Scientific Committee on Food

SCF/CS/FLAV/FLAVOUR/30 Final
9 April 2003

Opinion of the Scientific Committee on Food on Isosafrole

(expressed on 4 April 2003)
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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 implication for human health of isosafrole in the diet.

Introduction

Previous evaluations

In its opinion of 21 September 1979 the Scientific Committee for Food proposed limits for isosafrole in food. For total saffrole and isosafrole, limits of 1 mg/kg for foods and for beverages were proposed with the following exceptions: 2 mg/kg in alcoholic beverages with less than 25% alcohol, 5 mg/kg in alcoholic beverages with more than 25% alcohol and 15 mg/kg in foods containing mace and nutmeg (SCF, 1979).

Isosafrole was evaluated by the International Agency for Research on Cancer (IARC) in 1975. It was concluded that isosafrole is carcinogenic in mice and rats, producing liver tumours following its oral administration (IARC, 1976). Based on this monograph, IARC in 1987 concluded for isosafrole that there were no adequate human carcinogenicity data but limited evidence of animal carcinogenicity, and isosafrole was classified in group 3, “not classifiable as to its carcinogenicity for humans” (IARC, 1987).

In 1981 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) also concluded that isosafrole is carcinogenic in rats and mice and no ADI was allocated (WHO, 1981).

In 1999, the Council of Europe Committee of Experts on Flavouring Substances (CEFS) was informed by the International Organisation of Flavour Industry that isosafrole does not occur in any natural source material for flavourings used by flavour industry and therefore the CEFS deleted isosafrole from the list of active principles (Council of Europe, 1999). In 2002 CEFS was informed
that isosafrole does occur in relevant source materials for flavourings and CEFS decided to reconsider isosafrole at its next meeting (Council of Europe, 2002).

Current regulatory status

Isosafrole is listed in the Directive 88/388/EEC on flavourings in Annex II with maximum limits for isosafrole (and safrole) of 1 mg/kg in foodstuffs and beverages with exceptions of 2 mg/kg and 5 mg/kg in alcoholic beverages with not more than and with more than 25% volume of alcohol, respectively, and 15 mg/kg in foodstuffs containing mace and nutmeg (EEC, 1988).

Chemical characterisation

Isosafrole is a propenylbenzene derivative.
Name: Isosafrole
Synonyms: 5-(1’-Propenyl)-1,3-benzodioxole; 1,2-(methylenedioxy)-4-(1’-propenyl) benzene
CAS no.: 120-58-1
Structure:

\[ \text{O} \]

\[ \text{O} \]

\[ \text{2'} \]

\[ \text{3'} \]

\[ \text{1'} \]

\[ \text{E} \] - Isosafrole

\[ \text{O} \]

\[ \text{O} \]

\[ \text{1} \]

\[ \text{2'} \]

\[ \text{3'} \]

\[ \text{Z} \] - Isosafrole

Isosafrole exists as a trans-(E-)isomer (beta-isosafrole) (CAS no: 4043-71-4) and as a cis-(Z-)isomer (alfa-isosafrole), (CAS no: 17627-76-8).

Exposure assessment

According to CEFS (1999) natural source materials for flavourings used by the flavour industry do not contain isosafrole. Former information on occurrence of isosafrole in ylang-ylang oil and products of sassafras could not be confirmed (MAFF, 1996; Carlson and Thompson, 1997). On the other hand, small amounts of isosafrole were found in some samples of nutmeg oils (range < 0.1 - 3.4%) and oleoresins (range < 0.1 - 2.7%) (MAFF, 1996). In contrast to this report, Lawrence
(1990) did not find isosafrole in nutmeg oil. In a study by Adam and Postel (1992) isosafrole was found in trace amounts (0.01 and 0.03 mg/kg) in two out of 24 alcoholic beverages.

According to available data isosafrole only occurs sporadically and then together with safrole but at much lower concentrations than safrole, roughly an order of magnitude lower (MAFF, 1996). It has therefore been speculated, whether isosafrole occurs as an artefact, formed due to heating during the analytical process and/or during the preparation of source material extracts, essential oils, processed foods and others, containing safrole. This supposition is backed up by the fact that isosafrole can be produced by isomerisation of safrole under hot alkaline conditions (Bert, 1941; Naves and Ardizio, 1957) and hence the possibility that traces of isosafrole may be formed even under neutral conditions (Council of Europe, 1999).

