FINAL

Report on Carcinogens Background Document for

Methyleugenol

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Meeting of the
NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee

Prepared for the:
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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Summary Statement

Methyleugenol

CASRN 93-15-2

Carcinogenicity

Methyleugenol is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of its carcinogenicity in experimental animals. Oral administration of methyleugenol to rats increased the incidences of benign and malignant tumors of the liver, stomach, kidney, mammary gland, and skin. Oral administration of methyleugenol to mice increased the incidences of benign and malignant tumors of the liver. Tumors of the stomach in male mice also were considered related to exposure to methyleugenol (NTP 1998). Earlier studies found that methyleugenol and two structurally related allylbenzenes, safrole and estragole, induced liver tumors in mice after intraperitoneal injection (IARC 1976, Miller *et al*. 1983). Safrole is classified by the International Agency for Research on Cancer as *possibly carcinogenic to humans* (Group 2B) and is listed as *reasonably anticipated to be a human carcinogen* in the National Toxicology Program's Report on Carcinogens.

No studies on the potential carcinogenicity of methyleugenol in humans have been reported.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Mechanistic data indicate that liver tumors induced by methyleugenol and structurally related allylbenzenes result from metabolism of these compounds to DNA-reactive intermediates. Methyleugenol may be bioactivated by three different pathways: (1) hydroxylation at the 1′ position of the allylic side chain to yield 1′-hydroxymethyleugenol, followed by sulfation of this intermediate to form 1′-hydroxymethyleugenol sulfate, (2) oxidation of the 2′,3′-double bond of the allylic side chain to form methyleugenol-2,3-oxide, and (3) *O*-demethylation followed by spontaneous rearrangement to form eugenol quinone methide. Formation of protein adducts and DNA adducts in the livers of animals treated with allylbenzenes and induction of liver tumors by these compounds have been attributed to activation via the hydroxylation pathway, because similar effects were produced by the 1′-hydroxy metabolites and because these effects were inhibited by pretreatment with sulfotransferase inhibitors (Miller *et al*. 1983, Boberg *et al*. 1983, Randerath *et al*. 1984, Gardner *et al*. 1996).

Methyleugenol, safrole, and estragole induced unscheduled DNA synthesis in rat hepatocytes, and their corresponding 1′-hydroxy metabolites are more potent genotoxic agents than the parent compounds (Howes *et al*. 1990, Chan and Caldwell 1992). Methyleugenol induced morphological transformation in Syrian hamster embryo cells

(Kerckaert *et al*. 1996), sister chromatid exchanges in Chinese hamster ovary (CHO) cells (NTP 1998), intrachromosomal recombination in yeast (Schiestl *et al*. 1989), and DNA repair in *Bacillus subtilis* (Sekizawa and Shibamoto 1982). Methyleugenol did not induce mutations in *Salmonella typhimurium* (NTP 1998) or *Escherichia coli* (Sekizawa and Shibamoto 1982), chromosomal aberrations in CHO cells (NTP 1998), or micronucleated erythrocytes in peripheral blood of mice (NTP 1998). A higher frequency of *β-catenin* mutations was observed in liver tumors from mice treated with methyleugenol than in spontaneous liver tumors from control mice (Devereux *et al*. 1999). Methyleugenol's lack of mutagenicity in bacteria may be due to the need for sulfation in the metabolic activation of methyleugenol to its ultimate mutagenic or carcinogenic form.

No data are available that would suggest that mechanisms thought to account for tumor induction by methyleugenol in experimental animals would not also operate in humans.

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1 Introduction

Methyleugenol was nominated for listing in the Report on Carcinogens (RoC) by the National Institute of Environmental Health Sciences (NIEHS) -National Toxicology Program (NTP) RoC Review Group (RG1) because two-year studies of methyleugenol conducted by the NTP showed clear evidence of carcinogenic activity in rats and mice of both sexes. In addition, methyleugenol is structurally related to safrole, an agent classified by the International Agency for Research on Cancer (IARC) as *possibly carcinogenic to humans* (Group 2B) and is listed as *reasonably anticipated to be a human carcinogen* in the NTP RoC.

Methyleugenol is used in its natural or synthetic forms as a flavoring agent in foods, an attractant in insecticides, and as a fragrance in perfumes and soaps. Methyleugenol was detected in 98% of 206 adult human serum samples analyzed in the Third National Health and Nutrition Examination Survey (NHANES III). Thus, human exposure is expected to be widespread. This document provides a qualitative evaluation of human exposure to methyleugenol and its potential carcinogenic risk.

1.1 Chemical identification

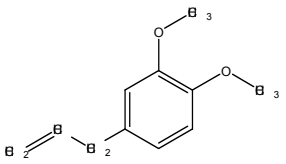
Methyleugenol ($C_{11}H_{14}O_2$, mol wt 178.2304, CASRN 93-15-2) also is known by the following names:

1,2-dimethoxy-4-(2-propenyl)benzene	1-allyl-3,4-dimethoxybenzene
4-allylveratrole	1,2-dimethoxy-4-allylbenzene
1-(3,4-dimethoxyphenyl)-2-propene	1,3,4-eugenol methyl ether
eugenol methyl ether	eugenyl methyl ether
allyl veratrole	veratrole methyl ether
dimethoxy-4-(2-propenyl)benzene	4-allyl-1,2-dimethoxybenzene
o-methyl eugenol ether	3,4-dimethoxyallylbenzene
2-methoxy-4-propenylphenol methyl ether	methyl eugenyl ether

Its RTECS code is CY2450000.

1.2 Physical-chemical properties

The structure of methyleugenol is illustrated in Figure 1-1, and its physical and chemical properties are summarized in Table 1-1. Methyleugenol is a colorless to pale yellow, oily liquid with a boiling point of 254.7°C and a melting point of -4°C. It has a delicate clover-carnation odor and a bitter burning taste. It forms azeotropic mixtures with ethylene glycol, eugenol, and benzoic acid. It slowly darkens and thickens when exposed to air and readily evaporates at room temperature (Lide 1998).



Source: ChemFinder 2000

Figure 1-1. Structure of methyleugenol

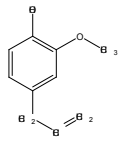
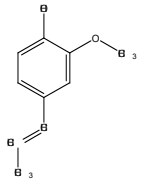
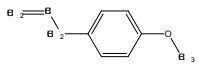
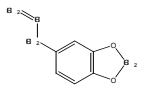
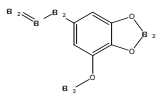
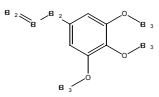
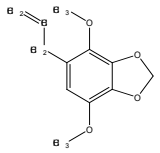
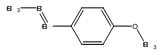
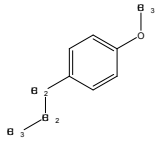
Table 1-1. Physical and chemical properties of methyleugenol

Property	Information	Reference
Molecular weight	178.2304	Budavari *et al.* 1996, ChemFinder 2000
Color	colorless to pale yellow	Budavari *et al.* 1996, Lide 1998, ChemFinder 2000
Odor	delicate clover-carnation odor	Lide 1998, HSDB 1996
Taste	bitter, burning taste	HSDB 1996
Physical state	liquid	Budavari *et al.* 1996, Lide 1998, ChemFinder 2000
Melting point (°C)	-4	Budavari *et al.* 1996, Lide 1998, HSDB 1996
Boiling point (°C)	254.7	Budavari *et al.* 1996, Lide 1998, HSDB 1996
Specific gravity (density) at 20°C or 4°C	1.0396	HSDB 1996
Refractive index	1.532	NTP 1998
Vapor pressure (mm Hg at 85°C)	1	HSDB 1996
Flash point (°C)	117	ChemFinder 2000
Solubility: Water at 19°C Ethanol Ether Chloroform Glycol Propylene glycol	 < 0.1 g/100 L soluble soluble soluble insoluble insoluble	 ChemFinder 2000 HSDB 1996 HSDB 1996 NTP 1998 NTP 1998 NTP 1998

1.3 Identification of structural analogs

Some structural analogs of methyleugenol are listed in Table 1-2.

