision 1999/217/EC (EU, 1999) and later amended. They are used because of their sweet taste (33-200 times sweeter than sucrose; Størmer *et al.*, 1993a; EFFA, 2001). As required by Commission Regulation (EC) No.1565/2000 (EU, 2000), specifications for marketed flavouring substances should be provided. Almost complete specifications were provided for ammonium glycyrrhizinate (for solubility in water only a qualitative statement was given; no data were provided for solubility in ethanol), but no specification data were provided for glycyrrhizinic acid.

Exposure Assessment

People may be exposed to glycyrrhizinic acid via food or other products that contain either the purified acid or its ammonium salt or via food in which the dried crude root extract of *Glycyrrhiza glabra* has been incorporated. Exposure to glycyrrhizinic acid may occur not only via the consumption of liquorice confectionery and sweets, but also through beverages, chewing gum, tooth paste and medicinal products. Other sources of intake may be sucking/chewing on dried *Glycyrrhiza* roots or chewing or smoking tobacco products.

<u>Exposure from the use as chemically defined flavouring substances</u>

The Upper Use Levels (UULs) of ammonium glycyrrhizinate and glycyrrhizinic acid in foods from various categories, as specified by EFFA (2001, 2003), are given in Annex I. According to EFFA (2001, 2003), the annual volumes of ammonium glycyrrhizinate and glycyrrhizinic acid for use as a chemically defined food flavouring substances are 1070

and 1965 kg, respectively. Based on these figures the Maximised Survey-Derived Intake (MSDI) [i] for ammonium glycyrrhizinate is (= 130 μg/person/day (~1.8 μg/kg bw/day) and the MSDI for glycyrrhizinic acid is 240 μg/person/day (~3.4 μg/kg bw/day).

When compared to the UULs for these substances, the MSDI calculations may result in unrealistically low intake figures for individuals who select to consume certain foods, flavoured at the UULs (see Annex I). For example, intake of 50 grams of candies, flavoured with ammonium glycyrrhizinate at the UUL would lead to an intake of ammonium glycyrrhizinate of 77.5 mg, while drinking of 1 liter of a glycyrrhizin-flavoured drink would provide an intake of 200 mg glycyrrhizinate. Both figures exceed the calculated MSDI by 3 orders of magnitude. Similarly, for glycyrrhizinic acid, consumption of 250 g of dairy products or edible ices would for both examples lead to an intake of 94 mg. For this substance, consumption of 20 g of fondant may already result in an intake of 100 mg.

Exposure via intake of liquorice confectionery

Glycyrrhizinic acid content

Størmer *et al.* (1993a) have given glycyrrhizinic acid levels which were determined in confectionery products marketed in Germany, Belgium and the United Kingdom. Glycyrrhizinic acid concentrations ranged from 0.29 to 7.9 mg/g liquorice confectionery; in the UK two products were above 3.5 mg/g and in Germany three products were above 4.2 mg/g. Certain "health" products such as liquorice-flavoured diet gum, and throat pearls may contain 15 and 47 mg/g, respectively**.**

In the Netherlands the mean glycyrrhizinic acid percentage in liquorice is 1.7 mg/g, based on an analysis of 19 samples, ranging from 0.3 to 5.1 mg/g. In 3 additional samples ("liquorice powder" and two brands of "bay liquorice") levels of 18 and 25-28 mg/g were found, respectively. "Bay liquorice" consists of 100% dried *Glycyrrhiza* root extract (Maas, 2000).

[i] Calculated as follows: [annual production volume * 10^9 μg]/ [population*survey correction factor*365 days]; with population = 10% (eaters only) of $375*10^6$ and survey correction factor = 0.6 (for under-reporting).

Consumption of liquorice confectionery and exposure to glycyrrhizinic acid

For Norway, Denmark, Iceland and Sweden average yearly consumption estimates of 1 to 2.5 kg of liquorice confectionery have been reported (Størmer *et al.*, 1993a). This average has been calculated for the entire population of consumers plus non-consumers.

The consumption of liquorice confectionery is not evenly distributed in the population. This is demonstrated by the intake data for liquorice confectionery in the Dutch population, based on the results from the 3rd Dutch food consumption survey (Kistemaker *et al.,* 1998). About 10% of the population that participated in this survey consumed liquorice confectionery at least once during the two days[ii]. The distribution of intake of liquorice confectionery within this group of regular consumers is shown in the table below:

percentile of regular consumers population	**Intake of liquorice confectionery (g/day)**	**intake of glycyrrhizinic acid* (mg/day)**
0	0,5	1
10	2	3
20	3	5
30	4	7
40	5	8
50	6	10
60	8	13
70	10	17
80	15	25
90	25	42
100	187.5	313

* calculation based on a mean glycyrrhizinic acid contents in liquorice confectionery of 0.17%

[ii] The survey method used is known to underreport consumption in occasional users. Hence the proportion of all consumers of liquorice (i.e. all eaters) in the entire Dutch population may be considerably larger. Additional data from the second survey (Hulshof and Kistemaker, 1995) indicated that about 60% of the population may eat liquorice at least once per month.

The average daily consumption among regular consumers was 11.5 g. Using the data of Maas (2000) that the mean content of glycyrrhizinic acid in liquorice confectionery in the Netherlands was 0.17%, this amount of would correspond to a daily intake of 19 mg glycyrrhizinic acid. About 3% of the regular consumers ate more than 50 g of liquorice confectionery per day. Sweet liquorice confectionery was the most popular type and the number of consumers of extra salted liquorice confectionery as well as the amounts consumed thereof were smaller. According to these data, about 2% of the regular consumers have a daily intake of glycyrrhizinic acid of over 100 mg/day (the maximum intake level, provisionally derived by the Committee in 1991).

Exposure via other sources

Herbal teas

Exposure to glycyrrhizinic acid via herbal teas may contribute significantly to the total daily intake of this substance. Maas (2000) described that of 33 brands of herbal teas, 13 contained < 10 mg/l, 16 contained 10 to 100 mg/l and 4 contained > 100 mg/l with a maximum of 450 mg/l (concentrations refer to the prepared beverage). Among these 33 brands, for 10 brands the label information stated liquorice plant material as ingredient, and for these teas the average glycyrrhizinic acid content was 126 mg/l (range 2 - 450 mg/l).

Beverages

Maas (2000) described that of the 18 brands of (herbal) alcoholic beverages studied, 6 contained < 10 mg/l, 2 contained 10 to 100 mg/l and 10 contained > 100 mg/l (range for all beverages: not detectable to 422 mg/l). Two non-alcoholic herbal drinks were also studied. One was a liquorice-syrup, which contained 411 mg glycyrrhizinic acid/l. In the other one no glycyrrhizinic acid could be detected (Maas, 2000).

Chewing gum

Based on the information provided in one case study, a brand of chewing gum is marketed which contains glycyrrhizinic acid in a concentration of 0.15% in a package size of 16 g. Consumption of the chewing gum in such a package in one day would result in a (maximum) intake of 24 mg of glycyrrhizinic acid/day (Rosseel and Schoors, 1993).

Tobacco products

Glycyrrhizinic acid in the form of liquorice extract (up to 4%) is also added to tobacco products, including chewing tobacco. Although exposure to glycyrrhizinic acid via chewing tobacco may elicit symptoms of pseudohyperaldosteronism (Blakey, 1998), for smoking tobaccos extensive exposure to glycyrrhizinic acid is not likely because of pyrolysis (Hoffmann and Hoffmann, 1977).

Hazard Assessment

The following text is a summary of the effects of glycyrrhizinic acid and its ammonium salt. More details on the individual studies, together with their reference citations, are contained in Annex II.

Glycyrrhizinic acid itself is hardly absorbed from the gastro-intestinal tract. Before absorption, glycyrrhizinic acid is hydrolysed to give glycyrrhetic acid, which is the ultimate biologically active metabolite. Bioavailability studies for glycyrrhetic acid have indicated that absorption from solutions (either as substance or as glycyrrhizinic acid) or from glycyrrhizinic acid in a liquorice matrix is equally effective and virtually complete. Due to excretion via the bile the substance is subject to entero-hepatic circulation, which may lead to prolonged maintenance of pharmacologically active plasma levels, especially in persons with slow gastrointestinal transit.

Glycyrrhetic acid is an inhibitor of the enzyme 11-beta-hydroxysteroid dehydrogenase-2 (11-BOHD-2), and via this inhibition it causes a cortisol-dependent increased sodium and water retention and an increased potassium excretion. When compensatory mechanisms (i.e. suppression of the renin-angiotensin-aldosterone axis) are surpassed, pseudohyper-aldosteronism may result, which is reflected in oedema, hypokalaemia and increased blood pressure. It can be concluded that not the acute but the repeated intake of glycyrrhizinic acid causes the effects and clinical symptoms may develop within exposure periods of a few days to weeks.

For the evaluation of the biological effects of glycyrrhizinic acid many studies in humans have been published, in addition to animal toxicity data. Based on all available data,

notwithstanding some *in vitro* and *in vivo* weak positive findings of questionable biological meaning, glycyrrhizinic acid and its hydrolysis product glycyrrhetic acid are considered to be non-genotoxic. In a limited chronic study with mice, no carcinogenic potential was detected. No carcinogenicity study in rats is available**.** In one developmental toxicity study with rats, indications were obtained for increased incidences of skeletal variants and ectopic kidneys at a dose level of 680 mg/kg bw/day (highest dose tested). Maternal effects were limited to increased water intake and a slight statistically insignificant decrease of plasma potassium. The authors of the study considered the findings of questionable relevance. However, in another study with rats at dose levels up to 1480 mg/kg bw/day the changes could not be confirmed, despite signs of (slight) maternal toxicity. An epidemiological study has indicated that while consumption of glycyrrhizinic acid at >499 mg/week is associated with a slight reduction in the length of the gestation period, teratogenicity was not associated with intake of glycyrrhizinic acid at levels up to 2464 mg/week.

The available information indicates that the pharmacological effects in humans and animals are similar (perturbation of sodium/potassium/water homeostasis) and in the past human dose-response data, mainly from case studies have been used to derive (provisional) recommendations with respect to maximum daily intake of glycyrrhizinic acid. Recently, two repeated-dose studies in human volunteers have been published from which two no-observed-adverse-effect levels (NOAELs) of 2 mg/kg bw/day (Bijlsma *et al.*, 1996) or 217 mg/person/day (Bernardi *et al*., 1994) can be derived. The NOAEL obtained in the study by Bijlsma *et al.* (1996) is considered the more appropriate because this study comprised larger groups of volunteers, included a placebo control group, and the exposure lasted for a longer period. At the next dose level above the NOAEL of 4 mg/kg bw/day (the highest dose tested), in 9 out of 11 volunteers, water retention, slight decreases in plasma potassium and suppression of the renin-angiotensin-aldosterone axis was observed. In addition in 1/11 volunteers clinical effects were observed as well.

Using a PBPK/PD model, Ploeger (2000a) predicted that at the intake level of 100 mg/day, 4 per 10^4 exposed persons (95% confidence limits: 4.6 per 10^6 - 3 per 10^2) might show signs of “pseudohyperaldosteronism”, but these should be considered as preliminary

results, because they were largely dependent on one fairly small study with human volunteers. 100 mg/day was the intake provisionally established by the Committee in 1991 that should not be exceeded on a regular basis. The model by Ploeger (2000a) also provides insight into the determinants of differences in sensitivity in humans. Gastrointestinal transit time, sensitivity of the target enzyme (11-BOHD-2) to glycyrrhetic acid and basal 11-BOHD-2 activity seem to be the most important determinants. Although there is no direct evidence, from the biomedical literature (Rose, 1994; Stewart, 2002) it is conceivable that the health of people with Cushing's syndrome, or other conditions related to hypertension, abnormal electrolyte or water homeostasis, may be adversely affected by exposure to glycyrrhizinic acid or its ammonium salt.

Conclusion

Previously, the Committee evaluated the toxicological information for glycyrrhizinic acid and concluded that the data were inadequate to derive an ADI (SCF, 1991). At that time, the Committee considered it prudent that regular ingestion should not exceed 100 mg/day, while it was explicitly mentioned that this was a provisional figure. Since then, new toxicological information, including data from human volunteer studies, has become available. Although these data provide a stronger basis for the upper limit for regular ingestion of glycyrrhizinic acid of 100 mg/day, the Committee still is of the opinion that an ADI for glycyrrhizinic acid and ammonium glycyrrhizinate cannot be derived, because the new human toxicity studies are too limited (small experimental groups, short duration). The Committee considers that this upper limit for regular ingestion of 100 mg/day provides a sufficient level of protection for the majority of the population. It is noted that this upper limit includes the intake of glycyrrhizinic acid via all products, liquorice confectionery as well as glycyrrhizinic acid- or ammonium glycyrrhizinate-flavoured products.

At the same time, the Committee realises that within the human population there are subgroups for which this upper limit might not offer sufficient protection. These subgroups comprise people with decreased 11-beta-hydroxysteroid dehydrogenase-2 activity (the target enzyme of glycyrrhizinic acid, for which genetic polymorphisms resulting in reduced basal activity have been described), people with prolonged gastrointestinal transit time, and people with hypertension or electrolyte-related or water homeostasis-related medical

conditions. A more extensive discussion on sensitive subgroups in the population can be found in Annex II (section on modeling "Pharmacokinetic-pharmacodynamic model").

The Committee notes that for ammonium glycyrrhizinate as well as for glycyrrhizinic acid, used as chemically defined flavouring substances, the Upper Use Levels in foods indicate that the Maximised Survey-Derived Intake (MSDI) exposure estimates (130 and 240 μg/person/day, respectively) may underestimate the intake for individuals who select to consume certain foods, e.g. foods flavoured at the UULs (see Annex I).

To complete the evaluation of glycyrrhizinic acid and ammonium glycyrrhizinate as chemically defined flavouring substances, the following information needs to be provided:

- for glycyrrhizinic acid: specifications as required by Commission Regulation (EC) No. 1565/2000.
- for ammonium glycyrrhizinate: adequate data on solubility, as required by Commission Regulation (EC) No. 1565/2000.
- data on occurrence of the 18-alpha isomer in the commercial product. The Committee notes that glycyrrhizinic acid and ammonium glycyrrhizinate are evaluated here irrespective of their chirality.
- for both glycyrrhizinic acid and ammonium glycyrrhizinate: more refined usage data (e.g market share data), as it seems these substances are used in many food categories, but within a given food category probably only in very few products.

References

Anonymous (1985). 21 CFR Parts 182 and 184 GRAS status of licorice, (Glycyrrhiza), Ammoniated glycyrrhizin and monoammonium glycyrrhizinate. Federal Register **50**, 21043-21044.

Bernardi, M., D'Intino, P.E., Trevisani, F., Cantelli-Forti, G., Raggi, M.A., Turchetto, E., Gasbarrini, G. (1994). Effects of prolonged ingestion of graded doses of liquorice by healthy volunteers. Life Sciences, **55**, 863-872.

Bijlsma, J.A., Van Vloten P., Van Gelderen C.E.M., Mensinga Tj.T., Mout, H.A., Elvers L.H., Van Leeuwen, F.X.R., Stolker, A.A.M., Van Ginkel, L.A., Looman, C.W.N., Van der Maas, P.J., Koomans, H.A. and Savelkoul, T.J.F. (1996). Onderzoek naar de effecten van verschillende doseringen glycyrrhizine bij gezonde vrouwelijke vrijwilligers. [Study into the effects of different dosages of glycyrrhizin in health female volunteers; in Dutch]. RIVM report 348801004, RIVM, Bilthoven, The Netherlands

Blakey, S.A. (1998). Hypokalemia in a tobacco chewer: licorice link. J. Pharm. Pract., **11**, 78-79.

EFFA (2001). Ammonium glycyrrhizinate. European Flavour and Fragrance Association (EFFA). Submission to SCOOP Working group on Chemically defined Flavouring Substances, document No: SCOOP/FLAV/8.4, SCF document No: SCF/CS/ADD/MsAd/203

EFFA (2003). European Flavour and Fragrance Association (EFFA) Submission to the European Commission, February 20th, 2003.

EU (1999). Commission Decision of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council of 28 October 1996 (notified under number C(1999) 399) (text with EEA relevance) (1999/217/EC). Official Journal of the European Communities, **L084**, 27/03/1999, 1 - 137.

EU (2000). Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council (Text

with EEA relevance). Official Journal of the European Communities, **L180**, 19/07/2000, 8-16.

Hoffmann, D. and Hoffmann, I.(1997). The chancing cigarette, 1950-1995. Journal of Toxicology and Environmental Health, **50**, 307-364.

Hulshof, K.F.A.M. and Kistemaker, C. (1995). The consumption of licorice and the intake of ammonium chloride among Dutch population groups (second Dutch National Food Consumption Survey). TNO-report V 94-599. July, 1995.

Kistemaker, C., Bouman, M. and Hulshof, K.F.A.M. (1998) De consumptie van afzonderlijke producten door Nederlandse bevolkingsgroepen. Voedselconsumptie-peiling 1997-1998. [The consumption of separate products by Dutch population sub-groups. Food Consumption Survey 1997-1998; in Dutch]. TNO-report V98.812, TNO-Voeding, Zeist, The Netherlands.

Maas, P.(2000). Zoethout in levensmiddelen: onderzoek naar het glycyrrhizine gehalte van thee, kruidenmengsels, dranken en drop. [Liquorice root in food stuffs: survey of the glycyrrhizin content of tea, herbal mixtures, alcoholic drinks and liquorice.; in Dutch]. De Ware(n) Chemicus, **30**, 65-74.

Ploeger, B.A. (2000a). Development and use of a physiologically based pharmacokinetic-pharmacodynamic model for glycyrrhizinic acid in consumer products. PhD thesis University of Utrecht.

Rose, B.D. (1994). Clinical physiology of acid-base and electrolyte disorders. McGraw-Hill, Health Profession Division, New York.

Rosseel, M. and Schoors, D. (1993). Chewing gum and hypokalaemia. The Lancet, **341**, 175.

SCF (1991). Reports of the Scientific Committee for Food (29th series). Commission of the European Communities, Food Science and Techniques. Report No EUR 14482 EN, CEC, Luxembourg.

Stewart, P.M. (2002). Adrenal cortex: renin-angiotensin-aldosterone axis and hypertension. In: Besser, G.M. and Thorner M.O. (eds) Comprehensive clinical endocrinology. Elsevier Scientific Publ. Amsterdam.

Størmer, F.C., Reistad, R., Alexander J. (1993a). Adverse health effects of glycyrrhizinic acid in liquorice. A risk assessment. Nordiske seminar- og Arbejdsrapporter 1993:526.

Størmer, F.C., Reistad, R., Alexander J. (1993b). Glycyrrhizinic acid in liquorice - evaluation of health hazard. Food and Chemical Toxicology, **31**, 303-312.

Van Gelderen, C.E.M., Bijlsma, J.A., Van Dokkum, W. and Savelkoul, T.J.F (2000). Glycyrrhizinic acid: the assessment of a no effect level. Human and Experimental Toxicolology, **19,** 434- 439.

Wang, Z.Y., Athar, M. and Bickers, D.R. (2000). Licorice in foods and herbal drugs: chemistry, pharmacology, toxicology and uses. In: Mazza, G. and Oomah B.D. (2000) Herbs, Botanicals and Teas. Technomic Publ. Co. Inc. Lancaster, UK. pp. 321-353.

ANNEX I

Upper Use Levels (UULs) for glycyrrhizinic acid and ammonium glycyrrhizinateas chemically defined flavouring substances in food

FOOD CATEGORIES		UUL*	
		Glycyrrhizinic acid	Ammonium glycyrrhizinate
1	Dairy products, excluding products of category 2	375	40
2	Fats and oils, and fat emulsions (type water-in-oil)		
3	Edible ices including sherbet and sorbet	375	95
4	Processed fruits and vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds		
1	Fruit		
2	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds		
5	Confectionery Chewing gum Sweets (candy) Fondant	$ 400 - 1500 - 5000	150 1550
6	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery		45
7	Bakery wares	200	65
8	Meat and meat products, including poultry and game	25	
9	Fish and fish products, including molluscs, crustaceans and echinoderms	20	300
10	Eggs and egg products		
11	Sweeteners including honey		100
12	Salts, spices, soups, sauce, salads, protein products, etc.		50
13	Foodstuffs intended for particular nutritional uses		60
14	Beverages, excluding dairy products	50	100@
1	Non-alcoholic ("soft") beverages	50	200@
2	Alcoholic beverages	135 - 550#	200@
15	Ready-to-eat savouries		150
16	Composite foods (e.g. casseroles, meat pies, mincemeat), foods that could not be placed in categories 1 to 15	10	80

* UULs are given in mg/kg product (EFFA 2001, 2003).

$ For these products only a rather poorly defined range of UULs was given.

A range of UULs was given for products like liquors, aromatised wines, aromatised wine-based drinks, aromatised wine-product cocktails. Spirits < 15% alcohol: coolers, light alcoholic drinks.

@ In several alcoholic and non-alcoholic drinks and in teas, levels of glycyrrhizinic acid have been detected which are higher than the UULs specified in this table (see main text, section on exposure). This is probably explained by the fact that these beverages and teas are not flavoured with glycyrrhizinic acid or ammonium glycyrrhizinate as such, but are produced with *Glycyrrhiza* plant extracts or with dried plant material.

References to Annex I

EFFA (2001). Ammonium glycyrrhizinate. European Flavour and Fragrance Association (EFFA). Submission to SCOOP Working group on Chemically defined Flavouring Substances, document No: SCOOP/FLAV/8.4, SCF document No: SCF/CS/ADD/MsAd/203

EFFA (2003). European Flavour and Fragrance Association (EFFA) Submission to the European Commission, February 20th, 2003.

ANNEX II

Overview of toxicological background information for glycyrrhizin and glycyrrhetic acid

Introduction

Since the opinion of the Committee 1991, important new information has become available. Not only the evaluation by the Nordic Council of Ministers (Størmer *et al.*, 1993a), but also new studies in human volunteers and some new case studies have been published. In addition, extensive physiologically based toxicokinetic-dynamic models have been developed, which contribute to a better understanding of the phenomena observed in humans exposed to glycyrrhizinic acid. Furthermore, studies have been published in which the matrix effect of liquorice on the availability of glycyrrhizinic acid has been studied.

In contrast to many other substances, for glycyrrhizinic acid (and several salts) a considerable amount of human data are available. A wealth of animal data are available, some of which is rather old, which contributes to the overall understanding of the effects that are elicited by these substances. The data also show that the effects observed in humans and animals are similar. Much of this information has been reviewed by Størmer *et al* (1993a) and Ploeger (2000a) and therefore it is only briefly mentioned here, with reference to these two sources. The opinion of the Committee of 1991 was based on human data. In the present document, only relevant animal data for toxicological end-points for which there are no (extensive) human data are presented.

Toxicokinetic and toxicodynamic data

Absorption

Glycyrrhizinate as such, whether taken as free acid or as ammonium salt is not or hardly absorbed from the GI tract. Only at very high dose levels is glycyrrhizinic acid detectable in rat or human plasma after oral administration. However, the substance is hydrolysed in the GI tract most likely by bacterial activity, and the free aglycone, glycyrrhetic acid (the biologically active form; see below), is readily absorbed and can be detected in plasma, even after low doses. The time at which maximum plasma levels of glycyrrhetic acid are reached after oral intake of glycyrrhizinic acid, is similar in rats and humans (12-16 h and

8-12 h, respectively). At higher dose levels (> 25 mg/kg) the rate of hydrolysis of glycyrrhizinic acid may become saturated, thereby limiting the relative amount of glycyrrhetic acid that can be absorbed from the GI-tract. In human volunteers, after oral administration of an extract of *Glycyrrhiza* plant material, absorption of glycyrrhetic acid was *ca* 75% compared to the absorption from orally administered pure glycyrrhizinic acid (Cantelli-Forti *et al.*, 1994). Other human data have indicated that the relative bioavailability of glycyrrhetic acid is independent of the form in which it was administered (either as pure aglycone, or as pure glycoside or as glycoside present in a liquorice matrix; Mensinga *et al.*; 1998 see below). According to data presented by Størmer (1993a), absorption of glycyrrhetic acid from the GI tract is virtually complete. Combination of the data from Størmer (1993a) and from Mensinga *et al.*(1998) would indicate that the absorption of glycyrrhetic acid from the gut is virtually complete, irrespective of the form (viz. glycoside or aglycone) or the matrix (pure substance or solid liquorice matrix) in which it is administered.

Pharmacokinetics of glycyrrhizinic acid in rat bile have been determined after a single oral administration. Peak levels of glycyrrhizinic acid in bile samples from rats treated orally with the pure substance (480 mg/kg bw) were approximately 5 times higher than peak levels observed after administration of the same amount of pure glycyrrhizinic acid in the form of a liquorice extract. In case of glycyrrhetic acid, concentrations were very low, often below the detection limit (0.2 μg/ml bile). Based on comparison of total biliary excretion over 16 h post-dosing, the study indicates that biliary excretion and therefore gastrointestinal absorption of glycyrrhizinic acid administered as pure substance is about 7-8 times higher than when administered as liquorice extract. No such comparison could be made for glycyrrhetic acid, the biologically acitive form. In addition, glycyrrhetic conjugates were not determined and in particular these are subject to entero-hepatic circulation. (Cantelli-Forti *et al*., 1997).

The Committee noted that the study provides only information on the biliary kinetics of glycyrrhizinic acid, administered in only one dose level at which intra-intestinal hydrolysis is likely to be saturated. It does not provide information on the kinetics of glycyrrhetic acid. The amount of glycyrrhizinic acid that was retrieved in the bile was only *ca.* 1% of the dose administered. The Committee considers the study by Mensinga *et al.* (1998) in human

volunteers, of greater importance.

Distribution, metabolism and elimination

Neither glycyrrhizinic acid nor glycyrrhetic acid are taken up by tissues to any major extent. The main compartment for distribution is the plasma, where they are bound to serum albumin. Human lysosomal enzymes can only hydrolyse one of the glucuronide moieties of glycyrrhizinic acid, while rat lysosomal enzymes can split off both glucuronide groups. From studies with bile duct cannulated rats it can be concluded that an intravenous dose of glycyrrhizinic acid is excreted predominantly unmetabolised via the bile. Only at very high dose levels, up to 5% of an iv dose can be found in the urine. With iv doses of glycyrrhetic acid, in rats it has been demonstrated that sulphate and monoglucuronide conjugates but hardly any parent substance are excreted into the bile. Complete biliary excretion of an intraperitoneal dose of glycyrrhetic acid could be demonstrated within 5 h post dosing. All of the dose had been metabolised. Enterohepatic circulation of glycyrrhetic acid has been demonstrated in rats. Human data with respect to biliary excretion are not available, but since glycyrrhetic acid metabolites can be hydrolysed by bacteria in the human gastro-intestinal tract, in humans such a circulation can be expected (Ploeger, 2000a). Time curve data for plasma glycyrrhetic acid in humans have also provided evidence that in humans enterohepatic circulation does occur (Mensinga *et al.,* 1998; see below*).*

Toxicodynamic data

The most prominent effect of glycyrrhizinic acid is elicitation of a syndrome known as "pseudohyperaldosteronism". Originally it was thought that this was the result of a direct interaction of glycyrrhizinic acid and the aglycone glycyrrhetic acid with the mineralocorticoid receptor. However, at the end of the 1980's it became clear that glycyrrhizinic acid and glycyrrhetic acid produce "pseudohyperaldosteronism" because of interference with the cortisol/cortisone homeostasis. The 18-beta- form of glycyrrhetic acid is an inhibitor of the enzyme 11-beta-hydroxysteroid dehydrogenase-2 (11-BOHD-2), which is responsible for the conversion of cortisol to cortisone. Under normal circumstances this inactivation allows aldosterone to control mineral and water homeostasis. However, when the enzyme is inhibited, aldosterone is displaced from the

mineralocorticoid receptors by cortisol, thereby increasing mineralocorticoid activity. This results in sodium and water retention, in potassium excretion and in metabolic alkalosis. In addition, decreased plasma aldosterone and renin activity are observed, which can be considered a compensatory reaction to the over-stimulation of the mineralocorticoid receptors. Clinical signs are oedema and hypertension. In severe cases destruction of skeletal muscles (rhabdomyolysis and myopathy), ventricular tachycardia and renal failure may occur (Størmer *et al.*, 1993a; Bijlsma *et al.*, 1996; Ploeger, 2000a).

Animal studies

Acute toxicity

According to EFFA (2001), no standard oral LD50 studies are available. In the Hazardous Substances DataBase (2002) an oral LD50 in the rat for glycyrrhizinic acid of 14.2 g/kg bw has been mentioned.

Groups of 4 female adult rats were dosed with 0.5, 1, 1.5 or 2 g/kg bw of either 18-alpha or 18-beta glycyrrhetic acid ammonium salt, via intraperitoneal injection. Dose levels up to 1.5 g/kg bw were well tolerated and no changes in electrocardiograms were observed. However, at 2 g/kg bw of the 18-alpha isomer, but not the 18-beta isomer, all animals died due to atrio-ventricular block. Post mortem examination of these animals revealed brain, cerebellum and lung oedema and haematic stasis in kidneys, adrenals, spleen and liver. In two rats calcium salt calculi were observed in the kidneys. Focal changes in the papillary cardiac muscles were reported, showing oedema, myolysis and cell distortion with granular cardiomyocytes with pyknotic nuclei. Apoptosis of papillary muscle cells was observed (Rossi *et al.*, 1999).

Repeated dose toxicity study

Groups of 40 female adult rats were orally dosed with 30 mg/kg bw glycyrrhizinic acid, 15 mg/kg bw 18-beta-glycyrrhetic acid or 15 mg/kg bw 18-alpha glycyrrhetic acid, dissolved in water for up to 30 days. A fourth group received only water. Per group, 10 rats were sacrificed before treatment, 15 rats were sacrificed after 15 and 30 days of treatment, and 10 rats were sacrificed at d30 days after cessation of treatment.

Damage of the cardiac muscles (milder than observed in the acute studies, see above) was observed with glycyrrhizinic acid and 18-alpha glycyrrhetic acid after 15 and 30 days of treatment, and this damage appeared to be irreversible. Both glycyrrhizinic acid and 18-alpha glycyrrhetic acid caused renal tubular calculi and slight expansion of the bronchus-associated lymphoid tissue in the lungs after 15 days. These changes were not observed with the 18-beta isomer. Urinary electrolyte levels showed increased sodium, potassium and calcium excretion after 15 days of dosing with 18-alpha glycyrrhetic acid, but not with glycyrrhizinic acid or the 18-beta-isomer. After 30 days increased potassium and calcium excretion was also seen with the 18-beta isomer, but to a lesser extent. Thirty days after cessation of treatment, urinary electrolyte excretion with the 18-beta isomer returned to normal but in the 18-alpha -isomer-treated animals the situation was worse. In the blood only an increase in plasma sodium was observed, with all three treatments after both 15 and 30 days, which was completely reversed after 30 days of withdrawal. The authors concluded that the 18-alpha glycyrrhetic acid is considerably more toxic than the 18-beta-isomer, but a mechanistic explanation was not provided. It was also not clear whether the cardiac effects were a primary effect or secondary to changes in the renin-angiotensin system (Rossi *et al.*, 1999).

Genotoxicity

The genotoxicity of glycyrrhizinic acid has been studied in *in vitro* and in *in vivo* test systems. It should be noted that in *in vivo* studies glycyrrhetic acid rather than glycyrrhizinic acid is tested, because of presystemic hydrolysis of the latter. In i*n vitro* studies conversion of glycyrrhizinic acid to glycyrrhetic acid is probably not as effective as after oral administration *in vivo*.

Disodium and trisodium glycyrrhizinate did not induce gene mutations in *Salmonella typhimurium* strains TA 92, TA 1535, TA100, TA1537, TA94 and TA98 in dose levels up to 5 or 10 mg/plate, respectively, with and without metabolic activation (Ishidate *et al.*, 1984).

Liquorice powder and ammonium glycyrrhizinate were not genotoxic at concentrations ranging from 0.01 to 0.5 mg/ml in TA97, TA98 and TA100 *Salmonella* tester strains either with or without metabolic activation (Cooper and Berry, 1988; only abstract available).
Disodium but not trisodium glycyrrhizinate induced structural chromosomal aberrations in Chinese hamster fibroblast cultures (no specification of percentage of gaps). With trisodium only polyploid cells were induced (both substances tested only in absence of metabolic activation; Ishidate *et al*., 1984).

Disodium or trisodium glycyrrhizinate did not induce micronuclei in bone marrow cells of male ddY mice after single intraperitoneal injections with doses ranging from 0-140 mg/kg bw or 0-2000 mg/kg, respectively. Absence of micronuclei induction was also found after 4 daily intraperitoneal injections with 20 mg/kg (disodium salt) or 500 mg/kg bw (trisodium salt). With the trisodium salt, signs of bone marrow toxicity were observed after the single dose of 2000 mg/kg bw and after 4 daily injections with 500 mg/kg bw. (Hayashi *et al.*, 1988).

Ammonium glycyrrhizinate induced statistically significant dominant lethal effects (dead implants in offspring) in male rats at the maximum tolerated dose (40 g/kg in the diet). In mice no increases in dominant lethal events were observed at a dietary levels of 22.5 g/kg (maximum tolerated dose). It did not induce heritable translocations in the same mice also treated at 22.5 g/kg in the diet (Sheu *et al.*, 1986).

Based on all available data, notwithstanding some *in vitro* and *in vivo* weak positive findings of questionable biological meaning, glycyrrhizinic acid and its hydrolysis product glycyrrhetic acid are considered to be non-genotoxic.

Carcinogenicity

In a carcinogenicity study with B6C3F1 mice, groups of 50-70 males and females of 8 weeks of age were exposed to disodium glycyrrhizinate via the drinking water for 96 weeks. After the exposure period the animals were maintained for another 14 weeks withdrawal period.Concentrations in the drinking water were 0, 40, 80 and 150 mg/l for the males and 0, 80, 150 and 300 mg/l for the females, resulting for the males in dose levels of

0, 71, 166 and 229 mg/kg bw/day and for the females in dose levels of 0,117, 217 and 407 mg/kg bw/day (Kobuke *et al.*, 1985). For both sexes the respective top dose was equal to the MTD as determined in a 10 week pre-test. In this pre-test 100% mortality occurred at drinking water concentrations of 600 or 1250 mg/l in both sexes. At 300 mg/l, one male died within the first 4 weeks, but in the females no mortality was seen at this concentration. All animals exposed to 300 mg/l or higher showed marked atrophy in all visceral organs, but no changes were seen at lower drinking water concentrations.

A dose-related reduction in drinking water intake was observed in both males and females. At week 78 survival was 40, 40, 33 and 50% for the males and 90, 76, 90 and 70% for the females. In total 49 males and 18 females survived until the end of the study (week 110). Effects on body weights were not observed. No effect on tumour incidence or "time to death with tumours" was observed in either sex. Commonly observed tumours were liver cell tumours in the males and lymphoid leukaemia in females, but without any relationship to treatment. In male animals older than 52 weeks amyloidosis was observed at a high incidence, frequently affecting spleen, liver, kidney and/or adrenals. This condition was observed in 51% and in 33% of control and treated males, respectively, but not in any of the female treatment groups. It is noted that the mortality in his study was rather high. In the males mortality occured at a more or less constant rate thoughout the study, irrespective of the dose level. Mortality rates in all female groups increased sharply at about week 80. In the females, mortality in the top dose group was increased during the first 80 weeks of the study. Due to insufficient detail in the paper a more thorough (statistical) analysis of the mortality data is not possible.

No carcinogenicity data are available for the rat.

Reproductive and developmental toxicity studies

No standard reproductive toxicity studies with (salts of) glycyrrhizinic acid are available. However, ammonium glycyrrhizinate has been tested in dominant lethal tests in mice and rats and a heritable translocation test in mice at dietary exposure levels up to the maximum tolerated dose for exposure periods of 8 or 10 weeks for male mice and rats, respectively. No effects on male fertility were observed (Sheu *et al.*, 1986).

Groups of 14-17 female rats were administered 0, 0.08, 0.4 and 2% disodium glycyrrhizinate via the diet (average dose levels equal to 0, 60, 290 and 1480 mg/kg bw/day) from day 0 to day 20 of gestation. At day 20, per group 9-12 of the dams were killed and their litters examined for external and skeletal and visceral malformations. The remaining dams were allowed to deliver and rear their litters. These litters were allowed to grow up to 8 weeks post partum. No significant effects were observed during pregnancy or postnatally, apart from a reduction in maternal body weight gain *post-partum* in the mid- and high dose group. No indications for a teratogenic potential for disodium glycyrrhizinate were obtained (Itami *et al.*, 1985).

In a study by Mantovani *et al.* (1988) 16-20 pregnant rats were given ammonium glycyrrhizinate during day 7-18 of gestation via the drinking water in concentrations of 0, 100, 1000 and 2500 mg/l (equal to 0, 21, 239 and 680 mg/kg bw/day). At day 20, the dams were bled and blood samples were studied for aldosterone, sodium and potassium. Maternal adrenals were collected for histological examination. The uteri were examined for signs of embryo- and foetotoxicity and the offspring were studied for external, skeletal and visceral malformations. The only clinical sign of an effect in the dams was an increased water intake in the mid and high dose groups. No significant effects were observed except for a statistically significant increase in total skeletal variants at the high dose. Sternebral variants were significantly increased in both mid and high dose groups. At the low and high dose, a statistically significant increase in external haemorrhages and haematomas was found, but not in the mid dose, where an increase in internal haemorrhages was seen. Soft tissue examination revealed a higher incidence in ectopic kidneys in the low and high dose group litters and a decrease in other kidney variants in the low dose group. The authors indicated that the skeletal variants should be considered as a reversible effect of doubtful toxicological significance, while ectopic kidney is (partly) subjective. According to the study authors, the data indicate a slight adverse effect of ammonium glycyrrhizinate on foetal rats. The NOAEL in this study was 239 mg/kg bw/day.

Human studies

Many case reports noting adverse effects have been summarised by Størmer *et al*. (1993a). Five of these cases consumed very large amounts of liquorice confectionery (up to 1.8 kg/week, equivalent to doses of glycyrrhizinic acid of *ca.* 500 mg/day) for a long time. In another case a patient had consumed a laxative providing a dose of approximately 150 mg glycyrrhizinic acid for two to three times a week during 2-3 years. In a clinical study with this patient, daily administration of the laxative, resulting in exposure levels of 94 mg/day, resulted in profound hypokalaemia with sodium retention and depression of the renin-angiotensin-aldosterone system. The potsssium loss may have been aggravated by the laxative effect (Cummings *et al*., 1988). Other case studies refer to adverse effects in people who took glycyrrhzinic acid at daily dose levels up to 130 mg for only several days to weeks.

In a sequence of clinical trials, body weight gain, water retention, oedema and increased blood pressure were seen in 17 persons taking 1560 mg glycyrrhizinic acid/day during an undefined period of time. In 6 of these volunteers, intake of 780 mg/day resulted in less pronounced body weight increases and in 4 of these 6 also increased blood pressure was observed. One very sensitive person from this group of 17 showed an increase in blood pressure, when 130 mg/day was taken for a longer (unspecified) period of time. These clinical studies were performed with ”succus liquoritiae”, containing 26% of glycyrrhizinic acid (Smorenberg-Schoorl and Vree, 1963).

In another clinical study, effects related to pseudohyperaldosteronism were seen in groups of 8 male and 8 female volunteers exposed to pure glycyrrhizinic acid at doses of 400 mg and 800 mg/day during 2-4 weeks. It was concluded that females were more sensitive than males (Van Vloten *et al.,*1989).

<u>New case reports</u>

In the past decade several new cases of more or less severe intoxications with glycyrrhizinic acid have been reported. In the present opinion, only those studies are cited that show that occasionally quite serious adverse effects may occur at exposure levels at, or (only slightly) below the maximum level provisionally advised by the Committee (SCF,

1991), or because they show that adverse effects do not only occur after consumption of liquorice confectionery, but also after consumption of chewing gum and of (large) amounts of liquorice tea.

A 42 year-old man was referred to the hospital in a soporific state. He had experienced worsening headaches, nausea, vomiting and sensitive neuropathy on the left side of the body. An onset of slight hypertension had been diagnosed 6 months earlier. Clinical examination showed a blood pressure of 200/140 mm Hg and decreased plasma potassium (2.9 mmol/l, while 3.8-5 mmol/l is normal) and aldosterone levels (180 pmol/l instead of 320-2000 pmol/l). The patient's plasma potassium could not be easily restored by supplementation, and it was noted that he ate about 50 g of liquorice confectionery a day (estimated to correspond to about 100 mg glycyrrhizinic acid). He was advised to stop and after two weeks he was discharged from hospital without symptoms. His blood pressure had fallen to 120/85 mm Hg, while his plasma potassium concentration had risen to 4 mmol/l (Russo *et al.* (2000).

