



EUROPEAN COMMISSION

HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions

C2 - Management of scientific committees; scientific co-operation and networks

**Scientific Committee on Food**

**SCF/CS/FLAV/FLAVOUR/6 ADD3 Final**

**9 January 2002**

**Opinion of the Scientific Committee on Food  
on the safety of the presence of safrole (1-allyl-3,4-  
methylene dioxy benzene) in flavourings and other food  
ingredients with flavouring properties**

(adopted on 12 December 2001)

---

B-1049 Bruxelles/ Brussel - Belgium

Telephone: direct line (+32-2) 295.4861, switchboard 299.11.11. Fax: (+32-2) 299.4891

Telex: COMEU B 21877. Telegraphic address: COMEUR Brussels

[http://europa.eu.int/comm/food/fs/sc/scf/index\\_en.html](http://europa.eu.int/comm/food/fs/sc/scf/index_en.html)

**Opinion of the Scientific Committee on Food on the safety of the presence of safrole (1-allyl-3,4-methylene dioxy benzene) in flavourings and other food ingredients with flavouring properties**

(adopted on 12 December 2001)

**Terms of reference**

The Committee is asked to advise the Commission on substances used as flavouring substances or present in flavourings or present in other food ingredients with flavouring properties for which existing toxicological data indicate that restrictions of use or presence might be necessary to ensure safety for human health.

In particular the Committee is asked to advise the Commission on the implications for human health of the presence of safrole (1-allyl-3,4-methylene dioxy benzene) in the diet.

**Introduction**

Previous evaluations

The Scientific Committee for Food proposed limits in its opinion of 21 September 1979 (SCF, 1979). For total safrole and iso-safrole, limits of 1 mg/kg for foods and beverages were proposed. The following exceptions were made: 5 mg/kg in alcoholic beverages with more than 25% alcohol by volume, 15 mg/kg in foods containing mace and nutmeg and 2 mg/kg in alcoholic beverages with less than 25% alcohol.

The evaluation of safrole by the International Agency for Research on Cancer (IARC) in 1976 and 1987 resulted in a classification in Group 2B (IARC, 1976 and 1987). In 1981 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) could not allocate an ADI due to the carcinogenicity (WHO, 1981).

In 1997 the Committee of Experts on Flavouring Substances (CEFS) of the Council of Europe evaluated safrole as follows: "Safrole is a weak hepatocarcinogen in experimental animal studies but is also a genotoxic and a transplacental carcinogen. Efforts should be made to reduce its consumption by foods and beverages as far as possible". CEFS proposed limits of 0.5 mg/kg for foods, 0.05 mg/kg for non-alcoholic beverages, 0.5 mg/kg for alcoholic beverages and an exceptional, not-specified limit for foods containing mace or nutmeg (Council of Europe, 1997). CEFS deleted the substance from the Blue Book,

meaning that the substance should not be added as such to foodstuffs (Council of Europe, 1992).

### Current Regulations

Annex II of Directive 88/388/EEC on flavourings sets the following maximum levels for safrole in foodstuffs and beverages to which flavourings or other food ingredients with flavouring properties have been added: 1 mg/kg in foodstuffs and beverages, with the exception of 5 mg/kg for alcoholic beverages with more than 25% alcohol by volume and 15 mg/kg for foods containing mace or nutmeg. Safrole may not be added as such to foodstuffs (EEC, 1988).

Safrole was voluntarily cancelled from the EPA pesticide regulation (1977) and has not been approved by the US Food and Drug Administration for use in foods (21 CFR 121-106).