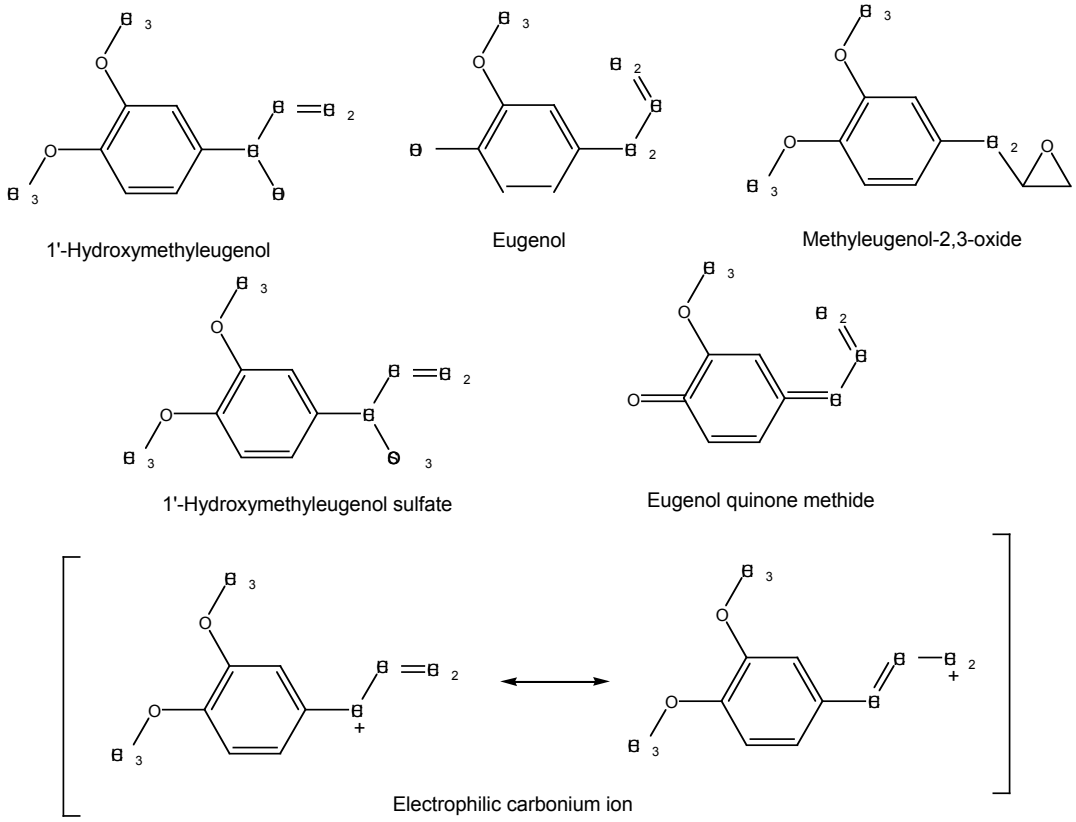
Table 1-2. Certain structural analogs of methyleugenol

Chemical name Formula Molecular weight	CASRN	Structure	Melting point (°C)	Boiling point (°C)	Solubility
Eugenol $C_{10}H_{12}O_2$ 164.20	97-53-0		15.44	487	practically insoluble in water
Isoeugenol $C_{10}H_{12}O_2$ 164.20	97-54-1		64.4	507	slightly soluble
Estragole $C_{10}H_{12}O$ 148.22	140-67-0		NA	216	insoluble
Safrole $C_{10}H_{10}O_2$ 162.19	94-59-7		11.2	232	insoluble in water
Myristicin $C_{11}H_{12}O_3$ 192.21	607-91-0		NA	173 at 40 mm Hg	NA
Elemicin $C_{12}H_{16}O_3$ 208.26	487-11-6		NA	NA	NA
Apiole $C_{12}H_{14}O_4$ 222.24	523-80-8		29.5	294	insoluble in water
trans-Anethole $C_{10}H_{12}O$ 148.20	4180-23-8		23	236	practically insoluble in water
p-Propyl anisole $C_{10}H_{14}O$ 150.22	104-45-0		NA	NA	NA

Sources: Budavari *et al*. 1996, Tice 1999
NA = not available.

1.4 Identification of metabolites

In mammals, metabolites of methyleugenol include 1′-hydroxymethyleugenol, eugenol, methyleugenol-2,3-oxide, and 1′-hydroxymethyleugenol sulfate (Gardner *et al.* 1996, Solheim and Scheline 1976, both cited in NTP 1998; Woo *et al.* 1997). The transient eugenol quinone methide and electrophilic carbonium ions of 1′-hydroxymethyleugenol sulfate also have been identified (Gardner *et al.* 1996). The structures of these metabolites are shown in Figure 1-2.



Source: Gardner *et al*. 1996

Figure 1-2. Structures of mammalian metabolites of methyleugenol

2 Human Exposure

2.1 Use

Methyleugenol is used as a flavoring agent in jellies, baked goods, nonalcoholic beverages, chewing gum, candy, pudding, relish, and ice cream. Methyleugenol has also been used as an anesthetic in rodents (NTP 1998). It also is used as an insect attractant in combination with insecticides (Hays and Laws 1991, cited in NTP 1998). Methyleugenol has been used as an agent in sunscreens (Radian 1991).

2.2 Production

Annual production of methyleugenol in 1990 was estimated at 25,000 lb (SRI 1990, cited in NTP 1998).

2.3 Analysis

The presence of methyleugenol in essential oils is determined by means of negative ion chemical ionization mass spectrometry. High-performance liquid chromatography also can be used to determine the identity, purity, and stability of methyleugenol (NTP 1998). Barr *et al.* (2000) have developed a method for quantifying methyleugenol in human serum through a solid-phase extraction followed by a highly specific analysis by means of isotope dilution gas chromatography/high resolution mass spectrometry. The limit of detection for this analytical method is 3.1 pg/g.

2.4 Environmental occurrence

Methyleugenol is a naturally occurring substance. It is present in many essential oils (Radian 1991). It is a component of rose, pimento, basil, hyacinth, citronella, anise, nutmeg, mace, cinnamon leaves, pixuri seeds, and laurel fruits and leaves. It also has been found in blackberry essence, bananas, black pepper, and bilberries (NTP 1998). Methyleugenol has been detected in the wastewater effluent from a paper mill (Moshonas and Shaw 1978, cited in NTP 1998).

2.5 Environmental fate

2.5.1 Atmospheric Fate

Methyleugenol exists as a vapor in the ambient atmosphere. Vapor-phase methyleugenol reacts with photochemically produced hydroxyl radicals and degrades with an estimated half-life of five hours (HSDB 1996).

2.5.2 Aquatic Fate

Methyleugenol adsorbs to suspended solids and sediments. It volatilizes from water with estimated half-lives of nine days for a model river and 68 days for a model lake. Methyleugenol bioconcentrates in aquatic organisms with a bioconcentration factor (BCF) of 120 (a BCF < 1000 is generally insufficient to result in bioaccumulation in aquatic organisms). Methyleugenol has a half-life of 34 hours in aquatic environments (HSDB 1996).

2.5.3 Terrestrial Fate

Methyleugenol is moderately mobile in soil and was demonstrated experimentally to be immobile in sand, silt clay, and loam. Volatilization may be an important fate process in moist soils, and biodegradation is expected to be the most important fate process in soils. Methyleugenol has a half-life in soil of 16 hours (HSDB 1996).

2.6 Environmental exposure

Although methyleugenol has been identified in various natural substances, no quantitative measurements have assessed nondietary environmental exposure to methyleugenol. The general population is exposed to methyleugenol via ingestion of essential oils and foodstuffs containing the compound (HSDB 1996).

Methyleugenol is used in commercial products as a flavorant at concentrations ranging from 5 ppm to 52 ppm and as a fragrance at concentrations from 0.002% to 0.3%. A subset of serum samples from human adults participating in NHANES III were analyzed for methyleugenol content (Barr *et al*. 2000). Methyleugenol was detected in 98% of the 206 samples analyzed. The mean methyleugenol concentration was 24 pg/g, and the highest concentration was 390 pg/g.

