Integrated Laboratory Systems

Estragole
[CASRN 140-67-0]

Review of Toxicological Literature

Prepared for

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EXECUTIVE SUMMARY

BASIC INFORMATION

Estragole [4-(2-propenyl)-1-methoxybenzene] was nominated by the National Institute of Environmental Health Sciences (NIEHS) based on limited carcinogenicity studies in nursing mice following subcutaneous (s.c.) injection, which resulted in a significant increase in hepatocellular carcinomas. It also is structurally similar to the known carcinogen safrole [4-(2-propenyl)-1,2-methylenedioxybenzene], and has widespread use in food and as a fragrance. Estragole is listed on the U.S. Environmental Protection Agency High Production Volume Chemicals list with an estimated annual production volume of 2.8 to 3.8 million pounds (1,300 to 1,700 metric tons).

TOXICOLOGICAL DATA

Estragole is metabolized via two major pathways *O*-demethylation and 1'-hydroxylation. In humans, 58% of an oral dose was excreted in the urine in 48 hours and 12% was exhaled as CO_2 in 8 hours. In CD-1 mice, 23% of an intraperitoneal (i.p.) dose was excreted as 1'-hydroxyestragole, as the glucuronide conjugate. In rodents, *O*-demethylation and 1'-hydroxylation are dose dependent with *O*-demethylation the major pathway at low doses and 1'-hydroxylation the major pathway at higher doses.

Acute toxicity values (LD_{50}) of about 1000 to 2000 mg/kg have been determined in the mouse and rat via i.p. and oral routes. Full strength application of estragole to the intact or abraded skin of rabbits was moderately irritating, but the dermal toxicity was low (LD_{50} >5000 mg/kg). In partially hepatectomized rats, estragole significantly increased liver regeneration. [No subchronic, chronic, and reproductive toxicity data were located.]

CARCINOGENICITY

Nursing CD-1 mice given three s.c. doses of estragole developed hepatocellular carcinomas (i.e., malignant hepatomas). Estragole induced hepatomas [note: term used by the authors; unspecified whether malignant or benign] in preweanling and 8-week-old CD-1 mice dosed i.p. or orally or when fed in the diet. In $B6C3F_1$ mice, estragole induced hepatomas within 18 months in 83% of males given three doses as nursing pups and in 95% of male mice in 10 months following a single i.p. injection on day 12 of age.

Of the metabolites identified in rodents and humans, only 1'-hydroxyestragole has been tested for carcinogenicity. Given s.c. to newborn CD-1 mice, hepatocellular carcinomas were induced by 12 months. Given i.p. or in the diet of mice, it induced hepatomas; susceptibility to hepatoma induction was found to be influenced by strain, sex, and age. Rats treated s.c. for 10 weeks did not have an increased incidence of hepatic carcinomas.

GENOTOXICITY

Estragole was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, in the presence or absence of metabolic activation. In

Escherichia coli strain WP2 *trp*$^-$, it was weakly or not mutagenic. Estragole failed to show mutagenic activity in the WP2s *uvrA* and *trpE* strains. In *Bacillus subtilis*, estragole induced DNA damage in strains PB1652 and PB1791, but not differential survival in strains H17$^+$ Rec$^+$ and M45 Rec$^-$. In cultured V79 mammalian cells, estragole was not clastogenic, with or without metabolic activation.

Estragole induced unscheduled DNA synthesis (UDS) in human skin fibroblasts and in cultured male Fischer 344 and male Wistar rat hepatocytes. All studies were conducted without exogenous metabolic activation. It also induced UDS in hepatocytes of treated rats and the formation of DNA adducts in the livers of mice.

STRUCTURAL ANALOGUES

Estragole, safrole, 1'-hydroxyestragole, 1'-hydroxysafrole, eugenol, and methyleugenol induced hepatocarcinomas in mice, while only safrole, 1'-hydroxysafrole, and methyleugenol induced a significant number of the tumors in rats. The preponderance of compounds in which increased incidences of liver, lung, and/or skin tumors have been observed were 2-propenyl compounds and their derivatives; however, this might simply be due to the testing of fewer 1-propenyl analogues.

The two major DNA adducts formed in mice given 1'-hydroxyestragole and estragole were N^2-(estragol-1'-yl)deoxyguanosine and N^2-(*trans*-isoestragol-3'-yl)deoxyguanosine. Two minor DNA adducts formed were N^2-(*cis*-isoestragol-3'-yl)deoxyguanosine and N^6-(*trans*-isoestragol-3'-yl)deoxyadenosine. With safrole, the same pattern of DNA adducts was formed in mice and in Chinese hamster ovary cells. Using ^{32}P-postlabeling, estragole, safrole, and methyleugenol formed DNA adducts in mouse liver, while the noncarcinogens, allylbenzene, anethole, myristicin, parsley apiol, dill apiol, and elemicin, were less active by 3- to 200-fold. Another metabolite of estragole, estragole 2',3'-epoxide, has been shown to form DNA adducts*in vitro*. These adducts are, however, not seen *in vivo* presumably because the epoxide is rapidly detoxified.

A comparison of the relative potencies of several estragole analogues in inducing liver tumors in 12-day-old male B6C3F$_1$ mice given a single i.p. injection with the potencies of known hepatic carcinogens was made. 1'-Hydroxy-2',3'-dehydroestragole and 1'-hydroxy-2',3'-dehydrosafrole were 5- and 10-fold, respectively, less active than diethylnitrosamine (DEN), the most active compound of the study. 1'-Hydroxyestragole, 1'-hydroxysafrole, precocene I, and *cis*-asarone were weaker carcinogens, with potencies approximately 35 to 275 times less than DEN.

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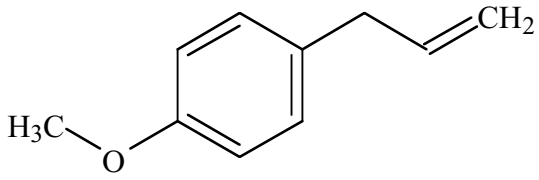
1.0 BASIS FOR NOMINATION

Estragole was nominated by the National Institute of Environmental Health Sciences (NIEHS) based on limited carcinogenicity studies in mice following a subcutaneous (s.c.) injection prior to weaning which resulted in a significant increase in hepatocellular carcinomas, its structural similarity to the known carcinogen safrole [4-(2-propenyl)-1,2-methylenedioxybenzene], and its widespread use in food and fragrances.

2.0 INTRODUCTION

Estragole
[140-67-0]



2.1 Chemical Identification

Estragole ($C_{10}H_{12}O$; mol. wt. = 148.22) is also called:

p-Allylanisole
4-Allylanisole
1-Allyl-4-methoxybenzene
4-Allylmethoxybenzene
4-Allyl-1-methoxybenzene
Anisole, *p*-allyl-
Benzene, 1-methoxy-4-(2-propenyl)-
Chavicol, *O*-methyl-
Chavicol methyl ether
Esdragol
Esdragole
Esdragon
Estragol
FEMA Number 2411
Isoanethole
p-Methoxyallylbenzene
4-Methoxyallylbenzene
3-(*p*-Methoxyphenyl)propene
Methyl chavicol
Tarragon

(HSDB, 1998)

2.2 Physical-Chemical Properties

Property	Information	Reference
Physical State	Colorless liquid	Budavari (1996)
Odor	Reminiscent of anise, differing from Anethole	HSDB (1998)
Boiling Point (^{o}C/^{o}F @ 764 mm Hg)	216/421	Budavari (1996); Esposito (1999)
Flash Point (^{o}C/ ^{o}F)	81/178	Radian (1991); Esposito (1999)
Refractive Index (@ 20 ^{o}C/D)	1.5195	Lide and Milne (1994)
Specific Gravity (@ 20 ^{o}C/4 ^{o}C)	0.9645	Karas and Piel (1994); Budavari (1996)
Water Solubility (g/L, @ 25 ^{o}C)	0.178; forms azeotropic mixtures	HSDB (1998); Budavari (1996)
Soluble in:	Ethanol, chloroform, and DMSO	Budavari (1996); Esposito (1999)

2.3 Commercial Availability

Estragole is produced by Givaudan-Roure Corporation, Specialty Division (Clifton, NJ), Aldrich Chemical Company (plant location not specified), and Penta Manufacturing Company (Fairfield, NJ) (SRI, 1998).

3.0 PRODUCTION PROCESSES AND ANALYSES

Synthetic estragole is prepared from 4-allylphenol (chavicol) by heating with methyl iodide in methanolic potassium hydroxide, or from allyl bromide and *p*-methoxyphenyl-magnesium bromide in ether (Furia and Bellanca, 1971). Estragole has been identified using the TAS method (thermomicroanalysis) [i.e., by thermally vaporizing the sample and analyzing the resulting vapor by thin layer chromatography (TLC)] (Liptak et al., 1980; cited by HSDB, 1998). Estragole may also be identified by headspace analysis of vapors above samples, using gas chromatography (GC) with mass spectroscopy (MS) and/or infrared spectroscopy (Williams et al., 1977; King and Knight, 1987). Estragole may also be isolated from American pine oil (Mookherjee and Wilson, 1994) and recovered from turpentine by distillation (Opdyke, 1976).

4.0 PRODUCTION AND IMPORT VOLUMES

Estragole is listed on the U.S. Environmental Protection Agency High Production Volume Chemicals list with an estimated annual production volume of 2.8 to 3.8 million lb (1.3 to 1.7 million kg) (U.S. EPA, 1998). In 1981, approximately 19,980 lb (9,080 kg) of estragole were produced and 17,370 lb (7,880 kg) imported (HSDB, 1998).

5.0 USES

Estragole is used as a flavoring agent in ice cream, non-alcoholic beverages, liqueurs, candy, and baked goods, and as a fragrance in perfumes, soaps, and detergents (HSDB, 1998; Budavari, 1996; Furia and Bellanca, 1971). It is also used as an antimicrobial agent against acid-tolerant food microflora (Lachowicz et al., 1998; Wan et al., 1998) and to produce synthetic anise oil (0.8% in Vietnamese variety; 5.5% in Chinese variety) (Mookherjee and Wilson, 1994).

Basil oil, which contains up to 85% estragole, has numerous claimed therapeutic uses including treatment of infections and joint pain. The German Commission E did not approve medicinal combinations with the herb as a component because of the lack of evidence for increased efficacy. The basil oil monograph noted the high concentration of estragole in basil oil and recommended that such preparations should not be used during nursing, by infants and small children, or over extended time periods (Blumenthal, 1998).

6.0 ENVIRONMENTAL OCCURRENCE AND PERSISTENCE

Estragole is the main constituent of tarragon oil (60-75%) (Furia and Bellanca, 1971; Bianchi et al., 1989 abstr.; Budavari, 1996) and has been reported to be a significant constituent of the oils of sweet basil (17-85%) (Bianchi et al., 1989 abstr.; Mookherjee and Wilson, 1994; Blumenthal, 1998), Russian anise, fennel, and turpentine (NCI, 1979; Budavari, 1996). Estragole is a monoterpene that has been occasionally reported in the foliar emissions of trees and other vegetation (Zimmerman, 1979; Isidorov et al., 1985; Winer et al., 1992; Arey et al., 1991; all cited by Guenther et al., 1994). Atmospheric persistence is low since its reactivity with respect to OH radical attack is high (< 1) (Atkinson, 1990; cited by Guenther et al., 1994). Concentrations of 10 to 45 g/L have been reported in kraft paper mill wastewaters in Georgia (Keith, 1976). Some plants containing estragole are listed in **Table 1**.