### **Chemical characterisation**

Name : Safrole

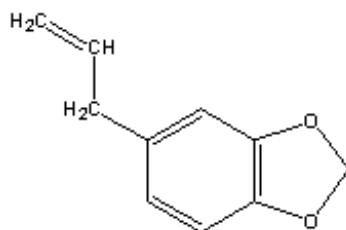
Synonyms : 1-allyl-3,4-methylene dioxy benzene; 5-allyl-1,3-benzodioxole;  
4-allyl-1,2-methylene dioxy benzene

CAS Name : 1,3-benzodioxole, 5(2-propenyl)

CAS No : 94-59-7

M.W.: 162.2

Structure :



### **Exposure assessment**

Safrole is a natural constituent of a number of spices such as nutmeg, mace, cinnamon, anise, black pepper and sweet basil. The most important dietary sources are nutmeg, mace and their essential oils. Safrole is also present in cola drinks (Council of Europe, 1997).

Intake estimates of flavouring substances are generally very poor because of the lack of data on the concentrations of these chemicals naturally occurring or voluntarily added in foodstuffs. Within the Council of Europe, UK and France provided calculations based on

their respective food consumption data and on concentration levels documented or assumed.

### Food consumption

There was no difference between the methodologies of food intake assessment as carried out by UK and France. The methodology was based on a seven days dietary record of adult individuals. The under-reporting subjects were excluded following the same method. In both surveys consumers were identified to assess the intake for consumers only. The respective methodologies have been described elsewhere (Council of Europe, 2001; Observatoire des consommations alimentaires, 1998).

### Flavour concentration

The estimate by the “Observatoire des Consommations Alimentaires” (1998) was based on maximum limits for flavourings substances in industrially prepared foods and therefore on the amount of safrole potentially added to foods. The information on the quantity of foods to which safrole can be added was provided by industry. It is assumed that a consumer consumes randomly both industrially prepared and home-made foodstuffs.

The exposure assessment was based on a selection of 28 food categories identified by industry to which safrole can be added. For these food categories, a concentration of 0.5 mg safrole/kg was assumed for food in general, a concentration of 2 mg/kg for food containing cinnamon and of 5 mg/kg for food containing nutmeg. For beverages, canned fish and chewing gum, the following concentrations were applied as specified by the Council of Europe (2001): beverages 5 mg/kg, canned fish 20 mg/kg and chewing-gum 10 mg/kg. Finally, the following correction factors were applied for the percentage of industrially prepared products. For beverages 4% of the market share will contain 5 mg/kg; for canned fish 30% of market share will contain 20 mg/kg and for chewing-gum 2% of market share will contain 10 mg/kg (Observatoire des consommations alimentaires, 1998).

### Intake estimate

Using the above assumptions the estimated average intake (for consumers only) amounts to 0.3 mg/day and the 97.5<sup>th</sup> percentile to 0.5 mg/day. It can be noted that in a previous evaluation (Council of Europe, 1995), a rough figure for estimating the intake of safrole was assumed to be 1 mg/person/day from food and spices and 1 mg/person/day from essential oils.

## **Hazard identification/characterisation**

### Absorption, distribution, metabolism and excretion

Safrole is rapidly absorbed from the gastrointestinal tract by passive absorption (Fritsch *et al.*, 1975 a,b). Extensive metabolism occurs in animals (rat, mouse, guinea-pig) (Kamienski and Casida, 1970; Oswald *et al.*, 1971; Borchest *et al.*, 1973 a,b; Stillwell *et al.*, 1974; Wislocki *et al.*, 1976; Levi *et al.*, 1977; Boberg *et al.*, 1983; Bolton *et al.*, 1994). The following metabolic transformations occur in all the investigated species, but the relative extent of each modification varies across species and strains:

- a) allylic hydroxylation to 1'-hydroxysafrole (HOS) and isomerisation to 3'-hydroxysafrole excreted in conjugated form;
- b) oxidation and cleavage of the methylene dioxy moiety, leading to 4-allyl catechol, easily oxidized to 4-allyl-o-quinone;
- c) epoxidation of the allylic side chain or, in small extent, of the aromatic ring;
- d) gamma oxidation of the allylic side chain leading to a carboxylic acid (piperonylic acid) further conjugated with glycine (piperonylglycine).

