London, 23 November 2005
Doc Ref: EMEA/HMPC/138363/2005

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**COMMITTEE ON HERBAL MEDICINAL PRODUCTS
(HMPC)**

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FINAL

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**PUBLIC STATEMENT ON THE USE OF HERBAL MEDICINAL
PRODUCTS CONTAINING METHYLEUGENOL**

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DISCUSSION IN THE HMPC	January 2005 March 2005
RELEASE FOR CONSULTATION	April 2005
DEADLINE FOR COMMENTS	June 2005
REDISCUSSION IN HMPC	November 2005
ADOPTION BY HMPC	November 2005

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Public Statement on the use of herbal medicinal products containing methyleugenol

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1. **Methyleugenol (CAS No. 93-15-2 ; $C_{11}H_{14}O_2$; MW 178.2)**

   Synonyms: 4-allyl-1,2-dimethoxybenzene; 4-allyl-veratrole; 4-allyl-3,4-dimethoxyben-zene; 1,2-dimethoxy-4-allylbenzene; 3,4-dimethoxyallylbenzene ENT 21040; 1-(3,4-di-methoxyphenyl)-2-propene; eugenol methylether; 1,3,4-eugenol methylether; veratrole methylether.

2. **Methyleugenol (ME)** is a natural constituent of the essential oils of a number of plants widely used in foodstuffs as flavouring agents.

<table>
<tr><td colspan="4">Occurrence of methyleugenol in medicinal plants and their essential oils fractions</td></tr>
<tr><th>Botanical name</th><th>Common name</th><th>Plant part used</th><th>Content</th></tr>
<tr><td>Acorus calamus L.</td><td>Sweet flag</td><td>-
rhizome</td><td>1.0%
1.025 ppm</td></tr>
<tr><td>Artemisia demissa L.</td><td></td><td>oil</td><td>8.5%</td></tr>
<tr><td>Artemisia dranunculus L.</td><td>Tarragon</td><td>plant</td><td>290-2900 ppm</td></tr>
<tr><td>Asarum canadense L.</td><td>Snake root</td><td>root oil</td><td>37%</td></tr>
<tr><td>Asarum heterotropoides Maekawa</td><td></td><td>root oil</td><td>47%</td></tr>
<tr><td>Asarum heterotropoides (Xixin) Maekawa</td><td></td><td>herb</td><td>0.51-2.43%</td></tr>
<tr><td>Canarium indicum</td><td></td><td>oil</td><td>300-700 ppm</td></tr>
<tr><td>Cymbopogon flexuosus W. Watson</td><td>Lemongrass</td><td>whole plant oil
leaves</td><td>77.6-82.4% ; 2%
500-1000 ppm</td></tr>
<tr><td>Cymbopogon nardus Rendl.</td><td>Citronella oil</td><td>plant</td><td>51-204 ppm</td></tr>
<tr><td>Cymbopogon winterianus Jow</td><td></td><td>oil
whole plant</td><td>1%
20-60 ppm</td></tr>
<tr><td>Echinophora tenuifolia</td><td></td><td>aerial part oil</td><td>24.8% (Turkey)</td></tr>
<tr><td>Elettaria cardamomum (L.) Maton.</td><td>Cardamom</td><td>oil</td><td>0.1%</td></tr>
<tr><td>Etlingera cevuga</td><td></td><td>rhizome oil</td><td>47.4%</td></tr>
<tr><td>Hyssopus officinalis L.</td><td>Hyssop</td><td>aerial part oil
plant</td><td>43.9% ; 0.54%
100 ppm</td></tr>
<tr><td>Illicium religiosum Sieb. et Zucc.</td><td>Shikimi</td><td>oil</td><td>9.8%</td></tr>
<tr><td>Laurus nobilis L.</td><td>Laurel tree</td><td>leaf</td><td>213-2.608 ppm</td></tr>
<tr><td>Melaleuca bracteata</td><td>Tee tree</td><td>leaf</td><td>2800-9000 ppm</td></tr>
<tr><td>Melaleuca leucadendron L.</td><td>Cajeput tree</td><td>ME-type oil</td><td>99%</td></tr>
<tr><td>Myristica fragrans L.</td><td>Nutmeg</td><td>seed</td><td>20-900 ppm</td></tr>
<tr><td>Myrtis communis L.</td><td>Myrtl</td><td>leaf oil</td><td>0.2-6% ; 2.3%</td></tr>
<tr><td>Ocimum basilicum L.</td><td>Sweet basil</td><td>plant</td><td>13-1.400 ppm</td></tr>
<tr><td>Ocimum suave Willd.</td><td></td><td>oil
leaf and flower oil</td><td>2%
65.49% ; 66.18%</td></tr>
<tr><td>Ocimum surave Willd.</td><td></td><td>shoots</td><td>2.240 ppm</td></tr>
<tr><td>Ocimum gratissimum L.</td><td></td><td>shoots</td><td>9.835 ppm</td></tr>
<tr><td>Ocimum tenuiflorum</td><td></td><td>plant
leaf</td><td>15-100 ppm
50 ppm</td></tr>
<tr><td>Ocotea pretiosa (Nees) Mez.</td><td></td><td>wood oil</td><td>0.1-78%</td></tr>
</table>

