SUMMARY OF DATA FOR CHEMICAL SELECTION

MYRISTICIN

CAS NO. 607-91-0

BASIS OF NOMINATION TO THE CSWG

Myristicin is presented to the CSWG for review because of its potential for widespread human exposure through foods and beverages and the possibility of adverse effects in diverse populations.

Information available on myristicin suggests a range of effects, some beneficial and others harmful. Myristicin is the hallucinogenic agent in nutmeg and mace. In quantities readily obtained from the local market, these spices have, at times, been drugs of abuse. On the other hand, nutmeg and mace are used as traditional medicines in Asia to treat stomach cramps, diarrhea, and rheumatism.

Myristicin is presented, rather than one of 20 spices, oleoresins, and oils containing myristicin, to permit exploration of its potential to be both a carcinogen and an anticarcinogen. Limited *in vivo* and *in vitro* studies suggest myristicin could inhibit tumor formation; in this regard its ability to prevent carbon tetrachloride liver toxicity in mice is interesting.

On the other hand, the probability that myristicin will be a liver carcinogen in experimental animals is extremely high. Myristicin is closely related to safrole, a liver carcinogen in mice and rats. Myristicin not only induces liver enzymes often associated with carcinogens, but also the corresponding mRNA. Like safrole, myristicin forms DNA adducts in mouse liver, although at a somewhat lower level. Experimental data on safrole suggest that myristicin may cause tumors from *in utero* exposure and through nursing. The increased incidence of liver tumors in mice given phenobarbital and safrole, if repeated for myristicin, is also of concern since this suggests an increased risk to certain vulnerable populations.

SELECTION STATUS

ACTION BY CSWG: 7/16/97

Studies requested:

- Carcinogenicity
- Mutagenicity assays
- Metabolism studies
- *In vitro* cytogenic analysis
- *In vivo* micronucleus assay

Priority: High

Rationale/Remarks:

- Lack of adequate chronic toxicity data
- Widespread exposure as an ingredient in natural products
- Suspicion of carcinogenicity
- Structural interest

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

Dr. Dan Benz, Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration (FDA) and Dr. Ed Matthews (formerly of CFSAN) provided information on myristicin from FDA's Priority-Based Assessment of Food Additives (PAFA) database.

CHEMICAL IDENTIFICATION

CAS Registry Number: 607-91-0

Chemical Abstract Service Name: 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- (9CI)

Synonyms and Trade Names: 6-Allyl-4-methoxy-1,3-benzodioxole; 5-allyl-1- methoxy-2,3-(methylenedioxy) benzene

Structural Class: Benzodioxole

Structure, Molecular Formula and Molecular Weight:

$C_{11}H_{12}O_3$ Mol. wt.: 192.20

Chemical and Physical Properties:

Description: Colorless oil (Budavari, 1996)

Boiling Point: 276-277%C (STN International, 1997)

Melting Point: <-20%C (STN International, 1997)

Specific gravity: 1.1437 at 20%C (Budavari, 1996)

Solubility: Insoluble in water; soluble in benzene and diethyl ether; slightly soluble in ethanol (STN International,

Vapor Pressure: 0.183 kPa at 100%C (CRC Press Inc., 1996)

Technical Products and Impurities: Myristicin is available in HPLC grade in research quantities with a purity ranging from 90-95% (Sigma Chemical Co., 1997).

EXPOSURE INFORMATION

Production and Producers: Nutmeg is the dried, ripe seed, and mace is the dried aril which envelops the shell containing the seed of trees of Myristica species, principally *Myristica fragrans* Houtt. The ground seed is the spice nutmeg; the ground aril is the spice mace. Oil of nutmeg and oil of mace are the essential oils obtained by steam distillation of nutmeg and mace, respectively. Nutmeg and mace owe their characteristic aroma to these essential oils. Mace oleoresin, a butter-like product obtained by pressing, consists chiefly (73%) of the fat trimyristin, about 12% of rather firmly bound essential oil, and smaller amounts of other fats, fatty acids, and unsaponified lipids. A similar product, nutmeg butter, can be pressed from nutmeg (FASEB, 1973). The volatile or "essential" oil of nutmeg consists of approximately 80% monoterpenes, approximately 5% monoterpene alcohols, an aromatic ether fraction, and small quantities of miscellaneous compounds; the aromatic ether fraction includes myristicin (Matthews *et al.*, 1974; Forrest & Heacock, 1972; Sammy & Nawar, 1968).