The main metabolic pathways in all animal species are a) and b). Both these pathways as well as epoxidation form reactive metabolites able to react with macromolecules. However epoxide formation is low, whereas HOS and 4-allyl catechol represent the main metabolites. HOS can be conjugated by sulphate by 3'-phosphoadenosine-5-phosphosulfate (PAPS) or acetate; both esters can be easily split producing, in the case of sulphate, an electrophilic carbonium ion. Small doses of safrole radiolabeled with <sup>14</sup>C in the 1'-position of the allylic side chain were absorbed rapidly and excreted almost completely via the urine in 24 hours in both man (0.165 mg or 1.655 mg) and rat (0.63 mg/kg). In rats treated with a higher dose (750 mg/kg) only 25% safrole was excreted in 24 hours. 1,2-Dihydroxy-4-allyl benzene was the main urinary metabolite in both species; HOS was detected in the urine of rat and not in the urine of man (Benedetti *et al.*, 1977). This does not exclude the formation of small amounts of HOS and subsequent binding to macromolecules (e.g. DNA) in the liver.

### Acute toxicity

The oral LD<sub>50</sub> was reported to be 1950 mg/kg bw for rats and 2350 mg/kg bw for mice (Jenner *et al.*, 1964).

### Short-term toxicity

Groups of young adult Osborne-Mendel rats of both sexes received safrole by oral intubation at doses of 250, 500 and 750 mg/kg bw/day up to 105 days. At doses of 750 mg/kg/d for 19 days 9/10 animals died; with 500 mg/kg/d only 1/10 animals died after 46 days; with 250 mg/kg/d no animal died within 34 days and the following effects were observed: liver hypertrophy and focal necrosis plus slight fibrosis,

fatty infiltration (steatosis), bile duct proliferation, adrenal enlargement with fatty infiltration (Hagan *et al.*, 1965).

### Long-term and Chronic toxicity

Groups of 25 male and 25 female Osborne-Mendel rats were fed 0, 100, 500, 1000 and 5000 mg/kg (0, 0.01, 0.05, 0.1 and 0.5 %) safrole in the diet for two years. The levels correspond approximately to average daily intakes of 0, 5, 25, 50, or 100 mg/kg bw/day. Changes in the liver including benign and malignant tumours (hepatic cell adenoma, hepatocholangioma, hepatic cell carcinoma and hepatocholangiocarcinoma) were observed. Reduced body weight gain was reported in both sexes at the highest dose; mild anemia and leukocytosis were also reported. The liver injury was rated as very slight at 100 mg/kg, slight at 500 mg/kg, slight to moderate at 1000 mg/kg, and moderate to severe at 5000 mg/kg. Tumour incidence was significantly increased at 5000 mg/kg (14 rats with malignant tumours, 5 with benign tumours versus 2 and 1, respectively, in the controls. Tumour incidence in the other groups was: 8 benign on 1000 mg/kg, 2 malignant and 1 benign on 500 mg/kg, and 1 benign on 100 mg/kg. (Long *et al.*, 1963).

### *Rats*

Weanling Osborne-Mendel rats were fed safrole in the diet at levels of 0 (35M & 35F), 1000 (10M & 10F), 2500 (10M & 10F), 5000 (25M & 25F) and 10000 mg/kg (10M & 10F) (0, 0.1, 0.25 and 1%) for two years. Growth was depressed in both sexes at the two highest doses and in the females at 1000 mg/kg. At 10000 mg/kg no rats survived beyond 62 weeks. These animals showed testicular atrophy, stomach atrophy and changes in the liver including tumour formation. The livers were enlarged, irregularly nodular, with single and multiple tumour masses. Microscopically, there was hepatic cell enlargement which resulted in nodule formation. The nodules tended to progress in: cystic necrosis, cirrhosis and hyperplasia leading to tumours. The liver damage was slight at 1000 mg/kg and lacked tumours and cirrhosis, moderate at 2500 but lacked cirrhosis and severe at 5000 mg/kg, where there was a statistically significant increase of malignant liver tumours. There was mild hyperplasia of the thyroid at 5000 mg/kg and an increase of chronic nephritis at lower doses (Hagan *et al.*, 1965; Hagan *et al.*, 1967).

