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Directorate C - Scientific Opinions
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Scientific Committee on Food

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Opinion of the Scientific Committee on Food on Thujone

(expressed on 2 December 2002)

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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 thujone in the diet.

Introduction

Thujone occurs in nature as a mixture of α-(-)- and β-(+)-diastereoisomers, the proportions varying with the source (Micali & Lanuza, 1995; Pinto-Sconamiglio, 1967). Synthetic α-thujone is also available commercially. Data on both isomers, or mixtures, are considered in this opinion.

Previous evaluations

Council of Europe (CoE):
Thujone has been evaluated by the CoE which allocated a TDI of 10 μg/kg bw/d based on a NOEL for convulsions of 5 mg/kg bw in the female rat, dosed by gavage on 6 days per week for 14 weeks, to which a safety factor of 500 was applied (Council of Europe, 1999).

Joint FAO/WHO Committee on Food Additives (JECFA):
Thujone has not yet been evaluated by the JECFA.

Current Regulatory Status

European Union:
Annex II of Directive 88/388/EEC (EEC, 1988) on flavourings sets the following maximum levels for thujone (α and β) in foodstuffs and beverages to which flavourings or other food ingredients with flavouring properties have been added: 0.5 mg/kg in foodstuffs and beverages with the exception of

5 mg/kg in alcoholic beverages with not more than 25% volume of alcohol
10 mg/kg in alcoholic beverages with more than 25% volume of alcohol
25 mg/kg in foodstuffs containing preparations based on sage
35 mg/kg in bitters.

Thujone may not be added as such to food.

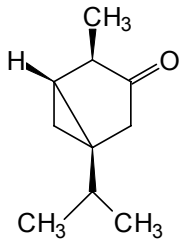
United States of America:
Thujone is not authorized for use as a flavouring substance in the USA.

Codex Alimentarius:
In 1979, the Codex Committee on Food Additives recommended restricting the use of α- and β-thujone to the following maximum levels in final products for consumption:

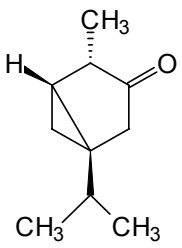
0.5 mg/kg in food and beverages
5 mg/kg in alcoholic beverages containing < 25% alcohol
10 mg/kg in alcoholic beverages containing > 25% alcohol
35 mg/kg in bitters (Codex Alimentarius Commission 1979)

Chemical characterisation

Name: Thujone
Synonyms: Thujon; α-thujone; (-)-thujone; (-)-isothujone; (1S, 4R, 5R)-(-)-3-thujanone; β-thujone; (+)-thujone
CAS Name: bicyclo(3,1,0)hexan-3-one, 4-methyl-1-(1-methylethyl)-(1S-(1-,4,5-α))
CAS Number: 546-80-5
Structure



alpha-thujone



beta-thujone

Exposure assessment

α- and β-thujone occur together in the essential oils and parts of the plants of *Artemisia absinthium* (wormwood), *Salvia officinalis* (sage), *Salvia sclarea* (clary), *Tanacetum vulgaris* (tansy) and in Juniperus and Cedris spp. The ratios of α- to β-thujone vary with source and the following have been reported:

Thujone content (%) in essential oils

Essential oil	α-thujone %	β-thujone %	Total (α + β) %	Reference
Cedar leaf	55.0	9.5	64.5	Pinto-Scognamiglio, 1967
Sage	28.3 ND*	14.5 ND*	42.5 55.2	Pinto-Scognamiglio, 1967 Farag *et al.*, 1986
Tansy	19.4	58.0	77.4	Pinto-Scognamiglio, 1967
Wormwood	0.53-1.22	17.5 - 42.3	ND*	Lawrence, 1995
Thyme	ND*	ND*	0.2	Farag *et al.*, 1986
Rosemary	ND*	ND*	4.2	Farag *et al.*, 1986

* Not determined

Estimates of intakes of thujone have been made in France and the United Kingdom. In France, the mean and 97.5th percentile daily intakes were estimated to be 15.6 and 44.3 μg/kg bw/day respectively. The intakes in the United Kingdom were estimated to be somewhat lower at 3.9 and 14.2 μg/kg bw/day respectively. Both estimates were based on the maximum limits proposed by the CoE (Council of Europe, 2000). The major dietary contribution to intake appeared to derive from sage and sage-flavoured products, and alcoholic beverages.

