RIVM report 340630001/2003

The health and addiction risk of the glycyrrhizic acid component of liquorice root used in tobacco products

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This investigation is carried out for the account of the Directorate for Public Health of the Ministry of Health, Welfare and Sports and of the Inspectorate for Health Protection and Veterinary Public Health, within the framework of project 340630

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Abstract

Reported here is the evaluation of the health effects and possible addictive effects of liquorice preparations as a conditioning and flavouring agent in tobacco products. Based on the literature survey, the evaluation describes the exposure to, and the pharmacology, pharmacokinetics and toxicology of glycyrrhizic acid, the main biologically active component of liquorice preparations. The report also goes into the addictive aspects of glycyrrhizic acid. The level of glycyrrhizic acid in cigarette smoke or in tobacco products is not known. Furthermore, it is improbable that the intake of glycyrrhizic acid during cigarette smoking will exceed the daily oral intake. A point of concern is the nature of the combustion products formed during cigarette smoking. It is unlikely that smoking-related exposure to glycyrrhizic acid will increase mineralocorticoid activity and result under some conditions in hypertension. The statement that glycyrrhizic acid acts as a bronchodilatator could not be confirmed from the currently available literature. The possibility that liquorice preparations directly or indirectly increase addiction to cigarettes can not be excluded. The health and addiction risks of adding liquorice preparations to tobacco products need to be studied further.

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Samenvatting

In dit rapport worden de gezondheids- en mogelijke verslavende effecten van glycyrrhizinezuur beschreven. Glycyrrhizinezuur is de belangrijkste biologisch actieve component in zoethoutpreparaten welke aan tabaksproducten worden toegevoegd als smaakstof. Dit literatuuronderzoek beschrijft de blootstelling, farmacologie, farmacokinetiek, toxicologie, interacties en verslavende aspecten van glycyrrhizinezuur.

Aan tabaksproducten worden concentraties tot 4% van zoethoutpreparaten toegevoegd. De hoeveelheid glycyrrhizinezuur in die preparaten varieert van 7 tot 39%. Een realistische schatting van de aan tabak toegevoegde hoeveelheid glycyrrhyzinezuur is door deze grote variatie niet te maken. Het lijkt echter niet waarschijnlijk dat de opname van glycyrrhizinezuur door het roken van tabaksproducten groter zal zijn dan de dagelijkse oraal ingenomen hoeveelheid. De veelal onbekende verbrandingsproducten die gevormd worden tijdens het roken van tabaksproducten zijn wel een reden voor bezorgdheid. Glycyrrhyzinezuur wordt in het maagdarmkanaal omgezet in zijn biologisch actieve metaboliet, glycyrrhetine zuur. Het is onwaarschijnlijk dat deze omzetting ook gebeurt na inhalatie. Ook is het onwaarschijnlijk dat een aan roken gerelateerde blootstelling aan glycyrrhizinezuur, in analogie met een overmatige drop consumptie, kan leiden tot een verhoging van de mineralocorticoïde activiteit en onder sommige omstandigheden tot hypertensie. Over de verslavende aspecten van de aan tabak toegevoegde stof glycyrrhizinezuur zijn geen gegevens beschikbaar. De bewering dat zoethoutpreparaten als smaakstof indirect de verslaving aan sigaretten verhogen kan niet worden uitgesloten.

De gezondheidsrisico’s van glycyrrhizinezuur, als bestanddeel van zoethoutpreparaten in tabaksproducten, lijken gering te zijn. Zoethoutpreparaten bevatten echter meer bestanddelen dan alleen glycyrrhizinezuur. Zo zijn er aanwijzingen dat toevoeging van zoethoutpreparaten aan tabak de hoeveelheid aldehyden in sigarettenrook verhogen. Vorming van aldehyden en andere verbrandingsproducten uit zoethoutpreparaten vormen punt van bezorgdheid en vereisen nader onderzoek. Daarnaast is het mogelijk dat glycyrrhizinezuur, als zoetstof, indirect bijdraagt aan het verslavende effect van tabaksproducten. Daarom is meer onderzoek nodig naar de rol van smaakverbeteraars op het experimenteer gedrag van kinderen met sigaretten.

Summary

Reported here is the evaluation of the health effects and possible addictive effects of liquorice preparations as a conditioning and flavouring agent in tobacco products. Based on the literature survey, the evaluation describes the exposure to, and the pharmacology, pharmacokinetics and toxicology of glycyrrhizic acid, the main biologically active component of liquorice preparations. The report also goes into the addictive aspects of glycyrrhizic acid.

Concentrations up to 4% of liquorice preparations are added to tobacco products. The level of glycyrrhizic acid in liquorice preparations vary from 7-39% w/w. A realistic estimate of the level of glycyrrhyzic acid in tobacco products can not be made due to this large variation. It seems unlikely that the intake of glycyrrhizic acid due to smoking will exceed the daily oral intake. A point of concern is the nature of the combustion products formed during cigarette smoking. More research is needed on this concern.

Glycyrrhyzic acid is metabolised in the gastrointestinal tract into glycyrrhetic acid, its biologically active metabolite. It is unlikely that this metabolisation can occur after inhalation. Therefore it is also unlikely that smoking-related exposure to glycyrrhizic acid, in analogy with excessive liquorice candy intake, will increase mineralocorticoid activity and result under some conditions in hypertension. The statement that glycyrrhizic acid acts as a bronchodilatator could not be confirmed in the available literature used for this evaluation. No data are available on the dependence potential of glycyrrhizic acid. The statement that liquorice preparations indirectly increase the addiction to cigarettes can not be excluded.

The health risks of adding glycyrrhizic acid, as liquorice preparations to tobacco products, seem small. Liquorice preparations contain, however, more constituents than glycyrrhizic acid alone. There are indications that the addition of liquorice preparations to tobacco products can increase the concentration of aldehydes present in mainstream cigarette smoke. This is a point of concern and needs further study. It is also possible that glycyrrhizic acid, as a sweetener, indirectly contributes to the addiction to tobacco products. More research is therefore needed to elucidate the effect of flavouring agents on experimentation with cigarette smoking among children and adolescents.

1. Introduction

Cigarette smoking is generally thought of as the main cause of early preventable death in humans. In 1997, smoking caused more than 23.000 deaths in the Netherlands (1). Hence, prevention and quitting smoking are major public health goals. Recently, more interest has been developed in the composition of cigarettes and the possibility of harm reduction. The European Commission developed directive 2001/037/EG concerning regulation of tobacco ingredients. One of the goals of the directive is to come to regulation, harmonisation and standardisation of ingredients added to tobacco.

At now a wide disparity exists in legal regulations. Moreover, in some Member States, additive rules for tobacco are assimilated to those for foodstuff (2). In this context, those compounds that are Generally Recognized As Safe (GRAS) food additives are regarded also to be safe for the use as additives to tobacco, that is to be smoked in the form of cigarettes, cigars, pipes, or hand-rolled cigarettes. This assumption of safety is encumbered by the fact that nonvolatile additives are at least partially pyrolysed during smoking, so that they may give rise to toxic and/or carcinogenic agents in the smoke (3).

Another concern regarding the use of additives involves the possible change in addiction properties of tobacco products due to the use of additives. This includes not only concerns for direct addictive effects of additives but also the indirect addictive effects due to taste improvement. The latter concerns the statement that if cigarettes are made to taste better, more people may start to smoke, continue to smoke, or decide not to quit. The use of sweeteners and other flavoring enhancers make cigarettes more palatable and ‘aspirational’, particular to children and the young (4).

Liquorice preparations are used as a conditioning and flavoring agent in tobacco products. So far more than 80 different constituents of liquorice preparations (flavonoids, chalcones and coumarines) have been identified. Glyzyrrhizic acid or glycyrrhizin is the main biologically-active compound of the liquorice root (5) (6), and therefore only this compound is discussed. Glycyrrhizin possesses a sweet taste and sweetness-potentiating characteristics and have been employed industrially for this reason (6). In this report glycyrrhizin’s environmental and smoking exposure will be reviewed, as well as it’s chemical, pharmacological, pharmacokinetic, toxicological, interaction and dependency properties. The purpose of this study is to evaluate whether glycyrrhizin itself or its combustion products alters the health and addictive effects of smoking tobacco products.

1.1 References

(1) Nationaal Kompas Volksgezondheid. 30-5-2002. See http://www.rivm.nl/vtv/data/site_kompas/index.htm

(2) Progress achieved in relation to public health protection from the harmful effects of tobacco consumption; Commission report to the European parliament, the council, the economic and social committee, and the committee for the regions. http://europa.eu.int/comm/health/ph/news/old/report.pdf . 2001.

(3) Hoffmann D, Hoffmann I. The changing cigarette, 1950-1995. Journal of Toxicology and Environmental Health 1997; 50:307-364.

(4) Bates C., Jarvis, M., Connolly, G. Tobacco Additives. Cigarette engineering and nicotine addiction, 1999. See http://www.ash.org.uk.

(5) Ploeger BA. Development and use of a physiologically based pharmacokinetic-pharmacodynamic model for glycyrrhizic acid in consumer products. 2000. PhD thesis, University of Utrecht.

