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Risk assessment of thujone in foods and medicines containing sage and wormwood – Evidence for a need of regulatory changes?

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ABSTRACT

Thujone is a natural substance found in plants commonly used in foods and beverages, such as wormwood and sage, as well as in herbal medicines. The current limits for thujone in food products are based on short-term animal studies from the 1960s, which provided evidence for a threshold-based mechanism, yet only allowed for the derivation of preliminary values for acceptable daily intakes (ADI) based on the no-observed effect level (NOEL). While the 2008 European Union Regulation on flavourings deregulated the food use of thujone, the European Medicines Agency introduced limits for the substance in 2009. The present study re-evaluates the available evidence using the benchmark dose (BMD) approach instead of NOEL, and for the first time includes data from a long-term chronic toxicity study of the National Toxicology Program (NTP). The NTP data provide similar results to the previous short-term studies. Using dose–response modelling, a BMD lower confidence limit for a benchmark response of 10% (BMDL10) was calculated as being 11 mg/kg bw/day for clonic seizures in male rats. Based on this, we propose an ADI of 0.11 mg/kg bw/day, which would not be reachable even for consumers of high-levels of thujone-containing foods (including absinthe). While fewer data are available concerning thujone exposure from medicines, we estimate that between 2 and 20 cups of wormwood or sage tea would be required to reach this ADI, and view that the short-term medicinal use of these herbs can also be regarded as safe. In conclusion, the evidence does not point to any need for changes in regulations but confirms the current limits as sufficiently protective for consumers.

1. Introduction

Thujone is a bicyclic monoterpene ketone that occurs in two stereoisomeric forms: α-thujone (CAS# 546-80-5) and β-thujone (CAS# 471-15-8). For regulatory purposes, the sum of both isomers is generally assessed (Lachenmeier et al., 2006); similarly, thujone in this article refers to the total thujone content of both isomers. Thujone is naturally found in a number of aromatic plants commonly used for flavouring of foods and beverages. This substance fell under scrutiny at the beginning of the 20th century, due to its association with the adverse effects following the consumption of the wormwood-flavoured spirit absinthe (Lachenmeier et al., 2004). Symptoms of so-called "absinthism" included convulsions, blindness, hallucinations and mental deterioration (Lachenmeier et al., 2006). Absinthe and the use of wormwood extracts for food purposes were prohibited around the years 1910–1920 in many countries (Padosch et al., 2006). It was not until the 1960s that the first systematic toxicological studies in animals were conducted; these demonstrated that the effects were threshold-based and allowed for the estimation of acceptable daily intakes of thujone (Surber, 1962; Margaria, 1963). In 1979, the Codex Alimentarius Commission proposed the following maximum thujone limits in food and beverages: 0.5 mg/kg for ready-to-eat foods and beverages in general; 5 mg/kg in alcoholic beverages containing less than 25% vol.; 10 mg/kg in alcoholic beverages above 25% vol.; 25 mg/kg in food containing sage; 35 mg/kg in bitters and 250 mg/kg in sage stuffings (Codex Alimentarius, 1979). With the exception of the 250 mg/kg limit for sage stuffings, the Codex Alimentarius proposal was introduced into the European Union law in 1988 (European Council, 1988), which re-legalised the production of absinthe from wormwood as well as the food use of other thujone-containing plants. This European regulation has recently been amended to now regulating only beverages and the 35 mg/kg limitation applying to all *Artemisia*-derived alcoholic beverages (and not only bitters) (European Parliament and Council, 2008). However, the specific limits for sage preparations and the general limit for foods were removed from the regulation, so that *Artemisia absinthium*, *Salvia officinalis* and other thujone-containing flavouring plants can now be used in foods without restrictions. Nevertheless, thujone as such (i.e., in chemically pure form) is not allowed to be added to foods (European Parliament and Council, 2008); it may only be indirectly introduced into foods by use of thujone-containing plants.

