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Public statement on the use of herbal medicinal products containing thujone

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1. Introduction (Problem statement)

During the assessments of *Artemisia absinthium* L. (monograph EMA/HMPC/234463/2008) and *Salvia officinalis* L. (monograph EMA/HMPC/331653/2008), it became apparent that the risk assessment of thujone, a major component in both herbal preparations, poses considerable uncertainties and difficulties. Thujone has been regarded as a severe neurotoxicant, although recently differing views have been expressed. Difficulties culminated in the determination of maximum limit of daily intakes of thujone, which in the case of Absinthii herba was set at 3 mg/person and in the case of sage leaf preparations 5 mg/person, both for a maximum duration of 2 weeks. Furthermore, the HMPC has concluded that the benefits of sage essential oil do not outweigh its risks (Public statement EMA/HMPC/41843/2009). Considering that thujone is a natural constituent of the essentials oils of a number of widely used plants, the HMPC decided to prepare a public statement on the use of herbal medicinal products containing thujone.

Thujone[2] occurs in nature as a variable mixture of α-thujone (CAS Number: 546-80-5) and β-thujone (CAS Number: 471-15-8). It is found in a number of medicinal plants and their essential oils, such as cedar leaf, sage, tansy, wormwood, thyme and rosemary (SCF 2002), in highly variable amounts.

Revision 1 contains editorial changes in sections 2.1. 2.2, 2.4, 2.5, 2.6, 2.7, 3 and 4 as regards presentation of available information taking into account recent publications in the field and additional comments from interested parties as reflected in the revised overview of comments (EMA/HMPC/732886/2010 Rev. 1).

2. Discussion

This evaluation benefited from extensive literature searches during the preparation of assessment reports of wormwood and sage. Furthermore, the relevant literature on thujone and thujone-containing preparations were searched principally via PubMed until November 2010.

2.1. Regulatory status of thujone or thujone-containing products

The Council of Europe (1999) allocated for thujone a tolerable daily intake (TDI) of 10 µg/kg body weight/day based on a NOEL for convulsions of 5 mg/kg body weight in the female rat, dosed by gavage on 6 days per week for 14 weeks, to which a safety factor of 500 was applied. The Council has confirmed these values in its later report (The Council of Europe 2007). The Scientific Committee on Food (SCF 2002) "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 studies should be reviewed when available."

According to the Directive 88/388/EEC 1988 as amended by Regulation (EC) No 1334/2008 a maximum thujone level of 10 mg/kg in alcoholic beverages, except those produced from Artemisia species, of 35 mg/kg in alcoholic beverages produced from Artemisia species and 0.5 mg/kg in non-alcoholic beverages produced from Artemisia species are allowed. As a worst-case calculation, an intake of 4-8 cl (40-80 ml) of absinthe corresponds to approximately 1.2-2.4 mg thujone per person. This is without any restriction in duration of use.

[2] Synonyms: Thujon; α-thujone; (-)-thujone; (-)-isothujone; (1S, 4R, 5R)-(-)-3-thujanone; β-thujone; (+)-thujone.

The use of thujone as such as a flavouring substance is not authorized in Europe (Regulation (EC) No 1334/2008). Also, thujone is not authorised for such a use in the USA.

According to the SCF (2002), in France and the United Kingdom the mean daily intake of thujone is estimated to be between 0.27 and 1.09 mg/person (70 kg). This is a worst-case scenario based on maximum limits.

2.2. Mechanism of toxic action of thujone

The mechanism of α-thujone neurotoxicity has been convincingly elucidated in experimental animal studies. α-thujone is a rapidly acting modulator of the GABA-gated chloride channel. The effect appears to be due to the parent compound (Höld et al. 2000; 2001]. α-thujone is more potent than β-thujone (about 2 to 3-fold) or 7-hydroxy-α-thujone (about 56-fold) in the $GABA_A$ receptor binding assay. The effective concentrations of α-thujone in various $GABA_A$ related assays were between 10 and 30 µM. The neuronal effect seems to be completely reversible. The highest measured brain concentrations of α-thujone and 7-hydroxy-α-thujone at 2.5 min after the intraperitoneal (i.p.) administration of α-thujone at the dose of 50 mg/kg were about 11 and 29 ppm, respectively, at the time of severe poisoning signs.

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.

