



EUROPEAN COMMISSION

HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions

C2 - Management of scientific committees II; scientific co-operation and networks

Scientific Committee on Food

SCF/CS/FLAV/FLAVOUR/6 ADD2 FINAL

26 September 2001

Opinion of the Scientific Committee on Food on Estragole (1-Allyl-4-methoxybenzene)

(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 estragole (1-allyl-4-methoxybenzene) in the diet.

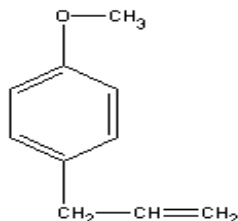
Introduction

Estragole was generally recognised as safe (GRAS) by the Expert Panel of the Flavor and Extract Manufacturers' Association (Hall and Oser, 1965) and is approved by the US Food and Drug Administration (FDA) for food use (21 CFR 121.1164). In 1981 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated estragole as follows: «....Estragole and its metabolites have been shown to be mutagenic in bacterial systems (Ames test) and to produce hepatomas in a susceptible strain of mice. The available toxicological studies were not adequate for evaluation. No ADI was allocated. The Committee requested additional long-term studies for evaluation of carcinogenic potential before an ADI can be established.» (WHO, 1981).

In 2000 the Committee of Experts on Flavouring Substances (CEFS) of the Council of Europe evaluated estragole as follows: «Available data show that estragole is a naturally occurring genotoxic carcinogen in experimental animals after chronic exposure or after few repeated doses. 1'-Hydroxyestragole, the supposed proximate carcinogen, has been found also in the urine of men dosed with 1 µg/kg bw, corresponding to the average exposure levels of humans (Sangster et al., 1987). In order to better assess the risk associated with estragole, then long-term carcinogenicity studies with rats and mice of both sexes and a wide range of dose levels of estragole are needed. In the mean time, a limit of 0.05 mg/kg (detection limit) is recommended.» (Council of Europe, 2000).

Chemical characterisation

Name: Estragole (1-Allyl-4-methoxybenzene)
Synonyms: 1-Methoxy-4-(2-propenyl)benzene; Estragol; Estragon; p-Allyl anisole; Chavicyl methyl ether; Isoanethole; Methyl chavicol
CAS No: 140-67-0
FEMA No: 2411
CoE No: 184
EINECS: 205-427-8
Structure:



Exposure assessment

Estragole occurs naturally in a variety of foods including tarragon (60-75% of essential oil), sweet basil (20-43% of essential oil), sweet fennel (5-20% of essential oil), anis vert (1% of essential oil), and anis star (5-6% of essential oil) (Council of Europe, 2000).

Intake estimates of flavouring substances are generally very poor because of the lack of data on the concentrations 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, 2001; Observatoire des consommations alimentaires, 1998).

Flavours concentration

The estimate provided by the "Observatoire des consommations alimentaires" (1998) was based on maximum limits for flavourings in industrially prepared foods and therefore on the amount of estragole potentially added to foods. The information on the quantity of foods to which estragole can be added was provided by industry. It was assumed that a consumer consumes randomly both industrially prepared and home-made foodstuffs.

The exposure assessment was based on a selection of 28 food categories identified by industry to which estragole can be added. For these food categories, a concentration of 10 mg estragole/kg food was assumed for food in general and a concentration of 50 mg/kg for food containing herbs and spices. For alcoholic beverages, canned fish and fats and oils, the following concentrations were applied as specified by the Council of Europe (2001): alcoholic beverages 100 mg/kg; canned fish 50 mg/kg; fats and oils 250 mg/kg. Finally, the following correction factors were applied for the percentage of industrially prepared products. For alcoholic beverages 4% of the market share will contain 100 mg/kg; for canned fish 30% of market share will contain 50 mg/kg and for fats and oils 1% of market share will contain 250 mg/kg (Observatoire des consommations alimentaires, 1998).

Intake estimate

Using the above assumptions, the estimated average intake (for consumers only) amounts to 4.3 mg/day and the 97.5th percentile to 8.7 mg/day. These intake estimates are in the same order of magnitude as those reported by the Council of Europe (1995). The Committee was unable to estimate the relative contributions to total exposure to estragole from food containing herbs and spices or from the use of added flavourings.

