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Opinion of the Scientific Committee on Food on Teucrin A, major component of hydroalcoholic extracts of *Teucrium chamaedrys* (wild germander)

(expressed on 5 March 2003)

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Terms of reference

The Committee is asked to advise the Commission on substances used as flavouring substances or present in flavourings or present in other food ingredients with flavouring properties for which existing toxicological data indicate that restrictions of use or presence might be necessary to ensure safety for human health.

In particular, the Committee is asked to advise the Commission on the implications for human health of the presence of Teucrin A in the diet.

Introduction

Wild germander (*Teucrium chamaedrys* L.) is a herbal plant of the Labiatae family that has been used since antiquity as folk medicine for its choleretic and antiseptic properties. Hydroalcoholic extracts of the plant are currently used in the preparation of flavoured wines, bitters and liqueurs. Teucrin A is the major component of the diterpenoid fraction of these extracts.

Previous evaluations

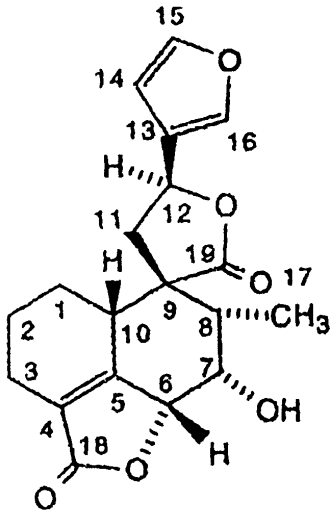
In 1999, the Council of Europe Committee of Experts on Flavouring Substances (CEFS) recommended a TDI of 0.002 mg Teucrin A/kg bw/day (corresponding to 0.12 mg/kg day for a 60 kg person) by applying a safety factor of 200 to a no-observed-adverse-effect-level (NOAEL) of 0.4 mg/kg bw/day. The NOAEL was derived for the hepatotoxicity observed in a 13 week oral study in rats treated with a hydroalcoholic extract of *T. chamaedrys* L. containing 7130 mg/L of Teucrin A and for which a NOAEL of 56 mg/kg bw/day of extract was derived (Council of Europe, 1999).
In addition, the CEFS recommended that Teucrin A should not be present in food and beverages, with the exception of alcoholic beverages, for which a limit of 0,05 mg/kg for every 1% of alcohol by volume was proposed (Council of Europe, 1999).
These recommendations were confirmed by CEFS at its 49th meeting (Council of Europe, 2001).

Current regulatory status

FDA (USA)-121.1163: in alcoholic beverages only.
No specific European Community regulations exist for Teucrin A in food.
In France all preparations containing germander as herbal medicine were prohibited (CFR 172-510, 1992). An analogous decision was taken by Italy (Ministero della Sanità, G.U. 181, 1996). Also Belgium prohibited the use of "*Teucrium chamaedrys*" and "*Teucrium polium* L" or their preparations in foodstuffs (Arrêté Royale du 29 Août 1997).

Chemical characterisation

Name:	Teucrin A
Systematic name:	Spiro(furan-3(2H),6'-(6H)naphtho(1,8-bc)furan)-2,2'(4'H)-dione,5-(3-furanyl)-3',4,5,5',5'a,7',8',8'a-octahydro-8'-hydroxy-7'-methyl-, (5aS-(5'alpha,6'beta(R*),7'beta,8'beta,8'alpha))-
Synonyms:	(5aS-(5'alpha,6'beta(R*),7'beta,8'beta,8'alpha))-5-(3-furanyl) 3',4,5,5',5'a,7',8',8'a-octahydro-8'-hydroxy-7'-methylspiro(furan-3(2H),6'-(6H)naphtho(1,8-bc)furan)-2,2'(4'H)-dione
CAS No:	12798-51-5 (*Teucrin A)*; 84929-80-6 (*Teucrium chamaedrys* extract)
MW:	344
Molecular formula:	$C_{19}H_{20}O_6$
Structure:	



