



VKM Report 2018: 09

Hazard assessment of glycyrrhizic acid from liquorice

Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food and Environment

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Norwegian Scientific Committee for Food and Environment (VKM)

Po 4404 Nydalen

N – 0403 Oslo

Norway

Phone: +47 21 62 28 00

Email: vkm@vkm.no

vkm.no

vkm.no/English

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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

Members of the project group that contributed to the drafting of the opinion (in alphabetical order after chair of the project group):

Inger-Lise Steffensen – Chair of the project group and chair of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health

Ragna Bogen Hetland – Member of the project group and vice-chair of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health

Gro Haarklou Mathisen – Project leader from the VKM secretariat. Affiliation: VKM

Members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics that contributed to the assessment and approval of the opinion:

In addition to Inger-Lise Steffensen and Ragna Bogen Hetland, these were (in alphabetical order):

Ellen Bruzell – Member of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Affiliation: 1) VKM; 2) Nordic Institute of Dental Materials

Berit Granum – Member of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health

Jens Rohloff - Member of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Affiliation: 1) VKM; 2) Norwegian University of Science and Technology

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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 18 α - and 18 β -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 tumorigenicity. 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 embryoletality. 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 glycyrrhetic 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 11 β -hydroxysteroid dehydrogenase type 2 (11 β -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 11 β -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 11 β -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 11 β -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 18 α - og 18 β -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 glykyrrhizinsyre 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.

Glykyrrhizinsyre har en hemmende effekt på 11 β -hydroksysteroid dehydrogenase type 2 (11 β -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 gravidenes 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 11 β -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 puberteten, kortisolnivåer, kognitive ferdigheter, psykiatriske symptomer etc. observert 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 11 β -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 11 β -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	adrenocorticotrophic 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
11 β -HSD	11 β -hydroxysteroid dehydrogenase
bw	body weight
CI	confidence interval
CRH	corticotropin releasing hormone
DOCA	desoxycorticosterone acetate
DPyr	deoxy pyridinoline
EFSA	European Food Safety Authority
EMA	European Medicines Agency
FAO	Food and Agriculture Organization of the United Nations
GA	glycyrrhetic acid
GC	glucocorticoid
GE	glycyrrhetic 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).

Glycyrrhetic acid: Synonym for glycyrrhetic acid.

Glycyrrhizates: 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 11 β -hydroxysteroid dehydrogenase type 2 enzyme (11 β -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 glycyrrhethinic 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 glycyrrhizic 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 glycyrrhizic 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. Glycyrrhizic 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 glycyrrhizic acid and its monoammonium salt as a natural constituent of liquorice (licorice) and its use as a flavouring substance in various food products. Glycyrrhizic acid and its monoammonium salt had not been evaluated previously by the Committee.

The Committee concluded that the safety evaluation of glycyrrhizic 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 (hypermineralocorticoidism 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.

