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Opinion of the Scientific Committee on Food on Methyleugenol (4-Allyl-1,2-dimethoxybenzene)

(adopted on 26 September 2001)

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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 implications for human health of methyleugenol (4-allyl-1,2-dimethoxybenzene) in the diet.

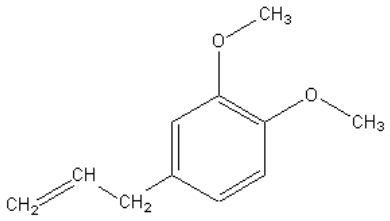
Introduction

In 1999 methyleugenol was evaluated by the Committee of Experts on Flavouring Substances of the Council of Europe. The conclusions of this Committee were: "Available data show that methyleugenol is a naturally-occurring genotoxic carcinogen compound with a DNA-binding potency similar to that of safrole. Human exposure to methyleugenol may occur through the consumption of foodstuffs flavoured with aromatic plants and/or their essential oil fractions which contain methyleugenol. In view of the carcinogenic potential of methyleugenol, it is recommended that absence of methyleugenol in food products be ensured and checked with the most effective available analytical method". (Council of Europe, 1999).

Chemical characterisation

Name:	Methyleugenol (4-Allyl-1,2-dimethoxybenzene)
Synonyms:	4-Allylveratrole; Eugenyl methyl ether; Methyl eugenol; 1,2-Dimethoxy-4-allylbenzene; 3,4-Dimethoxyallylbenzene
CAS No:	93-15-12
FEMA No:	2475
CoE No:	185
EINECS:	202-223-0

Structure:



Exposure assessment

Methyleugenol is a natural constituent of a number of plants such as nutmeg, pimento, lemongrass, tarragon, basil, star anise and fennel. Methyleugenol is used as a flavouring agent in jellies, baked goods, non-alcoholic beverages, chewing gum, relish and ice cream, and as a fragrance in several cosmetic products (Council of Europe, 1999).

Intake estimates of flavouring substances are very poor because of the lack of data about the concentration of these chemicals naturally occurring or voluntarily added in foodstuffs. Within the Council of Europe, UK and France provided calculations based on their respective food consumption data and on concentration levels documented or assumed.

Food consumption

There was no difference between the methodologies of food intake assessment as carried out by UK and France. The methodology was based on a seven days dietary record of adult individuals. The under-reporting subjects were excluded following the same method. In both surveys consumers were identified to assess the intake for consumers only. The respective methodologies have been described elsewhere (Council of Europe, 2001a and 2000b; Observatoire des consommations alimentaires, 1998).

Flavours concentration

The intakes of methyleugenol estimated by the UK (Council of Europe, 2001a and 2000b) were based on maximum use levels of methyleugenol obtained from source materials, i.e. essential oils. These use levels were provided by the International Organisation of the Flavour Industry (Council of Europe, 2000).

The following food categories were considered: non-alcoholic beverages (including all soft drinks and fruit juices), alcoholic beverages (liqueurs only), ices (including ice cream and ice lollies), candy (excluding chocolate), baked goods, gelatine-based desserts, meat products and condiments and relishes (including sauces and spreads).

Intake estimate

Using the above assumptions the average intake (for consumers only) amounts to 13 mg/person/day and the 97.5th percentile was 36 mg/person/day. If expressed on a body weight basis these values correspond to 0.19 and 0.53 mg/kg bw/day, respectively.

Hazard identification/characterisation

Absorption, distribution, metabolism and excretion

Methyleugenol belongs to the chemical class of alk-2-enylbenzenes comprising, among others, safrole, estragole, eugenol and myristicin. It is rapidly absorbed following oral administration to rats and mice. Kinetics data in rats are consistent with a rapid clearance from the blood, metabolism in the liver mediated by the P450 system, and excretion in the urine. Following administration of a single oral dose of methyleugenol (200 mg/kg bw) to rats, several urinary metabolites were identified (Solheim and Scheline, 1973). The major metabolic pathways included the oxidation of the allylic side chain, the formation of the hydroxyl acid via epoxidation of the double bond followed by hydroxylation of the benzene ring and O-demethylation. Excretion of metabolites was found in bile (Delaforge et al., 1980). The hydroxylation of C-1' of the allylic side chain, with formation of 1'-hydroxymethyleugenol is catalysed by CYP2E1 and most probably by CYP2C6, but not by CYP3A, CYP1A2, CYP2D1 or CYP2C11 (Gardner et al., 1997). Administration of high doses of methyleugenol to rats (at least 30 mg/kg bw/day for 25 days) caused dose-dependent auto-induction of 1'-hydroxylation of methyleugenol, mediated by various cytochrome P450 isozymes.

The auto-induction was not observed in rats treated at a lower dose (10 mg/kg bw/day for 5 days). The same authors showed that the rate of 1'-hydroxylation of methyleugenol *in vitro* in 13 human liver samples varied markedly (by 37-fold), with the highest activities being similar to that evident in control rat liver microsomes. This suggests that the risk posed by dietary ingestion of methyleugenol varies markedly in the human population.

