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Thujone and thujone-containing herbal medicinal and botanical products: Toxicological assessment

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ABSTRACT

Thujone, a major component of the notoriously famous absinthe drink, is neurotoxic, although the current view rather downgrades its risk to humans. In animal studies, thujone inhibits the gamma-aminobutyric acid A ($GABA_A$) receptor causing excitation and convulsions in a dose-dependent manner, although there are uncertainties about the doses required in humans. Toxicity of thujone has been extensively studied. Neurotoxicity is the principal toxic outcome in acute and chronic studies. There is some equivocal evidence of carcinogenicity in rats. Metabolism of thujone has been elucidated both in vitro and in vivo in several species and in vitro in human liver preparations. CYP2A6 is the principal metabolic enzyme, followed by CYP3A4 and, to a lesser extent, CYP2B6. CYP-associated metabolism may give rise to some potential pharmacogenetic and metabolic interaction consequences. Although the data base for determining exposure limits is of variable usefulness, the best estimates for allowable daily intakes via herbal preparations and diet are of the order of 3–7 mg/day. There are still important gaps in the knowledge required to assess thujone toxicity, the most important ones being human dose-concentration-effect relationships including the elucidation of bioavailability, and the actual toxicological consequences of potential pharmacogenetic variations and environmental factors.

1. Introduction

Thujone, a monoterpene ketone, is present in variable amounts in a large number of plants and consequently it is a significant component in many dietary botanical supplements and herbal medicinal products. Thujone has been regarded as a severe neurotoxicant, mainly on the basis of past notoriety of the once-popular drink absinthe, although recently differing views have been expressed (Lachenmeier et al., 2006a,b). During the assessments of *Artemisia absinthium* L. (EMA/HMPC, 2008a) and *Salvia officinalis* L. (EMA/HMPC, 2008b) it became apparent that the risk assessment of thujone, a major component in both herbal preparations, poses considerable uncertainties and difficulties. A case-by-case assessment led to a difference in the maximum limit of daily intakes of thujone, which in the case of Absinthii herba was set at 3 mg/day/person and in the case of sage leaf preparations 5 mg/day/person, both for a maximum duration of 2 weeks. Furthermore, EMA/HMPC (EMA/HMPC, 2009) has concluded that the benefits of sage essential oil do not outweigh its risks. Considering that thujone is a natural constituent of the essentials oils of a number of plants widely used, the HMPC decided to prepare a public statement on the use of herbal preparations containing thujone (EMA/HMPC, 2010). This public statement constituted a starting point for the development of this review article. However, inclusions and interpretations of scientific evidence about thujone toxicity are the authors' own and do not represent the official views of respective authorities.

Abbreviations: ADI, acceptable daily intake; BMDL, benchmark dose (lower confidence limit); CNS, central nerve system; CYP, cytochrome P450; EMA, European Medicines Agency; $GABA_A$, gamma-aminobutyric acid type A receptor; GC–FID, gas chromatography–flame ionization detector; GC–MS, gas chromatography–mass spectrometry; HMPC, Committee of Herbal Medicinal Products; HS-SPME, head-space solid phase micro extraction; LC/MS, liquid chromatography–mass spectrometry; LC–TOF, liquid chromatography–time of flight; NLM, National Library of Medicine; NOEL, no-observed-effect-level; NTP, National Toxicology Program; SCF, Scientific Committee on Food; TDI, tolerable daily intake; TMDI, theoretical maximum daily intake.

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This review benefited from extensive literature searches during the preparation of assessment reports of wormwood and sage and the elaboration of the public statement on thujone (see above). Furthermore, the relevant literature on thujone and thujone-containing preparations were searched principally via PubMed until June 2012 (search terms: thujone, alpha-thujone, beta-thujone, racemic thujone, *Artemisia*, *Salvia*).

2. Thujone in plants and preparations

2.1. Thujone in plants

Thujone (synonyms: Thujon; α-thujone; (−)-thujone; (−)-isothujone; (1S, 4R, 5R)-(−)-3-thujanone; β-thujone; (+)-thujone) occurs in nature as a variable mixture of α-thujone (CAS No. 546-80-5) and β-thujone (CAS No. 471-15-8). Thujone is a natural constituent of the essentials oils of a number of plants widely used for food and/or medicinal purposes (Table 1). Thujone has also been reported to occur in other plants, e.g. *Juniperus* spp, *Cedrus* spp., but its content has not been determined.

2.2. Thujone in various commodities

Annex III of Regulation (EC) No. 1334/2008 on flavourings sets levels for thujone in some beverages as a naturally occurring substance present in flavourings or other food ingredients to which flavouring properties have been added, but it may not be added as such to food (European Union, 2008).

The Council of Europe confirmed in its report from 2008 (Council of Europe, 2008) the limit of total thujone in food and beverages (0.1 mg/kg) set in 2005 (Council of Europe, 2005), and defined a TMDI of 0.01 mg/kg body weight/day. The toxicological data on thujone were considered to be insufficient to set a TDI/ADI.