A second patient (46 year-old male) who consumed about 40 g of liquorice confectionery a day was hospitalised with deep asthenia, headaches and somnolence. Two weeks earlier hypokalaemia was observed (2.3 mmol/l) which did not respond to potassium supplementation. The patient was sluggish, did not respond to environmental stimuli and showed muscle weakness. His blood pressure was 215/125 mm Hg and an electrocardiogram showed sinus bradycardia and T-wave levelling. Plasma clinical chemistry was normal except for potassium, aldosterone and renin, which were all depressed. Despite treatment with potassium supplementation and anti-hypertensives, his condition only improved when he stopped eating liquorice confectionery. Upon discharge, his blood pressure had fallen to 150/80 mm Hg (Russo *et al.,* 2000). According to the study authors, similar cases of encephalopathy have been reported previously only after consumption of 500 g of liquorice confectionery which would correspond to 1000 mg glycyrrhizinic acid. This is far above the tentatively derived human LOAEL of 100 mg/day (Størmer *et al.*, 1993a), which is associated with hypertension without complications. According to the authors a significant inter-individual variation in sensitivity occurs within

the human population. It was further speculated that this variability might be associated with a partial 11-BOHD-2 deficiency.

In another case report a woman (41 years of age) was diagnosed with "essential hypertension" and treated with anti-hypertensives, to which her condition did not respond. Her blood pressure was 210/115 mm Hg. After adding a diuretic to the medication, she developed a hypokalaemia (1.9 mmol/l), with muscle cramps and muscle weakness. The plasma potassium level did not sufficiently respond to potassium supplementation. The woman also suffered from polyuria (4 l/day) but denied eating liquorice sweets. However, she drank 3 l of liquorice tea a day. After the consumption of this tea was stopped, blood pressure and potassium level gradually improved in the next two months. The study authors did not analyse the particular tea for glycyrrhizinic acid, but based on the average level of 126 mg/l in liquorice teas (range 2-450 mg/l), which can be derived from the data by Maas (2000), daily intake of glycyrrhizinic acid may have been 375 mg (Brouwers and Van der Meulen, 2001; range 6.5-1350 mg).

A 55-year old male patient was referred to hospital with atypical abdominal pain, which developed after he had quit smoking, but started to use chewing gum. He had an increased blood pressure, despite taking anti-hypertensives, a low plasma renin activity and a hypokalaemia, but the plasma aldosterone level was normal. Upon enquiry he told that he used two packs of a chewing gum (a brand not known to contain nicotine) , which according to the manufacturer contained 24 mg of glycyrrhizinic acid per pack of 16 g. Hence the patient was taking about 50 mg glycyrrhizinic acid/day. After quitting the use of chewing gum, the patient's blood pressure returned to normal (without the use of anti-hypertensives) with normal plasma potassium levels and no abdominal pains (Rosseel and Schoors; 1993).

New volunteer studies

The bioavailability of glycyrrhetic acid from liquorice was studied in 16 human volunteers (8 males, 8 females), who were exposed to equimolar amounts of A: 130 mg glycyrrhetic acid in an aqueous suspension, B: 225 mg glycyrrhizinic acid in an aqueous solution; C: 225 mg glycyrrhizinic acid in 150 g of sweet liquorice candy and D: 225 mg glycyrrhizinic

acid in 150 g of a salted liquorice candy. Every participant received all treatments, with a dose interval sufficiently long to avoid interference between the various treatments. Plasma levels of glycyrrhetic acid were observed for up to 56 hr post dosing and reached peak values after 3 hr (treatment A) or 8-10 hr (treatments B-D). No intra-individual differences were observed in the relative bioavailability of glycyrrhetic acid from the 4 different treatments. However, between the participants, Cmax and AUC values varied by factors of about 4 to 5, respectively: e.g. with treatment C: range Cmax 0.4 to 1.6 mg/l and range AUC: 4.8 tot 23.1 mg/l*hr. With respect to elimination of glycyrrhetic acid from the body, even within this small group of participants two sub-populations could be distinguished: one (9 persons) in which the plasma glycyrrhetic acid levels declined mono-exponentially with time and a second one in which elimination followed a biphasic pattern with very long terminal plasma half-lives (up to 35 hr) and occurrence of a secondary plasma peak at 18-32 hr post dosing, indicative of enterohepatic circulation. No differences in kinetics were observed between male and female participants (Mensinga *et al.*, 1998).

Sigurjónsdóttir *et al.* (2001) studied the relationship between intake of glycyrrhizinic acid and hypertension in human volunteers. These volunteers were divided into three different groups. A high dose group consisting of 9 women and 1 man (540 mg glycyrrhizinic acid/day), a mid dose group with 19 women and 11 men (270 mg glycyrrhizinic acid/day) and a low dose group with 12 women and 12 men (75 mg glycyrrhizinic acid/day). The glycyrrhizinic acid was administered in the form of sweet liquorice confectionery, which was obtained from two different producers (low dose from one supplier, and mid and high dose from another). A placebo control group was not studied, and the volunteers were fully aware of their treatment. The volunteers were studied in three separate experiments. Blood pressure was the only parameter studied.

The study authors reported that for intake of glycyrrhizinic acid and hypertension a linear dose-response relationship could be established. The regression co-efficient for this dose-response relationship amounted to 0.011 and 0.014 for 2 and 4 weeks of liquorice consumption, respectively. Based on the graphically presented data, the relationship depended heavily on 2 out of 64 volunteers; one with low and one with high glycyrrhizinic acid intake. No other parameters indicative for pseudohyperaldosteronism were studied and

no indication was provided as to the bioavailability of the glycyrrhetic acid from the different brands of liquorice. There are so many flaws in the study design that it can only be concluded that the claim for the weak linear dose-response relationship is scientifically insufficiently underpinned.

Four groups of 10 healthy female volunteers received orally 0, 1, 2 or 4 mg pure glycyrrhizinic acid/kg bw/day for 8 weeks after an acclimatisation period of two weeks (Bijlsma *et al*., 1996; Van Gelderen *et al*., 2000). The study was done according to a placebo-controlled, randomised, double blind design. The exposure period was followed by a wash-out period of two weeks. Criteria for study enrolment were age 18-40 years, body weight 50-90 kg, not taking oral contraceptives, not being pregnant or breast-feeding, plasma potassium > 3.5 mmol/l, and a positive outcome of a physical examination, including blood electrolytes and kidney function tests. During the study the volunteers were not allowed to smoke, to consume any glycyrrhizinic acid-containing food product or to take alcoholic drinks, medication or drugs. The study was performed with female volunteers, because in a pilot study with higher dose levels (Van Vloten *et al.,*1989) women appeared to be more sensitive to glycyrrhizinic acid than men. In the study the following parameters were investigated: physical examination, physical condition, body weight, blood pressure, oedema, plasma-potassium, sodium, chloride, calcium, bicarbonate, plasma renin, aldosterone and atrial natri-uretic peptide and urinary potassium, sodium and creatinine. In addition plasma glycyrrhetic acid levels were determined. The volunteers filled out a diary for physical complaints every day and a dietary questionnaire had to be filled out every other week.

During the study one volunteer from the 2 mg/kg group dropped out because of hypokalaemia (not cross-checked but reversed after one week) and one volunteer from the 4 mg group left the study because of loss of concentration and general discomfort, oedema, body weight gain and raise of blood pressure. Headache, nausea, change in defecation pattern, swollen face and tickling in arms and legs occurred somewhat more often in the 4 mg/kg group, but the overall number of complaints diminished in all groups during the experiment. Although at the beginning of the exposure period an increase in body weight of about 1 kg was observed in 23/28 participants in the 1, 2 and 4 mg/kg bw groups, statistical significance was only observed in the 1 and 4 mg/kg groups. No consistent

changes in body weight were found later in the study. Average plasma glycyrrhetic acid levels increased gradually during the first 7 days of the exposure period and remained about constant during the next 8 weeks at about 0, 0.16, 0.26 and 0.94 mg/l in the 0, 1, 2 and 4 mg/kg bw/day groups, respectively. After the two weeks post-exposure period, glycyrrhetic acid could no longer be detected in any of the volunteers.

The highest dose of 4 mg/kg bw/day caused a significant reduction of plasma renin activity and aldosterone concentration, while at the dose of 2 mg/kg bw/day only a non-significant decrease in aldosterone concentration could be observed. The atrial natri-uretic peptide was significantly increased at 4 mg/kg at the end of the exposure period, but decreased to normal values after two weeks of wash-out. Average plasma potassium levels decreased significantly during exposure in the 4 mg/kg bw/day group and non-significantly in the 2 mg/kg bw/day group, but were never outside the normal physiological range. In the 4 mg/kg bw group, the plasma bicarbonate concentration was significantly increased as compared to the control group. The mean systolic and diastolic blood pressures in the 4 mg/kg-group remained more or less constant but as the values decreased in the control group, a statistically significant difference with the control group was observed. Both systolic and diastolic blood pressures in all groups remained within the normally accepted range. Volume expansion was observed, which may lead to hypertension at a later stage. No relevant dose-related differences between control and exposed groups were observed in any of the other parameters studied.

The body weight changes in the 1 mg/kg group were not accompanied by changes in the renin-angiotensin-aldosterone system and are therefore considered not treatment-related. In this study the NOAEL for glycyrrhizinic acid can be established at 2 mg/kg bw/day (on average equal to *ca.* 130 mg/person/day (Bijlsma *et al.*, 1996; Van Gelderen *et al.*, 2000). At this dose level, the plasma glycyrrhetic acid level was never higher than 800 microg/l in any of the volunteers.

Bernardi *et al.* (1994) administered daily doses of 108, 217, 380 and 814 mg glycyrrhizinic acid, as 'liquorice pills' during 4 weeks to four groups of 3 male and 3 female healthy volunteers. The liquorice was a dried aqueous root extract. The glycyrrhizinic acid content,

as assayed by HPLC, was 7.64% w/w ratio. Different dose levels were achieved by giving different numbers of pills to the participants in the respective groups. A control group was not incorporated in the study, but changes were detected by comparison with baseline values. Note that in this study the statistical evaluation of the data was performed by comparison of group averages at baseline level with group averages after 1, 2, or 4 weeks of treatment. The study might have gained in sensitivity if the baseline data and post exposure data would have been compared on an individual basis. The following parameters were studied: body weights, wrist, ankle and mid-arm muscle circumference, triceps skin fold, heart rate, mean arterial pressure, creatinine clearance, daily diuresis, daily renal sodium and potassium excretion, serum sodium and potassium, plasma renin and aldosterone concentrations, blood glucose and haematocrit.

Three persons were withdrawn from the experiment at the end of week 2. One female from the group administered 380 mg experienced continuous headache. Another female from the group administered 814 mg complained of headache, increase in body weight and peripheral oedema. She also showed borderline arterial hypertension and hypokalaemia. It should be noted that she was taking an oestrogenic-progestagenic drug at the same time. The remaining drop-out was a man at 814 mg who developed arterial hypertension. His family history was positive for this effect. These adverse effects subsided within 24-48 hrs after treatment suspension. The two remaining females at 814 mg showed mild periorbital oedema and body weight increase through the second week which was their pre-menstrual period. Afterwards, oedema disappeared and body weights decreased again, though not completely to the baseline value. There were signs of sodium retention in the volunteers of the 380 and 814 mg dose groups, but the changes in renal sodium excretion reached statistical significance only in the 380 mg group after 3 weeks. It was noted that renal sodium excretion showed marked temporal variability among the subjects. Plasma renin-activity was significantly decreased in volunteers at 380 and 814 mg. A decline in plasma aldosterone concentration was also observed in these two groups, but the decrease reached statistical significance only at 814 mg. In the highest dose group the volunteers showed an increase in body weight and a reduction in serum potassium concentration which reached statistical significance at week 2 and week 1, respectively. However, the serum potassium concentration in the 814 mg dose group was consistently lower than in any of the other

groups at any time point (1, 2 or 4 weeks). The NOAEL based on the study report was 217 mg/person/day. At higher dose levels sodium retention and depression of plasma renin and aldosterone levels were observed. According to the study authors, the female participants were slightly more sensitive to glycyrrhizinic acid than the males. (Bernardi *et al.*, 1994).

Epidemiological studies

Reproductive toxicity

In an epidemiological study (Strandberg *et al.*, 2001), the influence of liquorice consumption on several gestation and offspring parameters was studied in 1049 pregnant women in Finland, who gave birth to singleton healthy babies between March and November 1998. Glycyrrhizinic acid intake was assessed from questionnaire information on liquorice consumption together with data on the course of the pregnancy and parturition. The cohort was split into three subgroups with respect to their glycyrrhizinic acid intake, with low (< 250 mg /wk; n=751), medium 250-499 mg/wk; n=145) and high (> 499 mg/wk; n=110) intakes, respectively. Of the entire cohort 2.3% reported not to use liquorice at all. Average glycyrrhizinic acid intake among the liquorice consumers amounted to 363 mg/wk (S.D. 348; range 1-2464 mg/wk). No correlation could be found between glycyrrhizinic acid intake and birth weight. When glycyrrhizinic acid intake was considered as a continuous independent variable, regression analysis showed that for every 500 mg increase in glycyrrhizinic acid intake, the length of gestation diminished by 1.26 days ($p = 0.009$, 95% confidence interval 0.31-2.24). When considered as three sub-cohorts (see above) a significant decrease of the length of the gestation period was only found in the high intake group. When the high intake group was compared to the low intake group, the odds ratio for delivery < 38 weeks was 2.5 (95% confidence interval 1.1-5.5; $p = 0.03$). No effects were observed on any of the other pregnancy parameters studied, on birth weight or on type of delivery, and no effects were observed on maternal blood pressure. Although the authors speculated about a causal relationship between glycyrrhizinic acid intake and reduced length of the gestational period, they could not definitely conclude that this relationship occurred. Confounding factors might have influenced the outcome of the study (e.g. a positive correlation was also observed between glycyrrhizinic acid intake and intake of chocolate, which was not corrected for). No comparison was made with a group of mothers who did not take liquorice during pregnancy.

Colley and Gibson (1982) studied the outcome of 6408 singleton pregnancies, all supervised by one hospital. 313 Mothers had taken cough mixtures during pregnancy, and 137 of these had used a medication ("S&A") which contained an unspecified amount of liquorice extract. No changes were observed in rates of congenital malformations or perinatal death. Non-significant increases in the rates of low birth weight, poor Apgar score and intrauterine growth retardation were observed for the users of "S&A", as compared to the control group, but it is impossible to attribute these changes to liquorice intake, because the intake of liquorice from other sources was not studied (neither for users nor for controls). No stratification for smoking, maternal age, premature birth (total incidence slightly decreased in the user group) or other confounding factors was applied. Because of these limitations, the study is not useful for the evaluation of the reproductive toxicity of glycyrrhizinic acid.

Pharmacokinetic-pharmacodynamic model

Ploeger and co-workers (2000b, 2001a, 2001b) have developed a physiologically based pharmacokinetic-pharmacodynamic (PBPK/PD) model for glycyrrhizinic acid, glycyrrhetic acid and occurrence of adverse clinical effects in humans. The model has been calibrated on the basis of various literature data. It can accurately predict plasma glycyrrhetic acid levels in human volunteers after oral intake of glycyrrhetic acid, glycyrrhizinic acid itself or two liquorice confectionery treatments (sweet and salted liquorice), which were determined in an independent clinical trial. Important features of the model are that it includes the presence of a gall bladder, it takes into account the rate of bowel movements and stomach emptying, hydrolysis of glycyrrhizinic acid in the GI-tract and enterohepatic circulation of glycyrrhetic acid. In addition, it can estimate the effects of intake of glycyrrhizinic acid (and subsequently glycyrrhetic acid) on the activity of 11-BOHD-2 by prediction of the ratio cortisol/cortisone in 24-h samples of human urine following repeated intake of glycyrrhetic acid, as proposed by Heilmann *et al.* (1999). The sub-model which predicts changes in urinary cortisol/cortisone ratios was also calibrated on the basis of literature data and validated against data that were obtained from independent experiments in human volunteers who received multiple oral administrations of either glycyrrhizinic acid or glycyrrhetic acid.

By using this PBPK/PD model Ploeger and co-workers (2000a, 2001b) demonstrated that the the most important factors to determine interindividual variability in the response to glycyrrhizinic acid with respect to perturbation of the cortisol/cortisone status were:

- motility of the gastrointestinal tract: slow bowel movements result in a prolonged residence time of glycyrrhizinic acid and glycyrrhetic acid in the enterohepatic circulation and therefore in a higher systemic exposure. Experimentally, this is confirmed by findings of Mensinga *et al.* (1998)
- variability of the absorption rate constant: a rapid absorption results in a more extensive enterohepatic circulation and in a higher internal exposure to glycyrrhetic acid.
- variability of the IC50 (plasma glycyrrhetic acid concentration at which 50% of the 11-BOHD-2 activity is lost). A lower IC50 results in an effect on the cortisol/cortisone status at lower plasma levels of glycyrrhetic acid.
- variability of the "base-line" activity of 11-BOHD-2. Indeed, several cases of congenital apparent mineralocorticoid excess, a condition which is similar to pseudo-hyperaldosteronism, have been identified, in which mutations in the gene encoding for the 11-BOHD-2 enzyme could be identified. These mutations cause partial or complete loss of enzyme activity (Mune *et al.,* 1995). In addition to this, "base-line" variability may also result from differences in the level of expression of the 11-BOHD-2 gene (Ferrari *et al.*, 2001).

These findings and the PBPK/PD model were further used in a Monte Carlo simulation to estimate the proportion of the population that might experience disturbances of the cortisol/cortisone status and, linked to this, biochemical or even clinical manifestations of intoxication at various intake levels of glycyrrhizinic acid. These calculations were based on the following starting points:

- at the NOAEL of 2 mg glycyrrhizinic acid /kg bw/day, the plasma level of glycyrrhetic acid is never higher than 800 μg/l, as observed in the study by Bijlsma *et al.* (1996).
- the population distribution of sensitivity to glycyrrhizinic acid with respect to biochemical and clinical manifestations at the LOAEL of 4 mg glycyrrhizinic acid/kg bw/day is adequately reflected by the distribution of responders vs. non-responders in

the Bijlsma study. In this study, at the LOAEL, 1/11 participants did not show any effect, while in 10/11 participants biochemical changes were observed, in one of whom also clinical signs were seen.

Ploeger (2000a) calculated that at the maximum intake level of 100 mg/day, provisionally established by the Committee in 1991 (SCF, 1991), about 18% (95% confidence limits: 1.6 - 73.5%) of the exposed population would experience plasma glycyrrhetic acid levels greater than 800 μg/l, while in 26% of the exposed population (95% confidence limits: 12 - 47%) disturbances of the cortisol/cortisone status would be detectable in the urine. Overt clinical adverse effects (hypertension) would occur in about 4 per 10^4 persons (95% confidence limits: 4.6 per 10^6 - 3 per 10^2). These estimates apply to intake of glycyrrhizinic acid from any source, hence to the entire population.

In addition to the sensitive groups that were identified by Ploeger (2000a) and co-workers, some other groups in the population can be expected to be adversely influenced exposure to glycyrrhizinic acid or its ammonium salt. Although there is no direct evidence showing this, from the biomedical literature (Rose, 1994; Stewart, 2002) it is conceivable that the health of people with Cushing's syndrome, or other conditions related to hypertension or abnormal electrolyte or water homeostasis, may be adversely affected by exposure to glycyrrhizinic acid or its ammonium salt.

References to Annex II

Bernardi, M., D'Intino, P.E., Trevisani, F., Cantelli-Forti, G., Raggi, M.A., Turchetto, E., Gasbarrini, G. (1994). Effects of prolonged ingestion of graded doses of liquorice by healthy volunteers. Life Sciences, **55**, 863-872.

Bijlsma, J.A., Van Vloten P., Van Gelderen C.E.M., Mensinga Tj.T., Mout, H.A., Elvers L.H., Van Leeuwen, F.X.R., Stolker, A.A.M., Van Ginkel, L.A., Looman, C.W.N., Van der Maas, P.J., Koomans, H.A. and Savelkoul, T.J.F. (1996). Onderzoek naar de effecten van verschillende doseringen glycyrrhizine bij gezonde vrouwelijke vrijwilligers. [Study into the effects of different dosages of glycyrrhizin in health female volunteers; in Dutch]. RIVM report 348801004, RIVM, Bilthoven, The Netherlands

Brouwers, A.J.B.W. and Van der Meulen, J. (2001). "Drophouthypertensie"; ook door zoethoutthee ["Liquorice hypertension"; also by liquorice tea; in Dutch]. Ned. Tijdschr. Geneeskd., 1**45**, 744-746.

Cantelli-Forti, G, Maffei, F., Hrelia, P., Bugamelli, F., Bernardi, M., D'Intino, P., Maranesi, P. and Raggi, M.A. (1994). Interaction of liquorice on glycyrrhizin pharmacokinetics. Environmental Health Perspectives, **102**, Suppl. 9, 65-68.

Cantelli-Forti, G., Raggi, M.A., Bugamelli, F., Maffei, F., Villari, A. and Trieff, N.M. (1997). Toxicological assessment of liquorice: biliary excretion in rats. Pharmacological Research, **35**, 463-470

Colley, D.P., Kay, J. and Gibson, G.T. (1982). Three common Australian cough mixtures: a study of their use in pregnancy. Australian Journal of Pharmacy (April 1982 issue), 213-217.

Cooper, E.P. and Berry, C.W. (1988). Mutagenic potential of Glycyrrhizin. Journal of Dental Research, **67**, abstract 339 (Special Issue, March).

Cummings, A.M.M., Boddy, K., Brown, J.J., Fraser, R., Lever, A.F., Padfield, P.L. and Robertson, J.I.S. (1980). Severe hypokalaemia with paralysis induced by small doses of liquorice. Postgraduate Medical Journal, **56**, 526-529.

EFFA (2001). Ammonium glycyrrhizate. European Flavour and Fragrance Association (EFFA). Submission to SCOOP Working group on Chemically defined Flavouring

Substances, document No: SCOOP/FLAV/8.4, SCF document No: SCF/CS/ADD/MsAd/203

Ferrari, P., Sansonnens, A., Dick, B. and Frey, F.J. (2001). In vivo 11-beta-HSD-2 activity; variability, salt-sensitivity, and effect of licorice. Hypertension, **38**, 1330-1336.

HSDB: Hazardous Substances Data Base (2002). Issued by the National Library of Medicine through Silver Platter International N.V., Ovid Technologies, Amsterdam.

Hayashi, M., Kishi, M., Sofuni, T. and Ishidate Jr., M. (1988). Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food and Chemical Toxicology, **26**, 487-500.

Heilmann, P., Heide, J., Hundertmark, S. and Schoneshofer, M. (1999). Administration of glycyrrhetinic acid: significant correlation between serum levels and the cortisol/cortisone ratio in serum and urine. Experimental and Clinical Endocrinology and Diabetes, **107**, 370-378.

Ishidate, M., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M. and Matsuoka, A. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology, **22**, 623-636.

Itami, T., Ema, M. and Kanoh, S. (1985). Effect of disodium glycyrrhizinate on pregnant rats and their offspring. Journal of the Food Hygiene Society of Japan, **26**, 460-464.

Kobuke, T., Inai, K., Nambu, S., Ohe, K., Takemoto, T., Matsuki, K., Nishina, H., Huang, I.-B. and Tokuoka, S. (1985). Tumorigenicity study of disodium glycyrrhizinate administered orally to mice. Food and Chemical Toxicology, **23**, 979-983.

Maas, P.(2000). Zoethout in levensmiddelen: onderzoek naar het glycyrrhizine gehalte van thee, kruidenmengsels, dranken en drop. [Liquorice root in food stuffs: survey of the glycyrrhizin content of tea, herbal mixtures, alcoholic drinks and liquorice.; in Dutch]. De Ware(n) Chemicus, **30**, 65-74.

Mantovani, A., Ricciardi, C., Stazi, V., Marci C., Piccioni, A., Badellino, E., De Vincenzi, M., Caiola, S. and Patriarca, M. (1988). Teratogenicity study of ammonium glycyrrhizinate in the Sprague-Dawley rat. Food and Chemical Toxicology, **26**, 435-440.

Mensinga, Tj.T, Sips, A.J.A.M., Van den Ham, W. and Meulenbelt, J. (1998) Gezondheidsrisico's veroorzaakt door het eten van drop. [Health risks caused by the consumption of liquorice confectionery; in Dutch]. RIVM report 236850003, RIVM, Bilthoven, The Netherlands

Mune, T.M, Rogerson, F.M., Nikkilä, H., Agarwal, A.K. and White, P.C (1995). Human hypertension caused by mutations in the kidney isozyme of 11-beta-hydroxysteroid dehydrogenase. Nat. Genet. **10**, 394-399.

Ploeger, B.A. (2000a). Development and use of a physiologically based pharmacokinetic-pharmacodynamic model for glycyrrhizinic acid in consumer products. PhD thesis University of Utrecht.

Ploeger, B.A., Meulenbelt, J. and De Jongh, J. (2000b). Physiologically based pharmacokinetic modelling of glycyrrhizic acid, a compound subject to presystemic metabolism and enterohepatic cycling. Toxicology and Applied Pharmacology, **162**, 177-188.

Ploeger, B.A., Mensinga, T., Sips, A., Seinen, W., Meulenbelt, J. and De Jongh, J. (2001a). The pharmacokinetics of glycyrrhizinic acid evaluated by physiologically based pharmacokinetic modelling. Drug Metabolism Reviews, **33**, 125-147.

Ploeger, B.A., Mensinga, T., Sips, A., Deerenberg, C., Meulenbelt, J. and De Jongh, J. (2001b). A population physiologically based pharmacokinetic/pharmacodynamic model for the inhibition of 11-beta-hydroxysteroid dehydrogenase activity by glycyrrhetic acid. Toxicology and Applied Pharmacology, **170**, 46-55.

Rose, B.D. (1994). Clinical physiology of acid-base and electrolyte disorders. McGraw-Hill, Health Profession Division, New York.

Rosseel, M. and Schoors, D. (1993). Chewing gum and hypokalaemia. The Lancet, **341**, 175.

Rossi, T., Fano, A., Castelli, M., Malagoli, M., Ruberto, A.I., Baggio, G., Zennaro, R., Migaldi, M. and Barbolino, G. (1999). Correlation between high intake of glycyrrhizin and myolisis of the papillary muscles: an experimental in vivo study. Pharmacology and Toxicology, **85**, 221-229.

Russo, S., Mastropasqua, M., Mosetti, M.A., Persegani, C. and Paggi, A. (2000). Low doses of liquorice can induce hypertension encephalopathy. American Journal of Nephrology, **20**, 145-148.

SCF (1991). Reports of the Scientific Committee for Food (29th series). Commission of the European Communities, Food Science and Techniques. Report No EUR 14482 EN, CEC, Luxembourg.

Sheu, C.W., Cain, K.T., Rushbrook, C.J., Jorgenson, T.A., Generoso, W.M. (1986). Tests for mutagenic effects of ammoniated glycyrrhizin, butylated hydroxytoluene, and gum arabic in rodent germ cells. Environmental Mutagenesis, **8**, 357-367.

Sigurjónsdóttir, H.Á., Franzon, L., Manhem, K., Ragnarsson, J., Sigurdsson, G. and Wallerstedt, S. (2001). Liquorice-induced rise in blood pressure: a linear dose-response relationship. Journal of Human Hypertension, **15**, 549-552.

Smorenberg-Schoorl, M.E. and Vree H.M. (1963). Het gebruik van dropjes en "essentiële" hypertensie [Consumption of liquorice candies and "essential" hypertension; in Dutch]. Ned. T. Geneesk., **107**, 2013.

Stewart, P.M. (2002). Adrenal cortex: renin-angiotensin-aldosterone axis and hypertension. In: Besser, G.M. and Thorner M.O. (eds) Comprehensive clinical endocrinology. Elsevier Scientific Publ. Amsterdam.

Størmer, F.C., Reistad, R., Alexander J. (1993a). Adverse health effects of glycyrrhizinic acid in liquorice. A risk assessment. Nordiske seminar- og Arbejdsrapporter 1993:526.

Strandberg, T.E, Järvenpää, A.-L., Vanhanen , H. and McKeigue, P.M. (2001). Birth outcome in relation to licorice consumption during pregnancy. American Journal of Epidemiology, **153**, 1085-1088.

Van Gelderen, C.E.M., Bijlsma, J.A., Van Dokkum, W. and Savelkoul, T.J.F (2000). Glycyrrhizinic acid: the assessment of a no effect level. Human and Experimental. Toxicology, **19,** 434- 439.

Van Vloten, P., Savelkoul, T.J.F., De Groot, G., Koops, R., Loeber, J.G., Van Leeuwen, F.X.R., Looman, C.W.N. and Van der Maas, P.J. (1989). Onderzoek naar de biologische effecten van verschillende doseringen glycyrrhizine bij menselijke

vrijwilligers (pilot study). [Investigation into the biological effects of various dose levels of glycyrrhizin in human volunteers (pilot study); in Dutch]. RIVM report 348801 001, RIVM, Bilthoven, The Netherlands.

Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment

VKM Report 2018: 09

Hazard assessment of glycyrrhizic acid from liquorice

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Hazard assessment of glycyrrhizic acid from liquorice

Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to answer the request from the Norwegian Food Safety Authority. The project group consisted of two VKM members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and a project leader from the VKM secretariat. Two external referees commented on and reviewed the manuscript. The VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics evaluated and approved the final opinion drafted by the project group.

Authors of the opinion

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) has at the request of the Norwegian Food Safety Authority (Mattilsynet, NFSA) identified and characterized potential adverse effects to the fetus and long-term effects to the child that can result from maternal consumption of glycyrrhizic acid from liquorice, including at which doses these adverse effects appeared.

Glycyrrhizic acid is isolated from extracts of the dried roots of *Glycyrrhiza glabra*, a herb native to central and south-western Asia and the Mediterranean region. Its natural sweetness comes from glycyrrhizic acid, which is present at a concentration of about 5–7% in the root, and is said to be 50 times sweeter than refined sugar. The fresh root contains about 20% water-soluble extracts, of which glycyrrhizic acid constitutes 10–25%. Glycyrrhizic acid is a conjugate of glycyrrhetic acid (aglycone) and two molecules of glucuronic acid. Both glycyrrhizic acid and glycyrrhetic acid can exist as 18α- and 18β-stereoisomers, with the β-isomer of glycyrrhetic acid being the main metabolite of glycyrrhizic acid. In this hazard assessment, the term glycyrrhizin is used as a more general term denoting glycyrrhizic acid and its metabolite glycyrrhetic acid in liquorice. When referring to specific studies, the terms used by the respective authors are used.

Liquorice has a long history of use in medicine and as a flavouring substance, and glycyrrhizic acid and its ammonium salt (ammonium glycyrrhizinate) are widely used as sweeteners and flavourings in confectionary, sweets, drugs, beverages, chewing gum, tobacco products and toothpastes.

The present hazard assessment of glycyrrhizic acid is based on previous risk assessments and articles retrieved from a literature search. Several previous risk assessments suggested 100 mg glycyrrhizic acid per day as a safe level expected to protect the majority of the population, but possibly not the most susceptible subpopulation.

Glycyrrhizic acid, both in free form and as the ammonium salt, is poorly absorbed from the gastrointestinal tract, but is hydrolysed by intestinal bacteria to glycyrrhetic acid, which is readily absorbed. This absorption is nearly complete regardless of whether glycyrrhetic acid is formed by hydrolysis of the glycyrrhizic acid or is present as the glycoside or the aglycone. However, at doses >25 mg/kg body weight (bw) of glycyrrhizic acid, the rate of hydrolysis of glycyrrhizic acid to glycyrrhetic acid by the gut microflora may become saturated and this may limit the relative amount of glycyrrhetic acid that can be absorbed from the gastrointestinal tract. Glycyrrhetic acid is conjugated in the liver before excretion in the bile, and the metabolites may undergo further hydrolysis by the gut microflora, leading to enterohepatic recycling. Neither glycyrrhizic acid nor glycyrrhetic acid are significantly taken up by tissues, however, both components adhere extensively to serum albumin in a saturable process. The plasma clearance of glycyrrhetic acid is dose-dependent when exceeding the saturation of serum protein binding. Glycyrrhetic acid is able to cross the placental barrier and can be detected in the fetus.

Studies in experimental animals showed mineralocorticoid effects, including increased blood pressure, of glycyrrhizic acid. Low acute toxicity of glycyrrhizin was demonstrated in mice and rats. Disodium glycyrrhizin given for 96 weeks in doses up to 229 mg/kg bw in male mice and 407 mg/kg bw in female mice showed no evidence of chronic toxicity or tumourigenicity. Regarding teratogenicity and effects on reproduction, the results from animal studies varied. In some studies, adverse effects were not reported, whereas others did. Ammonium glycyrrhizinate administered to rats on days 7-17 of pregnancy induced a slight but significant dose-related increase in embryolethality. The prevalence of external hemorrhages and hematomas and the rate of affected litters were significantly higher after 21 and 680 mg/kg bw per day, minor skeletal anomalies were dose-relatedly increased with 239 and 680 mg/kg bw, and renal ectopy was significantly increased at 680 mg/kg bw. When pregnant rats were given glycyrrhetinic acid from day 13 of gestation until term substantially impaired fetal lung maturation was observed. A study indicated that a crude 95% ethanol extract of *Glycyrrhiza glabra* had estrogenic effects in mice.

Glycyrrhizin was considered not to be mutagenic or genotoxic.

The human studies included in this assessment of effects on the fetus or child after their mother's intake of liquorice during pregnancy reported that high glycyrrhizin exposure versus a lower exposure did not significantly affect birth weight or maternal blood pressure (Strandberg et al., 2001). However, high glycyrrhizin exposure was significantly associated with shorter gestational duration (Strandberg et al., 2001), more than twofold increased risk of preterm (<37 weeks) delivery, and an even stronger association with early preterm (<34 weeks) delivery (Strandberg et al., 2002). When the children reached a mean age of 8.1 years, they were reported to have poorer cognitive performance, externalising symptoms and attention problems after high exposure (Räikkönen et al., 2009). They also had higher salivary cortisol awakening peak, salivary cortisol awakening slope, salivary cortisol awakening AUC and baseline TSST-C salivary cortisol levels (Räikkönen et al., 2010). At mean age 12.5 years, girls, but not boys, were taller, heavier and had higher body mass index for age, were closer to adult height and had more advanced pubertal development (Räikkönen et al., 2017). Both girls and boys scored lower on tests of intelligence quotient, had poorer memory and had higher odds of attention deficit/hyperactivity disorder problems.

In VKM's opinion, an inhibitory effect on the 11ß-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme by glycyrrhizic acid, leading to overexposure of the fetus to cortisol, is a plausible mechanism for the adverse effects reported in the human studies in this assessment. The findings in these studies are indicative of potential adverse effects of glycyrrhizic acid on the offspring from liquorice intake during pregnancy. However, the levels of exposure of the fetus to glycyrrhizic acid are too uncertain based on the available data to be able to draw firm conclusions on cause and effects relationships. One of the uncertainties is the actual intake of glycyrrhizic acid by the mothers during pregnancy.

Based on the studies by Strandberg et al. (2001; 2002) and Räikkönen et al. (2009; 2010; 2017), the negative health effects on the mothers or their fetus or child were found with glycyrrhizin intake ≥500 mg/week, corresponding to approximately 250 g/week of liquorice,

compared with lower intake (0-499 mg/week). Therefore, from these studies 500 mg/week (71.4 mg/day) of glycyrrhizin, which corresponded to average 13.7 mg/kg bw for the mothers at delivery, can be regarded as the lowest observed adverse effect level (LOAEL) (Räikkönen et al., 2017). This intake is lower than 100 mg/day suggested as a safe level in several previous risk assessments. However, this external dose level is uncertain because of inherent weaknesses in these studies as discussed in this assessment. Several toxicokinetic factors affect the internal dose of glycyrrhetic acid that eventually reach the placenta, thus determining whether the actual level of glycyrrhetic acid is sufficient to inhibit the placental 11β-HSD2 enzyme.

In these above-mentioned human studies, no recording of glycyrrhizin intake in various parts of the pregnancy was done. Thus, there is also uncertainty regarding whether the exposure to glycyrrhizin occurred in critical periods during pregnancy relevant for the effects on puberty, cortisol levels, cognitive performance, psychiatric symptoms etc. observed in the children.

There is observed large interindividual variation in sensitivity to glycyrrhizic acid. Women appeared to be more sensitive to glycyrrhizic acid than men. There is also reported considerable variation in the 11β-HSD2 activity between human placentas.

Patients with decreased liver function or hypokalemia, women with preeclampsia or persons with apparent mineralocorticoid excess (AME), an inherited rare form of hypertension caused by mutations in the 11β-HSD2 gene, may be especially susceptible to excessive intake of liquorice. Glycyrrhizin is also shown to interact with various drugs, such as prednisolone and hydrocortisone, and prolonged intake of glycyrrhizin may result in accelerated metabolism of co-administered drugs via the induction of various metabolic enzymes.

In VKM's opinion, there is still not sufficient data to establish an acceptable daily intake (ADI) for glycyrrhizic acid.

Since no data were available on liquorice or glycyrrhizic acid intake in Norway, it was not possible to perform an exposure characterization. Therefore, a risk characterisation of glycyrrhizic acid from liquorice intake in Norway could not be performed.

VKM concludes that because of the large uncertainty associated with the relationship between the exposure dose and the observed adverse effects, a safe level cannot be established with certainty for glycyrrhizic acid or for the amount of liquorice that the pregnant mothers can consume without causing negative effects on the fetus or child.

Sammendrag på norsk

Vitenskapskomiteen for mat og miljø (VKM) har på forespørsel fra Mattilsynet identifisert og karakterisert mulige negative helseeffekter som mors inntak av glykyrrhizinsyre fra lakris kan ha for fosteret og hvilke langtidseffekter det kan føre til for barnet, inkludert ved hvilke doser disse effektene ble observert.

Glykyrrhizinsyre er isolert fra ekstrakter av de tørkede røttene av planten *Glycyrrhiza glabra*, en urt fra det sentrale og sør-vestlige Asia og Middelhavsregionen. Rotens naturlige søthet kommer fra glykyrrhizinsyre, som er til stede i en konsentrasjon på rundt 5-7% i roten, og som sies å være 50 ganger søtere enn raffinert sukker. Den friske roten inneholder ca. 20% vannløselige ekstrakter, hvorav glykyrrhizinsyre utgjør 10-25%. Glykyrrhizinsyre er et konjugat av glykyrrhetinsyre (aglykon) og to molekyler glukuronsyre. Både glykyrrhizinsyre og glykyrrhetinsyre kan eksistere som 18α- og 18β-stereoisomerer. For glykyrrhizinsyre er β-isomeren den viktigste metabolitten. I denne farekarakteriseringen brukes begrepet glykyrrhizin som en generell betegnelse som omfatter glykyrrhizinsyre og dens metabolitt glykyrrhetinsyre i lakris. Når det refereres til bestemte publikasjoner, har VKM brukt det samme uttrykket som forfatterne.

Lakris har en lang historie i medisinsk bruk og som et aromastoff. Glykyrrhizinsyre og ammoniumsaltet (ammoniumglykyrrhizinat) brukes i stor grad som søtningsmidler og aromastoffer i konfekt, søtsaker, medisiner, drikkevarer, tyggegummi, tobakk og tannkrem.

Denne farekarakteriseringen av glykyrrhizin er basert på tidligere risikovurderinger og artikler hentet fra litteratursøk. Flere tidligere risikovurderinger foreslo 100 mg glykyrrhizinsyre per dag som et trygt nivå som ble forventet å beskytte flertallet av befolkningen, men muligens ikke den mest følsomme gruppen.