Aerial parts of *T. chamaedrys* have been investigated for their chemical constituents. These include saponins, glycosides, flavonoids and a number of furan-containing neo-clerodane diterpenoids, the most abundant of which is Teucrin A (Popa and Reinbold, 1972; Savona *et*

al., 1982; Rodriguez *et al.*, 1984; Piozzi *et al.*, 1987). Structure and stereochemistry of Teucrin A have been described by Popa *et al.* (1973).
According to Galli (2001), the chemical composition of the diterpenoid fraction determined in the total hydroalcoholic extract (70% ethanol in water) of *T. chamaedrys* is: Teucrin A (70%), Teuflin (19.1%), Chamaedroxide (3.4%), Teucroxide (3.4%), Teugin (2.5%) and Dihydroteugin (2.6%). Teucrin A (not commercially available) has been extracted from germander and purified by means of chromatographic techniques. The average content of Teucrin A determined in 18 samples of hydroalcoholic infusions of *T. chamaedrys* is: 2338 mg/L (S.D. 740 mg/L; C.V. 32%; Max. 3445 mg/L; Min. 999 mg/L). A concentrated hydroalcoholic extract containing 7130 mg Teucrin A/L was used for the sub-acute and sub-chronic rat studies; a hydroalcoholic extract containing Teucrin A at a concentration of 1200 mg/L was used for the genotoxicity tests.

Exposure assessment

As reported in the datasheet of CEFS (Council of Europe, 2001), *T. chamaedrys* extracts are only known to be used in flavoured wines, bitters and liqueurs; no other food uses are known.
According to CEFS (1999, 2001), the levels of Teucrin A found in alcoholic beverages range from 1 to 5 mg/L. This is in agreement with findings of Galli (2001) who reported a level of 6.1±0.8 mg/L (n=10) for Teucrin A in bitter alcoholic beverages prepared with herbs containing *T. chamaedrys*.
According to the 1986/87 Dietary and Nutritional Survey of British Adults, the daily consumption of vermouths and liqueurs by the mean and by the 97.5th percentile consumer is 18.5 g/day and 80.4 g/day, respectively (Gregory *et al.,* 1990). If it were assumed that these two kinds of beverages each contained Teucrin A at a level of 5 mg/L, which is the upper value of the concentration range reported by CEFS, the mean and 97.5th percentile daily intakes of Teucrin A would be 0.001 and 0.005 mg/kg bw, respectively.

Hazard identification and characterisation

Standard toxicological *in vivo* studies (sub-acute toxicity, sub-chronic toxicity) as well as *in vitro* and *in vivo* genotoxicity assays have been carried out with hydroalcoholic extracts with known Teucrin A contents. Short-term hepatotoxicity studies in the mouse have been performed on germander, on a germander tea lyophilisate, on a diterpenoid fraction and also on Teucrin A. Other *in vitro* mechanistic studies are also available.

Metabolism, toxicokinetics

The neo-clerodane diterpenoids are transformed by cytochrome P450 enzymes (specifically CYP3A) into unidentified hepatotoxic metabolites, probably epoxides (Lekehal *et al.*, 1996; Loeper *et al.*, 1994)

Acute toxicity

No LD_{50} values are available.

A single intragastric administration of a germander tea lyophilisate (1250 mg/kg bw) or the furan neo-clerodane diterpenoid fraction (125 mg/kg bw) produced similar midzonal liver cell necrosis at 24 hours in mice (Loeper *et al.*, 1994). Toxicity was prevented by pre-treatment with troleandomycin (specific inhibitor of CYP3A) and enhanced by dexamethasone or clotrimazole (two inducers of CYP3A). Toxicity was reduced by pre-treatment with hydroxyanisole or clofibrate (inducers of microsomal epoxide hydrolase) and markedly increased by phorone-induced glutathione depletion. Review of liver slices from mice with germander-induced hepatitis (Loeper *et al.*, 1994) showed several apoptotic bodies, in addition to the predominant liver cell necrosis.