Table 1. Plants Containing Estragole

Species	Part	Concn. (ppm)
Foeniculum vulgare MILLER Fennel	Fruit	64,000
Ocimum basilicum L. Basil	Plant	9,000
Artemisia dracunculus L. Tarragon	Shoot	7,763
Piper betel L. Betel Pepper	Leaf	6,130
Limonia acidissima L. Elephant Apple, Manzana De Elefante, Wood-Apple	Leaf	0-6,570

Table 1. Plants Containing Estragole (Continued)

Species	Part	Concn. (ppm)
Hyssopus officinalis L. Hyssop	Leaf	80
	Flower	18
Petroselinum crispum (MILLER) NYMAN ex A.W. Hill Parsley	Leaf	1.6
Glycyrrhiza glabra L. Common Licorice, Licorice, Smooth Licorice	Root	1
Agastache foeniculum (PURSH) KUNTZE Giant Hyssop	Plant	not given
Agastache rugosa (FISCHER & C. Meyer) KUNTZE	Plant	not given
Dictamnus albus L. Akgiritotu, Burning Bush, Dittany, Gas Plant, Gazelotu	Plant	not given
Illicium verum HOOK. f. Star-Anise	Fruit	not given
Juniperus virginiana L. Red Cedar	Leaf	not given
Malus domestica BORKH. Apple	Essential Oil	not given
Ocimum gratissimum L. Agbo, Shrubby Basil	Shoot	not given
Origanum majorana L. Marjoram	Plant	not given
Pimpinella anisum L. Anise, Sweet Cumin	Fruit	not given
Pinus sylvestris L. Scotch Pine	Plant	not given

Source: Duke and Beckstrom-Sternberg (1999)

7.0 HUMAN EXPOSURE

Ingestion of estragole occurs from its use as an additive, flavoring agent, and fragrance in a variety of foods and cleaning and cosmetic products, and from the occasional consumption of the herbs tarragon, basil, and fennel (Drinkwater et al., 1976; Opdyke, 1976; HSDB, 1998). It has been estimated that approximately 15,000 lb of estragole are consumed in food in the United States annually (Hall, personal communication; cited by Drinkwater et al., 1976). The following products contain the amounts of estragole as indicated in the final product: non-alcoholic beverages - 10 ppm; ice cream, ices, etc. - 11 ppm; candy - 36 ppm; baked goods - 41 ppm; chewing gum - 50 ppm; condiments - 2.0 ppm; soap - usually 200 ppm (max. 2500 ppm); lotions - usually 100 ppm (max. 300 ppm); detergents - usually 20 ppm (max. 300 ppm); and perfume - usually 800 ppm (max. 3000 ppm). The average adult intake from dietary sources is estimated at 70-72 µg/day (Zangouras et al., 1981; Sangster et al., 1987).

The National Institute for Occupational Safety and Health (NIOSH) 1981-1983 National Occupational Exposure Survey (NOES) estimated that approximately 9,128 workers (6,777 female) in 668 facilities were potentially exposed to estragole annually (RTECS, 1998).

8.0 REGULATORY STATUS

Federal regulations pertaining to estragole are summarized in **Table 2**.

Table 2. Regulations Relevant to Estragole

	Regulation	Summary of Regulation
F D A	21 CFR 172	Subpart F Flavoring Agents and Related Substances. ⁄172.515 Synthetic flavoring substances and adjuvants. Synthetic estragole can be safely used in food in accordance with the conditions of this subpart. The substances should be used in the minimum quantity to produce their intended effect.
	21 CFR 182	Subpart A Substances That Are Generally Recognized as Safe. ⁄182.20 Essential oils, oleoresins (solvent-free), and natural extractives (including distillates): Estragole (esdragol, esdragon, tarragon) from *Artemisia dracunculus* is listed as a substance generally recognized as safe for its intended use, within the meaning of Section 409 of the Federal Food, Drug, and Cosmetic Act.

9.0 TOXICOLOGICAL DATA

9.1 General Toxicology

9.1.1 Human Data

No human data were located.

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

In vivo, estragole is metabolized by hydroxylation of the C-1 position of the allyl side-chain to yield the urinary metabolite 1'-hydroxyestragole (Solheim and Scheline, 1973; Rostron, 1977; Zangouras et al., 1981; Anthony et al., 1987). Estragole is also metabolized by several other pathways, including *O*-demethylation (to give chavicol and CO_2), epoxidation of the double bond, and oxidative degradation of the side-chain to carboxylic acids (Solheim and Scheline, 1973; Delaforge et al., 1980; cited by Zangouras et al., 1981).

Human Metabolism

The details of these studies are presented in **Table 3**.

In a study by Sangster et al. (1987), estragole (100 µg; 0.675 µmol) administered orally was eliminated primarily in the urine and as CO_2 in expired air. In urine, five metabolites 1'-hydroxyestragole (0.3%), 4-methoxyhippuric acid (12%), 4-methoxyphenyllactic acid (4%), 4-methoxycinnamoylglycine (0.8%), and 4-methoxyphenylacetic acid (0.5%) were identified.

Metabolism in Animals

The details of these studies are presented in **Table 4**.

Approximately 23% of a single dose of estragole (0.274 mg/g body weight; 1.85 μmol/g body weight) was excreted as a 1'-hydroxyestragole conjugate in 21-day-old and adult CD-1 mice within 24 hours (Drinkwater et al., 1976). Zangouras et al. (1981) found that both *O*-demethylation and 1'-hydroxylation are dose-dependent in both Wistar rats and CD-1 mice, with a proportional decrease in *O*-demethylation and an increase in 1'-hydroxylation as the dose increased from 0.05 to 1000 mg/kg. In another study, Anthony et al. (1987) also found that the major metabolic pathways for estragole (i.p., 0.05-1000 mg/kg; 0.3-6,750 mol/kg) in adult male CD-1 mice and female Wistar albino rats (oral intubation, 0.05-1000 mg/kg; 0.3-6,750 mol/kg) were dose-related. At doses up to 50 mg/kg (0.34 mmol/kg), demethylation predominated and urinary excretion of the radiolabel was of minor importance. As the dose increases, the demethylation became a minor route of excretion and 1'-hydroxylation predominated. The proposed metabolic pathways of estragole in rats and mice are depicted in **Figure 1**.

Species Comparison

At low doses, the amount of 1'-hydroxyestragole (excreted as the glucuronide conjugate) accounted for 0.3% of the administered dose (100 g) in humans (Sangster et al., 1987) and almost 1% in rats and mice given 50 g/kg (Zangouras et al. 1981). For doses above 250 mg/kg, rats and mice excrete 8-23% as conjugated 1'-hydroxyestragole (Drinkwater et al., 1976; Anthony et al. 1987; Zangouras et al. 1981).

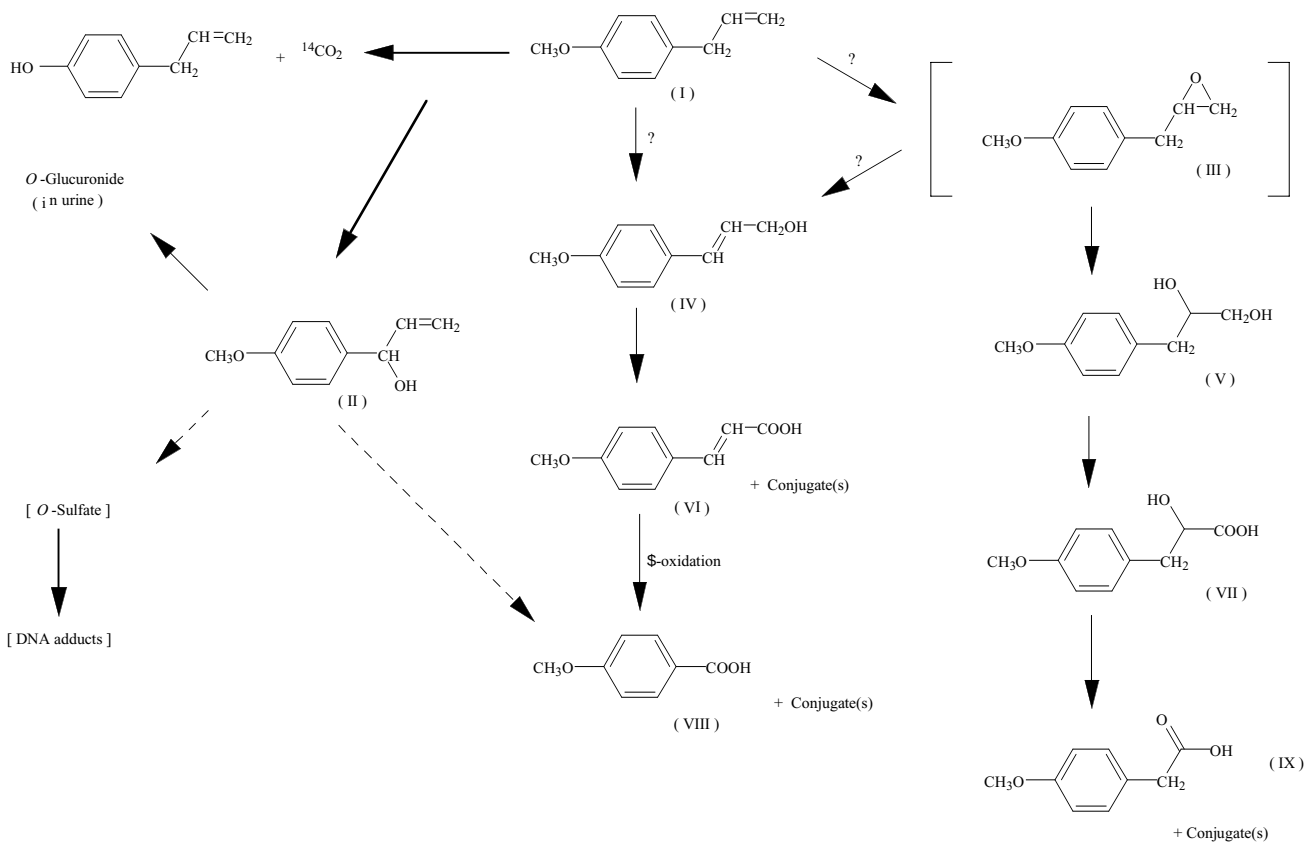


Figure 1. Proposed Metabolic Pathways of Estragole in the Rat and Mouse. Putative intermediates not isolated are shown in square brackets. Broken lines and ? indicate potential but uncertain pathways to isolated metabolites. The compounds identified are: I, estragole; II*, 1'-hydroxyestragole; III, estragole 2',3'-oxide; IV*, 4-methoxycinnamyl alcohol; V, 2',3'-dihydroxy-4-propylanisole; VI*, 4-methoxycinnamic acid (830-09-1); VII, 4-methoxyphenyllactic acid; VIII*, 4-methoxybenzoic acid (100-09-4); and IX*, 4-methoxyphenylacetic acid. (Adapted from Anthony et al., 1987)

*Known human metabolite (Source: Sangster et al., 1987)

Table 3. Metabolism of Estragole in Humans

Age of Subjects	Number and Sex of Subjects	Chemical Form, Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
35-yr-old and 47-yr-old	2 M volunteers	[*methoxy*-^{14}C]Estragole (sp. act. 62 µCi/mg), radiochemical purity >99%	Oral; 100 µg (0.675 µmol; containing 5-10 µCi) dissolved in 0.25 mL trioctanoin in a gel capsule	Urine collected at hourly intervals up to 8 h, and after from 8-12, 12-24, and 24-48 h.	Most of the administered dose was excreted as exhaled $^{14}CO_2$ within 8 h, and urinary excretion was complete within 12 h. There was no fecal elimination. The major metabolites identified were 4-methoxyhippuric acid (12%), 4-methoxyphenyllactic acid (VII) (4%), 4-methoxycinnamoylglycine (0.8%), 4-methoxyphenylacetic acid (IX) (0.5%), and 1'-hydroxyestragole (II) (0.3%).	Sangster et al. (1987)

Abbreviations: h = hours; M = male; sp. act. = specific activity; yr = years

Note: Roman numerals refer to the structures in **Figure 1**.