(6) Fenwick GR, Lutomski J, Nieman C. Liquorice, *Glycyrrhiza glabra* L.- Composition, Uses and Analysis. Food Chemistry 1990; 38:119-143.

2. Method

Publications on glycyrrhizin were identified through Medline, Toxline and Current Contents and from electronic citations in the Merck Index (2001), DOSE (1), RTECS (2), HSDB (3), BIG (4), SAX Dangerous Properties of Industrial Materials (2001) and Comprehensive Toxicology (2001). Additional information was derived from the references cited in these publications and from publications on Internet.

2.1 References

(1) The Dictionary of Substances and their Effects (DOSE); The Royal Society of Chemistry; 2001.

(2) The Registry of Toxic Effects of Chemical Substances (RTECS); The National Institute for Occupational Safety and Health (NIOSH); 2001.

(3) Hazardous Substances Data Bank (HSDB); The National Library of Medicine; 2001.

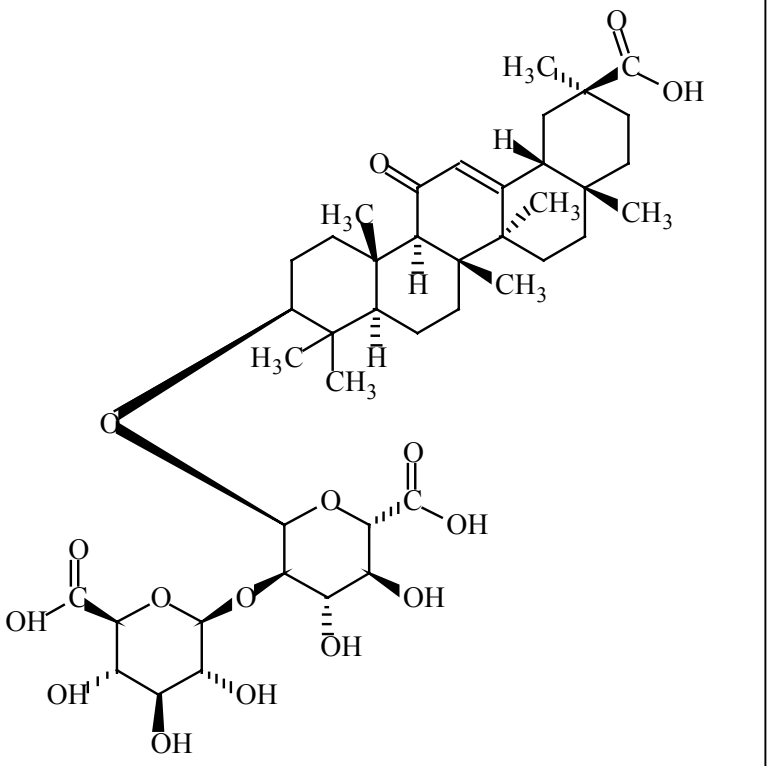
(4) Brandweer Informatiecentrum voor Gevaarlijke stoffen (BIG) (Firedepartment Informationcentre for Hazardous substances); versie 10 (10th edition).

3. Results

3.1 Glycyrrhizic acid

GENERAL
IUPAC systematic name: no data available.
Synonyms: glycyrrhizin (prime name), 20ß-carboxy-11-oxo-30-norolean-12-en-3ß -yl-2-o-ß-d-glucopyranuronosyl-α-d-glucopyranosiduronic acid; α-d-glucopyranosiduronic acid, (3ß,20ß)-20-carboxy-11-oxo-30-norolean-12-en-3-yl-2-o-ß-d-glucopyranuron osyl; glycyron; glycyrrhetinic-acid-glycoside; glycyrrhizic-acid; glycyrrhizinic acid; liquorice; sweet-root; licorice-root-extract (1).
Molecular formula: $C_{42}H_{62}O_{16}$ (1)

Molecular structure



Molecular weight: 823 g/mol (2)
Alifatic: yes
Aromatic: no
N containing: no
Halogen containing: no
CAS registry no.: 1405-86-3 (3)
Storage: no data available
R/S classification: no data available
dangercode (transport): no data available
Properties:
➢ melting point: no data available
➢ boiling point: no data available

- density: no data available
- refractive index: no data available
- solubility: freely soluble in hot water, alcohol (1).
- substance description:
  - color: no data available
  - liquid/gas/powder: liquid or dry extract (1)
  - odor/taste: light, slightly spiced scent, intensively sweet (1)
- volatility: no data available
- pK_a: no data available
- PA: no data available
- flammability: no data available
  - FP = no data available
  - FL Limits = no data available
  - IT = no data available
- decomposition temperature: 220 °C (1)
- stability: see conclusion.
- vapour pressure/ vapour tension (20 °C): no data available
- vapour pressure (50 °C): no data available
- relative density: no data available
- octanol water partition coefficient, log P: no data available, log K_{OW}: 2.80 (1)
- conversion factor: no data available

Critical assessment

Glycyrrhizic acid is a monodesmoside: a glycosylated compound in which one sugar-containing part is linked to an aglycon. The sugar moiety has a strongly polar character, while the aglycan has a largely apolar part (modulated with a polar carboxylic group). In general, this type of compounds exhibits characteristic surface active properties (foaming and sometimes haemolytic activity).
The OH-groups of the sugar moiety form potential linking points to acyl groups.
The presence of three carboxyl groups causes the compound to have an acidic character.
The sugar moiety is a potential source for dehydration and successive decomposition upon heating.

Conclusion

Glycyrrhizic acid is a nitrogen free, acidic compound, with structural features that will result in dehydration and decomposition upon heating.

FUNCTION IN TOBACCO

Liquorice preparations are used in tobacco as a conditioning and flavouring agent. Liquorice root is used in food products for its extremely sweet taste, it is 170 times sweeter than sucrose (2). Glycyrrhizic acid is the main biologically active component of liquorice root and therefore only glycyrrhizic acid is studied (4).

Glycyrrhizic acid

AMOUNT IN TOBACCO PRODUCTS

According to the Tobacco Industry concentrations up to 4% of extract of liquorice root are added to cigarettes (5). The composition of liquorice block extract differ depending on a variety of factors, including the root source and method of processing. The amount of glycyrrhizic acid from different countries in liquorice extracts varied from 7-39 % w/w (4). Because both the concentration of glycyrrhyzic acid in liquorice extract varies enormously and the amount of extract added to tobacco products is uncertain, the final concentration of glycyrrhizic acid in tobacco products is unknown.

AMOUNT IN SMOKE

Hoffmann and Hoffmann state that is it likely that liquorice root will decompose to unknown compounds in the course of smoking a cigarette (6).

- **main stream**

No data available

- **side stream**

No data available

SOURCE

Glycyrrhizic acid is an extract of the root of the liquorice plant (*Glycyrrhiza glabra L.*) In general, only the root and underground stems of the liquorice plant are harvested. After drying, the roots are macerated and extracted with hot water and this extract is finally concentrated to a product with a moisture content of about 20 % to obtain liquorice block extract. In general, this block extract forms the primary product of liquorice confectionery, but it may also be purified by further treatment with sulphuric acid following neutralization with diluted ammonia to obtain ammoniated glycyrrhizic acid. Among glycyrrhizic acid, being the main biological active compound of the liquorice root, more than 80 different constituents (flavonoids, chalcones and coumarines) have been identified (4). The glycyrrhizic acid content ranges from 22 mg to 32 mg per gram liquorice root plant (2).

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

The daily intake of glycyrrhizic acid in the Dutch population has been estimated to be approximately 20 mg/day, in Belgium and Denmark 5-10 mg/day, in the United Kingdom and the USA 1 mg/day and 3.3 mg/day respectively. In the Netherlands the consumption of glycyrrhizic acid consists mainly of liquorice sweets. The concentration of glycyrrhizic acid in liquorice in the Netherlands (based on dry weight) ranges from 0.03-0.27% (mean 0.15%) (7). Liquorice is used in a wide variety of other food products such as chocolate, chewing gum, sweets and in some alcoholic beverages. About 90 % of the liquorice consumed in the USA is applied by the tobacco industry. Liquorice is also present in various health products, like liquorice tea or throat pastilles and in medicinal preparations (2).

COMBUSTION PRODUCTS

When heated to decomposition it emits unknown compounds resulting in acrid smoke and irritating fumes (1;6).

CONSENSUS REPORTS

No data available.

Glycyrrhizic acid

STANDARDS AND RECOMMENDATIONS
ADI: 200 mg/ person a day (2); European Union: <100 mg/ person a day (2).
TWA_{NL} = MAC: no data available
TWA_{D} =MAK: no data available
TWA_{USA}: no data available
$STEL_{NL}$: no data available
$STEL_{USA}$: no data available
LTEL: no data available
TLV-C: no data available
TLV-CARCINOGENICITY: no data available
MAK-REPRODUCTION: no data available

Others:

Reference value:
no data available

CLASS
EG Carc. Cat.: no data available
IARC-category: no data available
CEC: no data available

Critical assessment
Glycyrrhizic acid is the main biologically active component of an extract of the root of the liquorice plant (*Glycyrrhiza glabra L.*). It is used as such an extract in cigarettes as a conditioning and flavouring agent. Concentrations up to 4 % of liquorice extract are added to tobacco products but the amount of glycyrrhizic acid in smoke or in tobacco products is not known. Little is known about the products formed during combustion. Only information about the oral intake of this substance is available.
Conclusion
No data are available on the exposure to glycyrrhizic acid through cigarette smoking. Research is needed to obtain information on the amount of glycyrrhizic acid in smoke and tobacco products and to obtain the nature of the combustion products formed during cigarette smoking.