In another *in vivo* study by Kouzi *et al.* (1994) the hepatotoxicity of germander and Teucrin A was investigated in male Swiss-Webster albino mice (20-25 g) treated by gavage. Single administration (2000-4000 mg/kg bw) of an ethanolic extract (dried and suspended in 0.5 mL of TWEEN 80/corn oil/saline 2:2:6) of germander to mice caused large increases of plasma alanine aminotransferases (ALT) levels as well as midzonal hepatic necrosis 24 hours after dosing. Single administration (500 mg/kg bw) of an acetone extract (dried and suspended in 0.5 mL of TWEEN 80/corn oil/saline 2:2:6) caused similar effects that were markedly attenuated by pre-treatment with the cytochrome P450 inhibitor piperonyl butoxide (PBO). Teucrin A caused midzonal hepatic necrosis at 150 mg/kg bw, comparable to that of the 500 mg/kg dose of the acetone extract. The extent of the necrosis also was significantly decreased by PBO. Pre-treatment of mice with buthionine sulfoximine (BSO), an inhibitor of glutathione synthesis, increased hepatotoxicity as indicated by a rise in ALT levels. These findings also suggest that bio-activation of Teucrin A by cytochromes P450 to reactive metabolite(s) is required for initiation of hepatocellular damage. Finally, tetrahydroteucrin A, an analogue of Teucrin A obtained by chemical reduction of the furan ring, was not hepatotoxic, suggesting that the furan ring moiety of the neoclerodane diterpenes is involved in the hepatotoxicity of germander.

Sub-acute toxicity (RBM SpA, 1997a)

A hydroalcoholic extract of *T. chamaedrys* containing Teucrin A in a concentration of 7130 mg/L (Galli, 2001) and diluted in de-ionised water, was given to Sprague Dawley Crl: CD (SD) BR rats by gavage, once a day, for 4 weeks at the dose levels of 14, 140 and 1400 mg/kg

bw/day, corresponding to about 0.1, 1 and 10 mg Teucrin A/kg bw/day ; control animals received the vehicle alone. The experimental groups consisted of 10 males and 10 females. Clinical observations, body weight recordings, food consumption measurements, ophthalmological examination and laboratory investigations (haematology, blood chemistry and urinalysis) were carried out during the study. At the end of the dosing period, all the animals were killed for pathology studies (gross pathology; organ weight and histology).
At 14 mg/kg bw/day (corresponding to about 0.1 mg Teucrin A/kg bw/day) no effects that could be related to the test article administration were found in either sex at the various clinical, laboratory and post-mortem investigations.
At 140 mg/kg bw/day (corresponding to about 1 mg Teucrin A/kg bw/day) the only modifications noted were a slight increase in gamma glutamyl transpeptidase serum activity in males and a slight increase in total serum protein in females.
At 1400 mg/kg bw/day (corresponding to about 10 mg Teucrin A/kg bw/day) the above-mentioned changes appeared to be slightly more evident; moreover biochemistry tests showed increases in urea and triglycerides and beta-globulins in the males. In both sexes a trend towards an increase in bilirubin was observed at urinalysis. Post-mortem examination revealed an increase in liver weight (both absolute and relative) accompanied by some histological changes which included slight or moderate hepatocellular hypertrophy with steatosis, basophilia of the cytoplasm and nuclear pleomorphism.
In addition in some males, necrosis of isolated hepatocytes, associated with subacute-inflammation and pigment-laden macrophages involving the centrilobular area, were seen, but these effects were generally slight in degree.
In conclusion, on the basis of the overall results obtained in this 4 week dose-range finding study on rats, it may be concluded that the test article *Teucrium chamaedrys* hydroalcoholic extract when given by oral route at the doses of 14 and 140 mg/kg bw/day (corresponding to about 0.1 and 1 mg Teucrin A/kg bw/day) was well tolerated and had no observable adverse toxicological effects.
At the dose of 1400 mg/kg bw/day (corresponding to about 10 mg Teucrin A/kg bw/day) the test compound induced liver modifications, generally slight, mainly of degenerative nature, associated with aspects of cellular hypertrophy. Moreover necrosis of isolated hepatocytes was found.

