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International Agency for Research on Cancer

World Health Organization

METHYLEUGENOL

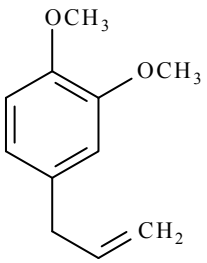
1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 93-15-2
Chem. Abstr. Name: 1,2-Dimethoxy-4-(2-propenyl)benzene
IUPAC Systematic Name: 1,2-Dimethoxy-4-prop-2-en-1-yl-benzene
Synonyms: 1-Allyl-3,4-dimethoxybenzene; 4-allyl-1,2-dimethyoxybenzene; 4-allyl-veratrole; benzene, 4-allyl-1,2-dimethoxy-; benzene, 1,2-dimethoxy-4-(2-propenyl)-; 1,2-dimethoxy-4-allylbenzene; 3,4-dimethoxyallylbenzene; 1-(3,4-dimethoxyphenyl)-2-propene;1,2-dimethoxy-4-(2-propen-1-yl) benzene; 1,3,4-eugenol methyl ether; eugenyl methyl ether; methyleugenol; methyl eugenol; *O*-methyl eugenol; veratrole methyl ether
EINECS No.: 202-223-0

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{11}H_{14}O_2$
Relative molecular mass: 178.23

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless to pale yellow liquid with a clove-carnation odour and a bitter taste (NTP, 2000)
Boiling-point: bp_{30}, 146–147 °C; bp_{760}, 244 °C (O'Neil *et al.*, 2006)
Melting-point: –2 °C (Lide, 2010)
Density: 1.0396 at 20 °C (Lide, 2010)
Solubility: Soluble in ethanol, ethyl ether, chloroform and most other organic solvents; insoluble in water, glycol and propylene glycol (NTP, 2000)
Volatility: Evaporates readily at room temperature (NTP, 2000)
Stability: Darkens and slowly thickens when exposed to air (NTP, 2000)
Octanol/water partition coefficient (P): log K_{ow}, 3.45 (Sangster, 2010)

1.1.4 Technical products and impurities

Methyleugenol is commercially available with the following specifications: purity, 98.0% min.; eugenol, 1.0% max. (Elan Chemical Company, 2007).

1.1.5 Analysis

The presence of methyleugenol in essential oils and aromatic plants can be determined by gas chromatography (GC)–mass spectrometry (MS) (Stanfill & Ashley, 1999; Miele *et al.*, 2001; Kothari *et al.*, 2004; Hamm *et al.*, 2005; Boussaada *et al.*, 2008; Verdian-rizi & Hadjiakhoondi, 2008; Zheljazkov *et al.*, 2008; Pino Benitez *et al.*, 2009; Lamas *et al.*, 2010) and high-performance liquid chromatography with ultraviolet (UV) detection (Chen *et al.*, 2009; Gursale *et al.*, 2010).

Methyleugenol has been determined in cosmetic creams applied to the skin by direct contact sorptive tape extraction–GC–MS (Sgorbini *et al.*, 2010).

Methyleugenol can be measured in human serum through solid-phase extraction followed by isotope dilution GC–high resolution MS, with a limit of detection of 3.1 pg/g (Barr *et al.*, 2000).

1.2 Production and use

1.2.1 Production

Methyleugenol is produced by the methylation of eugenol (Burdock, 2005).

The annual production of methyleugenol in the United States of America in 1990 was estimated at 11.4 tonnes (NTP, 2000).

Information available in 2010 indicated that methyleugenol was manufactured by 19 companies in the USA, four companies in the People's Republic of China, two companies each in Germany and China (Hong Kong SAR), and one company each in France, India, Indonesia, Japan and the United Kingdom (Chemical Sources International, 2010). HSDB (2010) reported three additional companies in the USA that produced methyleugenol.

1.2.2 Use

Methyleugenol is used as a flavouring agent in jellies, baked goods, non-alcoholic beverages, chewing gum, candy, puddings, relishes and ice cream. It is also widely used as a fragrance ingredient in perfumes, toiletries and detergents. Methyleugenol has been used as an anaesthetic in rodents. It also is used as an insect attractant in combination with insecticides (NTP, 2000; HSDB, 2010).

Methyleugenol is a component of several essential oils that are sold for use in aromatherapy, massage oils and alternative medicines (Government of Canada, 2010).

For centuries, fennel fruits have been used as a traditional herbal medicine in Europe and China. It is administered as a carminative to infants in private homes and in maternity clinics and is highly appreciated for its mild flavour and good tolerance. In several European Union (EU) countries, sweet fennel herbal tea is traditionally used for the treatment of symptoms in digestive upsets. In Germany, bitter fennel herbal tea is used by most of the population as a remedy for colds (European Medicines Agency, 2008).

Some essential oils, including citronella (*Cymbopogon* spp.), basil (*Ocimum* spp.), bay (*Laurus nobilis*) and tea tree (*Melaleuca* spp.), that may contain a high percentage of methyleugenol are used as fragrances in consumer products, such as personal care products and household cleaners (Environment Canada, 2010).

Citronella oil, which may contain methyleugenol, is an active ingredient in some commercially available personal insect repellent lotions and sprays that are applied to the skin. It is also used in outdoor candles and torches as an ambient insect repellent (Environment Canada, 2010).

Methyleugenol is used as a fragrance in perfumes (0.3–0.8%), creams and lotions (0.01–0.05%), and soaps and detergents (0.02–0.2%) (NTP, 2000).

1.3 Occurrence

1.3.1 Natural occurrence

Methyleugenol is a natural constituent of a large number of essential oils of plant origin and, in some cases, may be the major constituent.

A comprehensive review of the methyleugenol content of essential oils from different botanical sources has been published (Burfield, 2004a). The data reported in this review were derived from a variety of sources, including unpublished data distributed to its members by the US Flavor and Extract Manufacturers' Association, those related to commercial oils analysed by the British Essential Oil Organization in 2001, those available on the International Fragrance Association web site (www.ifraorg.org,) and those reported in the Agricultural Research Services database (www.ars-grin.gov).

Overall, 118 analytical determinations of methyleugenol in essential oils were considered in the review by Burfield (2004a). In four cases, for example, in *Cinnamomum camphora* (camphor oil, white from China), the substance was not detected. In 73 cases, the reported methyleugenol content was below 2%, for example, in *Artemisia dracunculus* French type (tarragon), *Syzygium aromaticum* (clove), *Daucus carota* (carrot), *Myrstica fragrans* (nutmeg) and *Rosmarinus officinalis* (rosemary). In 10 cases, high methyleugenol contents were reported: *Anasarum canadense* (snakeroot), 36–45%; *Artemisia dracunculus* (tarragon oil Russian type), 5–29%; *Dacrydium franklinii (*Huon pine oil), up to 98%; *Echinophora tenuifolia* from Turkey, 17.5–50%; *Melaleuca bracteata*, up to 50%; *Melaleuca leucadendron*, up to 97%; *Ocotea pretiosa* (Brazilian sassafras), up to 50%; and *Pimenta racemosa* var. racemosa (bay leaf), up to 48.1%. The methyleugenol content of *Ocimum basilicum* (basil) varies considerably according to the reported chemotype, from 1.6% in some products up to 55–65% in the case of var. 'grand vert' and var. minimum (known as small basil) (Burfield, 2004a). Methyleugenol is also the main constituent of the essential oil of *Melaleuca bracteata* F.v.M. leaves (90–95%) and *Cinnamomum oliveri* Bail. leaves (90–95%) (Burdock, 2005). Other data on the content of methyleugenol in aromatic plants have been reported (De Vincenzi *et al.*, 2000).

The amount of methyleugenol in an essential oil extracted from a given type of plant differs according to the variety, plant maturity at the time of harvesting, the method of harvesting, storage conditions and the method of extraction (Smith *et al.*, 2002).

The European Medicines Agency (2005) has reviewed the methyleugenol content of the parts of plants that are generally used. Thyme species are widely used as medicinal herbs (ElHadj Ali *et al.*, 2010), and are increasingly used in perfumery, cosmetic and medicinal applications. The mean content of methyleugenol in the essential oil of *Thymus algeriensis* leaves ranged between < 0.01 and 6.9%.