Per capita consumption of methyleugenol in foods was estimated by the World Health Organization to be 0.073 mg/day (WHO 1981, cited in NTP 1998) and, more recently, 0.26 mg/kg body weight (Strofberg and Grundschober 1987, NAS 1989, both cited in NTP 1998).

2.7 Occupational exposure

Occupational exposure to methyleugenol occurs through dermal contact, inhalation, and ingestion. Through the National Occupational Exposure Survey (1981 to 1983), the National Institute for Occupational Safety and Health estimated that 2,824 workers (including 877 females) were potentially exposed to methyleugenol (NTP 1998).

2.8 Biological indices of exposure

Three major pathways describe the metabolism of methyleugenol in humans. These include oxidation of the allylic side chain, formation of the hydroxy acid via epoxidation of the double bonds followed by hydration, and *O*-demethylation and hydroxylation of the benzene ring (NTP 1998). Although there is now a sensitive and accurate method to determine methyleugenol concentration in blood, detailed pharmacokinetic studies will be required in order to determine the relationship between methyleugenol intake and human serum methyleugenol concentrations (Barr *et al.* 2000).

2.9 Regulations

The U.S. Environmental Protection Agency (EPA) regulates methyleugenol under the Federal Insecticide, Fungicide, and Rodenticide Act. EPA allows for exemption from normal tolerances when methyleugenol is used in Oriental fruit fly eradication programs. The U.S. Food and Drug Administration (FDA) regulates methyleugenol, allowing it to be used as a synthetic flavoring substance and adjuvant for direct addition to food for human consumption. Table 2-1 summarizes EPA regulations, and Table 2-2 summarizes FDA regulations.

Table 2-1. U.S. EPA Regulations

Regulatory action	Effect of regulation and other comments
40 CFR 180—PART 180—TOLERANCES AND EXEMPTIONS FROM TOLERANCES FOR PESTICIDE CHEMICALS IN OR ON RAW AGRICULTURAL COMMODITIES. Promulgated: 36 FR 22540, 11/25/71. U.S. Codes: 21 U.S.C. 346a, 371a.	Part 180 provides procedural regulations and specific tolerances for various pesticides. Exemptions from tolerances also are given in this part.
40 CFR 180—PART 180 Subpart D—Exemptions From Tolerances. Promulgated: 47 FR 9002, 03/03/82. U.S. Codes: 21 U.S.C. 321(q), 346(a) and 371.	The insect attractant methyleugenol is exempt from the requirement of tolerances on all raw agricultural commodities when used in combination in Oriental fruit fly eradication programs under the authority of the U.S. Department of Agriculture. The maximum actual dosage per application per acre shall be 28.35 g (one ounce avoirdupois) methyleugenol.

Source: The regulations in this table have been updated through the 1999 Code of Federal Regulations 40 CFR, 1 July 1999.

Table 2-2. U.S. FDA Regulations

Regulatory action	Effect of regulation and other comments
21 CFR 172—PART 172—FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION. Promulgated: 42 FR 14491 03/15/77. U.S. Codes: 21 U.S.C. 321, 341, 342, 348, 371, 379e	The regulations in Subparts A through I govern the amounts of food additives allowed for human consumption.
21 CFR 172—Subpart F—Flavoring Agents and Related Substances. Promulgated: 61 FR 14245, 04/01/96.	Methyleugenol may be safely used in food provided it is used in the minimum quantity required to produce the intended effect, and otherwise in accordance with all the principles of good manufacturing practice.

Source: The regulations in this table have been updated through the 1999 Code of Federal Regulations 21 CFR, 1 April 1999.

3 Human Cancer Studies

No studies of the relationship of human cancer with exposure to methyleugenol have been reported.

4 Studies of Cancer in Experimental Animals

4.1 Oral administration study in rats

The carcinogenic potential of methyleugenol was evaluated in a cancer bioassay in rats of both sexes (NTP 1998). In this study, five to six week-old F344/N rats (50 per sex) received methyleugenol in 0.5% methylcellulose by gavage at doses of 37, 75, or 150 mg/kg body weight (b.w.), five days per week for 105 weeks. Other groups of F344/N rats (60 per sex) received methyleugenol in 0.5% methylcellulose by gavage at 300 mg/kg b.w., five days per week for 53 weeks, followed by 0.5% methylcellulose (vehicle) only for the remaining 52 weeks. Another group of F344/N rats (60 per sex) was administered 0.5% methylcellulose only and served as controls. At six and 12 months, five control rats and five rats in the 300-mg/kg exposure group were sacrificed. Moribund animals were sacrificed throughout the study. At the end of the study, all surviving animals were sacrificed. The tissues and organs of all animals were examined histopathologically.

Mean body weights of rats administered methyleugenol were lower than those of the vehicle control animals throughout most of the study. All male rats in the 150- and 300-mg/kg groups died before the end of the study. Survival of female rats was slightly lower in the 150-mg/kg group than in the control group. The survival rates are shown in Table 4-1.

Table 4-1. Survival rates of male and female F344/N rats administered methyleugenol by gavage for up to 105 weeks

	Dose level (mg/kg per day)				
Sex	**0[a]**	**37**	**75**	**150**	**300[a,b]**
Males	20/60	16/50	15/50	0/50	0/60
Females	22/60	25/50	22/50	11/50	16/60

Source: NTP 1998

[a]Five rats per group were euthanized at 6 and 12 months of the study.

[b]Stop-exposure group (53 weeks of exposure to methyleugenol followed by 52 weeks of vehicle-only treatment).

Histopathological examination of the tissues revealed benign and/or malignant tumors at various sites, including the liver, glandular stomach, kidney, mammary gland, and skin. The incidences of hepatocellular adenomas, hepatocellular carcinomas, hepatocholangiomas, hepatocholangiocarcinomas, neuroendocrine tumors of the glandular stomach, renal adenomas, malignant mesotheliomas, mammary gland fibroadenomas, and subcutaneous tissue fibromas were significantly increased in rats given methyleugenol. Tumor incidences and their statistical significance are shown in Table 4-2 for male rats and in Table 4-3 for female rats.

Table 4-2. Incidences of neoplastic lesions in male F344/N rats administered methyleugenol by gavage for up to 105 weeks

Tumor type	Tumor incidence/no. rats examined (Poly-3 est. neoplasm rates)[a]				
	Dose level (mg/kg per day)				
	0	37	75	150	300[b]
Liver					
Hepatocellular adenoma	5/50	12/50*	23/50**	38/50**	32/50**
Hepatocellular carcinoma	2/50	3/50	14/50**	25/50**	36/50**
Hepatocellular adenoma or carcinoma	7/50 (17)	14/50 (34)*	28/50 (64)**	43/50 (94)**	45/50 (99)**
Hepatocholangioma	0/50	0/50	0/50	1/50	6/50**
Hepatocholangiocarcinoma (includes multiples)	0/50	0/50	1/50	1/50	7/50**
Hepatocholangioma or hepatocholangiocarcinoma (includes multiples)	0/50 (0)	0/50 (0)	1/50 (3)	2/50 (6)	13/50 (44)**
Glandular stomach					
Malignant neuroendocrine tumor	0/50	0/50	0/50	4/50	2/50
Benign neuroendocrine tumor	0/50	0/50	0/50	3/50*	2/50
Benign or malignant neuroendocrine tumor	0/50	0/50	0/50	7/50**	4/50*
Kidney: Adenoma[c]	4/50 (10)	6/50 (16)	17/50 (44)**	13/50 (37)**	20/50 (65)**
Mammary gland: Fibroadenoma	5/50	5/50	15/50**	13/50**	6/50
Skin (subcutaneous):					
Fibroma	1/50	9/50**	8/50*	5/50	4/50
Fibroma or fibrosarcoma	1/50	12/50**	8/50*	8/50**	4/50
All organs: Mesothelioma	1/50	3/50	5/50	12/50**	5/50*

Source: NTP 1998

*$P \leq 0.05$; significantly different from vehicle controls (Poly-3 test).

