SUMMARY OF DATA FOR CHEMICAL SELECTION

α-Thujone
546-80-5

BASIS OF NOMINATION TO THE CSWG

Thujone is presented to the CSWG for review because of its potential for widespread human exposure through spices, herbs, and essential oils containing thujone. This compound is also presented to the CSWG as a representative of a structurally related group of natural compounds that contain six-member rings constrained by a bridge across the 2 and 4 positions. This bridge has profound conformational effects on the cyclohexane ring that would be expected to influence toxicity of the molecule. Other ingredients in spices and herbs with this structure include sabinene, umbellulone, and thujene.

Thujone was identified through a review of direct food additives given "GRAS" status by the Food and Drug Administration (FDA). Although thujone has known toxicity that has caused it to be banned from some products, 24 direct food additives in the FDA Priority-Based Assessment of Food Additives (PAFA) database contain thujone. Tens of thousands of workers are also potentially exposed to Dalmatian sage oil and cedarleaf oil, both of which contain a high concentration of thujone.

SELECTION STATUS

ACTION BY CSWG: 12/10/97

Studies requested:

- *In vitro* cytogenetics
- *In vivo* micronucleus
- Neurotoxicity studies
- Mouse lymphoma assay (NCI Short-Term Test Program)

Priority: Moderately high

Rationale/Remarks:

- Although thujone is banned in some food additives, potential for widespread consumer and worker exposure remains.
- Confer with FDA/NCTR regarding neurotoxicity studies.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

Dr. Dan Benz, Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration (FDA) and Dr. Ed Matthews (formerly with CFSAN) provided information on α-thujone from the FDA PAFA database.

Ms. Joellen Putnam, Scientific Project Manager, Flavor and Extract Manufacturers' Association (FEMA) provided a copy of the FEMA monograph on α-thujone.

CHEMICAL IDENTIFICATION

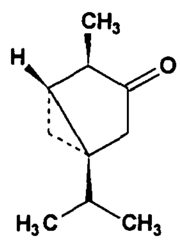
CAS Registry Number: 546-80-5

Chemical Abstract Service Name: Bicyclo(3.1.0)hexan-3-one, 4-methyl-1-(1-methylethyl)-, (1S-(1α, 4α, 5α))- (9CI)

Synonyms and Trade Names: (-)-Isothujone; (1S, 4R, 5R)-(-)-3-thujanone; thujon; thujone; (-)-thujone

Structural Class: Monoterpene ketone

Structure, Molecular Formula and Molecular Weight:



$C_{10}H_{16}O$ Mol. wt.: 152.24

Chemical and Physical Properties:

Description: Colorless or almost colorless liquid with a menthol-like odor (Albert-Puleo, 1978; Budavari, 1996)

Boiling Point: 201°C (FEMA, 1997)

Flash Point: 148°F, CC (FEMA, 1997)

Density: 0.9109 at 25°C/4°C (Budavari, 1996)

Solubility: Insoluble in water; soluble in ethanol, diethyl ether and chloroform (Albert-Puleo, 1978; Lewis, 1993)

Vapor Pressure: ~0.2 mm Hg @ 20°C (FEMA, 1997)

Technical Products and Impurities: α-Thujone is available at a purity of ~99% from Fluka. It is also available as a technical grade product containing 17.8% fenchone, 10.5% bornyl acetate, 3.1%

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camphor, 2% p-cymene and 1.2% limonene from Aldrich. Mixtures of α- and β-thujone isomers are available from TCI America and Fluka (Aldrich Chemical Company, Inc., 1996; TCI America, 1996; Fluka Chemical Corp., 1997).

EXPOSURE INFORMATION

Production and Producers: Thujone occurs in nature as a mixture of α- and β-isomers (Micali & Lanuzza, 1995). The equilibrium mixture consists of 33% α-thujone, 67% β-thujone (Budavari, 1996). α-Thujone can be isolated from natural oils with bisulfite, or via fractional distillation and crystallization, and it has been synthesized (Albert-Puleo, 1978). According to recent chemical catalogs and directories, α-thujone is manufactured and/or distributed by Aldrich and Fluka (Aldrich Chemical Company, Inc., 1996; Fluka Chemical Corp., 1997).