Table 4. Metabolism of Estragole in Animals

Species, Strain, and Age	Number and Sex of Animals	Chemical Form, Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Mice						
CD-1, 21-day-old and 9- to 12-wk-old	5 M/group	Estragole, >99% purity	i.p.; 185 µmol (27.4 mg)/100 g bw in trioctanoin	Urine collected up to 24 h in metabolism cages	Both 21-day-old and adult mice excreted approximately 23% of a dose of estragole in the urine as a conjugate (presumably the glucuronide) within 24 h of dosing.	Drinkwater et al. (1976)
CD-1 (age n.p.)	At least 3 M/group	[*methoxy*-^{14}C]Estragole (sp. act. g. 1 mCi/mmol [6.7 µCi/mg]), radiochemical purity >99%	i.p.; 2 µCi/20 g bw in trioctanoin, equivalent to 0.05, 5, 500, and 1000 mg/kg (0.3, 34, 3370, and 6747 mol/kg)	Urine collected up to 24 h in metabolism cages	As the dose increased, excretion of exhaled CO_2 fell (from a mean of 38% to 22%), while urinary excretion rose (from a mean of 29% to 50%). The amount of 1'-hydroxyestragole in the urine increased as the dose increased (from 1.3% to 9.5%).	Zangouras et al. (1981)
CD-1 mice (age n.p.)	At least 4 M/dose	[*methoxy*-^{14}C]Estragole (sp. act. 62 µCi/mg), radiochemical purity >99%	i.p.; 0.05, 1, 5, 50, 100, 250, 500, and 1000 mg/kg (0.3, 7, 34, 340, 675, 1690, 3370, and 6747 mol/kg)	Urine, feces, and expired air collected up to 48 h in metabolism cages; urinalysis was performed on the 50 mg/kg dose group samples.	The main route of elimination was expired CO_2; urine was a minor route; trace amounts were excreted in the feces. Elimination of ^{14}C was essentially complete within 24 h. In the 50 mg/kg urine samples, the major metabolite was 4-methoxyhippuric acid (6.7% of dose), followed by 1'-hydroxyestragole (II) (5.2%), 4-methoxyphenaceturic acid (4-methoxyphenylacetylglycine) (3.3%), 4-methoxyphenyllactic acid (VII) (3.0%), and 4-methoxycinnamyl alcohol (IV) (1.5%). The remaining metabolites comprised <1% of dose.	Anthony et al. (1987)

Table 4. Metabolism of Estragole in Animals (Continued)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form, Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats						
Wistar albino (age n.p.)	4 F/dose	[*methoxy*-^{14}C]Estragole (sp. act. 62 µCi/mg), radiochemical purity >99%	Oral intubation; 0.05, 0.5, 5, 50, 100, 500, and 1000 mg/kg (0.3, 3.4, 34, 340, 675, 3370, and 6747 mol/kg)	Urine, feces, and expired air collected up to 48 h in metabolism cages; urinalysis was performed on the 50 mg/kg dose group samples.	The main route of elimination was expired CO_2; urine was a minor route; trace amounts were excreted in the feces. Significant excretion of ^{14}C was still significant for the 500 and 1000 mg/kg dose groups. In the 50 mg/kg urine samples, the major metabolite was 4-methoxyhippuric acid (8.2% of dose), followed by 1'-hydroxyestragole (II) (5.4%), 4-methoxyphenyllactic acid (VII) (4.5%), 4-methoxycinnamyl alcohol (IV) (2.9%), and 4-methoxyphenaceturic acid (1.2%). The remaining metabolites comprised <1% of dose.	Anthony et al. (1987)
Wistar albino (age n.p.)	At least 3 F/dose	[*methoxy*-^{14}C]Estragole (sp. act. g. 1 mCi/mmol [6.7 µCi/mg]), radiochemical purity >99%	i.p.; 20 µCi/200 g bw in trioctanoin, equivalent to 0.05, 5, 500, and 1000 mg/kg (0.3, 34, 3370, and 6747 mol/kg)	Urine collected up to 24 h in metabolism cages	As the dose increased, excretion of exhaled CO_2 fell (from a mean of 34% to 20%), while urinary excretion rose (from a mean of 26% to 53%). The amount of 1'-hydroxyestragole in the urine increased as the dose increased (from 0.9% to 8.0%).	Zangouras et al. (1981)

Abbreviations: bw = body weight; F = females; h = hours; i.p. = intraperitoneal injection; M = males; n.p. = not provided; sp. act. = specific activity; wk = week(s)

Note: Roman numerals refer to the structures in **Figure 1**.

9.1.3 Acute Exposure

Acute toxicity values for estragole are presented in **Table 5**.

Table 5. Acute Toxicity Values for Estragole

Route	Species (sex and strain)	LD_{50}	Reference
Dermal	Rabbit (species, sex n.p.)	LD_{50} > 5000 mg/kg (30 mmol/kg)	RTECS (1998); Moreno (1972; cited by Opdyke, 1976)
i.p.	Mouse (species, sex n.p.)	LD_{50} = 1260 mg/kg (8.501 mmol/kg)	RTECS (1998)
	Rat (species, sex n.p.)	LD_{50} = 1030 mg/kg (6.949 mmol/kg)	RTECS (1998)
Oral	Mouse (species, sex n.p.)	LD_{50} = 1250 mg/kg (8.433 mmol/kg)	RTECS (1998); Jenner et al. (1964; cited by Opdyke, 1976)
	Rat (species, sex n.p.)	LD_{50} = 1230 mg/kg (8.298 mmol/kg); 1820 mg/kg (12.28 mmol/kg)	RTECS (1998); Moreno, (1972); Jenner et al. (1964); both cited by Opdyke (1976)

Abbreviations: i.p. = intraperitoneal; LD_{50} = dose lethal to 50% of test animals; n.p. = not provided

Rats administered four daily oral doses of estragole (605 mg/kg; 4.08 mmol/kg) showed liver discoloration and mottling and blunting of lobe edges, representing minor liver damage (Taylor et al., 1964).

Estragole, applied full strength, was moderately irritating to the intact or abraded skin of rabbits following 24-hour application under occlusion (Moreno, 1972; cited by Opdyke, 1976).

9.1.4 Short-Term and Subchronic Exposure

The effect of estragole and estragole-containing essential oils (percent estragole n.p.) administered in peanut oil at a volume of 0.5 mL was evaluated in partially hepatectomized male and female Charles River rats (Gershbein, 1977). Estragole (50 mg/rat/day), tarragon oil, fennel oil, and anise oil significantly increased liver regeneration in male and female rats administered the substances via s.c. injection for 7 days following partial hepatectomy, then sacrificed after 10 days. In another experiment, tarragon oil administered 0.50% in the diet for 10 days after partial hepatectomy had no effect on liver regeneration in male or female rats.

No other short-term or subchronic toxicity data were located.

9.1.5 Chronic Exposure

No chronic toxicity data were located.

9.2 Reproductive and Teratological Effects

No reproductive toxicity data were located.

9.3 Carcinogenicity

This subsection describes rodent bioassays of estragole and its metabolite 1'-hydroxyestragole. To facilitate discussion of structure-activity relationships among these two compounds and several structural analogues discussed in section 10, the specific experiments have been coded. The codes appear in the Reference column.

9.3.1 Estragole

The details of these studies are presented in **Table 6**.

Estragole induced hepatocellular carcinomas in 23 and 39% of CD-1 mice administered total doses of 4.4 or 5.2 μmol (650 or 770 μg), respectively, s.c. prior to weaning (1-22 days of age) (Drinkwater et al., 1976). In this study, only mice that survived to at least 12 months of age were included in the analysis and the animals were sacrificed at 15 months of age. Estragole (i.p.; 0.75 μmol/g in 10 L trioctanoin/g body weight; 110 μ g/g body weight) induced hepatomas in 95% of male B6C3F_1 mice within 10 months when administered at 12 days of age (Wiseman et al., 1987). Intraperitoneal administration of pentachlorophenol, a potent sulfotransferase inhibitor, prior to treatment with estragole reduced the incidence of animals developing hepatomas to control levels.

Estragole induced hepatomas in 73 and 65% of nursing male CD-1 mice when administered either perorally [p.o.; 2.5 μmol (370 μg)/g body weight, 2x/wk for 5 weeks] or intraperitoneally [i.p.; 9.45 μmol (1400 μg), four doses over 22 days], respectively (Miller et al., 1983). Eighty-three percent of male B6C3F_1 mice administered estragole [4.75 μmol (704 μg), given four i.p. injections over 22 days] developed hepatomas within 18 months. In addition, estragole (0.23 or 0.46% in the diet for 12 months; equivalent to 15.5 or 31 mmol/kg) induced

Table 6. Carcinogenicity of Estragole in Mice

Strain and Age	Number and Sex of Animals	Chemical Form, Purity	Route/Dose/ Duration	Observation Period	Results/Comments	Reference
CD-1 (newborn)	Low-dose group: 79 M High-dose group: 19 M	Estragole, >99% purity	s.c.; total doses of 4.4 and 5.2 µmol (650 or 770 µg), respectively, intermittently over 22 days	15 mo	Estragole induced hepatocellular carcinomas by 12 mo in 23% (14/60) and 39% (7/18) low-dose and high-dose group survivors, respectively, compared to 12% (6/51) in the trioctanoin-treated controls.	Drinkwater et al. (1976) D1
B6C3F$_1$ (12-days-old)	39 and 40 M/group	Estragole, purity n.p.	i.p.; 0.75 µmol (110 g)/g bw with and without i.p. injection of pentachloro-phenol (PCP) 45 min prior to test compound	10 mo	Estragole induced hepatomas in 95% of mice, with a mean number of 6.6 tumors/mouse. Pretreatment with PCP, a potent sulfotransferase inhibitor, completely inhibited the formation of estragole-induced hepatomas (only 18% tumors compared to 17% in solvent-only controls).	Wiseman et al. (1987) W5
CD-1 (preweanling)	55 M, 49 F	Estragole, at least 98% purity	p.o., via stomach tube; 2.5 µmol (370 µg)/g bw, 2x/wk for 5 wk, beginning on day 4 following birth	11-14 mo	Estragole induced hepatomas in 73% and 9% of M and F mice, respectively, compared to 24% and 2% of M and F control animals, respectively. The results were significant in the males.	Miller et al. (1983) M1
CD-1 (preweanling)	52 M	Estragole, at least 98% purity	i.p.; 9.45 µmol (1400 µg) administered on days 1, 8, 15, and 22	12 mo	Estragole induced hepatomas in 65% of mice, compared with 26% of solvent-injected control mice and 15% of non-injected control mice.	Miller et al. (1983) M2

Table 6. Carcinogenicity of Estragole in Mice (Continued)

Strain and Age	Number and Sex of Animals	Chemical Form, Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
$B6C3F_1$ (preweanling)	47 M	Estragole, at least 98% purity	i.p.; 4.75 µmol (704 µg) administered on days 1, 8, 15, and 22	13-18 mo	Estragole induced hepatomas in 83% of mice within 18 mo, compared with 41% of solvent control mice.	Miller et al. (1983) M4
CD-1 (8-wk-old)	50 F/dose level	Estragole, at least 98% purity	Oral, daily in diet for 12 mo; 0.23% or 0.46% (15.5 or 31 mmol/kg)	20 mo	Estragole induced hepatomas in 56% and 71% of low-dose and high-dose mice, compared to 0% of control animals.	Miller et al. (1983) M5
A/J (8-wk-old)	25 F	Estragole, at least 98% purity	i.p.; 1 mol (148 g)/g bw 2x/wk for 12 wk	8 mo	Estragole did not induce lung adenomas (only 6% with adenomas).	Miller et al. (1983) M8

Abbreviations: bw = body weight; F = females; i.p. = intraperitoneal; M = males; mo = months; n.p. = not provided; p.o. = peroral; s.c. = subcutaneous; wk = weeks

hepatomas in 56 or 71% of female CD-1 mice, respectively, within 18 months. Estragole did not induce lung adenomas when administered to female A/J mice at 1 μmol (148 μg)/g body weight twice weekly for 12 weeks; mice were examined at 8 months.