Sub-chronic toxicity (RBM SpA, 1997b)

The hydroalcoholic extract of *T. chamaedrys* containing 7130 mg Teucrin A/L and diluted in deionised water, was given to Sprague Dawley Crl: CD (SD) BR rats by gavage, once a day, for 13 consecutive weeks. Dose levels were 56, 280 and 1400 mg/kg bw/day, corresponding to about 0.4, 2 and 10 mg Teucrin A/kg bw/day. The experimental groups consisted of 20 males and 20 females. At the end of the treatment period, 5 animals/sex/group were maintained without further treatments and sacrificed after 6 weeks, while the others were sacrificed for the pathological examinations. After 13 weeks of administration by oral route in Sprague Dawley rats, the test article, *T. chamaedrys* hydroalcoholic extract, proved to be well tolerated at 56 mg/kg bw/day (i.e. 0.4 mg Teucrin A/kg bw/day). At this dose the compound induced

minor effects on body weight of both males and females and slight, reversible liver changes, confined to females, which mainly consisted of hepatocellular hypertrophy. This modification, in absence of other morphological findings, can be considered an adaptative metabolic, rather than toxic, change. At the dose of 280 mg/kg bw/day (i.e. 2 mg Teucrin A/kg bw/day) the tolerability of the compound was still satisfactory from the clinical point of view. However, at the morphological examination, liver changes, even though reversible, appeared more evident and diffuse than at the lowest dose, and involved both males and females; hepatocellular steatosis was also present.
At the dose of 1400 mg/kg bw/day (i.e. 10 mg Teucrin A/kg bw/day) the test article was poorly tolerated causing severe toxic effects on liver and related clinical alterations (mainly in blood chemistry parameters). The main target in males appeared to be hepatocytes (hypertrophy associated with diffuse steatosis and other degenerative changes, including necrosis), while in females the most important changes occurred in the hepatobiliary system (bile duct hyperplasia, associated with sclerosis and or dilation and interstitial fibrosis and inflammation).
Three females died towards the end of the treatment period in consequence of the above-mentioned liver changes.
Furthermore, vacuolation of the adrenal cortex was seen in males. This change, consistent with increased intracytoplasmatic accumulation of fat droplets, may, according to the authors, be interpreted as an effect of the test article on the lipid metabolism.
After 6 weeks of withdrawal, the liver changes showed a clear trend toward recovery, while the above-mentioned adrenal cortex modification had completely reverted.
Since no adverse toxicological signs occurred in this 13-week study at a dose level of 56 mg hydroalcoholic extract/kg bw/day (corresponding to 0.4 mg Teucrin A/kg bw/day), this value can be considered as a NOAEL for the hepatotoxicity of the extract.

Chronic toxicity /carcinogenicity

No data available.

Genotoxicity

A number of genotoxicity tests were carried out with a hydroalcoholic extract containing Teucrin A at a concentration of 1200 mg/L.

The test extract did not show any mutagenic activity using *Salmonella typhimurium* TA1535, TA1537, TA98, TA100 and TA102 as tester strains with and without S9 mix. The levels used, expressed as quantities of the test extract per plate, were 3, 9, 27, 80 and 240 µg/plate, respectively. The highest dose assayed corresponding to 1.7 µg Teucrin A/plate, was the highest technically applicable, corresponding to the addition to the soft agar of 0.2 mL extract/plate (RBM SpA, 1996).

