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Evaluation of Medicines for Human Use

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COMMITTEE ON HERBAL MEDICINAL PRODUCTS (HMPC)

FINAL

PUBLIC STATEMENT ON THE USE OF HERBAL MEDICINAL PRODUCTS CONTAINING ESTRAGOLE

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Public statement on the use of herbal medicinal products containing estragole

1. **Estragole (CAS no. 140-67-0 ; $C_{10}H_{12}O$; MW 148.2)**

   Synonyms: 1-allyl-4-methoxybenzene; 1-methoxy-4-(2-propenyl)-benzene estragol; estragon; p-allylanisole; chavicyl methylether; methylchavicol; chavicol methylether; isoanethole.

2. Estragole (ES) is a natural constituent of a number of aromatic plants and their essential oil fractions including among others tarragon, sweet basil, sweet fennel and anise star.

Occurrence of estragole in aromatic plants and/or essential oils

Botanical name	***Common name***	***Plant part used***	***Content***
Agastache foeniculum (*Lophantus anisatus*) (Pursh) Kunze	Giant Hyssop	plant essential oil	555-12.160 ppm 43.7%
Agastache rugosa Kuntz.		essential oil	90%
Agastache sp.		essential oil	46.7-94.6%
Amomum pavieanum		essential oil (rhizome)	92%
Anthriscus cerefolium (L.) Hoffm.	Garden cheroil	essential oil (herb)	75%
Artemisia dranunculus L.	Tarragon	plant	172-7000 ppm
Clausena anisata Hook. f.		essential oil (leaf)	92.7%
Collinsonia anisata		essential oil	80%
Cuminum cyminum L.	Cumin	fruit	30 ppm
Dictamnus albus L.	White fraxinella	shoot (leaf)	200-605 ppm
Escholtzia flava		essential oil	40.5%
Feronia elephantum (F. limonia) Correa		essential oil	92%
Foeniculum vulgare Mill.	Fennel	fruit essential oil	70-4.018 ppm 0.8 - >80%
Hyssopus officinalis L.	Hyssop	shoot	1-260 ppm
Illicium verum Hook f.	Chinese star anise	fruit essential oil	280-6.500 ppm 0.6-6%
Myrtus communis L.	Myrtle	plant	58-88 ppm
Ocimum basilicum L.	Sweet basil	plant essential oil	238-8.780 ppm 5-85%
Ocimum canum Sims.	Schrubby basil	essential oil	52%
Ocimum nudicaule		essential oil	98%
Ocimum selloi		essential oil essential oil (leaf) essential oil (flower)	51.1% 94.95% 92.54%
Ocimum tenuiflorum L.	Anise scanted basil	leaf	39.950 ppm
Origanum majorana L.	Sweet majoram	plant	96-550 ppm
Orthodon methylchavicoliferum		essential oil	75%

Occurrence of estragole in aromatic plants and/or essential oils (continued)			
Botanical name	***Common name***	***Plant part used***	***Content***
Persea americana var. *drymifolia* Mill.	Avocado	essential oil (leaf)	3-85%
Pimenta dioica (L.) Merr.	Jamaica pepper	leaf	3 ppm
Pimenta racemosa (Mill.) Moore	Pimento	leaf	30-10.745 ppm
Pimpinella anisum L.	Anise	fruit	1050 ppm
Piper betle L.	Bayrum tree	essential oil essential oil (leaf)	1.02-4.0% 8%
Solidago odora Ait.	Blue mountain tree	essential oil	75%
Tagetes filifolia		essential oil	61.2%
Tagetes lucida Cav.		essential oil	45%
Vanillosmopsis arborea		essential oil (wood bark)	36%

ES has also been reported to occur in the following plants, but its content has not been mentioned: *Achillea fragrantissima* Del., *Acorus calamus* L.*, Agathosma cerefolium,* Bartl .et Wendl, *Anethum graveolens* L.*, Boswellia serrata* Roxb.*, Cinnamomum aromaticum* Nees., *Cinnamomum verum* J. Presl.*, Commiphora mukul* Hook., *, Dictamnus hispanicus, Glycyrrhiza glabra* L.*, Hyacinthus orientalis* L.*, Magnolia denudata* Desr.*, Magnolia fargesii* Cheng.*, Magnolia kobus* D.C.*, Melilotus officinalis* Desf.*, Micromeria congesta* Boiss. et Hausskn.*, Micromeria myrtifolia* Boiss. et Hohen*, Ocimum gratissimum* L.*, Ocimum sanctum* L.*, Pelargonium sidoides, Pelargonium remiforme, Pinus sp., Pseudocaryophyllus sp., Syzygium aromaticum* (L.) Merr. et Perry.

3. ES was generally recognised as safe (GRAS) by the Expert Panel of the Flavour and Extract Manufacturer's Association (FEMA) and is approved by the US Food and Drug Administration (FDA) for food use (21 CFR (Code of Federal Regulation) 121.1164).
   In 1981 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated ES, and no ADI (Acceptable Daily Intake) was allocated.
   In 2000 the Committee of Experts on Flavouring substances (CEFS) of the Council of Europe evaluated ES and recommended a limit of 0.05 mg/kg (detection limit).

   There are a number of processed foodstuffs including baked foods, frozen dairy, meat products, soft candy and non-alcoholic beverages to which the ES containing plants or their essential oils may be added as flavourings.