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While the restrictions for foods have been lowered, the opposite has occurred for medicines. In addition to food and beverage use, *A. absinthium* L. and especially *S. officinalis* L. are common medicines and are sold as such, or as preparations or extracts (e.g., sage tea). The European Medicines Agency (EMA) has recently evaluated herbal medicinal products containing both plant species (EMA, 2009a,b). Although the assessors judged the toxicological data on thujone and the quality of available studies to be insufficient to set a maximum daily intake, the EMA nevertheless proposed a daily intake of 3.0 mg/person as acceptable for a maximum duration of use of 2 weeks in the *A. absinthium* monograph (EMA, 2009a). An increased acceptable daily intake (ADI) of 5.0 mg/person was later implemented in the *S. officinalis* monograph (EMA, 2009b). As these new EMA-ADI values also question the current practices for food (e.g., the intake of 3 mg could be reached by drinking less than 100 ml of a spirit containing 35 mg/kg of thujone), our intention with this article is to re-evaluate the toxicological evidence concerning thujone. In addition, we will update the risk assessment using the "benchmark dose" (BMD) approach that is currently preferred by international agencies and in the scientific literature over the previously used "no-observed (adverse) effect level" (NO(A)EL) (see, e.g., Filipsson et al., 2003; IPCS, 2009; Bi, 2010).

The BMD is the point on the dose–response curve that characterises adverse effects. The values are based on data from the entire dose–response-curve for the critical effect, whereas the standard NOAEL approach can be regarded as a special, simplified case of dose–response analysis, as it identifies a single dose that is assumed to be without an appreciable adverse effect (IPCS, 2009). The BMD approach was developed by Crump (1984), and has since then been adopted by the US Environmental Protection Agency (US EPA, 1995) as well as the European Food Safety Authority (EFSA, 2005), primarily for risk assessment of genotoxic carcinogens. Only recently, the BMD approach was widened to include other agents with a wide range of effects (e.g. pesticides, mycotoxins and natural toxins) (Muri et al., 2009) as well as macroconstituents in foods, such as sugar and fat (Bi, 2010). As the BMD approach incorporates the shape of the dose–response-curve and the variability in the data, as mentioned above, it could be especially effective in the case of thujone, for which only animal data with limited experimental design existed (Surber, 1962; Margaria, 1963). In addition to the old studies from the 1960s, this article is the first to include data from a recent long-term chronic toxicity study conducted by NTP (2009) for regulatory evaluation. Our results of BMD-modelling will be used to assess about the risk of thujone-containing foods and medicines.

2. Methods

Our literature review was based on previous monographs regarding the toxicity of thujone (WHO, 1981; SCF, 2003; NTP, 2005; Committee of Experts on Flavouring Substances, 2005; EMA, 2009a), which was compounded by a computer-assisted literature search for the key-words "thujone", "*Artemisia*", and "*Salvia*" in combination with "toxicity", "ADI", "NO(A)EL", "BMD" in the following databases: PubMed (US National Library of Medicine, Bethesda, MD), Web of Science (Thomson Reuters, Philadelphia, PA), and Scopus (Elsevier B.V., Amsterdam, Netherlands). The references, including abstracts, were imported into Reference Manager V.11 (Thomson Reuters, Carlsbad, CA) and the relevant articles were manually identified and purchased in full text. The reference lists of all articles were checked for relevant studies not included in the databases.

BMD-modelling was conducted using international guidelines (US EPA, 1995; EFSA, 2005; IPCS, 2009; EFSA, 2009). The benchmark response (BMR) was set at 10%. For clarity, we use the abbreviation BMD10 to designate the BMD at a BMR of 10%. Different models, as detailed in the results section, were evaluated. In addition to the BMD10, the Benchmark Dose Lower Confidence Limit (BMDL10) was calculated. The BMDL10 is a statistical lower confidence bound on the true value of the BMD, at a BMR of 10% and at a confidence level 95%. It is used to characterise the uncertainty inherent in the point estimator of the BMD, as calculated from the data.

All calculations were conducted using the US EPA's BMDS 2.1.1-software (available at the US Environmental Protection Agency website: http://www.epa.gov/ncea/bmds/index.html). The calculations were conducted strictly according to the EPA criteria following the tutorial on the EPA website (US EPA, 2008) and in accordance with the International Programme on Chemical Safety document Principles for Modelling Dose–Response for the risk assessment of chemicals (IPCS, 2009) as well as the recent guidance from EFSA (2009). Further background on BMD method was provided by Filipsson et al. (2003) and Sand et al. (2008). All parameters were set at default values (e.g., for slope, intercept). The risk type was set to "extra risk". All dichotomous models available in the US EPA software were evaluated. The best-fitting model was selected according to *p*-value and Akaike's information criterion. The goodness-of-fit was also visually confirmed in the model graphs. Finally under consideration of an uncertainty factor (UF), the ADI was calculated according to IPCS (2009) as ADI = BMDL10/UF.