9.3.2 Estragole Metabolites

The details of these studies are presented in **Table 7**.

Of the metabolites identified in rodents and humans, only one (1'-hydroxyestragole; 1-HE) has been tested for carcinogenicity in rodent bioassays. 1-HE induced hepatocellular carcinomas in mice treated by s.c., i.p., or in the diet, and examined after 15 months (Drinkwater et al., 1976; Wiseman et al., 1987; Miller et al., 1983). Susceptibility to hepatoma induction was influenced by mouse strain, sex, and age (Wiseman et al., 1987). However, mice treated i.p. for 12 weeks and examined after 8 months did not show a significant increase in the incidence of lung adenomas (Miller et al., 1983). Rats given 1-HE s.c. for 10 wk did not have an increased incidence of hepatoma when examined at 20-24 months (Miller et al., 1983).

Tumor induction by compounds that have been proposed as estragole metabolites is discussed in section 10.

Table 7. Carcinogenicity of Estragole Metabolite 1'-Hydroxyestragole

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Route/Dose	Duration	Results/Comments	Reference
Mice						
CD-1 (newborn)	67 M	1'-Hydroxyestragole (1-HE), >99% purity	s.c., Intermittently over 22 days; total dose of 4.4 µmol	15 mo	Hepatocellular carcinomas were induced by 12 mo in 70% of 1-HE treated mice, compared to 12% in controls.	Drinkwater et al. (1976) D1
$B6C3F_1$ (preweanling)	35 M	1'-Hydroxyestragole (1-HE), at least 98% purity	i.p.; 1.87 mol, administered on days 1, 8, 15, and 22	12 mo	1-HE induced hepatomas in 93% of mice, compared to 15% of non-injected control animals.	Miller et al. (1983) M3
$B6C3F_1$ (preweanling)	Low-dose group: 63 M Mid-dose group: 44 M High-dose group: 49 M	1'-Hydroxyestragole (1-HE), at least 98% purity	i.p.; 1.90, 2.85, and 4.65 µmol, administered on days 1, 8, 15, and 22	18 mo	At 13 mo, hepatomas were induced in 95, 88, and 100% of mice dosed with 1.90, 2.85, and 4.65 µmol 1-HE, respectively, compared with 5% of solvent-injected control mice and 12% of non-injected control mice. For mice dead or killed from 13-18 mo, 98-100% of 1-HE-injected mice developed hepatomas, compared with 41% of solvent-injected mice and 28% of non-injected control mice.	Miller et al. (1983) M4
CD-1 (8-wk-old)	50 M	1'-Hydroxyestragole (1-HE), at least 98% purity	Oral, daily in diet for 12 mo, 0.25% (15.5 mmol/kg)	20 mo	1-HE induced hepatomas in 56% of dosed mice, compared to 0% of control animals.	Miller et al. (1983) M5
A/J (8-wk-old)	25 F	1'-Hydroxyestragole (1-HE), at least 98% purity	i.p.; 0.5 or 1 µmol/g bw twice/wk for 12 wk	8 mo	The incidence of lung adenomas (23%) was not increased significantly.	Miller et al. (1983) M2
$B6C3F_1$ (12-days-old)	Low-dose group: 47 M High-dose group: 43 M	1'-Hydroxyestragole (1-HE), purity n.p.	i.p. 0.04 mol and 1.9 mol in trioctanoin	13 mo	Hepatoma formation occurred in 51% of the low-dose mice and 95% of the high-dose mice, significantly different compared to the solvent-only-treated group.	Wiseman et al. (1987) W4

Table 7. Carcinogenicity of Estragole Metabolite 1'-Hydroxyestragole (Continued)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Route/Dose	Duration	Results/Comments	Reference
C3H/HeJ and C57BL/6J (preweanling)	C3H/HeJ: 38 M, 34 F C57BL/6J: 36 M, 36 F	1'-Hydroxyestragole (1-HE), purity n.p.	i.p.; 0.1 µmol/25µL trioctanoin (day 1); and 0.04 µmol/10 µL/g bw (day 8); 0.04 mol/5 L/g bw (day 15); 0.08 mol/7 L/g bw (day 22)	14 mo	Male C3H/He mice treated with 1-HE were more susceptible to the formation of hepatomas than were male C57BL/6J mice treated with 1-HE (hepatoma formation in 76% versus 14% of 1-HE-dosed mice, respectively). Female mice of both species were resistant to the induction of hepatomas in this study.	Wiseman et al. (1987) W1
B6C3F$_1$ (1- or 12-days-old)	35-48 M/dose	1'-Hydroxyestragole (1-HE), purity n.p.	i.p.; 0.05, 0.10, or 0.15 mol/g bw	14 mo	Mice dosed at 12-days-old showed 2- to 3-fold greater susceptibility to hepatoma formation than those dosed at 1-day-old. The hepatoma-bearing mice (58-66%) dosed at 1-day-old had an average of 0.9 to 1.8 hepatomas per mouse. The hepatoma-bearing mice (74-89%) dosed at 12-days-old had an average of 1.9 to 4.5 hepatomas per mouse.	Wiseman et al. (1987) W2
B6C3F$_1$ (12-days-old)	Low-dose group: 37 M High-dose group: 39 M	1'-Hydroxyestragole (1-HE), purity n.p.	single i.p.; 0.01 mol/g bw or 0.1 mol/g bw in 10 L trioctanoin	12 mo	Hepatomas occurred in 95% of the high-dose group and 22% of the low-dose group. In the former, one mouse also had a pulmonary adenoma; in the latter, one mouse had a hemangiosarcoma in the liver.	Wiseman et al. (1987) W3
Rats						
Fischer (5-wk-old)	20 M	1'-Hydroxyestragole (1-HE), at least 98% purity	s.c.; 0.05 mmol 2x/wk for 10 wk	24 mo	The incidence of hepatic carcinomas (observed in only 1 rat) was not significant.	Miller et al. (1983) M9r

Abbreviations: bw = body weight; F = females; i.p. = intraperitoneal; M = males; mo = months; n.p. = not provided; s.c. = subcutaneous; wk = week(s)

9.4 Genotoxicity

The details of these studies are presented in **Table 8**.

9.4.1 Prokaryotic Systems

In the majority of studies, estragole, at doses up to 300 µg/plate (2.02 µmol/plate), was not mutagenic in *Salmonella typhimurium* tester strains TA1535, TA100, TA1537, TA1538, and/or TA98 in the absence or presence of metabolic activation (Dorange et al., 1977; Sekizawa and Shibamoto, 1982; Zeiger et al., 1987; Zani et al., 1991). However, To et al. (1982) reported an increase in induced mutations in tester strain TA1535 only, with and without metabolic activation, with estragole tested at concentrations up to 50 g/plate (340 nmol/plate).

Similarly, estragole at 30-300 µg/plate (0.20-2.02 µmol/plate) was not mutagenic in *Escherichia coli* strain WP2 *trp*$^-$ in either the absence or presence of metabolic activation (Sekizawa and Shibamoto, 1982).

Estragole (4 mg/disk; 0.03 mmol/disk) did not induce differential survival in *Bacillus subtilis* strains H17$^+$ Rec$^+$ and M45 Rec$^-$, in the presence or absence of metabolic activation (Sekizawa and Shibamoto, 1982). However, the essential oil (10 or 30 µL) extracted from the plant *Artemisia dracunculus* L. (containing 77.5% estragole) induced DNA damage in the *Bacillus subtilis rec*$^-$ assay, in the presence or absence of metabolic activation (Zani et al., 1991).

9.4.2 Lower Eukaryote Systems

Estragole in tarragon oil (60%) but not basil oil (16.5%) was mutagenic in *Saccharomyces cerevisiae* strain D7.

9.4.3 Mammalian Systems *In Vitro*

Estragole (0.1-10 mol/mL) was not clastogenic in V79 cells, either in the presence or absence of metabolic activation (M ller et al., 1994).

Estragole induced unscheduled DNA synthesis (UDS) in human skin fibroblasts at 0.001 M (0.148 g/L) (Francis et al., 1981), and in cultured rat hepatocytes at 0.01 M (1.48 g/L) (Howes et al., 1990; Chan and Caldwell, 1992; M ller et al., 1994). All studies were conducted in the absence of metabolic activation. Basil oil, when tested at doses equivalent to 0.01 M (1.48 g/L) estragole, also induced a marked UDS response in cultured rat hepatocytes (M ller et al., 1994).

9.4.4 Mammalian Systems *In Vivo*

In *in vivo* UDS experiments, estragole and basil oil (at a dose equivalent to 2.0 g/kg or 0.013 mol/kg estragole) induced a positive response in hepatocytes isolated 4 or 12 hours following oral administration of the test compound to male Wistar rats (M ller et al., 1994).

Estragole, administered at 0.25, 0.5, 1.0, and 3.0 µmol (37, 74, 150, and 440 µg) on days 1, 8, 15, and 22, respectively, to newborn $B6C3F_1$ male mice, induced DNA adducts in the livers of mice sacrificed on days 23, 29, and 43 (Phillips et al., 1984).

9.5 Immunotoxicity

Estragole tested at 3% in petrolatum produced no sensitization in 25 volunteers using the Kligman maximization test (Opdyke, 1976).

Table 8. Genetic Toxicity of Estragole

Test System	Biological Endpoint	+/- S9	Chemical Form, Purity	Dose	Endpoint Response	Reference
9.4.1 Prokaryotic Systems						
Salmonella typhimurium strains TA1535, TA100, TA1537, TA1538, TA98	Histidine revertants	-	Estragole, purity n.p.	Up to 200 nmol/plate (29.6 g/plate)	-	Dorange et al. (1977)
S. typhimurium strains TA1535, TA100, TA1537, TA1538, TA98	Histidine revertants	+/-	Estragole, 99.9% purity	30-300 µg/plate (0.2-2.2 µmol/plate)	-	Sekizawa and Shibamoto (1982)
S. typhimurium strains TA1535, TA100, TA1537, TA1538, TA98	Histidine revertants	+/-	Estragole, purity n.p.	0.1-100 mg/mL (0.7-675 µmol/mL)	TA1535, +/-S9: + ($p<0.05$); all other strains: -	To et al. (1982)
S. typhimurium strains TA100, TA1535, TA1537, TA98	Histidine revertants	+/-	Estragole, purity n.p.	1-200 µg/plate (7-1350 nmol/plate)	-	Zeiger et al. (1987)
S. typhimurium strains TA1535, TA100, TA1537, TA98	Histidine revertants	+/-	Estragole, approximately 77.5% in essential oil extracted from *Artemisia dracunculus* L.	0.06-0.5 µL oil/plate (0.06-0.5 mg/plate; 0.4-3.4 mol/plate)	-	Zani et al. (1991)
Escherichia coli strain WP2 *trp*$^-$	Trp$^-$ revertants	+/-	Estragole, 99.9% purity	30-300 µg/plate (0.2-2.02 µmol/plate)	-	Sekizawa and Shibamoto (1982)

Table 8. Genetic Toxicity of Estragole (Continued)