Både i fri form og som ammoniumsalt tas lite glykyrrhizinsyre opp fra mage-tarmkanalen. Glykyrrhizinsyre hydrolyseres til glykyrrhetinsyre av tarmbakterier, og glykyrrhetinsyre tas lett opp. Opptaket av glykyrrhetinsyre er nesten fullstendig, og det gjelder uansett om glykyrrhetinsyre dannes ved hydrolyse av glykyrrhizinsyre eller om det er tilstede som glykosid eller aglykon. Ved doser av glykyrrhizinsyre >25 mg/kg kroppsvekt, kan tarmfloraens maksimale hydrolyse av glykyrrhizinsyre til glykyrrhetinsyre være nådd, og det vil kunne begrense mengden av glykyrrhetinsyre som tas opp fra mage-tarmkanalen. Glykyrrhetinsyre blir konjugert i leveren før den skilles ut i gallen. De konjugerte metabolittene fra leveren kan igjen bli hydrolysert av tarmbakteriene, noe som fører til enterohepatisk resirkulering. Hverken glykyrrhizinsyre eller glykyrrhetinsyre tas opp av vev i vesentlig grad, men begge stoffene bindes i stor grad til serum-albumin i en metningsprosess. Fjerningen av glykyrrhetinsyre fra plasma er doseavhengig over det nivået hvor serum protein-bindingen er mettet. Glykyrrhetinsyre er i stand til å krysse morkakebarrieren og kan påvises i fosteret.

Studier i forsøksdyr har vist at glykyrrhizinsyre har mineralokortikoide effekter, noe som blant annet innebærer at det kan øke blodtrykket. Studier i rotter og mus har vist at glykyrrhizin har lav akutt toksisitet. Det var ingen tegn på kronisk toksisitet eller kreft av toverdig natriumsalt av glykyrrhizin som ble gitt i 96 uker til mus i doser på opptil 229 mg/kg kroppsvekt til hanner og 407 mg/kg kroppsvekt til hunner. Når det gjelder fosterskader og effekter på reproduksjon, ga dyreforsøkene varierende resultater. Noen studier rapporterte ingen slike effekter, mens andre gjorde det. Ammoniumsaltet av glykyrrhizin førte til en liten, men signifikant doserelatert økning i embryodødelighet, da det ble gitt til rotter på dag 7-17 av graviditeten. Forekomsten av eksterne blødninger og hematomer, og frekvensen av berørte kull, var signifikant høyere etter doser på 21 og 680 mg/kg kroppsvekt per dag, mindre anomalier i skjelettet viste dose-relatert økning etter 239 og 680 mg/kg kroppsvekt, og unormal beliggenhet av nyrer (ektopi) var signifikant økt ved 680 mg/kg kroppsvekt. Når gravide rotter fikk glykyrrhetinsyre fra dag 13 av svangerskapet, ble det observert en signifikant svekket modning av lungene hos avkommet. En studie indikerte at et ekstrakt med 95 prosent etanol fra *Glycyrrhiza glabra* hadde østrogene effekter i mus.

Glykyrrhizin ble vurdert å ikke være mutagent eller gentoksisk.

De humane studiene som ble inkludert i denne vurderingen av effekter på fosteret eller barnet etter mors inntak av lakris under graviditeten rapporterte at høy glykyrrhizin-eksponering sammenlignet med en lavere eksponering ikke påvirket fødselsvekten eller mors blodtrykk signifikant (Strandberg et al., 2001). Men høy eksponering for glykyrrhizin var signifikant forbundet med lavere svangerskapsvarighet (Strandberg et al., 2001), mer enn doblet risiko for prematur fødsel (<37 uker) og en enda sterkere assosiasjon med prematur (<34 ukers) fødsel (Strandberg et al., 2002). Ved en gjennomsnittlig alder på 8,1 år ble det rapportert at barna hadde dårligere kognitive ferdigheter, mer utagerende adferd og oppmerksomhetsproblemer (Räikkönen et al., 2009), og også høyere nivåer av kortisol i spytt i flere ulike tester (Räikkönen et al., 2010). Ved en gjennomsnittsalder på 12,5 år var jenter, men ikke gutter, høyere, tyngre, hadde høyere kroppsmasseindeks for alderen, var nærmere voksen høyde og hadde mer fremskredet pubertetsutvikling (Räikkönen et al., 2017). Både jenter og gutter scoret lavere på tester av intelligenskvotient, hadde dårligere hukommelse og mer oppmerksomhets- og hyperaktivitetsforstyrrelser.

Glykyrrhetinsyre har en hemmende effekt på 11ß-hydroksysteroid dehydrogenase type 2 (11β-HSD2)-enzymet i morkaken, og når dette enzymet hemmes fører det til overeksponering av fosteret for kortisol. VKM anser at dette er en sannsynlig mekanisme for de negative effektene som er rapportert i de humane studiene som er inkludert i denne vurderingen. Funnene indikerer at gravides inntak av glykyrrhizinsyre fra lakris har potensielle negative effekter på fosteret eller barnet. Men det er ikke mulig å trekke sikre konklusjoner om årsak- og effektforhold, fordi det ut i fra de foreliggende studiene er for usikkert hvilke mengder glykyrrhizinsyre fostrene faktisk ble eksponert for. Dette skyldes blant annet usikkerhet knyttet til de gravides faktiske inntak av glykyrrhizinsyre.

Strandberg et al. (2001; 2002) og Räikkönen et al. (2009; 2010; 2017) observerte negative helseeffekter på mødre eller deres foster eller barn, når den gravides inntak av glykyrrhizin

var ≥500 mg/uke sammenlignet med lavere inntak (0-499 mg/uke). Inntak ≥500 mg/uke av glykyrrhizin tilsvarer et lakrisinntak på ca. 250 g/uke. Ut ifra dette kan 500 mg/uke (71,4 mg/dag) av glykyrrhizin betraktes som det laveste dosenivået med en observert negativ effekt (LOAEL) (Räikkönen et al., 2017). Dette inntaket er lavere enn 100 mg/dag som har blitt foreslått som et trygt nivå i flere tidligere risikovurderinger. Imidlertid er det usikkerhet knyttet til de gravides faktiske lakrisinntak i disse studiene på grunn av de iboende svakhetene i disse studiene som er diskutert i denne vurderingen.

Flere faktorer, som tarmbakterienes evne til å omdanne glykyrrhizinsyre til glykyrrhetinsyre, grad av enterohepatisk resirkulering og av binding av glykyrrhizinsyre og glykyrrhetinsyre til serum albumin, påvirker hvor mye glykyrrhetinsyre som til slutt når morkaken, og dermed hvorvidt nivået av glykyrrhetinsyre er tilstrekkelig til å hemme 11β-HSD2-enzymet i morkaken.

Inntak av glykyrrhizin i ulike deler av svangerskapet var ikke registrert i de humane studiene som er inkludert i denne vurderingen. Dermed er det også usikkerhet om hvorvidt eksponeringen for glykyrrhizin skjedde under kritiske perioder av svangerskapet som er relevante for effektene på pubertet, kortisolnivåer, kognitive ferdigheter, psykiatriske symptomer etc. observerte hos barna.

Det er observert stor variasjon i hvor følsomme ulike individer er for glykyrrhizinsyre. Kvinner ser ut til å være mer følsomme for glykyrrhizinsyre enn menn. Det er også rapportert betydelig variasjon i aktiviteten av 11β-HSD2-enzymet mellom kvinners morkaker.

Pasienter med nedsatt leverfunksjon eller lavt nivå av kalium, kvinner med svangerskapsforgiftning eller personer med mineralokortikoid overskuddssyndrom (AME), en arvelig sjelden form for hypertensjon forårsaket av mutasjoner i 11β-HSD2-genet, kan være spesielt sårbare for overdrevet inntak av lakris. Det er også vist at glykyrrhizin kan påvirke effekten av legemidler, for eksempel prednisolon og hydrokortison, og at langvarig inntak av glykyrrhizin kan føre til økt omdannelse av legemidler som inntas samtidig via induksjon av ulike metabolske enzymer.

VKM mener at det fremdeles ikke er tilstrekkelige data for å etablere et akseptabelt daglig inntak (ADI) for glykyrrhizinsyre.

Siden det ikke forelå data om inntak av lakris eller glykyrrhizinsyre i Norge, var det ikke mulig å utføre en eksponeringskarakterisering. Dermed kunne det heller ikke gjøres en risikokarakterisering av glykyrrhizinsyre fra inntak av lakris i Norge.

VKM konkluderer med at det på grunn av den store usikkerheten knyttet til forholdet mellom eksponeringsdosen og de observerte negative helseeffektene kan det ikke med sikkerhet fastsettes et trygt nivå av glykyrrhizinsyre eller av mengden lakris som gravide kan innta uten at det fører til negative effekter på fosteret eller barnet.

Abbreviations and glossary

Abbreviations

ACTH	adrenocorticotropic hormone
ADHD	attention deficit/hyperactivity disorder
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AME	apparent mineralocorticoid excess
ANP	atrial natriuretic peptide
AUC	area under the curve
11β-HSD	11ß-hydroxysteroid dehydrogenase
bw	body weight
CI	confidence interval
CRH	corticotropin releasing hormone
DOCA	desoxycorticosterone acetate
DPyr	deoxypyridinoline
EFSA	European Food Safety Authority
EMA	European Medicines Agency
FAO	Food and Agriculture Organization of the United Nations
GA	glycyrrhetinic acid
GC	glucocorticoid
GE	glycyrrhetinic acid
GI	gastrointestinal
GR	glucocorticoid receptor
GRAS	generally recognised as safe
HPA/HPAA	hypothalamic-pituitary-adrenal (adrenocortical) (axis)
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD	lethal dose
LOAEL	lowest observed/observable adverse effect level
MD	mean difference
MGL	monoammonium glycyrrhizinate
MR	mineralocorticoid receptor
NaG	disodium glycyrrhizinate
NFSA	Norwegian Food Safety Authority
NOAEL	no observed adverse effect level
NOEL	no observed effect level
OR	odds ratio
Pyr	pyridinoline
s.c.	subcutaneous
SCF	Scientific Committee for Food

SD	standard deviation
TSST-C	Trier Social Stress Test for Children
VKM	Norwegian Scientific Committee for Food and Environment
WHO	World Health Organization

Glossary

Glycyrrhizin: In some of the literature, the name glycyrrhizin has been used interchangeably with glycyrrhizic acid. This is not technically correct, and historically the term glycyrrhizin has been used to describe the crude acid extract of the roots of liquorice plants (JECFA/IPCS, 2006). Thus, glycyrrhizic acid is a component of glycyrrhizin. The name glycyrrhizin may also be used for the potassium, calcium and magnesium salts of glycyrrhizic acid isolated from the extracts, as well as for the ammonium salt which usually is the commercial preparation. Thus, this term is often used as a more general term denoting these substances in liquorice.

Glycyrrhizic acid: Composed of glycyrrhetic acid and two molecules of glucuronic acid. May be in free form or as a salt.

Glycyrrhizinic acid: Synonym for glycyrrhizic acid.

Glycyrrhetic acid: The active form that is absorbed after hydrolysis of glycyrrhizic acid by the intestinal microflora (the aglycone of glycyrrhizic acid).

Glycyrrhetinic acid: Synonym for glycyrrhetic acid.

Glycyrrhizinates: Various salts of glycyrrhizic acid.

Background as provided by the Norwegian Food Safety Authority

Glycyrrhizic acid is the flavour that gives the characteristic taste to liquorice products such as sweets and drinks. Several Finnish studies show long-time adverse effects in children exposed prenatally to glycyrrhizic acid caused by the mother's consumption of liquorice during pregnancy.

Consequently, the food and health authorities in Finland recommend that pregnant women should avoid large consumption of liquorice confectionery. Likewise, the Norwegian Food Safety Authority (NFSA) advises pregnant women against eating large amounts of liquorice.

In order to describe the dietary recommendation further and to have a scientific basis for assessing if other measures are necessary, NFSA asked the Norwegian Scientific Committee for Food and Environment (VKM) to assess which intake of glycyrrhizic acid by the mother is likely to cause adverse effects in the fetus or child.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) ask the Norwegian Scientific Committee for Food and Environment (VKM) to identify and characterize potential adverse effects to the fetus and long-term effects to the child that can result from maternal consumption of glycyrrhizic acid from liquorice, including at which doses these adverse effects appear, if such data are available.

Assessment

1 Introduction

Glycyrrhizic acid is a natural constituent of liquorice and is isolated from extracts of the dried roots of *Glycyrrhiza glabra.* This herb is native to central and south-western Asia and the Mediterranean region. The genus name *Glycyrrhiza* is derived from the Greek words "glycos", meaning sweet, and "rhiza" meaning root. The sweetness comes from glycyrrhizic acid, which is present at a concentration of about 5–7% in the root, and is said to be 50 times sweeter than refined sugar. The fresh root contains about 20% water-soluble extracts, of which glycyrrhizic acid constitutes 10–25%. Liquorice has a long history of medicinal use and as a flavouring, and glycyrrhizic acid and its ammonium salt (ammonium glycyrrhizinate) are widely used as sweeteners and flavourings in confectionary, sweets, drugs, beverages, chewing gum, tobacco products and toothpastes (Isbrucker and Burdock, 2006; JECFA/IPCS, 2006).

Glycyrrhizic acid is an environmental chemical from food, which mimics mineralocorticoids (MR) in its action and may disturb the MR-regulated physiological processes (cortisol-induced MR activation, hypertension, sodium retention, altered vascular function, direct enzyme inhibition and enhanced exposure of the fetus to glucocorticoids). The study of glucocorticoid and mineralocorticoid disruptors is an emerging field of research, and the identification of relevant xenobiotics and their underlying mechanisms of toxicity remains a major challenge (Odermatt and Gumy, 2008).

There is a large amount of scientific literature describing the biological effects of glycyrrhizin on humans (EMA, 2013; Isbrucker and Burdock, 2006; JECFA/IPCS, 2006). Apparent associations between mother's intake of glycyrrhizin during pregnancy and potential effects on the fetus or child have been published (Räikkönen et al., 2009; 2010; 2017; Strandberg et al., 2001; 2002). A possible mechanism for potential adverse effects is excess exposure of the fetus to maternal cortisol. It is well known that absorbed glycyrrhetic acid inhibits the enzyme 11ß-hydroxysteroid dehydrogenase type 2 enzyme (11β-HSD2) that converts cortisol to cortisone, resulting in a cortisol-induced mineralocorticoid action (Omar et al., 2012).

In this evaluation, VKM has used the terms glycyrrhizic acid (not the synonym glycyrrhizinic acid) and glycyrrhetic acid (not the synonym glycyrrhetinic acid), in addition to the more general term glycyrrhizin. When referring to specific studies, the terms used by the respective authors are used (see also Glossary).

2 Hazard identification and characterisation

2.1 Literature

2.1.1 Previous risk assessments

Scientific Opinion on the safety and efficacy of glycyrrhizic acid ammoniated (chemical group 30, miscellaneous substances) when used as a flavouring for all animal species. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). European Food Safety Authority (EFSA), Parma, Italy, 2015.

The conclusions from the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) were that glycyrrhyzic acid ammoniated is safe at the concentration of 1 mg/kg complete feed for all species, except chickens for fattening and laying hens. For these two categories, a safe concentration of 0.3 mg/kg complete feed applies. The FEEDAP Panel could not conclude on the safety of the additive used in water for drinking. The FEEDAP Panel considered that the use of glycyrrhizic acid ammoniated in animal nutrition would not measurably increase consumer exposure. In the absence of data on user safety, the FEEDAP Panel considered it prudent to treat glycyrrhizic acid ammoniated as an irritant to skin, eyes and respiratory tract and as a skin sensitizer.

Assessment report on *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch., radix. European Medicines Agency (EMA). European Medicines Agency/Committee on Herbal Medicinal Products (EMA/CHMP), London, United Kingdom, 2013.

The overall conclusion of the assessment was that there are no clinical data in the scientific literature to support a "well-established medicinal use". Short-term use (not more than 4–6 weeks) of liquorice preparations was considered safe. Serious side-effects such as hypokalemia and hypertension following chronic use of high dose of liquorice root were reported. More rarely, cardiac rhythm disorders could occur. Furthermore, in susceptible people prolonged daily intake even of low doses of liquorice (corresponding to 80–100 mg of glycyrrhizic acid) was referred to as being able to provoke severe hypertension. It was also stated that there was insufficient data to support the safety of liquorice root during pregnancy and lactation, in children and adolescents (<18 years). Therefore, the use is not recommended for these groups.

Flavouring Group Evaluation 36, (FGE.36). Two triterpene glycosides from the priority list. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC). European Food Safety Authority (EFSA), Parma, Italy, 2008.

The Panel was asked to evaluate two flavouring substances in the Flavouring Group Evaluation FGE.36 (FGE.36) using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. The FGE.36 dealt with two triterpene glycosides, glycyrrhizic acid [FL-no: 16.012] and glycyrrhizic acid, ammoniated [FL-no: 16.060] from chemical group 30, Annex I of the Commission Regulation (EC) No 1565/2000. The two substances were presented without specification of the stereoisomeric composition. The Panel agreed with the evaluation by the Scientific Committee on Food (SCF, 2003) which concluded that "an acceptable daily intake (ADI) for glycyrrhizic acid and ammonium glycyrrhizinate cannot be derived, because the new human toxicity studies are too limited (small experimental groups, short duration). The Committee considers that this upper limit for regular ingestion of 100 mg/day provides a sufficient level of protection for the majority of the population. It is noted that this upper limit includes the intake of glycyrrhizic acid via all products, liquorice confectionery as well as glycyrrhizic acid- or ammonium glycyrrhizinate-flavoured products. At the same time, the Committee realises that within the human population there are subgroups for which this upper limit might not offer sufficient protection."

Safety evaluation of certain food additives. Prepared by the Sixty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO food additives series: 54. World Health Organisation, Geneva, 2006.

The monographs contained in this volume were prepared at the sixty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met at WHO Headquarters in Geneva, Switzerland, 8–17 June 2004. These monographs summarize the safety data on selected food additives reviewed by the Committee, including glycyrrhizinic acid and its monoammonium salt as a natural constituent of liquorice (USA, 'licorice') and in its use as a flavouring substance in various food products. Glycyrrhizinic acid and its monoammonium salt have not been evaluated previously by the Committee. The conclusion of the safety evaluation in this monograph is identical to the conclusion referred in the report in the next paragraph (JECFA, 2005).

Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organisation, Geneva, 2005.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) was asked to comment on the safety of glycyrrhizinic acid and its monoammonium salt as a natural constituent of liquorice (licorice) and its use as a flavouring substance in various food products. Glycyrrhizinic acid and its monoammonium salt had not been evaluated previously by the Committee.

The Committee concluded that the safety evaluation of glycyrrhizinic acid should be based on the data from humans. It was observed that there is a sensitive subset of the population who appear to show signs of pseudohyperaldosteronism at lower exposures than those which produce effects in the general population, but the available data did not allow the Committee to adequately characterize this subgroup, and hence the data could not be used

to assign an ADI. The available data suggested that an intake of 100 mg/day would be unlikely to cause adverse effects in the majority of adults. The Committee recognized that, in certain highly susceptible individuals, physiological effects could occur at intakes somewhat below this figure. The data indicated that consumers with a high intake of liquorice confectionery or herbal tea containing liquorice may have an intake of glycyrrhizinic acid of >100 mg/day. A toxicological monograph was prepared.

Opinion of the Scientific Committee on Food on glycyrrhizinic acid and its ammonium salt. European Commission, Scientific Committee on Food. Brussel, Belgium, 2003.

The Committee was asked to consider if the opinion of the Committee expressed in 1991 (SCF, 1991) on glycyrrhizin was still valid in the light of additional information resulting from toxicological and clinical studies published since then on both glycyrrhizinic acid and its salts. The Committee was asked to take into account dietary exposure from all known sources, including contributions due to its natural occurrence in liquorice and through the ingestion of food products to which it is added as a flavouring substance. The Committee was also asked to evaluate ammonium glycyrrhizinate as a chemically defined flavouring substance for the possible acceptability of its inclusion in the Community Register.

Previously, the Committee evaluated the toxicological information for glycyrrhizinic acid and concluded that the data were inadequate to derive an ADI (SCF, 1991). At that time, the Committee considered it prudent that regular ingestion should not exceed 100 mg/day (provisional figure). Although new data provide a stronger basis for the upper limit for regular ingestion of glycyrrhizinic acid of 100 mg/day, the Committee still is of the opinion that an ADI for glycyrrhizinic acid and ammonium glycyrrhizinate cannot be derived. The Committee considered that this upper limit for regular ingestion of 100 mg/day provides a sufficient level of protection for the majority of the population. At the same time, the Committee realised that within the human population there are subgroups for which this upper limit might not offer sufficient protection.

The health and addiction risk of the glycyrrhizic acid component of liquorice root used in tobacco products. The National Institute for Public Health and the Environment (RIVM), The Netherlands, 2003.

RIVM published in 2003 the report "The health and addiction risk of the glycyrrhizic acid component of liquorice root used in tobacco products". In this report, the authors refer to an ADI of 200 mg/person a day, based on the PhD thesis "Development and use of a physiologically based pharmacokinetic-pharmacodynamic model for glycyrrhizic acid in consumer products" by Ploeger B.A., University of Utrecht, 2000.

Adverse health effects of glycyrrhizic acid in licorice – a risk assessment. Nordiske Seminar- og Arbejdsrapporter, 1993:526. Nordic Council of Ministers, Copenhagen, Denmark, 1993.

The authors of this report concluded that it was not possible, based on the referred data, to precisely determine the minimum level of glycyrrhizic acid required to produce the described symptoms (hypermineralocorticidism resulting in sodium retention and potassium loss, oedema, increased blood pressure and depression of the renin-angiotensin-aldosterone system). They also concluded that there apparently is a great interindividual variation in the susceptibility to glycyrrhizic acid. It was noted that much of the database consisted of case reports and studies on the same patients, thus the possibility of representing a group of particularly sensitive individuals was present. However, studies in healthy adults showed effects in the same dose range. Altogether, adverse effects occurred at a regular daily intake of about 100 mg glycyrrhizic acid in the most sensitive individuals. Thus, a regular intake of 100 mg/day was established as a provisional lowest observable adverse effect level (LOAEL) for adults.

2.1.2 Regulations

The European Food Safety Authority agreed with the evaluation by the Scientific Committee on Food (SCF) (SCF, 2003) that the intake of up to 100 mg/person per day would not give rise to safety concerns. However, there could be safety concerns with intake above this level. Therefore, specific conditions of use were set for glycyrrhizic acid (FL 16.012) and its ammoniated form (FL 16.060) as flavouring substances by the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF) in the Regulation (EC) 1334/2008 (EU Commission, 2008). In this regulation, not more than 1500 mg/kg of glycyrrhizic acid or ammoniated glycyrrhizic acid may be used in confectionary products. The same restrictions are also used in Norway (HOD, 2011).

The U.S. Food and Drug Administration (2017) has affirmed extract from licorice (glycyrrhiza) root and ammoniated glycyrrhizin as generally recognised as safe (GRAS). The maximum level, specified as percent content of glycyrrhizin in foods as served, is 16% for hard candy and 3.1% for soft candy. The maximum allowable levels in other foods vary from 0.05% to 0.15%.

2.1.3 Literature search and publication selection

Literature searches were performed in Medline, Embase, ISI Web of Science, Scopus, Cochrane Database of Systematic Reviews and Epistemonikos in order to retrieve publications on adverse effects caused by glycyrrhizin. These databases were chosen to ensure comprehensive study retrieval. No restrictions in language or time period were used in the search. The literature searches were performed by a librarian on September 27, 2017. The search strategy is included in Appendix 1.

The literature search identified 569 articles after duplicates was removed. In the primary screening, titles and abstracts of all publications retrieved were screened against the inclusion criteria checklist.

Inclusion and exclusion criteria checklist:

- Inclusion criteria:
    - Negative health effects that maternal consumption of glycyrrhizin may cause to fetus or child
    - Human study designs – all included
    - Experimental animal studies
    - Mechanistic *in vitro* studies
    - Publication type – primary research studies, relevant commentaries, review papers, systematic reviews, meta-analyses and risk assessments
    - English, Norwegian, Swedish, Danish or German language

- Exclusion criteria:
    - Studies reporting exclusively preventive/beneficial effects
    - Studies reporting effects on the mother not likely to affect the fetus
    - Editorials

Articles that did not appear to meet the inclusion criteria were excluded from further analysis. In situations where it was unclear whether the publication was of relevance to the study, it was retained for further screening. The primary screening was performed independently by two persons.

The full text of articles that passed the primary screening was retrieved for secondary screening. In this screening, the full text articles were reviewed and compared against the inclusion criteria checklist. The secondary screening was performed by two persons.

The secondary screening resulted in 88 full text articles. Of these, 29 papers were included in the hazard identification and characterization section, i.e. reporting adverse effects of glycyrrhizin relevant for evaluating effects on fetus or child from the mother's consumption of liquorice during pregnancy. In addition, 18 relevant papers were found by snowballing. In total, 47 papers were included in the hazard identification and characterization section. The rest of the included references provided information used in other sections of this assessment.

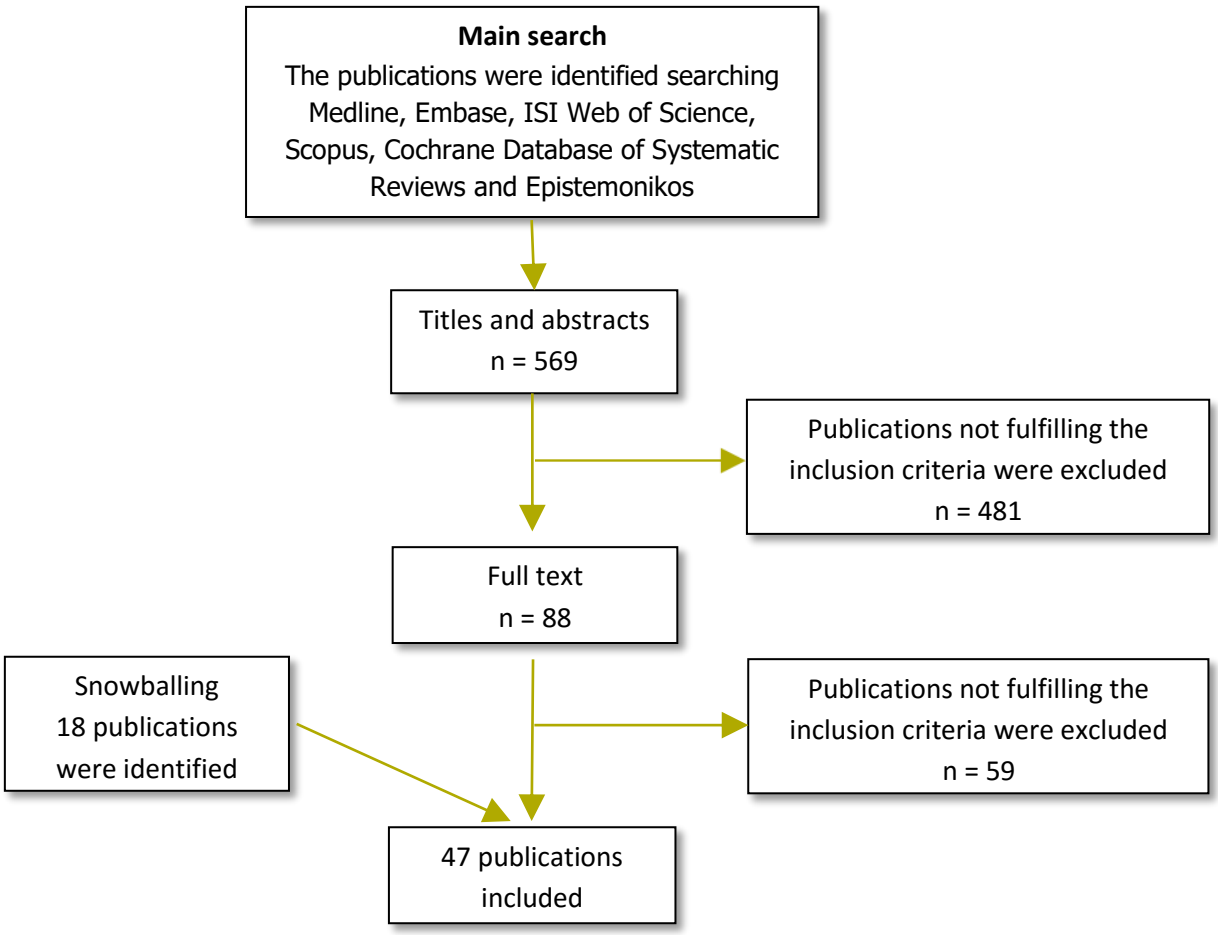

Figure 2.1.2-1: Flowchart for the literature search for glycyrrhizin and the subsequent publication selection of papers included in the hazard identification and characterization section of this assessment.

2.2 General information

2.2.1 Chemistry

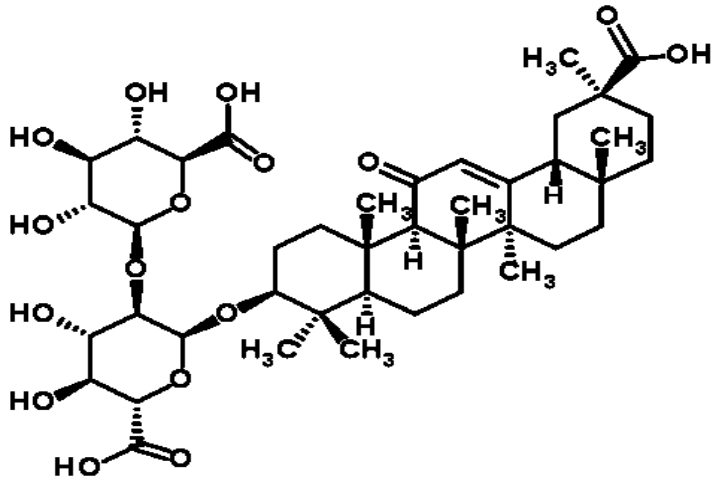

Figure 2.2.1-1. The structural formula of glycyrrhizic acid (http://www.chemspider.com/ImageView.aspx?id=14263).

Glycyrrhizic acid is a natural triterpenoid saponin with the molecular formula $C_{42}H_{62}O_{16}$ and molecular weight 822.93 g/mol (CAS no. 1405-86-3). Glycyrrhizic acid is a conjugate of glycyrrhetic acid (aglycone) and two molecules of glucuronic acid. Glycyrrhetic acid, with the molecular formula $C_{30}H_{46}O_4$ and molecular weight 471 g/mol (CAS no. 471-53-4), can be released upon cleavage with glucuronidase. Both glycyrrhizic acid and glycyrrhetic acid can exist as 18α- and 18β-stereoisomers, with the β-isomer of glycyrrhetic acid being the main metabolite of glycyrrhizic acid. The α-isomer of glycyrrhetic acid may be present in up to 13%, is shown to interact with the glucocorticoid receptor, but its pharmacological and toxicological activity is not well studied. However, data in rats indicated that the 18α-isomer of glycyrrhetic acid may be more toxic that the 18β-isomer. Glycyrrhizic acid can form a variety of salts and occurs naturally as calcium and potassium salts, whereas ammonium salt is manufactured from liquorice extract as a flavouring substance (CIR Expert Panel, 2007; EMA, 2013; Isbrucker and Burdock, 2006; JECFA/IPCS, 2006; Nordic Council of Ministers, 1993). The structural formula of glycyrrhizic acid is shown in Figure 2.2.1–1.

2.2.2 Occurrence

Glycyrrhizic acid is extracted from the root system of the plant *Glycyrrhiza glabra* native to central and south-western Asia and the Mediterranean region. The fresh root contains about 20% of water-soluble extracts, of which glycyrrhizic acid constitutes 10–25%. The number of other chemicals in the liquorice root extracts can be high and is influenced by genetic, environmental and processing factors. (CIR Expert Panel, 2007; Isbrucker and Burdock, 2006; JECFA/IPCS, 2006).

Furthermore, the European Medicine Agency (EMA, 2013) refers that a standardised (European Pharmacopoeia 2010 under minor revision) liquorice ethanolic liquid extract (70% v/v), containing 3-5% of 18β-glycyrrhizic acid, is produced from the herbal drug. However, it was referred that no information was available on products on the market containing this standardised liquorice ethanolic liquid extract.

Glycyrrhizin is used as sweeteners and flavourings in foods and drinks such as candy, chewing gum, cookies, ice creams, syrups, herbal tea, alcoholic and non-alcoholic beverages, and chewing tobacco, and is present in traditional and herbal medicinal products. However, the main source of glycyrrhizin is most likely liquorice confectionery (EFSA, 2008; Isbrucker and Burdock, 2006; JECFA/IPCS, 2006).

2.3 Absorption, distribution, metabolism and excretion (ADME) in humans and experimental animals

Based on the available information, ADME appears to be relatively similar in experimental animals and humans. Glycyrrhizic acid, both in free form and as the ammonium salt, is poorly absorbed from the gastrointestinal (GI) tract. Glycyrrhizic acid is, however, hydrolysed by intestinal bacteria to glycyrrhetic acid (the aglycone of glycyrrhizic acid) which is readily absorbed. Glycyrrhetic acid is extensively absorbed from the gut. At high dose levels of glycyrrhizic acid (>25 mg/kg bw), the rate of hydrolysis of glycyrrhizic acid to glycyrrhetic acid by the gut microflora may become saturated and this may limit the relative amount of glycyrrhetic acid that can be absorbed from the GI tract. The absorption of the glycyrrhetic acid from the human gut, however, is nearly complete regardless of whether it is formed by hydrolysis of the glycyrrhizic acid or initially is present as the glycoside or the aglycone in a food matrix (JECFA/IPCS, 2006; SCF, 2003). Glycyrrhetic acid is conjugated in the liver before excretion in the bile. Thus, the metabolites provide a substrate for further hydrolysis by the gut microflora, leading to enterohepatic recycling. This has been shown in rats, and is presumed to take place in humans (CIR Expert Panel, 2007; EFSA, 2015; EMA, 2013).

Neither glycyrrhizic acid nor its hydrolysis product glycyrrhetic acid are taken up by tissues to any significant extent. However, both components adhere extensively to human and rat serum albumin in a saturable process (Isbrucker and Burdock, 2006; JECFA/IPCS, 2006).

The plasma clearance of glycyrrhetic acid is dose-dependent when administered to rats and humans at levels that exceed the saturation of serum protein binding. Significantly decreased plasma clearance has been demonstrated in patients with compromised liver function, thus a hepatic-related capacity-limited process for the metabolism and excretion of glycyrrhetic acid is suggested. The plasma concentration of glycyrrhetic acid shows several peaks during the subsequent time (up to 50 h) after oral administration of glycyrrhizic acid, glycyrrhetic acid or liquorice. This is explained by the enterohepatic recycling of glycyrrhetic acid and the varying emptying of the metabolites of glycyrrhetic acid from the gallbladder into the intestine. For example, in one study where 16 healthy adults consumed liquorice containing 225 mg glycyrrhizin, a peak plasma glycyrrhetic acid concentration of 1 µg/ml was reached after 10 h. A second and a third peak of approximately 0.2 and 0.1 µg/ml appeared after 30

and 50 hours, respectively. Since the complete elimination of glycyrrhetic acid takes several days, the potential for accumulation becomes more apparent when administration occurs on a daily basis (Isbrucker and Burdock, 2006).

It has been shown in rats that glycyrrhetic acid is to a certain degree able to cross the placental barrier and can be detected in the fetus (Isbrucker and Burdock, 2006). Dams were fed 100 mg glycyrrhetic acid/kg bw per day starting on the 13th day of gestation. The maternal plasma glycyrrhetic acid concentration was approximately 100 µg/ml at day 17, 19 and 21 of gestation, whereas the corresponding fetal concentrations were 5, 18 and 32 µg/ml, respectively.

2.4 Toxicological data/Adverse effects

2.4.1 Animal studies

2.4.1.1 Acute toxicity

In mice, oral LD_{50} values of extracts of *Glycyrrhiza* sp. were >7.5 g/kg bw for both sexes, whereas the LD_{50} values were 14.2 and 18.0 g/kg bw in male and female rats, respectively. A similar acute toxicity in rats and mice, with LD_{50} values between 4.0 and 4.4 g/kg bw, was reported after s.c. administration of extract of *Glycyrrhiza* sp. containing approximately 53% glycyrrhizic acid (Isbrucker and Burdock, 2006). Oral LD_{50} values for salts of glycyrrhizic acid in mice have been reported to be in the range of 1220–12700 mg/kg bw. Additionally, no deaths were reported in mice given a maximum oral dose of 610 mg/kg bw of glycyrrhetic acid (reviewed in JECFA/IPCS, 2006).

2.4.1.2 Short-term toxicity

Toxic effects of short-term liquorice extract administration to male and female Wistar rats have been examined. Rats were administered 0.31, 0.63, 1.25 or 2.5 g liquorice extract/kg bw per day by gavage for 90 days with liquorice extract estimated to contain 53% glycyrrhizin. Body weight (bw) gain was slightly inhibited in animals that received 2.5 g/kg bw per day. Hematological evaluation revealed a significant decrease in the red blood cell counts with decrease in hematocrit of the male rats receiving the two highest doses of liquorice extract. Male rats also had a slightly, but significantly, elevated neutrophil and decreased lymphocyte count at the highest dose. Total protein, albumin, aspartate transaminase (AST) and alanine transaminase (ALT) were significantly elevated in the male rats receiving the highest doses, whereas the same parameters were significantly decreased in the female rats administered the highest doses. Serum cholesterol was also decreased in both male and female rats with a 40% decrease in the female rats administered 2.5 g liquorice extract/kg bw per day. Although the average liver and kidney weights increased in the groups given 1.25 and 2.5 g/kg bw per day, there were no significant histological changes observed in these organs. Histology performed on the highest dose group revealed a slight atrophy of the thymus medulla, along with some lymphofollicular formations, as well

as some atrophy and catarrh of the stomach mucosa. These changes were not considered significant, because recovery was seen upon withdrawal of the liquorice extract. The authors considered the no observed effect level (NOEL) to be 0.31–0.63 g extract/kg bw (approximately 165–334 mg glycyrrhizin/kg bw) for 90 days of treatment (Komiyama et al., 1977, cited in Isbrucker and Burdock 2006).

In a range-finding study preliminary to a chronic, two year toxicity study, 0, 0.08, 0.15, 0.3, 0.6 or 1.25% disodium glycyrrhizin in drinking water (0, 200, 375, 750, 1500 or 3125 mg/kg bw) was administered to male and female B6C3F1 mice for 10 weeks. None of the animals receiving the two highest doses of glycyrrhizin survived, with animals showing histological signs of marked starvation atrophy. From this study, the authors determined that the maximum tolerated dose of disodium glycyrrhizin in drinking water was 0.15% for male and 0.3% for female mice (Kobuke et al., 1985, cited in Isbrucker and Burdock, 2006).

Possible neurobehavioural effects of ammoniated glycyrrhizin involving the pituitary–adrenal axis were investigated in male Sprague–Dawley rats fed 0, 2, 3 or 4% ammoniated glycyrrhizin (80% purity) in chow, providing approximately 0, 1.23 ± 0.02, 1.87 ± 0.03 or 2.55 ± 0.03 g/kg bw per day for 4–6 months (Sobotka et al., 1981, cited in Isbrucker and Burdock (2006)). Expected changes in the basic physiological measurements were noted, including hypertension, increased kidney and heart weight, polydipsia and bradycardia. Motor coordination and balance were unaffected by the glycyrrhizin treatment. Behavioral studies demonstrated that there was no effect on the passive avoidance or fixed interval responses, indicating that glycyrrhizin had no obvious effect on response inhibition, learning, retention or shock sensitivity. The conditioned avoidance response was found to be facilitated at the 4% glycyrrhizin dose, unaffected by the 3% dose and depressed in those animals administered the 2% dose. Although these data do not provide information on the neuropharmacological mechanism of glycyrrhizin, the authors do note that its actions are specific, rather than general, and that they are similar to those associated with other neuropeptides such as adrenocorticotropic hormone (ACTH).

2.4.1.3 Chronic toxicity

The chronic effects of disodium glycyrrhizin consumption were studied in male and female B6C3F1 mice (Kobuke et al., 1985, cited in Isbrucker and Burdock, 2006). A preliminary, sub-chronic, range-finding study had determined the maximum tolerated doses to be 0.15% (~375 mg/kg bw) for male mice and 0.3% (~750 mg/kg bw) for female mice. Glycyrrhizin was administered in drinking water for 96 weeks at concentrations of 0, 0.04, 0.08, 0.15 or 0.3%, delivering an approximate daily dose of 0, 71, 166 or 229 mg/kg bw to the male mice and 0, 117, 217 or 407 mg/kg bw to the female mice. Glycyrrhizin treatment did not significantly affect average bw, cumulative mortality rates and mean time to death, incidence, types or distribution of tumours. The authors concluded that the long-term daily administration of glycyrrhizin to these mice did not provide any evidence of chronic toxicity or tumourigenicity.