3. Results

A number of anecdotal reports have been published concerning toxicity associated with overdosing with extracts of *Salvia* or *Artemisia* in humans; however, none of these confirms these effects to be due exclusively to thujone (Smith, 1862, 1863; Robinson, 1889; Whitling, 1908; Millet et al., 1981; Centini et al., 1987; Weisbord et al., 1997; Tong et al., 2003). No epidemiological studies were identified in the course of our literature research. Two studies in humans about the ingestion of thujone in alcoholic beverages were identified (Dettling et al., 2004; Kröner et al., 2005), but these provided no conclusions sufficient for risk assessment (see Section 4). Thus, due to the lack of human data, thujone risk assessment could only be based on dose–response information derived from animal studies. The major effect reported in animals was epileptiform convulsion (Keith, 1931; Sampson and Fernandez, 1939; Wenzel and Ross, 1957; Pinto-Scognamiglio, 1967; Millet et al., 1979, 1981; Steinmetz et al., 1980), which was proposed to be based on γ-aminobutyric acid type A ($GABA_A$) receptor modulation (Höld et al., 2000). Two short-term animal experiments conducted in the 1960s with rats (Surber, 1962; Margaria, 1963), and two more recent chronic long-term studies with rats and mice (NTP, 2009) were identified in the literature as having data suitable for dose–response modelling.

In the work of Margaria (1963), four groups of 20 rats (10 male and female) received thujone in doses of 0, 5, 10 or 20 mg/kg by gavage 6 days per week for 14 weeks. Convulsions were observed after dosing in many instances in nine female and six male animals in the top dose group, while a single female animal from the 10 mg/kg dose group had one convulsion on the 38th day. One male and three female rats in the top dose group died of convulsions. At termination, no significant differences were observed between groups with respect to weight gain, haematology, or weights of heart, liver, spleen, kidney and adrenals. No treatment-dependent gross pathological or histopathological lesions were observed. The no-effect level (NOEL) was 5 mg/kg/day for females and 10 mg/kg/day for males.

Surber (1962) administered a commercial mixture of α- and β-thujone by gavage to weanling rats in groups of 20 (male and

female, respectively) at doses of 0, 12.5, 15.0 and 50.0 mg/kg/day for 13 weeks. Doses were given in five daily increments as a suspension in aqueous agar. Five rats (four males and one female) died during acclimatisation and three others (one male from each of the low and middle dose groups; one female control) died from a viral infection during treatment. Post-treatment convulsions were frequently observed. No effects were observed on body weight gain, or haematology, and histopathological examination at termination did not reveal any dose-related lesions. The NOEL for males was 12.5 mg/kg/day; a NOEL could not be established for female rats since one rat in the lowest dose group displayed convulsions on two occasions. The results from Margaria (1963) and Surber (1962) are summarised in Table 1.

Recently, the US National Toxicology Program (NTP) conducted several animal experiments with thujone (a mixture of α- and β-thujone), including short-term toxicity studies (2 weeks and 13 weeks) in rats and mice (10 animals/sex/species), as well as a long-term carcinogenicity study (50 animals/sex/species). In this evaluation, we considered only the long-term study, which is most significant for regulatory toxicology (NTP, 2009). Modelling of the short-term studies showed BMD10 values of the same order of magnitude as the long-term study (data not shown). The NTP provided results for several endpoints (including incidence of neoplastic and non-neoplastic lesions, as well as clinical observations such as excitability, clonic and tonic seizures, eye abnormality, diarrhoea, head tilt, lethargic, different masses, nasal/eye discharge, ruffled fur, thinness, ulcers/abscesses of different areas). No significant dose–response relationship was detected for any endpoint besides mortality and the clonic and tonic seizures during clinical observation (see summary in Tables 2 and 3).

The results of our BMD-modelling for seizures and mortality are shown in Table 4. Only the result of the best-fitting model (selected according to *p*-value and Akaike's information criterion) are presented. For almost all studies and selected endpoints, a significant dose–response was proven; most cases showed an excellent fit with *p*-values above 0.9. In Figs. 1 and 2, the modelling of tonic seizures in the NTP rat study is shown as an example. Some of the data were problematic to model, as only one dose group (the highest) showed a response; nevertheless, we decided to show these results in brackets for comparative purposes. The only modelled endpoint with non-significant dose–response was the tonic seizures in female rats from the NTP study. In that case, the incidence was lower (4/50) in the highest dose group than in the dose group below (15/50).