**$P \leq 0.01$; significantly different from vehicle controls (Poly-3 test).

[a]Poly-3 estimated neoplasm rates, which adjust for intercurrent mortality, are given in parentheses.

[b]Stop-exposure group (53 weeks of exposure to methyleugenol followed by 52 weeks of vehicle-only treatment).

[c]Combined standard and extended evaluations of renal tubule adenoma.

Table 4-3. Incidences of neoplastic lesions in female F344/N rats administered methyleugenol by gavage for up to 105 weeks

Tumor type	Tumor incidence/no. rats examined (Poly-3 est. neoplasm rates)[a]				
	Dose level (mg/kg per day)				
	0	37	75	150	300[b]
Liver					
Hepatocellular adenoma	1/50	8/50*	11/49**	33/49**	43/50**
Hepatocellular carcinoma	0/50	0/50	4/49	8/49**	22/50**
Hepatocellular adenoma or carcinoma	1/50 (3)	8/50 (20)*	14/49 (33)**	34/49 (77)**	43/50 (97)**
Hepatocholangioma	0/50	0/50	0/49	0/49	8/50**
Hepatocholangiocarcinoma (includes multiple)	0/50	0/50	0/49	3/49	9/50**
Hepatocholangioma or hepatocholangiocarcinoma (includes multiples)	0/50 (0)	0/50 (0)	0/49 (0)	3/49 (8)	17/50 (43)**
Glandular stomach					
Benign neuroendocrine tumor	0/50	0/50	13/50**	9/50**	5/50**
Malignant neuroendocrine tumor	0/50	1/50	12/50**	26/50**	36/50**
Benign or malignant neuroendocrine tumor	0/50 (0)	1/50 (2)	25/50 (59)**	34/50 (80)**	41/50 (82)**
Forestomach: Squamous cell papilloma or carcinoma	0/50	0/50	1/50	3/50	1/50

Source: NTP 1998

*$P \leq 0.05$; significantly different from vehicle controls (Poly-3 test).

**$P \leq 0.01$; significantly different from vehicle controls (Poly-3 test).

[a]Poly-3 estimated neoplasm rates, which adjust for intercurrent mortality, are given in parentheses.

[b]Stop-exposure group (53 weeks of exposure to methyleugenol followed by 52 weeks of vehicle-only treatment).

The NTP concluded that under the conditions of this bioassay, there was *clear evidence of carcinogenic activity* for methyleugenol in male and female F344/N rats. This conclusion was based on increased incidences of liver neoplasms and neuroendocrine tumors of the glandular stomach in male and female rats and increased incidences of kidney neoplasms, malignant mesotheliomas, mammary gland fibroadenomas, subcutaneous fibromas, and fibromas or fibrosarcomas (combined) in male rats (NTP 1998).

4.2 Oral administration study in mice

The carcinogenic potential of methyleugenol was evaluated in a cancer bioassay in mice of both sexes (NTP 1998). In the study, 6- to 7-week-old $B6C3F_1$ mice (50 per sex) received methyleugenol in 0.5% methylcellulose by gavage at doses of 37, 75, or 150 mg/kg b.w. per day, five days per week for 104 weeks. Other groups of $B6C3F_1$ mice (50 per sex) were administered 0.5% methylcellulose vehicle only and served as controls.

The mean body weights of the female mice administered methyleugenol were lower than those of the vehicle control mice by week 17 of the study. In male mice, mean body weights were generally less than those of the vehicle control groups after weeks 81, 41, and 17 for the 37, 75, and 150 mg/kg groups, respectively. Although the survival rate was similar in all male groups (exposed and control), it was significantly lower among exposed females. The survival rates are summarized in Table 4-4.

Table 4-4. Survival of male and female B6C3F$_1$ mice administered methyleugenol by gavage for up to 104 weeks

Sex	Dose level (mg/kg per day)			
	0	37	75	150
Males	38/50	36/50	37/50	35/50
Females	31/50	18/50	18/50[a]	2/50

Source: NTP 1998

[a]Two animals died during the last week of the study

Histopathological examination of the tissues revealed malignant and/or benign tumors in the liver and glandular stomach. The incidences of hepatocellular adenoma and hepatocellular carcinoma were significantly increased in male mice. The incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma were significantly increased in female mice. Two malignant neuroendocrine tumors of the glandular stomach found in the high-dose male mice were considered to be related to methyleugenol exposure because of the rarity of these tumors in control mice.

In addition, a retrospective analysis of tissues from this study, using an assay based on polymerase chain reaction–restriction fragment length polymorphism, found *Helicobacter hepaticus* in 4 of 14 mice in the methyleugenol study. *H. hepaticus* is associated with the development of oval-cell hyperplasia. However, in the female mice administered methyleugenol, the incidence of this tumor exhibited a dose-response pattern (a pattern not seen in nine other studies in which *H. hepaticus* was found in retrospective analyses). In addition, both treatment and control groups were relatively equally affected. Thus, the oval-cell hyperplasia observed in this study was considered to be related to methyleugenol exposure and unrelated to the presence of *H. hepaticus* (Nyska *et al.*1997).

Tumor incidences and their statistical significance are shown in Table 4-5 for male and female mice.

Table 4-5. Incidences of neoplastic lesions in male and female B6C3F$_1$ mice administered methyleugenol by gavage for up to 104 weeks

	Tumor incidence/no. mice examined (Poly-3 est. neoplasm rates)[a]			
	Dose level (mg/kg per day)			
Tumor type	0	37	75	150
Males				
Liver				
Hepatocellular adenoma	26/49	43/50**	38/50**	39/50**
Hepatocellular carcinoma	10/49	20/50*	19/50*	9/50
Hepatocellular adenoma or carcinoma	31/49 (65)	47/50 (97)**	46/50 (96)**	40/50 (86)**
Hepatoblastoma	0/49	0/50	1/50	3/50
Glandular stomach				
Carcinoma	0/49	0/48	0/49	1/50
Malignant neuroendocrine tumor	0/49	0/48	0/49	2/50
Females				
Liver				
Hepatocellular adenoma	20/50	48/50**	46/49**	41/50**
Hepatocellular carcinoma	7/50	37/50**	47/49**	47/50**
Hepatocellular adenoma or carcinoma	25/50 (55)	50/50 (100)**	49/49 (100)**	49/50 (100)**
Hepatoblastoma	0/50	6/50**	11/49**	15/50**
Hepatocholangiocarcinoma	0/50	0/50	0/50	2/50

Source: NTP 1998

*$P \leq 0.05$; significantly different from vehicle controls (Poly-3 test).

**$P \leq 0.01$; significantly different from vehicle controls (Poly-3 test).

[a]Poly-3 estimated neoplasm rates, which adjust for intercurrent mortality, are given in parentheses.

The NTP concluded that under the conditions of this bioassay, there was *clear evidence of carcinogenic activity* for methyleugenol in male and female B6C3F$_1$ mice, based on increased incidences of liver neoplasms in both sexes. Neuroendocrine tumors of the glandular stomach in males also were considered to be related to methyleugenol exposure (NTP 1998).

4.3 Intraperitoneal injection study in mice

Methyleugenol, dissolved in trioctanoin, was administered by intraperitoneal (i.p.) injection to male B6C3F$_1$ mice on days 1, 8, 15, and 22 of age (Miller *et al.* 1983). The total administered dose per mouse was 4.75 μmol (0.85 mg). Livers were examined by laparotomy at 13 months, and the surviving mice were sacrificed at 18 months. In a similar study, 1′-hyroxymethyleugenol was administered to male B6C3F$_1$ mice at a total

dose of 2.85 μmol (0.55 mg). Exposure to methyleugenol or 1′-hyroxymethyleugenol increased liver tumor incidence and multiplicity (Table 4-6).