4. No data are available on the acute, sub-acute and sub-chronic toxicity of ES.

5. Although no studies of the long-term health effects of human exposure to ES were reported, several studies have demonstrated the carcinogenic effects of ES in mice.
   ES or its metabolites administered to adult or newborn mice of different strains, through different routes of administration, produced malignant liver tumours.
   Administration of ES to adult female CD-1 mice via the diet for 12 months induced increased incidences of hepatocellular carcinomas compared with control mice.

   Administration of ten doses of ES by oral intubation to newborn CD-1 mice produced increased incidences of liver tumours in males, but not females. ES administered by multiple intraperitoneal or subcutaneous injections to newborn male CD-1 mice or multiple intraperitoneal injections to male B6C3F1 mice resulted in high incidences of hepatocellular carcinoma. A single

intraperitoneal dose of ES administered to newborn male B6C3F1 mice was also found to be sufficient to induce a high incidence of liver cancer.

1'-Hydroxyestragole, the putative proximate toxic metabolite of ES, also induced high incidences of liver tumours when administered by subcutaneous injection to newborn CD-1 mice or via intraperitoneal injection to newborn male CD-1, B6C3F1, CeH/HeJ, or C57B1/6J mice, or in the diet for 12 months to adult female CD-1 mice.
Other metabolites of ES (i.e. estragole-2',3'-oxide and 1'-hydroxy-estragole-2',3'-oxide) and synthetic derivatives (i.e. 1'-acetoxyestragole, 1'-hydroxy-2',3'-dehydroestragole, and 1'-acetoxy-2',3'-dehydroestragole) were also potent carcinogens in mice.
The carcinogenicity of ES has not been investigated in the rat, although one subcutaneous injection study of derivatives of ES in male rats did not observe any treatment-related increases in tumours.

6. No data on reproductive toxicity and teratogenicity are available. Several data on the putative mutagenicity of ES have been reported.
ES and its metabolites produced genotoxic effects in bacteria, yeasts, and mammalian cells. Results of mutagenicity testing of ES in *Salmonella typhimurium* were generally negative, likely due to the complex metabolism required for bio activation *in vivo*.
Positive results were reported for ES in strain TA1535 with the addition of the sulphation cofactor 3'-phospho-adenosine-5'-phosphosulphate (PAPS). The putative toxic metabolites of ES, namely 1'-hydroxyestragole and epoxides of ES, were generally positive in mutagenicity assays with or without exogenous activation.
ES produced mixed results in a DNA repair test, exhibiting dose-related DNA damage in *Bacillus subtilis* in one study and exhibiting negative results in *B. subtilis* and *Escherichia coli* in another.
ES and its metabolites induced unscheduled DNA synthesis (UDS) in several studies in human and rat cell lines or *ex vivo* in the livers of rats treated orally with ES.
ES or its metabolite, 1'-hydroxyestragole, administered to mice binds readily to DNA; several DNA adducts have been characterized. The level of binding and the adducts formed are equivalent to those produced by safrole, a structurally related carcinogen.

7. Pharmacokinetic data and metabolic characterization of ES are available.
ES belongs to the class of alk-2-enylbenzenes comprising among others, safrole, methyleugenol, eugenol and myristicin.
The major metabolic pathways of ES have been established in rats and mice. At low doses ES mainly undergoes O-demethylation of which CO_2 is the terminal metabolite, but as the dose is increased, the proportion of O-demethylation falls and other pathways, notably 1'-hydroxylation, came into prominence. Single doses of ES 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 CO_2. At higher doses (500 and 1000 mg/kg bw) CO_2 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 of 0.05 to 50 mg/kg bw or for 11.4-13.7% in the dose range of 500-1000 mg/kg bw.
Comparable dose fractions were excreted as 1'-hydroxyestragole and CO_2 by CD-1 mice dosed intraperitoneally with 0.05 to 50 mg/kg bw ES. These data indicate that O-demethylation was more important than 1'-hydroxylation in the low dose range.

Concerning human studies it has been reported that after oral administration of ES 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.

Conclusions and Recommendations

- Available toxicological data show that ES is a naturally occurring genotoxic carcinogen with a DNA potency similar to the one of safrole.
- The hazard determination uses a mechanism-based approach in which production of the hepatotoxic sulfate conjugate of the 1'-hydroxy metabolite is used to interpret the pathological changes observed in different species of laboratory rodents in chronic and subchronic studies. In the risk evaluation, the effect of dose and metabolic activation on the production of the 1'-hydroxy metabolite in humans and laboratory animals is compared to assess the risk to humans from use of ES is naturally occurring component of a traditional diet and as added flavouring substance.
- Both the qualitative and quantitative aspects of the molecular disposition of ES and its associated toxicological sequelae have been relatively well defined from mammalian studies. Several studies have clearly established that the profiles of metabolism, metabolic activation, and covalent binding are dose dependent and that the relative importance diminishes markedly at low levels of exposure (i.e. these events are not linear with respect to dose). In particular, rodent studies show that these events are minimal probably in the dose range of 1-10 mg/kg body weight, which is approximately 100-1000 times the anticipated human exposure to this substance.
- For these reasons it is concluded that the present exposure to ES resulting from consumption of herbal medicinal products (short time use in adults at recommended posology) does not pose a significant cancer risk.
- Nevertheless, further studies are needed to define both the nature and implications of the dose-response curve in rats at low levels of exposure to ES. In the meantime exposure of ES to sensitive groups such as young children, pregnant and breastfeeding women should be minimised.
- Toxicological assessment of preparations for topical and external use needs further investigation because data on absorption through the skin are missing.

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