PHARMACODYNAMICS
The data reported are all data on oral intake, except when reported otherwise.
Mechanism of action
Glycyrrhizic acid and glycyrrhetic acid (after oral administration glycyrrhizic acid is hydrolyzed to glycyrrhetic acid by commensal bacteria; glycyrrhetic acid is completely absorbed) are potent competitive inhibitors of the enzyme 11-ß-hydroxysteroid dehydrogenase (11-ß-HSD2). Under physiological circumstances, 11-ß-HSD2 converts cortisol into cortisone and allows endogenous mineralocorticoid aldosterone to occupy the mineralocorticoid receptors to regulate the physiological mineralocorticoid activity. However, if 11- ß-HSD2 activity is absent or impaired, cortisol will displace aldosterone from the mineralocorticoid receptors and will act as a potent mineralocorticoid. Consequently the mineralocorticoid activity will increase.

Glycyrrhizic acid

In vitro, glycyrrhetic acid is about 200-1000 times more potent than glycyrrhizic acid. It has also been hypothesized that the 3ß-glucoride metabolite of glycyrrhetic acid causes the adverse effects (2).
Glycyrrhizic acid binds minimally to estrogen and androgen receptors and not at all to the progesterone receptor in uterine cytosol. It exhibits a moderate binding activity to glucocorticoid receptors in liver cytosol. Glycyrrhizic acid also exhibits weak binding activity to both cortico-steroid-binding globulin and sex-hormone-binding sites (8).

Pulmonary system

- **breathing frequency**: No data available.
- **tidal volume**: No data available.
- **lung compliance**: No data available.
- **airway resistance**: No data available.

Cardiovascular system

- **blood pressure**: The systolic blood pressure in humans was raised by 3.1-14.4 mm Hg following the daily ingestion of 50-200 g liquorice for 2-4 weeks, corresponding to a daily intake of 75-540 mg glycyrrhizic acid, the active substance in liquorice. The increase in blood pressure varied considerably among the subjects (9). In a prior experiment it was demonstrated that the blood pressure returned to the normal values within 3-4 weeks after cessation (10). In another study the systolic blood pressure increased following ingestion of 500 mg glycyrrhizic acid during 3 days (human data). However the systolic blood pressure was not significantly different form the basal value when the glycyrrhizic acid ingestion was continued for another 4 days. It has been suggested that the glycyrrhizic acid induced hypertension might only become apparent after daily glycyrrhizic acid intake of high doses (>75 mg/day) for more than 10 days (2).
  Glycyrrhizic acid in Male Sprague-Dawley rats (200 mg/kg/day oral, for 5 weeks), is able to induce hypertension, and inhibits the transcriptions of both 11ß-HSD2 and CYP11B2 (cytochrome P450 isozyme) in the vasculature, leading to lower aldosterone and higher corticosterone production in vessels, and increased vasoconstrictor responses to norepinephrine (11). See also toxicology.
- **heart rate**: The ingestion of large quantities of glycyrrhizic acid, whether as a drug or a sweetener, is known, in susceptible subjects in some cases, to induce hypokaliaemia-induced arrythmias (12). See also toxicology.

Renal system

The ingestion of large quantities of glycyrrhizic acid (>140 mg per day for a person of 70 kg), whether as a drug or a sweetener, is known, in susceptible subjects, to induce a syndrome similar to hypermineralocorticoidism, with bouts of hypertension, hypokaliaemia and rabdomyolysis, sometimes associated with severe renal failure and hypokaliaemia-induced arrythmias (human data). Also decreased plasma renin activity and decreased plasma aldosterone have been reported.
Glycyrrhizic acid is mainly absorbed as its metabolite glycyrrhetic acid. This metabolite is a potent inhibitor of the enzyme 11-ß-hydroxysteroid dehydrogenase (11-ß-HSD2), which is mainly present in the kidney. Under physiological circumstances, 11-ß-HSD2 converts cortisol into its inactive metabolite cortisone and allows the endogenous mineralocorticoid aldosterone to occupy the mineralocorticoid receptors. In case of absent or impaired 11-ß-HSD2 activity, cortisol will displace

aldosterone from these receptors, which will increase the mineralocorticoid activity. This will influence the osmolarity of plasma by retention of sodium. As a result, the urine production will decline and the volume of the extracellular fluid will expand. The plasma renin activity will decline due to the increased mineralocorticoid activity. This will lower the aldosterone excretion via the renin-angiotensine-aldosterone system. ANP (Atrial natiuretic peptide) will induce natiuresis and diuresis by increasing the glomular filtration, inhibition of sodium reabsorption in the proximal tubule and the collecting duct, and reduction of aldosterone secretion. When these counteracting physiological mechanisms may fail (partly) hypertension and symptoms associated with electrolyte disturbances will become apparent. In addition, hypokaliaemia may occur due to excretion of more potassium to maintain the electrolyte balance. This may finally result in clinical manifestations like proximal myopathy, lethargy and muscle cramps (2).
Glycyrrhizin attenuates renal ischemia-reperfusion injury when given 30 minutes after the onset of reperfusion in a rabbit model (glycyrrhizin infusion for 72 h), measured as a lower mean blood urea nitrogen creatinine ratio (13).
Glycyrrhizin is also known to isomerize into the glycyrrhetic (or glycyrrhetinic) acids 18 α - and 18ß-. These metabolites cause bouts of hypertension and reduction in diuresis at doses of 15 mg/kg/day orally in the rat. In particular, the α isomer causes significant elimination of the calcium ion in the urine. 18 α -glycyrrhetic acid is more toxic than either glycyrrhizin or the ß isomer (12). See also toxicology.

- **diuresis**: see above.
- **saluresis**: see above.

Nervous system
There are no *in vivo* data available.

- **central nervous system**: In the brain 11-β-HSD2 is also present. The mineralocorticoid receptors in the brain may participate in mineralocorticoid hypertension, but the role of cerebral 11-β-HSD2 remains to be solved (2).
  Glycyrrhizic acid and deoxycorticosterone have similar effects on corticotrophin releasing factor and beta-endorphin containing neurons at the paraventricular nucleus (14).
- **autonomic system**:
  No data available.

Other
Glycyrrhizic acid accelerates both Interleukin 2 (IL-2) production and proliferation of mature T lymphocytes (cellculture of mice thymocytes 200 µg/ml). In immature thymocytes glycyrrhizic acid promotes IL-2 production/IL-2 receptor expression but inhibits cell growth. Glycyrrhizic acid mediated growth inhibition of thymocytes is not due to the cytotoxic action of glycyrrhizic acid that induces cell death or DNA fragmentation. Glycyrrhizic acid promotes the tyrosine phosphorylation of p56 but suppresses the phosphorylation of p40 induced by anti-CD3 (CD = clusters of differentiation). Moreover, glycyrrhizic acid and anti-CD3 show a combination effect suppressing the transcription of c-fos, which was promoted by anti-CD3 alone or glycyrrhizic acid alone. It is suggested that whereas mature and immature T cells share a common signal pathway for IL-2 production augmented by the action of glycyrrhizic acid, they have signalling steps for DNA synthesis which are under different mechanisms receiving the modulation effects of glycyrrhizic acid in

opposite directions (15).
Glycyrrhizic acid (intraperitoneally, 10 mg/kg of body weight 1 day before infection and 1 and 4 days postinfection, 21-day experimental period) may protect mice exposed to a lethal amount of influenza virus through the stimulation of IFN-gamma production by T cells, because T cells have been shown to be producer cells of IFN-gamma stimulated with the compound (16).
Glycyrrhizic acid has anti-inflammatory activity and has been used for the treatment of chronic viral hepatitis (80-200 mg intravenously daily for 4 to 8 weeks). Glycyrrhizic acid inhibits the cytolytic activity of complement via the activation of both the classical and alternative pathways, while it has no effect on immune adherence, suggesting that it blocks C5 or a later stage of the complement cascade (human serum, 0-4.8 mM glycyrrhizic acid). Glycyrrhizic acid inhibits the lytic pathway in which the membrane attack complex (MAC) is formed. This mechanism suggests that glycyrrhizic acid may prevent tissue injury caused by MAC not only in chronic hepatitis but in many autoimmune and inflammatory diseases (17).

Critical assessment
Glycyrrhizic acid and glycyrrhetic acid are potent competitive inhibitors of 11-ß-HSD2. Glycyrrhetic acid is *in vitro*, about 200-1000 times more potent than glycyrrhizic acid. This may result in an increased mineralocorticoid activity with as consequence hypertension, hypokalaemia, reduction of diuresis, decreased plasma renin and decreased plasma aldosteron levels. Also anti-inflammatory activity has been reported. Neither smoking related exposure nor smoking related pharmacological data are available.
Conclusion
Following inhalation it is unclear if glycyrrhizic acid is metabolised into glycyrrhetic acid and increases in mineralocorticoid activity resulting in e.g. hypertension seem unlikely to occur. Smoking related exposure to glycyrrhizic acid and its pharmacological effects need to be studied.