Table 4-6. Incidences of hepatoma in male B6C3F$_1$ mice administered methyleugenol or 1′-hyroxymethyleugenol by i.p. injections on days 1, 8, 15 and 22 of age[a]

Compound	Total dose (μmol/mouse)	No. of mice examined	Hepatoma-bearing mice (%)	Mean no. of hepatomas/ mouse
Trioctanoin (vehicle control)	–	58	41	0.5
Methyleugenol	4.75	58	96*	3.2*
1′-Hyroxymethyleugenol	2.85	44	93*	3.5*

Source: Miller *et al.* 1983

*$P < 0.001$; significantly different from the vehicle control group (Fisher's exact test).

[a]Mice were observed for up to18 months.

4.4 Summary

Methyleugenol administered orally was found to be carcinogenic in rats and mice. Methyleugenol significantly increased the incidences of liver neoplasms and neuroendocrine tumors of the glandular stomach in male and female rats and the incidences of kidney neoplasms, malignant mesotheliomas, mammary gland fibroadenomas, and subcutaneous fibromas or fibrosarcomas (combined) in male rats. Methyleugenol also significantly increased the incidences of liver neoplasms in mice of both sexes. Neuroendocrine tumors of the glandular stomach in male mice also were considered to be related to methyleugenol exposure. Methyleugenol and its metabolite 1′-hyroxymethyleugenol induced liver tumors in mice that had received four i.p. doses before weaning.

5 Genotoxicity

Though limited, the peer-reviewed published literature indicates both positive and negative results in standard assays for genotoxicity. DNA-damaging effects, as evidenced by unscheduled DNA synthesis in mammalian hepatocyte systems, have been observed under certain conditions. The NTP has performed a battery of genotoxicity evaluations of methyleugenol, including assays for the following effects: gene mutation in *Salmonella typhimurium*, sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells *in vitro*, chromosomal aberrations in CHO cells *in vitro*, and micronucleus formation in erythrocytes in mouse blood *in vivo*. This section contains genotoxicity information from the literature and from the NTP Technical Report on the Toxicology and Carcinogenesis Studies of Methyleugenol (CAS No. 93-15-12) in F344/N Rats and B6C3F_1 Mice (Gavage Studies) (NTP 1998).

5.1 Prokaryotic Systems

5.1.1 Gene mutation in Salmonella typhimurium

Methyleugenol, tested up to a maximum concentration of 666 μg/plate, did not induce reverse mutations in *S. typhimurium* strain TA98, TA100, TA1535, TA1537, or TA1538, with or without S9 microsomal metabolic activation (Sekizawa and Shibamoto 1982, Mortelmans *et al*. 1986, Kettering and Torabinejad 1995, NTP 1998).

5.1.2 Gene mutation in Escherichia coli

Methyleugenol did not induce reverse mutations in *E. coli* WP2 *uvr*A *trp*$^-$ either with or without S9 metabolic activation (Sekizawa and Shibamoto 1982).

5.1.3 DNA repair in Bacillus subtilis (rec assay)

At a concentration of 1 mg/disk, methyleugenol tested positive in the *B. subtilis* DNA repair test (rec assay), with a difference of 6.5 mm between the inhibition zones for rec$^-$ and rec$^+$ (Sekizawa and Shibamoto 1982).

5.2 Non-mammalian eukaryotic systems

5.2.1 Intrachromosomal recombination in Saccharomyces cerevisiae

Methyleugenol induced positive, dose-related responses in intrachromosomal recombination studies in the yeast *S. cerevisiae,* both with and without S9 metabolic activation (Schiestl *et al.* 1989, Brennan *et al.* 1996). Methyleugenol at a concentration of 1.0 mg/mL induced a 12.5-fold increase in deletion recombination. The effect was nonlinear, with a threshold between 0.3 and 0.6 mg/mL. The threshold corresponded to the lowest concentration associated with any cytotoxic effect. The authors noted that this type of nonlinear, threshold-dependent response has been reported as characteristic of carcinogens that test negative for gene mutation in *S. typhimurium,* and of some oxidative mutagens (Brennan *et al.* 1996).

5.3 Mammalian Systems

5.3.1 In vitro *assays*

5.3.1.1 Chromosomal aberrations in CHO cells

Methyleugenol did not induce chromosomal aberrations in cultured CHO cells in either the presence or the absence of S9. The methyleugenol concentrations tested were limited by cytotoxicity to a high of 233 μg/mL (NTP 1998).

5.3.1.2 Sister chromatid exchange in CHO cells

Methyleugenol induced SCEs in cultured CHO cells in each of two replicate trials with S9 metabolic activation at concentrations between 17 and 250 μg/mL. The increases in SCEs per chromosome relative to solvent controls ranged from 17.5% to 69.6%. In the absence of S9 activation, no significant increase in SCEs was observed (NTP 1998).

5.3.1.3 Unscheduled DNA synthesis in rat hepatocytes

Methyleugenol induced unscheduled DNA synthesis (UDS) in rat hepatocytes in primary cultures. Dose-related increases in UDS were observed at methyleugenol concentrations from 10^{-4} M to 10^{-3} M, with DNA synthetic activity exceeding control values by as much as 2.7 times (a ratio of 1.5 indicates a positive response) (Howes *et al.* 1990, Chan and Caldwell 1992).

The methyleugenol metabolite 1′-hydroxymethyleugenol was more potent than its parent compound as an inducer of UDS in rat hepatocytes. Dose-related increases in UDS were observed at 1′-hydroxymethyleugenol concentrations from 10^{-5} M to 10^{-4} M, about an order of magnitude lower than methyleugenol concentrations that produced a similar degree of DNA damage and repair. 1′-Hydroxymethyleugenol also was more cytotoxic than methyleugenol (by about an order of magnitude), as measured by the lactate dehydrogenate leakage viability assay (Chan and Caldwell 1992, Gardner *et al.* 1997).

5.3.1.4 Transformation of Syrian hamster embryo (SHE) cells

Methyleugenol at concentrations of 185 to 250 μg/ml for 24 hours induced morphological transformation of cultured SHE cells. The transformation frequency for exposed cultures was approximately four times the frequency for control cultures, and the response was not dose related.

5.3.2 In vivo *assays*

5.3.2.1 Mouse micronucleus test

Methyleugenol administered by gavage to male and female $B6C3F_1$ mice at doses of 10 to 1,000 mg/kg for 14 weeks (at unspecified intervals) did not increase the frequency of micronucleated normochromatic erythrocytes in peripheral blood. In the same study, methyleugenol did not affect the percentage of polychromatic erythrocytes in the blood, indicating no detectable bone marrow toxicity at the doses tested (NTP 1998).

Table 5-1. Genetic and related effects of methyleugenol exposure

Test system	End point	Results	References
S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	gene mutation	negative with or without S9	Sekizawa and Shibamoto 1982, Mortelmans *et al.* 1986, Kettering and Torabinejad 1995, NTP 1998
E. coli WP2	gene mutation	negative	Sekizawa and Shibamoto 1982
B. subtilis	DNA repair (*rec*-assay)	positive	Sekizawa and Shibamoto 1982
S. cerevisiae	intrachromosomal recombination	positive	Schiestl *et al.* 1989, Brennan *et al.* 1996
CHO cells	chromosomal aberrations	negative with or without S9	NTP 1998
CHO cells	SCE	positive with S9	NTP 1998
Rat hepatocyte primary cultures	UDS	positive	Howes *et al.* 1990, Chan and Caldwell 1992
SHE cells	morphological transformation	positive	Kerckaert *et al.* 1996
$B6C3F_1$ mice, exposed *in vivo* by gavage	micronucleus test	negative	NTP 1998

5.4 Summary

Table 5-1 summarizes the genetic and related effects of methyleugenol. Methyleugenol did not induce gene mutations in *S. typhimurium* or *E. coli* WP2, either with or without liver S9 metabolic activation. However, methyleugenol tested positive in the rec assay for DNA repair in *B. subtilis* and induced intrachromosomal recombination in *S. cerevisiae*. Methyleugenol did not induce chromosomal aberrations in CHO cells *in vitro,* but it did induce SCEs in CHO cells incubated with rat liver S9. Methyleugenol also induced DNA repair, measured as unscheduled DNA synthesis, in rodent hepatocytes *in vitro* and tested positive in the SHE cell morphological transformation assay. Methyleugenol administered by gavage for 14 weeks did not induce micronuclei in the erythrocytes of $B6C3F_1$ mice.