Test System	Biological Endpoint	+/- S9	Chemical Form, Purity	Dose	Endpoint Response	Reference
B. subtilis strains $H17^{+}$ Rec^{+} and M45 Rec^{-}	DNA damage	-	Estragole, 99.9% purity	4 mg/disk (0.03 mmol/disk)	-	Sekizawa and Shibamoto (1982)
Bacillus subtilis strains PB1652 and PB1791	DNA damage	+/-	Estragole, approximately 77.5% in essential oil extracted from *Artemisia dracunculus* L.	10 or 30 µL	+	Zani et al. (1991)
9.4.2 Lower Eukaryotic Systems						
Saccharomyces cerevisiae strain D7		n.p.	Estragole (60%) in tarragon oil and (16.5%) in basil oil	n.p.	Tarragon oil: + Basil oil: -	Bianchi et al. (1989 abstr.)
9.4.3 Mammalian Systems In Vitro						
V79 cells	Chromosomal aberrations	+/-	Estragole, 98% purity; basil oil, with specific estragole content of 88.2%	0.1-10 mol/mL; concentration of basil oil was 0.0114 M	-	M ller et al. (1994)
Normal human skin fibroblasts	Unscheduled DNA Synthesis (UDS) measured using the 5-bromodeoxy-uridine photolysis assay	-	Estragole, purity n.p.	0.001 M (0.148 g/L)	+	Phillips et al. (1984)
Isolated Fischer 344 rat hepatocytes	UDS measured autoradio-graphically	-	Estragole, purity n.p.	Up to 0.01 M (1.48 g/L)	+ (dose-dependent; cytotoxicity observed at $\geq$ 0.005 M)	Howes et al. (1990)

Table 8. Genetic Toxicity of Estragole (Continued)

Test System	Biological Endpoint	+/- S9	Chemical Form, Purity	Dose	Endpoint Response	Reference
Isolated male Fischer 344 rat hepatocytes	UDS	-	Estragole, >99% purity	Up to 0.01 M (1.48 g/L)	+ (dose-dependent; cytotoxicity observed at $\geq$ 0.005 M)	Chan and Caldwell (1992)
Isolated male Wistar rat hepatocytes	UDS	-	Estragole, 98% purity; basil oil, with specific estragole content of 88.2%	Up to 0.01 M (1.48 g/L) concentration of basil oil was 0.0114 M	+ (dose-dependent; cytotoxicity observed at 0.01 M)	M ller et al. (1994)
9.4.4 Mammalian Systems In Vivo						
Male Wistar rat hepatocytes	UDS	-	Estragole, 98% purity; basil oil, with specific estragole content of 88.2%3	2.0 g/kg (0.013 mol/kg)	+	M ller et al. (1994)
B6C3F1 mice (newborn)	DNA adducts	n.p.	Estragole, purity n.p.	i.p.; 0.25, 0.5, 1.0, and 3.0 mol (37, 74, 150, and 440 g) in trioctanoin on days 1, 8, 15, and 22, respectively, after birth	+	Phillips et al. (1984)

Abbreviations: n.p. = not provided; + = positive; - = negative

10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Besides the estragole metabolite discussed in section 9.1.2, the following structural analogues are considered. They are divided into three groups: 1) oxidized derivatives of estragole (synthetic or naturally occurring); 2) analogues that resemble estragole in containing the allyl (2-propenyl) or other alkenyl side chain in which the double bond is not conjugated with the aromatic ring; and 3) analogues containing the 1-propenyl side chain in which the double bond in the side chain is conjugated with the aromatic ring. The structures of some of these compounds are provided in **Figures 2** and **3**.

10.1 Carcinogenicity

Tables 9, **10**, and **11** present in detail additional carcinogenicity studies conducted by Miller et al. (1983), Drinkwater et al. (1976), and Wiseman et al. (1987), as well as a few others of the various structural analogues of estragole in the above groups. Only those giving positive results are listed. **Table 12** provides an overall summary of the results (both positive and negative) of the studies from **Tables 6** and **7** (carcinogenesis of estragole and 1'-hydroxyestragole, respectively) and **Tables 9-11**.

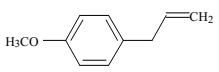
Estragole, safrole, 1'-hydroxyestragole, 1'-hydroxysafrole, and methyleugenol induced hepatocarcinomas in mice, while only safrole, 1'-hydroxysafrole, and methyleugenol induced the same tumors in rats. As can be seen in **Table 12**, the majority of compounds inducing increased incidences of liver, lung, and/or skin tumors were 2-propenyl compounds and their derivatives (detailed results in **Tables 6**, **7**, **9**, and **10**). However, this might simply be due to the testing of fewer 1-propenyl analogues.

Wiseman et al. (1987) compared the relative potencies of several estragole analogues in inducing liver tumors in 12-day-old male B6C3F_1 mice given a single i.p. injection, with the potencies of known hepatic carcinogens. In this system, the potencies of diethylnitrosamine (DEN), aflatoxin B_1, vinyl carbamate, benzo[*a*]pyrene, and ethyl carbamate were expressed as an average of 1100, 350, 250, 27, and 7 hepatomas per mol carcinogen per gram body weight, respectively. Values for estragole analogues were 1'-hydroxy-2',3'-dehydroestragole, 220; 1'-hydroxy-2',3'-dehydrosafrole, 110; 1'-hydroxyestragole, 32; 1'-hydroxysafrole, 20; precocene I, 10; and *cis*-asarone, 4 average hepatomas/ mol carcinogen/g body weight.

10.2 DNA Adduct Formation

Drinkwater et al. (1976) showed that estragole and its metabolite 1'-hydroxyestragole induced hepatomas in CD-1 mice when administered s.c. prior to weaning. Phillips et al. (1981) showed that the two major DNA adducts formed in mice given 1'-hydroxyestragole are N^2-(estragol-1'-yl)deoxyguanosine and N^2-(*trans*-isoestragol-3'-yl)deoxyguanosine. Two minor DNA adducts formed are N^2-(*cis*-isoestragol-3'-yl)deoxyguanosine and N^6-(*trans*-isoestragol-3'-yl)deoxyadenosine. With safrole, the same pattern of DNA adducts were formed in mice (Phillips et al., 1981) and in Chinese hamster ovary cells (Daimon et al., 1997). Using ^{32}P-postlabeling, Randernath et al. (1984) showed that estragole, safrole, and methyleugenol formed adducts *in vivo* in mouse-liver DNA while the noncarcinogens, allylbenzene, anethole, myristicin, parsley apiol, dill apiol, and elemicin, formed DNA adducts in the same tissue at 3- to 200-fold lower levels. Estragole 2',3'-epoxide is also a metabolite of estragole (Solheim and Scheline, 1973; Swanson et al., 1981) and has been shown also to form DNA adducts *in vitro* (Luo and Guenthner, 1995; Phillips et al., 1981). These adducts are not seen *in vivo* presumably because the epoxide is rapidly detoxified (Luo et al., 1992; Luo and Guenthner, 1994). The DNA adducts that have been identified for estragole, 1'-hydroxyestragole, safrole, and methyleugenol are listed in **Table 13**.

Figure 2. Oxidized Derivatives of Estragole



Estragole

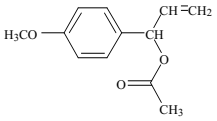
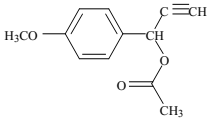
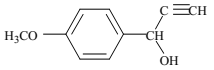
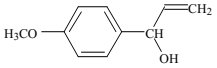
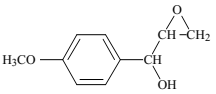
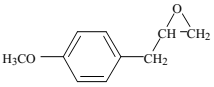
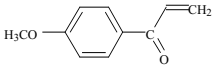
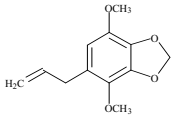
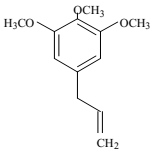
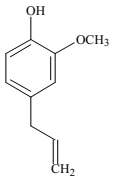
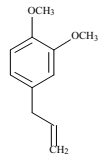
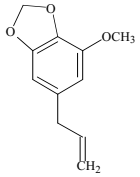
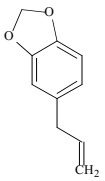
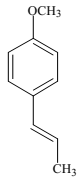
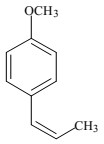
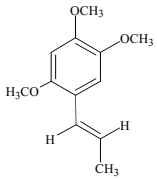
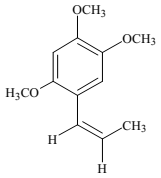
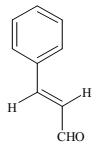
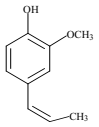
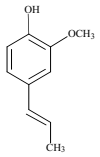
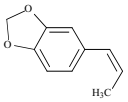
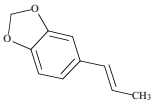
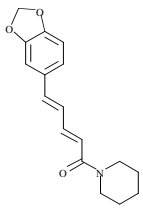
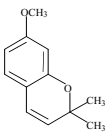
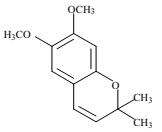
 1'-Acetoxyestragole	 1'-Acetoxy-2',3'-dehydroestragole	 1'-Hydroxy-2',3'-dehydroestragole
 1'-Hydroxyestragole (51410-44-7)	 1'-Hydroxyestragole 2',3'-oxide	 Estragole 2',3'-oxide
	 1'-Oxoestragole	

Figure 3. Other Estragole Structural Analogues[a]

2-Propenyl Analogues of Estragole		
 **Apiole** (523-80-8) $C_{12}H_{14}O_4$ MW: 222.24 m.p.: 29.5 b.p.: 294 Insoluble in water	 **Elemicin** (487-11-6) $C_{12}H_{16}O_3$ MW: 208.26	 **Eugenol** (97-53-0) $C_{10}H_{12}O_2$ MW: 164.20 d_4^{20} : 1.0664 m.p.: -9.2 to -9.1 b.p.: 255 Practically insoluble in water
 **Methyleugenol** (93-15-2) $C_{11}H_{14}O_2$ MW: 178.23 m.p.: -4 b.p.: 254.7	 **Myristicin** (607-91-0) $C_{11}H_{12}O_3$ MW: 192.21 d_{20}^{20} : 1.1437 b.p.: 173 at 40 mm Hg	 **Safrole** (94-59-7) $C_{10}H_{10}O_2$ MW: 162.19 d^{20}: 1.096 m.p.: ~11 b.p.: 232-234 Insoluble in water

1-Propenyl Analogues of Estragole		
 **_trans_-Anethole** (4180-23-8) $C_{10}H_{12}O$ MW: 148.20 d_4^{20} : 0.9883 m.p.: 21.4 b.p.: 81-81.5 at 2.3 mm Hg Practically insoluble in water	 **_cis_-Anethole** $C_{10}H_{12}O$ MW: 148.20 d_4^{20} : 0.9878 b.p.: 79-79.5 at 2.3 mm Hg	 **" -Asarone** $C_{12}H_{16}O_3$ MW: 208.26 m.p.: 62-63 b.p.: 296 Practically insoluble in water
 **$-Asarone** $C_{12}H_{16}O_3$ MW: 208.26 density: 1.073 Practically insoluble in water	 **Cinnamaldehyde** (104-55-2) C_9H_8O MW: 132.16 d_{25}^{25} : 1.048-1.052 m.p.: -7.5 b.p.: 246.0 Dissolves in ~700 parts water	 ***cis*-Isoeugenol** (5912-86-7) $C_{10}H_{12}O_2$ MW: 164.20 d_4^{20} : 1.008 b.p.: 133 at 11 mm Hg
 ***trans*-Isoeugenol** (5932-68-3) $C_{10}H_{12}O_2$ MW: 164.20 d_4^{20} : 1.087 m.p.: 33 b.p.: 140 at 12 mm Hg	 ***cis*-Isosafrole** $C_{10}H_{10}O_2$ MW: 162.19 d_4^{20} : 1.1182 m.p.: -21.5 b.p.: 77-79 at 3.5 mm Hg	 ***trans*-Isosafrole** $C_{10}H_{10}O_2$ MW: 162.19 d_4^{20} : 1.1206 m.p.: 8.2 b.p.: 253
 **Piperine** (94-62-2) $C_{17}H_{19}NO_3$ MW: 285.34 m.p.: 130 Almost insoluble in water (40 ppm)	 **Precocene I** $C_{12}H_{14}O_2$ MW: 190.24 b.p.: 120 at 6 mm Hg	 **Precocene II** $C_{13}H_{16}O_3$ MW: 220.27 m.p.: 47.5 b.p.: 136 at 6 mm Hg

[a] Temperatures are in degrees Celsius.