A rough intake estimate for isosafrole could be based on the estimated average safrole intake (for consumers only) (SCF, 2001), assuming that the intake of isosafrole is one tenth of the safrole intake. Using this assumption, the estimated average per capita intake of isosafrole amounts to 0.03 mg/day and the 97.5% percentile to 0.05 mg/day.

Hazard identification / characterisation

Absorption, distribution, metabolism and excretion

In an in vitro study with epithelial cells from adult rat liver, the major metabolite of isosafrole was 1’, 2’-dihydro- 1’, 2’-dihydroxyisosafrole with lesser amounts of 1’, 2’-epoxyisosafrole and 1’-hydroxyisosafrole. The metabolites were identified by gas chromatography-mass spectrometry, but no quantitative data are given (Janiaud et al., 1976).

At 1 mmol (162 mg) isosafrole (“Cis-trans mixture”)/kg bw given to three male albino rats (Wistar strain derived) by stomach tube, metabolite excretion accounted for 89 % of the dose in urine, in 72 hours. Demethylation leading mainly to 1,2-dihydroxy-4-(1’-propenyl)benzene was the most prominent reaction (92% of the urinary metabolites were demethylated) but also allylic hydroxylation and epoxide-diol pathway took place. Allylic hydroxylation took place at the 3’-position, but this was a minor pathway in the present rat study. Only 1.3% of the dose was recovered as 3’-hydroxyisosafrole and, contrary to the above in vitro study, no 1’-hydroxyisosafrole was detected. However, the authors did not exclude that some formation of this may occur when very large doses of isosafrole are administered (Klungsøyr and Scheline, 1982). This is supported by the study by Peele and Oswald (1978) who did in fact demonstrate excretion of traces of 1’-hydroxyisosafrole in the urine of rats to which they had administered 3’-hydroxyisosafrole. It has also been shown that 1’-hydroxyisosafrole may undergo chemical rearrangement to 3’-hydroxyisosafrole (Borchert et al., 1973b) and that this equilibrium between the two isomers strongly favours 3’-hydroxyisosafrole (Peele and Oswald, 1978).
**Acute toxicity**

LD$_{50}$ oral (mice): 2.47 g/kg bw. LD$_{50}$ oral (rats): 1.34 g/kg bw (Jenner et al., 1964; Hagan et al., 1965).

**Subacute/subchronic toxicity**

*Rat*

Rats (Osborne-Mendel strain, 10 males and 10 females) given 10,000 mg isosafrole/kg in diet showed growth retardation in both sexes. No rats survived beyond 11 weeks of treatment. The livers were enlarged and microscopically slight hepatic cell hypertrophy, which was usually focal and resulted in the formation of nodules, was shown (Hagan et al., 1965; Hagan et al., 1967). Daily doses of 460 mg isosafrole/kg bw given by stomach tube for four days to rats (3 males and 3 females) produced severe liver lesions consisting of discoloration, enlargement, and loss of normal texture. No histopathology was performed. Two of the rats died during the test period. Apparently, no control group was included in the study (Taylor et al., 1964). Oral intubation to young Osborne-Mendel rats of both sexes of 500 mg isosafrole/kg bw/day for 41 days resulted in mortality ratios of 8/10 rats and of 250 mg isosafrole/kg bw/day for 34 days of 2/10 rats. All ten rats of the control group survived. The following effects were observed: liver hypertrophy and slight focal necrosis and fibrosis, slight degree of focal fatty metamorphosis and bile duct proliferation (Hagan et al., 1965).

**Chronic toxicity/Carcinogenicity**

*M*ouse

In two strains of mice (C57BL/6 x C3H/Anf)F$_1$ and (C57BL/6 x AKR)F$_1$ (group size 18 males and 18 females) isosafrole (vehicle: water) was given by stomach tube from 7 days of age until 28 days of age at 215 mg/kg bw, then subsequently fed *ad libitum* at 517 mg/kg diet for up to 82 weeks. The study included control groups of up to 18 males and up to 18 females per strain. Liver cell tumours occurred in 5/18 males and 1/16 females and in 2/17 males and 0/16 females, pulmonary tumours in 3/18 males and 1/16 females and in 0/17 males and 0/16 females, and lymphomas in 1/18 males and 0/16 females, and in 1/17 males and 0/16 females; of the two strains, respectively. The difference from controls was only statistically significant for the liver tumours (P=0.05) in (C57BL/6 x C3H/Anf)F$_1$ mice (males and females combined) (Innes et al., 1969).

