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WORKING PARTY ON HERBAL MEDICINAL PRODUCTS

FINAL POSITION PAPER ON THE USE OF HERBAL MEDICINAL PRODUCTS CONTAINING ESTRAGOLE

DISCUSSION IN THE HMPWP	October 2001, July 2002, November 2002, February 2003
RELEASE FOR CONSULTATION	July 2003
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RE-DISCUSSION IN THE HMPWP	November 2003
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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 constitutent of a number of aromatic plants and their essential oil
    fractions including among others tarragon, sweet basil, sweet fennel and anise star.

<table>
<tr><th colspan="4">Occurrence of estragole in aromatic plants and/or essential oils</th></tr>
<tr><th>Botanical name</th><th>Common name</th><th>Plant part used</th><th>Content</th></tr>
<tr><td>Agastache foeniculum (Lophantus anisatus) (Pursh) Kunze</td><td>Giant Hyssop</td><td>plant
essential oil</td><td>555-12.160 ppm
43.7%</td></tr>
<tr><td>Agastache rugosa Kuntz.</td><td></td><td>essential oil</td><td>90%</td></tr>
<tr><td>Agastache sp.</td><td></td><td>essential oil</td><td>46.7-94.6%</td></tr>
<tr><td>Amomum pavieanum</td><td></td><td>essential oil (rhizome)</td><td>92%</td></tr>
<tr><td>Anthriscus cerefolium (L.) Hoffm.</td><td>Garden cheroil</td><td>essential oil (herb)</td><td>75%</td></tr>
<tr><td>Artemisia dranunculus L.</td><td>Tarragon</td><td>plant</td><td>172-7000 ppm</td></tr>
<tr><td>Clausena anisata Hook. f.</td><td></td><td>essential oil (leaf)</td><td>92.7%</td></tr>
<tr><td>Collinsonia anisata</td><td></td><td>essential oil</td><td>80%</td></tr>
<tr><td>Cuminum cyminum L.</td><td>Cumin</td><td>fruit</td><td>30 ppm</td></tr>
<tr><td>Dictamnus albus L.</td><td>White fraxinella</td><td>shoot (leaf)</td><td>200-605 ppm</td></tr>
<tr><td>Escholtzia flava</td><td></td><td>essential oil</td><td>40.5%</td></tr>
<tr><td>Feronia elephantum (F. limonia) Correa</td><td></td><td>essential oil</td><td>92%</td></tr>
<tr><td>Foeniculum vulgare Mill.</td><td>Fennel</td><td>fruit
essential oil</td><td>70-4.018 ppm
0.8 - >80%</td></tr>
<tr><td>Hyssopus officinalis L.</td><td>Hyssop</td><td>shoot</td><td>1-260 ppm</td></tr>
<tr><td>Illicium verum Hook f.</td><td>Chinese star anise</td><td>fruit
essential oil</td><td>280-6.500 ppm
0.6-6%</td></tr>
<tr><td>Myrtus communis L.</td><td>Myrtle</td><td>plant</td><td>58-88 ppm</td></tr>
<tr><td>Ocimum basilicum L.</td><td>Sweet basil</td><td>plant
essential oil</td><td>238-8.780 ppm
5-85%</td></tr>
<tr><td>Ocimum canum Sims.</td><td>Schrubby basil</td><td>essential oil</td><td>52%</td></tr>
<tr><td>Ocimum nudicaule</td><td></td><td>essential oil</td><td>98%</td></tr>
<tr><td>Ocimum selloi</td><td></td><td>essential oil
essential oil (leaf)
essential oil (flower)</td><td>51.1%
94.95%
92.54%</td></tr>
<tr><td>Ocimum tenuiflorum L.</td><td>Anise scanted basil</td><td>leaf</td><td>39.950 ppm</td></tr>
<tr><td>Origanum majorana L.</td><td>Sweet majoram</td><td>plant</td><td>96-550 ppm</td></tr>
<tr><td>Orthodon methylchavicoliferum</td><td></td><td>essential oil</td><td>75%</td></tr>
</table>

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 alo 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 Flavor 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 new-born mice of different strains, through different routes of administration, produced malignant liver tumors.
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 tumors in males, but not females. ES administered by multiple intraperitoneal or subcutaneous injections to newborn male CD-1 mice or multiple intraperitoneal injectioins to

male B6C3F1 mice resulted in high incidences of hepatocellular carcinoma. A single intraperitoneal dose of ES adminstered 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 tumors 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 tumors.

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 genotixic 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 bioactivation *in vivo*.

Positive results were reported for ES in strain TA1535 with the addition of the sulfation cofactor 3'-phospho-adenosine-5'-phosphosulphate (PAPS). The putative toxic metaboli-tes 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 largerly (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 inportant 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 occuring 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 sudies 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 children, pregnant and breastfeeding women should be minimised.
- Toxicological assessment of preparations for topical and external use need further investigation because data on absorption through the skin are missing.

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