Table 9. Carcinogenicity of Estragole Oxidized Derivatives Other Than 1'-Hydroxyestragole (Unconjugated Side Chain)

Species, Strain, and Age at Start of Expt.	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
Mice						
B6C3F$_1$ (12-days-old)	39 M weaned	1'-Acetoxyestragole (1-AE), purity n.p.	i.p. at 12 days of age in 10 µL of trioctanoin/g bw. Dose: 0.1 µmol/g bw	12 mo	Development of hepatomas occurred in 100% of mice dosed with 1-AE.	Wiseman et al. (1987) W6
B6C3F$_1$ (1-day-old)	42 M weaned	1'-Acetoxyestragole (1-AE), purity n.p.	i.p. on days 1, 8, 15, and 22 (in dose vol. 25-75 µL). Total dose: 1.9 mol	13 mo	Significant development of hepatomas occurred in 88% of 1-AE-treated mice.	Wiseman et al. (1987) W4
A/J (8- to 12-days-old)	M and F (55 and 47 weaned)	1'-Acetoxy-2',3'-dehydroestragole (1-A-2,3-DHE); purity n.p.	i.p.; 0.05 µmol/g bw, treated either on days 8 and 12 or on day 12 only	9 mo	Significant incidence of lung adenomas compared to solvent controls (39-40% of mice vs. 12%).	Wiseman et al. (1987) W3
B6C3F$_1$ (preweanling)	33 M	1'-Hydroxy-2',3'-dehydroestragole (1-H-2,3-DHE), at least 98% purity	i.p.; 1.86 µmol administered on days 1, 8, 15, and 22	13 to 18 mo	For mice dead or killed from 13-18 mo, 97% of 1-H-2,3-DHE-injected mice developed hepatomas, compared with 41% of solvent-injected mice and 28% of non-injected control mice.	Miller et al. (1983) M4
B6C3F$_1$ (1-day-old)	42 M weaned	1'-Hydroxy-2',3'-dehydroestragole (1-H-2,3-DHE), purity n.p.	i.p. on days 1, 8, 15, and 22 (in dose vol. 25-75 µL). Total dose: 0.4 µmol	13 mo	Significant development of hepatomas occurred in 95% of treated mice.	Wiseman et al. (1987) W3

Table 9. Carcinogenicity of Estragole Oxidized Derivatives Other Than 1'-Hydroxyestragole (Unconjugated Side Chain)

Species, Strain, and Age at Start of Expt. (Continued)	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
$B6C3F_1$ (12-days-old)	36-40 M/dose group weaned	1'-Hydroxy-2',3'-dehydroestragole (1-H-2,3-DHE), purity n.p.	i.p. at 12 days of age in 10 µL of trioctanoin/g bw. Dose: 0.1, 0.05, and 0.01 µmol/g bw	12 mo	Development of hepatomas occurred in 94-100% of mice dosed with 1-H-2,3-DHE.	Wiseman et al. (1987) W4
CD-1 (8-wk-old)	40 F	1'-Hydroxyestragole 2',3'-oxide (1-HE-2,3-O), at least 98% purity	Dermal; 11.2 mol 4 days/wk for 6 wk; 0.15 mL of 0.6% croton oil in acetone topically applied 2x/wk 1 wk after the last dose of epoxide	40 wk	Benign skin tumors were induced in 25 and 44% of 1-HE-2,3-O treated animals observed at 30 and 40 wk, respectively, as compared with 3 and 7%, respectively, in animals receiving only croton oil treatments. The incidence was significant.	Miller et al. (1983) M7
A/J (8-wk-old)	25 F	1'-Hydroxyestragole 2',3'-oxide (1-HE-2,3-O), at least 98% purity	i.p.; 2x/wk for 12 wk at 0.5 or 1 µmol/g bw	Up to 8 mo after first injection	Significant incidences of lung adenomas were found (47%), only at the high-dose level.	Miller et al. (1983) M8

Table 9. Carcinogenicity of Estragole Oxidized Derivatives Other Than 1'-Hydroxyestragole (Unconjugated Side Chain)

Species, Strain, and Age at Start of Expt. (Continued)	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
CD-1 (8-wk-old)	40 F	2',3'-Oxide of estragole (2,3-OE), at least 98% purity	Dermal; 11.2 mol 4 days/wk for 6 wk; 0.15 mL of 0.6% croton oil in acetone topically applied 2x/wk 1 wk after the last dose of epoxide	40 wk	Benign skin tumors were induced in 10 and 33% of 2,3-OE treated animals observed at 30 and 40 wk, respectively, as compared with 3 and 7%, respectively, in animals receiving only croton oil treatments.	Miller et al. (1983) M7
B6C3F$_1$ (1-day-old)	33 and 22 M/ dose group weaned	1'-Oxoestragole (1-OE), purity n.p.	i.p.; on days 1, 8, 15, and 22 (in dose vol. 25-75 µL). Total doses: 1.4 and 1.9 µmol	13 mo	Significant development of hepatomas occurred in 43% of 1-OE-treated mice at the higher dose.	Wiseman et al. (1987) W3

Abbreviations: bw = body weight; F = females; i.p. = intraperitoneal; M = males; mo = month(s); n.p. = not provided; s.c. = subcutaneous; wk = week(s)

Table 10. Carcinogenicity of 2-Propenyl Analogues of Estragole (Unconjugated Side Chain) and Their Derivatives

Species, Strain, and Age at Start of Expt.	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
Mice						
Elemicin						
$B6C3F_1$ (1-day-old)	48 M weaned	1'-Acetoxyelemicin (1-AEM), purity n.p.	i.p.; on days 1, 8, 15, and 22 (in dose vol. 25-75 µL). Total dose: 9.5 µmol	13 mo	Significant incidence of hepatomas was observed in 52% of 1-AEM-treated mice compared to 10% in controls.	Wiseman et al. (1987) W3
$B6C3F_1$ (1-day-old)	45 M weaned	1'-Hydroxyelemicin (1-HEM), purity n.p.	i.p.; on days 1, 8, 15, and 22 (in dose vol. 25-75 µL). Total dose: 9.5 µmol	13 mo	Significant development of hepatomas occurred in 51% of 1-HEM treated mice.	Wiseman et al. (1987) W3
Eugenol						
$B6C3F_1$ (age n.p.)	50 M and 50 F	Eugenol, >99% purity	Oral, daily in the diet for 2 yr. Dose: 3,000 and 6,000 ppm	2 yr	Equivocal evidence of carcinogenic activity in male and female mice increased incidence of both hepatocellular adenomas and carcinomas in low-dose males; dose-related positive trend in combined liver neoplasms in females.	NTP TR-223 (1983) N223
CD-1 (8 wk)	40 F	Eugenol 2',3'-oxide, at least 98% purity	Topical, 4 days/wk for 6 weeks; 11.2 mol/application	30 and 40 wk	25% of mice examined after 30 weeks showed an average of 0.4 skin tumors per mouse. 40% of mice examined after 40 weeks exhibited an average of 0.9 skin tumors per mouse.	Miller et al. (1983) M7

Table 10. Carcinogenicity of 2-Propenyl Analogues of Estragole (Unconjugated Side Chain) and Their Derivatives (Continued)

Species, Strain, and Age at Start of Expt.	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
Methyleugenol						
$B6C3F_1$ (1-day-old)	59 M weaned	Methyleugenol, at least 98% purity	i.p.; on days 1, 8, 15, and 22. Total dose: 4.75 : mol	18 mo	56 of 58 mice (96%) had hepatomas with an average of 3.2 hepatomas per mouse.	Miller et al. (1983) M4
$B6C3F_1$	50 M and 50 F	Methyleugenol, at least 98% purity	gavage; 37, 75, and 150 mg/kg bw for 104 wk	24 mo	Clear evidence of carcinogenic activity based on increased incidences of hepatocellular neoplasms in both sexes. Neoplasms included hepatocellular adenomas and carcinomas, and hepatoblastoma (significantly increased in all dosed groups of females and slightly increased in high-dose males). High-dose females showed a significant increase of hepatochol-angiocarcinoma. Male development of neuroendocrine tumors of the glandular stomach was also exposure-related.	NTP TR-491 (1998 draft) N491
$B6C3F_1$ (1-day-old)	44 M weaned	1-Hydroxy-methyleugenol, at least 98% purity	i.p.; on days 1, 8, 15, and 22. Total dose: 2.85 : mol	18 mo	41 of 44 mice (93%) had hepatomas with an average of 3.5 hepatomas per mouse.	Miller et al. (1983) M4
Safrole						
CD-1 (preweanling)	61 M and 53 F weaned	Safrole, at least 98% purity	p.o., via stomach tube; 2.5 µmol/g bw, 2x/wk for 5 wk, beginning on day 4 following birth	11-14 mo	Safrole induced hepatomas in 61% male mice, compared to 24% of male controls. The incidence of hepatomas was not significant in female mice. Mice weaned at 35 days.	Miller et al. (1983) M1
CD-1 (preweanling)	55 M weaned	Safrole, at least 98% purity	i.p.; 9.45 µmol administered on days 1, 8, 15, and 22	12 mo	Significant hepatomas were induced in 67% of male mice dosed with safrole compared to 26% in the controls.	Miller et al. (1983) M2

Table 10. Carcinogenicity of 2-Propenyl Analogues of Estragole (Unconjugated Side Chain) and Their Derivatives (Continued)

Species, Strain, and Age at Start of Expt.	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
CD-1 (8-wk-old)	50 F/dose level	Safrole, at least 98% purity	Oral; fed at 25 and 50% of final levels for 2 successive 10-day periods: 0.25 and 0.50% safrole in the diet	20 mo	Safrole induced hepatomas in 72% of low-dose and 80% of high-dose mice, compared to 0-7% of control animals.	Miller et al. (1983) M5
CD-1 (8-wk-old)	30 F/dose level	Safrole, at least 98% purity	Oral; fed at 15, 30 and 67% of the final levels for first 3 successive 10-day periods; 0.50% safrole in grain diet	18 mo	Hepatomas were observed in 70% of mice dosed with safrole, which was significantly higher than controls (7%). The average number of hepatomas per mouse was 1.8.	Miller et al. (1983) M6
B6AKF$_1$ mice (age n.p.)	18 M and 18 F	Safrole, purity n.p.	Gavage; 464 mg/kg bw at 7-days-old and daily until 28-days-old, then 1112 mg/kg of diet for 82 wk	82 wk	Hepatocellular tumors occurred in both the male and female animals.	IARC (1976); TRI (1997) T1
BALB mice (age n.p.)	M (number n.p.)	Safrole, purity n.p.	Oral; fed in the diet for 52 wk; dose(s) n.p.	75 wk	Hepatocellular adenomas and carcinomas occurred in the animals.	CCRIS (1997; cited by TRI, 1997) T2
B6C3F1 mice (age n.p.)	F (number n.p.)	Safrole, purity n.p.	Intubation 2x/wk for a total of 180x; dose(s) n.p.	90 wk	Renal tumors occurred in the female mice exposed *in utero*. Hepatocellular tumors were found in male offspring of nursing mothers and in the intubated adult females.	Vesselino-vitch et al. (1979; cited by TRI, 1997) T3

Table 10. Carcinogenicity of 2-Propenyl Analogues of Estragole (Unconjugated Side Chain) and Their Derivatives (Continued)