Parsley (*Petroselinum sativum*) is a member of the edible Umbelliferae plants. It is extensively used as a culinary herb for garnishing and seasoning. Parsley leaf oil (14% myristicin) obtained by steam distillation from the fresh leaves possesses a superior taste and aroma of the plant and is used primarily in food flavorings and seasonings, as well as perfumery and pharmaceutical preparation. Although myristicin is present in small quantities in fresh parsley (0.007%), the cumulative consumption of this compound from other edible plants such as celery, dill, mace, and nutmeg may be substantial (Zheng *et al.*, 1992).

Prolonged storage of nutmeg resulted in changes in the volatile composition, as determined by gas chromatography. The variation in composition of the nutmeg constituents appears to be mainly a function of their volatilization. Myristicin, having a lower volatility than some nutmeg constituents, was a more persistent component (Sanford & Heinz, 1971).

No data were reported for myristicin by the USITC in the ten most recent volumes of *Synthetic Organic Chemicals, US Production and Sales,* and no other quantitative information on annual production was found in the available literature.

Use Pattern: Myristicin itself has no particular use pattern. The foods, spices, oils and other commodities in which it occurs have many uses.

In traditional medicine, nutmeg is used for several purposes. It is a well known carminitive and stomachic. In China, the powdered nut is used as a warming agent and astringent remedy against dysentery, a remedy for stomach cramps and colic and also as a stimulant for the treatment of chronic rheumatism. In Indochina, powdered seeds in boiled rice are used as a remedy against dysentery, anorexia, and colic. It is further used to treat malarial debility. In Indonesia, mace is also used as an analgesic and as medicine for rheumatism (Ozaki *et al.,* 1989; Janssens *et al.,* 1990).

Human Exposure: There is potential for exposures to myristicin in occupational, consumer, and environmental settings by inhalation, ingestion, and dermal contact. Table 1 presents the human exposure estimate data from the FDA Priority-Based Assessment of Food Additives (PAFA) database for the spices and/or their components in which myristicin is found (Matthews, 1994).

Table 1. Human exposure estimate data from the FDA PAFA database

FASP[a] Number	Common Name	Exposure Estimate (lbs.)
1707	Anise oil	38,667
1708	Anise	255,000
1709	Star anise	11,283
1710	Star anise oil	7,117
2025	Dill oil	210,000
2089	Common fennel	1,585,000
2090	Sweet fennel	37,167
2091	Sweet fennel oil	6,550
2318	Mace	308,333
2319	Mace oil	10,667
2320	Mace oleoresin	6,967
2455	Nutmeg	2,366,667
2456	Nutmeg oil	265,000
2457	Nutmeg oleoresin	63
2507	Parsley oil	817
2508	Parsley oleoresin	2,083
2509	Parsley	7,733,333
2527	Black pepper oil	8,383
2528	Black pepper oleoresin	321,667
2529	Black pepper	32,333,333

[a]FASP = food additive safety profile

No reports of occupational exposure to myristicin during its production or processing were found in the available literature. No listing was found for myristicin in the National Occupational Exposure Survey (NOES).

Environmental Occurrence: Myristicin is a naturally occurring benzodioxole compound found in anise, Star anise, black pepper, carrot, common fennel, mace, nutmeg, sweet fennel, many natural oils, and flavoring agents (Jeong & Yun, 1995; Matthews, 1994).

Regulatory Status: No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of myristicin. The American Conference of Governmental Industrial Hygienists (ACGIH) has not recommended a threshold limit value (TLV) or biological exposure index (BEI) for myristicin.