Hazard identification/characterisation

Absorption, distribution, metabolism and excretion

Estragole belongs to the class of alk-2-enylbenzenes comprising, among others, safrole, methyleugenol, eugenol and myristicin. The major metabolic pathways of estragole have been established in rats and mice (Anthony et al., 1987). At low doses estragole mainly undergoes O-demethylation, of which CO₂ is the terminal metabolite, but as the dose is increased, the proportion of O-demethylation falls and other pathways, notably 1'-hydroxylation, come into prominence. Single doses of estragole in the range of 0.05 to 50 mg/kg bw administered to female Wistar albino rats by oral intubation, were largely (52-58%) excreted as CO₂. At higher doses (500 and 1000 mg/kg bw) CO₂ excretion only accounted for 28-29% of the administered dose. The metabolite 1'-hydroxyestragole excreted in the urine accounted for 1.3-5.4% of the dose in the range 0.05 to 50 mg/kg bw or for 11.4-13.7% in the dose range 500-1000 mg/kg bw. Comparable dose fractions were excreted as 1'-hydroxyestragole and CO₂ by CD-1 mice dosed i.p. with 0.05 to 50 mg/kg bw estragole. These data indicate that O-demethylation was more important than 1'-hydroxylation in the low dose range (Anthony et al., 1987; Zangouras et al., 1981). Concerning human studies it has been reported that after oral administration of estragole to two volunteers (100 µg/day for 6 months) the excretion of 1'-hydroxyestragole in the urine amounted to 0.2 and 0.4% of the administered dose (Sangster et al., 1987).

Acute/sub-acute/sub-chronic toxicity

No data were found.

Chronic toxicity/carcinogenicity

It has been reported that estragole and its metabolite 1'-hydroxyestragole, induce hepatic tumours in CD-1 or B6C3F1 mice either after dietary chronic exposure or after i.p. or s.c. injections prior to or after weaning (males appear to be more susceptible than females) (Drinkwater et al., 1976; Miller et al., 1983; Wiseman et al., 1987).

The results of a series of studies carried out by Miller et al. (1983) can be summarised as follows:

Mice

- 1) Pre-weanling CD-1 mice (group of about 50 animals) were administered 370 mg/kg bw of estragole ten times, twice weekly by gavage. The study was terminated at 14 months. The mice bearing hepatomas were 36/49 (73%) in the males versus 14/59 (24%) in the controls, and 4/44 (9%) in the females versus 1/47 (2%) in the controls. A small increase of lung adenomas was induced only in the males: 7/49 (15%) versus zero in the controls.
- 2) Pre-weanling CD-1 male mice (groups of about 50 animals) were administered a total dose of 70 mg/kg bw of estragole or estragole 2',3'-oxide, distributed by four weekly i.p. injections in the first three weeks of life. At 12 months the mice bearing hepatomas treated with estragole were 30/46 (65%) versus 11/42 (24%) in the controls. 40% of the mice treated with estragole 2',3'-oxide showed hepatomas versus 26% in the controls. The mice with lung adenomas were 4/46 (9%) versus 1/42 (2%) in the controls.
- 3) Groups of 50 CD-1 female mice, approximately 8 weeks old, were maintained for 12 months on grain diets containing 2300 or 4600 ppm estragole or 2500 ppm 1'-hydroxyestragole. According to the authors the dietary levels corresponded to an average daily intake of 150-300 and 300-600 mg/kg bw for the mice treated with estragole and 180-360 mg/kg bw for the mice treated with 1'-hydroxyestragole. The incidences of hepatoma-bearing mice at the end of the study were: 56% and 71% for mice treated with estragole and 56% for mice treated with 1'-hydroxyestragole. No hepatomas were found in the controls. Four mice treated with estragole at the highest dose (4600 ppm in the diet) showed angiosarcomas.
- 4) Groups of 40 CD-1 female mice were treated topically four days/week for 6 weeks with two epoxides, estragole 2',3'-oxide and 1'-hydroxyestragole 2',3'-oxide (11.2 µmol). 1 week after the last dose, croton oil was applied. The study was terminated at 40 weeks and showed a significant increase of benign skin tumours (papillomas and keratoacanthomas): 33% versus 7% in the controls for estragole 2',3'-oxide and 44% versus 7% for 1'-hydroxyestragole 2',3'-oxide.
- 5) Pre-weanling B6C3F₁ male mice (groups of about 50 animals) were administered a total dose of 31.8 mg/kg bw of estragole by four i.p. injections with the same schedule as described in 2). The study was terminated at 18 months. The mice bearing hepatomas were 34/41 (83%) versus 24/58 (41%) in the controls.
- 6) In another group (49 animals) the reactive metabolite 1'-hydroxyestragole was administered with the same schedule (total dose 15.6 mg/kg bw) and the study was terminated at 12 months. The hepatoma-bearing mice were 25/27 (93%) versus 5/32 (15%) in the controls.