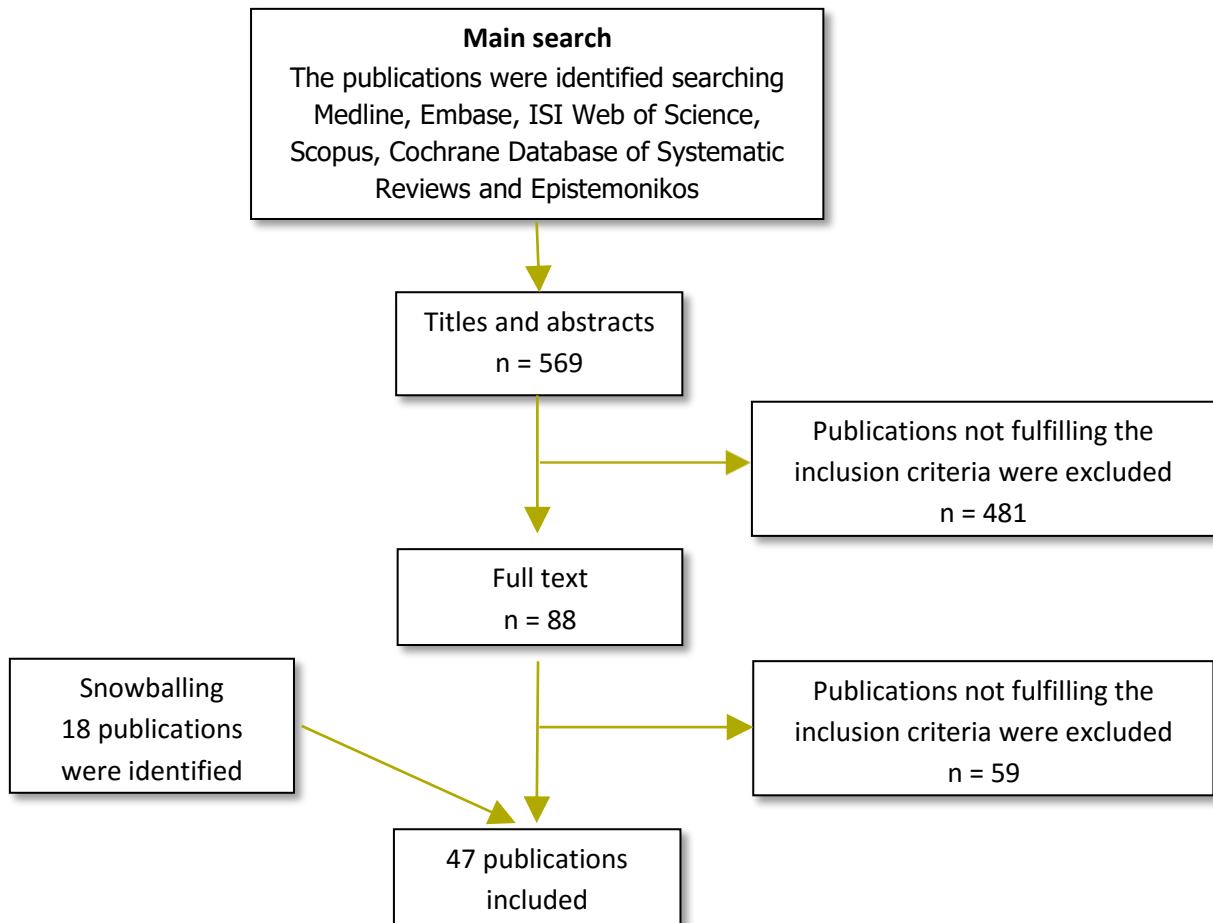


Figure 2.1.2-1: Flowchart for the literature search for glycyrrhizin and the subsequent publication selection of papers included in the hazard identification and characterization section of this assessment.

2.2 General information

2.2.1 Chemistry

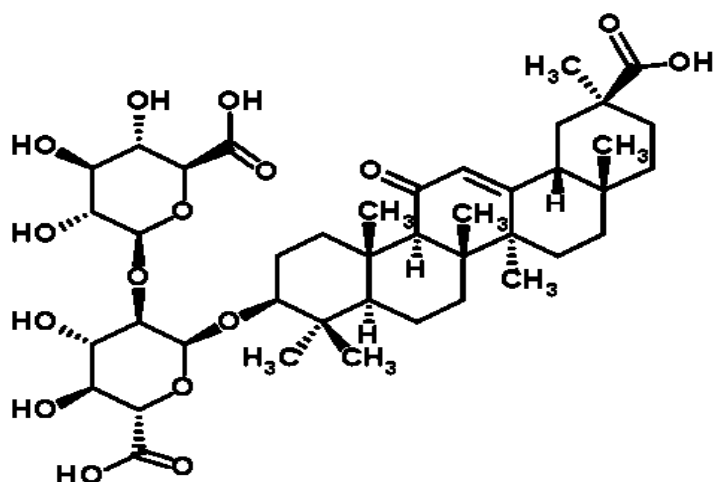


Figure 2.2.1-1. The structural formula of glycyrrhizic acid (<http://www.chemspider.com/ImageView.aspx?id=14263>).