Botanical name	Common name	Plant part used	Content
Pelargonium sidoides		leaf oil	4.3%
Peumus boldus Mol.	Boldo	leaf	100-125 ppm
Pimenta dioica (L.) Merr.	Jamaica pepper	leaf	190 ppm
Pimenta officinalis Lindl.		oil	5.0-8.8% ; 10%
Pimenta racemosa (Mill.) Moore	Pimento	leaf	4.31-14.65 ppm
Piper betle L.	Bayrum tree	leaf oil	4.1%
Pseudocaryophyllus guili		leaf oil ; fruits	5%
Sassafras albidum Nees.	Sassafras	root bark oil	1.10%
Satureja montana L.		plant	25-415 ppm
Syzygium aromaticum (L.) Merr. et Perry	Clove tree	flowers	310-340 ppm
Vanillosmopsis arborea		bark oil	5.9%

ME has also been reported to occur in the following plants, but its content has not been determined: *Acacia senegal* Willd.*, Achillea abrotanoides, Agastache rugosa* Kuntz.*, A. foeniculum* (Pursch) Kunze*, A. nepetoides, Alpinia galanga* Willd.*, Artemisia glabella, A. glauca, Asarum sieboldii* Miguel*, Brachanthemum baranovii, Cinnamomum septentrionalis, C. platyphyllum, C. rigidissimum, C. verum* J. Presl.*, Cymbopogon citratus* D.C.*, Daucus carota* L.*, Eucalyptus robusta, E. resinifera* Sm.*, E. gomphocephala, Eugenia caryophyllata* Thunb.*, Gentiana lutea* L.*, Hamamelis virginiana* L.*, Hoslundia opposita* Vahl.*, Hyptis suaveolens* Poit.*, Lippia laxibracteata, L. junelliana, L. integrifolia, L. turbinata* Gris*, Melissa officinalis* L.*, Michelia hedyosperma, Mosla sp., Myristica odorata, Nicotiana tabacum* L.*, Ocimum micranthum, Ocotea odorifera, Perilla fructescens* Britton.*, Piper nigrum* L.*, Pistacia lentiscus* L.*, Pluchea sagittalis* Cabr.*, Rosmarinus officinalis* L.*, Tagetes filifolia, T. mandonii, T. florida* Cav.*, Thapsia sp., Thymus serpylloides ssp. serpylloides, Zieria sp., Zingiber officinale* Rosc.

3. ME was classified by the FDA in 1975 as generally recognised as safe under the conditions of use and approved for use in foods such as jellies, baked goods and ice cream as a flavouring agent and as a fragrance in soaps, lotions and perfumes.

ME is a central nervous system depressant with anaesthetic, hypothermic, myorelaxant and anticonvulsant properties.

4. Little is known about the acute toxicity of ME.

The reported oral LD_{50} values are 850 to 1560 mg/kg for rats and 540 mg/kg for mice.

5. Several data on subchronic toxicity are available.

In a 14-week study, female and male rats were administered by gavage ME (0, 10, 30, 100, 300 or 1000 mg/kg) in 0.5% methylcellulose 5 days/week. A significant reduction of weight gain was observed in all groups administered 300 or 1000 mg/kg. Hepatocellular damage and adrenal hypertrophy at doses of 100 mg/kg or more, cholestasis or other altered hepatic functions, reduced serum total protein and albumin, and atrophic gastritis at doses of 300 or 1000 mg/kg were observed in both sexes.

A similar experiment on B6C3F1 mice showed a significant reduction of body weight gain in both males and females at 300 mg/kg. Liver weights at 30-1000 mg/kg in males and 300-1000 mg/kg in females were significantly increased. Moreover, increased incidences of cytological alteration, necrosis, bile duct hyperplasia, and subacute inflammation were observed in the liver of male mice at 1000 mg/kg and at 300 and 1000 mg/kg in females. Atrophy, degeneration, necrosis, oedema, mitotic alteration, and cystic glands of the fundic region of the glandular stomach were observed at 30 mg/kg in mice.