No data were reported for α-thujone by the US International Trade Commission (USITC) in the ten most recent volumes of *Synthetic Organic Chemicals, US Production and Sales*, for the years 1983-1993 and no other quantitative information on annual production was found in the available literature.

α-Thujone is listed in the EPA's Toxic Substances Control Act (TSCA) Inventory (NLM, 1997a).

Use Pattern: Thujone is the primary constituent of essential oils derived from a variety of plants including wormwood (*Artemisia absinthium*), mugwort (*Artemisia vulgaris*), sage (*Salvia officinalis*), clary (*Salvia sclarea*), tansy (*Tanacetum vulgare*), and yellow cedar (cedarleaf oil) (*Thuja occidentalis*) (Albert-Puleo, 1978). Thujone itself has a limited use pattern; Lewis (1993) cites a solvent use only. However, the essential oils in which thujone occurs are used in herbal medicine and as flavorings, fragrances, and rodent and mite repellants.

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Herbal medicine: Essential oils containing thujone have been used in traditional medicine for the following purposes: abortifacient, female hormone activity, and emmenagogue (sage, cedar, tansy, mugwort); digestive problems and carminative (sage, cedar, tansy, mugwort); anthelmintic (tansy, wormwood, mugwort); and corns, warts, venereal warts, acne, fever, cough, rheumatism, scurvy, dropsy (cedar) (Albert-Puleo, 1978; Millet *et al.*, 1981; Ishida *et al.*, 1989; Kim *et al.*, 1992; Hui *et al.*, 1994; Dupont, 1995; Anon., 1997a; Grieve, 1997). Cedarleaf oil has been used for decades for treating the common cold in products such as Vick's Vaporub™ (Anon., 1997b).

Flavorings: Thujone is banned as a food additive in the US and its presence in foods and beverages is regulated in several countries. However, many of the thujone-containing plant oils are used as flavoring substances in the alcoholic drink industry. Absinthe, made from wormwood, is available in Spain, Denmark and Portugal. Vermouth, chartreuse, and benedictine all contain small amounts of thujone and wormwood is popular as a flavoring for vodka in Sweden (Galli *et al.*, 1984; Baggott, 1993; Micali & Lanuzza, 1995). Sage oil is an important food flavor, especially in sausages, meats, condiments, and sauces (Rogers, 1981).

Fragrances: Cedarleaf oil is used for the scenting of various technical preparations such as shoe polish (Rogers, 1981). It is also used in the men's fragrances Ralph Lauren Safari™ and Hugo Boss (Anon., 1997b). Europeans have used the essential oil of mugwort in perfumery (Kim *et al.*, 1992).

Pest repellants: A paint containing 25-31% of cedarleaf oil has been patented as a rodent repellant (Harding, 1987). A new OFF™ product, based on cedarleaf oil and used to repel mites in closets, is soon to be marketed (Anon., 1997b).

Human Exposure: There is potential for exposures to α-thujone in occupational, consumer, and environmental settings by inhalation, ingestion, and dermal contact. Thujone (α- and/or β-) was found in 41 direct food additive substances listed in the Priority-Based Assessment of Food Additives (PAFA) database (FDA, 1994). In an updated analysis of PAFA, the number of thujone occurrences reported was reduced to 24 (FDA, 1997a). No listing was found for thujone in the National Occupational Exposure Survey (NOES).

Dalmation sage oil and cedarleaf oil contain approximately 40-60% and 60% thujone (isomer not specified), respectively (Rogers, 1981). NOES, which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 10,944 workers, including 6,777 female workers, were potentially exposed to Dalmation sage oil in the workplace and that 38,433 workers, including 10,450 female workers, were potentially exposed to cedarleaf oil in the workplace. The NOES database does not contain information on the frequency, level or duration of exposure to workers of any chemical listed therein (NLM, 1997b,c).