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 than 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 18 β -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 μ g/ml was reached after 10 h. A second and a third peak of approximately 0.2 and 0.1 μ g/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 LD₅₀ values of extracts of *Glycyrrhiza* sp. were >7.5 g/kg bw for both sexes, whereas the LD₅₀ 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 LD₅₀ 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 LD₅₀ 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 adrenocorticotrophic 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 <i>Glycyrrhiza glabra</i> (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 \pm 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 \pm 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 embryoletality; 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 glycyrrhizic acid	No GE in the diet	Reduction/loss of pulmonary 11 β -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 CrI: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 \pm 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 sternbrae 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 \pm 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 embryoletality; 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, glycyrrhetic acid (GE), in pregnant Wistar rats ($n = \sim 6$) 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 11 β -HSD activity in the late fetal lung; levels of plasma 11-dehydrocorticosterone were also high. Reduction/loss of pulmonary 11 β -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 11 β -HSD for lung maturation was confirmed with the same results found in lungs from 11 β -HSD1 $-/-$ knockout mice (Hundertmark et al. (2002b)). The authors concluded that the data emphasized the importance of 11 β -HSD1 in amplifying key GC-dependent maturational processes in the late fetal lung. Whether a high intake of liquorice, leading to inhibition of 11 β -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 F₀ generation was 25 mg/kg bw, and the NOELs for the effects on the parental F₁ generation and on the development of the offspring (F₂ 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 glycyrrhetic 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 (F₁ and F₂ 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 11 β -hydroxysteroid dehydrogenase (11 β -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 glycyrrhetic 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		
<p>Randomized double-blind intervention (pilot) study</p> <p>van Gelderen et al. (2000)</p>	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)	<p>Symptoms similar to apparent mineralocorticoid excess (AME).</p> <p>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.</p>
<p>Randomized double-blind intervention study</p> <p>van Gelderen et al. (2000)</p>	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	<p>Symptoms similar to apparent mineralocorticoid excess (AME).</p> <p>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</p>

Study design/reference	Participant characteristics	Country	Treatment and number in experimental groups		Dose(s)	Main endpoint(s), observed effects
			Glycyrrhizin or carbenoxolone	Control		
						<p>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</p>

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, <i>n</i> = 751), moderate (250–499 mg/week, <i>n</i> = 145) and heavy (≥500 mg/week, <i>n</i> = 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; <i>P</i> = 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, <i>n</i> = 751), moderate (250–499 mg/week, <i>n</i> = 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 11 β -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 \pm 1.3 years (mean \pm 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 \pm 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 the 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 variable, 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 were 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 HPA 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 11 β -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 11 β -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 glycyrrhetic 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 11 β -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 11 β -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 11 β -HSD2 isozyme were demonstrated in rat and human osteosarcoma cell lines, and expression in osteoblasts of

normal human fetal bone, whereas the 11 β -HSD1 isozyme was expressed in human osteoblast cultures (Condon et al., 1998). Further, 11 β -HSD expression was characterized in fresh normal adult human bone, where both 11 β -dehydrogenase (cortisol-to-cortisone conversion) and reductase (cortisone-to-cortisol conversion) activities were demonstrated (Cooper et al., 2000). Carbenoxolone inhibits both 11 β -dehydrogenase and 11 β -oxoreductase, unlike liquorice, which inhibits only 11 β -dehydrogenase (Stewart et al., 1990).

The effect of 11 β -HSD on bone metabolism was also assessed *in vivo* using the synthetic liquorice derivative carbenoxolone in eight normal male volunteers aged 21.5 \pm 1.3 years (mean \pm 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 \pm 1.2 μ mol/L, indicating activity of 11 β -HSD1. The 11 β -HSD1 isozyme was expressed in cells of the osteoblast lineage and in osteoclasts, whereas the 11 β -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 \pm 0.55 (mean \pm SE), for DPyr/creatinine -0.4 \pm 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 11 β -HSD isozyme expression and activity may explain individual variation in susceptibility to glucocorticoid-induced osteoporosis.