It is concluded from the results of the 14-week study that the primary sites for the toxicity of ME in rats are the liver and the glandular stomach. The lesions observed in the submandibular salivary

glands of rats were considered secondary to the chemical-related effects in the liver and the glandular stomach.

Based on mortality, body weight gain, clinical chemistry, and microscopic evaluation of mice and rats tissues, the NOEL of ME for both species is 10 mg/kg.

6. Chronic toxicity data on ME show the compound to be a genotoxic carcinogen.

Intraperitoneal injection of ME or its 1'-hydroxy metabolite in pre-weaning mice induced significantly hepatic tumour incidence. In the other 2-year studies both rats and mice were administered 37, 75 or 150 mg/kg by gavage 5 days/week. In all male rat dose groups, increased incidences of liver neoplasms, malignant mesothelioma, mammary gland fibroadenoma and subcutaneous fibroma and fibroma or fibrosarcoma were observed. A marginal increase in squamous cell neoplasms of the forestomach may have been related to ME administration in female rats. In all dosed groups of male and female mice, the incidences of hepatocellular neoplasms and the multiplicity of neoplasms were generally greater than in the vehicle controls. The incidences of hepatoblastoma were significantly increased in all dosed groups of females and slightly increased in 150 mg/kg males. Hepatocholangiocarcinoma was observed in 150 mg/kg females. The incidences of eosinophilic foci, oval cell proliferation, bile duct hyperplasia, and hemosiderin pigmentation were significantly increased in two or more dosed groups of male and/or female mice.

7. No data on reproductive toxicity and teratogenicity are available. On the contrary, several data on the putative mutagenicity of ME have been reported.

ME was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537, with or without exogenous metabolic activation (S9).

In cytogenetic tests with cultured Chinese hamster ovary cells, ME induced sister chromatid exchanges in the presence of S9, but no induction of chromosomal aberrations was noted in cultured Chinese hamster ovary cells following exposure to ME, with or without C9. ME did not show any activity on the *E. coli* Wp^2 reversion test and on the micronucleus test in mice after oral treatment with ME for 14 weeks. However, ME was found to be mutagenic in the yeast assay, in the *Bacillus subtilis* DNA-repair test and in the UDS assay.

In vivo, no increase in the frequency of micronucleated monochromatic erythrocytes was seen in male or female mice administered ME by gavage for 14 weeks.

The metabolites, 2,3-epoxy-ME and 1'-hydroxy-ME, were mutagenic in the Ames-test and UDS assay, respectively. Moreover, ME readily formed adducts with DNA and proteins in both rabbits and mice.

8. Pharmacokinetic data, including metabolism of ME are available.

The high liphophilicity and the extremely rapid absorption of ME may explain the toxicity of this chemical to the liver and the stomach of rats and mice. The octanol-water partition coefficient for ME is estimated at 800, indicating that ME can pass cell membranes easily. Furthermore, the toxicokinetic data for rats and mice show that the time to achieve maximum concentration in the blood is short (app. 5-15 min.).

Because maximum blood concentrations are reached long before the stomach could have emptied it is concluded that ME is absorbed from the stomach. This absorption suggests that ME is transported via the portal vein directly to the liver, resulting in severe hepatic effects observed in dosed rats and mice. In the liver ME is metabolised by the cytochrome P_{450} system, involving 0-demethylation, side-chain hydrolysis and epoxide diol formation. Of the metabolites formed, the two reactive metabolites 1'-hydroxy-ME and the epoxide-diol are most likely to be responsible for the toxic effects at these two sites.

Besides to the liver, ME is after absorption also distributed to the spleen, ovaries, fat and stomach; 85% of the absorbed ME is eliminated within 72 h as metabolites, no unchanged ME is detected in the urine.

Elimination from the bloodstream is rapid, with initial and terminal half-lives in the order of 5 and 120 min., respectively. It is also suggested that metabolic induction may occur to a greater extent in male than in female rats.

Conclusions and Recommendations

- Available toxicological data show that ME 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 ME as naturally occurring component of a traditional diet and as added flavouring substance.
- Both the qualitative and quantitative aspects of the molecular disposition of ME and 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 ME 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 ME. In the meantime, exposure of ME 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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