Overall, the BMDL10 values fell within a range between 7 and 26 mg/kg bw/day. In some endpoints (convulsions), females appeared to be slightly more sensitive than males, but under consideration of the BMD-modelling uncertainties, no clear difference between the sexes was obvious.

As indicated anecdotally from intoxication cases, seizures are probably the major adverse effect in humans (e.g., see Weisbord et al., 1997). For this reason, we decided to use the lowest BMDL10 value from the long-term NTP studies, at 11 mg/kg bw/day from clonic seizures in male rats, as a departure point for our regulatory considerations. Although dose–response modelling of the mortality data shows results in the same order of magnitude as those for the seizures, we did not consider these further in our evaluation because of the high mortality, even at the control and low doses. This may have been caused by viral infection, as in the study of Surber (1962); however, no further explanations were currently found on the NTP website (NTP, 2009).

To calculate an ADI, the traditional uncertainty factor (UF) of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was chosen (100). This assumes that the human being is 10 times more sensitive than the test animal and that the difference in sensitivity within the human population lies within a 10-fold range (IPCS, 1987). According to IPCS (2009), the ADI based on the composite UF of 100 has been accepted by international institutions and countries as a health-based guidance value. The Committee of Experts on Flavouring Substances (2005) used an increased UF of 500 because of the poor data quality at the time, but we feel that the quality of the new NTP data justifies the use of the standard UF of 100.

With a BMDL10 of 11 mg/kg bw/day, the ADI would therefore be 0.11 mg/kg bw/day, corresponding to an ADI of 6.6 mg/day for a 60-kg human. To reach this ADI, 189 ml of an absinthe containing the maximum-allowed amount of 35 mg/l of thujone would have to be consumed. Given that the average thujone content in absinthe manufactured before the ban in 1915 was 25 mg/l (Lachenmeier et al., 2008), a volume of 264 ml would have to be consumed to exceed the ADI.

Population-based intake estimates for thujone in food and beverages were provided by the Scientific Committee on Food (SCF, 2003) for France and the UK. The major dietary contribution appeared to derive from sage and sage-flavoured products, as well as alcoholic beverages. 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 UK were estimated at 3.9 and 14.2 μg/kg bw/day, respectively. The Committee of Experts on Flavouring Substances (2005) confirmed these intakes for the UK, and commented that the most important single source of intake is sweets spiced with sage. Further contributors to total thujone intake are sage-flavoured sausages and other meat products, sage

Table 1
Studies on short-term thujone administration to rats.

Sex	Administration to rats by gavage on 6 days per week for 14 weeks (Margaria, 1963)[a]			Administration to weanling rats by gavage in five increments daily for 13 weeks (Surber, 1962)[b]		
	Thujone dose (mg/kg bw/day)	Endpoint: convulsions	Endpoint: mortality	Thujone dose (mg/kg bw/day)	Endpoint: convulsions[c]	Endpoint: mortality[c]
Male	0	0/10	0/10	0	0/20	0/20
Female		0/10	0/10		0/20	0/20
Male	5	0/10	0/10	12.5	0/16	4/20
Female		0/10	0/10		0/20	0/20
Male	10	0/10	0/10	25	0/18	2/20
Female		1/10	0/10		7/18	2/20
Male	20	6/10	1/10	50	10/12	8/20
Female		9/10	3/10		7/8	12/20

[a] The Margaria (1963) study is unpublished. The data was taken from a summarisation in WHO Food Additives Series 16 (WHO, 1981).
[b] The Surber (1962) study was not available in full text. The data was taken from a summarisation in WHO Food Additives Series 16 (WHO, 1981), which without specific citation apparently includes the raw data table from Surber (1962), this was verified in the thujone monograph of the Committee of Experts on Flavouring Substances (2005).
[c] Data for month 3 are shown. A no-effect level cannot be established for female rats since one rat in the lowest dose group displayed convulsions on two occasions at month 2 (WHO, 1981).

Table 2
NTP chronic long-term study in F344/N rats by gavage (NTP, 2009).

Sex	Thujone dose (mg/kg bw/day)	Endpoint: clonic seizures	Endpoint: tonic seizures	Endpoint: mortality
Male	0	1/50	0/50	24/50
Female		1/50	0/50	15/50
Male	12.5	5/50	0/50	25/50
Female		3/50	0/50	17/50
Male	25	43/50	2/50	33/50
Female		47/50	15/50	31/50
Male	50	50/50	18/50	50/50
Female		50/50	4/50	50/50

Table 3
NTP chronic long-term study in B6C3F1 mice by gavage (NTP, 2009).