No hepatocarcinogenic activity was found in male B6C3F1 mice given a single i.p. injection (solvent: trioctanoin) of isosafrole (52 % cis-/48 % trans-isomer) to a group of 29 animals or trans-isosafrole (90 % trans-/10 % cis-isomer) to a group of 32 animals, at 12 days of age (dose: 0.75 mmol/kg bw equal to 122 mg/kg bw) and killed at 10 months. Thirty-two animals only given the solvent, served as a control group (Wiseman et al., 1987).
**Rat**

Isosafrole was given in the diet for two years to Osborne-Mendel rats at 0, 1,000, 2,500 or 5,000 mg isosafrole/kg (control group: 35 males and 35 females). At the two lowest dose levels (10 males and 10 females per group) there was slight growth retardation in females. Microscopically, slight hepatic cell hypertrophy but no primary hepatic tumours were reported. At 2,500 mg/kg in the diet there was also slight hyperplasia in the thyroid. At the highest dose, 5,000 mg/kg in the diet (25 males and 25 females) growth retardation was reported in both sexes. The liver was enlarged and microscopically slight hepatic cell hypertrophy, which was usually focal and resulted in the formation of nodules, was shown. Five rats had primary hepatic tumours (two adenomas and three carcinomas). Slight hyperplasia in the thyroid and an increase in the incidence of chronic nephritis were demonstrated. An increased number of interstitial cell tumours was found in testes. The study is poorly reported, and it does not allow a clear NOEL to be established (Hagan et al., 1965; Hagan et al., 1967).

No local tumours were observed in 18 male rats (Charles River CD, random bred) given a total of 20 (twice weekly) s.c. injections, each of 3 mg isosafrole (trioctanoin solution) per rat. The rats were examined after surviving 18 months (Borchert et al., 1973a).

**Genotoxicity**

*In vitro*

Isosafrole (19.7% cis/78.2% trans isomer) did not induce gene mutations in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 or in *Escherichia coli* WP 2 uvr A with or without S9. It was negative in a *Bacillus subtilis* DNA repair test (Rec assay) without S9 (Sekizawa and Shibamoto, 1982). In contrast to safrole, estragole and methyleugenol, it did not induce UDS in cultured rat hepatocytes (Howes et al., 1990).

**DNA adduct formation**

DNA adduct formation using $^{32}$P post labelling analysis was studied in livers from adult female CD1 mice, isolated 24 hours after i.p. administration of different alkenylbenzenes, including isosafrole (2 or 10 mg/mouse). After administration of isosafrole, only low binding to the mouse liver DNA was demonstrated, with the two major DNA adducts formed in the N²-position of guanine. The low DNA binding could also be expressed by the covalent binding index (CBI) value of about 1 for isosafrole. For comparison the CBI values for safrole, estragole and methyleugenol were all about 30 (Randerath et al., 1984).

**Reproduction and developmental toxicity**

No data available.
Human data

No data available.

Other studies

Isosafrole is a known inducer of some of the liver enzymes of the cytochrome P-450 group in rodents, especially CYP1A2 (cf. Ryan et al., 1980; Waxman et al., 1985; Murray and Reidy, 1989; Ishida et al., 1998; Allis et al., 2002).

Summary of hazard identification / characterization

Isosafrole is an efficient inducer of some of the liver cytochrome P-450’s, and is a weak liver carcinogen in rats and mice. Liver DNA adduct formation ($^{32}$P post labelling) is low. All genotoxicity tests are negative. Overall, these data provide support for a non-genotoxic mechanism of hepatocarcinogenicity associated with hepatic enzyme induction, but the available data from carcinogenicity studies in mouse and rat do not allow establishing a clear NOEL.

Finally, it cannot be completely excluded that high exposure to isosafrole may give rise to some isomerisation of 3’-hydroxyisosafrole to 1’-hydroxysafrole, the anticipated proximate carcinogen of safrole.

Conclusion

Isosafrole is a weak rodent hepatocarcinogen; the carcinogenicity is probably mediated by a non-genotoxic mechanism. Isosafrole metabolites may give rise to very low binding to liver DNA in mice. It cannot be excluded that high exposure to isosafrole may give rise to isomerisation of 3’-hydroxy-isosafrole to 1’-hydroxysafrole, the proximate carcinogen metabolite of safrole. However, generally exposure to isosafrole is estimated to be very low.

A clear NOEL could not be demonstrated for hepatic effects in the long-term studies. Therefore, the Committee could not establish a TDI. The Committee notes that isosafrole occurs together with safrole, but at much lower concentrations. Any measure to restrict exposure to safrole in food would also cover isosafrole.
References