6 Other Relevant Data

6.1 Absorption, distribution, metabolism, and excretion

Methyleugenol is rapidly absorbed following oral administration to F344/N rats or $B6C3F_1$ mice (NTP 1998). Rats and mice were administered methyleugenol by gavage at dose levels of 36, 75, 150, or 300 mg/kg b.w., either as single doses or for five days per week for 6, 12, or 18 months, or they received single intravenous doses of 36, 75, 150, or 300 mg/kg b.w. The kinetic data are consistent with rapid clearance from the blood, metabolism in the liver, and elimination of metabolites in the urine.

In rats of both sexes, plasma levels of methyleugenol peaked within the first five minutes at all dose levels. Methyleugenol was preferentially distributed to the liver within 72 hours of administration. Elimination of orally administered methyleugenol from the bloodstream was rapid and multiphasic, with terminal half-lives on the order of 15 to 30 minutes in both sexes. Absorbed methyleugenol was rapidly and extensively metabolized, and 85% of its metabolites were eliminated in the urine within 72 hours. The majority of the excreted metabolites were identified as hydroxylated, sulfated, and glucuronidated compounds (NTP 1998).

In mice, plasma levels of methyleugenol peaked within the first five minutes at all dose levels in both sexes. However, methyleugenol was preferentially distributed to the ovaries, stomach, fat, spleen, and liver within 72 hours of administration. Elimination of orally administered methyleugenol from the bloodstream was rapid and multiphasic, with terminal half-lives on the order of 15 to 30 minutes. Absorbed methyleugenol was rapidly and extensively metabolized, and 85% of its metabolites were eliminated in the urine within 72 hours. Although the nature of the major urinary metabolite was unknown, the minority portion contained hydroxylated, sulfated, and glucuronidated compounds (NTP 1998).

6.2 Bioactivation

Methyleugenol is metabolized by the cytochrome P-450 system (Borchert *et al.* 1973, cited in NTP 1998) by three different pathways: *O*-demethylation, side-chain hydroxylation, or side-chain epoxidation. Of the various metabolites formed, 1′-hydroxymethyleugenol and methyleugenol-2′,3′-oxide, were considered to be the ones most likely responsible for the toxic effects of methyleugenol in the liver (Solheim and Scheline 1976, cited in NTP 1998; Woo *et al.* 1997).

The metabolic bioactivation of methyleugenol to its DNA- and protein-reactive intermediates is a two-step process. The first step involves hydroxylation at the 1′ position of the allyl side chain to yield 1′-hydroxymethyleugenol. 1′-Hydroxymethyleugenol is subsequently sulfated to yield 1′-sulfoxy metabolites, which decompose spontaneously in an aqueous environment to electrophilic carbonium ions that can bind covalently to DNA and other cellular macromolecules, including protein (Gardner *et al.* 1996, 1997). A proposed scheme for the bioactivation of methyleugenol is shown in Figure 6-1 (Gardner *et al.* 1996).

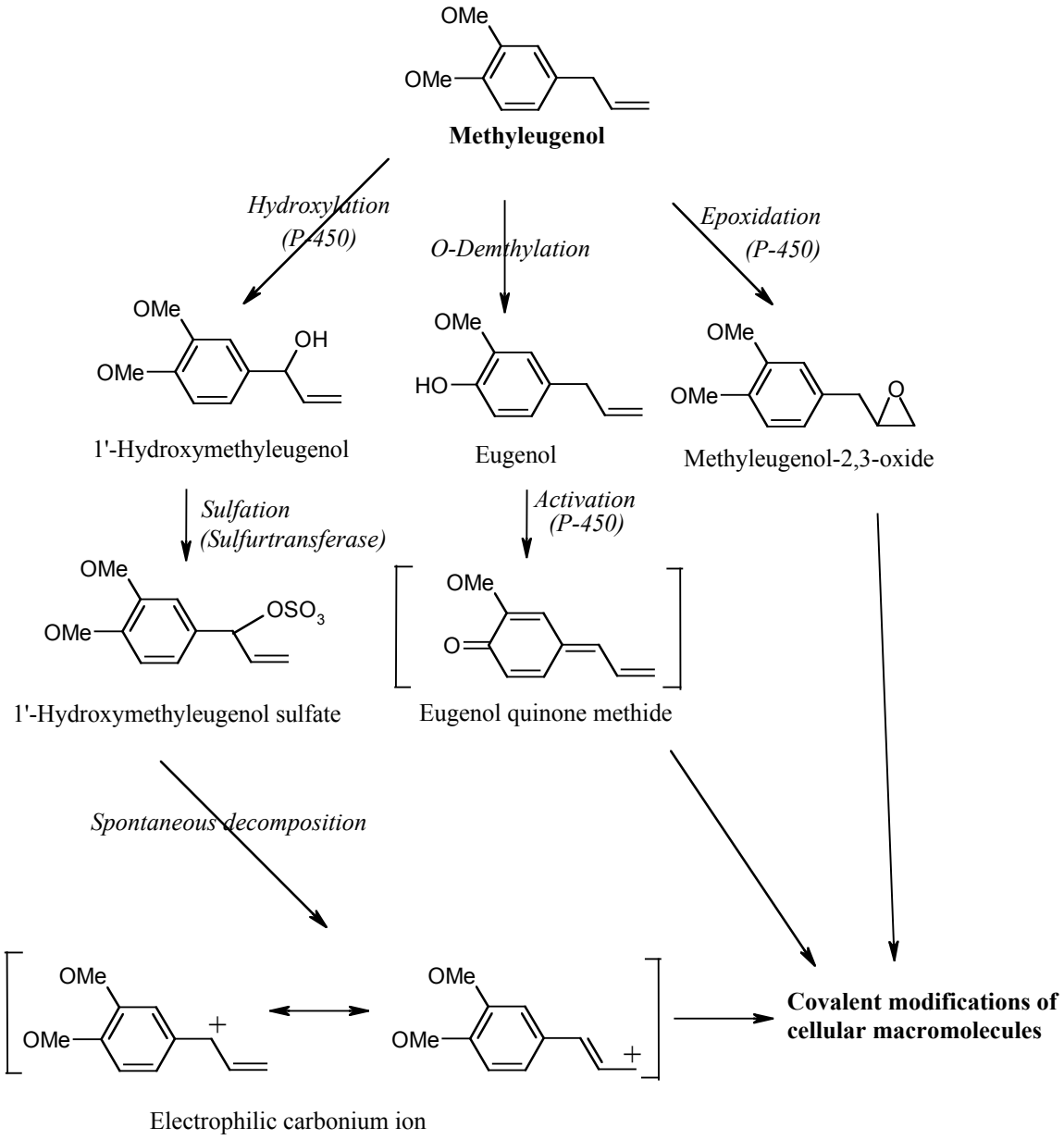
Based on DNA binding data and studies of liver tumor induction by allylbenzenes and their metabolites, it is apparent that bioactivation of methyleugenol via the pathway of side-chain hydroxylation followed by sulfation (Figure 6-1) is an important step in liver tumor induction by this chemical. Bioactivation via the side-chain epoxidation or *O*-demethylation pathways may also contribute to the cancer process.

The lack of correlation between *in vitro* mutagenicity and carcinogenicity of methyleugenol may be due to the requirement of sulfation in the metabolic activation of this compound (Randerath *et al.* 1984, Boberg *et al.* 1983, cited in NTP 1998). In studies with analog allylbenzenes, pretreatment with sulfotransferase inhibitors inhibited the binding of safrole to mouse liver DNA and the tumorigenicity of 1′-hydroxysafrole (Miller *et al.* 1983, Boberg *et al.* 1983, Randerath *et al.* 1984, Gardner *et al.* 1996).