Comments from VKM: Since 11 β -HSD isozyme expression was demonstrated in fetal bone, and glycyrrhizin in liquorice has the same inhibitory effect on the 11 β -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 11 β -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 11 β -HSD activity in microsomal preparations. Placental 11 β -HSD was inhibited by β -glycyrrhetic 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 glycyrrhetic 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 glycyrrhetic 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 μ M, 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 16 β -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 11 β -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 11 β -HSD2 activity. Heterozygotes appear clinically normal, although experimental studies have suggested that heteromeric 11 β -HSD2 formation may compromise overall 11 β -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 11 β -HSD, which interconverts cortisol to cortisone. The study by McCalla et al. (1998) aimed to determine the role of human placental 11 β -HSD activity in preeclampsia, which is a hypertensive disorder in pregnancy. The 11 β -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. 11 β -Dehydrogenase (oxidation activity of 11 β -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 11 β -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 11 β -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 11 β -hydroxysteroid dehydrogenase (11 β -HSD) isozymes, which catalyses the conversion of glucocorticoids, but not the mineralocorticoid aldosterone, to inactive metabolites. More specifically, it interconverts active cortisol and inactive cortisone, and regulates the access of cortisol to both MR and GR in humans. 11 β -Hydroxysteroid dehydrogenase is an enzyme complex consisting of 11 β -dehydrogenase activity (catalysing cortisol to cortisone (inactive form)) and 11 β -oxoreductase activity (catalysing cortisone to cortisol (active form)). Two isoforms of 11 β -HSD have been described. The type 1 NAD(H)-dependent dehydrogenase/oxoreductase has bi-directional activity, however, *in vivo* the enzyme predominantly functions as a low affinity (μ M) 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 selectivity (Hammer and Stewart, 2006; Stewart et al., 1994). 11 β -HSD1 is expressed in liver, adipose tissues, lung, gonads and brain. 11 β -HSD2 is expressed in kidney, colon, salivary gland and placenta. 11 β -HSD2, but not 11 β -HSD1, is found in fetal tissues, at least at midgestation (Stewart et al., 1990). The widespread distribution of 11 β -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 11 β -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 11 β -HSD2.

Thus, during pregnancy, the fetus is protected from high glucocorticoid levels in the mother by the action of the placental barrier enzyme 11 β -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 11 β -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).