2.4.1.4 Reproductive/developmental toxicity

Since the objective of this hazard assessment was to evaluate adverse effects of glycyrrhizin to the fetus and child, the animal experiments investigating reproductive, teratogenic or other adverse effects on the fetus of glycyrrhizin or the synthetic liquorice derivative carbenoxolone were described in more detail and summarized in Table 2.4.1.4-1.

Table 2.4.1.4-1. An overview of animal experimental studies investigating reproductive, teratogenic or other potentially adverse effects on the fetus of glycyrrhizin or the synthetic liquorice derivative carbenoxolone.

Reference	Study	Dose and number in treatment group		Conclusion with regard to adverse effects
		Glycyrrhizin or carbenoxolone	**Control**	
Shihata and Elghamry (1963)	Estrogenic activity	Crude extracts of the plant *Glycyrrhiza glabra* (powdered and Soxhlet-extracted with 95% ethanol for 6 hours and evaporated to solid) given as 25 mg in 3 daily s.c. doses (total dose 7.5 g extract per kg bw) to 3-week old female mice (n = 100, strain not stated)	The same dose of ethanol	Estrogenic activity was found by the extract, i.e. the uteri were greatly enlarged with the mean uterine weight significantly increased ($P = 0.0027$) and the treated mice showed vaginal opening whereas the control mice did not. The extract had an inhibitory influence on the spontaneous movement of the uterus during di-estrus, estrus and pregnancy.
Itami et al. (1985)	Teratogenicity	Disodium glycyrrhizinate (NaG) in 60, 290 and 1480 mg/kg bw per day given in the diet to Wistar rats on day 0 to 20 of pregnancy, total NaG intake of 0.30 ± 0.01, 1.47 ± 0.04 and 7.34 ± 0.18 g (mean ± SE)	The same diet with no NaG	NaG had no teratogenic effects on the rat fetus at doses up to 1480 mg/kg bw per day given on gestational day 0 to 20
Mantovani et al. (1988)	Teratogenicity, embryotoxicity	Ammonium glycyrrhizinate (AG) administered in the drinking water to Sprague-Dawley rats on days 7-17 of pregnancy. The doses were (mean ± SE) 0, 21.33 ± 1.22, 238.75 ± 17.50 and 679.94 ± 69.87 mg/kg bw per day (groups 0, 1, 2 and 3, respectively)	Drinking water without AG	AG caused excessive thirst in the dams, but no signs of toxicity. There was a slight but significant increase in embryolethality; the prevalence of resorptions was significantly related to the dose ($P < 0.03$). The prevalence of external hemorrhages and hematomas, and the rate of affected litters, were significantly higher ($P < 0.01$ and $P < 0.001$, respectively) in groups 1 and 3 compared with that of the controls. Skeletal examination revealed a dose-related increase in the two highest dose groups in minor anomalies, especially in the sternebral variants ($P <0.001$). Renal ectopy also

Reference	Study	Dose and number in treatment group		Conclusion with regard to adverse effects
		Glycyrrhizin or carbenoxolone	**Control**	
				increased significantly at the highest dose. These results indicate possible embryotoxicity of AG.
Langley-Evans (1997)	Adverse effects (birth weight, blood pressure)	Pregnant Wistar rats were injected s.c. with 12.5 mg/kg bw carbenoxolone daily either throughout pregnancy (day 0-22), or in early (days 0-7), mid (days 8-14) or late (days 15-22) gestation.	Not relevant	In rats exposed to the inhibitor over days 8-14, 15-22 or 0-22, systolic blood pressure at 4 weeks was significantly higher than in controls. The greatest elevation of pressure was associated with treatment in late (days 15-22) gestation, indicating that adverse effects on offspring may be dependent on the time period during pregnancy exposure occurs.
van Gelderen et al. (2000) **(originally published as two RIVM reports in Dutch in 1984)**	Study I: rats Study II: rats	I: Effects of glycyrrhizic acid were compared with the effects of desoxycorticosterone acetate (DOCA), a mineralocorticoid, and a control group. All groups were studied with or without extra NaCl added to the diet during 10 weeks. II: Effects of glycyrrhizic acid on the sodium and natrium balance were studied in more detail; glycyrrhizic acid groups were compared to control groups with or without extra NaCl. No further information were available on these studies.	Control group present in both experiments	Both rat experiments confirmed the mineralocorticoid effects of glycyrrhizic acid. In these two rat studies, a NOAEL could not be established, because the lowest dose of glycyrrhizic acid tested (0.5 g/kg food, approximately 25 mg/kg bw) induced effects.
Hundertmark et al. (2002a)	Maturation of fetal lungs	Pregnant Wistar rats were given 10, 100 or 1000 mg/kg bw per day of glycyrrhetinic acid	No GE in the diet	Reduction/loss of pulmonary 11β-HSD1 activity in GE-treated rats substantially impaired fetal lung

Reference	Study	Dose and number in treatment group		Conclusion with regard to adverse effects
		Glycyrrhizin or carbenoxolone	**Control**	
		(GE) in the diet from day 13 of gestation until term.		maturation. Lungs from GE-exposed rats had lower surfactant protein-A (mRNA and protein) levels and reduced amniotic fluid lecithin/sphingomyelin ratios. There was a marked depletion of lung surfactant before and after birth, as detected by both light and electron microscopy.
Yoshida et al. (2011)	Reproductive and developmental toxicity	Monoammonium glycyrrhizinate (MGL) given by i.v. injection to Crl:CD (SD) rats were used in fertility and early embryonic development study (4-1-1 study), rat pre- and postnatal development study (4-1-2 study), rat and rabbit embryo-fetal development studies (4-1-3 study), and toxicokinetic study of pregnant and lactating rats. Animals (20 per sex/group) were administered at doses of 25, 75 or 225 mg/kg bw, and 150 mg/kg bw was added for the post-weaning assessment.	Vehicle (0.9% saline)	The reproductive toxicity tests performed at up to the toxic range of MGL did not show any influence on fertility and reproductive performance, 2) no embryotoxic or fetotoxic effects were found and no influence on progeny (F_1 and F_2 generation) was noted, and 3) none of the tests revealed any teratogenic effects.
Diao et al. (2013)	Reproduction (embryo implantation)	Glycyrrhizic acid (100 mg/kg bw) given by i.p. injection to pregnant wild-type Lpar3$^{(+/-)}$ and Lpar3$^{(-/-)}$ mice, both with normal embryo implantation, on gestation day 3 a few hours before embryo attachment to the uterine luminal epithelium	Carbenoxolone was injected i.p. (100 mg/kg bw) or via local uterine fat pad (10 mg/kg bw)	Glycyrrhizic acid, which shares similar structure and multiple properties, such as inhibition of 11β-HSD and anti-inflammation, with carbenoxolone, but is ineffective in blocking gap junctions, did not affect embryo implantation. The carbenoxolone treatment disrupted embryo implantation, suggesting local effects of carbenoxolone in the uterus.

Shihata and Elghamry (1963) showed that crude extracts of the plant *Glycyrrhiza glabra* (powdered and Soxhlet-extracted with 95% ethanol for 6 hours and evaporated to solid, however, content of glycyrrhizic acid was not stated) given as 25 mg in 3 daily s.c. doses (total dose 7.5 g extract per kg bw) to 3-week old female mice (n = 100, strain not stated) showed estrogenic activity. Control mice were given the same dose of ethanol. The uteri were greatly enlarged with the mean uterine weight significantly increased ($P = 0.0027$) and the treated mice showed vaginal opening whereas the control mice did not. The dose of 50 mg extract daily for 3 days did not show a corresponding increase in uterine weight and a diminished response. The extract had an inhibitory influence on the spontaneous movement of the uterus during di-estrus, estrus and pregnancy.

Itami et al. (1985) examined the teratogenicity of disodium glycyrrhizinate (NaG) in Wistar rats. Pregnant rats were fed diet containing 0% (n = 8), 0.08% (n = 10), 0.4% (n = 11) or 2% (n = 9) NaG *ad libitum* from day 0 to 20 of pregnancy. The daily intake in the dams during the period of administration was 60, 290 and 1480 mg/kg bw, respectively, for the three dietary groups. This administration gave an estimated total NaG intake of 0.30 ± 0.01, 1.47 ± 0.04 and 7.34 ± 0.18 g (mean ± SE), respectively. A comparison of the control and all groups of rats treated with NaG revealed no significant differences in food intake and in bw gain during pregnancy. However, bw gains after delivery were significantly lower in the two highest NaG dose groups. No significant differences between the control and NaG-treated groups were found in the numbers of corpora lutea and implants of dams, in the number of live fetuses and intrauterine dead fetuses per litter, in the sex ratios, in fetal bw of both sexes, in the placental weight, in the degrees of ossification in the sternebrae and caudal vertebrae of the fetuses, or in the live birth index, survival rate or bw gain of the offspring within 8 weeks after birth. Several kinds of skeletal variation of the fetus were observed in all the groups treated with NaG, but the incidences showed no significant differences compared with the controls. Except for one fetus with dilatation of the renal pelvis in the 0.08% group, no fetus with external, skeletal or internal malformations was found at any level examined. From these results, the authors concluded that NaG had no teratogenic effects on the rat fetus at least in doses up to 1480 mg/kg bw per day given on day 0 to 20 of pregnancy.

In a study by Mantovani et al. (1988), ammonium glycyrrhizinate (AG), a commercial salt of glycyrrhizic acid (with 99% purity), was administered in the drinking water to Sprague-Dawley rats on days 7-17 of pregnancy. The actual intakes were (mean ± SE) 0, 21.33 ± 1.22, 238.75 ± 17.50 and 679.94 ± 69.87 mg AG/kg bw per day for groups 0, 1, 2 and 3, respectively. The number of dams per group was 18, 19, 20 and 16 in groups 0, 1, 2 and 3, respectively. AG caused polydipsia (excessive thirst) in the dams, but no signs of toxicity were evident, based on bw increase, feed consumption and biochemical and histological parameters. Fetuses from the treated litters did not present an increase in external malformations, a decrease in weight or a decrease in the degree of ossification. However, there was a slight but significant increase in embryolethality; the prevalence of resorptions was significantly related to the dose ($P < 0.03$). The prevalence of external hemorrhages and hematomas, and the rate of affected litters, were significantly higher ($P < 0.01$ and $P <$

0.001, respectively) in groups 1 and 3 compared with that of the controls. Skeletal examination revealed a dose-related increase in the two highest dose groups in minor anomalies, especially in the sternebral variants (P <0.001). Renal ectopy also increased significantly at the highest dose. The authors concluded that these results indicated that the possible embryotoxicity of aromatizing compounds, such as glycyrrhizic acid derived from liquorice, should be considered.

In a study by Langley-Evans (1997), conducted in compliance with the British Home Office Animals (Scientific Procedures) Act 1986, pregnant Wistar rats (n = 4-6 per group) were injected subcutaneously (s.c.) with carbenoxolone, an inhibitor of 11β-hydroxysteroid dehydrogenase. Injections of 12.5 mg/kg bw carbenoxolone were administered daily either throughout pregnancy (day 0-22), or targeted to specific periods in early (days 0-7), mid- (days 8-14) or late (days 15-22) gestation. Control animals were given saline on the same days. Fetal exposure to carbenoxolone at any period in gestation resulted in lower weight at birth. In rats exposed to the inhibitor over days 8-14, 15-22 or 0-22, systolic blood pressure at 4 weeks was significantly higher than in control animals. The greatest elevation of pressure was associated with carbenoxolone treatment in late (days 15-22) gestation. Increased fetal exposure to maternal glucocorticoids because of downregulated 11β-hydroxysteroid dehydrogenase impairs fetal growth and programmes elevated blood pressure in later life. If the situation is similar in humans, this study indicates that adverse effects on offspring may be dependent on the time period during pregnancy exposure to carbenoxolone, and possibly glycyrrhizic acid, occurs.

In the Netherlands, two experiments in rats were reported as two RIVM reports in 1984 written in the Dutch language. However, these studies were described in a paper written in English by van Gelderen et al. (2000). In the first rat study, the effects of glycyrrhizic acid were compared with the effects of desoxycorticosterone acetate (DOCA), a mineralocorticoid, and a control group. All groups were studied with or without extra NaCl added to the diet during 10 weeks. The second rat experiment was performed to study the effects of glycyrrhizic acid on the sodium and natrium balance in more detail; glycyrrhizic acid groups were compared to control groups with or without extra NaCl. Both rat experiments confirmed the mineralocorticoid effects of glycyrrhizic acid. In these two rat studies, a no observed adverse effect level (NOAEL) could not be established, because in the lowest dose of glycyrrhizic acid tested (0.5 g/kg food, approximately 25 mg/kg bw), effects were observed.

Glucocorticoids (GC) induce surfactant synthesis in the late fetal lung. Deficient GC action causes respiratory distress syndrome. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inert cortisone (11-dehydrocorticosterone in rodents) into active cortisol (corticosterone), thus amplifying intracellular GC action. Hundertmark et al. (2002a) investigated 11β-HSD1 in the late fetal lung using the liquorice-derived inhibitor, glycyrrhetinic acid (GE), in pregnant Wistar rats (n = ~6) on day 13 of gestation until term. The dams were given 0, 10, 100 or 1000 mg/kg bw of GE per day in the diet. The GE treatment, in all doses, had no apparent effects upon the general development of fetuses.

There was no increase in malformations or fetal death rate. Control fetal mice and rats showed high 11β-HSD activity in the late fetal lung; levels of plasma 11-dehydrocorticosterone were also high. Reduction/loss of pulmonary 11β-HSD1 activity in GE-treated rats substantially impaired fetal lung maturation. Lungs from GE-exposed rats had lower surfactant protein-A (mRNA and protein) levels and a dose-dependent decrease in amniotic fluid lecithin/sphingomyelin ratios. There was a marked depletion of lung surfactant before and after birth, as detected by both light and electron microscopy. The importance of 11β-HSD for lung maturation was confirmed with the same results found in lungs from 11β-HSD1 -/- knockout mice (Hundertmark et al. (2002b). The authors concluded that the data emphasized the importance of 11β-HSD1 in amplifying key GC-dependent maturational processes in the late fetal lung. Whether a high intake of liquorice, leading to inhibition of 11β-HSD1, could impair fetal lung development also in humans, is not known.

Yoshida et al. (2011) investigated the influence of monoammonium glycyrrhizinate (MGL) by intravenous (i.v.) injection on reproductive and developmental toxicity in Crl:CD (SD) rats in several studies; fertility and early embryonic development study (4-1-1 study), rat pre- and postnatal development study (4-1-2 study), rat and rabbit embryo-fetal development studies (4-1-3 study), and toxicokinetic study of pregnant and lactating rats. All studies were carried out under the GLP regulations. Animals (20 per sex/group) were administered at dose levels of 25, 75 or 225 mg/kg bw, and 150 mg/kg bw was added for post-weaning assessment. In 4-1-1- study, the NOELs for the maternal generation and for the fetuses were 25 mg/kg bw and 75 mg/kg bw, respectively. In 4-1-2 study, the NOEL for the parental F_0 generation was 25 mg/kg bw, and the NOELs for the effects on the parental F_1 generation and on the development of the offspring (F_2 generation) were both above 150 mg/kg bw. In 4-1-3 study, the NOELs for the dams and for the fetuses were 75 mg/kg bw each in rats and 25 and 75 mg/kg bw in rabbits, respectively. In the toxicokinetic study, MGL administration caused low glycyrrhizin and glycyrrhetinic acid levels in the milk of lactating rats, and resulted in low exposure of the pups. The authors concluded that 1) the reproductive toxicity tests performed at up to the toxic range of MGL did not show any influence on fertility and reproductive performance, 2) no embryotoxic or fetotoxic effects were found and no influence on progeny (F_1 and F_2 generation) was noted, and 3) none of the tests revealed any teratogenic effects.

Gap junctions have an important role in cell-to-cell communication, a process obviously required for embryo implantation. The uterine luminal epithelium is the first contact for an implanting embryo and is critical for the establishment of uterine receptivity. To determine the potential function of uterine gap junctions in embryo implantation, carbenoxolone, a broad gap junction blocker, was injected i.p. (100 mg/kg bw) or via local uterine fat pad (10 mg/kg bw) into pregnant mice on gestation day 3 at 1800 h, a few hours before embryo attachment to the uterine luminal epithelium (Diao et al., 2013). All methods used were approved by the Animal Subjects Programs of the University of Georgia. Wild-type $Lpar3^{(+/-)}$ and $Lpar3^{(-/-)}$ mice, both with normal embryo implantation, were used. The carbenoxolone treatment disrupted embryo implantation, suggesting local effects of carbenoxolone in the uterus. However, i.p. injection of glycyrrhizic acid (100 mg/kg bw), which shares similar

structure and multiple properties, such as inhibition of 11β-hydroxysteroid dehydrogenase (11β-HSD) and anti-inflammation, with carbenoxolone but is ineffective in blocking gap junctions, did not affect embryo implantation.

Comments from VKM: Most of these animal studies on reproductive, teratogenic or other potentially adverse effects on the fetus of glycyrrhizin or carbenoxolone were quite old and not performed according to guidelines.

2.4.2 Genotoxicity

Previous assessments have reported that the majority of bacterial genotoxicity studies have demonstrated an absence of mutagenic and genotoxic effects from glycyrrhizin, glycyrrhizinate compounds or liquorice extracts in the Ames Salmonella assay (Isbrucker and Burdock, 2006; JECFA/IPCS, 2006; Nordic Council of Ministers, 1993). One positive mutagenic response was reported for liquorice extract in *S. typhimurium* TA100 at all concentrations tested, but not in TA98 (Martinez et al., 1999, cited in Isbrucker and Burdock, 2006). However, this response was not clearly concentration-dependent suggesting either some toxicity or influence on the DNA repair mechanisms at the higher concentrations. Pre-incubation of the extract with rat liver S9 fraction did not change the responses. Genotoxicity studies using *Escherichia coli* WP2 (Stanford Research Institute (SRI), 1979, cited in Isbrucker and Burdock, 2006) or *Saccharomyces cerevisiae* D-3 (Green, 1977, cited in Isbrucker and Burdock, 2006) were also reported to show an absence of mutagenic effects to glycyrrhizin (information about metabolic activation was not referred).

In mammalian cells *in vitro*, chromosome aberrations have been reported in Chinese hamster lung fibroblasts treated with glycyrrhizin or sodium glycyrrhizinate, but not in human embryonic lung cells treated with ammoniated glycyrrhizin. Glycyrrhizic acid trisodium salt was negative in tests for sister chromatid exchange and micronucleus formation in Chinese hamster cell cultures and human fibroblastic cell lines. Liquorice extracts were reported to produce negative results in the assay for unscheduled DNA synthesis assay in rat hepatocytes, but reportedly produced positive results in mouse lymphoma cells (JECFA/IPCS, 2006).

One *in vivo* genotoxic study of 39 food additives, including glycyrrhizin, has been performed (Sasaki et al., 2002, cited in Isbrucker and Burdock, 2006). A single oral dose of 2000 mg glycyrrhizin/kg bw was administered to male ddY mice and DNA damage was measured in various organs three and 24 h later by the COMET assay. Glycyrrhizin did not increase DNA damage in any of the 8 organs examined.

Comments from VKM: Regarding *in vitro* mutagenicity, only one positive test in *S. typhimurium* TA100 without metabolic activation was found with liquorice extract, which did not show clear dose-response, whereas the other reported mutagenicity tests were negative with or without activation. Regarding *in vitro* genotoxicity, positive results in tests of chromosomal aberrations in Chinese hamster lung fibroblasts with glycyrrhizin or sodium

glycyrrhizinate, and in mouse lymphoma cells treated with liquorice extracts, were reported, whereas the other reported *in vitro* genotoxicity tests were negative. However, the positive *in vitro* genotoxicity results were not confirmed *in vivo*, since negative results of high doses in the COMET assay in mice were reported.

Based on these results, VKM considers these substances to be non-mutagenic and non-genotoxic.

2.4.3 Human experimental and observational studies

Two human experimental (intervention) studies on effects of glycyrrhizic acid on healthy female and male volunteers were identified. Six observational studies on effects of glycyrrhizin on pregnant women and on their children, five in Finland and one in Korea, were found in the literature search (Table 2.4.3-1). In addition, a human intervention study on effects on bone of carbenoxolone, a synthetic 18β-hemisuccinate derivative of glycyrrhetinic acid, was found.

Table 2.4.3-1. An overview of human experimental and observational studies investigating health effects of glycyrrhizin or the synthetic liquorice derivative carbenoxolone.

Study design/reference	Participant characteristics	Country	Treatment and number in experimental groups		Dose(s)	Main endpoint(s), observed effects
			Glycyrrhizin or carbenoxolone	Control		
Randomized double-blind intervention (pilot) study **van Gelderen et al. (2000)**	16 healthy volunteers (8 women, 8 men) aged 19-30 years, weight range 58-71 kg for women and 56-91 kg for men	The Netherlands	Glycyrrhizic acid in capsules, study period 8 weeks; 2-week adaptation period, glycyrrhizic acid for 4 weeks, and then 2-week washout period		400 and 800 mg per day (about 6.6-13.3 mg/kg bw)	Symptoms similar to apparent mineralocorticoid excess (AME). One man and two women of the 800 mg group and one woman of the 400 mg group withdraw from the study because of edema (weight gain 0.6-6 kg), headache and general discomfort. In total 9 persons showed edema after 4-7 days of ingestion. Serum potassium concentration decreased in all volunteers, especially in the women. Aldosterone concentration and plasma renin activity were decreased considerable, again more marked in women. Effects were observed in both dose groups.
Randomized double-blind intervention study **van Gelderen et al. (2000)**	39 healthy female volunteers aged 19-40 years with bw 55-83 kg	The Netherlands	Glycyrrhizic acid in capsules for 8 weeks, after 2-week adaptation and with 2-week wash-out period		0 (n = 10), 1 (n = 9), 2 (n = 9) or 4 (n = 11) mg/kg bw	Symptoms similar to apparent mineralocorticoid excess (AME). Withdrawals: one in the 2 mg group after 2 weeks, (potassium concentration decreased below 3.0 mmol/l), one in the 4 mg group after 6 weeks (concentration difficulties, general discomfort and slight increase in blood pressure). Serum aldosterone

Study design/reference	Participant characteristics	Country	Treatment and number in experimental groups		Dose(s)	Main endpoint(s), observed effects
			Glycyrrhizin or carbenoxolone	Control		
						concentration significantly lower in the 4 mg group ($P < 0.001$) vs. controls after 2, 4, 6 and 8 weeks, whereas 1 and 2 mg groups did not differ from controls. Plasma renin activity decreased in a similar pattern, being significant in the 4 mg group only ($P < 0.001$). The atrial natriuretic peptide (ANP) concentration decreased significantly in the 4 mg group ($P < 0.001$) after the wash-out period, but was not significant in the 1 and 2 mg groups. Systolic and diastolic blood pressure in the 2 and 4 mg groups were unchanged. The bw showed no difference between any dose groups. Plasma potassium concentration decreased significantly in the 4 mg group compared with controls from week 2 to 4 ($P < 0.01$), gradually increasing to baseline. The decrease in plasma potassium concentration was not significant in the 2 mg group. The daily questionnaire showed an inconsistent picture, most observed effects were subclinical. Only for headache, nausea and vomiting, a dose-related increase was observed, and the 4 mg group differed from the controls. For change of defecation pattern, swollen face and tickling in arms and

Study design/reference	Participant characteristics	Country	Treatment and number in experimental groups		Dose(s)	Main endpoint(s), observed effects
			Glycyrrhizin or carbenoxolone	Control		
						legs, the 4 mg group differed from the other groups, however, without a clear dose-response.
Cross-sectional cohort study **Strandberg et al. (2001)**	1049 pregnant women and their healthy singleton children	Finland	Glycyrrhizin intake was calculated from detailed questionnaires on liquorice consumption answered by the mothers in the maternity ward		Mothers' intake during pregnancy: Low (<250 mg/week, *n* = 751), moderate (250–499 mg/week, *n* = 145) and heavy (≥500 mg/week, *n* = 110) intake of glycyrrhizin (75, 14 and 11% of the births, respectively)	Birth weight and gestational duration. No significant effects found on birth weight or maternal blood pressure, but glycyrrhizin exposure during pregnancy was significantly associated with shorter gestational duration OR for birth before 38 weeks' gestation was 2.5 (95% CI: 1.1, 5.5; *P* = 0.03).
Case-control study **Strandberg et al. (2002)**	Finnish women	Finland	95 women who delivered singleton babies before 37 or 34 weeks	107 women who delivered babies after normal gestational	Mothers' intake during pregnancy: Low (<250 mg/week, *n* = 751), moderate (250–499 mg/week, *n* = 145) and	Preterm (<37 weeks) and early preterm (<34 weeks) births. Heavy consumption versus the combination of moderate and low levels of consumption was associated with >2-fold increased risk of preterm (<37 weeks) delivery (OR = 2.28, 95% CI: 1.01, 5.14). The association was

Study design/reference	Participant characteristics	Country	Treatment and number in experimental groups		Dose(s)	Main endpoint(s), observed effects
			Glycyrrhizin or carbenoxolone	Control		
			Glycyrrhizin intake from Strandberg et al., 2001	duration in the same hospital	heavy (≥500 mg/week, *n* = 110) intake of glycyrrhizin (75, 14 and 11% of the births, respectively)	stronger for preterm (<34 weeks) births (OR = 3.07, 95% CI: 1.17, 8.05).
Cohort study **Räikkönen et al. (2009)**	321 Finnish children 8.1 (range 7.4 – 8.8) years of age born as healthy singletons at 35-42 weeks of gestation	Finland	Glycyrrhizin intake was calculated from detailed questionnaires on liquorice consumption answered by the mothers in the maternity ward		Mothers' intake during pregnancy: Low (<250 mg/week, *n* = 751), moderate (250–499 mg/week, *n* = 145) and heavy (≥500 mg/week, *n* = 110) intake of glycyrrhizin (75, 14 and 11% of the births, respectively)	Cognitive performance (subtests of the Wechsler Intelligence Scale for Children III as well as the Children's Developmental Neuropsychological Assessment and the Beery Developmental Test of Visual-Motor Integration) and psychiatric symptoms (Child Behavior Checklist). High maternal liquorice consumption compared with zero-low consumption during pregnancy was associated with poorer cognitive performance (range of mean differences in standard deviation (SD) units, -0.31 to -0.41; $P < 0.05$) and with externalizing symptoms and attention problems (range of ORs, 2.15 to 3.43; $P < 0.05$) in the offspring. The effects on cognitive performance appeared dose-related.

Study design/reference	Participant characteristics	Country	Treatment and number in experimental groups		Dose(s)	Main endpoint(s), observed effects
			Glycyrrhizin or carbenoxolone	Control		
Cohort study **Räikkönen et al. (2010)**	Children born healthy, singleton and who were not severely preterm or suffered from major perinatal disorders(*n* = 321, mean age 8.1 years, SD = 0.3 years)	Finland	Glycyrrhizin intake was calculated from detailed questionnaires on liquorice consumption answered by the mothers in the maternity ward		Low (<250 mg/week, *n*=751), moderate (250–499 mg/week, *n*=145) and heavy (≥500 mg/week, *n*=110) intake of glycyrrhizin (75, 14 and 11% of births, respectively)	Diurnal salivary cortisol and salivary cortisol during the Trier Social Stress Test for Children (TSST-C). Versus zero-low exposure, children with high exposure had 19.2% higher salivary cortisol awakening peak, 33.1% higher salivary cortisol awakening slope, 15.4% higher salivary cortisol awakening area under the curve (AUC), 30.8% higher baseline TSST-C salivary cortisol levels, and their salivary cortisol levels remained high throughout the TSST-C protocol (*P*-values <0.05). These effects appeared dose-related.
Cohort study **Räikkönen et al. (2017)**	Children (mean age 12.5 (SD 0.4) years; *n* = 378), both genders	Finland	Glycyrrhizin intake was calculated from detailed questionnaires on liquorice consumption answered by the mothers in the maternity ward		Mothers' intake during pregnancy: low (<250 mg/week, *n*=751), moderate (250–499 mg/week, *n*=145) and heavy (≥500 mg/week, *n*=110) intake of glycyrrhizin (75,	Pubertal maturation (height, weight, body mass index for age, difference between current and expected adult height, Tanner staging, score on the Pubertal Development Scale), neuroendocrine function (diurnal salivary cortisol, dexamethasone suppression), cognition (neuropsychological tests), and psychiatric problems (as measured by the Child Behavior Checklist). Girls with heavy maternal glycyrrhizin consumption were taller (mean difference (MD) = 0.4 SD, 95% CI: 0.1, 0.8), were heavier (MD = 0.6 SD, 95% CI: 0.2, 1.9), and

Study design/reference	Participant characteristics	Country	Treatment and number in experimental groups		Dose(s)	Main endpoint(s), observed effects
			Glycyrrhizin or carbenoxolone	Control		
					14 and 11% of births, respectively).	had higher BMI for age (MD = 0.6 SD, 95% CI: 0.2, 0.9). They were also 0.5 SD (95% CI: 0.2, 0.8) closer to adult height and reported more advanced pubertal development ($P <$ 0.04). There were no consistent associations between maternal liquorice consumption during pregnancy and pubertal maturation in boys at this age. Girls and boys exposed to heavy maternal glycyrrhizin consumption scored 7 (95% CI: 3.1, 11.2) points lower on tests of intelligence quotient, had poorer memory ($P <$ 0.04), and had 3.3-fold (95% CI: 1.4, 7.7) higher odds of ADHD problems compared with children whose mothers consumed little to no glycyrrhizin. No differences in cortisol levels were found.
Prospective cohort study **Choi et al. (2013)**	Singleton pregnant mothers	The Republic of Korea	185 mothers taking over-the-counter or naturopathic formulations containing liquorice.	370 age-matched controls that were not exposed to liquorice	Median dose 250.0 mg/day (range 0.93-2104.1), cumulative dose 16.7 mg/kg bw (range 0.06-971.1), exposure between day 4	Stillbirths, malformations. The rate of stillbirths was marginally higher among women who took liquorice than those who did not (OR = 7.9; 95% CI 0.9-71.5; $P =$ 0.048), and significantly higher when compared to the general population (OR = 13.3; 95% CI 4.9-35.8; $P <$ 0.001). Other fetal outcomes assessed were not significantly different between the two study groups, e.g.,

Study design/reference	Participant characteristics	Country	Treatment and number in experimental groups		Dose(s)	Main endpoint(s), observed effects
			Glycyrrhizin or carbenoxolone	Control		
					and week 25 of gestation	the OR for the difference of major malformations between the groups was 3.9 (95% CI 0.4-43.5; *P* = 0.27).
Case report **Hauksdottir et al. (2015)**	18-year-old healthy primigravida with high blood pressure and proteinuria at 18 weeks gestation with strong family history of pre-eclampsia	Iceland	Consumption of considerable amounts of liquorice at least up to the end of the first trimester on a close to daily basis		Sensitivity to liquorice confirmed with challenge test with liquorice	Pre-eclampsia. In healthy women with a familial or genetic (such as defect 11β-HSD2) susceptibility for pre-eclampsia, liquorice consumption may aggravate the course of the disease.
Intervention study **Cooper et al. (2000)**	Normal male volunteers aged 21.5 ± 1.3 years (mean ± SD), *n*=8	UK	Oral doses of carbenoxolone- a synthetic liquorice derivative		100 mg carbenoxolone 3 times per day for 7 days (in total 2100 mg)	Bone metabolism. A significant decrease in the bone resorption markers, pyridinoline (Pyr) and deoxypyridinoline (DPyr) (change in urinary Pyr/creatinine -1.55 ± 0.55 (mean ± SE), for DPyr/creatinine -0.4 ± 0.14 nmol/mmol; *P* < 0.05 for both), with no overall change in the bone formation markers C- and N-terminal propeptides of type I collagen (PICP and PINP).

In the Netherlands, a pilot study in humans was published as a RIVM report in 1989 written in the Dutch language. However, this study was described in a paper written in English by van Gelderen et al. (2000). The pilot study was a randomized double-blind study performed in 16 healthy volunteers (8 women, 8 men) aged 19-30 years, weight range 58-71 kg for women and 56-91 kg for men. Both genders were equally present in both dose groups given 400 and 800 mg glycyrrhizic acid per day (about 6.6-13.3 mg/kg bw) administered orally in capsules. The study lasted 8 weeks; starting with a two-week adaptation period (no alcohol, glycyrrhizic acid containing products or smoking), glycyrrhizic acid for 4 weeks, and at the end a two-week washout period. Effects were observed in both dose groups. One man and two women of the 800 mg group and one woman of the 400 mg group withdraw from the study because of edema (weight gain 0.6-6 kg), headache and general discomfort. In total, 9 persons showed edema after 4-7 days of ingestion. The serum potassium concentration decreased in all volunteers, especially in the women. The aldosterone concentration and plasma renin activity were reported to show a considerable decrease, again more marked in women. The symptoms observed were similar to the state of apparent mineralocorticoid excess (AME). Also interindividual differences were observed, but the dose of glycyrrhizic acid seemed to be of no influence on the severity of symptoms per individual.

In the pilot study, women appeared to be more sensitive to glycyrrhizic acid than men. Therefore, a follow-up study with lower doses was performed in women only, described in van Gelderen et al. (2000). Oral doses of 0 (n = 10), 1 (n = 9), 2 (n = 9) or 4 (n = 11) mg glycyrrhizic acid/kg bw in capsules were administered in a randomized double-blind fashion for 8 weeks to 39 healthy female volunteers aged 19-40 years with bw 55-83 kg. The experiment lasted 12 weeks including an adaptation and a "wash-out" period. The participants filled out a dietary questionnaire during 3 days every fortnight and a questionnaire on their physical condition every day.

One woman of the 2 mg group was withdrawn after two weeks, because the plasma potassium concentration decreased below 3.0 mmol/l (reference value 3.8-5.0 mmol/l), normalizing one week after withdrawal (van Gelderen et al., 2000). One woman of the 4 mg group was withdrawn after 6 weeks because of concentration difficulties, general discomfort and a slight increase in blood pressure (7 mm Hg systolic and diastolic) and in bw (3 kg) – both blood pressure and bw returning to normal after discontinuation. The aldosterone concentration in serum was significantly lower in the 4 mg/kg group ($P < 0.001$) than those of the control group after 2, 4, 6 and 8 weeks, whereas in the 1 and 2 mg/kg bw groups they were not difference from the controls. Plasma renin activity decreased in a similar pattern, the changes being significant in the 4 mg/kg group only ($P < 0.001$). The atrial natriuretic peptide (ANP) concentration decreased significantly in the 4 mg/kg bw group ($P < 0.001$) after the wash-out period of two weeks, but did not change significantly in the 1 and 2 mg/kg bw groups. The systolic and diastolic blood pressure in the 2 and 4 mg/kg bw groups remained unchanged during the administration period. However, as there was a slight decrease in systolic and diastolic blood pressure in the control group, blood pressure in the 2 and 4 mg/kg bw groups was increased relatively compared to the control group; however, the changes were significant only in the 4 mg/kg bw group ($P = 0.018$). The bw of

the women showed no difference between the four dose groups. The plasma potassium concentration decreased significantly in the 4 mg/kg bw group compared with controls from week 2 to 4 ($P < 0.01$), gradually increasing to baseline during the experiment. The decrease in plasma potassium concentration was not significant in the 2 mg/kg bw group. The daily questionnaire showed an inconsistent picture, most observed effects were subclinical. Only for headache, nausea and vomiting, a dose-related increase was observed, and the 4 mg/kg bw group differed from the control group. For change of defecation pattern, swollen face and tickling in arms and legs, the 4 mg/kg bw group differed from de other groups, however, without a clear dose-response relationship. In all dose groups, the overall number of complaints decreased during the study.

From this study, van Gelderen et al. (2000) proposed a NOEL of 2 mg/kg bw, and an ADI of 0.2 mg/kg bw was extrapolated with a safety factor of 10. This corresponded to consumption of 12 mg glycyrrhizic acid per day for a person with bw 60 kg, which would be equal to 6 g liquorice per day, assuming that liquorice contained 0.2% glycyrrhizic acid.

Based on a suspected role for glucocorticoids in the etiology of low birth weight, Strandberg et al. (2001) tested whether maternal consumption of glycyrrhizin in liquorice affected birth weight in a cross-sectional study in humans. A sample of 1049 Finnish women and their healthy singleton infants was studied in 1998. Glycyrrhizin intake was calculated from detailed questionnaires on liquorice consumption answered by the mothers while being in the maternity ward. Weekly glycyrrhizin intake was calculated from the reported quantity (in grams) and frequency (never, seldom, weekly, daily) of consumption of liquorice (as brand names). A list of all brands of liquorice-containing confectionery on sale in Finland, based on a report prepared by the National Food Administration in 1993, and updated with information from manufacturers, was used. This information was obtained from 1006 of the 1049 women. Glycyrrhizin intake was analysed both as a continuous variable and as a categorical variable grouped into three levels: low (<250 mg/week; $n = 751$), moderate (250-499 mg/week; $n = 145$), and heavy (≥500 mg/week; $n = 110$), which comprised 75, 14 and 11% of the births, respectively. Birth weight and gestational duration (the term gestational age was used by the authors) were obtained from hospital records. Gestational duration was obtained from fetal ultrasound measurements of biparietal diameter during the first trimester for 90% of the mothers and from the mothers' self-report of the last menstrual period for the rest of the women.

Babies with heavy prenatal exposure to glycyrrhizin were not significantly lighter at birth (either with glycyrrhizin intake as continuous or categorical variable), but they were significantly more likely to be born earlier (Strandberg et al., 2001). When glycyrrhizin intake was considered as a continuous variable (adjusted for sex and maternal age), the slope of the relation was equivalent to a reduction in gestational duration of 1.26 days (95% confidence interval (CI); 0.31, 2.24, $P = 0.009$) for every 500 mg/week increase in glycyrrhizin intake. When it was treated as a categorical viable, the odds ratio for being born before 38 weeks' gestation was 2.5 (95% CI: 1.1, 5.5; $P = 0.03$) (adjusted for sex, maternal age, parity, smoking, coffee consumption and systolic blood pressure). Although the effect of

heavy glycyrrhizin intake on mean duration of gestation was small (2.52 days) when expressed as an effect on the mean, this shift to the left of the distribution of duration of gestation was sufficient to double the risk of being born before 38 weeks. The association remained in multivariate analyses. The authors concluded that heavy glycyrrhizin exposure during pregnancy did not significantly affect birth weight or maternal blood pressure, but that it was significantly associated with shorter gestational duration.

Comments from VKM: A reduction in gestational duration of 1.26 days for every 500 mg/week increase in glycyrrhizin intake seems small, and appear to be within normal variation (Jukic et al., 2013). Also the effect of heavy glycyrrhizin intake on mean duration of gestation was small (2.52 days) when expressed as an effect on the mean. However, as pointed out by the authors this shift to the left of the distribution of duration of gestation was sufficient to double the risk of being born before 38 weeks.

Since heavy liquorice (glycyrrhizin) consumption was associated with shorter gestational duration in the study in 2001, Strandberg et al. examined whether this association also applied to preterm births (delivery <37 weeks) in a case-control study (Strandberg et al. 2002). In 2000–2001, a sample of 95 Finnish women who delivered preterm singletons was compared with controls (n = 107) who delivered babies of normal gestational duration in the same hospital. Glycyrrhizin intake was calculated from questionnaires containing detailed items on liquorice consumption (i.e. collected retrospectively), similar to in Strandberg et al. (2001). Glycyrrhizin exposure was grouped into three levels: low (<250 mg/week), moderate (250–499 mg/week) and heavy (≥500 mg/week). The heavy intake of 500 mg/week of glycyrrhizin was reported to correspond to approximately 250 g/week of liquorice.

Heavy consumption versus a lower level (moderate and low levels combined) of consumption was associated with a more than twofold increased risk of preterm (<37 weeks) delivery (Strandberg et al., 2002). The association was stronger when only the 40 births classified as early preterm delivery (<34 weeks) were included (odds ratio (OR) = 3.07, 95% CI: 1.17, 8.05) for the fully adjusted model (mother's age, sex, parity and smoking). The authors concluded that heavy glycyrrhizin exposure was associated with preterm delivery and might be a novel marker of this condition. The hypothesis was that glycyrrhizin inhibits the local breakdown of cortisol in placenta, leading to increased cortisol levels that may affect prostaglandins. A local increase in prostaglandins in the uterus during pregnancy could lead to contractions.