Sex	Thujone dose (mg/kg bw/day)	Endpoint: clonic seizures	Endpoint: tonic seizures	Endpoint: mortality
Male	0	0/50	0/50	10/50
Female		1/50	0/50	13/50
Male	3	0/50	0/50	8/50
Female		1/50	0/50	17/50
Male	6	0/50	0/50	9/50
Female		0/50	0/50	11/50
Male	12	0/50	0/50	13/50
Female		0/50	0/50	9/50
Male	25	41/50	35/50	37/50
Female		50/50	40/50	50/50

stuffings, salad dressings, vermouth, liqueurs and bitters. The Committee found that the thujone intake from wormwood, and in particular from absinthe, appears to be very limited. The total intake of thujone from all sources was estimated to be approximately 0.25 mg/person/day for mean consumers and up to 1 mg/person/day for high-level consumers. None of these intake estimations would exceed the ADI value proposed in this study.

The exposure assessment for medicines is more difficult, as no systematic data exist for the typical thujone content of preparations containing *Artemisia* or *Salvia*. We assume that the most typical use is as a tea infusion. If a tea is prepared with 3 g of herbal substance containing 0.6% oil with 17.6% thujone (average for *A. absinthium* (Lachenmeier and Nathan-Maister, 2007)), a typical cup of tea would contain 3 mg thujone (assuming the unlikely case of complete extraction), so that approximately two cups of wormwood tea per day could be consumed without reaching the ADI. According to Lima et al. (2005), sage tea contains 2.0 μg/ml of thujone (2 g in 150 ml boiling water, steep for 5 min), which corresponds to 0.3 mg per cup. On this basis, 22 cups of this sage tea per day could be consumed without reaching the ADI.

4. Discussion

4.1. The use of studies in humans to postulate regulatory limits for thujone

Much of the evidence regarding the detrimental effects of thujone on humans is anecdotal. Historical reviews show that most, if not even all, of the effects of absinthe may have been due to its alcohol content or toxic adulterants but not to thujone (Padosch et al., 2006; Luauté, 2007). As seizures are a well-known effect of ethanol (Brust, 2008; Samokhvalov et al., 2010), their occurrence may have been wrongly attributed to thujone. For public health risk assessment, the limited nature of the available evidence from the 19th century renders it unusable, as there is no control for the confounding effects between alcohol and thujone. Current data (e.g., animal experiments) also fail to account for the combined exposure to thujone and alcohol. Therefore, a limitation of the present study is that it can assess only the risk of thujone, independent of possible confounding effects induced by alcohol or other food ingredients.

It is striking that in human intoxications with pure wormwood or sage oil, seizures were reported (similar to the anecdotal reports from the 19th century); in these cases, thujone could be the cause as it is often one of the major constituents in the oil. The results from the animal experiments mentioned in the results section, as well as the mechanistic evidence of $GABA_A$ receptor mediation, further increase the plausibility of thujone causing seizures in humans. Therefore, we have chosen seizures as the endpoint for deriving our ADI value.

It would be clearly preferable to use human data for any health risk assessment. However, based on our literature review, the modern literature offers no reports about adverse effects of thu-

Table 4
Dose–response modelling results for thujone in different animal experiments (data from Tables 1–3).

Study, animal model	Endpoint	Sex	Model[a]	*p*-Value[b]	BMD10[c] (mg/kg bw/day)	BMDL10[d] (mg/kg bw/day)
Margaria (1963), Rats	Convulsions	Male	LogProbit	(1.0000)[e]	(16.7)	(9.4)
		Female	LogProbit	0.9997	10.0	7.3
	Mortality	Male	LogProbit	(1.0000)[e]	(20.0)	(12.0)
		Female	LogProbit	(1.0000)[e]	(18.1)	(9.7)
Surber (1962), Rats	Convulsions	Male	LogProbit	(1.0000)[e]	(39.7)	(26.3)
		Female	LogProbit	0.7204	17.9	13.3
	Mortality	Male	Weibull	0.3762	11.2	7.4
		Female	LogProbit	0.9765	25.4	18.5
NTP (2009), Rats	Clonic seizures	Male	Gamma Multi-Hit	0.9796	13.0	11.0
		Female	LogProbit	0.9954	13.8	12.2
	Tonic seizures	Male	LogProbit	0.9795	31.8	26.1
		Female	–[f]	–	–	–
	Mortality	Male	Log–Logistic	0.9801	23.0	16.4
		Female	Gamma Multi-Hit	0.9064	18.7	12.4
NTP (2009), Mice	Clonic seizures	Male	LogProbit	(1.0000)[e]	(19.6)	(14.2)
		Female	Weibull	0.5679	18.9	12.9
	Tonic seizures	Male	LogProbit	(1.0000)[e]	(20.2)	(14.6)
		Female	LogProbit	(1.0000)[e]	(19.7)	(14.2)
	Mortality	Male	LogProbit	0.8729	12.1	8.3
		Female	Weibull	0.2917	19.0	12.2