2.3. Single dose toxicity

The most prominent symptoms associated with acute intoxication are epileptiform convulsions, which are in line with the proposed mechanism of action. The oral LD_{50} of a mixture from α- and β-thujone has been reported with 192 mg/kg in rats, 230 mg/kg in mice and 396 mg/kg in guinea pigs (Margaria 1963). The subcutaneous (s.c.) LD_{50} of α-thujone was given with 134 mg/kg in mice and that of β-thujone with 442 mg/kg in mice (Rice and Wilson 1976). In rats, i.p. administrations of thujone led to both convulsant and lethal effects at a dose of 180 mg/kg body weight (Pinto-Scognamiglio 1967, SCF 2002). The i.p. LD_{50} of α-thujone in mice was 45 mg/kg body weight (Höld et al. 2000). The intravenous (i.v.) LD_{50} in the rat was 0.031 mg/kg body weight (NTP 2003).

2.4. Repeat dose toxicity

Thujone was administered to rats by gavage at doses of 0, 5, 10 or 20 mg/kg/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 in males and 5 mg/kg in females; no changes were reported in haematologic or histopathologic examinations (Margaria 1963).

Thujone was tested in the framework of the National Toxicology Program (NTP) of the US Department of Health and Human Services (TR 570, NTP 2011). α-thujone and isomeric mixture were administered by gavage to B6C3F1 mice and to Fischer 344 rats at doses of 0, 1, 3, 10, 30 or 100 mg/kg for 14 days. In both species, the increased mortality observed in the top dose group was associated with indications of neurotoxicity (hyperactivity, tremors, tonic seizures).

The 3-month NTP study was essentially similar to the 2-week study except the duration. α-thujone and isomeric mixture were administered by gavage to B6C3F1 mice and to Fischer 344 rats at doses of 0, 6.25, 12.5, 25, 50 or 100 mg/kg for 13 weeks. In both species, the increased mortality observed in the higher dose groups (from 25 or 50 and greater) was associated with seizures.

2.5. Chronic toxicity

The National Toxicology Program study on thujone toxicity and carcinogenicity has recently been finalized (TR 570, NTP 2011). An isomeric mixture of thujone was administered by gavage to B6C3F1 mice at doses of 0, 3, 6, 12, and 25 mg/kg body weight/day and to Fischer 344 rats at doses of 0, 12.5, 25, and 50 mg/kg body weight/day for 2 years. In both species, increased mortality was observed in the top dose group, and in the rat also in the middle dose group. Clonic and tonic seizures were observed in the middle and top dose groups in rats and in the top dose group in mice. A small increase in clonic seizures was observed also in the low dose group in rats. The administration of α, β-thujone resulted in increased incidences of nonneoplastic lesions in the brain and spleen of male and female F344/N rats, the kidney of male F344/N rats and the pituitary gland of female F344/N rats usually at the two highest dose levels. In the rat, the NOEL value was 12.5 mg/kg for mortality and tonic seizures (no NOEL for clonic seizures). In the mouse, the NOEL was 12 mg/kg body weight for seizures and mortality.

Reproductive toxicity studies have not been performed.

2.6. Genotoxicity and carcinogenicity of thujone

In connection with the NTP study (NTP 2011, TR No. 570), the genotoxic potential of racemic thujone (used in the carcinogenicity study) and α-thujone were investigated according to the NTP protocols. The Ames test results of both compounds were negative in the presence or absence of the activating enzyme system. *In vivo*, daily exposure by gavage to racemic thujone (6.25, 12.5, 25, 50, or 75 mg α, β-thujone/kg body weight) for 3 months did not result in an increase in micronucleated erythrocytes in the peripheral blood of male B6C3F1 mice. However, female mice had a small but significant increase in micronucleated erythrocytes in the peripheral blood at the end of the 3-month study. Racemic thujone did not induce bone marrow toxicity.

According to the NTP report (TR No 570, NTP 2011) on 2-year gavage studies with rats (dose levels 12.5, 25, and 50 mg/kg) and mice (dose levels 3, 6, 12, 25 mg/kg), there was some evidence of carcinogenic activity of α, β-thujone in male F344/N rats based on increased incidences of preputial gland neoplasms at the dose level of 25 mg/kg (all rats at 50 mg/kg died before the end of the study); increased incidences of benign pheochromocytoma of the adrenal medulla may have been related to administration of α, β-thujone in male F344/N rats administered 12.5 or 25 mg/kg. There was no evidence of carcinogenic activity of α, β-thujone in female F344/N rats administered 12.5 or 25 mg/kg. There was no evidence of carcinogenic activity of α, β-thujone in male or female B6C3F1 mice administered 3, 6, or 12 mg/kg.