6.3 Formation of protein and DNA adducts

Methyleugenol protein adducts were detected by immunochemical methods (enzyme-linked immunosorbent assay and immunoblotting) in the livers of male F344 rats injected i.p. with methyleugenol (dissolved in tricaprylin) at doses of 10 to 300 mg/kg b.w. per day for five days. A 44-kDa adduct was the major adduct found in the livers of the rats given higher doses of methyleugenol (100 or 300 mg/kg) and the only adduct detected in the livers of rats given low doses of methyleugenol (10 or 30 mg/kg) (Gardner *et al.* 1996).

Methyleugenol-DNA adducts were detected by a modified ^{32}P-postlabeling analysis in the livers of female CD-1 mice injected i.p. with methyleugenol (in trioctanoin) at doses of 2 or 10 mg per mouse. The livers of the mice were sampled at 24 hours and 7, 28, 58, 98, and 140 days after exposure. The major adducts identified were 3′,5′-biphosphate of a N^2-(*trans*-propenylbenzene-3′-yl) deoxyguanosine and a derivative of a N^2-(allylbenzen-1′-yl)deoxyguanosine, and the minor adducts may represent N^6-adenine derivatives. A fourth adduct, which was not characterized, represented 2% to 3% of the total adducts formed (Randerath *et al.* 1984). Similar results were obtained in ^{32}P-postlabeling studies with C57BL/6J and A/J mice (Levy and Weber 1988) and the offspring of C57B1 female mice mated with male C3H mice (B6C3F$_1$) (Phillips *et al.* 1984). Methyleugenol also was shown to bind to protein and DNA *in vitro* in assays using microsomal fractions or rat liver slices in the presence of metabolic activation (Gardner *et al.* 1996, NTP 1998, Williams 1997, Woo *et al.* 1997).



Source: Gardner *et al*. 1996.

Figure 6-1. Pathways of bioactivation of methyleugenol

6.4 Oncogene activation

*6.4.1 Activation of H-*ras *oncogene*

Methyleugenol did not induce any detectable H-*ras* codon 61 mutations in 29 methyleugenol-induced hepatocellular neoplasms in $B6C3F_1$ mice from the NTP two-year gavage study (NTP 1998, Devereux *et al.* 1999).

6.4.2 Activation of β-catenin *oncogene*

Upregulation of Wnt signaling, the result of *β-catenin* activation, is an important event in the development of certain human and rodent cancers (Gumbiner 1997). A study of methyleugenol-induced hepatocellular neoplasms obtained from the NTP two-year carcinogenesis study in $B6C3F_1$ mice (NTP 1998) found point mutations at codons 32, 33, 34, or 41, indicative of *β-catenin* activation, in 20 of the 29 tumors analyzed (Devereux *et al.* 1999). This incidence was significantly greater than the incidence of *β-catenin* mutations in liver tumors from controls (2 of 22). *β-catenin* mutations were detected almost equally in adenomas and carcinomas, indicating that this is an early event in carcinogenesis. The *β-catenin* mutation frequency in these tumors did not appear to be related to methyleugenol dose.

6.5 Structure-activity relationships

Eugenol, safrole, isosafrole, and estragole are allylbenzene compounds that are metabolized via pathways similar to those for methyleugenol to yield analogous side-chain hydroxylation and side-chain epoxidation products (Borchert *et al.* 1973, Stillwell *et al.* 1974, Solheim and Scheline 1976, Delaforge *et al.* 1980a, b, Sims and Grover 1974, Miller *et al.* 1983, all cited in NTP 1998).

Methyleugenol, estragole, and safrole produce similar levels of DNA, RNA, and protein binding. Through ^{32}P-postlabeling analysis, N^2-(estragol-1′-yl)deoxyguanosine and N^2-(*trans*-isoestragol-3′-yl)deoxyguanosine were found to be the major DNA adducts in the livers of CD-1 mice treated with [^{3}H]1′-hydroxyestragole, the proximate carcinogenic metabolite of estragole. N^2-(*cis*-isoestragol-3′-yl)deoxyguanosine and N^6-(*trans*-isoestragol-3′-yl)deoxyguanosine were identified as the minor DNA adducts. DNA adducts that had similar elusion profiles and were assigned analogous structures were obtained from the livers of mice treated with [^{3}H]1′-hydroxysafrole, the proximate carcinogenic metabolite of safrole. The similarity of chromatographic migration (polyethyleneimine-cellulose) maps of the DNA adducts for estragole, safrole, and methyleugenol suggests that the pathway for the activation of all three allylbenzenes may be similar. Anethole, elemicin, parsley and dill apioles, and myristicin were less active in the formation in DNA adducts. No DNA adducts were detected for eugenol (Phillips *et al.* 1984, Randerath *et al.* 1984).

The reduced ability of other structural analogs of methyleugenol (eugenol, elemicin, myristicin, dill apiole, and parsley apiole) to bind DNA or induce carcinogenesis has been suggested to result from the differences in the substitution positions and substituent groups. Thus, methyleugenol, safrole, and estragole, which have methoxy and/or methylenedioxy substitutions at the 4-position or at both the 3-position and 4-position, exhibit the greatest DNA-binding capacity and carcinogenic potential *in vivo*. Elemicin, and myristicin, with substitutions at the 3-position, 4-position, and 5-position, have intermediate DNA-binding capacity, but the levels and/or persistence of the DNA adducts formed apparently are inadequate to induce significant incidences of cancers. Dill apiole and parsley apiole, which have substitutions at the 5-position and at the 2-position, have low DNA-binding capacities and reduced ability to cause cancers. Eugenol, which has a

hydroxy substitution at the 1-position, exhibits no potential to bind DNA (Phillips *et al.* 1984).

6.6 Genotoxicity of some compounds structurally related to methyleugenol

Table 6-1 summarizes genetic and related effects of eugenol, safrole, and estragole. Similarly to methyleugenol, these compounds did not induce mutations in *S. typhimurium* but did induce UDS in rat hepatocytes. In addition, eugenol and safrole induced SCEs in CHO cells, and safrole did not induce chromosomal aberrations.

Table 6-1. Genetic and related effects of allylbenzene compounds structurally related to methyleugenol

Compound	Test system	End point	Results	References
Eugenol	*S. typhimurium*	gene mutations	negative w/ or w/out S9	Sekizawa and Shibamoto 1982, Haworth *et al*. 1983
Safrole			negative w/ or w/out S9	Zeiger and Haworth 1985
Estragole			negative w/ or w/out S9	Zeiger *et al*. 1987
Eugenol	*S. cerevisiae*	intrachromosomal recombination	positive	Schiestl *et al*. 1989
Safrole			–	–
Estragole			–	–
Eugenol	*Drosophila melanogaster*	sex-linked recessive lethal mutation or reciprocal translocation	negative	Foureman *et al*. 1994
Safrole			negative	Zimmering *et al*. 1989
Estragole			–	–
Eugenol	CHO cells	chromosomal aberrations	positive	Galloway *et al*. 1997
Safrole			negative	Gulati *et al*. 1985
Estragole			–	–
Eugenol	CHO cells	SCE	positive	Galloway *et al*. 1997
Safrole			positive	Gulati *et al*. 1985
Estragole			–	–
Eugenol	Rat hepatocyte primary cultures	UDS	positive	Howes *et al.* 1990, Chan and Caldwell 1992
Safrole			positive	Howes *et al.* 1990, Chan and Caldwell 1992
Estragole			positive	Howes *et al.* 1990, Chan and Caldwell 1992
Eugenol	L5178Y mouse lymphoma cells	gene mutation	positive	Myhr and Caspary 1991, Sofuni *et al*. 1996
Safrole			positive	Mitchell *et al*. 1988
Estragole			positive	Myhr and Caspary 1988
Eugenol	mice,	micronucleus test	negative	Shelby *et al*. 1993

Compound	Test system	End point	Results	References
	i.p. injection			
	mice, i.p. injection		negative	Hayashi *et al*. 1988, Maura *et al*. 1989
			positive	Woolverton *et al*. 1986
	rats, oral exposure		negative	Hayashi *et al*. 1988, Maura *et al*. 1989
	rats, gavage		positive	Woolverton *et al*. 1986
Safrole	–		–	–
Estragole	–		–	–
Eugenol	$B6C3F_1$ mice, gavage	chromosomal aberrations	equivocal	NTP 2000
Safrole			–	–
Estragole			–	–

6.7 Carcinogenicity of some compounds structurally related to methyleugenol

6.7.1 Induction of liver tumors

Safrole, estragole, and their 1′-hydroxy metabolites induced liver tumors in preweanling mice when administered via gavage or i.p. injection (Miller *et al.* 1983). Eugenol did not induce tumors in these studies. Dietary administration of safrole, estragole, and 1′-hydroxyestragole to mice for 12 months induced liver tumors. The tumorigenicity of these allylbenzene compounds and some of their metabolites in B6C3F$_1$ and CD-1 mice is summarized in Table 6-2 for i.p. administration and in Table 6-3 for dietary administration.