[a] Data from best-fitting models selected with BMDS 2.1.1-software according to US EPA (2008) criteria are presented.
[b] A *p*-value greater than 0.1 indicates that the model fits the data (*p*-value 1.0 = perfect fit).
[c] BMD10: benchmark dose for a benchmark response of 10%.
[d] BMDL10: lower one-sided confidence limit of the BMD.
[e] No proven dose–response due to only one positive dose group. The results of such calculations are shown in brackets.
[f] No significant dose–response.

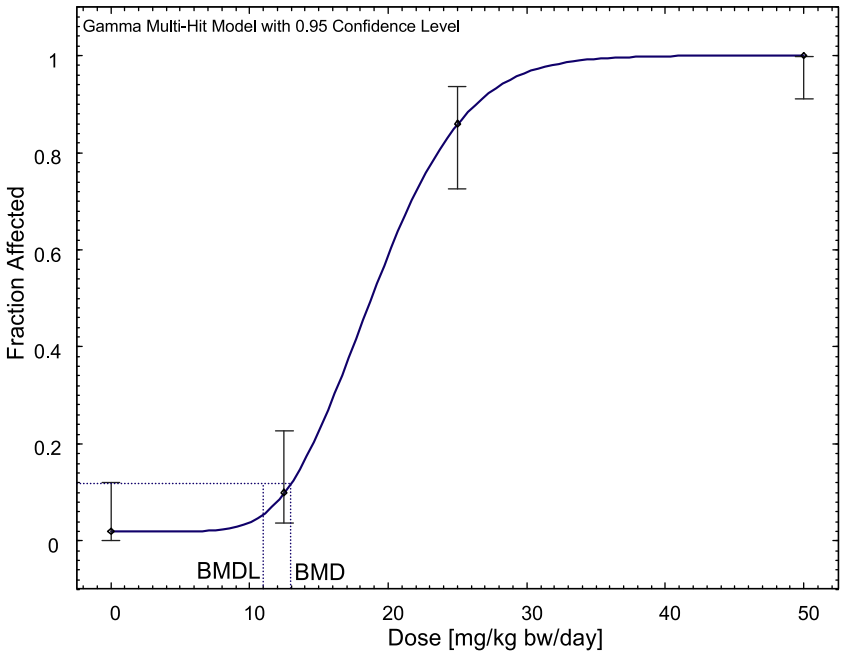

Fig. 1. BMD-modelling for clonic seizures in a chronic long-term study with male B6C3F1 rats. Original data from NTP (2009).