PHARMACOKINETICS
Absorption
After oral administration in humans as well as in rats glycyrrhizic acid is hydrolyzed to glycyrrhetic acid by commensal bacteria. Glycyrrhetic acid is completely absorbed. No systemic hydrolysis is expected, as no glycyrrhetic acid was detected in plasma after glycyrrhizic acid injection into the portal vein in rats (2). No data are available on absorption after inhalation.

Bioavailability
In humans no glycyrrhizic acid was detected in plasma following an oral dose of 100-800 mg glycyrrhizic acid. In rats glycyrrhizic acid plasma levels were only detectable after high oral doses of 50-500 mg/kg, demonstrating low oral bioavailability (4% after 200 mg/kg). Glycyrrhetic acid, the aglycon of glycyrrhizic acid was detected in both rat and humans (2).

Distribution
The distribution of glycyrrhizic acid and glycyrrhetic acid among erythrocytes and

the remaining body tissues of the rat were reported to be minimal. The main compartment for distribution for both compounds is the blood serum, where they are bound to serum albumin (2).

Metabolism

In lysosymes of rodents and humans glycyrrhizic acid was hydrolyzed by glucuronidases into 18-β-glycyrrhetic acid monoglucuronide (GM) (2) and 18α-glycyrrhetic acid (12). Glycyrrhetic acid is metabolised in the liver to glucuronide and GM. Glycyrrhetic acid and it's metabolites are subject to enterohepatic cycling (2).

In male Sprague-Dawley rats glycyrrhizic acid (23 mg/kg oral, for 6 days) caused increases in specific activities of p-nitrophenol UDP-glucuronosyltransferase by 96 %, and UDP-glucuronic acid by 484 %. Glycyrrhizin activated glucuronidation and this suggests the possibility that glycyrrhizic acid may influence detoxification of xenobiotics in rat liver in male Sprague-Dawley rats (18).

Glycyrrhizic acid and glycyrrhetic acid reduce alanine transaminase (ALT) and aspartate transaminase (AST) values in the serum (19).

The repeated (10 days) oral administration of high doses glycyrrhizic acid to mice and rats (240 or 480 mg/kg bw) induced the cytochrome P450 isozymes CYP3A, CYP2B1 and CYP2A1. However, single dose treatment did not result in an induction of CYP-isoenzymes (20;21).

Excretion

Glycyrrhizic acid and glycyrrhetic acid are excreted mainly with bile, but also with urine (2). Only 1 % of the glycyrrhetic acid is excreted with urine (22). With an increasing dose, the cumulative fraction of the dose that is excreted into the bile decreased, indicating a dose dependent biliary excretion of glycyrrhizic acid. Model simulations demonstrate that especially in subjects with prolonged gastrointestinal residence times, glycyrrhetic acid may accumulate after repeated liquorice consumption (2).

Kinetic parameters

The plasma clearance of glycyrrhizic acid was 16-25 ml/h kg and its volume of distribution at steady state (38-64 ml/kg) appeared to be close to the mean volume of human serum (43 ml/kg). The time (T_{max}) at which glycyrrhetic acid reached it's maximum plasma levels (C_{max}) after oral administration of glycyrrhizic acid ranged from 8-12 h in humans and 12-16 h in rats (2).

Critical assessment

No data are currently available on absorption of glycyrrhizic acid after inhalation. After oral administration glycyrrhizic acid is hydrolyzed to the active metabolite glycyrrhetic acid in the gastro-intestinal and glycyrrhetic acid is completely absorbed. The bioavailability of glycyrrhizic acid after relatively high oral doses is low. Glycyrrhizic acid is metabolised in the liver and is subject to enterohepatic cycling. The excretion is mainly with bile, but also with urine.

Conclusion

There are no kinetic data on glycyrrhizic acid after inhalation exposure. Following inhalation it is unclear if glycyrrhizic acid is metabolised into glycyrrhetic acid. The kinetics of smoking related exposures to glycyrrhizic acid needs further study.

Glycyrrhizic acid

TOXICOLOGY

Acute toxicity
Human
No data available.
Animal
LD50 Rat oral 14200 mg/kg (1)
LD50 Rat ip 1420 mg/kg (1)
LD50 Rat sc 4200 mg/kg (1)
LD50 Mouse ip 1500 mg/kg (1)
LD50 Mouse sc 4000 mg/kg (1)
Groups of 4 female adult Sprague Dowley rats were dosed with 0.5, 1, 1.5 and 2 g/kg of glycyrrhizin, 18α-glycyrrhetic acid or 18β-glycyrrhetic acid ammonium salts, administered intraperitoneally. Dose levels up to 1.5 g/kg were well tolerated and the electrocardiogram did not reveal any significant lesion(s). On the other hand, at 2 g/kg of only the α-isomer, all animals died of atrio-ventricular block. Histopathological study revealed selective damage to the myocardium with oedema, myolysis, apoptosis and blistering of the sarcoplasm. Progressive impairment of cardiac function can be seen 60 minutes after acute treatment (ECG tracing) resulting in death after 130-140 minutes (12).

Local tolerance
Human
No data available.
Animal
No data available.

Repeated dose toxicity
Subacute
Groups of 40 female Sprague Dowley rats were dosed 30 mg/kg/ day glycyrrhizic acid, 15 mg/kg/day 18α-glycyrrhetic acid, or 15 mg/kg/ day 18β-glycyrrhetic acid, dissolved in water for 30 days. The fourth group received only water. In the course of the treatment (30 days) myolisis of the papillary muscles manifests itself in a milder form (than seen in the acute treatment group) appearing from day 30 of treatment with glycyrrhizin (30 mg/kg/day orally) or with 18 α-glycyrrhetic acid (15 mg/kg/day orally). In the group treated with 18β-glycyrrhetic acid (15 mg/kg/day orally) this myolisis was not seen. No ECG tracing was carried out. In the groups treated with glycyrrhizic acid and with18 α-glycyrrhetic acid, tubular calculi and slight expansion of bronchus associated lymphoid tissue are observed in the lungs after day 15. The renal calculi appear to be connected with the enhanced calcium ion excretion. An increase was found in the amount of calcium, sodium and potasium excreted with the urine from day 15 of treatment with 18α-glycyrrhetic acid, in the other groups the increase was not reached until day 30 of treatment (12). *Semichronic*
Human data:
In a pilotstudy, in which 8 male and 8 female volunteers consumed 400 and 800 mg glyccyrhizic acid daily for 4 weeks, clinical effect like headache, edema and an increase in weight were observed in 5 of the 16 subjects. In addition a decrease in the plasma renin and aldosterone activity was observed. The results of this study indicated that females are more sensitive for glycyrrhizic acid adverse effects than male subjects (23). In a dose-response study adverse effects of increasing doses of

glycyrrhizic acid administered to 39 healthy female volunteers for 8 weeks were studied. After the oral treatment of 2 mg/kg glycyrrhizic acid, no adverse effects were observed in any of the subjects, whereas in the higher dose group (4 mg/kg glycyrrhizic acid), glycyrrhizic acid induced adverse effects, such as a decrease in plasma renin activity and plasma aldosterone levels. In addition, the plasma potassium levels were decreased in this higher dose group. As a result, a human NOAEL of 2 mg glycyrrhizic acid per day per kg was concluded. For a subject with a bodyweight of 70 kg for instance, this level would be equal to 140 mg per day (23).

A 41-year-old woman, who presented with (apparent) essential hypertension, was treated with atenolol and candesartan. This treatment, however, was unsuccessful. After the addition of hydrochlorothiazide (HCT) to the combination, she developed hypokalaemia with muscle cramps and weakness. This hypokalaemia persisted for more than 4 weeks after discontinuation of HCT and starting potassium suppletion. As a result of polyuria (> 4000 ml/day) found in a 24-hour urine collection, it was discovered that the patient drank at least 3 litres of liquorice tea a day. She had denied eating liquorice sweets, a well-known cause of hypertension in the Netherlands, but no one had thought of asking her if she drank liquorice tea. Blood pressure and serum potassium normalised about 2 months after she stopped drinking liquorice tea, and medication was withdrawn (24). The dose can be estimated at 375 mg a day (personal communication with W. Mennes, RIVM), based on an average level of 126 mg/l in liquorice tea (range 2-450 mg/l) (7).

Bernardi *et al.* (1994) (25) administered graded daily doses of dried, aqueous extract of licorice root, containing 108, 217, 380 and 814 mg of glycyrrhizic acid, to 4 groups of 6 healthy volunteers of both sexes for 4 weeks. No significant effects occurred in groups of 108 and 217 mg glycyrrhizic acid. After 2 weeks, side effects leading to withdrawal from the protocol occurred in a female in the group of 380 mg glycyrrhizic acid (headache). In the group of 814 mg glycyrrhizic acid, a male with a family history of hypertension (arterial hypertension), and a female also taking oral contraceptives (hypertension, hypokalaemia and peripheral edema) retreated. In the group of 814 mg glycyrrhizic acid, 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 the groups of 380 and 814 glycyrrhizic acid. 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. The NOAEL based on this study was 217 mg/person/day (25).