1.3.2 Occupational exposure

The National Institute for Occupational Safety and Health, in its National Occupational Exposure Survey conducted in 1981–83, estimated that, among the 4490 establishments surveyed (522 industry types, employing approximately 1 800 000 workers), 2824 workers (including 877 women) were potentially exposed to methyleugenol in the USA (NIOSH, 1990).

Methyleugenol was registered as an active pesticide ingredient in the USA in 2006 (EPA, 2006). It is an insect parapheromone, which is attractive to male fruit flies (Australian Government, 2005), and is used in insect traps to attract certain species such as the oriental fruit fly. According to the US Environmental Protection Agency (EPA), because methyleugenol is used for the control of fruit flies in traps, no contact is expected by workers using the traps, but may

occur for workers who prepare the pesticide and methyleugenol mixture or fill the traps with the mixture.

Moreover, aromatherapists are liable to be exposed repeatedly to methyleugenol-containing oils through dermal contact (Burfield, 2004b).

1.3.3 Dietary exposure

(a) Occurrence in food

Some examples of common culinary herbs and spices that contain methyleugenol are basil, tarragon, lemon grass, bay leaf, nutmeg, allspice, cloves and mace (Environment Canada, 2010).

Methyleugenol is also contained in edible fruit such as grapefruit, bananas and some forest fruit at a level of less than 0.1 mg/kg (TNO, 2010). Methyleugenol was identified as a volatile flavour compound in the juice of Kogyoku apples (Yagima *et al.*, 1984).

The methyleugenol content of basil has been studied very extensively (Tsai & Sheen, 1987; Green & Espinosa, 1988, cited by Smith *et al.*, 2002; Lawrence *et al.*, 1988; Bobin *et al.*, 1991; Sheen *et al.*, 1991; Lawrence & Shu, 1993)

(b) Occurrence in food as a flavouring substance or as constituent of essential oils

Processed foodstuffs can be flavoured with essential oils or extracts of specific plants that contain methyleugenol such as sassafras (*Sassafras albidum)* leaf extracts, tarragon (*Artemisia dracunculus* L.), laurel (*Laurus nobilis L.*) and Ceylon citronella (*Cymbopogon nardus*) (Burdock, 2005).

It has been reported to be added as a flavouring agent to baked goods (27–40 mg/kg), chewing gum (10–45 mg/kg), condiments and relishes (3–7 mg/kg), frozen dairy products (15–17 mg/kg), gelatins and puddings (15–17 mg/kg), hard candy (0.6 mg/kg), non-alcoholic beverages (9–12 mg/kg) and soft candy (19–24 mg/kg) (Burdock, 2005).

Some brands of cookies available in the USA were found to contain approximately 3.3 mg/kg methyleugenol as an added flavouring, i.e. 18 µg/cookie. Lower concentrations were found (in decreasing order) in other brands of gingersnaps, cinnamon-flavoured oatmeal, vinaigrette salad dressing, cinnamon-flavoured mints, chewing gum, cake doughnuts and cola beverages. In 20 other brands of gingersnaps and other cookies, doughnuts, colas and foods flavoured with cinnamon, nutmeg or ginger, methyleugenol was either not detected or was found at concentrations < 0.05 mg/kg (Schecter *et al.*, 2004).

Methyleugenol was measured in a limited number of well defined food products purchased on the Italian national market, and was found to be present in tomato sauce containing basil (0.01–0.33 mg/kg) and in Vienna sausage (0.10–0.14 mg/kg), probably due to the addition of nutmeg (Siano *et al.*, 2003).

(c) Estimates of dietary exposure

Ocimum basilicum cv. Genovese Gigante is by far the most popular basil cultivar used in the production of a typical Italian sauce called pesto. Pesto is traditionally prepared with basil that is 10–12 cm in height, when the percentage of methyleugenol in the essential oil is generally more than 40%. Considering that, at this stage of growth, the amount of essential oil in *O. basilicum cv.* Genovese Gigante corresponds to ~0.5% and that one portion of pesto contains ~10 g of basil, the resulting dietary exposure to methyleugenol could reach 250 µg/kg body weight (bw) per meal in adults and 500 µg/kg bw per meal in children (Miele *et al.*, 2001).

Smith *et al.* (2002) estimated dietary exposure to methyleugenol using data on the annual volumes of plant materials with methyleugenol-containing essential oils (principally spices), methyleugenol-containing essential oils used as flavour ingredients and neat methyleugenol used as a flavouring substance imported and

consumed in the USA in 1999. The dietary intake of methyleugenol was estimated for a 60-kg bw adult and the assumption that methyleugenol would be consumed by 10% of the population of the USA. The plant materials considered were bananas and spices (anise, basil (dried and fresh), mace, nutmeg, pimento berry (allspice) and tarragon) and lead to an estimated dietary exposure of 0.50 μg/kg bw per day, mainly from basil (assuming an average concentration of methyleugenol of 2.6% in dried basil and 0.11% in fresh basil), nutmeg and allspice. The essential oils considered were basil, bay (leaves and sweet oil), citronella, clove (bud), nutmeg, pimento berry and pimento leaf and lead to an estimated dietary exposure of 0.16 μg/kg bw per day, mainly from nutmeg oil. The estimated dietary exposure from methyleugenol used as an added flavouring substance was 0.11 μg/kg bw per day. Thus, the overall dietary exposure was estimated to be 0.77 μg/kg bw per day (Smith *et al.*, 2002).

The Joint FAO/WHO Expert Committee on Food Additives published an evaluation of a group of alkoxy-substituted allylbenzenes, including methyleugenol (JECFA, 2009). Per-capita dietary exposure was assessed by dividing the volumes of spices, herbs and oils in the USA and Europe, as reported by industry, by the total population (320 million in Europe and 280 million in the USA), and considering the range of concentrations of methyleugenol in oil samples. The lower/upper limits and mean values for dietary exposure to methyleugenol were: 2.5/424 and 80.5 μg per day (USA) and 0.6/39 and 9.6 μg per day (Europe) (Williams & Mattia, 2009).

Based on annual production volumes of 77 kg methyleugenol in the USA (Gavin *et al.*, 2007), per-capita intake as flavouring agents for the whole population was estimated to be 0.8 μg per day in the USA. Estimated total dietary exposure in the USA would therefore amount to 81.3 μg per day versus 9.6 μg per day in Europe (Smith *et al.*, 2010).

[The per-capita intake assessed by Williams & Mattia (2009) considered the whole population as consumers. Estimates obtained by assuming that 10% of the population were consumers would lead to a total dietary exposure of 813 μg per day in the USA and 96 μg per day in Europe, i.e. 13.5 and 1.6 μg/kg bw per day, respectively, for a 60-kg adult.]

The intake of methyleugenol estimated by the United Kingdom delegation to the Council of Europe was considered by the Scientific Committee on Food (European Commission, 2001). The average intake (for consumers only) amounted to 13 mg/person per day and the 97.5th percentile was 36 mg/person per day. If expressed on the basis of adult size, these values correspond to 0.19 mg/kg bw per day and 0.53 mg/kg bw per day, respectively. These estimates were based on maximum used levels of methyleugenol provided by the European Flavour and Fragrance Association from a global industry survey. [It should be noted that this estimate pre-dated the decision to remove the use of methyleugenol as a flavouring additive from the EU register. In this study, it was assumed that methyleugenol was maximally added to the following food categories: 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, gelatin-based desserts, meat products and condiments and relishes (including sauces and spreads). A United Kingdom survey performed through 7-day dietary records on 2197 adults (16–65 years old) was used as the basis for consumption estimates.]

Smith *et al.* (2010) assessed dietary exposure to methyleugenol using the theoretical added maximum daily intake technique, assuming that maximum levels of methyleugenol in food were those regulated by the EU (European Commission, 2008a). The calculation was made assuming a concomitant daily consumption of 324 g of beverages in general (containing

1 mg/kg methyleugenol), 133.4 g of food in general (20 mg/kg methyleugenol), 20 g of sauces and condiments (60 mg/kg methyleugenol) and 20 g of ready-to-eat savouries (20 mg/kg methyleugenol). A maximum intake of 4.6 mg methyleugenol per day was calculated for the European adult population which, assuming an average body weight of 70 kg, would be equivalent to 66 µg/kg bw per day.