Some critical comments have been raised by Hughes et al. (2003) towards Strandberg et al. (2002). They includes the fact that lifestyle factors such as body mass index or blood pressure where not measured in the study on children born in 2000-2001 (Strandberg et al., 2002). The mothers' ages were fairly similar, although the mothers associated with preterm births were statistically significantly younger (30.2 years, standard deviation (SD) 5.1 vs. 32.3 years, SD 5.3; P = 0.007). Slattery and Morrison (2003) commented upon the fact that the percentage of women with heavy glycyrrhizin intake was not significantly different between the cases (20.0%) and controls (10.3%) (P = 0.06).

Räikkönen et al. (2009) studied whether prenatal exposure to glycyrrhiza in liquorice exerts detrimental effects on cognitive performance (subtests of the Wechsler Intelligence Scale for Children III as well as the Children's Developmental Neuropsychological Assessment and the Beery Developmental Test of Visual-Motor Integration) and psychiatric symptoms (Child Behavior Checklist) in 321 Finnish children 8.1 (range 7.4 – 8.8) years of age born in 1998 as healthy singletons at 35-42 weeks of gestation invited to participate in this follow-up study in 2006.

In comparison to the group with zero-low glycyrrhiza exposure (0-249 mg/week), those with high exposure (≥500 mg/week) had significant decrements in verbal and visuospatial abilities and in narrative memory (range of mean differences in standard deviation units, -0.31 to -0.41; $P < 0.05$) and significant increases in externalizing symptoms and in attention, rule-breaking and aggression problems (range of odds ratios, 2.15 to 3.43; $P < 0.05$) (Räikkönen et al., 2009). Thus the key findings were that high maternal liquorice consumption compared with zero-low consumption during pregnancy was associated with poorer cognitive performance (range of mean differences in SD units, -0.31 to -0.41; $P < 0.05$) and with externalizing symptoms and attention problems (range of ORs, 2.15 to 3.43; $P < 0.05$) in offspring at 8.1 years of age. The effects on cognitive performance appeared dose-related (a graded, linear association was found). The effects found in this study were independent of length of gestation, birth weight or head circumference. The authors concluded that the data were compatible with adverse fetal "programming" by overexposure to glucocorticoids and cautioned against excessive intake of liquorice-containing foodstuffs during pregnancy.

Comment from VKM: The children's own liquorice intakes were not reported in this study by Räikkönen et al. (2009).

From the same cohort as in the previous study, Räikkönen et al. (2010) studied if maternal consumption of glycyrrhizin in liquorice associates with HPAA function in children born healthy, singleton and who were not severely preterm or suffered from major perinatal disorders. In addition, according to the authors there were no exposure-level group differences in maternal health during pregnancy, all factors that could have compromised the internal validity of the study findings. Diurnal salivary cortisol and salivary cortisol during the Trier Social Stress Test for Children (TSST-C) were measured in children contacted in 2006 (n = 321, mean age 8.1 years, SD = 0.3 years) whose mothers consumed varying levels of glycyrrhizin in liquorice during pregnancy; exposure-level groups were denoted high (≥500 mg/week), moderate (250–499 mg/week) and zero-low (0–249 mg/week) and were reported in 1998.

In comparison to the zero-low exposure group, children in the high exposure group had 19.2% higher salivary cortisol awakening peak, 33.1% higher salivary cortisol awakening slope, 15.4% higher salivary cortisol awakening area under the curve (AUC), 30.8% higher baseline TSST-C salivary cortisol levels, and their salivary cortisol levels remained high throughout the TSST-C protocol (P - values <0.05) (Räikkönen et al., 2010). These effects

appeared dose-related. The authors concluded that their findings lended support to prenatal 'programming' of hypothalamic-pituitary-adrenocortical axis (HPAA) function by overexposure to glucocorticoids.

Comment from VKM: The children's own liquorice intakes were not reported in this study by Räikkönen et al. (2010).

Prenatal glucocorticoid exposure influences the timing of puberty in animal models, but the human relevance of those findings is unknown. Räikkönen et al. (2017) studied whether voluntary consumption of liquorice, which contains glycyrrhizin, by pregnant women (reported in 1998) was associated with pubertal maturation (height, weight, body mass index for age, difference between current and expected adult height, Tanner staging, score on the Pubertal Development Scale), neuroendocrine function (diurnal salivary cortisol, dexamethasone suppression), cognition (neuropsychological tests) and psychiatric problems (as measured by the Child Behavior Checklist) in their children. The children were born in 1998 in Helsinki, Finland, and examined during 2009–2011 (mean age = 12.5 (SD = 0.4 years; *n* = 378). The adolescents' own liquorice consumption (never, less than once a week, once a week, 2-4 days a week, daily, no answer) was reported in the follow-up assessment. The average weekly glycyrrhizin content (per kg bw at delivery) in liquorice products consumed by the mothers was 2.3 mg/kg bw (range 0 – 4.5 mg/week) in the zero-low exposure group and 13.7 mg/kg bw (range 6.4 – 41.4 mg/ week) in the high exposure group. A range of potential confounders were adjusted for (maternal education as proxy for maternal intelligence, maternal self-reported age at menarche (years) as a crude proxy for the genetic component of pubertal development, maternal age and BMI, maternal smoking, alcohol consumption and coffee, tea, cacao, chocolate and salt consumption, stress during pregnancy, highest educational level of either parent, adolescents' age, gestational length, birth weight and the adolescents' own liquorice consumption).

Girls exposed to high maternal glycyrrhizin consumption (≥500 mg/week) vs. zero-low consumption (≤249 mg/week) were taller (mean difference (MD) = 0.4 SD, 95% CI: 0.1, 0.8), were heavier (MD = 0.6 SD, 95% CI: 0.2, 1.9), and had higher body mass index for age (MD = 0.6 SD, 95% CI: 0.2, 0.9) (Räikkönen et al., 2017). They were also 0.5 standard deviations (95% CI: 0.2, 0.8) closer to adult height and reported more advanced pubertal development ($P < 0.04$). There were no consistent associations between maternal liquorice consumption during pregnancy and pubertal maturation in boys at this age. Girls and boys (tested combined because sex X exposure level group interactions were not significant) exposed to high (≥500 mg/week) maternal glycyrrhizin consumption scored 7 (95% CI: 3.1, 11.2) points lower on tests of intelligence quotient, had poorer memory ($P < 0.04$), and had 3.3-fold (95% CI: 1.4, 7.7) higher odds of attention deficit/hyperactivity disorder (ADHD) problems compared with children whose mothers consumed little to no glycyrrhizin (≤249 mg/week). No differences in cortisol levels were found. The authors concluded that liquorice consumption during pregnancy might be associated with harm for the developing offspring.

Criticisms of this and previous studies were given in an invited commentary (Keyes and Susser, 2017):

The 'zero-low' category includes persons whose liquorice intake ranges up to the 75th percentile of maternal liquorice consumption, combining those with no exposure to glycyrrhizin and those consuming up to 249 mg of glycyrrhizin per week - a rather heterogeneous group. A 'high-consumption' category includes persons with liquorice intake above the 91st percentile of maternal consumption. Those with liquorice consumption between these two categories were omitted, as was done in some but not all previous studies. Another limitation is that the study included at its start fewer than half the women from the original sampling frame, and they were not systematically selected (Räikkönen et al., 2009); in addition, the current study has an approximately 45% rate of retention of those included at the start. Inverse probability weighting is used to account for differences in loss to follow-up, but there is little information on the outcomes of the 55% of women who were not followed and whether those outcomes are related to exposure. It is difficult to draw strong conclusions under these conditions, especially based on small numbers. It should also be noted that the measurement of liquorice consumption at the time of birth is not ideal, as previous studies have shown that post-pregnancy recall of pregnancy-related exposures is imperfect, especially for exposures that might be less salient (such as, presumably, the quantity and frequency of liquorice consumption).

The unadjusted results in supplemental tables in Räikkönen et al. (2017) limited to liquorice consumers suggested a linear relationship between liquorice consumption and pubertal timing. However, the existing literature on cortisol exposure and other stress-reactivity measures in relation to pubertal staging and dynamics suggests that the relationship is non-linear and complex (Ellis et al., 2011; Saxbe et al., 2015; Shi et al., 2011). Keyes and Susser (2017) also claim that 'it is premature to accept the finding on cognitive ability as being established' and 'that in light of the present analyses, the finding on mental disorders is not interpretable'.

Comments from VKM: It should be noted that in the studies by Strandberg et al. (2001) and Räikkönen et al. (2009; 2010; 2017) all mothers and their children are from one original cohort (children born in 1998), whereas a separate cohort was included in Strandberg et al. (2002) (children born in 2000-2001). The estimated glycyrrhizin intake in the mothers during pregnancy used in the follow-up studies of the children were from interviews of the same original cohort of mothers answering questionnaires in 1998 (Strandberg et al., 2001). The women in the cohort used in Strandberg et al. (2002) reported their liquorice intake, but lifestyle factors such as body mass index or blood pressure were not reported.

In none of these human studies was the effect of glycyrrhizin on the activity of the 11β-HSD2 enzyme actually measured. No adjustments were done for food intake other than coffee, tea, cacao, chocolate, or for protein intake, which may be confounders. Apparently, nor was there any recording of use of other sources of glycyrrhizin, such as chewing

tobacco, cough medicines or use of traditional and herbal medicines with liquorice, in any of these human studies.

Keeping in mind the weaknesses above, the negative health effects reported on the mothers or their offspring were found with estimated glycyrrhizin intake of the mothers of ≥500 mg/week, reported to correspond to approximately 250 g/week of liquorice, compared with lower intake (0-499 mg/week). Therefore, 500 mg/week of glycyrrhizin can be regarded as the lowest observed adverse effect level (LOAEL). The average weekly glycyrrhizin content (per kg bw at delivery) in liquorice products consumed by the mothers was 2.3 mg/kg bw (range 0 – 4.5 mg/week) in the zero-low exposure group and 13.7 mg/kg bw (range 6.4 – 41.4 mg/ week) in the high exposure group (Räikkönen et al., 2017).

In a prospective cohort study, Choi et al. (2013) studied the outcome of 185 singleton pregnancies who took over-the-counter or naturopathic formulations containing liquorice during their pregnancy, and 370 age-matched singleton pregnant controls that were not exposed to any potential teratogen. The indication in 56.8% of the women taking liquorice was for cough and cold control, with the maximum dose of 2104 mg/day and exposure occurring between the 4th day and 25th week of gestation. The rate of stillbirths was marginally higher among women who took liquorice than those who did not (OR = 7.9; 95% CI 0.9-71.5; $P = 0.048$), and significantly higher when compared to the general population in the Republic of Korea (OR = 13.3; 95% CI 4.9-35.8; $P < 0.001$). Other fetal outcomes assessed in the study were similar between the two study groups, e.g., the OR of major malformations was 3.9 (95% CI 0.4-43.5; $P = 0.27$). The authors concluded that the present study suggested that liquorice is not a major teratogen. However, whether liquorice may increase the risk of stillbirths requires careful consideration in further studies with a larger sample size.

Major criticisms were raised towards this study by MacLennan and Koog (2014):

The data collection method used provided an opportunity for memory bias regarding their use of herbal medicines with liquorice (as is the case also in the papers reported above regarding liquorice intake by Finnish women). The presence of acute illness during early gestation may have influenced the data of the women in the experimental groups, since more than half of the women received herbal medication to treat acute illness, including influenza, and maternal infections may cause high maternal fever, respiratory distress or other systematic reactions, thus contributing to the death of the fetus. It is uncertain whether all the women in the experimental group actually received medication containing glycyrrhiza. In addition, there was concern about the correctness of the data analysis. The data on stillbirth was reported with OR = 7.9 (95% CI: 0.9 – 71.5), $P = 0.048$. However, since the 95% CI contains the value 1, it is non-significant, and the P-value was shown to be 0.065 on recalculation, indicating no significant difference in stillbirths between the cases and controls.

Comment from VKM: The level of exposure to glycyrrhiza in the study by Choi et al. (2013) is too uncertain for it to be used in a quantitative hazard assessment of liquorice intake, and it will not be considered further in this assessment.

Other effects on the mothers that may harm the fetus

Hypertensive activity that may add to the complication of preeclampsia is reported for various herbs, including liquorice (Newall et al., 1996). Glycyrrhizin inhibits the maternal 11β-HSD2 enzyme and therefore increases cortisol access to renal mineralocorticoid receptors, potentially causing maternal hypertension, which may harm the fetus (see also 2.4.8).

Hauksdottir et al. (2015) presented the case of very early onset preeclampsia, possibly aggravated by liquorice consumption, in Iceland. Preeclampsia at less than 20 weeks gestation is extremely rare and is usually not seen until after 24 weeks. An 18-year-old healthy primigravida was presented with high blood pressure and proteinuria at 18 weeks gestation. She had a strong family history of preeclampsia and was consuming considerable amounts of liquorice at least up to the end of the first trimester on a close to daily basis. A diagnosis of severe preeclampsia/hemolysis, elevated liver enzymes and low platelet count was confirmed. The pregnancy was terminated. Extensive investigation ruled out underlying diseases and autopsy revealed a normal fetus. In three consecutive pregnancies, she developed milder forms of preeclampsia, but delivered healthy babies, when abstaining from liquorice consumption. A challenge test with liquorice (100 g liquorice (150 mg glycyrrhetinic acid) daily for two weeks) was performed 6 months after delivery in the second pregnancy. The test resulted in inhibition of serum and urinary levels of aldosterone, and inhibition of serum levels of renin. It also resulted in inhibition of the enzymatic activity of 11β-HSD2, reflected in an increase in the urinary ratio of cortisol/cortisone metabolites, although it was still within normal reference values. The authors concluded that in healthy women with a familial or genetic (such as defect 11β-HSD2) susceptibility for preeclampsia, liquorice consumption may aggravate the course of the disease.

Other case reports briefly mentioned in van Gelderen et al. (2000): chronic intoxications were described after intake of 60-100 g liquorice per day (equivalent to 120-200 mg glycyrrhizic acid assuming a content of 0.2%), consumption of 25-200 g liquorice a day (50-400 mg glycyrrhizic acid), 48 mg glycyrrhizic acid per day from two packs of chewing gum, and a patient who suffered from hypokalemia and a depression of the renin-aldosterone axis by a prescription of 40 mg glycyrrhizic acid per day by his physician.

Hyperglycemic activity that might complicate gestational diabetes is also found in liquorice (Newall et al., 1996).

Effects on bone metabolism

Glucocorticoids have an essential role in skeletal development and function, but are detrimental in excess. Previously, expression and activity of the 11β-HSD2 isozyme were demonstrated in rat and human osteosarcoma cell lines, and expression in osteoblasts of

normal human fetal bone, whereas the 11β-HSD1 isozyme was expressed in human osteoblast cultures (Condon et al., 1998). Further, 11β-HSD expression was characterized in fresh normal adult human bone, where both 11β-dehydrogenase (cortisol-to-cortisone conversion) and reductase (cortisone-to-cortisol conversion) activities were demonstrated (Cooper et al., 2000). Carbenoxolone inhibits both 11β-dehydrogenase and 11β-oxoreductase, unlike liquorice, which inhibits only 11β-dehydrogenase (Stewart et al., 1990).

The effect of 11β-HSD on bone metabolism was also assessed *in vivo* using the synthetic liquorice derivative carbenoxolone in eight normal male volunteers aged 21.5 ± 1.3 years (mean ± SD) in United Kingdom (UK) (Cooper et al., 2000). They were given oral doses of 100 mg carbenoxolone three times per day for 7 days (in total 2100 mg). There was considerable interindividual variation in the dehydrogenase, but not reductase, activity (Cooper et al., 2000). In bone homogenates, activity was NADP-dependent with a *K*m for cortisol of 4.8 ± 1.2 µmol/L, indicating activity of 11β-HSD1. The 11β-HSD1 isozyme was expressed in cells of the osteoblast lineage and in osteoclasts, whereas the 11β-HSD2 isozyme was expressed only in osteoblasts and at a low level. Ingestion of carbenoxolone by the volunteers resulted in a significant decrease in the bone resorption markers, pyridinoline (Pyr) and deoxypyridinoline (DPyr) (change in urinary Pyr/creatinine -1.55 ± 0.55 (mean ± SE), for DPyr/creatinine -0.4 ± 0.14 nmol/mmol; $P < 0.05$ for both), with no overall change in the bone formation markers C- and N-terminal propeptides of type I collagen (PICP and PINP). These data suggested that local tissue metabolism of glucocorticoids is likely to be important in determining the sensitivity of both osteoblasts and osteoclasts to glucocorticoids. In particular, variation in 11β-HSD isozyme expression and activity may explain individual variation in susceptibility to glucocorticoid-induced osteoporosis.

Comments from VKM: Since 11β-HSD isozyme expression was demonstrated in fetal bone, and glycyrrhizin in liquorice has the same inhibitory effect on the 11β-HSD enzyme as carbenoxolone, it is plausible that glycyrrhizin may affect bone metabolism in fetal bone. However, at which doses of glycyrrhizin the net effect will be positive or negative for the bone formation in the fetus is not known.

2.4.4 Human *ex vivo*/*in vitro* studies

Human placenta exhibits high levels of 11β-HSD oxidative activity. The enzymatic activity was tested in tissue slices, homogenates, microsomes and CHAPS (steroidal detergent)-solubilized microsomal protein of spontaneously delivered fresh human placenta (Blum et al., 1995). Compared to liver and kidney, the placenta exhibits the highest specific 11β-HSD activity in microsomal preparations. Placental 11β-HSD was inhibited by β-glycyrrhetinic acid. The authors concluded that the placenta constitutes an important barrier for 11-OH steroids during pregnancy between the maternal and fetal organism.

In isolated perfused human placenta lobules it was shown that glycyrrhetinic acid transfers from the maternal to the fetal circulations without detectable metabolism during 6 hours of perfusion (Dodds et al., 1997).

2.4.5 *In vitro* studies

Yamaguchi et al. (2010) analysed the effects of glycyrrhetinic acid (GA) on the induction of anoikis-like death and cytoskeletal disruption in the central nervous system tumorigenic cells (SFME and r/m HM-SFME-1 cells). GA was cytotoxic in time- and dose-dependent manners, and the tumorigenic cells shed floating cells upon the GA treatment and even some of the adherent cells were easily detached from the fibronectin-coated culture dish by gentle shaking and aspiration. Reculture of the detached cells revealed that the longer the duration of GA exposure, the less the number of the proliferatable cells. These results indicated that GA perturbed cell adhesion and induced anoikis-like cell death. Further, GA also induced morphologic changes and disturbed cytoskeletal proteins. The concentration of GA that affected the tumor cells in this study was 10 µM, a concentration apparently also reached in the plasma of humans ingesting moderate to high levels of liquorice (de Groot et al., 1988). Whether GA may also affect normal cells in a similar manner leading to potential adverse effects, for instance in a fetus, is not known.

2.4.6 Interactions

Interactions between herbal products containing liquorice and drugs may be important also during pregnancy. Glycyrrhizin is shown to interact with various drugs, such as prednisolone and hydrocortisone (EMA, 2013). Prolonged intake of high liquorice extract or glycyrrhizin may result in accelerated metabolism of co-administered drugs, via the induction of various metabolic enzymes (i.a. CYP3A, CYP2B1, CYP1A2 and CYP2B9) and 16β-testosterone hydroxylase. Hypertension, edema and hypokalemia have been reported as adverse effects of interactions between drugs and intake of glycyrrhizin. Oral contraceptive use may increase sensitivity to glycyrrhizin.

Herbal products may contain allergens (Newall et al., 1996), however, no data was found on liquorice or glycyrrhizic acid and allergy.

2.4.7 Other vulnerable groups

Significant decreased plasma clearance of glycyrrhetic acid has been demonstrated in patients with compromised liver function, indicating a hepatic-related capacity-limited process for the metabolism and excretion of glycyrrhetic acid. Therefore, patients with decreased liver function may be more susceptible to adverse effects of glycyrrhetic acid compared with healthy persons (EMA, 2013). Consumption of liquorice is contraindicated also for patients with hypokalemia, for instance in persons taking cardiac glycosides.

Apparent mineralocorticoid excess (AME) is a rare form of hypertension, which is inherited in an autosomal recessive fashion, which untreated may lead to damage to organs such as kidneys, cardiovascular system, retina and the central nervous system (Hammer and Stewart, 2006). This condition is caused by mutations in 11β-HSD2 gene, located on chromosome 16q22. More than 30 different mutations have been defined within this gene in

approximately 60 kindreds to cause type I and type II AME. The latter is a milder variant of AME, with less severe clinical phenotype of hypertension, but which also includes a defect in 11β-HSD2 activity. Heterozygotes appear clinically normal, although experimental studies have suggested that heteromeric 11β-HSD2 formation may compromise overall 11β-HSD2 activity. Excess liquorice intake is regarded as the acquired counterpart to the inherited AME syndrome (see also Hauksdottir et al. (2015)). The prevalence of AME is difficult to estimate and likely varies between populations depending on the level of consanguinity. Less than 100 cases have been reported in the literature so far. The prevalence of AME is reported internationally as <1/1 000 000 (see http://www.orpha.net/consor/cgi-bin/index.php), but the prevalence in Norway is not known.

AME and liquorice-induced hypertension, both hypertensive disorders, have been attributed to a defect in the enzyme 11β-HSD, which interconverts cortisol to cortisone. The study by McCalla et al. (1998) aimed to determine the role of human placental 11β-HSD activity in preeclampsia, which is a hypertensive disorder in pregnancy. The 11β-HSD activity was determined in placentas of 17 normotensive and 11 preeclamptic patients matched for gestational age at 34-42 weeks. Cortisol levels in umbilical venous and arterial sera were also determined for both groups. 11β-Dehydrogenase (oxidation activity of 11β-HSD) activity was significantly lower in placentas of preeclamptic compared to normotensive patients (0.19 ± 0.09 vs. 0.26 ± 0.08 mmoles/min/placenta, $P = 0.02$). Cortisol level in umbilical cord blood was significantly higher in the preeclamptic group (14.99 ± 14.08 vs. 6.71 ± 3.69 g/dl, $P = 0.02$). The decreased 11β-HSD activity was accompanied by an expected increase in umbilical cord blood cortisol levels and decrease in fetal weights. The authors concluded that the results indicated that the decreased 11β-HSD activity in the placenta was related to decreased fetal growth in preeclampsia and that this enzyme may play an important role in influencing fetal growth.

2.4.8 Mode of action for adverse effects

Mineralocorticoids are a class of corticosteroids, which are a class of steroid hormones (Hammer and Stewart, 2006). Mineralocorticoids are produced in the adrenal cortex and influence electrolyte (salt) and water balances, and binds to the mineralocorticoid receptor (MR). The primary endogenous mineralocorticoid is aldosterone, although several other endogenous hormones (including progesterone and deoxycorticosterone) have mineralocorticoid function. Glucocorticoids are another class of corticosteroids, which bind to the glucocorticoid receptor (GR), which is expressed in most fetal tissues from mid-gestation onwards, as well as in placenta and fetal membranes. Glucocorticoids are distinguished from mineralocorticoids and sex steroids by their specific receptors, target cells, and effects. Glucocorticoids regulate multiple physiological and pharmacological processes including glucose metabolism, immune activity and the stress response. Cortisol is the most important human glucocorticoid.

Glucocorticoids have many essential roles in the body, including regulation of fetal growth, brain development and organ maturation to prepare the fetus for extra-uterine life, but may

be detrimental in excess (Figure 2.4.8-1). In several tissues, glucocorticoid action is dependent upon the expression of 11β-hydroxysteroid dehydrogenase (11β-HSD) isozymes, which catalyses the conversion of glucocorticoids, but not the mineralocorticoid aldosterone, to inactive metabolites. More specifically, it interconvert active cortisol and inactive cortisone, and regulates the access of cortisol to both MR and GR in humans. 11β-Hydroxysteroid dehydrogenase is an enzyme complex consisting of 11β-dehydrogenase activity (catalysing cortisol to cortisone (inactive form)) and 11β-oxoreductase activity (catalysing cortisone to cortisol (active form)). Two isoforms of 11β-HSD have been described. The type 1 NAPD(H)-dependent dehydrogenase/oxoreductase has bi-directional activity, however, *in vivo* the enzyme predominantly functions as a low affinity (μM) oxoreductase and consequently facilitates GR-mediated hormone action leading to tissue-specific modulation of cortisol concentrations. The second isoform is a high affinity (nM) type 2 NAD(H)-dependent dehydrogenase, which serves to protect the MR from cortisol to ensure aldosterone selectively (Hammer and Stewart, 2006; Stewart et al., 1994). 11β-HSD1 is expressed in liver, adipose tissues, lung, gonads and brain. 11β-HSD2 is expressed in kidney, colon, salivary gland and placenta. 11β-HSD2, but not 11β-HSD1, is found in fetal tissues, at least at midgestation (Stewart et al., 1990). The widespread distribution of 11β-HSD2 in placenta and fetal tissues suggests that it has an important role in fetal development. It may serve to protect developing tissues from cortisol excess or may modulate the permissive actions of glucocorticoids. Since 11β-HSD2 expression was demonstrated in fetal tissues, and also in placenta, and glycyrrhetic acid in liquorice has an inhibitory effect on this enzyme, it is plausible that glycyrrhetic acid may affect the fetus provided that the concentration is sufficiently high in these tissues to inhibit 11β-HSD2.

Thus, during pregnancy, the fetus is protected from high glucocorticoid levels in the mother by the action of the placental barrier enzyme 11β-HSD2 (Bertram and Hanson, 2002; Khulan and Drake, 2012; Reynolds, 2013). The main adverse effect of liquorice via its ingredient glycyrrhizin is to disrupt the ability of placental 11β-HSD2 enzyme to inactivate cortisol (by converting it to cortisone) before it reaches the fetus, leading to higher levels of fetal cortisol exposure. The fetal levels of cortisol are generally 10-15% of the maternal levels. The concern is that the fetal overexposure to cortisol may in turn modify fetal development by 'reprogramming' the fetal hypothalamic-pituitary-adrenal (HPA) axis, possibly via epigenetic modifications. These perturbations are associated with low birth weight and lasting adverse effects such as type 2 diabetes, cardiovascular disease and other manifestations of metabolic syndrome over the life-course, and even transgenerationally (Achard et al., 2006). Thus liquorice consumption may serve, in some ways, to mimic maternal stress, acting via the same mechanisms (Reynolds et al., 2013).

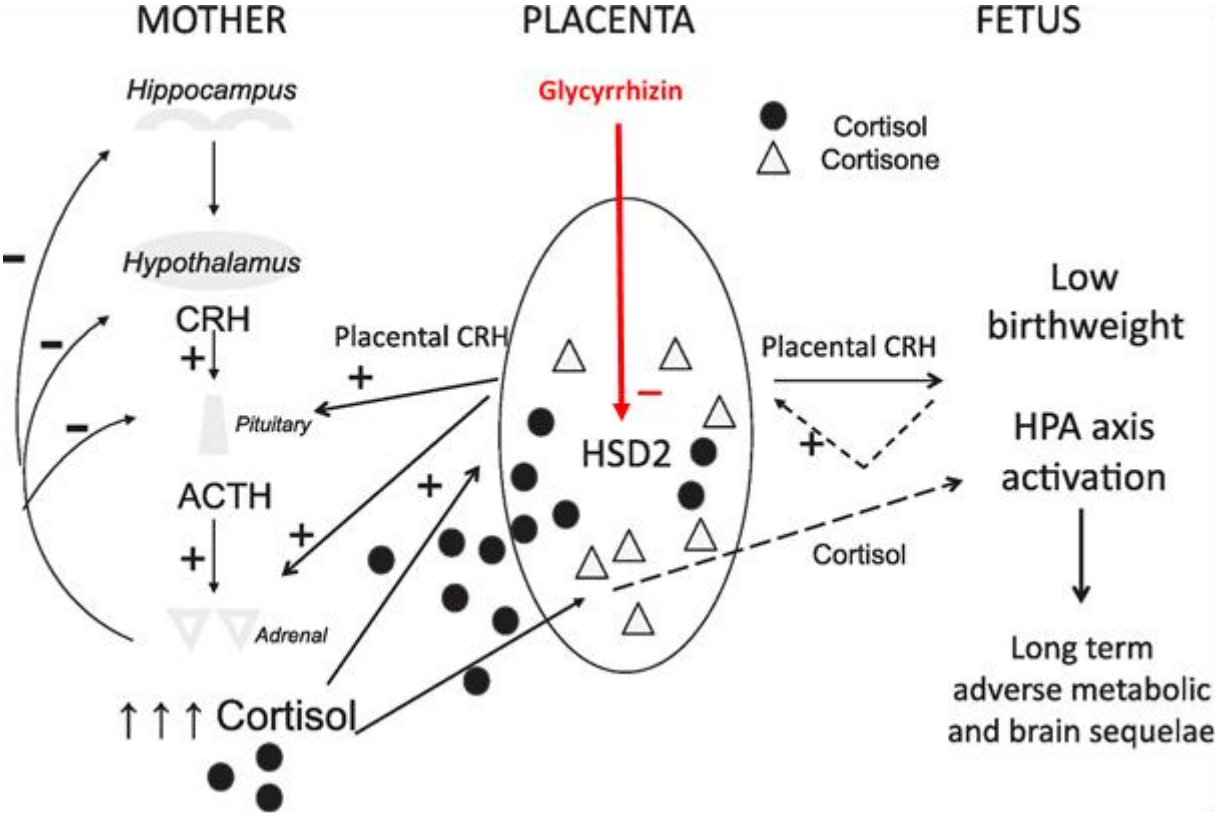

Figure 2.4.8-1. Glucocorticoid signalling between mother, placenta and fetus. The figure shows interaction between maternal, placental and fetal compartments during pregnancy leading to overexposure of the developing fetus to glucocorticoids. Activation of the maternal hypothalamic–pituitary–adrenal (HPA) axis during pregnancy leads to increased circulating levels of cortisol (filled circles). Placental corticotropin releasing hormone (CRH) also directly stimulates the maternal pituitary and adrenal to further increase cortisol levels, while maternal cortisol also stimulates placental CRH production. Maternal cortisol passes through the placenta where it is broken down by the enzyme 11β hydroxysteroid dehydrogenase type 2 (HSD2) into inactive cortisone (grey triangles). The fetus can also signal to the placenta to increase production of placental CRH when fetal metabolic demands increase. Overexposure of the developing fetus to excess cortisol leads to fetal HPA axis activation which is associated with low birth weight and long-term adverse programmed outcomes including metabolic and brain sequelae. ACTH – adrenocorticotropin hormone. The figure is modified from Reynolds (2013).

Impaired 11β-HSD2 activity can be caused by genetic mutations in its gene or by inhibitors such as glycyrrhetic acid and carbenoxolone. The mineralocorticoid receptors in the distal nephron, which are normally protected from cortisol by the 11β-HSD2 activity, are then activated by cortisol, leading to increased transcription of MR target genes (Hammer and Stewart, 2006). In this way, cortisol mimics aldosterone; resulting in increased sodium reabsorption from, and potassium excretion into, the urine. Increased sodium resorption depresses the renin-angiotensin-aldosterone axis. As a reaction to increased atrial stretch caused by fluid retention the serum concentration of ANP increases. Thus, glycyrrhizic acid can cause a state of apparent mineralocorticoid excess.

Both glycyrrhetinic acid and carbenoxolone may inhibit both human 11β-HSD1 and 11β-HSD2 (Ma et al., 2011). Both substances are more potent inhibitors of 11β-HSD2 than 11β-HSD1.

2.5 Summary of hazard identification and characterisation

2.5.1 Summary of previous risk assessments

The safe levels suggested in previous risk assessments of glycyrrhizic acid are summarized in Table 2.5.1-1.

Table 2.5.1-1. Summary of suggested safe levels in previous risk assessments of chronic intake of glycyrrhizic acid and its salts in adults.

Suggested safe levels	Reference
Not possible to establish ADI	EMA, 2013
100 mg/day*	EFSA, 2008
100 mg/day*	JECFA, 2005; JECFA/IPCS, 2006
100 mg/day*	SCF, 2003
Provisional LOAEL = 100 mg/day	Nordic Council of Ministers, 1993
GRAS	US FDA, 2017
200 mg/day	RIVM, 2003

*The suggested safe levels were expected to protect the majority of the population, but possibly not the most susceptible subpopulation.

2.5.2 Summary of ADME

ADME of glycyrrhizin appears to be relatively similar in experimental animals and humans. Glycyrrhizic acid, both in free form and as the ammonium salt, is poorly absorbed from the GI, but is hydrolysed by intestinal bacteria to glycyrrhetic acid, which is readily absorbed. At doses >25 mg/kg bw of glycyrrhizic acid, the rate of hydrolysis of glycyrrhizic acid to glycyrrhetic acid by the gut microflora may become saturated and this may limit the relative amount of glycyrrhetic acid that can be absorbed from the GI tract. The absorption of the glycyrrhetic acid from the human gut, however, is nearly complete regardless of whether it is formed by hydrolysis of the glycyrrhizic acid or initially is present as the glycoside or the aglycone (JECFA/IPCS, 2006; SCF, 2003). Glycyrrhetic acid is conjugated in the liver before excretion in the bile. Thus, the metabolites provide a substrate for further hydrolysis by the gut microflora, leading to enterohepatic recycling. This has been shown in rats, and is presumed to take place in humans (CIR Expert Panel, 2007; EFSA, 2015; EMA, 2013).

Neither glycyrrhizic acid nor its hydrolysis product glycyrrhetic acid are taken up by tissues to any significant extent. However, both components adhere extensively to human and rat serum albumin in a saturable process (Isbrucker and Burdock, 2006; JECFA/IPCS, 2006). The plasma clearance of glycyrrhetic acid is dose-dependent when administered to rats and humans at levels that exceed the saturation of serum protein binding (Isbrucker and

Burdock, 2006). It has been shown in rats that glycyrrhetic acid is to a certain degree able to cross the placental barrier and can be detected in the fetus (Isbrucker and Burdock, 2006).

2.5.3 Summary of animal experiments

Low acute toxicity of glycyrrhizin was demonstrated in mice and rats. In a 90-day toxicity study of liquorice extract in Wistar rats, the NOEL was 0.31–0.63 g extract/kg bw (approximately 165–334 mg glycyrrhizin/kg bw) (Komiyama et al., 1977, cited in Isbrucker and Burdock 2006). In a range-finding 10-week study in B6C3F1 mice, the maximum tolerated dose of disodium glycyrrhizin was 750 mg/kg bw for males and 1500 mg/kg bw for females (Kobuke et al., 1985, cited in Isbrucker and Burdock, 2006). Further, when disodium glycyrrhizin given for 96 weeks in doses up to 229 mg/kg bw in male mice and 407 mg/kg bw in female mice, there were no evidence of chronic toxicity or tumourigenicity.

When neurobehavioural effects of ammoniated glycyrrhizin involving the pituitary–adrenal axis were investigated in male Sprague–Dawley rats fed approximately 0, 1.23 ± 0.02, 1.87 ± 0.03 or 2.55 ± 0.03 g/kg bw per day for 4–6 months (Sobotka et al., 1981, cited in Isbrucker and Burdock 2006), there was no effect on the passive avoidance or fixed interval responses, indicating that glycyrrhizin had no obvious effect on response inhibition, learning, retention or shock sensitivity. However, the conditioned avoidance response was found to be facilitated at 2.55 g/kg bw, unaffected by 1.87 g/kg bw and depressed in those animals administered 1.23 mg/kg bw. The authors speculated that this behavioural profile may be caused by interaction of ammoniated glycyrrhizin with the pituitary system.

Studies in rats also showed mineralocorticoid effects, including increased blood pressure, of glycyrrhizic acid and carbenoxolone (Langley-Evans, 1997; van Gelderen et al., 2000).

Regarding teratogenicity, Itami et al. (1985) reported no teratogenic effects on the rat fetus up to 1480 mg/kg bw per day of disodium glycyrrhizinate given on gestational day 0 to 20. Up to 225 mg/kg bw of monoammonium glycyrrhizinate in rats and/or rabbits did not show any influence on fertility and reproductive performance, embryotoxic or fetotoxic effects, no influence on F_1 and F_2 generation and no teratogenic effects (Yoshida et al., 2011). However, ammonium glycyrrhizinate administered to rats on days 7-17 of pregnancy induced a slight but significant dose-related increase in embryolethality (Mantovani et al., 1988). In this study, the prevalence of external hemorrhages and hematomas, and the rate of affected litters, were significantly higher after 21 and 680 mg/kg bw per day, minor skeletal anomalies were dose-relatedly increased with 239 and 680 mg/kg bw, and renal ectopy was significantly increased at 680 mg/kg bw. I.p. injection of glycyrrhizic acid (100 mg/kg bw) into pregnant mice on gestation day 3 a few hours before embryo attachment to the uterine luminal epithelium did not affect embryo implantation, whereas the same dose of carbenoxolone did (Diao et al., 2013).

When pregnant rats were given glycyrrhetinic acid from day 13 of gestation until term substantially impaired fetal lung maturation was observed. Lungs from the exposed rats had

lower surfactant protein-A levels and a dose-dependent (10, 100 and 1000 mg/kg bw per day) decrease in amniotic fluid lecithin/sphingomyelin ratios (Hundertmark et al., 2002a).

A study indicated that a crude 95% ethanol extract of *Glycyrrhiza glabra* had estrogenic effects in mice (Shihata and Elghamry, 1963).

Based on the *in vitro* test results on mutagenicity and the *in vitro* and *in vivo* test results on genotoxicity, VKM considers glycyrrhizin to be non-mutagenic and non-genotoxic.

2.5.4 Summary and discussion of human studies

Glycyrrhizin inhibits the maternal 11β-HSD2 enzyme and therefore increases cortisol access to renal mineralocorticoid receptors, potentially causing maternal hypertension. Hypertensive activity that may add to the complication of preeclampsia is reported for liquorice (Newall et al., 1996). Hyperglycemic activity that might complicate gestational diabetes is also found in liquorice (Newall et al., 1996). Such adverse effects of glycyrrhizin on the pregnant mother may be detrimental to the fetus.

From the randomized double-blind study on adult volunteers, observing symptoms similar to the state of AME, van Gelderen et al. (2000) proposed a NOEL of 2 mg/kg bw, and an ADI of 0.2 mg/kg bw was extrapolated with a safety factor of 10. This corresponded to consumption of 12 mg glycyrrhizic acid per day for a person with bw 60 kg, which would be equal to 6 g liquorice per day, assuming that liquorice contained 0.2% glycyrrhizic acid. Women appeared to be more sensitive to glycyrrhizic acid than men (van Gelderen et al. (2000).

Strandberg et al. (2001) correlated glycyrrhizin intake of mothers during pregnancy with outcome in the offspring, and found that heavy glycyrrhizin exposure during pregnancy did not significantly affect birth weight or maternal blood pressure, but was significantly associated with shorter gestational duration. In a separate cohort, Strandberg et al. (2002) found that heavy liquorice consumption versus a lower consumption was associated with a more than twofold increased risk of preterm (<37 weeks) delivery. The association was stronger when only the 40 births classified as early preterm delivery (<34 weeks) were included (OR = 3.07, 95% CI: 1.17, 8.05) for the fully adjusted model (mother's age, sex, parity and smoking). Possibly relevant for the risk of under-developed lungs in premature children, it was shown that reduction/loss of pulmonary 11β-HSD1 activity in rats treated with glycyrrhetinic acid substantially impaired fetal lung maturation (Hundertmarkt et al., 2002a).

Follow-up studies were conducted with the children included in the original Finnish cohort reported in Strandberg et al., (2001). Räikkönen et al. (2009) reported that high maternal liquorice consumption compared with zero-low consumption during pregnancy was associated with poorer cognitive performance (range of mean differences in SD units, -0.31 to -0.41; $P < 0.05$) and with externalizing symptoms and attention problems (range of ORs, 2.15 to 3.43; $P < 0.05$) in offspring 8.1 years of age. The effects on cognitive performance

appeared dose-related. In a further study of this cohort (Räikkönen et al., 2010), in comparison to the zero-low exposure group, children in the high exposure group had 19.2% higher salivary cortisol awakening peak, 33.1% higher salivary cortisol awakening slope and 15.4% higher salivary cortisol awakening AUC. In addition, they had 30.8% higher baseline TSST-C salivary cortisol levels, and their salivary cortisol levels remained high throughout the TSST-C protocol (*P* - values <0.05). These effects also appeared dose-related. In these two studies, the liquorice intake by the children was not reported.