Environmental Occurrence: Thujone is found in nature as a constituent of essential oils where it exists as a mixture of the α- and β-isomers (Micali & Lanuzza, 1995). Thujone levels in various essential oils are shown in Table 1.

Table 1. 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 --	14.5 --	42.5 55.17	Pinto-Scognamiglio, 1967 Farag *et al.*, 1986
Tansy	19.4	58.0	77.4	Pinto-Scognamiglio, 1967
Wormwood	0.53-1.22	17.50-42.30	--	Lawrence, 1995
Thyme	--	--	0.2	Farag *et al.*, 1986
Rosemary	--	--	4.17	Farag *et al.*, 1986

Thujone has been identified as a volatile organic compound emitted by vegetation to the atmosphere (Guenther *et al.*, 1994). Arey and coworkers (1995) identified α- and β-

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thujone in the emissions of California sagebrush. α-Thujone has been reported in emissions from kitchen and garden waste (Wilkins & Larsen, 1995).

Regulatory Status: No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of α-thujone. Thujone was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a threshold limit value (TLV) or biological exposure index (BEI) are made. Thujone is potentially toxic and the presence of α- and β-thujone in food and beverages is regulated by law in several countries (Micali & Lanuzza, 1995). The use of thujone as a food additive has been banned in the United States (Rogers, 1981; Galli *et al.*, 1984). The natural flavoring substances wormwood, white cedar, and oak moss may be used in food under the condition that the finished food is thujone-free. Tansy may only be used in alcoholic beverages provided that the finished product is thujone-free, and yarrow use is specified as beverages only, thujone-free (FDA, 1997b).

In 1979, the FAO/WHO Codex Committee on Food additives restricted the use of α- and β-thujones to the following maximum levels in final products ready for consumption: 0.5 ppm in food and beverages, 10 ppm in alcoholic beverages containing more than 25% alcohol, 5 ppm in alcoholic beverages containing less than 25% alcohol, and 35 ppm in bitters (Galli *et al.*, 1984).

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EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposure to α-thujone and cancer risks in humans were identified in the available literature.

At a concentration of 4%, α-thujone was negative in dermal irritation and maximization tests in humans (FEMA, 1997).

Commercial preparations of essential oils of sage, thuja, and cedar which contain thujone have caused central nervous system effects. The following reported poisonings occurred in France and all victims had taken large doses of these thujone-containing oils or hyssop oil for their therapeutic benefits. The clinical intoxications were characterized by tonico-clonic or solely clonic convulsions. The outcomes were satisfactory and electro-encephalograms made after the crisis were all normal. The cumulative effect of treatment was shown in the case of a 50-year-old woman who took 20 drops of undiluted thuja oil twice a day for 5 days uneventfully, but 30 minutes after the tenth dose suffered a tonic seizure (Millet *et al.*, 1981).

Thujone has been suggested as the neurotoxic cause of absinthism. This syndrome characterized by addiction, hyperexcitability, and hallucinations was caused by chronic use of absinthe, an alcoholic drink made with an extract from wormwood; thujone makes up 40-90% (by weight) of the essence of wormwood. The debilitating illness suffered by Vincent Van Gogh during the last decade of his life has been linked to absinthism. The toxicity of thujone was responsible for the eventual ban of absinthe at the beginning of this century (Bonkovsky *et al.*, 1992; Baggott, 1993).

In a recent case, a male subject drank about 10 ml of essential oil of wormwood under the impression that it was in fact absinthe which he had learned of on the Internet/World Wide

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Web. He was found several hours later agitated, incoherent, and disoriented. He improved with treatment but subsequently acute renal failure developed (Reese, 1997).

Animal Data: No 2-year carcinogenicity studies of α-thujone in animals were identified in the available literature. Toxicity information was limited to acute data. Acute toxicity values are shown in Table 2.