In conclusion, dietary exposure to methyleugenol may arise from: (i) the ingestion of fruits and vegetables containing methyleugenol (minor source), (ii) the ingestion of herbs and spices containing methyleugenol (primary source), (iii) the ingestion of essential oils and extracts of herbs and spices used to flavour food and beverages and (iv) the ingestion of methyleugenol added directly as a flavouring to food and beverages (outside Europe). Average total dietary exposures are in the range of 8–81.3 µg per day, i.e. 0.13 –1.35 µg/kg bw per day for a 60-kg bw adult. Total dietary exposures assessed for regular consumers of food containing methyleugenol are in the range of 66–514 µg/kg bw per day (Smith *et al.*, 2010).

1.3.4 Environmental occurrence

(a) Releases

Methyleugenol was detected at a concentration of 5 ppb [0.005 mg/L] in wastewater from a New Jersey (USA) publicly owned treatment works facility located at an industrial site (industrial contribution to the influent is 18%) (Clark *et al.*, 1991), and in the raw and partially treated effluent of one unbleached kraft paper mill at concentrations of 0.001–0.002 mg/L, but not in the final effluent (Keith, 1976).

Shaver & Bull (1980) described the fate of methyleugenol in the environment after its use in a fruit fly control programme; the study was carried out on soil, water and tomatoes, a representative crop that might be affected by the use of methyleugenol. Moreover, the proposed use of methyleugenol in a male anihilation programme that involved the aerial distribution of cigarette filters saturated with the lure and malathion over fruit fly-infested areas was investigated. Methyleugenol was shown to dissipate rapidly from both soil and water. At 32 °C, 98% of the material was lost within 96 hours, and 77% and 81% were lost from water and soil, respectively, after 96 hours at 22 °C. Methyleugenol had a half-life of approximately 6 hours in soil and water at 32 °C and 16 hours and 34 hours in soil and water at 22 °C, respectively. The persistence of methyleugenol in water was very similar for treatment rates of 1 and 10 mg/100 mL water. Methyleugenol disappeared rapidly from the surface of field-grown tomatoes treated topically with 1 mg/fruit of the fruit fly control product. Only 3.8% of the dose was recovered in the external wash after 24 hours, and none was detected 3, 7 and 14 days after treatment. No methyleugenol was detected in tomatoes from plants that had been exposed to a cigarette filter containing 0.5 mL methyleugenol placed at the base of the plant (Shaver & Bull, 1980).

The California Department of Food and Agriculture began an oriental fruit fly (*Dacus dorsalis*) eradication programme in California (USA) in 1988 (Turner *et al.*, 1989) using methyleugenol (Dorsalure ME) and the pesticide Naled (Dibrom 14 Concentrate). Methyleugenol is used to attract male oriental fruit flies to bait stations that are set up during eradication programmes and to traps placed in fruit trees to detect new infestations.

In October 1988, the Environmental Hazards Assessment Program of the California Department of Food and Agriculture determined the concentrations of methyleugenol in ambient air and fruit during oriental fruit fly trapping for this programme. In Los Angeles county, the air in the vicinity of insect traps baited with methyleugenol was analysed for the presence of the substance 0–5 days after the bait stations were set up. During the first application,

methyleugenol was detected in samples on days 0 (353–1050 ng/m^3) and 1, but not on day 5, at a distance of 5 m from the traps. During the fourth application, concentrations did not decrease significantly over time. The variability in methyleugenol concentrations found during the fourth application is believed to be due to microclimate variations within each site and variable bait application. Whole citrus fruit samples were collected from a detection area in September 1988 in Sacramento County. Methyleugenol was detected in several fruit from two of the four sites sampled at concentrations ranging from 70 to 210 ppb [µg/kg] (Turner *et al.*, 1989).

(b) Terrestrial fate

Based on its physical properties (see Section 1.1.3), methyleugenol is expected to be highly mobile in soil. However, the compound was immobile in a silty loam, Lufkin fine sandy loam, Houston clay and Brazos river bottom sand from Texas (USA) using soil thin-layer chromatography (Shaver, 1984).

The volatilization of methyleugenol from moist soil surfaces is expected to be an important process (HSDB, 2010). Dissipation half-lives of 6 and 16 hours in soil and water at 32 and 22 °C, respectively, have been measured (Shaver & Bull, 1980). Methyleugenol is not expected to volatilize from dry soil surfaces based upon its vapour pressure (Perry & Green, 1984, cited by HSDB, 2010). Biodegradation may be an important environmental process in soil (HSDB, 2010).

(c) Aquatic fate

Based on its physical and chemical properties, methyleugenol is not expected to adsorb to suspended solids and sediment. Volatilization from water surfaces is expected (Lyman, 1990, cited by HSDB, 2010), with half-lives for a model river and model lake of 9 and 69 days, respectively. Dissipation half-lives of 6 and 34 hours in water at 32 and 22 °C, respectively, have been measured (Shaver & Bull, 1980). Its potential for bioconcentration in aquatic organisms is low, but biodegradation may be an important environmental process in water (HSDB, 2010).

(d) Atmospheric fate

Methyleugenol is expected to exist almost entirely as a vapour in the ambient atmosphere (HSDB, 2010). Vapour-phase methyleugenol is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 5 hours at an atmospheric concentration of 5×10^5 hydroxyl radicals per cm^3 (Meylan & Howard, 1993). The rate constant for the vapour-phase reaction of methyleugenol with photochemically produced hydroxyl radicals has been estimated to be 7.5×10^{-11} cm^3/molecule.s at 25 °C using a structure estimation method, which corresponds to an atmospheric half-life of approximately 5 hours (Meylan & Howard, 1993). The rate constant for the vapour-phase reaction of methyleugenol with ozone has been estimated to be 1.2×10^{-17} cm^3/molecule.s at 25 °C using a structure estimation method (Meylan & Howard, 1993), which corresponds to an atmospheric half-life of about 1 day at an atmospheric concentration of 7×10^{11} ozone molecules per cm^3 (Atkinson & Carter, 1984). Methyleugenol is not expected to undergo hydrolysis in the environment due to its lack of hydrolysable functional groups (Lyman, 1990) nor to photolyse directly due to its lack of absorption in the environmental UV spectrum (> 290 nm) (cited by HSDB, 2010).

(e) Environmental exposure models

Based on its physical and chemical properties and taking into consideration its estimated half-lives in air (5 hours), water (measured as 8 days), soil (8 days, estimated to be the same as that in water) and sediments (32 days, estimated at four times that in water), methyleugenol is expected to reside mainly in the environmental compartment into which it is released (Environment Canada, 2010).

1.3.5 Exposure of the general population

The general population may be exposed to methyleugenol via inhalation and by dermal contact with consumer products that contain methyleugenol.

(a) Indoor exposure

Fragrances are present in household products, air fresheners, insecticides and cosmetics, and, because of their nature, inhalation exposure should be considered as an important pathway, especially in indoor environments (Lamas *et al.*, 2010). Because people in developed countries spend up to 90% of their time indoors, inhalation of indoor air is potentially the most important exposure pathway to many pollutants (Brown *et al.*, 1994; Molhave *et al.*, 1997).

(b) Cosmetic ingredients

Potential exposure to methyleugenol from the use of personal care products made with essential oils that contain methyleugenol was assessed by Environment Canada (2010) using consumer exposure modelling software. For adult women, estimated daily systemic exposure to methyleugenol as a result of dermal exposure only through the aggregate use of four types of personal care products (body lotion, face moisturizer, skin cleanser and fragrance) formulated with various essential oils that contain methyleugenol was 1.5 µg/kg bw per day (1 µg/kg bw per day from fragrance, 0.2 µg/kg bw per day from body lotion, 0 µg/kg bw per day from face cream and 0.3 µg/kg bw per day from skin cleanser). These estimates were based on the following assumptions: (i) methyleugenol was present at the upper level authorized in the EU (SCCNFP, 2000) and Canada (Health Canada, 2010); (ii) the dermal absorption of methyleugenol was 40% for products applied to the skin; and (iii) the permeability coefficient was 0.0221 cm/h for skin cleanser that was washed off. The estimates of exposure from the use of personal care products are not expected to differ appreciably across age groups. The concentration of methyleugenol in plant-derived material is quite variable, and there is significant uncertainty associated with these estimates (Environment Canada, 2010).