A 42-year old man was referred to the hospital in a sopoforic state. He had experienced worsening headaches, nausea, vomiting and sensitive neuropathy on the left side of his body. An onset of light hypertnesion had been diagnosed 6 months earlier.Clinical examination showed a blood pressure of 200/140 mm Hg, decreased plasma potasium level (2.9 mmol, while 3.8-5.0 mmol/l is normal) and plasma aldosterone of 180 pmol/l (normal 320-2,000). The patients plasma potassium level could not be easily restored with supplements. It was noted that he ate 50 g of liquorice a day. Two weeks after he stopped, his blood pressure had fallen to 120-85 mm Hg and his plasma potassium level had risen to 4 mmol/l (26)

A second patient was a 46-year old man with asthenia, headaches and somnolence. Two weeks earlier hypokaliema (2.3 mmol/l) was observed that remained despite oral potassium supplementation. The man ate about 40 g of liquorice a day. Clinical examination showed a man who was sluggish, and did not respond to environmental

stimuli. He had generally muscle weakness. His blood pressure was 215/125 mm Hg, and an electrocardiogram showed sinus bradycardia and T wave leveling. Plasma clinical chemistry was normal except for potassium, aldosterin and renin, which were all depressed. Despite treatment with potassium supplementation and anti-hypertensives, his condition only improved when he stopped eating liquorice. When he was discharged he had a blood pressure of 150/80 mm Hg (26). According to the authors of the study, eating 50 g of liquorice corresponds with 100 mg glycyrrhyzic acid, the human LOAEL, which is associated with hypertension without complications. They speculated that there exists a significant individual variation in the susceptibility to glycyrrhyzic acid. Also partial deficiencies of 11-βHSDB have been suggested (26).

Animal data:

An increase in right atrial pressure as well as thickening of the pulmonary vessels (suggesting pulmonary hypertension), was seen in a study with male Sprague-Dawley rats (body weight 200 g) receiving drinking water containing 0.1 mg/ml and 1.0 mg/ml glycyrrhizic acid for 12 weeks. Rats drink 20- 30 ml (27) a day resulting in a dose of 10-15 mg/kg body weight per day and 100-150 mg/kg body weight per day (28).

Chronic

The adverse effects of liquorice are recognised as mineralocorticoid like. Hypertension and electrolyte disturbances will become apparent. Hypokaleamia occurs due to the excretion of more potassium to maintain the electrolyte balance. This finally results in clinical manifestations like oedema and hypertension. In cases of (severe) hypokalaemia, destruction of skeletal muscles or myopathy, ventricular tachycardia and renal failure was reported. Also hyperprolactinaemia, decrease in serum testosterone levels and discoloration of the skin are reported (human data) (2) (23). In most cases, the patients recovered completely within 1 to 4 months after cessation of glycyrrhizic acid intake. There exist substantial inter-individual differences in susceptibility for glycyrrhizic acid induced adverse effects (2).

Repeated intake of 500 mg glycyrrhizic acid for 7 days by healthy male subjects resulted in a considerable decrease of the serum tesosterone levels. Glycyrrhizic acid inhibits 17-ß-HSD and 17,20 lyase (2).

In a case report, a 85 year old tobacco chewer was described with classical features of exogenous mineralocorticoid excess: hypokalemia, hypertension, renal potassium wasting, metabolic alkalosis, sodium retention, and depressed renin. The intake of glycyrrhizic acid was estimated between 0.88 and 1.33 g a day for about 50 years. This patient swallowed the saliva and expectorated only the well-chewed tobacco leaf (29).

Carcinogenicity

Human

Anti-tumor effects have been reported (see beneficial effects).

Animal

Anti-tumor effects have been reported (see beneficial effects).

Reproduction toxicology

Human

Due to the presence of 11-β-HSD2 in the placenta, it has been hypothesised that inhibition of placental 11-β-HSD2 increases the fetal exposure to maternal glucocorticoids after exposure to glycyrrhizic acid. In the literature contradictory data have been reported, such as no effect on birth weight or maternal blood pressure and

decreased birth weight and lower gestational age (2;30).
Animal
No data available

Mutagenicity
Human
No data available.
Animal
No data available

Other

Critical assessment
The acute toxicity of glycyrrhizic acid is low. In a semichronic or chronic exposure duration glycyrrhizic acid may result in an increased mineralocorticoid activity with as consequence hypertension, hypokalaemia, reduction of diuresis, decreased plasma renin and decreased plasma aldosteron levels. After cessation of glycyrrhizic acid intake most patient recovered completely within 1 to 4 months. There are no data on local tolerance.
Conclusion
The toxicological effects of glycyrrhizic acid after oral exposure are the same as seen in a syndrome of hypermineralocorticoidism. More research is needed on the toxicological effects of glycyrrhizic acid after inhalation.

INTERACTIONS
Chemical
Hydrolysis of one mole glycyrrhizic acid yields one mole of glycyrrhetic acid and 2 moles of glucuronic acid (1). Due to its polar and apolar regions, the molecular structure is open for interactions related to polarity.

In vivo
Glycyrrhizic acid is structurally and chemically similar to aldosteron and desoxycorticosterone (1). Glycyrrhizic acid increases the plasma prednisolone concentrations by inhibiting the metabolism of prednisolone and it potentiates pharmacological effects of prednisolone (intravenous administration 0.075 mg/kg of prednisolone, with or without 200 mg of glycyrrhizic acid, human data) (31).
Glycyrrhizic acid is structurally similar to many cyclopentanophenanthrene steroids (1).
Known drug interactions include potassium loss due to thiazide diuretics, as well as increased sensitivity to digitalis glycosides (1).
The repeated (10 days) oral administration of high doses glycyrrhizic acid to mice and rats (240 or 480 mg/kg bw) induced the cytochrome P450 isozymes CYP3A, CYP2B1 and CYP2A1. However, single dose treatment did not result in an induction of CYP-isoenzymes (20;21).

Critical assessment
Chemical
The chemical structure results in surface active properties.
In vivo
Glycyrrhizic acid may potentiate pharmacological effects of prednisolone. It also may

cause an increased sensitivity to digitalis glycosides due to potassium losses. Induction of CYP-isoenzymes occur after repeated high oral doses of glycyrrhizic acid.

Conclusion

Chemical

The compound has surface active properties and decomposes readily upon heating.

In vivo

No conclusions could be drawn on smoking related glycyrrhizic acid exposure.

DEPENDENCY

No data available.

Effects of smoking cessation

No data available.

Critical assessment

Not relevant.

Conclusion

Not relevant.

COMMERCIAL USE

Glycyrrhizic acid is used as a foaming agent in root beer and mouthwashes, as sweetener in chocolate, cocoa, and chewing gum; and as a taste-masking agent in pharmaceuticals such as aspirin (1). It is commonly used in traditional medicine for instance as an expectorant, antitussive and mild laxative (1).

BENEFICIAL EFFECTS

Glycyrrhizic acid has been reported to protect against chronic cadmium (Cd) toxicity in rats (10 mg/kg) (32). Glycyrrhizic acid and glycyrrhetic acid reduce alanine transaminase (ALT) and aspartate transaminase (AST) values in the serum. This hepatoprotective effect has recently been explained as the inhibitory effects of glycyrrhizic acid and glycyrrhetic acid on immune-mediated cytotoxicity against hepatocytes (murine CD4+ cell line) and on nuclear factor (NF)-kappa B (murine liver), which activates genes encoding inflammatory cytokines in the liver (19).

In Japan, long term administration of a medicine consistent of 0.2 % glycyrrhizin, 0.1% cysteine and 2.0% glycine was effective in preventing liver carcinogenesis in chronic hepatitis C patients (21).

Glycyrrhizic acid inhibits human colon tumour cell arylamine N-acetyltransferase activity and DNA adduct formation (2,4,6 and 8 mM) (33).

Oral feeding of glycyrrhizic acid to Sencar mice resulted in substantial protection against skin tumorigenesis caused by 7,12-dimethyl-benz [a]anthracene (DMBA) initiation and 12-O-tetradecanoylphorbol-13-acetate (TPA) promotion. The latent period prior to the onset of tumor development was considerably prolonged in glycyrrhizic acid-fed animals compared with animals not fed glycyrrhizic acid and resulted in significant decrease in the number of tumors per mouse. Oral feeding of glycyrrhizic acid in drinking water also resulted in inhibition in the binding of topically applied [3H]benzo[a]pyrene and [3H]DMBA to epidermal DNA. The

possible mechanism(s) of the anti-tumor-initiating activity may be due to the involvement of glycyrrhizic acid as inhibitor of the carcinogen metabolism followed by an inhibition of DNA adduct formation (34).

Also anti-inflammatory effects have been reported (see pharmacodynamics and toxicology).

Critical assessment
Glycyrrhizic acid may have anti-inflammatory and anti-tumour effects.
Conclusion
No conclusions could be made on smoking related glycyrrhizic acid exposure.