Hazard Identification and Characterisation

Absorption, metabolism and excretion

After oral administration to male rabbits of a mixture of α- and β-thujone (ratio 9:2) at a dose level of about 650-800 mg/kg bw, two neutral urinary metabolites were identified as 3-β-hydroxy-α-thujane and 3-β-hydroxy-β-thujane indicating that the reduction was stereospecific in spite of the different configurations of the methyl group (Ishida *et al.,* 1989).

α-thujone was rapidly metabolised by mouse liver microsomes forming 7-hydroxy-α-thujone as the major metabolite with five minor products (4-hydroxy-α-thujone, 4-hydroxy-β-thujone, two other hydroxythujones and 7,8-dehydro-α-thujone).

Incubation of α-thujone with rabbit (but not mouse) liver cytosol yielded the reduction products, thujol and neothujol, in low yield (Höld *et al.,* 2000a,b).

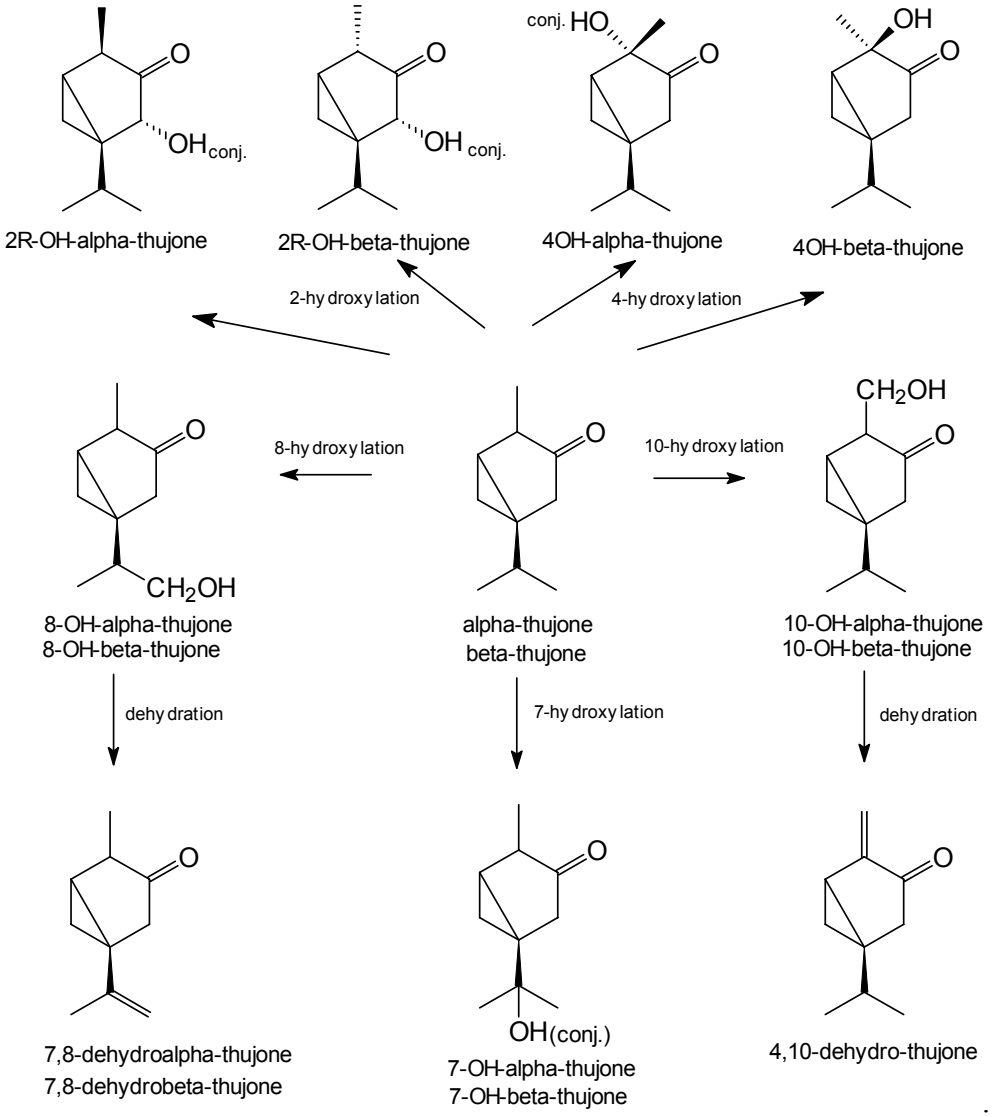
Site specificity and species differences in metabolism of the thujone diastereoisomers were observed in mouse, rat and human liver microsomes and in rats and mice *in vivo.* 2-hydroxylation was observed only in mice where the conjugated metabolite was a major urinary metabolite. 4-hydroxylation of α- and β-thujones is another major pathway and 4-hydroxy thujone is the major urinary metabolite in rats. 7-hydroxylation is another important pathway of metabolism but the conjugated product is a minor urinary metabolite except for β-thujone in the mouse. Site specificity in glucuronidation favours conjugation of the (2R)-hydroxy- and 4-hydroxythujone glucuronides rather than the other three hydroxy thujones. 7,8- and 4,10-dehydro metabolites have been identified *in vitro* and as urinary metabolites respectively (Höld *et al.,* 2001).