These estimates do not include exposure arising from the use of dental or oral hygiene products. For example, clove flower oil is licensed for sale in Canada as a non-prescription dental analgesic (Environment Canada, 2010).

In a study in which the skin (stratum corneum) of volunteers was treated with a cream of known composition, approximate permeation of methyleugenol through skin was reported to be 14.5% (Sgorbini *et al.*, 2010). Within a survey of hand soaps performed in Denmark, three products that contained scent of roses were analysed for methyleugenol (methyleugenol is a natural component of rose oil). All were below the limit of detection (10 mg/kg) (Danish EPA, 2006).

(c) Insect repellent

Health Canada (2004) assessed the exposure to methyleugenol due to its presence in citronella oil that is used as a personal insect repellent. Assuming that 1 mg/cm^2 citronella oil is applied, that 25% of the body surface is treated (i.e. 4610 cm^2 in a 70-kg adult and 1641 cm^2 in a 15-kg child) [based on this estimation, the Working Group calculated that each application would result in 66 and 109 mg/kg bw citronella oil for adults and children, respectively] and that the concentration of methyleugenol in the product would be 0.0002%, an exposure of 0.13 µg/kg bw for adults and 0.21 µg/kg bw for children would occur.

(d) Tobacco smoke

In a study of eight commercial brands of cigarettes in the USA, methyleugenol was identified in the smoke particulate of unblocked cigarettes at a level above the limit of detection (1.1 ng/cigarette) in only one brand (average of three measurements: 46.5 ng in the smoke

particulate of one cigarette) (Stanfill & Ashley, 2000). The effect of blocking ventilation holes in the cigarette filter was assessed in another brand (containing 81 ng/cigarette). Methyleugenol was not detected in the unblocked cigarette smoke, whereas it was detected in the smoke when the holes were partially or fully blocked (6.4 ng and 10.8 ng in the smoke particulate of one cigarette, respectively).

Bidi cigarettes (small hand-rolled cigarettes produced primarily in India) are sold in the USA in a wide variety of exotic (e.g. clove and mango) and candy-like flavours (e.g. raspberry, dewberry, chocolate and clove) and are popular among adolescents. Certain tobacco flavourings contain alkenylbenzenes, including methyleugenol (Stanfill *et al.*, 2003, 2006).

Methyleugenol was detected in 11/20 *bidi* cigarettes purchased in the USA and in Indian *bidi* cigarettes at levels ranging from 0.49 µg/g to 61 µg/g. The highest levels were found in Kailas Strawberry brand (47–61 µg/g) followed by Darshan Clove brand (5.1–12 µg/g). Lower levels of methyleugenol were observed in US cigarettes, ranging from 0.018 to 0.021 µg/g (Stanfill *et al.*, 2003).

In a study by Stanfill *et al.* (2006), compounds in the burnable portions of the filler and wrapper material actually consumed during the smoking of *bidis* and US cigarettes were analysed. Methyleugenol was not detected (< 6.3 µg/cigarette) in the three US cigarettes, and was detected in only two *bidis*: Azad clove brand (not detected–7.52 µg/cigarette) and Azad herbal brand (27.7–36.6 µg /cigarette).

In Canada, exposure to methyleugenol through cigarette smoking is liable to decrease because, in May 2009, the Government of Canada introduced amendments to the Tobacco Act to prohibit the sale of cigarettes, little cigars and blunt wraps (leaf-wrapped tobacco) with flavours and additives that taste like candy (Government of Canada, 2009).

1.3.6 Total human exposure

According to the Government of Canada (2010), exposure to methyleugenol is dominated by the ingestion of food and beverages, with smaller contributions from the use of personal care products, cosmetics and citronella-based insect repellents.

(a) Biomonitoring data

The Centers for Disease Control and Prevention in the USA measured methyleugenol in a non-representative subset of adult serum samples collected as a part of the Third National Health and Nutrition Examination Survey conducted during 1988–94 (Barr *et al.*, 2000). The mean methyleugenol concentration in this subset was approximately 24 pg/g serum, and ranged from < 3.1 to 390 pg/g serum. Methyleugenol was detected in 98% of the 206 adult human serum samples analysed, indicating that human exposure in the USA is ubiquitous. Only four individuals had methyleugenol concentrations below the limit of detection (< 3.1 pg/g). The 5–95% distribution was 5–78 pg/g in serum. Bivariate and multivariate analyses using selected demographic variables showed only marginal relationships between race/ethnicity and sex/fasting status and methyleugenol serum concentrations. The data on integrated exposure to methyleugenol derived from biomonitoring indicate that serum levels as high as 390 pg/g have been measured and may be higher in some individuals depending on such factors as diet, genetics and body weight (Barr *et al.*, 2000).

In a study by Schecter *et al.* (2004) involving nine volunteers, the highest blood levels after consumption of about 216 µg methyleugenol (contained in 12 gingersnap cookies) corresponding to 3.16 µg/kg bw were about 100 pg/g. About 15 minutes after ingesting the gingersnaps, the median concentration of methyleugenol peaked at 54 pg/g serum (range, 25–100 pg/g), then fell to a mean level of about 25 pg/g serum

(whole weight) after 2 hours. The results of this study suggest that methyleugenol is present in the blood after oral intake and that levels rapidly decline.

A comparison of the results of these two studies (Barr *et al.*, 2000; Schecter *et al.*, 2004) suggest that levels of exposure observed in the general population are higher than those obtained after the ingestion of about 3.16 µg/kg bw methyleugenol (Robison & Barr, 2006).

[The mean level of methyleugenol observed in adults in the USA (24 pg/g serum) corresponds to the mean level reached 2 hours after ingestion of 3.16 µg/kg bw methyleugenol, which is 25% of the estimated high exposure level. However, as stated by the authors, the methods used for the analysis of gingersnap cookies have not been validated for accuracy, reproducibility or detection limits (Schecter *et al.*, 2004).]

1.4 Regulations and guidelines

In the USA, methyleugenol was affirmed as generally recognized as safe by the US Food and Drug Administration as a food additive under 21 CFR §172.515 (FDA, 2004). It is also permitted for direct addition to food for human consumption as a synthetic flavouring substance in the USA (FDA, 2010).

In the EU, EC Regulation 1334/2008, which became effective in January 2011, prohibits the addition of methyleugenol to foods and restricts the concentration of methyleugenol in compound foods that have been prepared with flavourings or food ingredients with flavouring properties. The permitted maximum concentrations were: dairy products, 20 mg/kg; meat preparations and meat products (including poultry and game), 15 mg/kg; fish preparations and fish products, 10 mg/kg; soups and sauces, 60 mg/kg; ready-to-eat savouries, 20 mg/kg; and non-alcoholic beverages, 1 mg/kg. However, if the only food ingredients with flavouring properties that have been added are fresh, dried or frozen herbs and spices, the maximum limits for methyleugenol do not apply. For instance, pesto made with basil is permitted in food preparations, regardless of its methyleugenol content (European Commission, 2008a).

In the EU, the technical product of citronella used on non-food crops to control for ragwort must contain no more than 0.1% of the manufacturing impurities methyleugenol and its structurally related methyl-isoeugenol (European Commission, 2008b).

The Government of Canada will propose a phase-out plan for insect repellents that contain citronella oil if further information to support their continued safety is not provided. The re-evaluation of citronella oil-based personal insect repellents by the Pest Management Regulatory Agency is on-going pending additional data to refine the proposed risk assessment published on 17 September 2004. Following the report from an Independent Science Panel on Citronella Oil as an Insect Repellent, the Pest Management Regulatory Agency required producers of skin application products that contain citronella oil to provide confirmatory data that the levels of methyleugenol do not exceed 0.0002% of the product formulation (Government of Canada, 2010).

In the EU and in Canada, methyleugenol should not be intentionally added as a cosmetic ingredient. When fragrance compounds containing methyleugenol that is naturally present in essential oils are used as components in cosmetic products, the highest concentration of methyleugenol in the finished products must not exceed 0.01% in fine fragrance, 0.004% in eau de toilette, 0.002% in a fragrance cream, 0.0002% in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products [such as skin cleanser] (SCCNFP, 2000; Health Canada, 2010).