When the same cohort of children was studied at mean age 12.5 years, girls exposed to high maternal glycyrrhizin consumption (≥500 mg/week) vs. zero-low consumption (≤249 mg/week) were taller (MD = 0.4 SD, 95% CI: 0.1, 0.8), were heavier (MD = 0.6 SD, 95% CI: 0.2, 1.9) and had higher body mass index for age (MD = 0.6 SD, 95% CI: 0.2, 0.9) (Räikkönen et al., 2017). They were also 0.5 standard deviations (95% CI: 0.2, 0.8) closer to adult height and reported more advanced pubertal development (*P* < 0.04). There were no consistent associations between maternal liquorice consumption during pregnancy and pubertal maturation in boys at this age. Girls and boys exposed to high (≥500 mg/week) maternal glycyrrhizin consumption scored 7 (95% CI: 3.1, 11.2) points lower on tests of intelligence quotient, had poorer memory (*P* < 0.04) and had 3.3-fold (95% CI: 1.4, 7.7) higher odds of ADHD problems compared with children whose mothers consumed little to no glycyrrhizin (≤249 mg/week). In this study, the liquorice intake by the children was adjusted for in the analyses.

Comment from VKM: One animal study reported neurobehavioural effects in adult male rats (Sobotka et al., 1981, cited in Isbrucker and Burdock (2006)). The conditioned avoidance response was found to be facilitated at 2.55 g/kg bw glycyrrhizin, unaffected by 1.87 g/kg bw and depressed after 1.23 g/kg bw per day for 4–6 months. However, the doses in this experiment were very high compared with the average dose of 13.7 mg/kg bw for the mothers at delivery in the human studies (Räikkönen et al., 2017).

It should be noted that in the studies by Strandberg et al. (2001) and Räikkönen et al. (2009; 2010; 2017), all mothers and their children are from one original cohort (children born in 1998), whereas a separate cohort was included in Strandberg et al. (2002) (children born in 2000-2001). The estimated glycyrrhizin intakes in the mothers during pregnancy used in the follow-up studies of the children were from interviews of the one original cohort of mothers answering questionnaires in 1998 (Strandberg et al., 2001). The women in the cohort used in Strandberg et al. (2002) reported their liquorice intake, but lifestyle factors such as body mass index or blood pressure where not reported.

In none of these human studies was the effect of glycyrrhizin on the activity of the 11β-HSD2 enzyme actually measured. No adjustments were done for food intake other than coffee, tea, cacao, chocolate, or for protein intake, which may be confounders, especially for birth weight (Hynes et al., 2012). Apparently, nor was there any recording of use of other sources of glycyrrhizin, such as chewing tobacco, cough medicines or use of traditional and herbal medicines with liquorice, in any of these human studies.

Nor were there any analyses or adjustments done for other potential environmental chemicals that may inhibit the 11β-HSD2 enzyme, such as phthalates, organotins and dithiocarbamates (Odermatt and Gumy, 2008; Ma et al., 2011).

Glucocorticoid action is dependent upon the expression of 11β-hydroxysteroid dehydrogenase (11β-HSD) isozymes, which interconverts active cortisol and inactive cortisone, and regulates the access of cortisol to both MR and GR (Hammer and Stewart, 2006). Two isoforms of 11β-HSD have been described. The widespread distribution of 11β-HSD2 in placenta and fetal tissues suggests that it has an important role in fetal development. During pregnancy, the fetus is protected from high glucocorticoid levels in the mother by the action of the placental barrier enzyme 11β-HSD2 (Bertram and Hanson, 2002; Khulan and Drake, 2012; Reynolds, 2013).

The main adverse effect of liquorice via glycyrrhizin is to disrupt the ability of placental 11β-HSD2 enzyme to inactivate cortisol (by converting it to cortisone) before it reaches the fetus, leading to higher levels of fetal cortisol exposure. The fetal levels of cortisol are generally 10-15% of the maternal levels. The concern is that the potential fetal overexposure to cortisol may in turn modify fetal development by 'reprogramming' the fetal HPA axis, possibly via epigenetic modifications. These perturbations are apparently associated with low birth weight and lasting adverse effects over the life course, and even transgenerationally (Achard et al., 2006).

In VKM's opinion, the suggested mechanism of an inhibitory effect on the 11β-HSD2 enzyme by glycyrrhizin, leading to overexposure of the fetus to cortisol with subsequent adverse effects, is regarded as plausible. The findings in these studies are indicative of potential adverse effects of glycyrrhizin on the offspring from liquorice intake during pregnancy.

Keeping in mind the weaknesses above, the negative health effects reported on the mothers or their offspring were found with estimated glycyrrhizin intake of the mothers of ≥500 mg/week, corresponding to approximately 250 g/week of liquorice, compared with lower glycyrrhizin intake (0-499 mg/week). Therefore, apparently 500 mg/week of glycyrrhizin can be regarded as the LOAEL. The average weekly glycyrrhizin content (per kg bw at delivery) in liquorice products consumed by the mothers was 2.3 mg/kg bw (range 0 – 4.5 mg/week) in the zero-low exposure group and 13.7 mg/kg bw (range 6.4 – 41.4 mg/week) in the high exposure group (Räikkönen et al., 2017).

This LOAEL value based on the Finnish studies by Strandberg et al. (2001; 2002) and Räikkönen et al. (2009; 2010; 2017), is uncertain. There is potential recall bias of liquorice consumption by the mothers and issues with the categorization of exposure levels used in these studies as discussed above (Keyes and Susser, 2017). The data on content of glycyrrhizic acid in the liquorice confectionary on sale in Finland was obtained from a report prepared by the National Food Administration (Blomberg and Hallikainen, 1993), updated with information from manufacturers. In this report, the average content of glycyrrhizic acid in the analysed samples (n = 102) was 0.2% (range 0.017% - 0.73%). Other studies have reported 0.26-7.90 g/kg (0.03-0.8%) in liquorice-containing confectionary in UK (Spinks and

Fenwick, 1990) and 0.85-1.05 g/kg (0.09-0.11%) in sweets in Czech Republic (Kvasnička et al., 2007). Based on these studies, the content of glycyrrhizic acid in liquorice-containing confectionary may vary approximately 47-fold.

However, the level of exposure of the fetus to glycyrrhizin is too uncertain based on the available data to be able to draw firm conclusions on a cause and effect relationship. One of main uncertainties is the actual intake of glycyrrhizin by the mothers during pregnancy, i.e. the external dose.

The glycyrrhizin intake in these human studies was reported as average weekly consumption of glycyrrhizin from liquorice confectionary intake during pregnancy, but no recording of glycyrrhizin intake in various parts of the pregnancy was done. Thus, there is also uncertainty regarding whether the exposure to glycyrrhizin occurred in critical periods during pregnancy relevant for the effects on puberty, cortisol levels, cognitive performance, psychiatric symptoms etc. observed in the children. A study in rats on carbenoxolone indicated that adverse effects on offspring may be dependent on the time period during pregnancy when exposure occurs Langley-Evans (1997). The same may be the case also for glycyrrhizic acid exposure.

Regarding the internal dose absorbed into the mothers' circulation, this is depending on the capacity limit of the intestinal microflora to hydrolyse glycyrrhizic acid to glycyrrhetic acid and of the enterohepatic recycling, the binding of glycyrrhizic acid and glycyrrhetic acid to serum albumin in a saturable process, and the percentage of glycyrrhetic acid eventually reaching the placenta. In isolated perfused human placenta lobules it was shown that glycyrrhetinic acid transfers from the maternal to the fetal circulations without detectable metabolism during 6 hours of perfusion (Dodds et al., 1997). Ultimately, the question is whether the actual level of glycyrrhetic acid is sufficient to inhibit the placental 11β-HSD2 enzyme. An *ex vivo* dual-perfusion method study of fresh, intact, human term placentas showed that even very low doses of glycyrrhetic acid potently and rapidly inhibited the placental glucocorticoid barrier function by inhibiting 11β-HSD2 (Benediktsson et al., 1997).

Räikkönen et al. (2017) stated that 'Because the associations between maternal glycyrrhizin intake and pubertal maturation in girls and cognition and attention deficit/hyperactivity disorder problems in both girls and boys were linear, it appears that no safe exposure during human pregnancy exists.' This suggested linear relationship between liquorice consumption and pubertal timing was apparently based on unadjusted results in supplemental tables in Räikkönen et al. (2017) limited to liquorice consumers (Keyes and Susser, 2017). However, the existing literature on cortisol exposure and other stress-reactivity measures in relation to pubertal staging and dynamics suggests that the relationship is non-linear and complex (Ellis et al., 2011; Saxbe et al., 2015; Shi et al., 2011).

There is observed large interindividual variation in sensitivity to glycyrrhizin (Nordic Council of Ministers, 1993; van Gelderen et al., 2000). A possible reason for this, at least partly, may be differences in the ability of the gut microflora to hydrolyse glycyrrhizic acid to glycyrrhetic

acid (Isbrucker and Burdock, 2006). There is also reported considerable variation in the 11β-HSD2 dehydrogenase activity, inactivating cortisol, between human placentas (Benediktsson et al., 1997).

Human *ex vivo* studies showed that human placenta exhibits high levels of 11β-HSD oxidative activity, which was inhibited by β-glycyrrhetinic acid (Blum et al., 1995). An *in vitro* study showed that glycyrrhetinic acid may perturb cell adhesion, induce anoikis-like cell death, induce morphologic changes and disturb cytoskeletal proteins in human relevant concentrations (Yamaguchi et al. (2010).

Glycyrrhizin is shown to interact with various drugs, such as prednisolone and hydrocortisone, and prolonged intake of high liquorice extract or glycyrrhizin may result in accelerated metabolism of co-administered drugs, via the induction of various metabolic enzymes (EMA, 2013).

Patients with decreased liver function and hypokalemia may be vulnerable at excessive intake of liquorice. Persons with AME, an inherited rare form of hypertension caused by mutations in 11β-HSD2 gene, may be susceptible to liquorice. If untreated, AME may lead to damage to various organs (Hammer and Stewart, 2006). Decreased 11β-HSD activity in the placentas of women with preeclampsia was related to lower fetal growth (McCalla et al., 1998).

3 Exposure

No data was available on glycyrrhizic acid intake, either as liquorice confectionery, from intake of other foods and drinks, or as a herbal product, among pregnant women in Norway.

A study in the Netherlands reported food consumption data through a two-day record (Hulshof and Kistemaker, 1994). Fourteen percent of the Dutch population consumed liquorice during the two record days, and the users had a mean daily consumption of 13 g liquorice. About 50% of the users consumed more than 50 g liquorice per day. In the United States, where liquorice confectionery is reported not to be popular, daily consumption levels of glycyrrhiza ranged from 1.6 mg to 215.2 mg (Isbrucker and Burdock, 2006). In reality, the intake of glycyrrhizic acid is not from liquorice consumption only. All sources need be taken into account for an accurate estimation of the glycyrrhizic acid intake in a population.

In Italy, 27.8% of 392 pregnant women interviewed at the maternity ward reported taking herbal products during pregnancy and liquorice was the second most frequent herb used, reported by 13.8% (Cuzzolin et al., 2010).

Since no data were available on liquorice or glycyrrhizic acid intake in Norway, it was not possible to perform an exposure characterization. Therefore, a risk characterization of glycyrrhizic acid from liquorice intake in Norway could not be performed.

4 Uncertainties

The level of exposure of the fetus to glycyrrhetic acid is too uncertain, based on the available studies, to be able to draw firm conclusions on cause and effects relationships. These uncertainties include:

- The actual intake of glycyrrhizic acid from liquorice by the mothers during pregnancy in the studies included in this assessment.
- The internal dose absorbed into the mothers' circulation depending on the capacity limit of the intestinal microflora to hydrolyse glycyrrhizic acid to glycyrrhetic acid and the degree of enterohepatic recycling.
- The binding of glycyrrhizic acid and glycyrrhetic acid to serum albumin in a saturable process.
- The percentage of glycyrrhetic acid reaching the placenta.
- Whether the actual level of glycyrrhetic acid reaching the placenta was sufficient to inhibit the placental 11β-HSD2 enzyme.
- Whether the exposure to glycyrrhizic acid occurred in critical periods during pregnancy relevant for the effects on puberty, cortisol levels, cognitive performance, psychiatric symptoms etc. observed in the children.

In addition, there are uncertainties regarding the safety of liquorice related to:

- The potential adverse effects of the 18α-isomer of glycyrrhetic acid.
- The potential adverse effects of other substances than glycyrrhizic acid in liquorice.

5 Conclusions (with answer to the terms of reference)

The Norwegian Food Safety Authority (NFSA) asked the Norwegian Scientific Committee for Food and Environment (VKM) to identify and characterize potential adverse effects to the fetus and long-term effects to the child that can result from maternal consumption of glycyrrhizic acid from liquorice, including at which doses these adverse effects appeared, if such data were available.

In VKM's opinion, an inhibitory effect on the 11ß-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme by glycyrrhizin, leading to overexposure of the fetus to cortisol, is a plausible mechanism for the adverse effects reported in the human studies in this assessment. These effects were shorter gestational duration, increased risk of preterm delivery, poorer cognitive performance, more externalising symptoms and attention problems, increased cortisol levels, more advanced pubertal development, lower scores on tests of intelligence quotient, poorer memory and higher odds of attention deficit/hyperactivity disorder problems. The findings in these studies are indicative of potential adverse effects of glycyrrhizic acid on the offspring from liquorice intake during pregnancy. However, the levels of exposure of the fetus to glycyrrhizin are too uncertain based on the available data to be able to draw firm conclusions on cause and effects relationships. One of the main uncertainties is the actual intake of glycyrrhizic acid by the mothers during pregnancy.

Based on the studies by Strandberg et al. (2001; 2002) and Räikkönen et al. (2009; 2010; 2017), the negative health effects on the mothers (i.e. hypertension) or their fetus or child were found with glycyrrhizin intake ≥500 mg/week, corresponding to approximately 250 g/week of liquorice, compared with lower intake (0-499 mg/week). Therefore, from these studies, 500 mg/week (71.4 mg/day) of glycyrrhizin, which corresponded to average 13.7 mg/kg bw for the mothers at delivery, can be regarded as the LOAEL (Räikkönen et al., 2017). This intake is lower than 100 mg/day suggested in several previous risk assessments (Table 2.5.1-1) as an upper limit for chronic ingestion of glycyrrhizic acid and its salts that provides a sufficient level of protection for the majority of the adult population, but possibly not the most susceptible subpopulation. However, this external dose level is uncertain because of inherent weaknesses in these studies as discussed in this assessment.

Several toxicokinetic factors affect the internal dose of glycyrrhetic acid that eventually reach the placenta, thus determining whether the actual level of glycyrrhetic acid is sufficient to inhibit the placental 11β-HSD2 enzyme.

In these human studies, no recording of glycyrrhizin intake in various parts of the pregnancy was done. Thus, there is also uncertainty regarding whether the exposure to glycyrrhizin occurred in critical periods during pregnancy relevant for the effects on puberty, cortisol levels, cognitive performance, psychiatric symptoms etc. observed in the children.

There is observed large interindividual variation in sensitivity to glycyrrhizic acid (Nordic Council of Ministers, 1993; van Gelderen et al., 2000). Women appeared to be more sensitive to glycyrrhizic acid than men (van Gelderen et al., 2000). There is also reported considerable variation in the 11β-HSD2 dehydrogenase activity between human placentas (Benediktsson et al., 1997).

Patients with decreased liver function or hypokalemia, women with preeclampsia or persons with apparent mineralocorticoid excess (AME), an inherited rare form of hypertension caused by mutations in the 11β-HSD2 gene, may be especially susceptible to excessive intake of liquorice (Hammer and Stewart, 2006; McCalla et al., 1998). Glycyrrhizin is also shown to interact with various drugs, such as prednisolone and hydrocortisone, and prolonged intake of glycyrrhizin may result in accelerated metabolism of co-administered drugs via the induction of various metabolic enzymes (EMA, 2013).

In VKM's opinion, there is still not sufficient data to establish an ADI for glycyrrhizic acid.

VKM concludes that because of the large uncertainty associated with the relationship between the exposure dose and the observed adverse effects, a safe level cannot be established with certainty for glycyrrhizic acid or for the amount of liquorice that the pregnant mothers can consume without causing negative effects on the fetus or child.

6 Data gaps

Hazard identification and characterization

- Well-designed dose-response studies in experimental animals to evaluate at which doses of glycyrrhizic acid various adverse effects may occur in different population groups are needed.
- There were no animal experiments of glycyrrhizic acid on pubertal maturation and the other effects observed after prenatal exposure in the human studies, and only one study in adult rats on neurobehavioural effects.
- There was not sufficient data to conclude definitely whether a NOAEL exists for the inhibiting effect of glycyrrhizic acid on the placental 11β-HSD2 enzyme or if there is a linear dose-response.
- There was no human data on whether adverse effects of glycyrrhizic acid may differ after exposure in various periods of the pregnancy.
- There is insufficient knowledge on the potential adverse effects of the 18α-isomer of glycyrrhetic acid.
- There is insufficient knowledge on the potential adverse effects of other substances than glycyrrhizic acid in liquorice.

Exposure characterization

- There were no data available on glycyrrhizic acid or liquorice intake in various population groups, including pregnant women or children, in Norway.
- Well-designed human studies of pregnant women with accurately estimated exposure (with good quality data on both intake of liquorice from all sources and the content of glycyrrhizic acid in the liquorice) are needed.

7 References

Achard V., Boullu-Ciocca S., Desbrière R., Grino M. (2006) Perinatal programming of central obesity and the metabolic syndrome: role of glucocorticoids. Metab Syndr Relat D 4(2):129-137.

Benediktsson R., Calder A.A., Edwards C.R.W., Seckl J.R. (1997) Placental 11β-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. Clin Endocrinol 46(2):161–166.

Bertram C.E., Hanson M.A. (2002) Prenatal programming of postnatal endocrine responses by glucocorticoids. Reproduction 124:459-467.

Blomberg K., Hallikainen A. (1993) Glycyrrhizic acid levels in liquorice confectionary. National Food Administration, Helsinki, Finland. Research Notes 10, 12 pages (in Finnish with English summary).

Blum S., Bühler H. Lichtenstein I., Siebe H. (1995) Characterization of human placental 11β-hydroxysteroid dehydrogenase, a key enzyme of corticosteroid metabolism. Cell Physiol Biochem 5(3):167-175.

Choi J.-S., Han J.-Y., Ahn H.-K., Ryu H.-M., Kim M.-Y., Chung J.-H., Nava-Ocampo A.A., Koren G. (2013) Fetal and neonatal outcomes in women reporting ingestion of licorice (*Glycyrrhiza uralensis*) during pregnancy. Planta Med 79:97-101.

Condon J., Gosden C., Gardener D., Nickson P., Hewison M., Howie A.J., Stewart P.M. (1998) Expression of type 2 11β-hydroxysteroid dehydrogenase and corticosteroid hormone receptors in early human fetal life. J Clin Endocr Metab 83(12):4490-4497.

Cooper M.S., Walker E.A., Bland R., Fraser W.D., Hewison M., Stewart P.M. (2000) Expression and functional consequences of 11β-hydroxysteroid dehydrogenase activity in human bone. Bone 27(3):375-381.

Cosmetic Ingredient Review (CIR) Expert Panel. (2007) Final report on the safety assessment of glycyrrhetinic acid, potassium glycyrrhetinate, disodium succinoyl glycyrrhetinate, glyceryl glycyrrhetinate, glycyrrhetinyl stearate, stearyl glycyrrhetinate, glycyrrhizic acid, ammonium glycyrrhizate, dipotassium glycyrrhizate, disodium glycyrrhizate, trisodium glycyrrhizate, methyl glycyrrhizate, and potassium glycyrrhizinate. Int J Toxicol 26(Suppl. 2):79-112. Cosmetic Ingredient Review (CIR) Expert Panel, USA, http://journals.sagepub.com/doi/pdf/10.1080/10915810701351228.

Cuzzolin L., Francini-Pesenti F., Verlato G., Joppi M., Baldelli P., Benoni G. (2010) Use of herbal products among 392 Italian pregnant women: focus on pregnancy outcome. Pharmacoepidem Drug S 9:1151-1158.

de Groot G., Koops R., Hogendoorn E.A., Goewie C.E., Savelkoul T.J.F., van Vloten P. (1988) Improvement of selectivity and sensitivity by column switching in the determination of glycyrrhizin and glycyrrhetic acid in human plasma by high-performance liquid chromatography. J Chromatog 456:71-81.

Diao H., Xiao S., Howerth E.W., Zhao F., Li R., Ard M.B., Ye X. (2013) Broad gap junction blocker carbenoxolone disrupts uterine preparation for embryo implantation in mice. Biol Reprod 89(2):article 31,1-10.

Dodds H.M., Taylor P.J., Johnson L.P., Mortimer R.H., Pond S.M., Cannell G.R. (1997) Cortisol metabolism and its inhibition by glycyrrhetinic acid in the isolated perfused human placenta lobule. J Steroid Biochem Mol Biol 62 (4):337-343.

Ellis B.J., Shirtcliff E.A., Boyce W.T., Deardorff J., Essex M.J. (2011) Quality of early family relationships and the timing and tempo of puberty: effects depend on biological sensitivity to context. Dev Psychopathol 23(1):85–99.

EU Commission. (2008) REGULATION (EC) No 1334/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC, http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R1334-20170713&qid=1522740388485&from=EN.

European Food Safety Authority (EFSA). (2008) Flavouring Group Evaluation 36, (FGE.36). Two triterpene glycosides from the priority list. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC). EFSA-Q-2003-172C. EFSA J 740:2-19, https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.740.

European Food Safety Authority (EFSA). (2015) Scientific Opinion on the safety and efficacy of glycyrrhizic acid ammoniated (chemical group 30, miscellaneous substances) when used as a flavouring for all animal species. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). EFSA J 13(1):3971, http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.3971/pdf.

European Medicines Agency (EMA). (2013) Assessment report on *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch., radix. EMA/HMPC/571122/2010 *Corr.* European Medicines Agency, London,

http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2012/08/WC500131285.pdf.

Hammer F., Stewart P.M. (2006) Cortisol metabolism in hypertension. Best Pract Res Clin Endocrinol Metab 20(3):337-353.

Hauksdottir D., Sigurjonsdottir H.A., Arnadottir M., Geirsson R.T. (2015) Severe, very early onset pre-eclampsia associated with liquorice consumption. Hypertens Pregnancy 34(2):221-226.

Helse- og omsorgsdepartementet (HOD). (2011) Forskrift om aroma og næringsmiddelingredienser med aromagivende egenskaper til anvendelse i og på næringsmidler (aromaforskriften), FOR-2011-06-06-669 (in Norwegian), https://lovdata.no/dokument/SF/forskrift/2011-06-06-669/*#*.

Hughes J., Sellick S., King R., Robbé I.J. (2003) Re: "Preterm birth and licorice consumption during pregnancy." (Letter). Am J Epidemiol 158(2):190-191.

Hulshof K.F.A.M., Kistemaker C. (1994) The consumption of licorice and the intake of ammonium chloride among Dutch population groups (second Dutch National Food Consumption Survey). TNO-report V 94.298.

Hundertmark S., Dill A., Bühler H., Stevens P., Looman K., Ragosch V., Seckl J.R., Lipka C. (2002a) 11β-hydroxysteroid dehydrogenase type 1: a new regulator of fetal lung maturation. Horm Metab Res 34(10):537-544.

Hundertmark S., Dill A., Ebert A., Zimmermann B., Kotelevtsev Y.V., Mullins J.J., Seckl J.R. (2002b) Foetal lung maturation in 11beta-hydroxysteroid dehydrogenase type 1 knockout mice. Horm Metab Res 34(10):545-549.

Hynes G., Cooper C., Dennison E. (2012) Chapter 62. Parental determinants of neonatal anthropometry. In Preedy V.R., Ed., *Handbook of Anthropometry. Physical Measures of Human Form in Health and Disease.* Springer Science+Business Media, ISBN 978-1-4419-1787-4.

Isbrucker R.A., Burdock G.A. (2006) Risk and safety assessment on the consumption of Licorice root (*Glycyrrhiza* sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. Regul Toxicol Pharmacol 46(3):167-192.

Itami T., Ema M., Kanoh S. (1985) Effect of disodium glycyrrhizinate on pregnant rats and their offspring. Studies on the fetal toxicity of food additives. III). J Food Hyg Soc Japan 26(5):460-464.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). (2005) Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 928. World Health Organisation, Geneva, 2005, http://apps.who.int/iris/bitstream/handle/10665/43141/WHO_TRS_928.pdf;jsessionid=EC2A640818F9E11001CA5144190AC205?sequence=1.

Joint FAO/WHO Expert Committee on Food Additives (JECFA)/International Programme on Chemical Safety (IPCS). (2006) Safety evaluation of certain food additives. WHO food additives series: 54. ISBN 92 4 166054 6, ISSN 0300-0923. WHO, Geneva, https://www.scribd.com/document/82676853/9241660546-eng.

Jukic A.M., Baird D.D., Weinberg C.R., McConnaughey D.R., Wilcox A.J. (2013) Length of human pregnancy and contributors to its natural variation. Hum Reprod 28(10):2848-2855.

Keyes K.M., Susser E. (2017) Invited Commentary: An ingenious approach to examining the relationship between maternal stress and offspring health? Am J Epidemiol 185(5):329-332.

Khulan B., Drake A.J. (2012) Glucocorticoids as mediators of developmental programming effects. Best Pract Res Clin Endocrinol Metab 26(5):689-700.

Kvasnička F., Voldrich M., Vyhnálek J. (2007) Determination of glycyrrhizin in liqueurs by on-line coupled capillary isotachophoresis with capillary zone electrophoresis. J Chromatogr A 1169:239-242.

Langley-Evans S.C. (1997) Maternal carbenoxolone treatment lowers birthweight and induces hypertension in the offspring of rats fed a protein-replete diet. Clin Sci 93(5):423-429.

Ma X., Lian Q.-Q., Dong Q., Ge R.-S. (2011) Environmental inhibitors of 11β-hydroxysteroid dehydrogenase type 2. Toxicology 285(3):83-89.

MacLennan, E., Koog Y.H. (2014) A re-analysis of studies regarding the use of *Glycyrrhiza* spp. in women during pregnancy. J Herb Med 4(2):106-110.

Mantovani A., Ricciardi C., Stazi A.V., Macri C., Piccioni A., Badellino E., De Vincenzi M., Caiola S., Patriarca M. (1988) Teratogenicity study of ammonium glycyrrhizinate in the Sprague-Dawley rat. Food Chem Toxicol 26(5):435-440.

McCalla C.O., Nacharaju V.L., Muneyyirci-Delale O., Glasgow S., Feldman J.G. (1998) Placental 11β-hydroxysteroid dehydrogenase activity in normotensive and pre-eclamptic pregnancies. Steroids 63(10):511-515.

National Institute for Public Health and the Environment (RIVM). (2003) The health and addiction risk of the glycyrrhizic acid component of liquorice root used in tobacco products. RIVM report 340630001/2003. The Netherlands, 2003, https://rivm.nl/bibliotheek/rapporten/340630001.pdf.

Newall C.A., Anderson L.A., Phillipson J.D. (1996) Herbal medicines: a guide for health-care professionals. London, The Pharmaceutical Press.

Nordic Council of Ministers. (1993) Adverse health effects of glycyrrhizic acid in licorice – a risk assessment. Nordiske Seminar- og Arbejdsrapporter 1993:526. ISBN 92 9120 196 0, ISSN 0906-3668.

Odermatt A., Gumy C. (2008) Glucocorticoid and mineralocorticoid action: Why should we consider influences by environmental chemicals? Biochem Pharmacol 76:1184-1193.

Omar H.R., Komarova I., El-Ghonemi M., Fathy A., Rashad R., Abdelmalak H.D., Yerramadha M.R., Ali Y., Helal E., Camporesi E.M. (2012) Licorice abuse: time to send a warning message. Ther Adv Endocrinol Metab 3(4):125-138.

Räikkönen K., Martikainen S., Pesonen A.-K., Lahti J., Heinonen K., Pyhälä R., Lahti M., Tuovinen S., Wehkalampi K., Sammallahti S., Kuula L., Andersson S., Eriksson J.G., Ortega-Alonso A., Reynolds R.M., Strandberg T.E., Seckl J.R., Kajantie E. (2017) Maternal licorice consumption during pregnancy and pubertal, cognitive, and psychiatric outcomes in children. Am J Epidemiol 185(5):317–328.

Räikkönen K., Pesonen A.-K., Heinonen K., Lahti J., Komsi N., Eriksson J.G., Seckl J.R., Järvenpää A.-L., Strandberg T.E. (2009) Maternal licorice consumption and detrimental cognitive and psychiatric outcomes in children. Am J Epidemiol 170(9):1137–1146.

Räikkönen K., Seckl J.R., Heinonen K., Pyhälä R., Feldt K., Jones A., Pesonen A.-K., Phillips D.I.W., Lahti J., Järvenpää A.-L., Eriksson J.G., Matthews K.A., Strandberg T.E., Kajantie E. (2010) Maternal prenatal licorice consumption alters hypothalamic – pituitary - adrenocortical axis function in children. Psychoneuroendocrinology 35:1587-1593.

Reynolds R.M. (2013) Glucocorticoid excess and the developmental origins of disease: Two decades of testing the hypothesis – 2012 Curt Richter Award Winner. Psychoneuroendocrinology 38(1):1-11.

Reynolds R.M., Labad J., Buss C., Ghaemmaghami P., Räikkönen K. (2013) Transmitting biological effects of stress *in utero*: Implications for mother and offspring. Psychoneuroendocrinology 38:1843-1849.

Saxbe D.E., Negriff S., Susman E.J., Trickett P.K. (2015) Attenuated hypothalamic-pituitary-adrenal axis functioning predicts accelerated pubertal development in girls 1 year later. Dev Psychopathol. 27(3):819–828.

Scientific Committee on Food (SCF). (1991) Reports of the Scientific Committee for Food (29th series). Commission of the European Communities, Food Science and Techniques. Report No EUR 14482 EN, CEC, Luxembourg.

Scientific Committee on Food (SCF). (2003) Opinion of the Scientific Committee on Food on glycyrrhizinic acid and its ammonium salt. European Commission, Health & Consumer Protection Directorate-General, https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out186_en.pdf.

Shi L., Wudy S.A., Buyken A.E., Maser-Gluth C., Hartmann M.F., Remer T. (2011) Prepubertal glucocorticoid status and pubertal timing. J Clin Endocr Metab 96(6):E891–E898.

Shihata I.M., Elghamry M.I. (1963) Estrogenic activity of Glycyrrhiza glabra with its effect upon uterine motility at various stages of sex cycle. Zentralblatt für Veterinärmedizin Reihe A 10(2):155-162.

Slattery M.M., Morrison J.J. (2003) Authors' reply: Risk factors for preterm delivery. Lancet 361:436-437.

Spinks E.A., Fenwick G.R. (1990) The determination of glycyrrhizin in selected UK liquorice products. Food Addit Contam 7(6):769-778.

Stewart P.M., Murry B.A., Mason J.I. (1994) Type 2 11β-hydroxysteroid dehydrogenase in human fetal tissues. J Clin Endocr Metab 78(6):1529–3152.

Stewart P.M., Wallace A.M., Atherden S.M., Shearing C.H., Edwards C.R. (1990) Mineralocorticoid activity of carbenoxolone: contrasting effects of carbenoxolone and liquorice on 11β-hydroxysteroid dehydrogenase activity in man. Clin Sci 78(1):49–54.

Strandberg T.E., Andersson S., Järvenpää A.-L., McKeigue P.M. (2002) Preterm birth and licorice consumption during pregnancy. Am J Epidemiol 156(9):803–805.

Strandberg T.E., Järvenpää A.-L., Vanhanen H., McKeigue P.M. (2001) Birth outcome in relation to licorice consumption during pregnancy. Am J Epidemiol 153(11):1085–1088.

U.S. Food and Drug Administration (FDA). (2017) CFR - Code of Federal Regulations Title 21. PART 184 -- DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE. Subpart B--Listing of Specific Substances Affirmed as GRAS.

Sec. 184.1408 Licorice and licorice derivatives. Revised as of April 1, 2017, page last updated 14.08.2017, https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=184.1408.

van Gelderen C.E.M., Bijlsma J.A., van Dokkum W., Savelkoul T.J.F. (2000) Glycyrrhizic acid: the assessment of a no effect level. Hum Exp Toxicol 19:434-439.

Yamaguchi H., Kidachi Y., Kamiie K., Noshita T., Umetsu H., Ryoyama K. (2010) Glycyrrhetinic acid induces anoikis-like death and cytoskeletal disruption in the central nervous system tumorigenic cells. Biol Pharm Bull 33(2):321-324.

Yoshida T., Hatta A., Akasaka Y., Takei M., Sato T., Leuschner J., Inoue H. (2011) Reproductive and developmental toxicity studies of monoammonium glycyrrhizinate by intravenous administration in rats and rabbits. Jpn Pharmacol Ther 39(3):309-327.

Appendix 1

Literature search strategy

The total result (after removal of duplicates) was 569. The search was performed 27 September 2017.

Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>. Result: 242

1	exp Glycyrrhiza/ or Glycyrrhizic Acid/ or Glycyrrhetinic Acid/	4637
2	(Glycyrrhiza or Glycyrrhizic or Glycyrrhetinic or glycyrrhizin or Glycyrrhetic or Glycyrrhizinic or Glycyrrhetinate or Glycyrrhetate or Glycyrrhetinyl or Glycyrrhizate or Glycyrrhizinate or Glycopyranosiduronic or Glucopyranosiduronic or "Olean-12-En-29-Oic Acid" or glycyrrhetyl or "3-Hydroxy-11-Oxo*" or licorice? or liquorice? or Liquiriti or liquiritiae or "sweet root" or "Glycyrrhizae extractum crudum" or "Gan Cao" or carbenoxolone or monoglucuronylglycyrrhetic or 3MGA or ketoglycyrrhetic or gan zao? or ganzao? or gancao? or kanzo or reglisse or sucsan or Glycyram or Glyciram or Rhetinic Acid or Uralenic Acid or Enoxolone or Arthrodont or "Po 12" or "gm 1292" or "gm 1658" or phytosome or biosone or glycerrhetinic or glycerrhitinic or glycerrhetic or glycyrrhetin or glycerrhizic or glycyrraizinic or glycyrrhizine or "radix glycyrrhizae").mp	8417
3	1 or 2	8417
4	exp Pregnancy Trimesters/ or Prenatal Diagnosis/ or exp Pregnancy/ or Pregnant Women/ or Pregnancy Complications/ or Prenatal Care/ or Fetus/ or exp Perinatal Care/	895235
5	(pregnan* or prenatal* or "ante natal" or antenatal or perinatal or gravidity or fetus* or fetal or foetal or foetus* or faetus* or maternal or maternity or maternities or embryonated or "in utero" or embryo or offspring* or pup or pups).tw,kw.	966044
6	4 or 5	1303571
7	3 and 6	242

Embase <1974 to 2017 September 2>. Result: 450

1	exp Glycyrrhiza/ or glycyrrhetinic acid/ or glycyrrhetinic acid derivative/ or glycyrrhetinic acid phospholipid complex/ or glycyrrhizic acid/ or glycyrrhizic acid derivative/	9763

2	(Glycyrrhiza or Glycyrrhizic or Glycyrrhetinic or glycyrrhizin or Glycyrrhetic or Glycyrrhizinic or Glycyrrhetinate or Glycyrrhetate or Glycyrrhetinyl or Glycyrrhizate or Glycyrrhizinate or Glycopyranosiduronic or Glucopyranosiduronic or "Olean-12-En-29-Oic Acid" or glycyrrhetyl or "3-Hydroxy-11-Oxo*" or licorice? or liquorice? or Liquiriti or liquiritiae or "sweet root" or "Glycyrrhizae extractum crudum" or "Gan Cao" or carbenoxolone or monoglucuronylglycyrrhetic or 3MGA or ketoglycyrrhetic or gan zao? or ganzao? or gancao? or kanzo or reglisse or sucsan or Glycyram or Glyciram or Rhetinic Acid or Uralenic Acid or Enoxolone or Arthrodont or "Po 12" or "gm 1292" or "gm 1658" or phytosome or biosone or glycerrhetinic or glycerrhitinic or glycerrhetic or glycyrrhetin or glycerrhizic or glycyrraizinic or glycyrrhizine or "radix glycyrrhizae").mp.	16081
3	1 or 2	16081
4	exp pregnancy/ or prenatal diagnosis/ or pregnant woman/ or prenatal care/ or maternal care/ or perinatal care/ or exp fetus/	855370
5	(pregnan* or prenatal* or "ante natal" or antenatal or perinatal or gravidity or fetus* or fetal or foetal or foetus* or faetus* or maternal or maternity or maternities or embryonated or "in utero" or embryo or offspring* or pup or pups).tw,kw	1127728
6	4 or 5	1389302
7	3 and 6	450

ISI Web of Science. Result: 226

# 3	226	#2 AND #1 *Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years*
# 2	832,900	TOPIC: ("pregnan*" or "prenatal*" or "ante natal" or "antenatal" or "perinatal" or "gravidity" or "fetus*" or "fetal" or "foetal" or "foetus*" or "faetus*" or "maternal" or "maternity" or "maternities" or "embryonated" or "in utero" or "embryo" or "offspring*" or "pup" or "pups") *Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years*
# 1	7,903	TOPIC: ("Glycyrrhiza" or "Glycyrrhizic" or "Glycyrrhetinic" or "glycyrrhizin" or "Glycyrrhetic" or "Glycyrrhizinic" or "Glycyrrhetinate" or "Glycyrrhetate" or "Glycyrrhetinyl" or "Glycyrrhizate" or "Glycyrrhizinate" or "Glycopyranosiduronic" or "Glucopyranosiduronic" or "Olean-12-En-29-Oic Acid" or "glycyrrhetyl" or "3-Hydroxy-11-Oxo*" or "licorice$" or "liquorice$" or "Liquiriti" or "liquiritiae" or "sweet root" or "Glycyrrhizae extractum crudum" or "Gan Cao" or "carbenoxolone" or "monoglucuronylglycyrrhetic" or "3MGA" or "ketoglycyrrhetic" or "gan zao$" or "ganzao$" or "gancao$" or "kanzo" or "reglisse" or "sucsan" or "Glycyram" or "Glyciram" or "Rhetinic Acid" or "Uralenic Acid" or "Enoxolone" or "Arthrodont" or "Po 12" or "gm 1292" or "gm 1658" or "phytosome" or "biosone" or "glycerrhetinic" or "glycerrhitinic"

		or "glycerrhetic" or "glycyrrhetin" or "glycerrhizic" or "glycyrraizinic" or "glycyrrhizine" or "radix glycyrrhizae") *Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years*

Scopus. Result: 202

13	#3 AND #12	202 document results
12	#4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11	10,150 document results
11	ABS (glycerrhitinic OR glycerrhetic OR glycyrrhetin OR glycerrhizic OR glycyrraizinic OR glycyrrhizine OR "radix glycyrrhizae")	234 document results
10	TITLE (glycerrhitinic OR glycerrhetic OR glycyrrhetin OR glycerrhizic OR glycyrraizinic OR glycyrrhizine OR "radix glycyrrhizae")	100 document results
9	ABS (ketoglycyrrhetic OR "gan zao*" OR ganzao* OR gancao* OR kanzo OR regliss e OR sucsan OR glycyram OR glyciram OR rhetinic AND acid OR uralenic AND acid OR enoxolone OR art hrodont OR "Po 12" OR "gm 1292" OR "gm 1658" OR phytosome OR biosone OR glycerrhetinic)	78 document results
8	TITLE (ketoglycyrrhetic OR "gan zao*" OR ganzao* OR gancao* OR kanzo OR regliss e OR sucsan OR glycyram OR glyciram OR rhetinic AND acid OR uralenic AND acid OR enoxolone OR art hrodont OR "Po 12" OR "gm 1292" OR "gm 1658" OR phytosome OR biosone OR glycerrhetinic)	6 document results
7	ABS ("Olean-12-En-29-Oic Acid" OR glycyrrhetyl OR "3-Hydroxy-11-Oxo*" OR licorice* OR liquorice* OR liquiriti OR liquiritiae OR "sweet root" OR "Glycyrrhizae extractum crudum" OR "Gan Cao" OR carbenoxolone OR monoglucuronylglycyrrhetic OR 3mga)	3,905 document results
6	TITLE ("Olean-12-En-29-Oic Acid" OR glycyrrhetyl OR "3-Hydroxy-11-Oxo*" OR licorice* OR liquorice* OR liquiriti OR liquiritiae OR "sweet root" OR "Glycyrrhizae extractum crudum" OR "Gan Cao" OR carbenoxolone OR monoglucuronylglycyrrhetic OR 3mga)	2,172 document results
5	ABS (glycyrrhiza OR glycyrrhizic OR glycyrrhetinic OR glycyrrhizin OR glycyrrhetic OR glycyrrhizinic OR glycyrrhetinate OR glycyrrhetate OR glycyrrhetinyl OR glyc	6,052 document results

	yrrhizate OR glycyrrhizinate OR glycopyranosiduronic OR glucopyranosiduronic)	
4	TITLE (glycyrrhiza OR glycyrrhizic OR glycyrrhetinic OR glycyrrhizin OR glycyrrhetic OR glycyrrhizinic OR glycyrrhetinate OR glycyrrhetate OR glycyrrhetinyl OR glycyrrhizate OR glycyrrhizinate OR glycopyranosiduronic OR glucopyranosiduronic)	3,659 document results
3	#1 or #2	1,225,924 document results
2	ABS (pregnan* OR prenatal* OR "antenatal" OR antenatal OR perinatal OR gravidity OR fetus* OR fetal OR foetal OR foetus* OR faetus* OR maternal OR maternity OR maternities OR embryonated OR "in utero" OR embryo OR offspring* OR pup OR pups)	1,022,416 document results
1	TITLE (pregnan* OR prenatal* OR "antenatal" OR antenatal OR perinatal OR gravidity OR fetus* OR fetal OR foetal OR foetus* OR faetus* OR maternal OR maternity OR maternities OR embryonated OR "in utero" OR embryo OR offspring* OR pup OR pups)	582,909 document results