The test extract also did not show any mutagenic activity when tested for the induction of 6-thioguanine resistant mutants in Chinese hamster V79 cells with and without S9 mix. The test extract concentrations assayed were: 0.01, 0.03, 0.1, 0.3, 0.9, 2.7 and 24 µg/mL. Concentrations in the range of 2.7-24 µg/mL (2.2-20 µL/mL of the hydroalcoholic extract, corresponding to 0.02–0.17 µg Teucrin A/mL) were highly cytotoxic to V79 cells. At 0.3 µg/mL without S9 mix and 0.9 µg/mL with S9 mix the plating efficiencies were reduced with 79% and 71%, respectively, in comparison with the negative control. At 0.1 µg/mL without S9 mix and 0.3 µg/mL with S9 mix the colony growth reduction was 26% and 13%, respectively. At the other concentrations assayed, no significant cytotoxic effects were detected. On the basis of these results, 0.3 and 0.9 µg/mL concentrations of the test compound were chosen as the highest doses to be tested. Three serial 1:3 dilutions of the test extract were assayed. The mutant frequency of each dose was within the confidence limit (95%) of the spontaneous mutant frequency, both with and without metabolic activation (RBM SpA, 1997c).

The clastogenicity of a hydroalcoholic extract of *T. chamaedrys* was assayed using cultured human lymphocytes from healthy donors, with and without rat liver S9 fraction as metabolising system. The test substance was assayed at concentrations of 0, 0.1, 0.3, 0.9, 2.7, 8 and 24 µg/mL. The highest level used was the highest technically applicable, corresponding to the addition of 0.2 mL of the extract to the incubation mixture of the lymphocytes. At levels of 8 and 24 µg/mL, corresponding to 0.06 and 0.17 µg Teucrin A/mL the reduction of the mitotic index was about 35% both with and without S9 fraction. The test was repeated in an independent assay. In both experiments no increase of chromosomal aberrations and no increase of polyploid or endoreduplicated cells was observed (RBM SpA, 1997d).

Male and female Sprague Dawley rats were treated by gavage with *T. chamaedrys* hydroalcoholic extract diluted in water at the single dosages of 2.5, 5 and 10 mg/kg bw, corresponding to 17.5, 35 and 70 µg Teucrin A/kg bw/day. The test substance did not induce any statistically significant increase in the frequency of micronucleated cells in the bone marrow 24 and 48 hours after the administration. Furthermore the ratio of polychromatic to normochromatic erythrocytes in both male and female animals remained unaffected, indicating that the test article is not toxic to or has not reached the bone marrow cells under the experimental conditions used (RBM SpA, 1997e). The Committee noted that the dose was selected on the basis of expected human exposure (according to the sponsor, approximately 1000 times the daily intake), rather than on the basis of MTD level.

Reproduction and developmental toxicity

No data available.

Human data

Germander, a herb used in teas or sold in tablets to facilitate weight loss, can occasionally cause acute cytolytic hepatitis (Castot and Larrey, 1992; Larrey *et al.*, 1992; Dao *et al.*, 1993). Twenty-six subjects (25 females, 1 male) developed acute hepatitis within 9 weeks after ingesting capsules containing a powdered herbal plant of *T. chamaedrys* at recommended doses of 600 to 1800 mg/day (Castot and Larrey, 1992).
In particular, 9/26 subjects developed hepatitis after taking "Arkogèlules Germandrèe", containing "270 mg par gelule" of only *T. chamaedrys* as powdered herbal plant (a minimum of 540 mg for two years). Other patients took mixtures of different herbs. Hepatitis was characterised by jaundice and high level of plasma-aminotransferases. Liver cell necrosis was the main lesion observed in biopsies. Viral hepatitis tests were negative. There was no relationship with the daily intake (number of capsules) and the durations of treatment. Recovery was obtained between 1.5 and 6 months after withdrawal. In 12 cases, re-administration was followed by prompt recurrence of hepatitis. According to the authors, liver injury induced by germander is non-specific and resembles that seen in cases of acute cytolytic hepatitis caused by viruses or drugs. The causal relationship with germander is probable, but the mechanism remains unclear. Using GC/MS, Galli (2001) measured the content of Teucrin A in the same brand of capsules with powdered herbal plant of *T. chamaedrys* ("Arkogèlules Germandrèe") that were ingested in France by the subjects who developed acute hepatitis. Each capsule contained 270 mg of the "herbal medicine". The content of Teucrin A measured in each capsule was 1 mg. A dose of 600 mg/day of *T. chamaedrys* (2 capsules), containing 2 mg of Teucrin A (equivalent to 0.03 mg/kg bw/day), was able to cause cytolytic hepatitis within 9 weeks of treatment.