The oxidative metabolic pathways identified are summarised in Figure 1.

Acute toxicity

The oral LD_{50} in the rat has been reported to be 192 mg/kg bw (Margaria, 1963) and 500 mg/kg bw (NLM, 1997). Thujone is much more acutely toxic after parenteral administration and the intravenous LD_{50} in the rabbit is stated to be 0.031 mg/kg bw (NLM, 1997). The symptoms associated with acute intoxication are epileptiform convulsions with general vasodilation, hypotension, lower cardiac rhythm and increased respiratory amplitude (Pinto-Scognamiglio, 1967). In rats, i.p. injections of thujone induced electro-cortical seizures associated with myoclonic activity and the convulsant and lethal effects occurred at similar doses of 0.2 mL/kg bw.

Figure 1: Oxidative metabolism of thujone



Sub-acute/subchronic studies

Thujone was administered to rats by gavage at doses of 12.5, 25 or 50 mg/kg bw/day on five days per week for 13 weeks. There was an increased lethality of 60% in females and 37% in males at the top dose level. The NOEL for convulsions in the males was 12.5 mg/kg bw but no NOEL could be established in females in this study (Surber, 1962).

In a further study, thujone was administered to rats by gavage at doses of 0, 5, 10 or 20 mg/kg bw/day 6 times per week for 14 weeks. There were 3 deaths in females and 1 in males associated with convulsions at the top dose level. The NOEL for convulsions was reported to be 10 mg/kg bw in males and 5 mg/kg bw in females; no changes were reported in haematologic or histopathologic examinations (Margaria, 1963).

α-thujone and a mixture of α- and β-thujone have been included in the NTP testing programme and studies are currently in progress. The in-life phase of the 14-day sub-acute and 13-week sub-chronic studies have been completed and the preliminary results of the 14-day study were available for consideration by the SCF.
In the 14-day study, α-thujone was administered by gavage to B6C3F1 mice and to Fischer 344 rats at doses of 0, 1, 3, 10, 30 or 100 mg/kg bw. In mice, mortality was 4/5 males and 5/5 females in the top dose group; mortality was not increased in the lower dose groups. The increased mortality was associated with indications of neurotoxicity (hyperactivity, tremors, tonic seizures). Histological changes observed only at the top dose level included only mild renal tubular dilatation/focal degeneration, increased haematopoiesis in spleen, and bone marrow myeloid cell hyperplasia. No increased mortality occurred in male rats but there was increased mortality (3/5 animals) in females of the top dose group. As in mice, the increased death rate was associated with convulsions/seizures.
In the 14-day study on the mixture of α- and β-thujone (detailed composition not available), similar doses were administered by gavage to mice and rats of the same strains. In mice, at the top dose level there was increased mortality in males (5/5) and females (2/5) but not associated with any notable gross or histopathological causation. In rats, there was death of 1/5 males in the highest dose group but gross and histological effects were minimal.
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Genotoxicity

No relevant genotoxicity data were available.

Long-term studies for chronic toxicity/carcinogenicity

The results of the 90-day and long-term studies are not yet available, but it is scheduled for inclusion in the NTP studies.