SUMMARY AND FINAL CONCLUSION
Glycyrrhizic acid is the main biologically active component of an extract of the root of the liquorice plant (*Glycyrrhiza glabra L.*). It is used as such an extract in tobacco as a conditioning and flavouring agent. According to its chemical features, glycyrrhizic acid is a nitrogen free, acidic compound, with structural features that will result in dehydration and decomposition upon heating. Concentrations up to 4% of liquorice extract are added to tobacco products but the amount of glycyrrhizic acid in smoke or in tobacco products is not known. Only data on the oral intake of this substance are available. Research is needed to obtain the amount of glycyrrhizic acid in smoke and tobacco products and to obtain the nature of the combustion products formed during cigarette smoking. The daily oral intake of glycyrrhizic acid in the Dutch population has been estimated to be approximately 20 mg/day, mainly due to liquorice sweets. The European Union recommends a daily intake of less than 100 mg/person (orally). No data are available on the exposure to glycyrrhizic acid through inhalation or on local effects in the pulmonary system. Further research on this route of exposure is necessary.
There are no kinetic data on glycyrrhizic acid after inhalation exposure. After oral administration glycyrrhizic acid is hydrolyzed to the active metabolite glycyrrhetic acid in the gastro-intestinal tract and glycyrrhetic acid is completely absorbed.
The kinetics of smoking related exposures to glycyrrhizic acid needs further study.
Glycyrrhizic acid and glycyrrhetic acid are potent competitive inhibitors of the enzyme 11-ß-hydroxysteroid dehydrogenase (11-ß-HSD2). Under physiological circumstances, 11-ß-HSD2 converts cortisol into cortisone and allows endogenous mineralocorticoid aldosteron to occupy the mineralocorticoid receptors to regulate the physiological mineralocorticoid activity. The increased mineralocorticoid activity may result in hypertension, hypokalaemia, reduction of diuresis, decreased plasma renin and decreased plasma aldosteron levels, after high doses. Also anti-inflammatory activity has been reported. Following inhalation it is unclear if glycyrrhizic acid is metabolised into glycyrrhetic acid and increases in mineralocorticoid activity resulting in e.g. hypertension seem unlikely to occur. The acute toxicity of glycyrrhizic acid is low. Glycyrrhizic acid may prevent and inhibit tumour growth. There are no data available on dependency or smoking cessation.
Beneficial effects might include anti-inflammatory and anti-tumour effects.

No conclusions can be drawn based on the available information to assess the health

risks of smoking related exposure to glycyrrhizic acid from extract of liquorice root. The health risks of addition of liquorice root to tobacco products need to be studied. These kind of studies should include studies to assess the amount of glycyrrhizic acid in smoke and tobacco products and studies to assess the nature of the combustion products formed of liquorice root during cigarette smoking. The local effects of glycyrrhizic acid or its combustion products on the pulmonary system, as well as their systemic effects through inhalation are points of concern.

DATE THIS SHEET WAS GENERATED
Based on literature available in June 2001.

REFERENCES

(1) The hazardous Substance Data Bank (HSDB). through 2000/10 . 2001. The National Library of Medicine (NLM).

(2) Ploeger BA. Development and use of a physiologically based pharmacokinetic-pharmacodynamic model for glycyrrhizic acid in consumer products. 2000. PhD thesis, University of Utrecht.

(3) Handbook of Chemistry and Physics. 79th edition . 1999. CRC, Electronic version by William Andrew Publishing, USA.

(4) Fenwick GR, Lutomski J, Nieman C. Liquorice, *Glycyrrhiza glabra* L.- Composition, Uses and Analysis. Food Chemistry 1990; 38:119-143.

(5) Additives in tobacco (a UK department of health report). 2001. See www.aoiko.net/useful/tabaccoadditives.asp.

(6) Hoffmann D, Hoffmann I. The changing cigarette, 1950-1995. Journal of Toxicology and Environmental Health 1997; 50(4):307-364.

(7) Maas P. Zoethout in levensmiddelen: onderzoek naar het glycyrrhizinegehalte van thee, kruidenmengsles, dranken en drop. De Ware(n) Chemicus 2000; 30:65-74.

(8) Tamaya T, Sato S, Okada H. Inhibition by plant herb extracts of steroid bindings in uterus, liver and serum of the rabbit. Acta Obstet Gynecol Scand 1986; 65(8):839-842.

(9) Sigurjonsdottir HA, Franzson L, Manhem K, Ragnarsson J, Sigurdsson G, Wallerstedt S. Liquorice-induced rise in blood pressure: a linear dose-response relationship. J Hum Hypertens 2001; 15(8):549-552.

(10) Sigurjonsdottir HA, Ragnarsson J, Franzson L, Sigurdsson G. Is blood pressure commonly raised by moderate consumption of liquorice? J Hum Hypertens 1995; 9(5):345-348.

(11) Wu P, Zhang Y, Liu Y, Wang X, Guo Z, Liang X et al. Effects of glycyrrhizin on production of vascular aldosterone and corticosterone. Horm Res 1999;

51(4):189-192.

(12) Rossi T, Fano RA, Castelli M, Malagoli M, Ruberto AI, Baggio G et al. Correlation between high intake of glycyrrhizin and myolysis of the papillary muscles: an experimental in vivo study. Pharmacol Toxicol 1999; 85(5):221-229.

(13) Subramanian S, Bowyer MW, Egan JC, Knolmayer TJ. Attenuation of renal ischemia-reperfusion injury with selectin inhibition in a rabbit model. Am J Surg 1999; 178(6):573-576.

(14) Ruszymah BH, Nabishah BM, Aminuddin S, Sarjit S, Khalid BA. Effects of glycyrrhizic acid and steroid treatment on corticotropin releasing factor and beta-endorphin containing neurons of the hypothalamus of the rat. Malays J Pathol 1999; 21(1):51-58.

(15) Zhang YH, Kato M, Isobe K, Hamaguchi M, Yokochi T, Nakashima I. Dissociated control by glycyrrhizin of proliferation and IL-2 production of murine thymocytes. Cell Immunol 1995; 162(1):97-104.

(16) Utsunomiya T, Kobayashi M, Pollard RB, Suzuki F. Glycyrrhizin, an active component of licorice roots, reduces morbidity and mortality of mice infected with lethal doses of influenza virus. Antimicrob Agents Chemother 1997; 41(3):551-556.

(17) Fujisawa Y, Sakamoto M, Matsushita M, Fujita T, Nishioka K. Glycyrrhizin inhibits the lytic pathway of complement--possible mechanism of its anti-inflammatory effect on liver cells in viral hepatitis. Microbiol Immunol 2000; 44(9):799-804.

(18) Moon A, Kim SH. Effect of Glycyrrhiza glabra roots and glycyrrhizin on the glucuronidation in rats. Planta Med ; 63 :2, 115 119 , 1997 1997; 63.

(19) Shibata S. A drug over the millennia: pharmacognosy, chemistry, and pharmacology of licorice. Yakugaku Zasshi 2000; 120(10):849-862.

(20) Paolini M, Barillari J, Broccoli M, Pozzetti L, Perocco P, Cantelli-Forti G. Effect of liquorice and glycyrrhizin on rat liver carcinogen metabolizing enzymes. Cancer Lett 1999; 145(1-2):35-42.

(21) Paolini M, Pozzetti L, Sapone A, Cantelli-Forti G. Effect of licorice and glycyrrhizin on murine liver CYP-dependent monooxygenases. Life Sci 1998; 62(6):571-582.

(22) Parke DV, Pollock S, Williams RT. The fate of tritium-labeled beta-glycyrrhetic acid in the rat. Pharmacology 1963; 15:500-506.

(23) Bijlsma JA, Van Vloten p, Van Gelderen CEM, Mensinga TT, Mout HA, Elvers LH et al. Onderzoek naar de effecten van verschillende doseringen glycyrrhizine bij gezonde vrouwlijke vrijwilligers (The effects of different doses of glycyrrhizic acid in healthy females) 1996. RIVM report 348801004.

(24) Brouwers AJ, van der Meulen J. 'Drophypertensie'; ook door zoethoutthee. ['Licorice hypertension' also caused by licorice tea]. Ned Tijdschr Geneeskd 2001; 145(15):744-747.

(25) Bernardi M, D'Intino PE, Trevisani F, Cantelli-Forti G, Raggi MA, Turchetto E et al. Effects of prolonged ingestion of graded doses of licorice by healthy volunteers. Life Sci 1994; 55(11):863-872.

(26) Russo S, Mastropasqua M, Mosetti MA, Persegani C, Paggi A. Low doses of liquorice can induce hypertension encephalopathy. Am J Nephrol 2000; 20(2):145-148.

(27) Stichting proefdierkundige informatie. Proefdierkunde, Biotechnisch handboek. 3th ed. Valkenswaard: Offset-service B.V., 1990.

(28) Ruszymah BH, Nabishah BM, Aminuddin S, Khalid BA. Effects of glycyrrhizic acid on right atrial pressure and pulmonary vasculature in rats. Clin Exp Hypertens 1995; 17(3):575-591.

(29) Blachley JD, Knochel JP. Tobacco chewer's hypokalemia: licorice revisited. N Engl J Med 1980; 302(14):784-785.

(30) Strandberg TE, Jarvenpaa AL, Vanhanen H, McKeigue PM. Birth outcome in relation to licorice consumption during pregnancy. Am J Epidemiol 2001; 153(11):1085-1088.