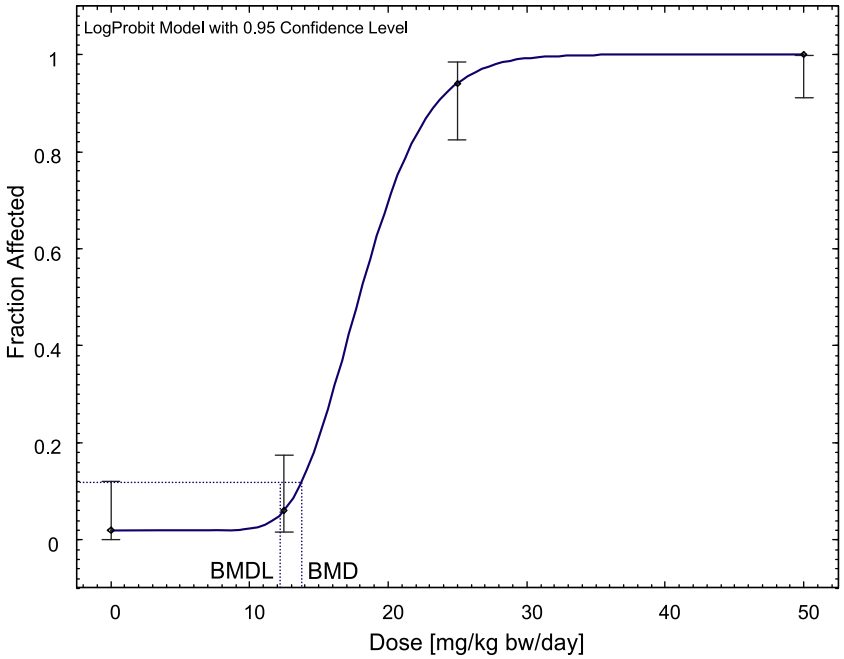

Fig. 2. BMD-modelling for clonic seizures in a chronic long-term study with female B6C3F1 rats. Original data from NTP (2009).

jone-containing foods and beverages. Kröner et al. (2005) were not able to detect thujone in the blood stream following ingestion of 110 ml absinthe containing 35 mg/l of thujone, which confirms the rapid metabolic detoxification detected in animal experiments. The metabolites were also found to be considerably less toxic than thujone itself (Höld et al., 2000).

The human study of Dettling et al. (2004) is interesting as it was used by EMA (2009a) for their proposal of an acceptable daily intake of 3.0 mg/person. Dettling et al. (2004) studied the attention performance and mood under the influence of thujone and alcohol. The studied thujone levels were 10 and 100 mg/l, which according to EMA (2009a), corresponded to approximately 1.5 and 15 mg/person. At the lower dose, no-effect was detected. The EMA (2009a) considered that three single doses of 1 mg of thujone per day would provide an adequate safety. While the EMA-ADI is in the same order of magnitude as our BMDL10-ADI, we disagree with the EMA (2009a) that the Dettling et al. (2004) study can be used to derive such a limit. Besides deficits in experimental design (not placebo controlled, not double blinded, limited non-homogenous collective ($n = 25$), no physiological parameters determined) that are normally required for regulatory toxicology, the study only researched thujone in combination with alcohol (no thujone only group was included). The result of Dettling et al. (2004), in which the high thujone group (in combination with alcohol) showed changes in attention performance, was used to postulate the requirement of a warning label in which "patients should not drive

or operate machinery after intake of Absinthii herba preparations" (EMA, 2009a). We agree that this warning is obvious for alcohol-containing products, but we question the scientific foundation of applying the warning to ingestion of pure thujone (e.g., in the form of aqueous extracts such as wormwood or sage tea). The experimental design deficits, the confounding with ethanol, as well the unclear dose–response relationship (in some cases, the low-dose group showed a non-significantly improved outcome compared with the control) make these data unusable for regulatory purposes (a purpose for which they were never intended).

It is striking that the EMA changed the rationale for the derivation of the limit following the public consultation of the *Salvia* monograph and subsequently increased the limit to 5 mg/day (EMA, 2009b). In our opinion, this new rationale is as scientifically problematic as the previous one. The comparably old report from SCF (2003) was used to postulate an acceptable daily intake of 0.08 mg/kg bw/day (which would be equal to 5 mg/day). It is striking that the SCF (2003) considered the available data inadequate to establish a TDI/ADI. The value of 0.08 mg/kg bw/day mentioned in the SCF report was not an ADI but a model calculation based on the consumption of as much as 1 l of an alcoholic beverage containing 5 mg/l, the maximum permitted level of thujone in alcoholic beverages with up to 25% alcohol (SCF, 2003). Both the human data from Dettling et al. (2004), as well as the experience of absinthe drinking, are unsuitable for BMD-modelling, which necessitated the basing of our evaluation purely on animal experiments.

4.2. Animal data and extrapolation to humans

Before publication of the NTP (2009) results, the available animal data were generally considered insufficient for deriving tolerable daily intakes (see, e.g., SCF (2003)). Not only were the studies of Surber (1962) and Margaria (1963) not published through peer review (one was an internal report), with data drawn from secondary sources, but both were short-term studies, conducted with few animals and few dose groups. Nevertheless, possibly for pragmatic reasons, the NOEL's from these studies were apparently used anyway, with the Codex Alimentarius (1979) maximum limits for food and beverages, also based on this evidence, in place for over 30 years. According to our literature research, the food safety of thujone-containing products has not been questioned in modern times, at least not in terms of acute effects such as seizures, while absinthism has not re-appeared since absinthe's re-legalisation in 1988.