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data:

*Acute Toxicity***.** Ingestion of five or more grams of nutmeg causes acute nutmeg poisoning, which includes giddiness, hallucinations, and feelings of depersonalization. Symptoms usually appear three to six hours after ingestion of 1-3 whole nutmegs or 5-15 gm of the grated spice. Recovery usually occurs within 24 hours. Nevertheless, duration of action may extend beyond several days and even include death (Painter *et al*., 1971; Green, 1959).

*Subacute/Subchronic Toxicity***.** A central problem in the pharmacology of nutmeg was identification of the active compound (Truitt, 1979). This is now believed to be myristicin, but the myristicin fraction of nutmeg oil lacks adequate potency to explain nutmeg intoxication completely.

Interest in the pharmacologic action of myristicin and nutmeg grew in the late 1960s and 1970s when nutmeg became a drug of abuse. Recognition that myristicin has a structure resembling mescaline and hallucinogenic amphetamines prompted investigations such as the behavioral studies described in the animal studies section.

*Chronic Studies***.** No epidemiological studies or case reports investigating the association of exposure to myristicin and cancer risk in humans were identified in the available literature.

Animal Data:

*Acute***.** Acutely toxic doses of myristicin can cause organ damage. Cats were especially sensitive to myristicin, exhibiting central excitation followed by coma. Oral administration of myristicin at 400 mg/kg to cats caused fatty degeneration of the liver. Rabbits and guinea pigs administered a subcutaneous (sc) dose of myristicin developed brain and liver lesions and metabolic derangement (NLM, 1997).

Acute toxicity data reported for myristicin are summarized below (NLM, 1997).

rat oral LD_{50} = 4260 mg/kg
cat oral LD_{Lo} = 400 mg/kg
rabbit sc LD_{Lo} = 900 mg/kg
guinea pig LD_{Lo} = 2 gm/kg

Subacute/Subchronic Studies. Ozaki and coworkers (1989) fractionated the ingredients in mace and administered them orally to rats with a carrageenin-induced edema. One fraction produced a lasting anti-inflammatory activity. It contained myristicin, suggesting the analgesic ingredient in mace is myristicin.

Myristicin and elemicin[1] are structurally similar to the hallucinogen, mescaline (Buchanan, 1978). All three compounds impaired the rope-climbing and bar-pressing performance of rats (Cesario de Mello & Carlini, 1973).

[1]Elemicin is present in the same nutmeg fraction as myristicin and probably accounts for some of the activity observed with nutmeg (Braun & Kalbhen,

Several tests have shown myristicin to be a weak monamine oxidase (MAO) inhibitor (Truitt, 1979). Synthetic myristicin showed considerable activity when samples were fresh. The distilled concentrate of oil of nutmeg was much less active than synthetic myristicin.

Myristicin can alter the toxicity of other compounds. For example, myristicin caused a change in the pathway of parathion degradation when fed to rats (Marcus & Lichtenstein, 1982). Myristicin also completely prevented carbon tetrachloride-induced hepatotoxicity in mice (Zhao & O'Brien, 1996).

*Chronic/Carcinogenicity Studies***.** No 2-year carcinogenicity studies of myristicin in animals were identified in the available literature.

Myristicin was one of 23 naturally occurring and synthetic alkenylbenzene derivatives assayed for hepatocarcinogenicity in mice (Miller *et al.*, 1983). Specifically, myristicin was administered intraperitoneally (ip) to male B6C3F_1 mice 24 hours after birth and at days 8, 15, and 22 for a total dose of 4.75 mol. At 13 months, 10% of the mice had hepatomas; at 18 months (end of experiment), 31% bore hepatomas (0.4 tumors/mouse) but the findings were not statistically significant compared with vehicle or untreated controls.

Myristicin was tested as an inhibitor of benzo[a]pyrene (B[a]P)-induced tumors in female A/J strain mice (Zheng *et al.*, 1992). B[a]P was given orally twice a week for four weeks at 1 mg per dose. Myristicin was given orally, 10 mg per dose, three times a week on days other than the B[a]P treatment and for one week before the first administration of B[a]P. Eighteen weeks after the first dose of B[a]P, the mice were killed for examination of lung and forestomach tumors. Myristicin significantly inhibited lung tumor multiplicity; the mean number of tumors in tumor-bearing animals was reduced by 65%. Myristicin slightly inhibited forestomach tumor multiplicity; the mean number of tumors in tumor-bearing animals was reduced by 31%.