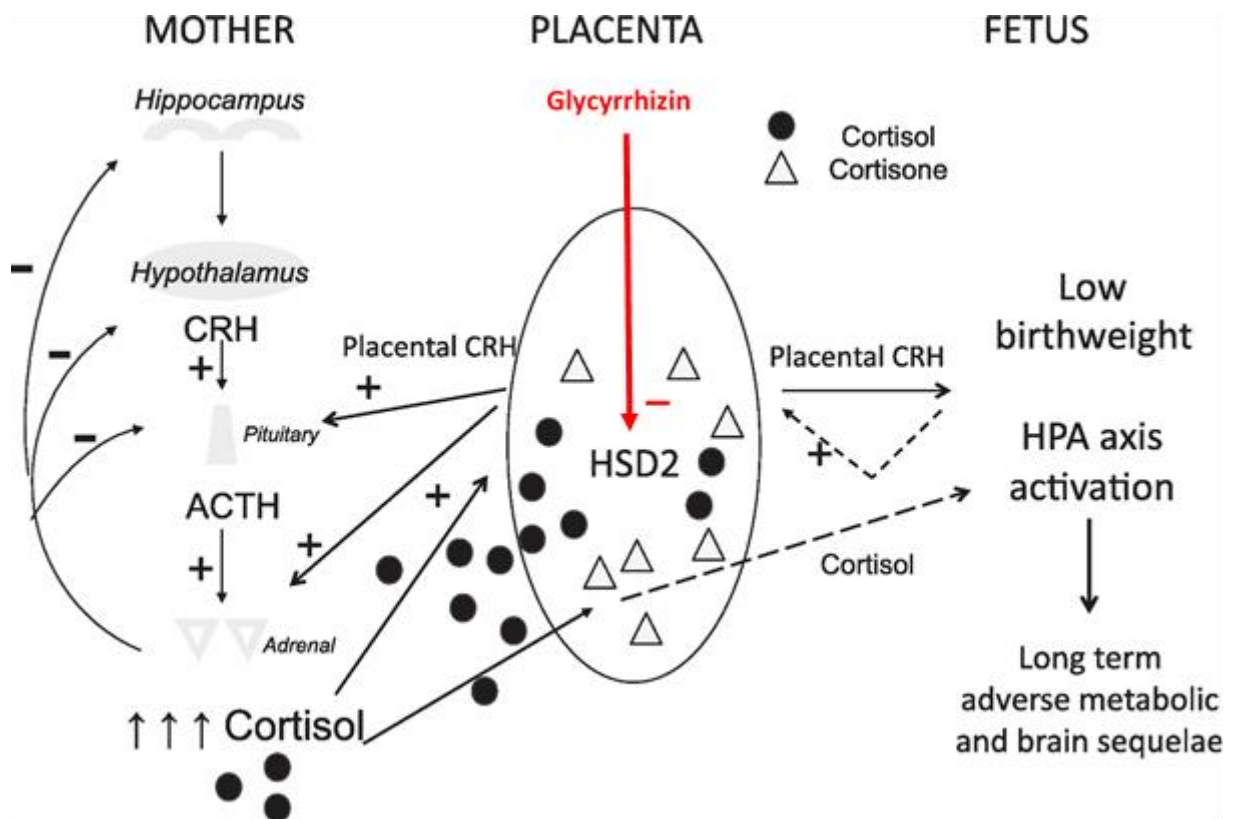


Figure 2.4.8-1. Glucocorticoid signalling between mother, placenta and fetus. The figure shows interaction between maternal, placental and fetal compartments during pregnancy leading to overexposure of the developing fetus to glucocorticoids. Activation of the maternal hypothalamic–pituitary–adrenal (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 11 β 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 11 β -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 11 β -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 glycyrrhetic acid and carbenoxolone may inhibit both human 11 β -HSD1 and 11 β -HSD2 (Ma et al., 2011). Both substances are more potent inhibitors of 11 β -HSD2 than 11 β -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 F₁ and F₂ 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 embryoletality (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 glycyrrhetic 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 11 β -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 11 β -HSD1 activity in rats treated with glycyrrhetic acid substantially impaired fetal lung maturation (Hundertmark 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 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, 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 11 β -HSD2 enzyme, such as phthalates, organotins and dithiocarbamates (Odermatt and Gummy, 2008; Ma et al., 2011).

Glucocorticoid action is dependent upon the expression of 11 β -hydroxysteroid dehydrogenase (11 β -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 11 β -HSD have been described. The widespread distribution of 11 β -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 11 β -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 11 β -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 11 β -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 glycyrrhetic 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 11 β -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 11 β -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 11 β -HSD2 dehydrogenase activity, inactivating cortisol, between human placentas (Benediktsson et al., 1997).

Human *ex vivo* studies showed that human placenta exhibits high levels of 11 β -HSD oxidative activity, which was inhibited by β -glycyrrhetic acid (Blum et al., 1995). An *in vitro* study showed that glycyrrhetic 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 11 β -HSD2 gene, may be susceptible to liquorice. If untreated, AME may lead to damage to various organs (Hammer and Stewart, 2006). Decreased 11 β -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 11 β -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 18 α -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 11 β -hydroxysteroid dehydrogenase type 2 (11 β -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 R  ikk  nen 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 (R  ikk  nen 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 11 β -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 11 β -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 11 β -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 11 β -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 18 α -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 11 β -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 11 β -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 11 β -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 11 β -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 glycyrrhizate. *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 glycyrrhetic 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 næringsmiddelingsredienser 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) 11 β -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., Vyhňálek J. (2007) Determination of glycyrrhizin in liqueurs by on-line coupled capillary isotachopheresis 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 11 β -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 11 β -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., Komsu 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 glycyrrhizic 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 11 β -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 11 β -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) Glycyrrhetic 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.