Reproduction and developmental studies

No experimental data available.

Special studies on mechanisms of toxicity

Several studies on the mechanism of the neurotoxicity of α-thujone indicate that it is a modulator of the GABA Type A receptor (Meschler & Howlett, 1999, Höld *et al.,* 2000a). It is a rapidly acting modulator of the GABA-gated chloride channel. The effects appear to be due to the parent compound and metabolism leads to detoxification (Höld *et al.,* 2000a).

Suggestions that thujone activates the CB1 cannabinoid receptor, based on structural similarities of thujone enol to tetrahydrocannibinol, have not been supported experimentally (Meschler & Howlett, 1999).

Other biological effects

Studies on primary cultures of chick embryo liver cells indicate that thujone is porphyrogenic, leading to accumulation of copro- and protoporphyrins. It induces 5-aminolaevulinic acid synthase in this test system (Bonkovsky *et al.,* 1992)

Thujone is reported to have antinociceptive activity in mice (Rice & Wilson, 1976) and the α-isomer is more potent and toxic than the β-isomer.

Effects in humans

There are several anecdotal and case study reports of the acute effects of essential oils containing thujone causing seizures in humans (see Anderson *et al.,* 1996; Burkhard *et al.,* 1999; Haines, 1998, Steinmetz *et al.,*1980; Strang *et al.,* 1999) indicating that the animal data are of relevance to humans. In most cases, the doses are not well determined but one case was associated with about twelve drops of the essential oil of sage, which caused a generalised tonic-clonic seizure followed by a postictal coma lasting for 15 minutes. However, there are no reliable studies of the long-term effects of sub-convulsive doses either on the nervous system or on the liver. In the latter context, it has been suggested that porphyria may be a consequence of long-term ingestion of absinthe but this is conjectural (Loftus & Arnold 1991).

Conclusion and Risk Characterisation

The metabolism of α- and β-thujone occurs mainly by 7-hydroxylation with lesser amounts of other hydroxylated metabolites or thujol/neothujol which are excreted as urinary conjugates. α -thujone appears to be more neurotoxic than β-thujone and metabolism results in detoxication.

The principal manifestation of intoxication by thujone is epileptiform convulsions in animals and man. The NOEL for convulsions in subchronic toxicity studies in female rats was 5 mg/kg bw but there are no long-term or reproductive toxicity data. No relevant genotoxicity studies are available on thujone.

The Committee considered the available data inadequate to establish a TDI/ADI but noted that some of the deficiencies in the database were being addressed in ongoing NTP studies and recommended that the results of these should be reviewed when available.

In the meantime, the Committee notes that cases of human intoxication have been reported from essential oils rich in thujone and the doses involved, although poorly specified, indicate that humans are at least as sensitive as rodents to the acute neurotoxic effects. Accordingly, the Committee does not consider it appropriate to use thujone as a chemically identified flavouring substance. The Committee supports the continued application of the current upper limits to the occurrence of α- and β-thujone in foods and beverages.

In this context, the Committee noted that the consumption of as much as 1 litre of an alcoholic beverage containing 5 mg/l, the maximum permitted level of thujone in alcoholic beverages with up to 25% alcohol, would result in an intake of about 0.08 mg thujone/kg bw for a 60 kg adult. This intake is about 100 times lower than the NOEL derived from a 14 week study in rats.

References

Anderson, I.B., Mullen, W.H., Meeker, J.E., Khojasteh-Bakht, S.C., Oishi, S., Nelson, S.D., and Blanc, P.D., 1996. Pennyroyal oil toxicity: measurement of toxic metabolite levels in two cases and review of the literature. *Ann. Intern. Med.,* **124**, 726-734.

Bonkovsky, H.L., Cable, E.E., Cable J.W., Donohue, S.E., White, E.C., Greene, Y.J., Lambrecht, R.W., Srivastava, K.K., and Arnold, W.N., 1992. Porphyrogenic properties of the terpenes, camphor, pinene and thujone (With a note on historic implications for absinthe and the illness of Vincent van Gogh). *Biochem. Pharmacol.,* **43**, 2359-2368.

Burkhard, P.R., Brukhardt, K., Haenggeli, C.A., and Landis, T., 1999. Plant-induced seizures: reappearance of an old problem. *J. Neurol.,* **246**, 667-670.