Rats

Groups of 20 male Fischer rats were administered by subcutaneous injection 1'-hydroxyestragole, 1'-hydroxyestragole 2',3'-oxide, twice weekly for 10 weeks (total dose: 900 mg/kg bw). Besides three sarcomas (1'-hydroxyestragole) and one sarcoma (estragole 2',3'-oxide), one hepatic carcinoma was induced by 1'-hydroxyestragole and one by estragole 2',3'-oxide. No tumours were found in the controls. In the same study 1'-hydroxysafrole (2 µmol/rat) induced 11 hepatic carcinomas (Miller et al., 1983).

Genotoxicity

In vitro

Estragole was non-mutagenic in the Ames test (Zeiger et al., 1987) and in the *Escherichia coli* WP2 uvrA reversion test and negative in the *Bacillus subtilis* repair test (Rec-assay) without S9 (Sezikawa and Shibamoto, 1982). Highly purified 1'-hydroxyestragole was mutagenic for strain TA100 in the absence of fortified liver microsomes. Supplementation with NADPH-fortified rat liver microsomes and cytosol increased the mutagenic activity of 1'-hydroxyestragole and estragole. The electrophilic 2',3'-oxides of 1'-hydroxyestragole and estragole showed dose-dependent mutagenic activities for strain TA1535 in the absence of fortified liver microsomes (Swanson et al., 1979). Estragole has been found to be very weakly mutagenic in TA1535 strain of *S. typhimurium*, however, the addition of 3'-phospho-adenosine-5'-phosphosulphate (PAPS) to the microsomal assay markedly increased the mutagenic activity of estragole (Leleng et al., 1982). This result suggests that estragole and related compounds (e.g. safrole) may be converted to DNA-binding sulphuric acid esters under these conditions.

Estragole (10^{-3} - 10^{-5} M) was unable to induce chromosomal aberrations in V79 Hamster cells in the presence and in the absence of exogenous metabolic activation (S9) or in primary rat hepatocytes. In the same range of concentrations estragole was able to induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes (Müller et al., 1994). Estragole, methyleugenol and their 1'-hydroxy metabolites induced UDS in cultured hepatocytes derived from male Fischer 344 rats (Chan and Caldwell, 1992). The 1'-hydroxy-derivatives were more potent genotoxins than their parent compounds.

In vivo

In an *in vivo* liver UDS assay rats were treated with estragole at doses of 500, 1000 and 2000 mg/kg bw. UDS was induced only at the highest dose (Müller et al., 1994).

DNA adducts

Randerath et al. (1984) showed two major DNA adducts and two minor DNA adducts in enzymatic digests of hepatic DNA from mice treated i.p. with either 1'-hydroxyestragole or 1'-hydroxysafrole. Two of these adducts were characterised as N²-(trans-isoestragol-3'-yl) deoxyguanosine and N⁶-(trans-isoestragol-3'-yl) deoxy-adenosine. Further characterisation of the DNA adducts formed by electrophilic esters of 1'-hydroxyestragole and 1'-hydroxysafrole *in vitro* and in mouse liver *in vivo*, including new adducts at C-8 and N-7 of guanine residues was reported by Wiseman et al. (1985). In a previous study Phillips et

al. (1984) found that estragole, methyleugenol and safrole induced adducts to liver DNA of newborn male B6C3F1 mice treated by i.p. injection on day 1, 8, 15 and 22 after birth at doses of 0.25, 0.5, 1.0 and 3.0 μmol per animal. The adduct levels with methyleugenol (72.7 pmol/mg DNA) were higher than those with estragole (30.0 pmol/mg DNA) and safrole (14.7 pmol/mg DNA).

Reproductive and developmental toxicity

No data were found.

Neurotoxicity

No data were found.

Human data

No epidemiological data were found.

Summary

Several studies, limited with respect to the standard long-term bio-assays, have shown that estragole is a weak inducer of hepatocarcinogenicity in mice treated orally, by i.p. or s.c. injection. The induction of liver tumours seems to depend on formation of 1'-hydroxymetabolites. Metabolic studies indicate that in the high dose range of carcinogenicity studies (150-600 mg/kg bw) the production of 1'-hydroxyestragole, expressed as percentage of the dose, is about 5-10 times higher than that at lower doses (0.05-50 mg/kg bw). 1'-Hydroxyestragole has been found also in the urine of men dosed with 100 μg estragole/day for 6 months. The limited genotoxicity studies show that estragole is not mutagenic or very weakly mutagenic in *S. typhimurium*; very likely this is due to lack of appropriate co-factors (e.g. PAPS) in the exogenous metabolic system. The electrophilic epoxides of estragole and 1'-hydroxyestragole are directly mutagenic in *S. typhimurium*. Both estragole and its 1'-hydroxy metabolite induced unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* and estragole also *in vivo*. The formation of hepatic DNA adducts by estragole and by the 1'-hydroxy metabolite of estragole has also been demonstrated in mice.

Conclusion

Estragole 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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