2.7. Acute (and chronic) toxicity to humans

Cases with severe intoxications in humans have been reported after consumption of essential oil rich in thujone (Centini et al. 1987, Milett et al. 1981, SCF 2002). Overdosage of alcoholic Absinthii herba preparations or the use of the essential oil may cause CNS disturbances which can lead to convulsions and ultimately to unconsciousness and death (Gessner 1974, Roth et al. 1994). Although it is difficult to determine exposing doses in these cases, SCF (2002) concluded that humans are at least as sensitive to thujone neurotoxicity as experimental animals.

In a “drinking trial” by Dettling et al. (2004), 25 volunteers were exposed to absinthe containing high (100 mg/l) and low (10 mg/l) concentrations of thujone. Approximate thujone amounts consumed were 0, 1.5 mg and 15 mg. The simultaneous administration of alcohol containing a high concentration of thujone had a negative effect on attention performance and some mood dimensions at the earliest

examination time (30 minutes). Alcohol alone or with a low concentration of thujone did not result in similar effects. The authors interpreted the observations at a high thujone dose as the antagonistic effect of thujone on the $GABA_A$ receptor.

In a pilot absinthe drinking study by Kröner et al. (2005), two subjects consumed 110 ml absinthe with 3.85 mg thujone (content of absinthe 35 mg/l) within 15 minutes, 15 and 30 minutes and then every 30 minutes until up to 2 hours after drinking, blood samples were drawn. Blood alcohol concentrations >1 g/l were observed whereas no thujone could be detected in blood samples (detection limit 0.34 ng/ml). Conjugates of thujone were not determined. The two subjects showed typical signs of alcohol effects (e.g. staggering, chattiness), while hallucinogenic effects were not described.

2.8. Pharmacokinetics of thujone

Metabolism of thujone has been investigated in mouse, rat and human liver preparations *in vitro* and in mice, rats and (partially) rabbits *in vivo*. Hydroxylations at various positions, followed to a different extent by glucuronidation, and reductions as minor reactions are principal metabolic pathways, although *in vitro* and *in vivo* metabolic profiles do not necessarily agree with each other (Ishida et al. 1989, Höld et al. 2000; 2001).

After *in vitro* liver microsomal incubations with α-thujone, 7-hydroxy-α-thujone seems to be a major metabolite in mice, rats and humans, whereas with β-thujone, formation of 4-hydroxy-β-thujone exceeded that of 7-hydroxymetabolite in all species. 2-hydroxy-thujone was observed only in mouse liver microsomes. Earlier studies indicated that among human recombinant P450 enzymes studied, CYP3A4 and CYP2D6 were the most active enzymes, producing 7-hydroxy-α-thujone, 4-hydroxy-thujone (in this order of abundance) and some minor metabolites. CYP1A2, CYP2C9, CYP2C19, and CYP2E1 were less active, catalysing only about 1% conversion in one hour of incubation (Höld et al. 2001, Jiang et al. 2006). The latest study (Abass et al. 2010) with a more comprehensive set of recombinant enzymes indicate that the principal CYP enzyme metabolising α-thujone is CYP2A6, followed by CYP3A4 and, to a small extent, CYP2B6. The major metabolites produced were 7- and 4-hydroxyl compounds. Extrapolation of microsomal metabolic clearances suggested that α-thujone is a liver blood flow–dependent substance.

Incubation of α-thujone with rabbit (but not mouse) liver cytosol led to the reduction products, thujol and neothujol, in low yield (Höld et al. 2000; 2001). 7,8- and 4,10-dehydro metabolites have been identified *in vitro* and as urinary metabolites respectively (Höld et al. 2001).

In mice, treated with α-thujone *in vivo*, surprisingly 2-hydroxy-α-thujone (mostly as a glucuronide) was the principal metabolite in urine, whereas 7-hydroxy-β-thujone was by far the most abundant urinary metabolite after β-thujone administration. In the rat, 4-hydroxy-thujones were principal urinary metabolites after thujone administrations (Höld et al. 2001).