Australia and the USA permit the use of methyleugenol in insect traps and lure products as an insect attractant in eradication

programmes and as an anaesthetic in rodents (Australian Government, 2005; EPA, 2010). In March 1982, an exemption from the requirement of a tolerance was established by the US EPA for a methyleugenol:malathion (3:1 ratio) combination impregnated on a carrier and used in US Department of Agriculture Oriental Fruit Fly Eradication Programs. This exemption was modified in April 2004 to define the carrier further. In February 2005, the US EPA published a Tolerance Reassessment Eligibility Document for methyleugenol and concluded "... there is a reasonable certainty that no harm to any population or subgroup will result from the dietary and water exposure to methyleugenol from uses specified in the existing exemption for the requirements for tolerance for methyleugenol under 40 CFR §180.1067." (EPA, 2006, 2010).

The Government of Canada will propose not to authorize the use of pure methyleugenol in natural health products (Government of Canada, 2010).

According to the Government of Canada (2010), oral use of methyleugenol present as a component of essential oils should not exceed 200 µg/kg bw per day.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Oral administration

See Table 3.1

3.1.1 Mouse

In a 2-year study, groups of 50 male and 50 female B6C3F$_1$ mice were administered methyleugenol (99% pure) in 0.5% methylcellulose by gavage at doses of 0 (control), 37, 75 or 150 mg/kg bw on 5 days a week for 105 weeks (Johnson *et al.*, 2000; NTP, 2000). Methyleugenol caused significant dose-related increases in the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma in both sexes. The incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) was significantly increased in all treated groups, and that of hepatocellular carcinoma was significantly increased in males administered 37 or 75 mg/kg and in all groups of treated females. In both sexes, the incidence of hepatoblastoma was increased, and that in all groups of treated females was dose-related and significantly increased. The incidence of hepatoblastoma in both sexes exceeded the historical control range for gavage studies. Tumours of the glandular stomach, including one carcinoma, developed in females and two malignant neuroendocrine tumours occurred in males.

[The Working Group noted that hepatoblastomas are rare spontaneous neoplasms, and that neuroendocrine tumours of the glandular stomach are extremely rare spontaneous neoplasms in experimental animals.]

3.1.2 Rat

In a 2-year study, groups of 50 male and 50 female F344/N rats received methyleugenol (99% pure) in 0.5% methylcellulose by gavage at doses of 37, 75 or 150 mg/kg bw on 5 days a week for 105 weeks (Johnson *et al.*, 2000; NTP, 2000). Groups of 60 males and 60 females that received the 0.5% methylcellulose vehicle alone served as controls. In a stop-exposure study, additional groups of 60 males and 60 females received 300 mg/kg bw methyleugenol in 0.5% methylcellulose by gavage for 52 weeks followed by the vehicle alone for the remaining 53 weeks of the study. Groups of five male and five female vehicle controls, and five males and five females administered 300 mg/kg were killed at 6 and

Table 3.1 Carcinogenicity studies of oral administration by gavage of methyleugenol to experimental animals

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance (poly-3 test)	Comments
Mouse, B6C3F$_1$ (M, F) 105 wk	0, 37, 75 or 150 mg/kg bw 0.5% methylcellulose, 5 d/wk 50/group	Liver (hepatocellular adenoma): M–26/49, 43/50*, 38/50*, 39/50** F–20/50, 48/50***, 46/49***, 41/50***	*$P \leq 0.01$, **$P = 0.003$, ***$P \leq 0.001$ $P = 0.006$ (trend M) $P \leq 0.001$ (trend F)	99% pure In all treated groups the incidence of glandular ectasia and neuroendocrine-cell hyperplasia was significantly increased.
		Liver (hepatocellular carcinoma): M–10/49, 20/50*, 19/50**, 9/50 F–7/50, 37/50***, 47/49***, 47/50***	*$P = 0.030$, **$P = 0.044$, ***$P < 0.001$ $P < 0.001$ (trend F)	
		Liver (hepatocellular adenoma or carcinoma combined): M–31/49, 47/50**, 46/50**, 40/50* F–25/50, 50/50***, 49/49***, 49/50***	*$P = 0.02$, **$P \leq 0.001$, ***$P \leq 0.001$ $P = 0.02$ (trend M) $P \leq 0.001$ (trend F)	
		Liver (hepatoblastoma): M–0/49, 0/50, 1/50, 3/50 (M) F–0/50, 6/50*, 11/49**, 15/50**	*$P = 0.009$, **$P < 0.001$ $P = 0.019$ (trend M) $P < 0.001$ (trend F)	
		Liver (hepatocholangiocarcinoma): F–0/49, 0/50, 0/50, 2/50	NS	
		Glandular stomach (carcinoma): M–0/49, 0/48, 0/49, 1/50 (M)	NS	
		Glandular stomach (malignant neuroendocrine tumour): M–0/49, 0/48, 0/49, 2/50	NS	
Rat, F344 (M, F) 105 wk	0[a], 37, 75, 150 or 300[a,b] mg/kg bw in 0.5% methylcellulose, 5 d/wk 50/group [a] 60/group [b] Stop exposure: 5 d/wk for 52 wks followed by vehicle	Liver (hepatocellular adenoma): M–5/50, 12/50*, 23/50**, 38/50**, 32/50** F–1/50, 8/50*, 11/49**, 33/49**, 43/50**	*$P \leq 0.05$, **$P \leq 0.001$ $P \leq 0.001$ (trend M, F)	99% pure Five M and F controls and five M and F receiving 300 mg/kg were killed at 6 and 12 mo. All M administered 150 or 300 mg/kg died before the end of the study. Mean body weights of all treated M and F were lower than those of the vehicle controls throughout most of the study.
		Liver (hepatocellular carcinoma): M–2/50, 3/50, 14/50*, 25/50*, 36/50* F–0/50, 0/50, 4/49, 8/49*, 22/50*	*$P \leq 0.001$ $P \leq 0.001$ (trend M, F)	
		Liver (hepatocellular adenoma or carcinoma combined): M–7/50, 14/50*, 28/50**, 43/50**, 45/50** F–1/50, 8/50*, 14/49**, 34/49**, 43/50**	*$P \leq 0.05$, **$P \leq 0.001$ $P \leq 0.001$ (trend M, F)	

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance (poly-3 test)	Comments
Rat, F344 (M, F) 105 wk (Contd.)		Liver (cholangioma): M–0/50, 0/50, 0/50, 0/50, 2/50	NS	
		Liver (hepatocholangioma): M–0/50, 0/50, 0/50, 1/50, 6/50* F–0/50, 0/50, 0/49, 0/49, 8/50*	*$P \leq 0.001$	
		Liver (hepatocholangiocarcinoma): M–0/50, 0/50, 1/50, 1/50, 7/50* F–0/50, 0/50, 0/49, 3/49, 9/50*	*$P \leq 0.001$	
		Liver (hepatocholangioma or hepatocholangiocarcinoma combined): M–0/50, 0/50, 1/50, 2/50, 13/50* F–0/50, 0/50, 0/49, 3/49, 17/50*	*$P \leq 0.001$	
		Glandular stomach (benign neuroendocrine tumour): M–0/50, 0/50, 0/50, 3/50, 2/50 F–0/50, 0/50, 13/50**, 9/50**, 5/50*	*$P \leq 0.05$, **$P \leq 0.001$ $P < 0.001$ (trend F)	In all treated groups, the incidence of neuroendocrine-cell hyperplasia of the glandular stomach was significantly increased. The incidence of these non-neoplastic lesions was increased in treated M and F at 6 and 12 mo and at 2 yr. In an extended step-section evaluation of the kidney, the incidence of renal tubule hyperplasia was increased in treated M.
		Glandular stomach (malignant neuroendocrine tumour): M–0/50, 0/50, 0/50, 4/50*, 2/50 F–0/50, 1/50, 12/50**, 26/50**, 36/50**	*$P \leq 0.05$, **$P \leq 0.001$ $P < 0.001$ (trend, F)	
		Glandular stomach (benign or malignant neuroendocrine tumour combined): M–0/50, 0/50, 0/50, 7/50**, 4/50* F–0/50, 1/50, 25/50**, 34/50**, 41/50**	*$P = 0.03$, **$P \leq 0.002$ $P \leq 0.001$ (trend M, F)	