Short-Term Tests: Few studies of the potential mutagenic effects of myristicin were identified in the available literature, perhaps because myristicin has bacteriocidal properties. Marcus and Lichtenstein (1982) mentioned that nutmeg

oil and myristicin showed no mutagenic activity in *Salmonella typhimurium* TA100 and TA98 at up to cytotoxic doses with and without S13 metabolic activation.

Damhoeri and coworkers (1985) conducted specialized mutagenicity tests on the oleoresins of pepper, shallot, and nutmeg fruit and related compounds. Streptomycin-dependent strains SD1018 and SD7823 isolated from *S. typhimurium* TA100 and TA98, respectively, were prepared for spot and plate incorporation tests. All assays were conducted without metabolic activation. In spot tests, nutmeg oleoresin and myristicin were positive in both SD1018 and SD7823 although the oleoresin tested negative in TA98, TA100, and *Escherichia coli* WP2. In plate incorporation assays, oleoresin of nutmeg kernels prepared from raw seeds produced a dose-responsive mutagenic effect. Oleoresin from dried and stored nutmeg was not active, suggesting the mutagenic substance is volatile.

Myristicin did not induce unscheduled DNA synthesis (UDS) in hepatocytes derived from male Fischer 344 rats in doses up to 0.01 molar, ten times the dose where cytotoxicity was noted (Hasheminejad & Caldwell, 1994).

Metabolism: There are several possible pathways for myristicin metabolism. Limited data for myristicin suggest the relative importance of these pathways may be dependent on dose, species, sex, length of exposure, and the like.

Kamienski and Casida (1970) demonstrated that one metabolic pathway for myristicin is cleavage of the methylene dioxyphenyl moiety through 4-oxidation, producing a polar product containing two hydroxy substituents on the benzene ring. Such molecules undergo glucuronidation and excretion. The methylene group on the five-member ring is exhaled as carbon dioxide.

Methylenedioxyphenyl compounds such as myristicin also interact with liver enzymes (Delaforge *et al.,* 1985). Mixed function oxidation is briefly inhibited, but this is followed by a period in which cytochrome P450 enzymes are induced. Both phenobarbital-induced cytochrome P450 and 3-methylcholanthrene-induced cytochrome P450 can metabolically convert methylenedioxybenzene compounds to their reactive “carbenes” and then interact with them to produce ligand

complexes. The oxidation of the allylic chain to epoxy- or hydroxy-derivatives enhances the affinity of these compounds for cytochrome P448, but not for cytochrome P450, as demonstrated for safrole and its metabolites.

Delaforge's study suggests that the rate and extent of oxidation of the allyl side chain on myristicin may be an important factor in whether or not myristicin is a carcinogen in chronic studies. Miller and coworkers (1983) have suggested that the ultimate carcinogen would be the sulfuric acid ester of the 1'-hydroxy derivative. Miller did not believe that epoxide metabolites were the major contributors to carcinogenic activity.

Because myristicin has hallucinogenic properties, Braun and Kalbhen (1973) suspected that the allyl group on myristicin might undergo amination. Using isolated perfused rat liver and rat liver homogenate, these authors reported detecting 3-methoxy-4,5- methylene dioxyamphetamine. These results seem speculative, but another research group reported finding 3-piperidyl-1,3'-methoxy-4',5'-methylenedioxyphenyl-1-propanone in rat urine and 3-pyrrolidinyl-1(3'-methoxy-4',5'-methylenedioxy-phenyl) 1-propanone in guinea pig urine following administration of myristicin (Oswald *et al.*, 1971).

To date, studies of each metabolic pathway for myristicin appear to have been conducted in isolation, with no intent to examine the importance of that pathway relative to other pathways. No available studies addressed which metabolic pathway predominates or whether this depends on dosing patterns or length of exposure.