There is some evidence that methyleugenol is eliminated more rapidly in males than in females after treatment of rats for 6 or 12 months. This suggests that metabolic induction may occur to a greater extent in males than in females (NTP, 1998).

Acute toxicity

Methyleugenol is moderately toxic. The median lethal oral doses were 810 to 1560 mg/kg bw for rats and 540 mg/kg bw for mice. The undiluted chemical (98% purity) was neither an eye irritant nor a skin irritant (NTP, 1998).

Subchronic toxicity

In a 14-week study (Abdo et al., 2001), a dose of 0, 10, 30, 100, 300 or 1000 mg/kg bw methyleugenol in 0.5% methylcellulose was administered to female and male F344/N rats by gavage for 5 days per week. All rats survived until the end of the study. A significant reduction in weight gain was observed in both sexes treated at the two highest doses. Clinical chemistry indicated hepatocellular damage in both sexes at doses of 100 mg/kg bw or higher. Cholestasis or other altered hepatic functions, hypoproteinemia and

hypoalbuminemia occurred in males and females treated with 300 or 1000 mg/kg bw Liver weights of 100, 300, 1000 mg/kg males and 300 and 1000 mg/kg females and testis weights of 1000 mg/kg males were significantly increased. Male and female rats administered 300 or 1000 mg/kg bw also showed atrophic gastritis; increased incidences of adrenal gland cortical hypertrophy occurred in rats treated with dose of 100 mg/kg bw and higher. The no-observed-effect level (NOEL) was estimated to be at 30 mg/kg bw/day.

A similar study was carried out with B6C3F1 mice (Abdo et al., 2001). All but one male and all female mice at 1000 mg/kg bw died before the scheduled end of the study. A significant reduction of body weight gain was observed in both sexes in the 300 mg/kg bw groups. Liver weights of 30, 100 and 300 mg/kg bw males and 300 mg/kg bw females were significantly increased. Increased incidences of cytological alteration, necrosis, bile duct hyperplasia, and subacute inflammation were observed in the liver of males treated at 1000 mg/kg bw and females treated at 300 and 1000 mg/kg bw. Incidences of atrophy, necrosis, oedema, mitotic alteration, and cystic glands of the fundic region of the glandular stomach were increased in one or more groups administered 30 mg/kg bw or more. The NOEL of methyleugenol was estimated to be at 10 mg/kg bw/day (Abdo et al., 2001).

Chronic toxicity/carcinogenicity

Pre-weanling B6C3F1 male mice were treated by i.p. injection of trioctanoin solution of methyleugenol twice weekly for 12 weeks. The total dose was 42.4 mg/kg bw. Data obtained at 18 months (N° of animals examined: 58 treated and 58 controls) showed a statistically significant increase in the percentage of mice bearing hepatomas (96% in the treated animals, 41% in the controls). In this study methyleugenol had an activity similar to that of its 1'-hydroxy metabolite (hepatoma-bearing mice 93% versus 41% in the controls) and to that of estragole (hepatoma-bearing mice 83% versus 41% in the controls). (Miller et al., 1983)

Methyleugenol was tested for toxicity/carcinogenicity in a 2-year NTP study in rats and mice (NTP, 1998; Johnson et al., 2000). F344/N rats and B6C3F1 mice (50 animals/ sex/dose/group) were given methyleugenol suspended in 0.5 % methylcellulose by gavage at doses of 37, 75, 150, mg/kg bw/day, 5 days per week, for 2 years. Control groups (60 rats/sex and 50 mice/sex) received only the vehicle. A stop-exposure group of 60 rats/sex received 300 mg/kg/day by gavage for 53 weeks followed by the vehicle only for the remaining 52 weeks. All male rats given 150 and 300 mg/kg/day died before the end of the study; survival of female rats given 150 mg/kg/day and of all treated female mice was decreased. Mean body weights of treated male and female rats and mice were lower than in the controls. Target organs included the liver, glandular stomach, forestomach (female rats) and kidney, mammary gland, and subcutaneous tissue (male rats). Liver neoplasms occurred in all dose groups of rats and mice and included hepatoadenoma, hepatocarcinoma, hepatocholangioma (rats only), hepatocholangiocarcinoma, and hepatoblastoma (mice only). Glandular stomach lesions in rats and mice included benign and malignant neuroendocrine tumours. In female rats, the forestomach showed a positive trend in the incidences of squamous cell papilloma or carcinoma

(combined). Male rats also exhibited kidney, mammary gland, subcutaneous tissue tumours as well as mesotheliomas.
The NTP concluded that there was clear evidence of carcinogenic activity in male and female F344/N rats, based on the increased incidences of liver neoplasms and neuroendocrine tumours of the glandular stomach in male and female rats and the increased incidences of kidney neoplasms, malignant fibroma and fibroma or fibrosarcoma (combined) in male rats. A marginal increase in squamous cell neoplasms of the forestomach may have been related to methyleugenol administration in female rats.
The NTP also concluded that there was clear evidence of carcinogenic activity of methyleugenol in male and female B6C3F1 mice based on the increased incidences of liver neoplasms in males and females. Formation of neuroendocrine tumours of the glandular stomach in male mice was also considered to be related to exposure to methyleugenol.
The findings of these studies indicate that even at the lowest dose (37 mg/kg bw/day) a carcinogenic effect (significant increase of hepatocellular carcinomas in male and female mice) was observed and that methyleugenol has to be considered a multisite, multispecies carcinogen.