Cochrane Database of Systematic Reviews: Issue 9 of 12, September 2017, Database of Abstracts of Reviews of Effect: Issue 2 of 4, April 2015, Cochrane Central Register of Controlled Trials: Issue 8 of 12, August 2017, NHS Economic Evaluation Database: Issue 2 of 4, April 2015, Health Technology Assessment Database: Issue 4 of 4, October 2016. Result: 2 (CDSR)

ID	Search	Hits
#1	[mh Glycyrrhiza]	92
#2	[mh ^"Glycyrrhizic Acid"]	48
#3	[mh ^"Glycyrrhetinic Acid"]	56
#4	(Glycyrrhiza or Glycyrrhizic or Glycyrrhetinic or glycyrrhizin or Glycyrrhetic or Glycyrrhizinic or Glycyrrhetinate or Glycyrrhetate or Glycyrrhetinyl or Glycyrrhizate or Glycyrrhizinate or Glycopyranosiduronic or Glucopyranosiduronic or "Olean-12-En-29-Oic Acid" or glycyrrhetyl or "3 Hydroxy 11 Oxo" or 3Hydroxy11Oxo* or licorice* or liquorice* or Liquiriti or liquiritiae or "sweet root" or "Glycyrrhizae extractum crudum" or "Gan Cao" or carbenoxolone or monoglucuronylglycyrrhetic or 3MGA or ketoglycyrrhetic or "gan zao" or "gan zaos" or ganzao* or gancao* or kanzo or reglisse or sucsan or Glycyram or Glyciram or "Rhetinic Acid" or "Uralenic Acid" or Enoxolone or Arthrodont or "Po 12" or "gm 1292" or "gm 1658" or phytosome or biosone or glycerrhetinic or glycerrhitinic or glycerrhetic or	480

	glycyrrhetin or glycerrhizic or glycyrraizinic or glycyrrhizine or "radix glycyrrhizae"):ti,ab	
#5	(Glycyrrhiza or Glycyrrhizic or Glycyrrhetinic or glycyrrhizin or Glycyrrhetic or Glycyrrhizinic or Glycyrrhetinate or Glycyrrhetate or Glycyrrhetinyl or Glycyrrhizate or Glycyrrhizinate or Glycopyranosiduronic or Glucopyranosiduronic or "Olean-12-En-29-Oic Acid" or glycyrrhetyl or "3 Hydroxy 11 Oxo" or 3Hydroxy11Oxo* or licorice* or liquorice* or Liquiriti or liquiritiae or "sweet root" or "Glycyrrhizae extractum crudum" or "Gan Cao" or carbenoxolone or monoglucuronylglycyrrhetic or 3MGA or ketoglycyrrhetic or "gan zao" or "gan zaos" or ganzao* or gancao* or kanzo or reglisse or sucsan or Glycyram or Glyciram or "Rhetinic Acid" or "Uralenic Acid" or Enoxolone or Arthrodont or "Po 12" or "gm 1292" or "gm 1658" or phytosome or biosone or glycerrhetinic or glycerrhitinic or glycerrhetic or glycyrrhetin or glycerrhizic or glycyrraizinic or glycyrrhizine or "radix glycyrrhizae") in Other Reviews, Technology Assessments and Economic Evaluationssobotka	8
#6	#1 or #2 or #3 or #4 or #5	520
#7	[mh "Pregnancy Trimesters"]	1690
#8	[mh ^"Prenatal Diagnosis"]	380
#9	[mh Pregnancy]	5738
#10	[mh ^"Pregnant Women"]	152
#11	[mh ^"Pregnancy Complications"]	1499
#12	[mh ^"Prenatal Care"]	1327
#13	[mh ^Fetus]	371
#14	[mh "Perinatal Care"]	548
#15	(pregnan* or prenatal* or "ante natal" or antenatal or perinatal or gravidity or fetus* or fetal or foetal or foetus* or faetus* or maternal or maternity or maternities or embryonated or "in utero" or embryo or offspring* or pup or pups) ti,ab	1486
#16	(pregnan* or prenatal* or "ante natal" or antenatal or perinatal or gravidity or fetus* or fetal or foetal or foetus* or faetus* or maternal or maternity or maternities or embryonated or "in utero" or embryo or offspring* or pup or pups) in Other Reviews, Technology Assessments and Economic Evaluations	3909
#17	#7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16	14233
#18	#6 and #17	2

Epistemonikos. Result: 1 (primary study)
(Glycyrrhiza OR Glycyrrhizic OR Glycyrrhetinic OR glycyrrhizin OR Glycyrrhetic OR Glycyrrhizinic OR Glycyrrhetinate OR Glycyrrhetate OR Glycyrrhetinyl OR Glycyrrhizate OR Glycyrrhizinate OR Glycopyranosiduronic OR Glucopyranosiduronic OR "Olean-12-En-29-Oic Acid" OR glycyrrhetyl OR "3 Hydroxy 11 Oxo" OR 3Hydroxy11Oxo* OR licorice* OR liquorice* OR Liquiriti OR liquiritiae OR "sweet root" OR "Glycyrrhizae extractum crudum" OR "Gan Cao" OR carbenoxolone OR monoglucuronylglycyrrhetic OR 3MGA OR ketoglycyrrhetic

OR "gan zao" OR "gan zaos" OR ganzao* OR gancao* OR kanzo OR reglisse OR sucsan OR Glycyram OR Glyciram OR "Rhetinic Acid" OR "Uralenic Acid" OR Enoxolone OR Arthrodont OR "Po 12" OR "gm 1292" OR "gm 1658" OR phytosome OR biosone OR glycerrhetinic OR glycerrhitinic OR glycerrhetic OR glycyrrhetin OR glycerrhizic OR glycyrraizinic OR glycyrrhizine OR "radix glycyrrhizae") AND (pregnan* OR prenatal* OR "ante natal" OR antenatal OR perinatal OR gravidity OR fetus* OR fetal OR foetal OR foetus* OR faetus* OR maternal OR maternity OR maternities OR embryonated OR "in utero" OR embryo OR offspring* OR pup OR pups)

PRIVILEGED AND CONFIDENTIAL
ATTORNEYS' WORK PRODUCT

Covington & Burling DRAFT -

December 8, 1993

SUMMARY OF DATA ON LICORICE

Abstract. Licorice is used in cigarettes both as a flavor and as a casing material to smooth the harsh taste of certain kinds of tobacco. Most cigarette tobacco blends contain less than 1% licorice. Licorice has been used in cigarettes for decades, and is approved for cigarette use in the United Kingdom, at levels up to 4%, and in Germany. It is also used as a food and flavor in other foods and beverages.

Acute, subchronic, and chronic tests have shown that licorice is toxic only at very high levels, far above the amount a heavy smoker would be exposed to each day. Studies have also not indicated that licorice is carcinogenic, or teratogenic, though mutagenicity tests have produced inconsistent results. No cardiovascular, respiratory, or immunotoxic effects have been demonstrated.

During smoking, licorice in the cigarette generally decomposes to glycyrrhetinic acid, which is found in the mainstream smoke in small amounts. A small amount of glycyrrhizic acid may also be found in the smoke. Inhalation studies have been conducted comparing the toxic effects of smoke from cigarette tobacco blends containing licorice at approximately five times the level used in a typical commercial cigarette to the effects of smoke from reference cigarettes. The tests indicate no substantial differences in acute toxicity or mutagenicity, and no consistent differences in a variety of physical, clinical chemistry, hematological, histological, and bronchioalveolar lavage fluid parameters. In addition, mutagenicity studies comparing cigarette smoke condensate from reference cigarettes to condensate from cigarettes containing 1.5, 3, or 6 times the level of licorice used in commercial cigarettes indicate that licorice does not alter the biological activity of the condensate.

Background. Licorice or glycyrrhiza (CAS No. 8008-94-4) is derived from the rhizomes and roots of a perennial leguminous wild plant, Glycyrrhiza glabra L., native to Southern Europe, Central Asia, and Asia Minor. The roots are

best suited for harvesting after 4 to 6 years of growth and contain from 6 to 14 percent glycyrrhizic acid, also known as glycyrrhizin. Spanish and Italian roots tend to contain lower amounts of glycyrrhizin than Oriental roots (Houseman, 1922-23; Muller, 1965). The licorice root is the only known vegetation to contain significant quantities of glycyrrhizin.

As a tribasic acid, glycyrrhizin can form a variety of salts, including mono-, di-, and tri-ammonium glycyrrhizinates and corresponding potassium salts. The name "ammoniated glycyrrhizin" is widely used for the most significant commercial product, and usually signifies a mixture of the three ammonium salts.

Besides glycyrrhizin, licorice contains reducing sugars and nonreducing sugars, starch, plant gums, resins, flavonoids, essential oils, inorganic salts and low levels of nitrogenous constituents such as proteins, amino acids, and nucleic acids (Muller, 1965; Vora, 1984; Frattini, 1977). Appendix I provides further information on the composition of licorice. The licorice root yields licorice for a variety of products, including block licorice, licorice powder, spray dried powder, drum dried pulverized pellets, liquid extracts and glycyrrhizin formulations.

Licorice has been known for over 3,000 years, as evidenced by the finding of the root in the tomb of the Egyptian Pharaoh Tutankhamen (Neiman, 1957). The Greeks referred to it as "sweet root" (Kirk-Othmer, 1963-1970).

Licorice products are widely used in foods as flavoring agents, flavor enhancers, and surfactants, and are also used in beverages and pharmaceuticals. Appendix II provides information on the use of licorice products in foods. Ammonium glycyrrhizinate has been reported as the sweetest substance on the present United States Food and Drug Administration (FDA) list of natural flavors that are generally recognized as safe (GRAS), and, in the presence of sucrose and other sugars, is 100 times sweeter than sugar alone (Cook, 1975). The synergistic effect has been described in a patent issued to Muller in 1966.

The Select Committee on GRAS Substances (SCOGS) believes the average daily intake from foods and beverages is about 27 mg licorice root and about 0.61 mg of licorice extract, calculated from the quantities of licorice imported (SCOGS, 1974).

Tobacco Uses. Licorice has been used since the 1880's as an additive in cigarette tobacco as well as in pi blends and snuff (Tilley, 1948). Glycyrrhizin is not a natural constituent of the tobacco plant. Current levels o licorice applied to cigarette tobacco range between 0 to 2% weights, and it is estimated that most cigarette tobacco blends contain less than 1% licorice by weight. Approximat nine million pounds of licorice root, fluid extract, and powder were used in the manufacture of cigarettes in the United States in 1986.

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Licorice and its derived products are added to cigarette tobacco as flavorants, and as enhancing, potentiating, and smoothing agents. They are also thought to act as surface active agents during the casing operation to help distribute flavors evenly on the blend (Vora, 1984). Licorice is used as an adjunct to boost the sweetness of tobacco (Givaudan Flavorist, 1970).

Regulatory Status. The Food and Drug Administration has affirmed that licorice (glycyrrhiza), ammoniated glycyrrhizin and monoammonium glycyrrhizinate are generally recognized as safe (GRAS) for use in human foods as flavoring agents, flavor enhancers, and surfactants, except they may not be used as components of sugar substitutes. 21 C.F.R. § 184.1408. The maximum permitted levels in food, expressed as percent glycyrrhizin content of food as served, range from 0.05% for baked foods to 16.0% for hard candy. 21 C.F.R. § 184.1408(c).

Licorice extract, licorice extract powder, and licorice root were included in the FEMA list of GRAS substances published in 1960 and 1965 (Ball, 1960; Hall, 1965). Glycyrrhizic acid (glycyrrhizin) is included on the Council of Europe List of substances permitted in foods, with a limit of 50 mg/kg (Council of Europe, 1981).

Licorice root and licorice root extracts in block, liquid or powdered forms are on the list of approved tobacco ingredients prepared by the United Kingdom's Independent

Scientific Committee on Smoking and Health (the Hunter Committee), at levels up to 4% by dry weight, or approximately 30 mg/cigarette. Licorice is also permitted as an ingredient in the manufacture of tobacco under the German Tobacco Ordinance, and licorice and licorice root extracts in block, liquid or powdered forms are included in the Canadian list of ingredients which may be used in cigarettes.

Metabolism. On oral administration of tritium-labelled monoammonium glycyrrhizinate to human subjects, Carlat (1959) determined that the substance was only slightly absorbed from the gastrointestinal tract, and was mainly hydrolyzed to form glycyrrhetic acid, which was excreted unchanged in the feces. Oral administration of labelled glycyrrhetic acid produced essentially the same results. However, when tritium-labelled B-glycyrrhetic acid was administered intraperitoneally (25 mg/kg) to male and female albino rats, an average of 100 percent of the label was absorbed and then excreted within 12 hours through the bile into the feces (Parke, 1963). The rate of excretion was slower when the substance was orally administered at a level of 60 mg per kg; an average of 83 percent of the label was excreted in the feces and one percent in the urine in one to three days. The bile contained three unidentified metabolites of glycyrrhetic acid. Parke suggested that Carlat and his colleagues might have made similar observations had they collected bile for more than 4 hours.

Oral administration of ammoniated glycyrrhizin (about 7 g/kg), monoammonium glycyrrhizinate (about 2 g/kg), and glycyrrhetic acid (about 1.5 g/kg) to bilaterally adrenalectomized rats significantly decreased sodium output and caused retention of urine (Hassan, 1954). The first two compounds had little or no effect on potassium output, but glycyrrhetic acid increased potassium retention. When given by any route to male albino rats, glycyrrhetic acid exhibits a strong antidiuretic effect and, when given orally (about 500 mg per kg), delays water absorption from the alimentary tract (Galal, 1955). Cats and rats administered glycyrrhetic acid intraperitoneally (200 mg and 125 mg/kg respectively) exhibited a marked antidiuretic action; however, there was an increase in urinary potassium excretion (Finney, 1958). Following oral administration of as much as 1.5 g/kg glycyrrhetic acid to male albino rats daily for 8 days, Linko and Vasama (1958) noted an increase in excretion of potassium, while the body weight of the rats increased.

In vitro experiments by Whitehouse (1967) have shown that glycyrrhetic acid is a potent uncoupler of oxidative phosphorylation in rat liver mitochondria. Kraus (1958) reported that when rats received 0.4 percent ammoniated glycyrrhizin in drinking water (about 500 mg per kg per day) for a week, their ability to mobilize glucose was decreased. The ability of mice receiving drinking water containing 0.4 percent ammoniated glycyrrhizin (about 800 mg/kg/day), to

withstand cold temperatures was decreased. These results led the investigator to suggest that glycyrrhizin decreased the output of adreno-cortico-trophic hormone (ACTH). Evdokimova and Kimilov (1967) found that potassium glycyrrhizinate (15 mg/kg daily), "injected internally" for two months, decreased experimental atherosclerosis in rabbits by decreasing the amount of cholesterol in the blood and reducing the cholesterol-lecithinic coefficient.

Gujral (1961) found that oral glycyrrhizin (100 to 1000 mg/kg per day) exhibits antiarthritic and anti-inflammatory effects in adrenalectomized rats with Brownlee's formaldehyde induced arthritis. Elmadjian (1956) found that monoammonium, glycyrrhizinate and hydrocortisone have synergistic effects in the adrenalectomized patient. Sasano (1966) reported that simultaneous intravenous administration to rats of glycyrrhizin with dexamethasone inhibits the dexamethasone-inducted atrophy of the adrenals, indicating adrenocortical stimulation by the glycrrhizin. Asanuma (1965) found that glycyrrhizin can either suppress or intensify the action of cortisone in adrenalectomized rats, depending on the immediate conditions, and can suppress the inhibitory action of dexamethasone on the pituitary.

The pharmacokinetic behavior of glycyrrhetinic acid was investigated in rats receiving intravenous doses of 2,5, or 12 mg/kg. A biexponential reduction in plasma concentration was observed at each dose. A greater-than-

proportional increase in plasma concentration and decrease in total body clearance was seen with increasing dose. The steady-state distribution pattern seemed unaffected by dose. A two-compartment distribution model glycyrrhizin was hypothesized, with a saturable elimination rate. When glycyrrhizin was administered at a dose level of 100 mg/kg before glycyrrhetinic acid, sustained levels of plasma glycyrrhetinic acid were observed; this was attributed to intestinal reabsorption of glycyrrhizin/glycyrrhetinic conjugates during enterohepatic recycling (Ishida et al., 1989). (An abstract of this study was reviewed).

Ishida et al. (1990) developed three physiologically-based pharmacokinetic models incorporating enterophepatic recycling to describe glycyrrhizin disposition in the rat and in humans. The rat model included fourteen compartments and assumed direct excretion of glycyrrhizin from the liver to the gut. For the human models a gallbladder compartment was added for excretion from the liver into the gut. The authors found that scale-up of the disposition kinetics from rats to man was successful. (An abstract of this study was reviewed).

Van Katwijk (1954) fed glycyrrhetic acid to two human subjects (one with Addison's disease and one with a jejunal ulcer) in amounts up to 2.5 g/day for unspecified periods. The urine of these patients showed no traces of glycyrrhetic acid. No data on fecal excretion were reported.

However, the investigators isolated an apparent metabolite of the acid in the urine which was unidentified except for its red color and absorption maximum (555-560nm) when treated with sulfuric acid.

Acute, Subchronic, and Chronic Toxicity. The LD_{50} of various glycyrrhizin salts administered to mice has been determined by Klosa (1957) and Fujimura, with results as shown in Table I.

TABLE I

Acute Toxicity of Glycyrrhizin Salts in Mice

Route	Glycyrrhizin salt	LD_{50}
		mg per kg
Oral	ammonium (crude)	12,700
	diammonium	9,600
	potassium (crude)	12,400
	monopotassium	1,220
	dipotassium	8,100
Intravenous	monopotassium	412
Intraperitoneal	ammonium (crude)	1,050
	monoammonium	1,070
	diammonium	1,250
	potassium (crude)	1,260
	dipotassium	1,400
Intramuscular	monopotassium	695
Subcutaneous	monopotassium	697

Finney (1958), using albino mice of both sexes, reported an intraperitoneal LD_{50} of 308 mg/kg for glycyrrhetic acid. Upon oral or subcutaneous administration, no deaths occurred with single doses as high as 610 mg/kg.

In a report on the acute and subacute toxicity of Glycyrrhiza extract, it was reported that licorice powder containing 48-58% glycyrrhizin gave LD_{50} values of 4.0-4.4, 1.42-1.70, and 14.2-18.0 g/kg in rats and mice after subcutaneous, intraperitoneal, and oral administration, respectively (Komiyama, 1977).

An acute toxicity assessment of licorice extract was performed by a single oral administration to Sprague-Dawley albino rats of various doses of an aqueous solution followed by a 14 day observation period. LD_{50} values of 3349 mg/kg (2679-4187; 95% confidence limits) for males, 2299 mg/kg (1752-3018) for females, and 2846 mg/kg (2321-3488) for combined sexes were determined.

Tocco (1923) observed that when pigeons received subcutaneous doses of glycyrrhizin of from 450 to 500 mg/kg of body weight, they became diarrheic within an hour, and showed depression lasting about 24 hours. Guinea pigs receiving glycyrrhizin subcutaneously in doses of 1,000 mg/kg rapidly became depressed and diarrheic, showed decreased urinary volume, and died within 24 hours. In dogs, intravenous doses of glycyrrhizin of about 500 mg/kg were fatal. The same dose given subcutaneously produced only a slight depression for up

to 3 hours; by the oral route, this dose produced almost no adverse reaction.

Over a 50-day period, Girerd (1958) gave oral doses to male Sprague-Dawley rats of (a) 10 g licorice/kg/day and (b) 1 g ammoniated glycyrrhizin/kg/day. The experimental animals showed a progressive increase in blood pressure to about 190 mm as compared to 125 mm for a control group. They also showed a significant depression of growth, which was greater in the licorice-treated rats than in the animals fed ammoniated glycyrrhizin. Both absolute and relative weight increases were noted in kidneys, adrenals, and hearts of treated animals, and weight losses in hypophyses and testes. Severe renal and cardiovascular lesions were found in the licorice-treated rats; milder lesions were noted in the ammoniated glycyrrhizin group. The survival rate, after 50 days, was 36 percent for licorice-treated rats and 77 percent for those receiving ammoniated glycyrrhizin, as compared to 100 percent for controls.

Macabies (1963a), administered glycyrrhizin orally to rats, at a level of 160 mg/day, on the following schedule: 70 days of treatment; 50 days without treatment; another 35 days of treatment; and a final 20 days without treatment. There was no effect on weight, but a 25 percent increase in blood pressure during glycyrrhizin administration was observed; blood pressure returned to normal when the treatment was discontinued. In another study, the same workers

(Macabies, 1963b) determined the hypertensive action of several licorice-related substances administered as shown below to male Wistar rats over a period of 10 to 25 days:

Route	Substance	Daily dose (mg per kg)
Intraperitoneal	Ammoniated glycyrrhizin	150 and 300
Intraperitoneal	Tripotassium glycyrrhizinate	150 and 300
Subcutaneous	Ammoniated glycyrrhizin	300
Oral	"Deglycyrrhizinated" licorice extract*	800
(Route unstated)	Glycyrrhetic acid (a and b isomers)	300

*Extract containing 3 to 4 percent glycyrrhizin, as compared to 20 to 25 percent in the original extract.

All of the glycyrrhizin salts increased the blood pressure, which returned to normal when the treatment was ended. The glycyrrhetic acid isomers also had a strong hypertensive effect, but the duration of action was shorter; the beta isomer particularly appeared to be more effective in this respect than the salts. The "deglycyrrhizinated" licorice extract had only a very weak hypertensive action.

An extensive study of the effect of ammonium glycyrrhizinate on blood pressure, electrolytes, and corticosterone was conducted by Gordon (1974). Dosing with technical ammonium glycyrrhizinate at 1000 and 2000 mg/kg/day

produced significant increases in the blood pressure of Sprague-Dawley rats within 2 to 3 weeks, but not in Osborne-Mendel rats over a 20 week period. There was a decrease in plasma corticosterone and increased kidney and heart weights at the 1000 mg/kg level. However, when the compound was fed at 4 percent of the diet (2000 mg/kg/day) for 5 weeks, plasma corticosterone, blood pressure, and organ weights all were increased.

Fujimura and Okamoto fed diammonium and dipotassium glycyrrhizinates at dietary levels of 0.1 (approximately 100 mg/kg per day) and 0.5 percent to rats for 90 days. At the higher level the male animals showed a slower rate of weight gain than did the controls; at autopsy, no gross or histological abnormalities were noted in the organs. Klosa, (1957) observed no differences in weight gain when rats were given potassium glycyrrhizinate (route unstated) at a level of 60 mg/kg per day for 8 months.

An assessment of the potential of licorice extract to induce primary irritation of mucosal membranes was performed by the deposition of 100 mg of the material into the right lower conjunctival sac of each of six New Zealand White rabbits (3 per sex). Daily observations conducted for a period of 7 days resulted in the classification of licorice extract as "mildly irritating."

Rats given 2.5 g powder/kg/day orally for 3 months showed decreased body weight gain, blood cell count, and

thymus weight, but these symptoms disappeared upon discontinuation of the oral administration. Oral administration of 0.31-0.63 g powder/kg for 90 days resulted in no toxic effect (Komiyama, 1977).

More recently, the oral administration of disodium glycyrrhizinate (DG) to mice for a treatment period of 96 weeks did not yield any evidence of chronic toxicity (Kobuke, 1985). The DG was administered in the drinking water in concentrations ranging from 0 to 0.15% (maximum tolerated dose) for males and from 0 to 0.3% (maximum tolerated dose) for females.

The Select Committee on GRAS Substances (SCOGS) reviewed the scientific literature from 1920 to 1974 with regard to the health aspects of licorice, glycyrrhiza and ammoniated glycyrrhizin (SCOGS-28, 1974), including some of the studies described above. The Committee concluded, based upon acute and short-term animal studies, that licorice and its derivatives are capable of eliciting a variety of pharmacological-effects, but only at levels considerably higher than those which are likely to be achieved in usual diets.

Acute and Subchronic Animal Studies. Teelucksingh et al., (1990) demonstrated that the enzyme 11ß-hydroxysteroid dehydrogenase, which catalyzes the conversion of cortisol to inactive cortisone in man and corticosterone to 11-dehydrocorticosterone in rodents, is inhibited by glycyrrhetinic acid. Glycyrrhetinic acid was shown to

potentiate the action of hydrocortisone and thus may be useful in targeting glucocorticoid therapy. (An abstract of this study was reviewed).

Monder et al., (1989) proposed that the mechanism by which glycyrrhetinic acid produces symptoms resembling those caused by excess mineralocorticoid secretion is inhibition of 11ß-dehydrogenase activity, not intrinsic mineralocorticoid activity and/or interaction with mineralocorticoid receptors. In vivo tests performed on rats measured the effect of ingested glycyrrhizin (in drinking water at ~75 mg/kg/day for 15 days and by gavage at 50 mg/kg/day for 12 days) on inhibition of 11ß-dehydrogenase activity. After sacrifice, renal cortical tissue was obtained from the rats and assayed for 11ß-dehydrogenase activity. In both studies, 11ß-dehydrogenase activity was inhibited by glycyrrhizin. In in vitro studies with renal tubules and rat kidney microsomes, glycyrrhetinic acid and its synthetic analog carbenoxolone inhibited the conversion of corticosterone to 11-dehydrocorticosterone by 11ß-dehydrogenase in a dose-dependent manner. The authors concluded that the effects of licorice on corticosteriod metabolism in the kidney are based on its inhibition of 11ß-dehydrogenase.

The tetradecanoylphorbol-13-acetate (TPA) - induced mouse ear edema assay is an assay used to test for anti-inflammatory effects of a material. Inoue et al. (1989) found

that, of the glycyrrhetinic acid derivatives examined, dihemiphthalate derivatives most strongly inhibited ear edema following both topical (1.6-2.0 mg/ear) an acute oral (25-200 mg/kg) administration. Also, glycyrrhetinic acid and its derivatives applied 30 minutes before TPA treatment were much more effective in inhibiting edema than when applied 30 minutes after TPA treatment. Glycyrrhetinic acid produced little inhibition at less than 200 mg/kg. The authors concluded that the dihemiphthalate derivatives of triterpenes derived from glycyrrhetinic acid by chemical modification are useful for the treatment of inflammatory skin diseases by both topical and oral administration.

Shibayama (1989) found that treatment of rats with glycyrrhizin (route and dosage unspecified) 20 hours before administration of carbon tetrachloride protected the liver against the development of pericentral hepatocellular necrosis. Glycyrrhizin treatment 2 hours preceding the administration of allyl formate also inhibited this type of liver damage. It did not protect against the development of endotoxin-induced focal and random hepatocellular necrosis, suggesting that glycyrrhizin can protect against hepatotoxicity induced by the direct action of carbon tetrachloride or allyl formate on hepatocytes, but has no protective effect following sinusoidal circulatory disturbance such as that caused by endotoxin. (An abstract of this study was reviewed).

Yano et al., (1989) found that the dihemiphthalate derivatives of glycyrrhetinic acid produced strong antiulcer activity when tested using stress-induced gastric lesions in mice and rats. The derivatives produced potent inhibition of lesion formation when dosed orally at 12 or 25 mg/kg, possibly by strengthening some gastric mucosal defensive mechanism. (An abstract of this study was reviewed).

A number of studies have described an anti-mutagenic and/or anti-carcinogenic effect of glycyrrhizin (Argawal et al., 1991; Mashiba and Matsunaga, 1990; Ngo, et al., 1992; Shankel and Clarke, 1990; Tanaka et al., 1987b; and Wang et al., 1991). Glycyrrhetinic acid is currently undergoing clinical chemopreventive trials conducted by the National Cancer Institute (Kelloff et al., 1992 and Vogel et al., 1992).

The mechanism of action by which glycyrrhetinic acid inhibits the formation of phorbol ester-induced tumors has not yet been elucidated. O'Brian et al., (1990) suggested that the anti-promoting activity may involve direct interaction with the tumor-promotor receptor. Since protein kinase C (PKC) is a phorbol ester tumor promoter receptor, the effects of glycyrrhetinic acid on PKC activity were assayed. Glycyrrhetinic acid, at a concentration of 1 mM, inhibited 90% of the PKC activity; a concurrent experiment showed that glycyrrhizin at 1 mM inhibited PKC by 23%. Glycyrrhetinic acid was found to inhibit PKC activity in purified rat brain

with a potency similar to its potency as a steroid receptor ligand. Therefore, the authors hypothesized that antagonism of tumor promotion in mouse skin may be a consequence of both its binding to steroid receptor and also its inhibition of PKC.

Acute Human Toxicity. Several studies have investigated the "mineralocorticoid effect" of licorice (Bannister et al., 1977; Cumming et al., 1980; Hayashi et al., 1992; and Koster and David, 1968). This effect, mainly associated with chronic ingestion of excessive amounts of licorice, has been well documented. In one study, 14 human volunteers received daily oral doses of 100 or 200 g licorice (equivalent to 0.7 and 1.4 g glycyrrhizinic acid) for one to four weeks. Effects such as decreased plasma renin activity, reduced urinary and plasma aldosterone, and decreased plasma angiotensin II levels were accompanied by significant reductions in plasma potassium concentrations, sodium retention and concomitant weight gain. Blood pressure did not rise significantly and, in all but the most severely affected subjects, hormonal values quickly returned to normal after licorice was withdrawn (Epstein et al., 1977). Morris et al., (1990) hypothesized that continual use of chewing tobacco containing licorice as a flavorant could result in hypertension, sodium retention and hypokalemia.

A combined therapy program of glycyrrhizinic acid plus interferon was used to treat patients positive for

hepatitis B. The most effective treatment was found to be glycyrrhizinic acid given for 4 weeks with continuous interferon-alpha treatment. For example, four months after glycyrrhizinic acid had been withdrawn, 6/12 patients were found to be negative for the hepatitis B antigens, as compared to 3/14 treated with interferon-beta plus glycyrrhizinic acid, and 0/10 untreated control patients (Hayashi et al., 1989 and 1990). (Abstracts of these studies were reviewed).

Glycyrrhizin sulfate was among several antiviral agents tested for its ability to inhibit four different strains of human immunodeficiency virus type 1 (HIV-1), a strain of type 2 (HIV-2), and human T-cell lymphotropic virus type I (HTLV-I). Glycyrrhizin sulfate inhibited cell-free viral infection and also blocked cell-to-cell infection by the cell fusion reaction. The extent of inhibition was not included in the abstract of this paper (Tochikura et al., 1989).

Carcinogenicity. As discussed above, Kobuke (1985) administered disodium glycyrrhizinate to mice for 96 weeks at levels from 0 to 0.3%. The authors reported that there were no differences between treated and control groups in tumor incidence, latent period before tumors appeared, or the distribution of different types of tumors. The authors concluded that the long-term oral administration of disodium glycyrrhizinate to mice did not yield any evidence of chronic tumorigenicity.

Genotoxicity. A mutagenic evaluation of ammoniated glycyrrhizin was made for the FDA by Litton Bionetics (Mutagenic Evaluation, 1972). The results from in vivo mutagenic tests in three systems were reported. The test systems included the host-mediated assay, cytogenetic studies and the dominant lethal assay. The compound was considered a possible mutagen in the host-mediated assay, but appeared to be negative on retesting. The other two test systems gave negative results for mutagenicity. Oral doses up to 5000 mg/kg were used in the tests.

More recently, the FDA reviewed two additional mutagenicity studies on glycyrrhizin. 48 Federal Register 54983 (1983). In one study a dominant lethal test in rats was used (Stanford Research Inst., 1977), and in the other a test for unscheduled DNA synthesis in mice was used. (Oak Ridge, 1983). Both tests were judged by FDA to be negative.

The mutagenic potential of licorice extract was assessed in an in vitro bacterial assay employing a battery of Ames' mutant Salmonella strains capable of detecting both frameshift (strains TA1537, TA1538 and TA98) and base pair-substitution (strains TA1535 and TA100) mutations. No mutagenic activity was detected in a range of doses from 1 to 10,000 ug licorice extract per plate (in triplicate), both in the presence and absence of a metabolic activation system consisting of an Aroclor-induced rat liver homogenate fraction (S9). Crebelli et al., (1990) report negative results in an

Ames Salmonella typhimurium assay in strains TA-98, TA-100, TA-1538, and TA-94, both with and without an S-9 metabolic acitivation system at doses up to 100 ul/plate.

A mammalian cell mutagenesis assay was conducted to determine the potential of licorice extract to induce forward mutations at the thymidine kinase (TK) locus in L5178Y $TK^{+/-}$ mouse lymphoma cells both with and without an induced mammalian liver metabolic activation system (S9). Licorice extract was found to be active in inducing significant increases in the mutant frequency at the TK locus, both with and without metabolic activation, at doses exhibiting moderate to high cytotoxicity and accompanied by acidic pH shifts in the culture medium. An attempt to control the acidic pH shifts caused by licorice extract was made in a repeat test by the addition of NaOH, but the test material was again shown to exhibit mutagenic activity in the assay both with and without metabolic activation. Neither the adequacy of this manipulation in controlling the source of potential erroneous positive results nor its effect on medium parameters other than pH (e.g., osmolality) was definitively assessed.

An in vitro CHO cell chromosome aberration assay was conducted to assess the clastogenic potential of licorice extract in mammalian cells, both in the presence and absence of a mammalian liver metabolic activation system (S9). Licorice extract was found to be positive in the chromosome aberration test only in the presence of the metabolic activa-

tion system at highly toxic dose levels ranging from 3.5 to 5 mg/ml. The effects of these concentrations of the test material on medium osmolality were not determined. (Unpublished data.)

The results of the mammalian cell mutagenesis assay, the CHO cell chromosome aberration assay, and other in vitro assays employing mammalian cells should be viewed with caution in light of recent data from the NTP and elsewhere indicating the propensity of these tests to produce erroneous positive results under conditions of altered osmolality, pH, or salt concentrations (Galloway, 1985; Ishidate, 1984; Brusick, 1984; Scott et al., 1991; Morita et al., 1992). Positive results for compounds assayed at high exposure concentrations limited only by cytotoxicity should therefore be regarded as tentative pending a more complete understanding of the phenomenon of pH- and osmolality-associated mutagenesis in mammalian in vitro systems and its relevance, if any, to genotoxic potential in vivo.

An additional in vitro CHO cell chromosome aberrations assay was conducted with licorice extract to further assess its clastogenic potential in mammalian cells, both in the presence and absence of a mammalian liver metabolic activation system (S9). In this assay, pH was maintained at ~7.5 and the osmolality of the test article in the medium was monitored and found not to vary significantly from that of the medium alone. Licorice was determined to be

positive at 2 dose levels (2.0 and 2.5 mg/ml) without S9 and at 1 dose level (5.0 mg/ml) with S9.

An in vitro CHO cell chromosome aberration assay was performed to assess the clastogenic potential of ammoniated glycyrrhizin in mammalian cells, both in the presence and absence of S9. No significant increases in cells with chromosomal aberrations were observed without metabolic activation except at the highest dose level of 2.28 mg/ml, where a weakly significant increase was observed. Significant increases in cells with chromosomal aberrations were observed under conditions of metabolic activation. Statistically significant, dose-related increases were observed at the 0.76 and 1.14 mg/ml dose levels; 11.0 and 62.5% of the cells had aberrations, respectively, as compared to the positive control (cyclophosphamide, 25.0 μg/ml which induced aberrations in 36.0% of the cells). The test article was found not to alter the assay medium osmolality at the dose levels tested. Ammoniated glycyrrhizin was therefore considered negative for inducing chromosomal aberrations in Chinese hamster ovary cells under nonactivation conditions, except at a single high dose level where the results were equivocal, but positive under conditions of metabolic activation.

In order to determine whether the indications from in vitro screening tests of a potential clastogenic effect of licorice extract were predictive of a similar potential in an intact animal model, an in vivo assay measuring chromosome

aberrations in mammalian bone marrow was subsequently performed in Sprague-Dawley rats. Groups of 15 males and 15 females were administered a single oral dose of licorice extract by gavage at levels of 5,000, 1,667, or 500 mg/kg. Bone marrow cells were harvested and analyzed at 6, 18 and 30 hours post-treatment. Licorice was clearly negative for inducing chromosome aberrations in this study.

Since no signs of toxicity were noted in the acute study, a repeated dosing regimen was chosen for further in vivo testing of licorice extract. Groups of 10 rats (5 males and 5 females) were dosed for 5 days with licorice extract at levels of 10,000, 7,500, and 5,000 mg/kg. These dose levels exceed those usually recommended for tests of this type and the high dose was the maximum deliverable dose of the test compound. Toxicity was apparent in the high dose males with weight loss in 4 of 5 test animals. Licorice was clearly negative for inducing chromosome aberrations in bone marrow cells of male and female rats under the conditions of this study.

Because of the possibility of false positive in vitro results for such a relatively nontoxic test article in assay systems sensitive to chromosome damage, an additional mutagenicity test sensitive to a different genetic endpoint was conducted. The genotoxic potential of licorice extract was assessed in an Unscheduled DNA Synthesis (UDS) assay employing primary hepatocytes derived from Sprague-Dawley

rats. The cell cultures were exposed for 18-20 hours to concentrations of licorice extract ranging from essentially nontoxic to highly toxic levels and DNA repair synthesis was assessed autoradiographically. No significant increase in net nuclear grain counts was observed at any dose level, and licorice extract was judged to be negative in the UDS assay. (Unpublished data.)

In summary, an examination of the in vitro genotoxicity profile of licorice extract in recently-performed tests reveals no indications of activity except in two assays (mouse lymphoma cell mutagenesis assay and CHO cell cytogenetics) which are sensitive to clastogens. A definitive follow-up evaluation of the in vivo clastogenic potential of licorice extract was completely negative. Taken together with its lack of genotoxic activity in several bacterial point mutation and mammalian hepatocyte UDS assays, as well as in a number of other in vivo tests which are sensitive to mutagens (dominant lethal and teratogenesis assessments), licorice extract does not appear to pose a genotoxic hazard to intact mammalian systems.

Teratogenicity and Reproductive Effects. Food and Drug Research Laboratories conducted an investigation into the teratologic effects of ammonium glycyrrhizinate in mice, rats, hamsters and rabbits (Food and Drug Research Labs., 1972). Ammonium glycyrrhizinate was administered daily by oral intubation to 109 pregnant adult female albino CD-1 outbred

mice at levels of 0, 27, 90, 300, and 1000 mg/kg beginning on the 6th day and continuing through the 15th day of gestation. Administration by the same method at the same dose levels was made to 106 pregnant adult female albino rats (Wistar derived stock) on the 6th day through the 15th day of gestation; to 111 pregnant adult female golden hamsters on the 6th through the 10th day of gestation; and to adult female Dutch-belted rabbits on the 6th through the 18th day of gestation. The mice, rats, hamsters and rabbits were subjected to Caesarean section on the 17th, 20th, 15th, and 29th day, respectively, and the numbers of implanation sites, resorption sites, and live and dead fetuses were recorded. Detailed examination of the urogenital tract of each animal was performed and the fetuses were examined for any abnormalities. It was concluded that ammonium glycyrrhizinate administered under the specified conditions exhibited no teratological effect nor unfavorably influenced maternal or fetal survival in any of the tested species.