Table 2. Acute toxicity data for a α-thujone

Route	Species	LD_{50} (mg/kg)	Reference
Oral	Rat	500	NLM, 1997a
Intravenous	Rabbit	0.031	NLM, 1997a
Subcutaneous	Mouse	2.157	NLM, 1997a
	Mouse	87.5	Rice & Wilson, 1976
	Rabbit	0.362	NLM, 1997a
Dermal	Rabbit	5000	FEMA, 1997

In rats, intraperitoneal injections of thujone induced electro-cortical seizures associated with myoclonic activity. The convulsant and lethal effects appeared together at a low dose (0.2 ml/kg) (Millet *et al.*, 1981).

Short-Term Tests: Thujone was tested at 1.5 and 3% in DMSO for its effect on the mutagenicity of aflatoxin B_1 in *Salmonella typhimurium* strain TA100. The plates treated with thujone showed evidence of colony damage which the researchers felt indicated some mutagenic activity on the part of thujone (Kim *et al.*, 1992).

Thujone concentrations of 1, 10, and 100 μg/ml were not cytotoxic to HeLa cells (Zolotovich *et al.*, 1967).

Metabolism: After oral administration of a mixture of α- and β-thujone (9:2), two neutral metabolites were identified in the urine of rabbits, β-hydroxy-α-thujane (neoisothujanol) and β-hydroxy-β-thujane (thujanol). As shown below, in the metabolism of α- and β-

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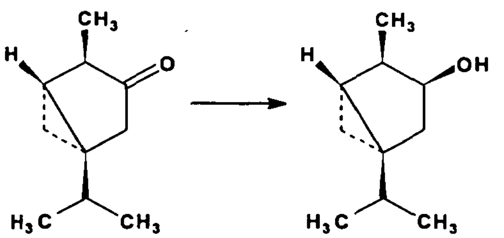
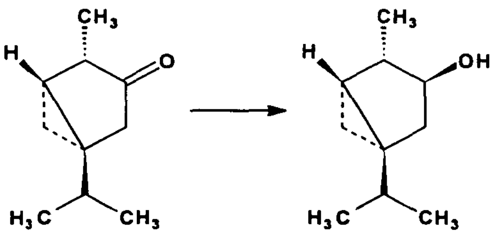
thujone, the carbonyl group was reduced to yield secondary alcohols. The configurations of these reduced hydroxyl groups in both metabolites are β-orientation in spite of the different configurations of the methyl group (Ishida *et al.*, 1989).



α-Thujone β-Hydroxy-α-thujane



β-Thujone β-Hydroxy-β-thujane

<u>Other Biological Effects</u>: Thujone (97% α-, 3% β-) produced an increase in porphyrin production in primary cultures of chick embryo liver cells. At the highest concentration tested (1 mM), thujone led to 80-100 pmol total porphyrin/mg protein (control=19-50). The accumulated porphyrins were predominantly copro- and protoporphyrins. Thujone produced inductions of 5-aminolevulinic acid synthase and benzphetamine demethylase. It also induced heme oxygenase by a heme-dependent mechanism. The results indicated that thujone would pose a threat to individuals with underlying defects in hepatic heme synthesis (Bonkovsky *et al.*, 1992).

The antinociceptive (pain-blocking) activity of α-thujone in mice was evaluated using the hot-plate and Nilsen Tests. In the hot-plate test, α-thujone (ED_{50} = 6.5 mg/kg) was found to be codeine-like and equipotent with (-)-Δ^9-tetrahydrocannabinol, the major psychoactive component of marijuana. Less antinociceptive activity was observed in the Nilsen test (ED_{50} = 14.1 mg/kg) (Rice & Wilson, 1976).

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Structure Activity Relationships: The unsymmetric bicyclic ring structure of thujone is characteristic of a group of natural products found widely in spices and herbs. Other examples include sabinene and umbellulone. None of these compounds appear to have any toxicity data relevant to a structure-activity analysis.

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