Genotoxicity

In vitro
In the NTP TR 491 (1998) it was reported that methyleugenol was not mutagenic in *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) with and without exogenous metabolic activation (S9). It was also reported that methyleugenol was unable to induce chromosomal aberrations in CHO cells while induced sister chromatid exchanges (SCEs) occurred only in the presence of metabolic activation (S9). Further studies confirmed the non-mutagenicity of methyleugenol in various strains of *S. typhimurium* and in the *Escherichia coli* WP2 uvrA strain with and without metabolic activation (S9) (Sezikawa et al., 1982).
Methyleugenol was found able to induce intra-chromosomal recombination in *Saccharomyces cerevisiae* with and without metabolic activation (Schiestl et al., 1989). Saturated and monofluoro analogues showed reduced genotoxic activity in *S. cerevisiae* (Brennan et al., 1996).
Methyleugenol, 1'-hydroxymethyleugenol and 2',3'-epoxymethyleugenol induced unscheduled DNA synthesis (UDS) in cultured rat hepatocytes (Chan and Caldwell, 1992). The 1'-hydroxy metabolite is a stronger inducer of UDS than the parent substance.

In vivo
Methyleugenol was reported negative in a micronucleus assay in mice treated by gavage with methyleugenol for 14 weeks at doses up to 1000 mg/kg bw (NTP, 1998).
In 20/29 mouse hepatocellular carcinomas induced by methyleugenol, mutations in the beta-catenin gene were observed, while only in 2/22 spontaneous liver tumours such mutations were found (Devereux et al., 1999). The activation of the beta-catenin gene, with the subsequent deregulation of the Wnt signal transduction route, has been

considered as an early event in chemically induced mouse hepatic carcinogenesis. These results represent a further indication of the genotoxic potential of methyleugenol.

DNA adducts

Methyleugenol formed adducts with DNA and proteins in human fibroblasts V79 cells transfected with human genes expressing sulfotransferase and in the mouse liver in vivo (Stening et al., 1997; Randerath et al., 1984; Phillips et al., 1984).

In particular, induction of liver DNA adducts was shown in a ^{32}P-post-labelling study (Randerath et al., 1984) in which adult CD-1 female mice was given by i.p. injection 100 or 500 mg/kg bw methyleugenol, estragole, safrole and other alkenylbenzene derivatives. The DNA binding activities of methyleugenol, estragole and safrole were higher than those of other alkenylbenzene derivatives.

In a related study (Phillips et al., 1984) newborn male B6C3F1 mice were treated by i.p. injection on day 1, 8, 15 and 22 after birth with the same series of compounds (doses: 0.25, 0.5, 1.0 and 3 μmol). The auto-radiographic map by a modified ^{32}P-post-labelling procedure showed a pattern very similar to that obtained with safrole and estragole, with liver DNA adducts prevalently on the N^2 of guanine and, with less extent, on the N^6 of adenine. The adduct level formed with methyleugenol (72.7 pmol/mg DNA) was higher than those induced by estragole (30.0 pmol/mg DNA) and safrole (14.7 pmol/mg DNA).

Reproductive and developmental toxicity

No information was found.

Human data

No epidemiological data were found.

Summary

Methyleugenol is a multisite, multispecies carcinogen. Both in mice and rats methyleugenol induces different types of liver tumours as well as neuroendocrine tumours in the glandular stomach. In rats, other types of tumours include neoplasms in the forestomach, kidney, mammary gland, subcutaneous tissue as well as mesotheliomas. Both in mice and rats, liver tumours were observed at the lowest dose used (37 mg/kg bw/day). Following oral administration to rats, the main metabolic reaction is the oxidation of the allylic side chain via 1'-hydroxylation and 2',3'-epoxide formation.

High doses of methyleugenol (at least 30 mg/kg bw for 25 days) cause auto-induction of 1'-hydroxylation by P450 cytochromes, with formation of the proximate carcinogen 1'-hydroxymethyleugenol. The auto-induction was not observed at lower doses (e.g. 10 mg/kg bw/day for 5 days). Methyleugenol was non mutagenic in bacterial cells and not clastogenic in mammalian cells. By analogy with estragole, this is probably due to insufficient metabolic activation. Methyleugenol and its two metabolites 1-hydroxymethyleugenol and 2',3'-epoxymethyleugenol induced unscheduled DNA synthesis (UDS) in vitro. In addition, methyleugenol formed DNA adducts *in vitro* and *in vivo*, similarly to safrole and estragole.

Conclusion

Methyleugenol has been demonstrated to be genotoxic and carcinogenic. Therefore the existence of a threshold cannot be assumed and the Committee could not establish a safe exposure limit. Consequently, reductions in exposure and restrictions in use levels are indicated.

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