Species, Strain, and Age at Start of Expt.	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
B6C3F$_1$ (12-days-old)	38 M weaned	1'-Acetoxysafrole (1-AS), at least 98% purity	i.p. at 12 days of age in 10 µL of trioctanoin/g bw. Dose: 0.1 µmol/g bw	12 mo	Development of hepatomas occurred in 79% of mice dosed with 1-AS.	Wiseman et al. (1987) W4
B6C3F$_1$ (12-days-old)	37 and 40 M/dose group weaned	1'-Hydroxy-2',3'-dehydrosafrole (1-H-2,3-DHS), purity n.p.	i.p. at 12 days of age in 10 µL of trioctanoin/g bw. Dose: 0.1 and 0.01 µmol/g bw	12 mo	Development of hepatomas occurred in 46% of low-dose mice and 100% of high-dose mice.	Wiseman et al. (1987) W4
CD-1 (1-day-old)	Control and low-dose group: 60 M weaned	1'-Hydroxysafrole (1-HS), <99% purity	s.c.; 0.17, 0.47, 0.95, and 2.84 mol on days 1, 8, 15, and 22. Total dose: 4.4 µmol	15 mo	Hepatocellular carcinomas were induced by 15 mo in 59% of treated mice alive at 12 mo, compared to 12% in controls.	Drinkwater et al. (1976) D1
B6C3F$_1$ (preweanling)	30 M weaned	1'-Hydroxysafrole (1-HS), at least 98% purity	i.p.; 3.75 µmol, administered on days 1, 8, 15, and 22	12 mo	1-HS induced hepatomas in 92% of mice, compared to 15% of non-injected control animals.	Miller et al. (1983) M3
CD-1 (preweanling)	52 M weaned	1'-Hydroxysafrole (1-HS), at least 98% purity	i.p.; 4.72 µmol administered on days 1, 8, 15, and 22	12 mo	Significant incidence of hepatomas was induced (in 65% of mice dosed with 1-HS).	Miller et al. (1983) M2

Table 10. Carcinogenicity of 2-Propenyl Analogues of Estragole (Unconjugated Side Chain) and Their Derivatives (Continued)

Species, Strain, and Age at Start of Expt.	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
C3H/He and C57BL/6J (preweanling)	33-50 M and F/ dose group	1'-Hydroxysafrole (1-HS), purity n.p.	i.p.; 0.1 µmol/ 25µL (day 1) and 0.08 µmol/7 µL/g bw (days 8, 15, and 22)	14 mo	Male C3H/He mice treated with 1-HS were more susceptible to the formation of hepatomas than were male C57BL/6J mice treated with 1-HS (hepatoma formation in 68% versus 33% of 1-HS-dosed mice). Hepatoma formation in male C57BL/6J mice treated with 1-HS did not differ significantly from solvent-only treated control mice. Female mice of both species were resistant to the formation of hepatomas in this study.	Wiseman et al. (1987) W1
B6C3F_1 (12-days-old)	38 M weaned and necropsied	1'-Hydroxysafrole (1-HS), purity n.p.	i.p. at 12 days of age in 10 µL of trioctanoin/g bw. Dose: 0.1 µmol/g bw	12 mo	Development of hepatomas occurred in 68% of mice dosed with 1-HS.	Wiseman et al. (1987) W4
CD-1 (preweanling)	56 M weaned	1'-Hydroxysafrole 2',3'-oxide (1-HS-2,3-O), at least 98% purity	i.p.; 9.45 µmol administered on days 1, 8, 15, and 22	12 mo	Incidence of hepatomas induced in 55% of mice dosed with 1-HS-2,3-O was significant compared to 26% in solvent-injected controls.	Miller et al. (1983) M2
CD-1 (8-wk-old)	40 F	1'-Hydroxysafrole 2',3'-oxide (1-HS-2,3-O), at least 98% purity	Dermal; 11.2 mol 4 days/ wk for 6 wk; 0.15 mL of 0.6% croton oil in acetone applied topically 2x/wk 1 wk after the last dose of epoxide	40 wk	Benign skin tumors were induced in 53 and 82% of animals observed at 30 and 40 wk, respectively, as compared with 3 and 7%, respectively, in animals receiving only croton oil treatments. Incidence at 40 wk was statistically significant.	Miller et al. (1983) M7
A/J (8-wk-old)	25 F	1'-Hydroxysafrole 2',3'-oxide (1-HS-2,3-O), at least 98% purity	i.p.; twice/wk for 12 wk at 0.5 or 1 µmol/g bw	Up to 8 mo after first injection	Significant incidences of lung adenomas were found (45%) only at the high-dose level.	Miller et al. (1983) M8

Table 10. Carcinogenicity of 2-Propenyl Analogues of Estragole (Unconjugated Side Chain) and Their Derivatives (Continued)

Species, Strain, and Age at Start of Expt.	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
CD-1 (8-wk-old)	40 F	2',3'-Oxide of safrole (2,3-OS), at least 98% purity	Dermal; 11.2 mol 4 days/wk for 6 wk; 0.15 mL of 0.6% croton oil in acetone applied topically 2x/wk 1 wk after the last dose of epoxide	40 wk	Benign skin tumors were induced in 21% and 36% of animals observed at 30 and 40 wk, respectively, as compared with 3 and 7%, respectively, in animals receiving only croton oil treatments. Incidence at 40 wk was statistically significant.	Miller et al. (1983) M7
Rats						
Methyleugenol						
Fischer 344/N	50 M and 50 F	Methyleugenol, ~99% purity	gavage; 37, 75, and 150 mg/kg bw for 105 wk	24 mo	Clear evidence of carcinogenicity based on increase of liver neoplasms and neuroendocrine tumors of the glandular stomach in both sexes and increased incidences of kidney neoplasms, malignant mesothelioma, mammary gland fibroadenoma, and subcutaneous fibroma and fibroma or fibrosarcoma (combined) in males.	NTP TR-491 (1998 draft) N491r
Safrole						
CD (age n.p.)	M (number n.p.)	Safrole, purity n.p.	Fed in the diet; dose(s) n.p.	22 mo	Hepatocellular carcinomas occurred in the rat. Enhanced response was seen if coadministered with phenobarbital.	Wislocki et al. (1977; cited by TRI, 1997) T5

Table 10. Carcinogenicity of 2-Propenyl Analogues of Estragole (Unconjugated Side Chain) and Their Derivatives (Continued)

Species, Strain, and Age at Start of Expt.	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
Osborne-Mendel (age n.p.)	25 M and 25 F	Safrole, purity n.p.	Oral; 100, 500, 1000, or 5000 mg/kg in the diet	2 yr	Liver tumors occurred in 19/47 autopsied rats fed the 5000 mg/kg dose (vs. 3/40 controls). Fourteen of the tumors were hepatocellular and cholangiocarcinomas. Eight mice given the 1000 mg/kg dose developed liver tumors. The incidences of liver tumors in rats fed the two lowest doses were similar to the controls.	IARC (1976); TRI (1997) T4
Fischer (5-wk-old)	20 M	1'-Acetoxysafrole, at least 98% purity	s.c. injections 2x/wk (total 20 injections) in rear hind leg; 0.03 mmol/injection	20 mo	Sarcomas were observed at the injection site in 4 of the 20 (20%) dosed rats. Tumors seen at other sites included one case of s.c. sarcoma, renal cell carcinoma, fibroadenoma (mammary gland), and hepatic carcinoma.	Miller et al. (1983) M9r
Fischer (5-wk-old)	20 M	1'-Hydroxysafrole, at least 98% purity	s.c. injections 2x/wk (total 20 injections) in rear hind leg; 0.05 mmol/injection	20 mo	Hepatic carcinomas were observed in 11 of 20 rats (55%) dosed with 1'-hydroxysafrole. Two cases of s.c. fibromas and one case of hemangioendotheliosarcoma (s.c.) were observed.	Miller et al. (1983) M9r
Fischer (5-wk-old)	20 M	1-Hydroxysafrole 2',3'-oxide, at least 98% purity	s.c. injections 2x/wk (total 20 injections) in rear hind leg; 0.01 mmol/injection	24 mo	Sarcomas at the injection site were reported in 4 of the 20 rats (20%). Also two cases of s.c. sarcomas and one case of s.c. fibroma were reported.	Miller et al (1983) M9r

Abbreviations: bw = body weight; F = females; i.p. = intraperitoneal; M = males; mo = month(s); n.p. = not provided; p.o. = peroral; s.c. = subcutaneous;

wk = week(s)

Table 11. Carcinogenicity of 1-Propenyl Analogues of Estragole (Conjugated Side Chain) and Their Derivatives

Species, Strain, and Age at Start of Expt.	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
Mice						
Anethole						
B6C3F$_1$ (1-day-old)	37 and 44 M/ dose group weaned	3'-Bromo-*trans*-anethole (3-BTA), purity n.p.	i.p.; on days 1, 8, 15, and 22, (in dose vol. 25-75 µL). Total doses: 1.4 and 1.9 µmol	13 mo	Significant development of hepatomas occurred in 80% of treated mice at the high dose.	Wiseman et al. (1987) W3
B6C3F$_1$ (12-days-old)	39 and 41 M/ dose group weaned	3'-Hydroxy-*trans*-anethole (3-HTA), purity n.p.	i.p. at 12 days of age in 10 µL of trioctanoin/g bw. Total doses: 0.1 and 2.5 µmol/g bw	12 mo	Development of hepatomas occurred in 13% and 36% of mice dosed with 3-HTA. No significant hepatoma formation at low dose.	Wiseman et al. (1987) W4
Asarone						
B6C3F$_1$ (1-day-old)	43 M weaned	*cis*-Asarone, purity n.p.	i.p.; on days 1, 8, 15, and 22 (in dose vol. 25-75 µL). Total dose: 4.8 µmol	13 mo	Significant development of hepatomas occurred in 83% of treated mice.	Wiseman et al. (1987) W3
B6C3F$_1$ (12-days-old)	30 M weaned	*cis*-Asarone, purity n.p.	i.p. at 12 days of age in 10 µL of trioctanoin/g bw. Total dose: 0.25 µmol/g bw	12 mo	Significant development of hepatomas occurred in 69% of mice dosed with *cis*-asarone.	Wiseman et al. (1987) W4

Table 11. Carcinogenicity of 1-Propenyl Analogues of Estragole (Conjugated Side Chain) and Their Derivatives (Continued)

Species, Strain, and Age at Start of Expt.	Number and Sex of Animals	Chemical Form and Purity	Route/Dose/ Frequency	Duration	Results/Comments	Reference
$B6C3F_1$ (12-days-old)	18-39 M/dose group	*cis*-Asarone, purity n.p.	i.p.; 0.25 µmol/g bw with and without i.p. injection of pentachloro-phenol (PCP), 0.5 µmol/g bw without PCP, 45 min prior to test compound	10 mo	Significant increase in hepatoma-bearing mice from all treatments: 94%, high dose-PCP; 62%, low dose-PCP; and 79%, low dose + PCP. PCP did not inhibit tumor induction.	Wiseman et al. (1987) W5
$B6C3F_1$ (1-day-old)	47 M weaned	*trans*-asarone, purity n.p.	i.p.; on days 1, 8, 15, and 22 (in dose vol. 25-75 µL). Total dose: 4.8 µmol	13 mo	Significant development of hepatomas occurred in 89% of treated mice.	Wiseman et al. (1987) W3
$B6C3F_1$ (12-days-old)	37 and 39 M weaned	*trans*-Asarone, purity n.p.	i.p.; 0.75 µmol/g bw with and without i.p. injection of PCP 45 min prior to test compound	10 mo	PCP pretreatment had no effect on *trans*-asarone induced hepatomas. The incidence of hepatoma-bearing mice was 85-86% in both expts.	Wiseman et al. (1987) W5
Precocene						
$B6C3F_1$ (12-days-old)	27-34 M/dose group weaned	Precocene I and II, purities n.p.	i.p. at 12 days of age in 10 µL of trioctanoin/g bw. Doses: 0.125-0.5 µmol/g bw	10 mo	Development of hepatomas occurred in 93-100% of mice dosed with precocene I and II.	Wiseman et al. (1987) W4

Abbreviations: bw = body weight; F = females; i.p. = intraperitoneal; M = males; mo = month(s); n.p. = not provided; PCP = pentachlorophenol; p.o. = peroral;
= subcutaneous; wk = week(s)

s.c.