(31) Chen MF, Shimada F, Kato H, Yano S, Kanaoka M. Effect of glycyrrhizin on the pharmacokinetics of prednisolone following low dosage of prednisolone hemisuccinate. Endocrinol Jpn ; VOL 37 , ISS 3, 1990 , P331 41 1990; VOL 37, ISS 3, 1990, P331 41.

(32) Shaikh ZA, Tang W. Protection against chronic cadmium toxicity by glycine. Toxicology 1999; 132(2-3):139-146.

(33) Chung JG, Chang HL, Lin WC, Wang HH, Yeh CC, Hung CF et al. Inhibition of N-acetyltransferase activity and DNA-2-aminofluorene adducts by glycyrrhizic acid in human colon tumour cells. Food Chem Toxicol 2000; 38(2-3):163-172.

(34) Agarwal R, Wang ZY, Mukhtar H. Inhibition of mouse skin tumor-initiating activity of DMBA by chronic oral feeding of glycyrrhizin in drinking water. Nutr Cancer 1991; 15(3-4):187-193.

4. Discussion

4.1 Exposure level

Glycyrrhizic acid is the main biologically active component of an extract of the root of the liquorice plant (*Glycyrrhiza glabra L.*) (1). It is used as such an preparation in cigarettes as a conditioning and flavouring agent (2). The amount of glycyrrhizic acid from different countries in liquorice preparations varies from 7-39 % w/w (1). Concentrations up to 4% of liquorice preparations are added to tobacco products. Because both the concentration of glycyrrhyzic acid in liquorice preparations varies enormously and the amount of preparation added to tobacco products is uncertain, the final concentration of glycyrrhizic acid in tobacco products is unknown.

No data are available on the exposure to glycyrrhizic acid through cigarette smoking. It is not likely, however that exposure levels of glycyrrhizic acid due to smoking will exceed the daily oral intake. The daily intake of glycyrrhizic acid in the Dutch population for instance, has been estimated to be approximately 20 mg/day (3). The European Union recommends a daily intake of less than 100 mg (orally) (2).

Data are missing on the combustion products of liquorice preparations formed during cigarette smoking. It is, however, likely that a large part will be burned during cigarette smoking.

Research is needed to obtain the amount of glycyrrhizic acid in smoke and in tobacco products and to obtain the nature of the combustion products formed during cigarette smoking.

4.2 Effects

Glycyrrhizic acid and it's metabolite glycyrrhetic acid are potent competitive inhibitors of the enzyme 11-ß-hydroxysteroid dehydrogenase (11-ß-HSD2) (2) (4). *In vitro*, glycyrrhetic acid is about 200-1000 times more potent than glycyrrhizic acid (2). Under physiological circumstances, 11-ß-HSD2 converts cortisol into cortisone and allows endogenous mineralocorticoid aldosteron to occupy the mineralocorticoid receptors to regulate the physiological mineralocorticoid activity. The increased mineralocorticoid activity may result in hypertension, hypokalaemia, reduction of diuresis, decreased plasma renin and decreased plasma aldosteron levels, after high doses (2) (4). Following inhalation it is unlikely that glycyrrhizic acid is metabolised into glycyrrhetic acid and increases in mineralocorticoid activity resulting in e.g. hypertension are unlikely to occur.

The acute toxicity of glycyrrhizic acid is low (5). Some beneficial effects of the compound have been described. Glycyrrhizic acid may prevent and inhibit tumour growth (6) (7). Also anti-inflammatory activity has been reported (8) (9) (10). The statement that glycyrrhizin acts as a bronchodilatator (11) could not be verified in the currently available literature.

Since glycyrrhizic acid is expected to decompose upon heating, the combustion products and its effects are points of concerns. It is unknown if glycyrrhyzic acid in unchanged form can

reach the airways. There are indications that the addition of liquorice preparations to tobacco products can increase the concentration of aldehydes present in mainstream cigarette smoke (12).
There are no data available on dependency or smoking cessation of the compound glycyrrhizic acid. It is sometimes stated that glycyrrhizin could enhance dependency indirectly because if cigarettes are made to taste better more people may start to smoke, continue to smoke or decide not to quit. The use of sweeteners and other flavourings could make cigarettes more palatable and aspirational particular to children and the young (11). No direct evidence for this statement is available at the moment. However it is known that cigarette smokers identify flavor as an important factor in the pleasure derived from smoking and for their choice of cigarette brand (13). Ratings of cigarette sweetness appear to be highly related to ratings of satisfaction and pleasantness (14). Moreover, the effects produced during a first tobacco episode may help to predict later regular use of tobacco. Retrospective studies indicate that heavy smokers experience less aversive effects than light smokers during their first cigarette do (15). More research is needed to elucidate the effect of less attractive cigarettes on experimentation with cigarette smoking among children and adolescents.

Acknowledgement

We would like to thank Dr T.T. Mensinga for reviewing this manuscript.

4.3 References

(1) Fenwick GR, Lutomski J, Nieman C. Liquorice, *Glycyrrhiza glabra* L.- Composition, Uses and Analysis. Food Chemistry 1990; 38:119-143.

(2) Ploeger BA. Development and use of a physiologically based pharmacokinetic-pharmacodynamic model for glycyrrhizic acid in consumer products. 2000. PhD thesis, University of Utrecht.

(3) Maas P. Zoethout in levensmiddelen: onderzoek naar het glycyrrhizinegehalte van thee, kruidenmengsles, dranken en drop. De Ware(n) Chemicus 2000; 30:65-74.

(4) Blachley JD, Knochel JP. Tobacco chewer's hypokalemia: licorice revisited. N Engl J Med 1980; 302(14):784-785.

(5) The hazardous Substance Data Bank (HSDB). through 2000/10 . 2001. The National Library of Medicine (NLM).

(6) Chung JG, Chang HL, Lin WC, Wang HH, Yeh CC, Hung CF et al. Inhibition of N-acetyltransferase activity and DNA-2-aminofluorene adducts by glycyrrhizic acid in human colon tumour cells. Food Chem Toxicol 2000; 38(2-3):163-172.

(7) Agarwal R, Wang ZY, Mukhtar H. Inhibition of mouse skin tumor-initiating activity of DMBA by chronic oral feeding of glycyrrhizin in drinking water. Nutr Cancer 1991; 15(3-4):187-193.

(8) Fujisawa Y, Sakamoto M, Matsushita M, Fujita T, Nishioka K. Glycyrrhizin inhibits the lytic pathway of complement--possible mechanism of its anti-inflammatory effect on liver cells in viral hepatitis. Microbiol Immunol 2000; 44(9):799-804.

(9) Utsunomiya T, Kobayashi M, Pollard RB, Suzuki F. Glycyrrhizin, an active component of licorice roots, reduces morbidity and mortality of mice infected with lethal doses of influenza virus. Antimicrob Agents Chemother 1997; 41(3):551-556.

(10) Zhang YH, Kato M, Isobe K, Hamaguchi M, Yokochi T, Nakashima I. Dissociated control by glycyrrhizin of proliferation and IL-2 production of murine thymocytes. Cell Immunol 1995; 162(1):97-104.

(11) Bates C., Jarvis, M., Connolly, G. Tobacco Additives. Cigarette engineering and nicotine addiction, 1999. See http://www.ash.org.uk.

(12) Carmines EL, Lemus R, Gaworski CL, Meisgen T, Rustemeier K, Van Miert E et al. Effects of the addition of liquorice extract to tobacco on the chemical composition and biologically activity of cigarette smoke. The Toxicologist 2003; 72(S-1):387.

(13) Levin ED, Behm F, Rose JE. The use of flavor in cigarette substitutes. Drug Alcohol Depend 1990; 26(2):155-160.

(14) Jaffe AJ, Glaros AG. Taste dimensions in cigarette discrimination: a multidimensional scaling approach. Addict Behav 1986; 11(4):407-413.

(15) Eisenberg T, Balster RL. Initial tobacco use eisodes in children and adolescnts: current knowledge, future directions. Drug Alcohol Depend 2000; 59(suppl. 1):S41-S60.

5. Conclusions and further considerations

No information is available on the health effects of glycyrrhizic acid after inhalation exposure. Therefore it difficult to assess the health risks of smoking related exposure to glycyrrhizic acid from liquorice preparations. Concentrations up to 4% of liquorice preparations are added to tobacco products. The level of glycyrrhizic acid in liquorice preparations vary from 7-39% w/w. A realistic estimate of the level of glycyrrhyzic acid in tobacco products can not be made due to this large variation. It seems unlikely that the intake of glycyrrhizic acid due to smoking will exceed the daily oral intake. A point of concern is the nature of the combustion products formed during cigarette smoking. More research is needed on this concern.

Glycyrrhyzic acid is metabolised in the gastrointestinal tract into glycyrrhetic acid, its biologically active metabolite. It is unlikely that this metabolisation can occur after inhalation. Therefore it is also unlikely that smoking-related exposure to glycyrrhizic acid, in analogy with excessive liquorice candy intake, will increase mineralocorticoid activity and result under some conditions in hypertension. The statement that glycyrrhizic acid acts as a bronchodilatator could not be confirmed in the available literature used for this evaluation. No data are available on the dependence potential of glycyrrhizic acid. The statement that liquorice preparations indirectly increase the addiction to cigarettes can not be excluded.