3. Conclusions and recommendations

Human intoxications by thujone-containing preparations have indicated that animal studies are of relevance to the human situation. However, dose-effect comparisons are uncertain. Few available studies suggest that low doses (of the order of 1.5 to 3.85 mg) have no effects attributable to thujone. Higher doses (15 mg) have been suggested to cause some subtle effects on attention and mood, but clearly further investigations are urgently needed.

There are no preclinical or clinical studies which would permit reliable scientific assessment of potential consequences regarding exposure of sensitive groups (i.e. pregnant women, children etc). Thus the use of thujone-containing herbal medicinal products in these groups should be minimised.

According to the current view, it is not possible to infer any useful conclusions for thujone toxicity from the phenomenon of absinthism (e.g. Lachenmeier et al. 2006).

Studies on human liver preparations and enzymes *in vitro* indicate that CYP2A6, and CYP3A4 and CYP2B6 to a lesser extent, are principal thujone-metabolising enzymes, at least *in vitro* (Höld et al. 2001, Jiang et al. 2006, Abass et al. 2010). Clearance calculations point to a possibility of a prominent first-pass metabolism. Induction and inhibition interactions with drugs after oral administration in humans are probably not likely because of multiple metabolising enzymes and a fairly rapid metabolism. However, metabolic and pharmacokinetic characteristics remain inadequately defined and need further studies.

Because maximum daily intake (acceptable daily intake (ADI) for food) is a measure of the amount of a specific substance that can be ingested (orally) over a lifetime without an appreciable health risk, for a limited intake, such as herbal medicinal preparations, the limit values of thujone can be set higher than the dietary intake. On this basis, the HMPC has recommended that the rat 14-week study would be taken as a basis of assessment, with an uncertainty factor of 100. This would mean a daily intake of 3.5 mg of thujone/person (70 kg) (EMA/HMPC/234463/2008). The content of thujone must be shown for every batch.

In the NTP 2-year study, there was no NOEL for rats, because it seems that even a low dosage induced seizures. Thus no safety factors can be applied. In mice the NOEL is given as 12 mg/kg and by using a safety of 100, a human equivalent dose is about 0.1 mg/kg meaning an ADI of 7 mg/day. However, it should be kept in mind that the rat seems to be more sensitive and normally the more sensitive species constitutes a a basis for safety factor calculations.

However, it has to be kept in mind that the average dietary daily intake might already be between 0.3 and 1 mg thujone/person, which would lead together with the intake of an HMP to a daily intake of 3.8-4.5 mg thujone/person. That would mean in comparison to the 14-weeks study in rats safety factors of 77-92. The intake by food cannot be ignored, because patients have no influence on it. The daily intake by food should be in general lower than the postulated TDI of 0.7 mg/person and by using the HMP, the daily dose to thujone is increased by at least 3 times. The basic thujone-impact by food needs some further consideration.

Recently, Lachenmeier and Uebelacker (2010) have performed a detailed re-evaluation of the available evidence using the benchmark dose (BMD) approach and found that the application of the appropriate dose-response modelling on the long-term chronic toxicity study of the NTP, using clonic seizures as a response, yielded a BMD lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) as 11 mg/kg body weight/day. Applying the uncertainty factor of 100, an ADI of 0.11 mg/kg was calculated, yielding a limit dose of 6.6 mg/day for a standard human being.

According to the study of Dettling et al. (2004) as a basis, a single dose of 0.28 mg/kg in men (20 mg/70 kg) and of 0.24 mg/kg (17 mg/70 kg) gives some evidence of an - effect of "borderline relevance", i.e. mainly related to driving, operating machinery, etc. A safety margin that covers the small number of subjects, repeated use, possible effects of other herbal constituents on metabolism, etc. is needed.

The "therapeutic margin" of thujone-containing herbal medicinal preparations, where effects may start at those borderline effects and end in seizures, is not known and its determination would need further studies. However, on the basis of the above mentioned limit doses of 3.5 and 6.6 mg/day, it is recommended that the amount of thujone in a preparation needs to be specified and that exposures in

the range between 3 and 7 mg/day do not pose special concerns (a range may allow a simpler analytical method for setting the specification). For higher concentrations, a case-by-case benefit/risk assessment would be necessary. The amount of dietary intake of 1 mg in average may not cause special concerns. However, for the upper limit of the additional intake from medicinal products, the highest safe amount was reduced by the possible intake by food, to give 6 mg as a limit of daily exposure.

Upon finalisation of this public statement, a revision of the monographs on wormwood and sage is envisaged.

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