Table 12. Summary of Carcinogenicity Tests for Estragole, Its Structural Analogues, and Their Derivatives[a,b,c]

Compound	*Study*																							
	Mice																			Rats				
	D1	M1	M2	M3	M4	M5	M6	M7	M8	N223	N491	T1	T2	T3	W1	W2	W3	W4	W5	W6	M9	N491	T4r	T5
Anethole[d]		-	-		-		-		-															
cis-																	-	-						
3'-Bromo-*trans*-																	+							
3'-Hydroxy-					-				-															
3'-Hydroxy-*trans*-																		-						
Asarone[d]																								
cis-																	+	+	-					
trans-																	+		-					
Cinnam-aldehyde[d]																								
trans-																	-							
Dill apiol[d]					-																			
Elemicin[e]					-																			
1'-Acetoxy-																	+							
1'-Hydroxy-					-												+	-						
Estragole	+	+m	+		+	+													+					
1'-Acetoxy-																	+	+		+l				
1'-Acetoxy-2',3'-dehydro-																				+l				
1'-Hydroxy-	+		-	+	+	+			+l						+m	+	+	+			-			
1'-Hydroxy-2',3'-dehydro-					+												+	+						
1'-Hydroxy-2',3'-oxide								+s	+l												-			
1'-Oxo-																	+							
2',3'-Oxide			-					+s																
Eugenol[e]		-	-				-			-														
2',3'-Oxide			-					+s													-			
Isosafrole[d]																		-						
trans-					+													-						
Methyleugenol[e]					+						+n											+n		
1'-Hydroxy-					+																			

Table 12. Summary of Carcinogenicity Tests for Estragole, Its Structural Analogues, and Their Derivatives[a,b,c] (Continued)

Compound	*Study*																						
	Mice																			Rats			
	D1	M1	M2	M3	M4	M5	M6	M7	M8	N223	N491	T1	T2	T3	W1	W2	W3	W4	W5	W6	M9	N491	T4r	T5
Myristicin[e]					-																			
1'-Hydroxy-				-																				
Parsley apiol[d]					-																			
Precocene (I and II)[d]																		+						
Safrole[e]		+m	+			+	+		-			+	+a	+r									+c	+
1'-Acetoxy-			+															+			+i			
1'-Acetoxy-2,3-oxide			-																					
2',3'-Dehydro-																			-					
1'-Hydroxy-	+		+	+					-						+			+			+			
1'-Hydroxy-2',3'-dehydro-																		+						
1'-Hydroxy-2',3'-oxide			+					+s	+l												+i			
2',3'-Oxide (epoxide)			-					+s	-												-			

Results Codes: a = hepatocellular adenomas; b = results for both rat and mouse species; c = hepatocellular and cholangiocarcinomas of the liver; h = hepatocarcinomas; i = injection site sarcomas; l = lung adenomas; m = males only; n = increase in neoplasms; r = renal tumors in females exposed in utero, hepatocellular tumors in male offspring of nursing mothers and in adult females; s = skin tumors

[a] Tumors were hepatomas unless otherwise specified.

[b] General descriptions of each test are given in Carcinogenicity **Tables 6**, **7**, and **9-11**. Test prefixes and their designations in the text and other tables are:

D = Drinkwater et al. (1976)
M = Miller et al. (1983)
N = NTP (1983) and NTP (1999)
T = TRI (1997)
W = Wiseman et al. (1987)

[c] More details of the positive experiments are given in **Tables 6**, **7**, and **9-11**.

[d] 1-propenyl analogue of estragole

[e] 2-propenyl analogue of estragole

= malignant tumors

Study Codes for the Preceding Carcinogenicity Summary Table (Table 12)

Code	Strain, Age and Sex of Animals; Dose; Duration of Study	Code	Strain, Age and Sex of Animals; Dose; Duration of Study
D1	CD-1 (1-day-old), M; s.c., total dose of 4.4 mol for all compounds and extra dose group with 5.2 mol of Estragole; 15 mo	T2	BALB (other information n.p.); fed in diet for 52 wk; 75 wk
M1	CD-1 (1-day-old), M and F; gavage, 2.5 mol/g bw 2x/wk for 5 wk starting on day 4; 11-14 mo	T3	$B6C3F_1$ (other information n.p.); females intubated 180 times, 2x/wk for 90 wk; duration n.p.
M2	CD-1 (1-day-old), M; i.p. 9.45 mol (3.72 mol for 1-HS) on days 1, 8, 15, and 22; 12 mo	W1	C3H/HeJ and C57BL/6J (1-day-old), M and F; i.p., 0.1 L/25 L trioctanoin (day 1), 0.04 L/7 L trioctanoin or 0.08 L/10 L trioctanoin per gram bw (days 8, 15, and 22); 14 mo
M3	$B6C3F_1$ (1-day-old), M; i.p., 1.87 (1-HE) and 3.75 (I-HS) mol on days 1, 8, 15, and 22; 12 mo	W2	$B6C3F_1$ (1-day-old), M; i.p., 0.05, 0.10 and 0.15 mol/g bw on day 1 or 12; 14 mo
M4	$B6C3F_1$ (1-day-old), M; i.p., total dose varied from 1.86 — 4.75 mol according to the compound on days 1, 8, 15, and 22; 13-18 mo	W3	$B6C3F_1$ (1-day-old), M; i.p., 0.04 — 9.5 mol in 25 — 75 L trioctanoin (amount varied with each compound) on days 1, 8, 15, and 22; 13 mo
M5	CD-1 (8-wk-old), F; oral in diet, 25 and 50% of final levels for the first and second 10-day periods; 20 mo	W4	$B6C3F_1$ (12-days-old), M; i.p., 0.01 — 2.5 mol/10 L trioctanoin (amount varied with each compound) per gram bw on day 1; 10 — 12 mo
M6	CD-1 (8-wk-old), F; oral in diet, 15, 30, and 67% of final levels for the first three successive 10-day periods, some mice given phenobarbitol as 0.05% of the drinking water from the beginning of the experiment, until termination; 18 mo	W5	$B6C3F_1$ (12-days-old), M; i.p., 0.25 — 7.5 mol/10 L trioctanoin (amount varied with each compound) per gram bw on day 1; 9 mo
M7	CD-1 (8-wk-old), F; topical treatment, 11.2 mol in 0.15 mL of redistilled acetone 4x/wk for 6 wk; 40 wk	W6	A/J (12-days-old), M and F; i.p., 0.05 mol/10 L trioctanoin (amount varied with each compound) per gram bw on day 1 or on day 8 and 12; 9 mo
M8	A/J (8-wk-old), F; i.p., 0.5 or 1.0 mol per g of bw in 5 L trioctanoin per g of bw 2x/wk for 12 wk; 8 mo	M9	Fischer (5-wk-old), M; s.c., injected 2x/wk for 10 wk in rear hind leg, 0.01 mmol/injection; 24 mo
N223	$B6C3F_1$ (age n.p.), M and F; oral in diet, 3,000 or 6,000 ppm for 103 wk; 48 mo	N491	Fischer 344/N rats (age n.p.), M and F; gavage, 37, 75, and 150 mg/kg bw 5x/wk for 105 wk; 24 mo
N491	$B6C3F_1$ (age n.p.), M and F; gavage, 37, 75, and 150 mg/kg bw for 104 wk; 48 mo	T4	Osborne-Mendel (other information n.p.); fed in the diet; 2 years
T1	$B6AKF_1$ (other information n.p.); gavaged for 1st mo then fed in diet, n.p.; duration n.p.	T5	CD (other information n.p.); fed in the diet for 22 mo, co-administration of phenobarbitol; 22 mo

Abbreviations: bw = body weight; F = females; i.p. = intraperitoneal; M = males; mo = month(s); n.p. = not provided; s.c. = subcutaneous; wk = week(s)

Table 13. DNA Adducts of 1'-Hydroxyestragole, Estragole, Safrole, and Methyleugenol

Chemical	DNA Adduct	Reference
1'-hydroxyestragole	N^2-(estragol-1'-yl)deoxyguanosine N^2-(*trans*-isoestragol-3'-yl)deoxyguanosine N^2-(*cis*-isoestragol-3'-yl)deoxyguanosine N^6-(*trans*-isoestragol-3'-yl)deoxyadenosine	Phillips et al. (1981)
estragole	N^2-(estragol-1'-yl)deoxyguanosine N^2-(*trans*-isoestragol-3'-yl)deoxyguanosine N^2-(*cis*-isoestragol-3'-yl)deoxyguanosine N^6-(*trans*-isoestragol-3'-yl)deoxyadenosine	Randernath et al. (1984)
safrole	N^2-(*trans*-isosafrol-3'-yl)deoxyguanosine N^2-(safrol-1'-yl)deoxyguanosine N^6-(*trans*-isosafrol-3'-yl)deoxyadenosine N^6-(safrol-1'-yl)deoxyadenosine	Randernath et al. (1984)
methyleugenol	N^2-(*trans*-isomethyleugenol-3'-yl)deoxyguanosine N^2-(methyleugenol-1'-yl)deoxyguanosine N^6-(*trans*-isosafrol-3'-yl)deoxyadenosine N^6-(methyleugenol-1'-yl)deoxyadeosine	Randernath et al. (1984)

11.0 ONLINE DATABASES AND SECONDARY REFERENCES

11.1 Online Databases

Chemical Information System Files

SANSS (Structure and Nomenclature Search System)
TSCATS (Toxic Substances Control Act Test Submissions)

DIALOG Files

CEH (Chemical Economics Handbook)

National Library of Medicine Databases

EMIC and EMICBACK (Environmental Mutagen Information Center)
CCRIS (Chemical Carcinogenesis Research Information System)

STN International Files

BIOSIS	HSDB
CANCERLIT	MEDLINE
CAPLUS	Registry
CHEMLIST	RTECS
EMBASE	TOXLINE

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicological Research Projects	CRISP
NIOSHTIC®	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Developmental and Reproductive Toxicology	DART

Databases Available on the Internet

Phytochemical and Ethnobotanical Databases

In-House Databases

CPI Electronic Publishing Federal Databases on CD-ROM
Current Contents on Diskette®
The Merck Index, 1996, on CD-ROM

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APPENDIX A: UNITS AND ABBREVIATIONS

bw = body weight

°C = degrees Celsius

°F = degrees Fahrenheit

µg/L = microgram(s) per liter

µg/mL = microgram(s) per milliliter

µM = micromolar

d = day(s)

DMSO = dimethyl sulfoxide

F = female(s)

g = gram(s)

g/mL = gram(s) per milliliter

GC = gas chromatography

h = hour(s)

i.p. = intraperitoneal(ly)

kg = kilogram(s)

LC_{50} = lethal concentration for 50% of test animals

LD_{50} = lethal dose for 50% of test animals

M = male(s)

mg/kg = milligram(s) per kilogram

mg/mL = milligram(s) per milliliter

mL/kg = milliliter(s) per kilogram

mm = millimeter(s)

mM = millimolar

mmol = millimole(s)

mmol/kg = millimole(s) per kilogram

mo = month(s)

mol. wt. = molecular weight

nm = nanometer(s)

n.p. = not provided

N/A = not applicable

p.o. = peroral(ly)

ppm = part(s) per million

s.c. = subcutaneous(ly)

wk = week(s)

yr = year(s)