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance (poly-3 test)	Comments
Rat, F344 (M, F) 105 wk (Contd.)		Kidney (renal tubule adenoma, single sections): M–3/50, 2/50, 6/50, 6/50, 8/50*	*$P = 0.018$	
		Kidney (renal tubule adenoma, step sections): M–2/50, 5/50, 14/50*, 11/50**, 13/50*	*$P \leq 0.001$, **$P = 0.002$ $P < 0.001$ (trend)	
		Kidney (renal tubule carcinoma, single sections): M–1/50, 0/50, 0/50, 0/50, 0/50	NS	
		Kidney (renal tubule adenoma or carcinoma, single sections): M–4/50, 2/50, 6/50, 6/50, 8/50*	*$P \leq 0.05$	
		Kidney (renal tubule adenoma, single and step sections): M–4/50, 6/50, 17/50**, 13/50*, 20/50**	*$P = 0.003$, **$P \leq 0.001$	
		Body cavities (malignant mesothelioma): M–1/50, 3/50, 5/50, 12/50*, 5/50**	*$P \leq 0.001$, **$P = 0.041$ $P < 0.001$ (trend)	
		Mammary gland (fibroadenoma): M–5/50, 5/50, 15/50*, 13/50*, 6/50	*$P \leq 0.01$ $P \leq 0.001$ (trend)	
		Skin (subcutaneous fibroma): M–1/50, 9/50**, 8/50*, 5/50, 4/50	*$P = 0.011$, **$P = 0.006$	
		Skin (subcutaneous fibroma or fibrosarcoma combined): M–1/50, 12/50**, 8/50*, 8/50***, 4/50 (M)	*$P = 0.011$, **$P < 0.001$, ***$P = 0.005$	
		Skin (subcutaneous fibrosarcoma): M–0/50, 3/50, 0/50, 3/50, 0/50	NS	

From Johnson *et al.* (2000); NTP (2000)

bw, body weight; d, day or days; F, female; M, male; mo, month or months; NS, not significant; wk, week or weeks; yr, year or years

12 months for histopathological evaluation. Methyleugenol induced rare benign and malignant neuroendocrine tumours of the glandular stomach in both sexes. A positive trend in the incidence of these tumours was observed in females, and the incidence in females administered 75 mg/kg and 150 mg/kg in the main study and 300 mg/kg in the stop-exposure study was significantly increased. The incidence of benign or malignant neuroendocrine tumours (combined) was increased in males administered 150 mg/kg in the main study and 300 mg/kg in the stop-exposure study, and that of malignant neuroendocrine tumours in male rats administered 150 mg/kg was significantly increased. Benign or malignant neuroendocrine tumours have not been observed in the glandular stomach of male or female historical controls in gavage studies. Positive trends were observed in the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) with significant increases in most of the treated groups, including the stop-exposure groups. The incidence of hepatocholangioma and hepatocholangiocarcinoma was significantly increased in the male and female stop-exposure (300 mg/kg) groups. At the 12-month interim histopathological evaluation, four males had hepatocellular adenomas, one male had a hepatocholangiocarcinoma and one female had a hepatocellular carcinoma. In males, a positive trend was observed in the incidence of renal tubule adenoma, which was significantly increased (single and step sections combined) in the 75-mg/kg and 150-mg/kg main study groups and in the 300-mg/kg (stop-exposure) group; the incidence in all groups exceeded the historical control range. [The Working Group noted the unusual incidence of renal tubule tumours in the controls that was higher than that of historical controls.] In males of the main study, a positive trend was observed in the incidence of malignant mesothelioma, and the incidence was significantly increased in 150-mg/kg males and stop-exposure males. The incidence in the 75-mg/kg, 150-mg/kg and stop-exposure (300 mg/kg) groups exceeded the historical control range. Mammary gland fibroadenoma occurred with a positive trend in males; the incidence in 75-mg/kg and 150-mg/kg males was significantly increased, and that in all groups of males exceeded the historical control range. The incidence of skin fibroma in 37- and 75-mg/kg males and that of fibroma or fibrosarcoma (combined) in 37-, 75- and 150-mg/kg males were significantly increased but not in a dose-related manner.

[The Working Group noted that tumours of the kidney, fibromas and fibrosarcomas of the skin, mesotheliomas and hepatocholangiocarcinomas are rare spontaneous neoplasms, and that neuroendocrine tumours of the glandular stomach are extremely rare spontaneous neoplasms in experimental animals. In the main and stop-exposure studies, there was consistency in the tumour response for cancer of the liver and of the glandular stomach in male and female rats, and for renal tubule tumours in male rats.]

3.2 Intraperitoneal injection

See Table 3.2

3.2.1 Mouse

Groups of male B6C3F$_1$ mice received intraperitoneal injections of 0 (controls) or 4.75 µmol methyleugenol/mouse (dissolved in tricotanoin) on lactation days 1, 8, 15 and 22, were weaned at 4 weeks and were then maintained on a purified diet for 18 months. Methyleugenol caused an increase in the incidence of hepatoma [hepatocellular adenoma] (Miller *et al.*, 1983). [The Working Group noted that the distinction between benign and malignant hepatomas had not been clearly defined at the time when the study was conducted.]

Table 3.2 Carcinogenicity studies of intraperitoneal administration of methyleugenol or 1′-hydroxymethyleugenol to experimental animals

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 18 mo	0 (control) or 4.75 µmol methyleugenol/mouse in tricotanoin on lactation d 1, 8, 15 and 22, weaning at 4 wk then purified diet for 18 mo	Liver (hepatoma [hepatocellular adenoma]): 24/58, 56/58	$P \leq 0.01$	Purity ≥ 98%
Mouse, B6C3F$_1$ (M) 18 mo	0 (control) or 2.85 µmol 1'-hydroxymethyleugenol/ mouse in tricotanoin on lactation d 1, 8, 15 and 22, weaning at 4 wk then purified diet for 18 mo	Liver (hepatoma [hepatocellular adenoma]): 24/58, 41/44	$P \leq 0.01$	Purity ≥ 98%

From Miller *et al.* (1983)

d, day or days; M, male; mo, month or months; NR, not reported; wk, week or weeks

3.3 Carcinogenicity of metabolites

See Table 3.2

3.3.1 Mouse

Groups of male B6C3F$_1$ mice received intraperitoneal injections of 0 (controls) or 2.85 µmol 1′-hydroxymethyleugenol (a metabolite of methyleugenol)/mouse (dissolved in tricotanoin) on lactation days 1, 8, 15 and 22, were weaned at 4 weeks and were then maintained on a purified diet for 18 months. 1′-Hydroxymethyleugenol caused an increase in the incidence of hepatoma [hepatocellular adenoma] (Miller *et al.*, 1983). [The Working Group noted that the distinction between benign and malignant hepatomas had not been clearly defined at the time when the study was conducted.]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

(a) Absorption, distribution and excretion

Schecter *et al.* (2004) studied nine volunteers who had a mean serum concentration of 16.2 pg/g wet weight methyleugenol after overnight fasting. The subjects then consumed gingersnap cookies (3.16 µg/g) that delivered a dose of ~216 µg methyleugenol/subject, which resulted in a peak serum concentration of 53.9 pg/g wet weight methyleugenol 15 minutes after consumption, with an estimated half-life of ~90 minutes. This peak level was within the range of < 3.1–390 pg/g noted in non-fasting subjects in a biomonitoring study (Barr *et al.*, 2000).

A study showed that methyleugenol had a 14.5% permeation rate 30 minutes after a cosmetic cream containing 50 ppm of the compound was applied to the skin of a human volunteer (Sgorbini *et al.*, 2010).

(b) Metabolism

Jeurissen *et al.* (2006) showed that incubation of methyleugenol with either supersomes that express individual human cytochrome P450 (CYP) or microsomes from pooled human livers resulted in the formation of the mutagenic metabolite 1′-hydroxymethyleugeol and that this reaction was catalysed by a variety of CYPs, including CYP1A2, –2C9, –2C19 and –2D6. However, when microsomes from the livers of 15 individuals were evaluated, only CYP1A2 and CYP2C9 were clearly important in the bioactivation of methyleugenol. Using microsomes from pooled human livers together with enzyme-specific inhibitors, Jeurissen *et al.* (2006) also showed that CYP1A2 was the most important enzyme for the hydroxylation of methyleugenol at concentrations found in biomonitoring studies. Other isoforms, such as CYP2C9 and CYP2C19, only had an effect at two- to fourfold higher concentrations of methyleugenol. The authors found a fivefold difference in catalytic activity among microsomal preparations from the 15 different donors. Gardner *et al.* (1997) showed that microsomes from 13 human liver samples exhibited a 37-fold difference in the conversion of methyleugenol to 1′-hydroxymethyleugenol; the highest activities were similar to those of control rat liver microsomes. Collectively, these data suggest that interindividual differences might be important in the sensitivity of humans to methyleugenol.