Cardiovascular and Respiratory Toxicity Function. The acute effects of licorice extract on cardiovascular and respiratory function were assessed by its intravenous administration to lightly-anesthetized young adult male beagle dogs at doses of 0.4, 0.8, and 2.0 mg/kg. A battery of tests monitored effects on cardiac function, systemic circulatory function, segmental vascular function, and respiratory parameters including rate, tidal volume, and minute volume. No

statistically significant changes in cardiovascular or pulmonary function were observed between the control and test animals, and the data indicated no physiological impairment of cardiovascular or pulmonary function due to the acute intravenous administration of licorice extract.

Hepatic Enzyme Induction. The capacity of licorice extract to induce hepatic microsomal enzyme activities was assessed following its oral administration at 2500 mg/kg (1/2 maximum tolerated dose) daily for 4 days to mice and rats of both sexes. No effect on hexobarbital-induced sleep times in mice was observed, and neither male nor female rats exhibited increases in relative liver weights. Statistically significant increases in liver p-nitroanisole-0-demethylase and aniline hydroxylase activities were recorded for licorice extract-dosed female and male rats, respectively, although these increases were less than those caused by phenobarbital, the positive control. Licorice extract may therefore be regarded as a weak and inconsistent inducer of selected rodent cytochrome P450 enzymes at extremely high doses.

Immunotoxicity. A screening test for immunosuppressive potential was conducted by the oral administration of licorice extract to male B6C3F1 mice at a dose of 2500 mg/kg (1/2 maximum tolerated dose) daily for 11 days. The animals were sensitized to sheep red blood cells (SRBC) by intraperitoneal injections on the third day of test material administration. A hemagglutination assay performed on the 12th day

following the initiation of dosing showed no suppression of the anti-SRBC primary immune response among licorice extract-treated mice, suggesting that this material lacks immunosuppressive potential even at levels far in excess of those of anticipated use.

Pyrolysis Chemistry Studies. The fate of glycyrrhizic acid and glycyrrhetinic acid during the smoking of cigarettes has been studied by adding these acids separately to cigarettes (Sakagami, 1974). It was found that glycyrrhizic acid decomposed to glycyrrhetinic acid and was transferred in the mainstream smoke as such. When glycyrrhetinic acid itself was added to cigarettes, it was transferred intact to the mainstream smoke. In both cases, the amount of glycyrrhetinic acid found in the smoke condensate was small, and it was concluded that the glycyrrhizic acid in licorice root used for tobacco flavoring was mostly decomposed on smoking.

Vora and Tuorto (1984) reported the presence of glycyrrhizic acid at low levels in smoke of cigarettes burned non-continuously and continuously, based on preliminary evidence using ultraviolet measurements at 254 and 280 nm.

Pyrolysis Toxicology Studies. A series of inhalation studies were performed comparing the toxicity of reference cigarettes to cigarettes to which approximately 21,000 parts per million licorice extract by weight of the tobacco (approximately 21 mg) had been added as one component

of a compounded flavorant mixture. An initial acute inhalation study was performed in order to establish maximally-tolerated exposure levels for subsequent subchronic studies. Groups of six female B6C3F1 mice or six male and six female Fischer 344 rats were exposed to the following low dose regimen in a one-day exposure: a standard two-second 35 ml puff diluted to 10% smoke concentration, thirty seconds smoke alternating with thirty seconds air over eight minutes, eight puffs/exposure, eight minute exposure alternating with an eight minute rest, nine exposures per day. High dose groups of six animals each were exposed to similar puffs at 10% smoke concentration, thirty seconds smoke alternating with thirty seconds air over eight minutes, eight puffs/exposure, two consecutive eight minute exposures alternating with an eight minute rest, twelve exposures per day. Sham-exposed (no cigarette) controls were treated simultaneously on another smoking machine. The animals exposed to smoke from both licorice-containing and reference cigarettes exhibited similar survival rates, carboxyhemoglobin levels, body weight changes, and general physical appearance.

Following the completion of the acute study, B6C3F1 mice were exposed subchronically (5 days/week for 6 weeks with 31.5 minutes per day total exposure to smoke and 130 minutes per day in smoke chamber) to the smoke of reference cigarettes or cigarettes containing approximately 21,000 ppm licorice extract by weight of the tobacco. Upon completion of the

study, the mice in the control and test groups were found to be essentially identical in a variety of hematological, clinical chemistry, histological, physical, and bronchioalveolar lavage parameters.

An additional 90-day subchronic inhalation study was then conducted to compare the toxicity of reference cigarettes to cigarettes to which approximately 21,000 parts per million licorice extract by weight of tobacco (approximately 21 mg) had been added as a component of a compounded flavorant mixture. Nose-only exposure of male and female Fischer 344 rats was followed by evaluation of an array of physical, clinical chemistry, hematological, histological, and bronchio-alveolar lavage fluid parameters. Occasional differences were noted between the licorice-supplemented and reference cigarette groups in various parameters, but these differences appeared to be attributable to normal biological variations in response and occurred with similar frequency in both the reference and the licorice-supplemented cigarette groups. None of the differences between the smoke-exposed and reference groups appeared to be toxicologically significant.

A final smoke inhalation study was performed to assess any differences in the capacity of reference cigarettes and cigarettes containing 21,000 ppm (approximately 21 mg) licorice extract by weight of the tobacco to induce sister chromatid exchanges (SCE) in bone marrow cells of B6C3F1 mice. No differences in SCE rates in mice were observed between

reference or licorice extract-containing cigarettes after exposure for 36 minutes per day for 10 days.

A mouse skin painting bioassay was conducted to compare the tumor-promoting potential of smoke condensate from reference cigarettes to that of cigarettes which contained licorice extract at 19,000 ppm tobacco as a major component of a flavorant mixture. Groups of 60 female ICR CD-1 mice received two subthreshold initiating doses of 7,12-dimethylbenz(a)anthracene followed by four weekly applications of 20 or 35 mg of the cigarette smoke condensate for 36 weeks. The condensates of the licorice extract-containing cigarette and the reference cigarette having no flavoring ingredients were comparable in tumor-promoting activity, with no significant differences in grossly-observed or histologically-confirmed tumor incidence at either dose (Unpublished).

A study was performed in which the effect of adding licorice block to cigarettes was evaluated by testing the resulting cigarette smoke condensate (CSC) in the Salmonella/ microsome (S/M) assay (Ames plate incorporation assay). Licorice block was dissolved in water and injected into University of Kentucky 1R4F Research Cigarettes at levels 1.5, 3.0, and 6.0 times those normally used in commercial cigarettes. The cigarettes were smoked, and the impaction-trapped CSC was tested in the S/M assay (strains TA98 and TA100) with and without metabolic activation. The results indicated that the addition of licorice block to

cigarettes, at any of the levels tested, did not alter the activity of the CSC.

APPENDIX I

Constituents of Licorice Extract

Glycyrrhizin	20%
Reducing sugars	5%
Nonreducing sugars	5%
Starch, dextrins, and gums	30%
Ash	8%
Moisture	17%

Source: Nieman 1957.

APPENDIX II

Typical Use (%) of Licorice-Related Substances in Foods

Food category	Licorice Root	Licorice Extract	Licorice Extract Powder	Ammoniated Gycyrrhizin
Alcoholic beverages	0.011	0.139	0.180	0.003
Baked goods, mixes	0.011	0.057	0.167	0.006
Chewing gum	—	2.880	0.582	0.072
Sugar confections	—	—	—	0.062
Frozen dairy desserts, mixes	0.052	0.047	0.020	0.005
Gelatins, puddings, fillings	—	0.019	—	0.005
Hard candy	24.400	0.446	0.401	0.040
Meat products	0.210	0.060	0.220	—
Non-alcoholic beverages	0.018	0.017	0.002	0.002
Soft candy	0.186	1.214	1.223	0.076
Sweet sauces, toppings, syrups	—	0.001	—	—

Source: National Research Council 1972

Ishidate, Jr., M., T. Sofuni, K. Yoshikawa, M. Hayashi, T. Nohmi, M. Sawada, A. Matsuoka, Primary Mutagenicity Screening of Food Additives Currently Used in Japan, Fd. Chem. Toxic. 22 623-636 (1984).

Kellogg, G., Boone, C., Malone, W., and Steele, V. 1992. Chemoprevention Clinical Trials. Mutation Research 267: 291.

Kirk-Othmer, Encyclopedia of Chemical Technology, 19 602 (2nd ed. 1963-1970).

Klosa, J., Beitrag zur therapeutischen wirkung der inhaltstoffe von succus liquiritiae, Pharm. Ztg. Ver. Apotheker-Ztg. 102 946-949 (1957).

Kobuke, T., K. Inai, S. Nambu, K. Ohe, T. Takemoto, K. Matsuki, H. Nishina, I.-B. Huang and S. Tokuoka, Food Chem. Toxicol. 23 (11) 979-983 (1985).

Komiyama, K., Y. Kawakubo, T. Fukushima, K. Sugimoto, H. Takeshima, Y. Ko, T. Sato, M. Okamoto, I. Umezawa, and Y. Nishiyama, OyoYakuri 14 (4) 535-548 (1977), Chem. Abstr. 88 69205e (1977).

Koster, M. and David, G. 1968. Reversible Severe Hypertension Due to Liquorice Ingestion. N. Engl. J. Med. 278: 1381.

Kraus, S.D., Glycyrrhizin-Induced Inhibition of the Pituitary-Adrenal Stress Response, J. Exp. Med. 108 325-328 (1958).

Linko, E., R. Vasama, Effect of Glycyrrhetenic Acid on the Adrenal Cortex, Ann. Med. Exp. Tenn. 36 97-107 (1958).

Macabies, J., A. Barbe, and P. Cristol, Action Hypertensive de la Glycyrrhizine chez le Rat, C.R. Soc. Biol. (Paris) 157 1665-1667 (1963a).

Macabies, J., A. Barbe., A. Orsetti, and P. Cristol, Hypertensive Action of Various Compounds Prepared from Licorice in the Normal Rat, C.R. Soc. Biol. (Paris) 157 2255-2257 (1963b).

Mashiba, H. and Matsunaga, K. 1990. Augmented Antiproliferative Effect of Tumor Necrosis Factor (Tnf), Lymphotoxin and Glycyrrihizin in Combined Use with Diethyldithiocarbamate on Meth A Tumor Cels In Vitro. Jap. J. Exp. Med.

Monder, C., Stewart, P., Lakshmi, R., Valentino, R., Burt, D., and Edwards, C. 1989. Licorice Inhibits Corticosteroid 11ß-Dehydrogenase of Rat Kidney and Liver: In vivo and In Vitro Studies. Endocrinology 125(2): 1046-1053.

Morris, D., Davis, E. and Latif, S. 1990. New Engl. J. Med. 322: 849.

Morita, T., T. Nagaki, I. Fukuda, and K. Okumura. Clastogenicity of Low pH to Various Cultured Mammalian Cells. Mutation Research 268: 297-305 (1992).

R.E. Muller, Tobacco Int. 161 (11) 12-15, 29 (1965).

R.E. Muller, U. S. Patent 3,282,706, (1966).

Mutagenic Evaluation of Compound FDA 71-1, Ammoniated Glycyrrhizin, Prepared for Food and Drug Administration by Litton Bionetics, Incorporated, PB-245-454 (1972).

Neiman, C., Licorice, Advances in Food Research 7 339-381 (1957).

Ngo, H., Teel, R., and Lau, B. 1992. Modulation of Mutagenesis, DNA Binding and Metabolism of Aflatoxin B_1 by Licorice Compounds. Nutrition Res. 12: 247.

Oak Ridge National Laboratory, Testing for Potential Mutagens by Use of Unscheduled DNA Synthesis (UDS) in the Germ Cells of Male Mice, Division of Biology, Oak Ridge, Tenn. (1982).

O'Brien, C., Ward, N., and Vogel., V. 1990. Inhibition of Protein Kinase C by the 12-O-Tetradecanoylphorbol-13-Acetate Antagonist Glycyrrhetic Acid. Cancer Letters 49: 9-12.

Parke, D.V., S. Pollock, R. T. Williams, The Fate of Tritium-Labelled B-glycyrrhetic acid in the rat, J. Pharm. Pharmacol 15 500-506 (1963).

Sakagami, H., Nippon Nogeikagaku Kaishi 47 623-626 (1973); Chem. Abstr. 80 93264d (1974).

Sasano, N., H. Miyazawa, K. Shimizu, K. Koizumi, Stimulation of Rat Adrenocortical Cells by Glycyrrhizin With Special Reference to its Inhibitory Effect on Dexamethasone-Induced Atrophy of the Adrenal Cortex, Tohoku J. Exp. Med. 90 391-403 (1966).

Scott, D., Galloway, S.M., Marshall, R.R., Ishidate, Jr., M., Brusick, D., Ashby, J. and Myhr, B. Genotoxicity under extreme culture conditions. A Report from ICPEMC Task Group 9. Mutation Research 257: 147-204 (1991).

Select Committee on GRAS Substances (SCOGS-28), Evaluation of the Health Aspects of Licorice, Glycyrrhiza and Ammoniated Glycyrrhizin as Food Ingredients, Prepared for Food and Drug Administration, PB-254-529 (1974).

Shankel, D. and Clarke, C. 1990. Specificity of Anti-Mutagens Against Chemical Mutagens in Microbial Systems. Basic Life Sci. 52: 457.

Shibata, S., Takahashi, K., Mori, T., Hirabayashi, K., Takeda, M., and Nagata, N. 1989. Antiulcer Activities of Glycyrrhetinic Acid Derivatives in Experimental Gastric Lesion Models. Chem. Pharm. Bull. 37(9): 2500-2504.

Shibayama, Y. 1989. Prevention of Hepatotoxic Responses to Chemicals by Glycyrrhizin in Rats. Exp. Mol. Pathol. 51: 48-55.

Stanford Research Institute, Study of Mutagenic Effects of Ammoniated Glycyrrhizin (71-1) by the Dominant Lethal Test in Rats, Menlo Park, Cal. (1977).

Tanaka, M., Mano, N. Akazai, E., Narui, Y., Kato, F. and Koyama, Y. 1987b. Inhibition of Mutagenicity by Glycyrrhiza Extract and Glycyrrhizin. Pharmacobio-dyn. 10: 685.

Teelucksingh, S., Mackie, A., Burt, D., McIntyre, M., Brett, L., and Edwards, C. 1990. Potentiation of Hydrocortisone Activity in Skin by Glycyrrehetinic Acid. Lancet 335(8697): 1060-1063.

Tilley, N. M., The Bright Tobacco Industry, 1860-1929, University of North Carolina Press, Chapel Hill, 511-512 (1948).

Tocco, L, Ricerche chimiche e farmacologiche sul principia attivoglicirizzina - della liquorizia (glycyrrhiza glabra, L. glycyrrhiza - tipica, Reg. e. Herd.) Arch. Intern. Pharmacodynamie 28 11-12 (1923).

Tochikura, T., Nakashima, H., and Yamamoto, N. 1989. Anti-Viral Agents with Activity Against Human Retroviruses. J. Acquired Immune Defic. Syndr. 2(5): 441-447.

Van Katwijk, V. M., L. G. Huis, In't Veld, Metabolism of Glycyrrhetinic Acid in Human Subjects, Nature 173 733-734 (1954).

Vogel, V., Newman, R., Ainslie, N., and Winn, R. 1992. Phase I Pharmacology and Toxicity Study of Glycyrrhetinic Acid as a Chemopreventive Drug. Proc. of the Amer. Assoc. for Cancer Res. 33: 208.

Vora, P. S. and R. M. Tuorto, Program Abstracts, Abstract No. 38, 38th Tobacco Chemists' Research Conference, Atlanta, Ga. (November 5-8, 1984).

Vora, P.S., Tobacco Int. 186 (9) 13-20 (1984).

Wang, Z., Agawal, R., Zhou, Z., Bickers, D., and Mukhtar, H. 1991. Inhibition of Mutagenicity in Salmonella Typhimurium and Skin Tumor Initiating and Tumor Promoting Activities in SENCAR Mice by Glycyrrhetinic Acid: Comparison of 18α- and 18 ß-Stereoisomers. Carcinogenesis 12(2): 187.

Whitehouse, M. W., P.D.G. Dean, T. G. Halshall, Uncoupling of Oxidative Phosphorylation by Glycyrrhetic Acid, Fusidic Acid, and Some Related Triterpenoid Acids, J. Pharm. Pharmacol. 19 533-544 (1967).

REFERENCES

Agarwal, R., Wang, Zu., and Mukhtar, H. 1991. Inhibition of Mouse Skin Tumor-Initiating Activity of Dmba by Chronic Oral Feeding of Glycyrrhizin in Drinking Water. Nutr. Cancer 15: 187.

Asanuma, Y., Effects of Glycyrrhizin on the Suppressive Action of Glucocorticoid on the Putuitary-Adrenal System, Nihon Naibunpitsu Gakkai Zasshi 41 623-632 (1965).

Bannister, B., Ginsburg, R., Shneerson, J. 1977. Cardiac Arrest Due to Liquorice Ingestion. N. Engl. J. Med. 278: 1381.

Brusick, D., Toxicology Forum Report, The Toxicology Forum, Washington, D.C., 139-152 (Feb. 1984).

Carlat, L.E., H.W. Margraf, H.H. Weathers, T.E. Weichselbaum, Human metabolism of orally ingested glycyrrhetinic acid and monammonium glycyrrhizinate, Proc. Soc. Exp. Biol. Med. 102 245-248 (1959).

Cook, M.K., The Use of Licorice and Other Flavoring Material in Tobacco, paper presented at the American Chemical Society Meeting, Philadelphia, Pa., (April 10, 1975).

Council of Europe, Flavouring Substances and Natural Sources of Flavourings, maisonneuve, Paris, France (3rd Ed. 1981).

Crebelli, R., Aquilina, G., Conti, G., and Carere, A. 1990. Microbial Mutagenicity Screening of Natural Flavoring Substances. Microbiologica 12(2): 115.

Cumming, A., Boddy, K., Brown, J. Severe Hypokalaemia with Paralysis Induced by Small Doses of Liquorice. Postgrad. Med. J. 56: 526.

Elmadjian, F., J.M. Hope, G. Pincus, The Action of Mono-Ammonium Glycyrrhizinate on Adrenalectomized Subjects and its Synergism with Hydrocortisone, J. Clin. Endocrinol. Metab. 16 338-349 (1956).

Epstein, M., Espiner, E., Donald, R., and Hughes, H. 1977. Effect of Eating Licorice on the Renin-Angiotensin Aldosterone Axis in Normal Subjects. Brit. Med. J. 1: 488.

Evdokimova, N.I., I.K. Kamilov, Farmakol. Alkaloidov, Glikozidov. 222-226 (U.S.S.R. 1967).

48 Federal Register 54983-54990 (December 8, 1983).

Finney, R.S.H., G.F. Sommers, J.H. Wilkinson, The Pharmacological Properties of Glycyrrhetinic Acid - A New Anti-Inflammatory Drug, J. Pharm. Pharmacol. 10 687-695 (1958).

Food and Drug Research Laboratories, Inc., Teratologic evaluation of FDA 71-1 in Mice, Rats, Hamsters and Rabbits (Unpublished report) (1972).

Fujimura, H., Test on Acute Toxicity of Crude Ammonium Glycyrrhizinate, Crude Potassium Glycyrrhizinate, and MonoAmmonium Glycyrrhizinate, unpublished report, Maruzen Pharmaceutical Co., Ltd. 5 pp.

Fujimura, H., and K. Okamoto, Toxicity test of Dipotasslum Glycyrrhizinate and Diammonium Glycyrrhizinate, unpublished report, Maruzen Pharmaceutical Co., Ltd. 14 PP.

C. Frattini, C. Bicchi, C. Barettini, G. M. Nano, J. Agri. Food Chem. 25 (6) 1238-1241 (1977).

Galal, E.E., The Effects of Glycyrrhetinic Acid on Salt and Water Metabolism, Brit. J. Pharmacol. 10 305-313 (1955).

Galloway, S.M., C.L. Bean, M.A. Armstrong, D. Deasy, A. Kraynak, M.O. Bradley, False Positive In Vitro Chromosome Aberration Tests With Non-Mutagens at High Concentrations and Osmolalities, Environ. Mutagenesis 7 Supp. 3 48-49 (1985).

Girerd, R.J., C.L. Rassaert, G. Di Pasquale, R.L. Kroc., Production of experimental hypertension and cardio-vascularrenal lesions with licorice and ammoniated glycyrrhizin, Amer. J. Physiol. 194 241-245 (1958).

Givaudan Flavorist, Licorice: An Old Fashioned Candy, No. 3, 3-5 (1970).

Gordon, L.R., Effects of Ammoniated Glycyrrhizin on Blood Pressure, Electrolytes and Corticosterone in Various Strains of Rats, Thesis., George Washington University, Washington, D.C. (1974).

Gujral, M. L., K. Sareen, D.P. Phukan, M. K. P. Amma, Antiarthritic Activity of Glycyrrhizin in Adrenalectomised Rats, Indian J. Med. Sci. 15 625-629 (1961).

Hall, R.L., B.L. Oser, Food Technology 19:2 151-197 (1965).

Hall, R.L., Food Technology 14:10 488-495 (1960).

Hassan, W.E., Jr., J.F. Palumbo, F. Elmadjian, The Electrolytic Effects of Licorice Preparations in the Adrenalectomized Rat, J. Amer. Pharm. Assoc. Sci. Ed. 43 551-554 (1954).

Hayashi, J., Kashiwagi, S., Noguchi, A., Ikematsu, H., Tsuda, H., and Motomura, M. 1990. Combination Therapy of Glycyrrhizin Withdrawal and Human Fibroblast Interferon for Chronic Hepatitis B. Clin. Ther. 11: 161-169.

Hayashi, J., Noguchi, kA., Nakashima, K., Hayashi, S., Kashiwagi, S., Mayumi, T., and Motomura, M. 1990. Human Lymphoblastoid and Fibroblast Interferon in the Treatment of Chronic Hepatitis B. Clin. Ther. 12: 12-21.

Hayashi, R., Maruyama, T., Maruyama, K., Yanagawa, S., Tako, K., and Yanagisawa, N. 1992. Myotonic and Repetitive Discharges in Hypokalemic Myopathy Associated with Glycyrrhizin-Induced Hypochloremia. J. Neurolog. Sci. 107: 74.

Houseman, P.A., J. Assoc. Off. Agric. Chem. VI (2) 191-196 (1922-1923).

Inoue, H., Mori, T., Shibata, S., and Koshihara, Y. 1989. Modulation by Glycyrrhetinic Acid Derivatives of TPA-Induced Mouse Ear Edema. Br. J. Pharmacol. 96: 204-210. Yano, S., Harada, M., Watanabe, K., Nakamaur, K., Hatakeyama, Y.

Ishida, S., Sakiya, Y., Ichikawa, T., and Awazu, S. 1989. Pharmacokinetics of Glycyrrhetinic Acid, A Major Metabolite of Glycyrrhizin, in Rats. Chem. Pharm. Bull. 37(9): 2509-2413.

Ishida, S., Sakiya, Y., Ichikawa, T., Taira, Z., and Awazu, S. 1990. Prediction of Glycyrrhizin Disposition in Rat and Man By a Physiologically Based Pharmacokinetic Model. Chem. Pharm. Bull. 38(1): 212-218.

SAFETY DATA SHEET

BARNET
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LICORICE PT-40

1. Identification

Product Name	Licorice Extract P-T 40
Supplier	Barnet Products
Address	920 Sylvan Avenue, Ste. 210 Englewood Cliffs, NJ 07632, USA
Telephone Number	201-346-4620
Emergency Information	CHEMTREC (Non-emergency calls cannot be serviced at this number) Within USA and Canada: 1-800-424-9300 Outside USA and Canada: +1 703 527 3887 (collect calls accepted)
Recommended Use	Raw material for cosmetic use
INCI Name	Glycyrrhiza Glabra (Licorice) Root Extract

2. Hazard Identification

Human Health Hazards	None

*This product is not classified as dangerous according to 1272/2008/EC as amended and GHS.

3. Composition/Information on Ingredients

Substance/Preparation	Preparation		
Chemical Name	**%**	**EINECS Number**	**CAS Number**
Glycyrrhiza Glabra (Licorice) Root Extract	100	283-895-2	97676-23-8/ 84775-66-6

4. First Aid Measures

Ingestion	Seek medical attention.
Inhalation	Remove to fresh air and keep at rest.
Skin Contact	Wash affected area with soap and water. If redness or irritation occurs, seek medical attention.
Eye Contact	Flush with plenty of water for 15 minutes. If redness or irritation occurs, seek medical attention.

5. Fire Fighting Measures

Extinguishing Media	
Suitable	CO_2. Dry chemical. Foam.
Special Firefighting Procedures	N/A
Hazardous Thermal (de) Composition Products	None
Protection of Firefighters	Self-contained breathing apparatus.

6. Accidental Release Measures

Personal Precautions	Use normal good practice.
Environmental Precautions	Use normal good practice.
Methods of Cleaning up	Use normal good practice.

7. Handling and Storage

Handling	Observe good manufacturing and handling procedures.
Storage	Keep container tightly closed and away from heat and light.
Shelf Life	2 years from date of manufacture if kept in original container.

SAFETY DATA SHEET

8. Exposure Control/Personal Protection

Respiratory System Protection	No special respirator needed.
Skin and Body Protection	Wear lab coat and apron.
Hand Protection	Wear impervious gloves.
Eye Protection	Wear goggles with side shields.

9. Physical and Chemical Properties

Physical State	Powder	**Flammability (solid, gas)**	Non-flammable
Color	Yellowish-brown to reddish-brown	**Vapor Pressure**	No data
Odor	Characteristic	**Vapor Density**	No data
pH	No data	**Relative Density**	No data
Melting Point	No data	**Solubility – Water**	Insoluble
Boiling Point	No data	**Partition Coefficient**	No data
Flash Point	No data	**Auto-ignition Temperature**	No data
Evaporation Rate	No data	**Decomposition Temperature**	No data
Viscosity	No data		

10. Stability and Reactivity

Conditions to Avoid	None expected if stored and handled properly.
Materials to Avoid	None expected if stored and handled properly.
Hazardous Decomposition Products	None

11. Toxicological Information

Skin Irritation	Not an irritant.
Eye Irritation	Fine dust particles might cause slight irritation.
Evaluation of Sensitizing Potential	Not a sensitizer.

12. Ecological Information

No ecotoxicological data currently available for this product.

13. Disposable Considerations

Method of Disposal	Follow all legal requirements.

14. Transport Information

Land – Road/Railway	N/A
Inland Waterways	N/A
Sea	N/A
Air	N/A
National Transport Regulation	N/A

15. Regulatory Information

Label Name	Licorice Extract PT-40

16. Other Information

History	
Date of Issue	December 9th, 2019 sr

1.Identification

Product name:	Licorice extract
Catalog#:	K148
IUPAC name:	Not available.
Product use restrictions:	Only for research and development use by, or directly under the supervision of, a technically qualified individual.
Company:	AK Scientific, Inc. 30023 Ahern Ave. Union City, CA 94587
Telephone:	(510) 429-8835
Fax:	(510) 429-8836
Website:	www.aksci.com
Emergency contact number:	1-800-633-8253 United States & Canada 1-801-629-0667 International

2.Hazard Identification:

GHS Classification

Skin irritation (Category 2)
Eye irritation (Category 2A)
Specific target organ toxicity - single exposure (Category 3), Respiratory system

Pictogram:

Signal word:

Warning

Hazard statement(s)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Precautionary statement(s):

P261	Avoid breathing dust/fume/gas/mist/vapors/spray.
P264	Wash skin thoroughly after handling.
P271	Use only outdoors or in a well-ventilated area.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P304+P340	IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P312	Call a poison center or doctor if you feel unwell.
P321	Specific treatment (see supplemental first aid instructions on this label).
P332+P313	If skin irritation occurs: Get medical advice/attention.
P337+P313	If eye irritation persists: Get medical advice/attention.
P362	Take off contaminated clothing and wash before reuse.
P403+P233	Store in a well-ventilated place. Keep container tightly closed.
P405	Store locked up.
P501	Dispose of contents/container to an approved waste disposal plant.

Hazards not otherwise classified (HNOC) or not covered by GHS:

None

3.Composition/Information on Ingredients

Synonyms:	Not available.
CAS#:	68916-91-6
Purity:	Not available.
EC:	272-837-1

4. First Aid Measures

General Information: Immediately remove any clothing contaminated by the product. Move out of dangerous area. Consult a physician and show this safety data sheet.
Inhalation: Move person to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Obtain medical aid.
Skin contact: Immediately flush skin with running water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Obtain medical aid immediately.
Eye contact: Immediately flush open eyes with running water for at least 15 minutes. Obtain medical aid immediately.
Ingestion: Do NOT induce vomiting without medical advice. Rinse mouth with water. Never administer anything by mouth to an unconscious person. Obtain medical aid immediately.
Most important symptoms and effects, both acute and delayed: No further information available. Please see sections 2 and 11.
Indication of any immediate medical attention and special treatment needed: No further information available.

5. Fire Fighting Measures

Suitable extinguishing media: Use water spray, dry chemical, carbon dioxide, or chemical foam.
Specific hazards arising from the chemical: .
Advice for firefighters: As in any fire, wear a NIOSH-approved or equivalent, pressure-demand, self-contained breathing apparatus and full protective gear. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion.

6. Accidental Release Measures

Personal precautions, protective equipment and emergency procedures: Wear protective equipment and keep unprotected personnel away. Ensure adequate ventilation. Remove all sources of ignition. Prevent further leak or spill if safe to do so. For personal protective equipment, please refer to section 8.
Environmental precautions: Do not let product enter drains, other waterways, or soil.
Methods and materials for containment and cleaning up: Prevent further leak or spill if safe to do so. Vacuum, sweep up, or absorb with inert material and place into a suitable disposal container. Consult local regulations for disposal. See section 13 for further disposal information.

7. Handling and Storage

Precautions for safe handling: Avoid contact with skin, eyes, and personal clothing. Wash hands thoroughly after handling. Avoid breathing fumes. Use only with adequate ventilation. Wear suitable protective clothing, gloves, and eye/face protection. Keep away from sources of ignition. Minimize dust generation and accumulation. Keep container tightly closed. Open and handle container with care. Do not eat, drink, or smoke while handling.
Conditions for safe storage, including any incompatibilities: Store in a tightly-closed

container when not in use. Store in a cool, dry, well-ventilated area away from incompatible substances. Keep away from sources of ignition.

8. Exposure Controls/Personal Protection

Exposure limits:

OSHA PEL: Not available.
NIOSH REL: Not available.
ACGIH TLV: Not available.

Appropriate engineering controls: Avoid contact with skin, eyes, and clothing. Wash hands before breaks and immediately after handling the product. Facilities storing or utilizing this material should be equipped with an eyewash fountain. Use adequate general and local exhaust ventilation to keep airborne concentrations low.

Personal protection

Eyes: Based on an evaluation of the eye or face hazards present, wear chemical splash-resistant safety glasses or goggles with side protection. A face shield may be appropriate in some workplaces. Use eyewear tested and approved under appropriate government standards such as OSHA 29 CFR 1910.133 or EU EN166.

Hands: Wear gloves selected based on an evaluation of the possible hazards to hands and skin, the duration of use, the physical conditions of the workplace, and the chemical resistance and physical properties of the glove material.

Skin and body: Protective clothing must be selected based on the hazards present in the workplace, the physical environment, the duration of exposure, and other factors. No fabric can provide protection against all potential hazards; therefore it is important to select the appropriate protective clothing for each specific hazard. At the minimum, wear a laboratory coat and close-toed footwear.

Respiratory: Respirators are not a substitute for accepted engineering control measures such as enclosure or confinement of the operation, general and local ventilation, and substitution of less toxic materials. When respiratory personal protective equipment is appropriate based on an assessment of respiratory hazards in the workplace, use a NIOSH- or CEN-certified respirator.

9. Physical and Chemical Properties

Pysical State:	Not available.
Molecular Formula:	-
Molecular Weight:	-
Odor:	Not available.
pH:	Not available.
Boiling Point Range:	Not available.
Freezing/Melting Point:	Not available.
Flash Point:	Not available.
Evaporation Rate:	Not available.
Flammability(solid,gas):	Please see section 2.
Explosive limits:	Not available.
Vapor Pressure:	Not available.
Vapor Density:	Not available.
Solubility:	Not available.
Relative Density:	Not available.
Refractive Index:	Not available.
Volatility:	Not available.
Auto-ignition Temperature:	Not available.
Decomposition Temperature:	Not available.
Partition Coefficient:	Not available.

10. Stability and Reactivity

Reactivity:	Not available.
Chemical stability:	Stable under recommended temperatures and pressures.
Possibility of hazardous reactions:	Not available.
Conditions to avoid:	Dust generation.
Incompatible materials:	Strong oxidizing agents.
Hazardous decomposition products:	.

11. Toxicological Information

RTECS#	Not available.
Acute toxicity:	Not available.
Routes of exposure:	Inhalation,eye contact,skin contact,ingestion.
Symptoms related to the physical,chemical and toxicological characteristics:	Skin contact may result in inflammation characterized by itching, scaling, reddening, blistering, pain or dryness. Eye contact may result in redness, pain or severe eye damage. Inhalation may cause irritation of the lungs and respiratory system. Overexposure may result in serious illness or death.

Carcinogenicity

IARC:	Not classified.
NTP:	Not listed.
OSHA:	Not listed.
Acute toxic effects:	Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

12. Ecological Information

Ecotoxicity:	Not available.
Persistence and degradability:	Not available.
Bioaccumulative potential:	Not available.
Mobility in soil:	Not available.
Other adverse effects:	Not available.

13. Disposal Considerations

Disposal of waste:	Chemical waste generators must determine whether a discarded chemical is classified as hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification. Observe all federal, state and local regulations when disposing of the substance.
Disposal of packaging:	Do not reuse containers. Dispose of as unused product.

14. Transportation Information

DOT(United States)

UN number:	not hazardous material.
Proper shipping name:	Not available.
Transport hazard class:	Not available.
Packing group:	Not available.

IATA

UN Number:	not dangerous goods.
Proper shipping name:	Not available.
Transport hazard class:	Not available.
Packing group:	Not available.

15. Regulatory Information

TSCA Chemical Inventory:
This product is NOT on the EPA Toxic Substance Control Act (TSCA) inventory. The product is supplied solely for use in research and development by or under the supervision of a technically qualified individual as defined in 40 CFR § 720 et seq. The health risks have not been fully determined. Any information that is or becomes available will be supplied on the SDS.

California Proposition 65:	Not listed.	
NFPA Rating:	Health:	Not available.
	Flammability:	Not available.
	Instability:	Not available.

16. Additional Information

Revision Date: 02/25/2019
Printed Date: 3/28/2019

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SAFETY DATA SHEET

May be used to comply with OSHA's Hazard Communication Standard, 29CFR 1910.1200. Standard must be consulted for specific regulations.

ACTIQUE LIC

Quick Identifier
Common Name (Used on Label and List)

SECTION I - IDENTIFICATION

Actique LIC

Manufacturer's Name	-	**JARCHEM INDUSTRIES, INC.**		
Address	-	**414 Wilson Avenue** **Newark, NJ 07105**		
Emergency Telephone #	-	**(973) 344-0600**	Secondary Telephone No.:	**CHEMTREC**
Other Information Calls	-	**(973) 344-0600**		**(800) 424-9300** **24 Hours a Day**
Date Prepared	-	**4/13/2016**		
Date Revised	-			

SECTION II - HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW

Non-Hazardous

HMIS HAZARD RATINGS

HEALTH	0
FLAMMABILITY	0
REACTIVITY	0

Pictograms

(See section for Toxicological Information)

SECTION III - COMPOSITION / INFORMATION ON INGREDIENT

PRODUCT NAME: Actique LIC

SYNONYMS:

CHEMICAL NAME: Glycyrrhiza Glabra (Licorice) Root Extra CAS#: 84775-66-6 EC#: 283-895-2

Ingredients	CAS#	% by Weight
Glycyrrhiza Glabra (Licorice) Root Extract	84775-66-6	>40(HPLC)
KANZOU FURABONOIDO (LICORICE FLAVONIDS)		<60

See sections on Exposure Guidelines and Regulatory Classifications.

SECTION IV - FIRST-AID MEASURES

EYES: Wash thoroughly with running water. Get medical advice if irritation develops.

SKIN: Not expected to require first aid measures.

INHALATION: Not expected to require first aid measures.

INGESTION: If large amounts were swallowed, give water to drink and get medical advice.

SECTION V - FIRE-FIGHTING MEASURES

NFPA Rating

FLAMMABLE PROPERTIES:

FLASH POINT:

HEALTH	0
FLAMMABILITY	0
REACTIVITY	0

EXTINGUISHING MEDIA AND INSTRUCTIONS:

Alcohol resistant foam, Carbon dioxide, Dry chemical Extinguishing media that must water

SECTION VI - ACCIDENTAL RELEASE MEASURES

STEPS TO BE TAKEN IN CASE OF SPILL OR LEAK:

SECTION VII - HANDLING AND STORAGE

USUAL SHIPPING CONTAINERS:

STORAGE/TRANSPORT TEMPERATURE:

STORAGE/TRANSPORT PRESSURE:

SECTION VIII - EXPOSURE CONTROLS / PERSONAL PROTECTION

ENGINEERING CONTROLS:

PERSONAL PROTECTIVE EQUIPMENT:

EYES: Safety glasses.

SKIN: Wash thoroughly after handling.

RERSPIRATORY PROTECTION: None Required

EXPOSURE GUIDELINES:

SECTION IX - PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE:	yellow-brown or reddish-brown powder
ODOR:	N/A
PHYSICAL STATE:	powder
pH:	N/A
VAPOR PRESSURE (mm Hg):	N/A
VAPOR DENSITY (AIR=1):	N/A
VISCOSITY:	N/A
BOILING POINT:	N/A
MELTING POINT:	N/A
POUR POINT:	N/A
SPECIFIC GRAVITY (H2O=1):	N/A

ORGANIC VOLATILE IMPURITIES:

FLAMMABILITY, FLASH POINT, LFL/UFL, AUTO IGNITION TEMP: See Section V

DECOMPOSITION TEMP: See Section X

SOLUBILITY IN WATER: N/A

SECTION X - STABILITY AND REACTIVITY

CONDITIONS TO AVOID: Extreme heat

INCOMPATABILITY WITH OTHER MATERIALS: Strong oxidixing or reducing agents

SECTION XI - TOXICOLOGICAL INFORMATION

EYES: N/A

SKIN: N/A

INHALATION: N/A

INGESTION: Rat: mus LD50: 7500mg/Kg: No toxic effects noted

REPRODUCTION: N/A

OTHER: N/A

SECTION XII - ECOLOGICAL INFORMATION

ECOTOXICOLOGICAL INFORMATION:

Disperse residue to reduce aquatic harm.

SECTION XIII - DISPOSAL CONSIDERATIONS

SPECIAL INSTRUCTIONS: Should not be considered hazardous waste.

SECTION XIV - TRANPORT INFORMATION

DOT DESCRIPTION:	Not regulated	Class	Packing Group
PROPER SHIPPING NAME:			
ICAO/IATA DESCRIPTION:	Not regulated	Class	Packing Group
IMDG DESCRIPTION:		Class	Packing Group
	EMS No.:		

SECTION XV - REGULATORY INFORMATION

US FEDERAL REGULATIONS

OSHA HAZARD COMMUNICATION STANDARD CLASSIFICATION:

TSCA INVENTORY LISTING: This material is not listed on the TSCA inventory.

SARA 302 Status:

SARA 311/312 CLASSIFICATION:

SARA 313 CHEMICALS:

CERCLA HAZARDOUS SUBSTANCE:

EUROEAN EINECS LISTING:	☐		
CANADIAN (DSL) LISTING:	☐	**CANADIAN (NDSL) LISTING:**	☐
CHINA INVENTORY LISTING:	☐	**TAIWAN LISTING:**	☐
JAPANESE (MITI) LISTING:	☐	**KOREAN INVENTORY LISTING:**	☐
AUSTRALIAN (AICS) LISTING:	☐	**NEW ZEALAND LISTING:**	☐
PHILIPPINES (PICCS) LISTING:	☐	**CALIFORNIA PROP. 65 LISTING:**	☐

SECTION XVI - OTHER INFORMATION

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