Sage containing meat preparations are thought to be the main source for the thujone intake from food, while for UK sweets spiced with sage are said to be the most important single source of intake (Lachenmeier and Uebelacker, 2010). But also bakery products, ice cream, fat and oils and alcoholic beverages may contain thujone, often in considerable amounts (Table 2 based on (Amberg-Müller, 2007).

In France the mean and 97.5th percentile daily intakes of total thujone were estimated to be 15.6 and 44.3 μg/kg/day, respectively (SCF, 2002). The SCF defined the total intake from all sources with approx. 0.25 mg/person/day for mean consumer and up to 1 mg/person/day for high-level-consumer (Lachenmeier and Uebelacker, 2010). While some authors postulated that the total intake of thujone from all sources appears to be well within the established TMDI value (Carratù et al., 2010), others maintained that for extreme consumption these limits might be exceeded (Amberg-Müller, 2007).

Thujone is also ingested by using herbal medicinal products. Until now several countries of the European Union used national limits of thujone content for such products. Companies had to provide data, that these limits are maintained. Sometimes the duration of use was graduated depending from the amount of thujone. By now the national limits are being replaced by the limits set in the individual monographs for special preparations or in the position paper concerning thujone in herbal medicinal products (see Section 1).

3. Measurement of thujone and its metabolites in various biological matrices

Gas chromatography techniques have been extensively applied to detect α- and β-thujone in a variety of matrices. Most of these studies have been conducted using GC–MS, including *Artemisia incana* oil (Çetin et al., 2009), absinthe (Lachenmeier et al., 2008, 2009), oil of *Artemisia cretica* ssp. *Carpatica*, (Pavlovic et al., 2010), *Artemisia herba-alba* oil (Mighri et al., 2010), *Salvia* species (Mossi et al., 2011; Rzepa et al., 2009), foods and medicines containing sage *S. officinalis* L. (Walch et al., 2011), and *Artemisia* essential oils (Lopes-Lutz et al., 2008).

GC–FID was employed to detect α-thujone and/or β-thujone in *Lychee* (Mahattanatawee et al., 2007), in Absinthe (Emmert et al., 2004), and in plant materials of *Thuja* twigs (Chizzola et al., 2004).

Few studies have been conducted using GC–MS for the detection of α- and β-thujone and their metabolites in biological matrices, including bacterial, human and rat cytochrome P450 enzyme incubation system (He and Ortiz de Montellano, 2004; Jiang et al., 2006; Jiang and Ortiz de Montellano, 2009a,b), and mouse, rat and human liver microsomes, human recombinant enzymes, and in orally treated mice and rats (Höld et al., 2000, 2001). By applying a validated GC/MS method and head-space solid-phase micro extraction (HS-SPME), thujone over the detection limit of 0.3 ng/ml could be detected in blood samples from humans (Kröner et al., 2005).

Recently, LC/MS has been applied for the detection of α- thujone and its metabolites in in vitro incubations with human liver microsomes and human recombinant enzymes (Abass et al., 2011). In this study, the initial screening and identification by accurate mass measurements were carried out using LC–time of flight (LC–TOF) equipped with a Z-spray ionization source. The quantification and fragmentation measurements were performed by LC combined with triple quadrupole MS. However, the low molecule weight of these compounds makes them rather difficult and tricky to work with electrospray ionization, but nevertheless they were possible to quantitate as a protonated dehydrated molecule.

4. Mechanism of toxic action of thujone

The mechanism of α-thujone neurotoxicity has been convincingly elucidated in experimental animals (Höld et al., 2000, 2001) and in cultured neuronal cells (Szczot et al., 2012) and expressed receptors (Hall et al., 2004). α-Thujone is a rapidly acting modulator of the GABA-gated chloride channel affecting preferentially both the gating properties and inhibitory postsynaptic current frequency. The effect appears to be due to the parent compound (Höld et al., 2000, 2001; Szczot et al., 2012). α-Thujone is more potent than β-thujone (about 2–3-fold) or 7-hydroxy-α-thujone (about 56-fold) in the $GABA_A$ receptor binding assay (Höld et al., 2001). The effective concentrations of α-thujone in various $GABA_A$ related assays were between 10 and 30 μM (Höld et al., 2000), although in cultured neurons higher concentrations (100–300 μM) were reported (Szczot et al., 2012). The neuronal effect seems to be completely reversible. The highest measured brain concentrations at 2.5 min of α-thujone and 7-hydroxy-α-thujone after the i.p. administration to mice of α-thujone at the dose of 50 mg/kg were about 11 (72 μM) and 29 ppm (about 200 μM), respectively, at the time of the most severe poisoning signs, seizures leading within a few minutes to death of almost all animals (Höld et al., 2000).

Other potential targets for thujone such as cannabinoid CB1 receptor (Meschler and Howlett, 1999) and serotonine 5-HT3 receptor (Deiml et al., 2004) have been suggested, but their significance remains to be demonstrated. In primary cultures of chick embryo liver cells, thujone induces 5-aminolevulinic acid synthase leading to the accumulation of copro- and protoporphyrins (Bonkovsky et al., 1992). This suggests that thujone may be porphyrinogenic.