4.1.2 Experimental systems

(a) Absorption, distribution and excretion

In the NTP (2000) rodent study, the mean ± standard deviation of methyleugenol plasma concentrations (wet weight) 15 minutes after gavage with the lowest dose (37 mg/kg bw) was 0.574 ± 0.229 µg/mL for male rats and 0.651 µg/mL (two samples; no standard deviation) for female

rats. The data for mice 10 minutes after gavage with the same dose were 0.417 ± 0.128 μg/mL for males and 0.681 ± 0.050 μg/mL for females.

(b) Metabolism

Gardner *et al.* (1997) found that CYP2E1 was the most important enzyme in the bioactivation of methyleugenol to 1′-hydroxymethyleugenol by microsomes from control F344 rat liver. In contrast, they showed that liver microsomes from methyleugenol-treated rats were more effective at catalysing this reaction, probably due to the induction of CYP2B and CYP1A2.

Fig. 4.1 shows a schema of the metabolism of allylbenzenes (of which methyleugenol is a member), which is complex but involves similar transformations for many chemicals of this class of agents. Three primary steps in the hepatic metabolism of the parent compounds, which are readily absorbed from the gastrointestinal tract, include *O*-demethylation, epoxidation and 1′-hydroxylation (Smith *et al.*, 2002). [The Working Group noted that formation of the 2′3′-epoxide of methyleugenol has not been firmly established.] The first two pathways account for formation of the majority of downstream metabolites, including numerous methoxy phenolic derivatives and 2′3′-diols that are rapidly and efficiently metabolized (Luo & Guenthner, 1996). Although 1′-hydroxylation is a minor pathway, subsequent sulfation is thought to produce highly reactive electrophiles that can react with cellular proteins and DNA (Gardner *et al.*, 1996). Glucoronidation of the 1′-hydroxy compounds has been demonstrated (Iyer *et al.*, 2003). With increasing doses of these compounds, the proportion of reactive 1′-hydroxy metabolites formed increases compared with *O*-demethylation products, presumably due to saturation of the enzyme systems responsible for *O*-dealkylation (Zangouras *et al.*, 1981).

(c) Models

Using the available experimental, in-silico and published data, Al-Subeihi *et al.* (2011) developed a physiologically based biokinetic model for methyleugenol in rats. Auerbach *et al.* (2010) used toxicogenomics and machine-learning to predict the rodent liver carcinogenicity of alkenylbenzene flavouring agents such as methyleugenol. Smith *et al.* (2010) analysed rodent data using the margin-of-exposure approach, which considers the relationship between a given point on the dose–response curve in animal and human exposure data. [The Working Group considered these to be risk assessment models, and not relevant to hazard identification.]

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group on the ability of methyleugenol to induce DNA adducts, DNA strand breaks, mutations, chromosomal effects, alterations in oncogenes or suppressor genes in tumours, or changes in gene expression in humans. However, methyleugenol induced DNA adducts in cultured human HepG2 hepatoma cells (Zhou *et al.*, 2007).

4.2.2 Experimental systems

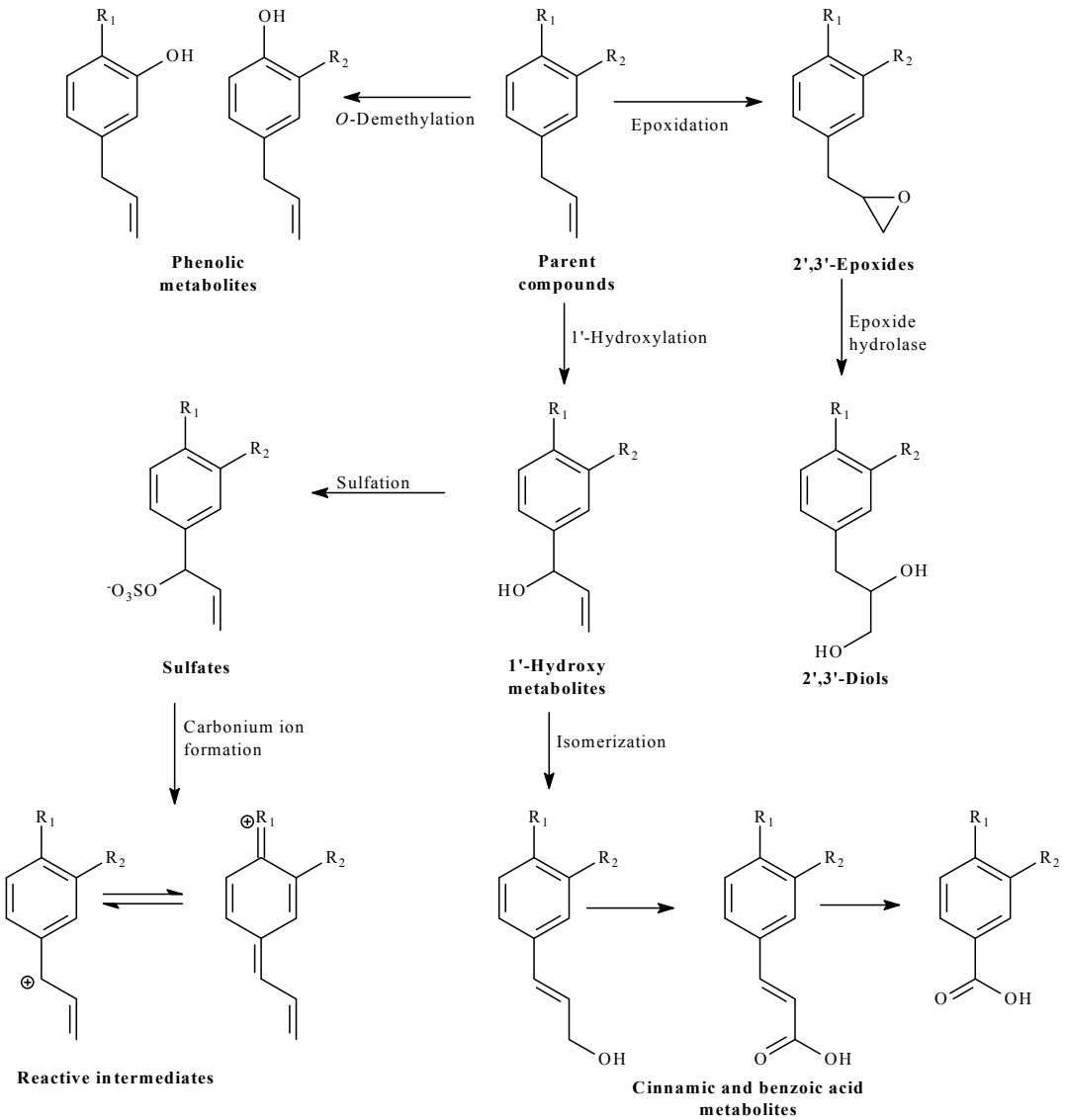
(a) DNA adducts

Intraperitoneal administration of methyleugenol induced DNA adducts in the livers of adult female CD-1 mice (Randerath *et al.*, 1984) and of newborn male B6C3F$_1$ mice treated on postnatal days 1, 8, 15 and 22 (Phillips *et al.*, 1984).

(b) DNA damage

Methyleugenol induced DNA damage in the absence of metabolic activation in the *Bacillus subtilis* rec assay (Sekizawa & Shibamoto, 1982) and in primary hepatocytes from mice and rats

Fig. 4.1 Schema of different metabolic pathways of allylbenzenes



in the unscheduled DNA synthesis assay (Howes *et al.*, 1990; Chan & Caldwell, 1992; Burkey *et al.*, 2000). Using various inhibitors, Burkey *et al.* (2000) showed that the DNA-damaging activity of methyleugenol in the unscheduled DNA synthesis assay was mediated by a sulfotransferase but did not involve epoxide formation.