Codex Alimentarius Commission, 1979. Report of the 13th Session of the Codex Committee on Food Additives. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, 11-17th September 1979, Alinorm 79/12-A

Council of Europe, 1999. Document RD 4.2/14-44. Revised Detailed Datasheet on Thujone.

Council of Europe, 2000. Document RD4.11/1-46 (revised).

EEC, 1988. Council Directive 88/388/EEC of 21 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production. *Official Journal of the European Communities*, 15.7.1988, L184/61-67.

Farag, R.S., Salem, H., Badei, A.Z.M.A., and Hassanein, D.E., 1986. Biochemical studies on the essential oils of some medicinal plants. *Fette, Seifen, Anstrichm.,* **88**, 69-72.

Haines, J.D. 1998. Absinthe – return of the green fairy. *Oklahoma State Medical Association Journal,* **91**, 406-407.

Höld, K.M., Sirisoma, N.S., Ikeda, T., Narahashi, T., and Casida, J.E., 2000a. α-thujone (the active component of absinthe); γ-aminobutyric acid type A receptor modulation and metabolic detoxification.

Höld, K.M., Sirisoma, N.S., Sparks, S.E., Quistad, G.B., and Casida, J.E., 2000b. α-thujone and absinthe: structural and metabolic aspects of neurotoxic action. TOXI 023, 219th ACS National meeting, San Francisco, CA, March 26-30th 2000.

Höld, K.M., Sirisoma, N.S., and Casida, J.E., 2001, Detoxification of α- and β-thujones (the active ingredients of absinthe): Site specificity and species differences in cytochrome P450 oxidation *in vitro* and *in vivo*. *Chem. Res. Toxicol.*, **14**, 589-595.

Ishida, T., Toyota, M., and Asakawa, Y., 1989. Terpenoid biotransformation in mammals. V. Metabolism of (+)-citronellal, (±)-7-hydroxycitronellal, citral, (-)-perillaldehyde, (-)-myrtenal, cuminaldehyde, thujone and (±)-carvone in rabbits. *Xenobiotica,* **19,** 843-855.

Lawrence, B.M. (ed.), 1995. *Natural Flavor and Fragrance Materials. "Perfumer & Flavorist". Essential Oils 1992-1994*. Carol Stream, IL., Allured Publishing Corp., pp 11-14.

Loftus, L.S., and Arnold, W.N., 1991. Vincent van Gogh's illness: acute intermittent porphyria? *Brit.Med.J.,* **303,** 1589-1591.

Margaria, R., 1963. Acute and sub-acute toxicity study on thujone. Unpublished report of the Istituto di Fisiologia, Università di Milano (cited from CoE Datasheet RD4.2/14-44, 1999).

Meschler, J.P., and Howlett, A.C., 1999. Thujone exhibits low affinity for cannabinoid receptors but fails to evoke cannabimimetic responses. *Pharmacology, Biochemistry and Behaviour,* **62**, 473-480.

Micali, G., and Lanuzza, F., 1995. HPLC determination of alpha- and beta-thujone, potentially toxic components of natural flavourings, in alcoholic beverages. *Flavour & Fragrance J.,* **10**, 329-333.

NLM, 1997. RTECS (Registry of Toxic Effects of Chemical Substances). Bethesda, MD. National Library of Medicine.

Pinto-Sconamiglio, W., 1967. Effetti del tujone sull'attività spontanea e sul comportamento condizionato del ratto. *Bull. Chim. Farm.,* **107,** 780-791.

Rice, K.C., and Wilson, R.S., 1976. (-)-3-Isothujone. A small nonnitrogenous molecule with antinociceptive activity in mice. *J .Med Chem.,* **19**, 1054-1057

Steinmetz *,* M.D.I., Tognetti*,* P., Mourgue, M., Jouglard, J.I., and Millet, Y., 1980. Toxicity of certain commercial essential oils of hyssop and oil of sage. *Plant Med. Phytother.,* **14**, 34-45

Strang, J., Arnold, W.N., and Peters, T.*,* 1999. Absinthe: what's your poison? *Brit. Med. J.,* **319**, 1590-1591.

Surber, W., 1962. Etude de toxicité sous-chronique de la thujone sur rats. Rapport Final. Institut Battelle: Genève.