Other Biological Effects: The correlation between induction of glutathione S-transferase (GST) and inhibition of tumorigenesis by anticarcinogens is well documented (Zheng *et al.*, 1992). On the other hand, the ability to form DNA adducts is a common property of most chemical carcinogens or their metabolites (Gupta *et al.*, 1993). Myristicin has induced GST and formed DNA adducts in rodent livers. Myristicin can also induce several families of rat liver P450, including the P450 1A enzyme which is also induced by PAHs, dioxins, and polychlorinated biphenyls.

When 10 mg of myristicin was given to female A/J mice every two days for a total of three doses, GST activity in the liver increased over 4-fold and in the small intestine over 3-fold (Zheng *et al.*, 1992). One of two class GST subunits was selectively induced, while GST-" activity was unchanged and GST- was moderately increased. These results suggested that myristicin might be a chemopreventive agent (Tobola *et al.*, 1996).

Jeong and Yun (1995) examined the effects of myristicin on rat liver P450 enzymes. Myristicin was administered ip to groups of four male Sprague-Dawley rats at doses of 10 to 5000 mol/kg. After 24 hours, the activities of P450 1A1/2, P450 2B1/2, and P450 2E1 were determined. Myristicin caused a dose-dependent increase of P450 activities, reaching a significant maximum at 500 mol/kg. The maximum activity was attained for P450 1A1/2 and P450 2B1/2 at 24 hours; for P450 2E1 the maximum activity occurred at 12 hours. The increase in P450 enzyme activities was accompanied by increases in P450 apoprotein content. Induction of P450 1A1/2 and P450 2B1/2 by myristicin was accompanied by a corresponding increase in mRNA encoding these proteins. In contrast, no significant change in P450 2E1 mRNA was observed.

In an earlier study, Iwasaki and coworkers (1986) examined species effects on cytochrome P-448 (P450 1A) induction by myristicin and safrole. Wistar rats, CD1 mice, and Syrian golden hamsters received ip injections of inducing agent for three days when the P-448 activity in liver, kidneys, and lungs was measured. Safrole, a liver carcinogen, produced a 9-fold increase in liver ethoxyresorufin O-deethylation (EROD) in rats, a 1-fold increase in hamsters, and a 3-fold decrease in mice. Myristicin caused a 4-fold increase in EROD activity in rat liver, nearly half the increase seen for safrole. Neither myristicin nor safrole caused an increase in EROD activity in the lung or kidney.

Randerath and coworkers (1984) examined DNA adduct formation in the livers of mice administered myristicin and other alkenylbenzenes. Randerath's studies were meant to complement carcinogenicity studies by Miller and coworkers (1983). Female CD-1 mice were given an ip injection of test compound (2 or 10 mg/mouse), and 24 hours later alkenylbenzene-DNA adducts in the liver were isolated. The mouse liver carcinogens safrole, estragol, and methyleugenol exhibited the strongest binding to mouse-liver DNA, followed by myristicin,

which was three to four times weaker. The adduct present in highest concentration was thought to be the 3',5'-bisphosphate of an N^2-(*trans*-propenylbenzene-3'-yl)deoxyguanosine adduct. The other DNA adduct was thought to be the corresponding derivative of an N^2-(allylbenzen-1'-yl)deoxyguanosine adduct.

The same research group analyzed DNA adducts formed in the livers of newborn mice given alkenylbenzenes, including myristicin (Phillips *et al.*, 1984). Male B6C3F_1 mice were given ip injections of test compound on days 1, 8, 15, and 22 after birth. Groups of three mice were killed on days 23, 29, and 43 to detect liver adducts. The highest levels of adducts were detected with the liver carcinogens, methyleugenol (72.7 pmol/mg DNA), estragole (30.0), and safrole (17.5), but significant DNA binding by myristicin was also found (7.8 pmol/mg DNA). Only low levels of adducts were detected with dill apiol and parsley apiol; no DNA binding was detected with eugenol.