2	(Glycyrrhiza or Glycyrrhizic or Glycyrrhetic 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 succan 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 glycyrrhetic or glycyrrhizic or glycyrrhetic or glycyrrhizin or glycyrrhizic or glycyrrhizic or glycyrrhizic 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	<u>226</u>	#2 AND #1 <i>Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years</i>
# 2	<u>832,900</u>	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") <i>Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years</i>
# 1	<u>7,903</u>	TOPIC: ("Glycyrrhiza" or "Glycyrrhizic" or "Glycyrrhetic" 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 "succan" 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 "glycyrrhetic" or "glycyrrhizic"

	or "glycerrhetic" or "glycyrretin" or "glycerrhizic" or "glycyrraizinic" or "glycyrhizine" or "radix glycyrrhizae") <i>Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years</i>
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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 glycyrretin OR glycerrhizic OR glycyrraizinic OR glycyrhizine OR "radix glycyrrhizae")	234 document results
10	TITLE (glycerrhitinic OR glycerrhetic OR glycyrretin OR glycerrhizic OR glycyrraizinic OR glycyrhizine OR "radix glycyrrhizae")	100 document results
9	ABS (ketoglycyrretic OR "gan zao*" OR ganzao* OR gancao* OR kanzo OR reglisse OR succin OR glycyram OR glyciram OR rhenic 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 glycerrhetic)	78 document results
8	TITLE (ketoglycyrretic OR "gan zao*" OR ganzao* OR gancao* OR kanzo OR reglisse OR succin OR glycyram OR glyciram OR rhenic 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 glycerrhetic)	6 document results
7	ABS ("Olean-12-En-29-Oic Acid" OR glycyrretyl 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 monoglucuronylglycyrretic OR 3mga)	3,905 document results
6	TITLE ("Olean-12-En-29-Oic Acid" OR glycyrretyl 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 monoglucuronylglycyrretic OR 3mga)	2,172 document results
5	ABS (glycyrrhiza OR glycyrrhizic OR glycyrretinic OR glycyrretin OR glycyrrhizin OR glycyrrhetic OR glycyrrhizinic OR glycyrrhizone OR glycyrrhetate OR glycyrrhetate OR glycyrrhetinyl OR glycyrrhetinyl)	6,052 document results

	yrhizate OR glycyrrhizinate OR glycopyranosiduronic OR glucopyranosiduronic)	
4	TITLE (glycyrrhiza OR glycyrrhizic OR glycyrrhetic 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 ^"Glycyrrhetic Acid"]	56
#4	(Glycyrrhiza or Glycyrrhizic or Glycyrrhetic 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 suncan or Glycyram or Glyciram or "Rhetic Acid" or "Uralenic Acid" or Enoxolone or Arthrodont or "Po 12" or "gm 1292" or "gm 1658" or phytosome or biosone or glycyrrhetic or glycyrrhizic or glycyrrhetic or	480

	glycyrrhizin or glycyrrhizic or glycyrrhizonic or glycyrrhizine or "radix glycyrrhizae"):ti,ab	
#5	(Glycyrrhiza or Glycyrrhizic or Glycyrrhizonic or glycyrrhizin or Glycyrrhizic or Glycyrrhizonic or Glycyrrhizinate or Glycyrrhizate or Glycyrrhizyl 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 succan or Glycyram or Glyciram or "Rhetinic Acid" or "Uralenic Acid" or Enoxolone or Arthrodon or "Po 12" or "gm 1292" or "gm 1658" or phytosome or biosone or glycyrrhizic or glycyrrhizonic or glycyrrhetic or glycyrrhizin or glycyrrhizic or glycyrrhizonic or glycyrrhizine or "radix glycyrrhizae") in Other Reviews, Technology Assessments and Economic Evaluations	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 Glycyrrhizonic OR glycyrrhizin OR Glycyrrhizic OR Glycyrrhizonic OR Glycyrrhizinate OR Glycyrrhizate OR Glycyrrhizyl 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 "Rhetic Acid" OR "Uralenic Acid" OR Enoxolone OR Arthrodon
OR "Po 12" OR "gm 1292" OR "gm 1658" OR phytosome OR biosone OR glycerrhetic 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)