There is a brief report of a fatal case of hepatitis in a 68-year old woman following ingestion of a preparation ("Tealine") containing wild germander and *Camellia thea*. The subject ingested the equivalent of 450 mg of wild germander daily for two weeks and repeated this dose after a period of 6 months and hepatitis occurred on the second occasion. It was reported that at some stage she also took dexfenfluramine but the details are unclear (Mostefa-Kara *et al.*, 1992).
Two cases of germander-induced hepatitis were reported in Canada (Laliberte and Villeneuve, 1996). In the first case, a 55-year-old-woman developed jaundice after taking germander 1600 mg daily for 6 months for hypercholesterolemia. Viral hepatitis tests were negative. Liver biopsy demonstrated necrosis, inflammation and mild portal fibrosis. When the intake of germander was discontinued, liver function tests improved steadily over the next 2 months. In the second case a 45-year-old-woman took germander (260 mg/day) with camellia and haricot bean for weight loss for 6 months before being hospitalized for jaundice and asthenia. Liver function tests were elevated. Serological tests for hepatitis A, B and C were negative. Upon stopping germander use, liver function tests improved.
Several other furan-containing compounds (e.g. furosemide, 2-substituted furans and thiophenes, menthofuran) are known to cause acute lethal cell injury, particularly in the liver (Mitchell *et al.*, 1974; McMurtry and Mitchell, 1977; Burka and Boyd, 1985; Gordon *et al.*,

1982; Carfagna *et al.*, 1993). Among them, the terpenoid menthofuran, a hepatotoxic metabolite of the monoterpene (R)-(+)- pulegone is a major constituent of the pennyroyal oil, another herbal preparation used in folk medicine as diaphoretic and emmenagogue, which caused severe hepatotoxicity and deaths in humans (Gunby, 1979; Sullivan *et al.*, 1979; Anderson *et al.*, 1996). The mechanism appears to involve, as in the case of germander, cytochrome P450-mediated formation of reactive electrophilic oxidative products of the furan ring (Thomassen *et al.*, 1992; Burka *et al.*, 1985; Ravindranath *et al.*, 1984; McClanalan *et al.*, 1989; Madyastha and Raj, 1990).

Mechanistic studies

Using isolated rat hepatocytes to determine the mechanisms of cell death, it has been shown (Fau *et al.*, 1997) that the diterpenoid fraction of germander as well as purified Teucrin A decreased cell glutathione, increased cytosolic Ca^{++}-dependent tissue transglutaminase forming a cross-linked protein scaffold, and caused internucleosomal DNA fragmentation and the ultrastructural features of apoptosis. Apoptosis was increased by CYP3A inducers (e.g. dexamethasone) and also by a diet deficient in sulfur aminoacids. Germander diterpenoids (100 µg/mL) did not significantly increase expression of p53 protein.

The observation that about half the French patients who had recovered from germander-induced cytolytic hepatitis showed a rapid recurrence after rechallenge with germander (Castot and Larrey 1992) suggested an immunologically based mechanism may be involved, at least in some subjects. De Berardinis *et al*. (2000) tested sera from four patients for anti-microsomal autoantibodies and found only antibodies directed against human microsomal epoxide hydrolase (hmEH). No such antibodies were found in control sera. Using a yeast strain expressing human CYP3A4 and hmEH it was shown that Teucrin A was metabolised to a reactive metabolite (possibly an epoxide) that formed adducts with hmEH. The antibodies in the sera from the patients also recognised this Teucrin A – hmEH adduct. The authors speculate that the direct hepatotoxicity of germander furanoditerpenoids produces apoptotic/necrotic hepatocytes and causes sensitation in susceptible subjects. Teucrin A reacts with hmEH, which was shown to be present at the outer surface of the human hepatocyte plasma membrane, forming the Teucrin A – hmEH adduct. This adduct is recognised by the anti-hmEH-adduct antibodies as well as cytotoxic T-cells starting the immune-mediated destruction of the hepatocytes, acting in concert with the direct hepatotoxicity of Teucrin A.