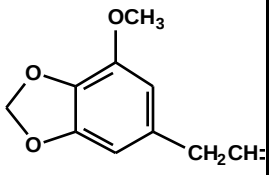
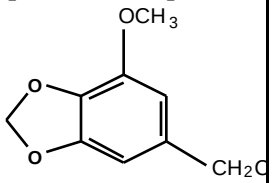
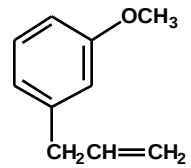
More recently, Randeranth and coworkers (1993) examined DNA adduct formation in the livers of ICR mice drinking a widely used non-diet cola instead of water for up to eight weeks. Adducts chromatographically identical to those induced by cola drinks were detected in mice treated with myristicin, extracts of nutmeg, mace or spices from the nutmeg tree. The predominant myristicin adduct accounted for about 80% of the total adducts. When pregnant mice were intubated with myristicin, myristicin adducts were also found in fetal liver which was of particular concern since the rapid division of fetal cells facilitates expression of such lesions. Adducts were not detected in hepatic DNA of mice given one of three different brands of a non-cola beverage.

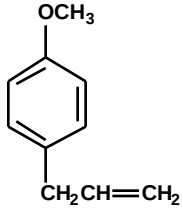
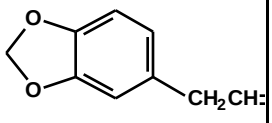
Structure/Activity Relationships: Four chemicals structurally similar to myristicin were screened for relevant information on carcinogenicity and genotoxicity, including *in vitro* tests for mutagenicity and information on DNA adduct formation (see Table 2 for summary information on myristicin and structurally similar compounds). Three (estragole, eugenol, and safrole) are found in spices, but dihydromyristicin was also examined because it has a saturated side chain. Three other compounds (methyleugenol, isoeugenol, and vinyl benzene) are discussed briefly. All compounds but dihydromyristicin contain an alkenyl side chain, believed by many to be the active site for carcinogenic activity.

A significant structural difference among the selected compounds is the number of substituents other than the alkenyl side chain. Vinyl benzene, the simplest member of the structural group has a covalent binding index 100 times lower than estragole, methyleugenol, and safrole, which are mouse liver carcinogens (Randerath *et al.*, 1984). Structural differences on the molecules at sites other than the alkenyl side chain may also influence metabolic pathways and alter carcinogenic potency.

Methyleugenol is on test at the National Toxicology Program (NTP), presently at the pathology assessment stage (NTP, 1997). Isoeugenol was nominated for testing in January 1997. Methyleugenol and its metabolite 1'-hydroxymethyleugenol were tested in weanling male B6C3F_1 mice. At 18 months, 96% of the methyleugenol group and 93% of the 1'-hydroxymethyleugenol had liver tumors compared with 41% of the controls. The number of tumors per tumor-bearing mouse was 3.2, 3.5, and 0.5, respectively. These results were significant at $P<0.001$ (Miller *et al.*, 1983).

Table 2. Summary of information on myristicin and structurally related compounds

Chemical [CAS No.]	Carcinogenicity Data	Genotoxicity
Myristicin [607-91-0] 	**Mouse** no increase in the incidence of liver tumors in male B6C3F$_1$ mice administered by ip injections on days 1, 8, 15, and 22 after birth (Miller *et al*., 1983) significantly inhibited B[a]P-induced lung tumorigenesis and multiplicity of lung tumors produced in Strain A mice when given one week before and 5 weeks after administration of B[a]P (Zheng *et al*., 1992)	negative in *S. typhimurium* TA98 and TA100 with or without S13 activation (Marcus & Lichtenstein, 1982) positive in SD1018 and SD7823, streptomycin-dependent strains isolated from *S. typhimurium* TA100 and TA98 (Damhoeri *et al*., 1985) negative in the UDS assay in cultured rat hepatocytes (Hasheminejad & Caldwell, 1994) DNA adducts formed in the livers of newborn male B6C3F$_1$ mice injected with myristicin (Phillips *et al*., 1984) DNA adducts in fetal liver and maternal liver when pregnant mice were intubated with myristicin (Randerath *et al*., 1993) DNA adducts in livers of adult female CD-1 mice 24 hours after ip injection of myristicin (Randerath *et al*., 1984)
Dihydromyristicin [52811-28-6] 	**Mouse** no significant decrease in B[a]P-induced lung or forestomach tumors in female Strain A mice gavaged three times a week for 5 weeks starting one week before administration of B[a]P (Zheng *et al*., 1992)	NDF
Eugenol [97-53-0] 	**Rat** no evidence of carcinogenicity when given in the diets of female F344/N rats (0, 0.6, or 1.25%) and male F344/N rats (0, 0.3, or 0.6%) for 2 years (NTP, 1983). **Mouse** equivocal evidence of carcinogenic	negative in *S. typhimurium* TA97, TA98, TA100, TA1530, TA1531, TA1532, TA1535, TA1537, TA1538, or TA1964 with or without metabolic activation (CCRIS, 1997; NTP, 1983) induced chromosome aberrations in CHO cells with activation and sister chromatid exchanges with or