It is striking that our BMDL10 value of 11 mg/kg bw/day calculated from NTP (2009) is in very good accordance with the NOEL values between 5 and 12.5 mg/kg bw/day of the previous studies. In light of the inherent uncertainties of BMD-modelling, differences of up to a factor of three are accepted as typical, even within the different mathematical dose–response models of the same experiment (US EPA, 2008). In our case, the BMDL10 values of the studies from the 1960s and the ones from NTP (2009) did not even differ by this factor. Our study therefore confirms that dose–response modelling enhances the ability to compare quantitatively different experiments, effects, and compounds within a common framework (IPCS, 2009). Therefore, we think that the data basis for the toxicological evaluation of thujone is now adequate for deriving an ADI. As our new values essentially confirm the old values from the 1960s, we see no need for regulatory changes, but think that the current limits are scientifically sound and can be enforced with a higher degree of validity.

4.3. Risk assessment and policy considerations

As there are no significant differences between the previous NOEL-based risk assessments and our BMDL10-ADI approach, we believe the status quo of the regulations for thujone is sufficient to protect consumers, with no need for regulatory changes.

For foods, the situation is relatively simple, as maximum limits have been in force for several years, with the previous risk assessments, based on higher uncertainty factors, finding the human exposure generally below current guidelines. For example, the Committee of Experts on Flavouring Substances (2005) derived a so-called theoretical maximum daily intake (TMDI) of 0.01 mg/kg bw/day based on the NOEL of 5 mg/kg bw/day from Margaria (1963) and a safety factor of 500. This increased safety factor was chosen due to the poor quality of data. Even this TMDI, which is 11-fold lower than our BMDL10-ADI, was considered as unreachable by mean intakes. Only high-level consumers (97.5th percentile), normally believed to be overestimated, slightly exceeded the TMDI. Our new ADI would not be exceeded by high-level consumers.

However, the situation is different for medicines. The EMA (2009a,b) has only recently proposed acceptable daily thujone intakes. As discussed above, the weak scientific rationale for the EMA limits of 3 mg/day or 5 mg/day would probably not be upheld if a manufacturer were to decide to take legal action (e.g., in the case of authorities prohibiting the marketing of the products based on this limit). However, these limits are in reasonably good agreement with our BMDL10-ADI based limit of 6.6 mg/day. While we did not find a systematic exposure assessment from medicines, the limited literature (e.g., about sage tea) offered no reason to assume a public health risk. The problem appears to be less significant for tea and other aqueous preparations, as thujone is less soluble in water than in ethanol (according to Tegtmeier and Harnischfeger (1994) only 8% of thujone is recovered in water compared to extraction in 90% vol. ethanol). Therefore, we expect less thujone in teas, than in, for example, spirits such as absinthe. For medicines, the restriction of use to a few days (i.e. the EMA assumes a maximum use of 14 days) must also be considered, while the ADI for foods is intended to provide safety for a lifelong daily ingestion. Additionally, a risk–benefit analysis appears necessary for these types of herbal medicines (Holden, 2003) as opposed to a complete safety requirement (as for foods). Wormwood, as well as sage, has been reported in several trials as possibly advantageous for the treatment of various disease conditions such as Crohn's disease (Omer et al., 2007; Krebs et al., 2010), stroke (Bora and Sharma, 2010) or Alzheimer's disease (Akhondzadeh et al., 2003). It is notable that the wormwood preparation used in the Crohn's disease trial was tested for acute (24 h), sub-acute (4 weeks) and chronic (6 months) toxicity (Omer et al., 2007). Five doses ranging from 0.575 to 5.812 g/kg were administered (thujone content less than 5 mg/kg). In the 6-months toxicity studies, body weight, organ weights and haematological findings did not indicate any toxicity. Teratogenic studies on rats after 6 months feeding also did not show any effects (Omer et al., 2007). The human study also did not report any side effects during a 6-weeks study period where 250 mg wormwood in capsules was administered three times a day (Krebs et al., 2010).

In conclusion, we currently see no risk associated with the occasional medicinal use of wormwood or sage (especially in the traditional use as herbal tea). However, we agree with the EMA (2009a,b) that the database regarding the thujone exposure via medicines is extremely limited. This database should be expanded in the future, preferably to include quantitative risk–benefit analyses (Lachenmeier, 2010).

Conflict of interest statement

The authors declare that there are no conflicts of interest. No funding was specific to the production of this manuscript. The salaries for authors were provided by the affiliated organisation.

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