(c) Mutation

Methyleugenol was not mutagenic in a variety of strains of *Salmonella typhimurium* (Sekizawa & Shibamoto, 1982; Mortelmans *et al.*, 1986) or in *Escherichia coli* WP2 *uvrA* (Sekizawa & Shibamoto, 1982) in the presence or absence of metabolic activation.

(d) Chromosomal effects

Methyleugenol induced intra-chromosomal recombination in yeast (Schiestl *et al.*, 1989; Brennan *et al.*, 1996). In Chinese hamster ovary cells, methyleugenol induced chromosomal aberrations in the presence but not in the absence of metabolic activation and gave weakly positive results for the induction of sister chromatid exchange in both the presence and absence of metabolic activation (NTP, 2000).

Methyleugenol also induced cell transformation in Syrian hamster embryo cells (Kerckaert *et al.*, 1996; NTP, 2002).

(e) Alterations in oncogenes and suppressor genes in tumours

Among 29 hepatocellular tumours from methyleugenol-treated mice, 20 (69%) had base-substitutions in the β-catenin gene at codon 32, 33, 34 or 41, all at sites that are also mutated in colon and other cancers in rodents and humans; only two of 22 spontaneous liver tumours (9%) had mutations in β-catenin. The authors also found a relatively high frequency of β-catenin gene mutations in mouse tumours induced by a variety of other chemicals, and noted that this gene was frequently mutated in human liver tumours (Devereux *et al.*, 1999). Mutations in this gene cause upregulation of proto-oncogene Wnt-signalling, which results in the stimulation of cell proliferation and the inhibition of apoptosis (Morin *et al.*, 1997).

(f) Changes in gene expression

Iida *et al.* (2005) analysed changes in gene expression in the livers of mice after 2 weeks of treatment with methyleugenol, and also the transcriptional profile in methyleugenol-induced mouse liver tumours. They found that methyleugenol upregulated several genes after 2 weeks of treatment during the early carcinogenic process, including p21, early growth response 1, Cyclin G1 and Dnase2a; it downregulated the fragile histidine trial and WW domain-containing oxidoreductase genes. In methyleugenol-induced mouse liver tumours, β-catenin, growth arrest and DNA-damage-inducible (*GADD45*), insulin-like growth factor-binding protein 1, Cyclin D1 and proliferating cell nuclear antigen genes were upregulated, and transcriptional repressor and fragile histidine trial genes were downregulated. These latter two genes, together with the WW domain-containing oxidoreductase gene, are involved in apoptosis, and their downregulation, especially at an early stage, suggests that methyleugenol causes a reduction in apoptosis soon after treatment. Iida *et al.*, (2007) also showed that the transcriptional repressor gene is a suppressor of *GADD45* gene expression.

4.3 Mechanistic data

4.3.1 Structure–activity relationships

Methyleugenol is an alkenylbenzene, as are the structurally related compounds estragole and safrole. Jeurissen *et al.* (2007) have shown that not all alkenylbenzenes are metabolized by human liver microsomes via the same CYP isoforms. Thus, methyleugenol, and to some extent estragole, are metabolized by CYP1A2,

whereas estragole and safrole are metabolized primarily by CYP2A6. Safrole is not metabolized by CYP1A2, and methyleugenol is not metabolized by CYP2A6. [The Working Group noted the possible importance of polymorphisms of CYP1A2 in the metabolism of methyleugenol by the human liver.]

4.4 Susceptibility

Among the many mutations identified to date in human *CYP1A2*, functional studies showed that three mutations decrease enzyme activity, and one enhances inducibility; no mutation increases enzyme activity (Karolinska Institutet, 2012). In addition, lifestyle factors, such as exposure to barbiturates, cruciferous vegetables, fried meat or cigarette smoke, can induce CYP1A2 and may play a critical role in the variation in catalytic ability found among human livers and, ultimately, in potential human susceptibility (Jiang *et al.*, 2006; Jeurissen *et al.*, 2007). This may also be relevant to susceptibility to the effects of methyleugenol.

4.5 Mechanisms of carcinogenesis

Clear differences in the metabolism of methyleugenol are dose-dependent. Data from studies in both humans and in animals are available for a comparison of plasma concentrations of methyleugenol in different settings (Barr *et al.*, 2000; NTP, 2000; Schecter *et al.*, 2004). [The Working Group acknowledged the large difference in plasma concentrations measured in experimental animals in the cancer bioassay and those generally observed in humans.]

The data suggest that the doses used in the rodent studies result in the metabolism of methyleugenol by specific CYPs that leads to the formation of high levels of 1′-hydroxymethyleugenol, which can form a reactive carbonium ion. This could then result in DNA damage, as indicated by the DNA adducts detected in human hepatocytes *in vitro* and in the liver of rats *in vivo*. Mutations have been found in genes such as β-catenin, which alters expression of the Wnt pathway. These effects, together with altered expression of other genes involved in apoptosis and other pathways, could then result in the liver tumours observed in rodent studies. Alterations in these pathways also appear to occur in humans. Thus, there is moderate evidence that a mutational mechanism underlies the formation of methyleugenol-induced tumours in rodents.

5. Summary of Data Reported

5.1 Exposure data

Methyleugenol occurs naturally in a variety of spices and herbs, including basil and tarragon. It is also produced by the methylation of eugenol and is used as a flavouring agent in a variety of foods, as a fragrance ingredient in perfumes, toiletries and detergents, and has been used as an insect attractant.

Daily human exposure to methyleugenol has been estimated at the microgram to milligram level through the ingestion of foods that contain the compound. Exposure can also occur via inhalation and dermal contact through the use of personal care products. Widespread exposure to methyleugenol occurs, as indicated by human biomonitoring data which show that methyleugenol is present in the blood serum of nearly all residents in the USA.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Methyleugenol was tested for carcinogenicity by oral administration by gavage in one study in mice and one study in rats and by intraperitoneal administration to mice in one study.

In mice, oral administration of methyleugenol caused a significantly increased incidence of liver tumours (hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma) in both sexes. In rats, oral administration of methyleugenol caused a significantly increased incidence of liver tumours (hepatocellular adenoma, hepatocellular carcinoma, hepatocholangioma and hepatocholangiocarcinoma) and benign and malignant neuroendocrine tumours of the glandular stomach in males and females, and renal tubule adenoma of the kidney, mammary gland fibroadenoma, skin fibroma, skin fibroma or fibrosarcoma (combined) and mesothelioma in males in the main and stop-exposure experiments. Tumours of the kidney, fibromas and fibrosarcomas of the skin, mesotheliomas, hepatoblastomas and hepatocholangiocarcinomas are rare spontaneous neoplasms, and neuroendocrine tumours of the glandular stomach are extremely rare spontaneous neoplasms in experimental animals. In the main and stop-exposure experiments in rats, there was consistency in the tumour response for cancers of the liver and glandular stomach in males and females, and of the kidney in males.

Intraperitoneal injection of methyleugenol caused a significantly increased incidence of hepatocellular adenoma in male mice.

1′-Hydroxymethyleugenol, a metabolite of methyleugenol, was tested for carcinogenicity by intraperitoneal injection in one study in mice, and caused a significantly increased incidence of hepatocellular adenoma in males.

5.4 Other relevant data

The three primary steps by which methyleugenol is metabolized by the liver are *O*-demethylation, epoxidation and 1′-hydroxylation. Although 1′-hydroxylation is a minor pathway, a subsequent sulfation reaction is thought to produce highly reactive electrophiles. Methyleugenol is metabolized by cytochrome P450 1A2, which is polymorphic in humans and is also induced by various dietary factors and cigarette smoking. Thus, a combination of phenotypic variation and lifestyle factors may play a role in the potential differential ability of humans to metabolize methyleugenol to reactive intermediates.

Although methyleugenol is not mutagenic in bacteria, it induces chromosomal aberrations *in vitro* and DNA adducts in the liver of rodents *in vivo*.

The enzymatic pathways by which methyleugenol is metabolized are similar in rodents and humans. Thus, there is moderate evidence that a mutational mechanism underlies the induction of tumours by methyleugenol in rodents.

The mechanistic data provide some additional support for the relevance of animal carcinogenicity data to humans.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of methyleugenol.

6.3 Overall evaluation

Methyleugenol is *possibly carcinogenic to humans (Group 2B).*

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