	activity when given in the diets of male and female $B6C3F_1$ mice (0, 0.3, or 0.6%) for 2 years; increased incidence of hepatocellular adenomas and hepatocellular carcinomas in low-dose males; dose-related positive trend in liver neoplasms in females (NTP, 1983) weak promoter of skin tumorigenesis initiated by 7,12-dimethylbenz[a]- anthracene in female ICR/Ha Swiss mice (NTP, 1983) not hepatocarcinogenic to weanling CD-1 mice dosed by stomach tube or ip injection for 1 month and held for 12 (ip) to 14 (oral) months (Miller *et al*., 1983)	without activation (NTP, 1983) did not induce DNA adducts in adult female CD-1 mouse livers or in the livers of newborn male $B6C3F_1$ after ip injection (Randerath *et al*., 1984; Phillips *et al*., 1984)
Estragole [140-67-0] 	**Mouse** significant increase in hepatocellular carcinomas in male CD-1 mice after four sc doses in one month, in female CD-1 mice given in the diet for 12 months, in weanling CD-1 mice intubated or ip injected 4 times in the first month (Miller *et al*., 1983; CCRIS, 1997)	negative in *S. typhimurium* TA98, TA100, TA1537, or TA1538 with or without metabolic activation; TA1535 reported negative without activation in three studies, negative with S9 in two studies, and positive with S9 in one study (CCRIS, 1997) induced UDS in rat hepatocytes *in vitro or in vivo* (CCRIS, 1997) induced mouse liver DNA adducts in adults and newborns (Phillips *et al*., 1984; Randerath *et al*., 1984)
Safrole [94-59-7] 	**Mouse** hepatocellular tumors in male and female $B6AKF_1$ mice gavaged the first month and then fed in the diet (IARC, 1976) hepatomas in male mice and lung tumors in female mice injected sc for three weeks (Epstein *et al*., 1970) hepatocellular adenomas and carcinomas in male BALB mice fed in the diet for 52 weeks and killed after 75 weeks (CCRIS, 1997)	negative in *S. typhimurium* TA98, TA100, TA102, TA1537, or TA1538 with or without metabolic activation; negative in TA1535 without activation but positive with activation (CCRIS, 1997) positive with but not without metabolic activation in the L5178Y mouse lymphoma cell assay (CCRIS, 1997) persistent liver DNA adducts induced in adult female mice and in newborn mice (Gupta *et al*., 1993; Phillips *et al*., 1984; Randerath *et*

	renal tumors in female $B6C3F_1$ mice exposed *in utero*; hepatocellular tumors in male offspring of nursing mothers and in adult females intubated 180 times, twice weekly for 90 weeks (Vesselinovitch *et al*., 1979) **Rat** hepatocellular carcinomas and cholangiocarcinomas of the liver in male and female Osborne-Mendel rats fed in the diet for two years (IARC, 1976) hepatocellular carcinomas in male CD rats fed in the diet for 22 months; enhanced response if coadministered phenobarbital (Wislocki *et al*., 1977)	*al*., 1984)

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