Table 6-2. Incidences of tumors in male mice administered allylbenzenes or their metabolites by i.p. injection on days 1, 8, 15, and 22 of age

Compound	Total dose (µmol/mouse)	No. of mice examined	Hepatoma-bearing mice (%)	Mean no. of hepatomas/ mouse
CD-1 mice observed for up to 12 months				
Trioctanoin (vehicle control)	–	42	26	0.5
Safrole	9.45	48	67**	1.9**
1′-Hydroxysafrole	4.72	46	65**	2.7*
Safrole 2′,3′-oxide	9.45	44	14	0.3
Estragole	9.45	46	65**	1.7**
Eugenol	9.45	45	24	0.6
B6C3F$_1$ mice observed for up to 12 months				
Trioctanoin	–	32	15	0.2
1′-Hydroxysafrole	3.75	26	92**	2.7*
1′-Hydroxyestragole	1.87	27	93**	2.7*
B6C3F$_1$ mice observed for up to 18 months				
Trioctanoin	–	58	41	0.5
Estragole	4.75	41	83**	2.4**
1′-Hydroxyestragole	1.90	60	98**	5.6*

Source: Miller *et al.* 1983

*$P < 0.05$; significantly different from the vehicle control group (Fisher's exact test).

**$P < 0.001$; significantly different from the vehicle control group (Fisher's exact test).

Table 6-3. Incidences of tumors in female CD-1 mice administered allylbenzenes in the diet for 12 months

Compound	% in diet[a]	No. of mice alive at 10 mo.	Hepatoma-bearing mice (%)	Mean no. of hepatomas/ mouse
Control	0	50	0	0
Safrole	0.25	47	72**	2.1**
Safrole	0.5	49	80**	2.3**
Estragole	0.23	48	56**	1.4**
Estragole	0.46	49	71**	1.8**
1′-Hydroxyestragole	0.25	43	56**	1.2*
Eugenol	0.5	29	0	0

Source: Miller *et al.* 1983

*$P < 0.05$; significantly different from the vehicle control group (Fisher's exact test).

**$P < 0.001$; significantly different from the vehicle control group (Fisher's exact test).

[a]After 12 months of dietary administration of the test compound, animals received control diets until the age of 20 months.

Several studies reviewed by IARC found safrole and its derivatives (isosafrole and dihydrosafrole) to be carcinogenic in rats and mice. Male CFN rats fed 1% safrole developed liver adenomas (Homburger *et al.* 1961, cited in IARC 1976). Hepatocellular sarcomas and cholangiosarcomas were diagnosed in 14 of the 19 liver tumors found in 47 autopsied Osborne-Mendel rats given safrole in the diet at a concentration of 1,000 or 5,000 mg/kg (ppm) (Long *et al.* 1963, Hagan *et al.* 1965, both cited in IARC 1976). Groups of 10 or 25 male and female Osborne-Mendel rats were given dihydrosafrole (Hagan *et al.* 1965, Long and Jenner 1963, both cited in IARC 1976) or isosafrole (Hagan *et al.* 1965, 1967, cited in IARC 1976) in the diet, both at concentrations of 1,000 to 10,000 mg/kg (ppm), for two years. Dihydrosafrole induced tumors of the esophagus at dietary concentrations of 2,500 to 10,000 ppm, and hepatocellular adenomas and carcinomas were reported in five of 50 rats that received isosafrole at a dietary concentration of 5,000 ppm.

Male and female (C57BL/6 x C3H/Anf)F_1 mice (18 per sex) administered safrole by gavage (464 mg/kg) for 4 weeks and then in the diet at a concentration of 1,112 mg/kg (ppm) for up to 82 weeks had higher incidences of liver tumors than controls (Innes *et al.* 1968, 1969, cited in IARC 1976). Increased incidences of hepatocellular carcinomas also were seen in CD-1 male mice given safrole in the diet at concentrations of 4,000 or 5,000 mg/kg (ppm) for 13 months (Borchert *et al.* 1973, cited in IARC 1976). Oral exposure to isosafrole or dihydrosafrole also increased the incidence of liver tumors in male and female (C57BL/6 x C3H/Anf)F_1 mice (Innes *et al.* 1968, 1969, cited in IARC 1976).

Eugenol was not carcinogenic in B6C3F_1 mouse pups when administered in the diet (Miller *et al.* 1983). However, in another study, dietary administration of eugenol to B6C3F_1 mice produced equivocal evidence of carcinogenicity based on marginally increased incidences of liver neoplasms (NTP 1983).

6.7.2 Induction of tumors at sites other than the liver

Neuroendocrine proliferation of the gastric mucosa in humans is an indirect effect of drugs that suppress gastric acid secretion and occurs secondary to hypergastrinemia (Bordi *et al*. 1997, 1998). Because gastrin regulates the function and growth of enterochromaffin-like (ECL) cells, chronic hypergastrinemia can induce ECL-cell hyperplasia and increase the risk of gastric cancer. However, factors that transform ECL cells to the neoplastic phenotype have not been fully determined. Analogous to the induction of gastric endocrine tumors in humans, the induction of benign and malignant neuroendocrine tumors of the glandular stomach in rats and mice exposed to methyleugenol was suggested to be due in part to induction of glandular stomach atrophy and consequent hypergastrinemia (NTP 1998). Mucosal atrophy, characterized by loss of glandular epithelial cells (particularly parietal and chief cells), and neuroendocrine cell hyperplasia were observed in the glandular stomach of rats and mice exposed to methyleugenol. The loss of glandular epithelial cells results in decreased gastric secretion, increased pH in the stomach, increased gastrin production, and gastrin-stimulated proliferation of ECL-like cells (Poynter and Selway 1991, Johnson *et al.* 1993, Thake *et al.* 1995, all cited in NTP 1998). With methyleugenol, the neoplastic conversion may also involve DNA-reactive intermediates formed via the bioactivation pathways shown in Figure 6-1.

Possible mechanisms of tumor induction by methyleugenol in other organs (kidney, mammary gland, and skin) are not known. Furthermore, it is not known whether tumor induction in other organs is affected by methyleugenol-induced alterations in the glandular stomach mucosa.

6.8 Summary

Methyleugenol is rapidly absorbed and cleared from the blood in experimental animals. Metabolism of methyleugenol occurs via the cytochrome P-450 system and involves side-chain hydroxylation, side-chain epoxide diol formation, and *O*-demethylation. Based on DNA binding data and studies of liver tumor induction by allylbenzenes and their metabolites, it is apparent that bioactivation of methyleugenol via the pathway of side-chain hydroxylation followed by sulfation (Figure 6-1) is an important step in liver tumor induction by this chemical. Activated *β-catenin* oncogenes were detected at higher frequencies in methyleugenol-induced mouse liver tumors than in tumors that arose spontaneously. Induction of benign and malignant neuroendocrine tumors of the glandular stomach in rats and mice exposed to methyleugenol may be due in part to induction of glandular stomach atrophy, reduced gastric acid secretion, hypergastrinemia, and gastrin-stimulated proliferation of ECL-like cells. DNA-reactive intermediates of methyleugenol metabolism also may be involved in the neoplastic transformation. Mechanisms of tumor induction by methyleugenol in other organs (kidney, mammary gland, and skin) or induction of mesotheliomas are not known.

7 References

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