The health risks of adding glycyrrhizic acid, as liquorice preparations to tobacco products, seem small. Liquorice preparations contain, however, more constituents than glycyrrhizic acid alone. There are indications that the addition of liquorice preparations to tobacco products can increase the concentration of aldehydes present in mainstream cigarette smoke. This is a point of concern and needs further study. It is also possible that glycyrrhizic acid, as a sweetener, indirectly contributes to the addiction to tobacco products. More research is therefore needed to elucidate the effect of flavouring agents on experimentation with cigarette smoking among children and adolescents.

LIST OF ABBREVIATIONS

- **CAS registry no.**: Chemical Abstracts Service Registry Number is a numeric designation assigned by the American Chemical Societys Chemical Abstracts Service and uniquely identifies a specific chemical compound. This entry allows one to conclusively identify a material regardless of the name or naming system used.
- **R**: Risk phrases: Warnings on the label about the harmful propertie(s) of the substance.
- **S**: Safety phrases: Directions on the label about the necessary safety precautions to handle the substance. See appendix 1.
- **PA**: proton affinity in the gas phase, kcal/mol
- **FP**: Flash point in °C, which is the minimum temperature at which the vapor pressure of a liquid is sufficient to form an ignitable mixture with air near the surface of the liquid.
- **FL Limits**: Flammable limits (often called explosive limits) in %, which specify the range of concentration of the vapor in air (in percent by volume) for which a flame can propagate. Below the lower flammable limit, the gas mixture is too lean to burn; above the upper flammable limit, the mixture is too rich. Values refer to ambient temperature and pressure and are dependent on the precise test conditions.
- **IT**: Ignition temperature (sometimes called autoignition temperature) in °C, which is the minimum remperature required for self-sustained combustion in the absence of an external ignition source.
- **ADI**: Acceptable Daily Intake.
- **TWA**: Time Weighed Average.
- **MAC**: Maximum Acceptable Concentration.
- **STEL**: Short-term exposure limit for airborne contaminants, which should not be exceeded for more than 15 min. A “C” following a value indicates a ceiling limit which should not be exceeded even for very brief periods because of acute toxic effects of the substance.
- **LTEL**: Long-Term Exposure Limit (8 hours exposure). Exposure limit: maximum concentration of a chemical agent as time-weighed average of a reference period (8 h/day; 40 h/week) above which no employee may be exposed.
- **TLV-C**: Treshold Limit Value.
- **MAK-reproduction**: Classification of substances on foetal harm according to the German MAK-Werte-Liste.
  - ➢ A = The substance is clearly able to cause foetal harm.
  - ➢ B = Possible risk on foetal harm.
  - ➢ C = In compliance with MAK-value, risk of foetal harm is not to be feared.
  - ➢ D = Foetal toxicity stil unclear. Based on the available information, classification in group A-C is not possible (yet).
- **IARC-category**:
  - ➢ Group 1: The agent is carcinogenic to humans.
  - ➢ Group 2A: The agent is probably carcinogenic to humans.
  - ➢ Group 2B: The agent is possibly carcinogenic to humans.
  - ➢ Group 3: The agent is not classifiable as to its carcinogenicity to humans.
  - ➢ Group 4: The agent is probably not carcinogenic to humans.
- **CEC**:
  - ➢ C = corrosive
  - ➢ E = explosive
  - ➢ F = highly flammable
  - ➢ F+ = extremely flammable

- O = oxidising
- T = toxic
- T+ = very toxic
- Xi = irritant
- Xn = harmful

RISK AND SAFETY CLASSIFICATION

Risk classification

- R1 Explosive when dry
- R2 Risk of explosion by shock, friction, fire or other sources of ignition
- R3 Extreme risk of explosion by shock, friction, fire or other source of ignition
- R4 Forms very sensitive explosive metallic compounds
- R5 Heating may cause an explosion
- R6 Explosive with or without contact with air
- R7 May cause fire
- R8 Contact with combustible material may cause fire
- R9 Explosive when mixed with combustible material
- R10 Flammable
- R11 Highly flammable
- R12 Extremely flammable

- R14 Reacts violently with water
- R15 Contact with water liberates extremely flammable gases
- R16 Explosive when mixed with oxidising substances
- R17 Spontaneously flammable in air
- R18 In use, may form flammable/explosive vapour-air mixture
- R19 May form explosive peroxides
- R20 Harmful by inhalation
- R21 Harmful in contact with skin
- R22 Harmful if swallowed
- R23 Toxic by inhalation
- R24 Toxic in contact with skin
- R25 Toxic if swallowed
- R26 Very toxic by inhalation
- R27 Very toxic in contact with skin
- R28 Very toxic if swallowed
- R29 Contact with water liberates toxic gas
- R30 Can become highly flammable in use
- R31 Contact with acids liberates toxic gas
- R32 Contact with acids liberates very toxic gas
- R33 Danger of cumulative effects
- R34 Causes burns
- R35 Causes severe burns
- R36 Irritating to eyes
- R37 Irritating to respiratory system
- R38 Irritating to skin
- R39 Danger of very serious irreversible effects
- R40 Limited evidence of a carcinogenic effect
- R41 Risk of serious damage to eyes
- R42 May cause sensitisation by inhalation
- R43 May cause sensitisation by skin contact

- R44 Risk of explosion if heated under confinement
- R45 May cause cancer
- R46 May cause heritable genetic damage

- R48 Danger of serious damage to health by prolonged exposure
- R49 May cause cancer by inhalation
- R50 Very toxic to aquatic organisms
- R51 Toxic to aquatic organisms
- R52 Harmful to aquatic organisms
- R53 May cause long-term adverse effects in the aquatic environment
- R54 Toxic to flora
- R55 Toxic to fauna
- R56 Toxic to soil organisms
- R57 Toxic to bees
- R58 May cause long-term adverse effects in the environment
- R59 Dangerous for the ozone layer.
- R60 May impair fertility
- R61 May cause harm to the unborn child
- R62 Possible risk of impaired fertility
- R63 Possible risk of harm to the unborn child.
- R64 May cause harm to breastfed babies
- R65 Harmful: may cause lung damage if swallowed
- R66 Repeated exposure may cause skin dryness or cracking
- R67 Vapours may cause drowsiness and dizziness
- R68 Possible risk of irreversible effects

Safety classification
- S1 Keep locked up
- S2 Keep out of the reach of children
- S3 Keep in a cool place
- S4 Keep away from living quarters
- S5 Keep contents under ... (appropriate liquid to be specified by the manufacturer)
- S6 Keep under ... (inert gas to be specified by the manufacturer)
- S7 Keep container tightly closed
- S8 Keep container dry
- S9 Keep container in a well-ventilated place

- S12 Do not keep the container sealed
- S13 Keep away from food, drink and animal feedingstuffs
- S14 Keep away from ... (incompatible materials to be indicated by the manufacturer)
- S15 Keep away from heat
- S16 Keep away from sources of ignition - No smoking
- S17 Keep away from combustible material
- S18 Handle and open container with care

- S20 When using do not eat or drink
- S21 When using do not smoke

- S22 Do not breathe dust
- S23 Do not breathe gas/fumes/vapour/spray (appropriate wording to be specified by the manufacturer)
- S24 Avoid contact with skin
- S25 Avoid contact with eyes
- S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
- S27 Take off immediately all contaminated clothing.
- S28 After contact with skin, wash immediately with plenty of ... (to be specified by the manufacturer).
- S29 Do not empty into drains
- S30 Never add water to this product

- S33 Take precautionary measures against static discharges

- S35 This material and its container must be disposed of in a safe way.
- S36 Wear suitable protective clothing
- S37 Wear suitable gloves
- S38 In case of insufficient ventilation, wear suitable respiratory equipment
- S39 Wear eye/face protection
- S40 To clean the floor and all objects contaminated by this material use ... (to be specified by the manufacturer)
- S41 In case of fire and/or explosion do not breathe fumes
- S42 During fumigation/spraying wear suitable respiratory equipment (appropriate wording to be specified by the manufacturer)
- S43 In case of fire use ... (indicate in the space the precise type of fire-fighting equipment. If water increases the risk add: Never use water).

- S45 In case of accident or if you feel unwell seek medical advice immediately (show the label where possible).
- S46 If swallowed, seek medical advice immediately and show this container or label.
- S47 Keep at temperature not exceeding ... °C (to be specified by the manufacturer).
- S48 Keep wetted with .... (appropriate material to be specified by the manufacturer).
- S49 Keep only in the original container.
- S50 Do not mix with ... (to be specified by the manufacturer)
- S51 Use only in well-ventilated areas
- S52 Not recommended for interior use on large surface areas
- S53 Avoid exposure - Obtain special instructions before use

- S56 Dispose of this material and its container to hazardous or special waste collection point.
- S57 Use appropriate containment to avoid environmental contamination

- S59 Refer to manufacturer for information on recovery/recycling
- S60 This material and its container must be disposed of as hazardous waste
- S61 Avoid release to the environment. Refer to special instructions/Safety data sheet
- S62 If swallowed, do not induce vomiting: seek medical advice immediately and show this container or label.

- S63 In case of accident by inhalation: remove casualty to fresh air and keep at rest.
- S64 If swallowed, rinse mouth with water, (only if the person is conscious).