Summary of the hazard characterisation

Experimental studies in rats and mice show that liver is the target organ for germander (*T. chamaedrys*) toxicity and human observations appear to confirm this. The diterpenoid fraction of germander, whose major component is Teucrin A, is believed to be responsible for the hepatotoxicity observed. *In vitro* studies and an *in vivo* mouse study indicate a metabolic conversion by cytochrome P450 enzymes, specifically CYP3A, into hepatotoxic metabolites (probably epoxides) that deplete cellular thiols and induce apoptosis.

Using cultured rat hepatocytes, it has been shown that the diterpenoid fraction of germander as well as purified Teucrin A induce apoptosis mainly through Ca^{++}-mediated changes, rather than through the induction of pro-apoptotic gene products, including p53.
Immunopurified germander-induced autoantibodies were shown to recognize human Teucrin A- microsomal epoxide hydrolase adduct located in the hepatocyte cell surface. In this way germander-induced autoantibodies could cooperate in triggering an immune-mediated liver cell cytolysis.

In mice a single intragastric administration of a germander tea lyophilisate (1250 mg/kg bw) or the diterpenoid fraction (125 mg/kg bw) produced midzonal liver cell necrosis as well as apoptosis at 24 hours. The hepatotoxicity of a germander tea lyophilisate in mice was entirely accounted for by substances recovered in a fraction containing the total of the diterpenoid fraction of the germander lyophilisate.
In another study in mice Teucrin A caused hepatic midzonal necrosis at 150 mg/kg bw, comparable to that of the 500 mg/kg dose of an acetone extract of germander. In the same study administration of an ethanolic extract of germander (2000-4000) mg/kg bw to mice also caused hepatic necrosis.

In a 13-week oral toxicity study on a *T. chamaedrys* hydroalcoholic extract in rats a NOAEL of 56 mg/kg bw/day and a LOAEL of 280 mg/kg bw/day for hepatotoxicity could be derived for this extract. Using the assumption that all hepatotoxicity can be attributed to Teucrin A, the NOAEL corresponds to 0.4 mg Teucrin A/kg bw/day.

A hydroalcoholic extract of germander was not genotoxic in conventional studies at gene and chromosome levels *in vitro* and in a limited *in vivo* micronucleus assay.

No data on chronic toxicity, carcinogenicity and reproductive and developmental toxicity are available.

Several cases of hepatitis associated with germander ingestion were reported, including a fatal case. In some of these cases there was a prompt reoccurrence of hepatitis on re-exposure after a withdrawal and recovery period.

Risk characterisation and conclusions

The toxicological data presently available on Teucrin A and the hydroalcoholic extract of *T. chamaedrys* (germander) are limited and the Committee was unable to establish an ADI.
A hydroalcoholic extract of germander is currently used in alcoholic beverages. Based on a survey on the daily consumption of vermouth and flavoured wines by British adults and considering the upper level of Teucrin A of 5 mg/L in alcoholic beverages (CEFS, 1999), the mean and 97.5th percentile daily intakes of Teucrin A from this source alone would be 0.001 and 0.005 mg/kg bw/day respectively. This is only 30 and 6 times lower, respectively, than the dose of Teucrin A of 0.03 mg/kg bw/day present in the capsules of germander that caused serious hepatotoxicity (cytolytic hepatitis) in humans. In view of these narrow margins and the fact that the exposure data relate only to alcoholic beverages, the Committee considers that the use of germander preparations including Teucrin A should not be extended beyond flavoured alcoholic beverages and that more data on current intakes of Teucrin A via alcoholic beverages are needed.

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