### *Dogs*

Two males and two female dogs were given orally safrole at 5 and 20 mg/kg bw for six years. Liver changes, and no tumours were observed at both doses. At the higher dose there was liver enlargement with a nodular surface. At the lower dose the liver changes were focal necrosis, bile-duct proliferation, fatty metamorphosis, hepatic cell atrophy and leucocytic infiltration (Hagan *et al.*, 1967).

## Carcinogenicity

### *Mice*

(C57BL/6 x C3Hanf)F<sub>1</sub> or (C57BL/6 x AKR)F<sub>1</sub> hybrid mice of 7 days of age (18M and 18 F per group) were administered safrole by stomach tube for 21 days (total dose: 464 mg/kg bw), followed by dietary administration (1112 mg/kg) for 82 weeks (total dose: 1265 mg/kg bw). Liver-cell tumours were found in 11/17 (65%) males and 16/16 (100%) females and 3/17 (18%) males and 16/17 (94%) females of the two strains, respectively, versus 8/79 (11%) males and 0/87 (0%) female and 7/90 (8%) male and 1/82 (1%) female controls, respectively (Innes *et al.*, 1969).

Groups of 35-40 male CD-1 mice were fed for 13 months with a diet containing 4000 or 5000 mg safrole per kg. The study was terminated at 16 months. Hepatocellular carcinomas were found in 23/87 (26%) surviving animals versus 7/70 (10%) in the controls (Borchest *et al.*, 1973 a).

The results of a large multipart study carried out by Miller *et al.* (1983) are summarized as follows:

1. Preweanling CD-1 mice (groups of about 100 animals of both sexes) were administered 400 mg/kg of safrole ten times, twice weekly by gavage. The study was terminated at 14 months. The mice bearing hepatoma were 30/49 (61%) versus 14/59 (24%) in controls in males, and 7/48 (13%) in females versus 1/47 (2%) in controls. The lung adenomas in males were 3/49 versus 0/59 in controls and in females 3/48 versus 2/47 in controls.
2. Preweanling male CD-1 mice (groups of about 50 animals) received i.p. injections of safrole (total dose: 81 mg/kg bw) and of its metabolite 1'-hydroxysafrole (total dose: 45 mg/kg bw) distributed by four weekly injections in the first three weeks of life. The experiment was terminated at 12 months. The mice bearing hepatoma were 33/48 (67%) versus 11/42 (26%) in the controls. The lung adenomas were 7/48 (14%) versus 1/42 (2%) in controls. For 1'-hydroxysafrole the mice bearing hepatoma were 31/46 (65%) versus 11/42 (26%) in controls. The lung adenomas were 5/46 (10%) versus 1/42 (2%) in controls.
3. Groups of 50 CD-1 female mice, approximately 8 weeks old, were maintained for 12 months on grain diets containing 2300 or 4600 mg/kg of safrole. According to the authors the dietary levels correspond to an average daily intake of 150-300 and 300-600 mg/kg bw. The experiment was terminated at 19 months. The mice bearing hepatomas were at the low dose 23/34 (68%) and at the high dose 27/39 (69%) versus 0/39 in controls.
4. Groups of 40 CD-1 female mice were treated topically four days/week for 6 weeks with safrole-2',3'-oxide and 1'-hydroxysafrole-2',3'-oxide (11.2 µMol). 1 week after the last dose croton oil was applied. The experiment was terminated at 40 weeks. Skin papillomas were 14/40 (35%) in mice treated with safrole-2',3'-oxide and 33/40 (82%) in mice treated with 1'-hydroxysafrole-2',3'-oxide, versus 3/40 (7%) papillomas in acetone controls.
5. Groups of 25 female A/J mice were administered by i.p. injections safrole (total dose 3900 mg/kg bw), 1'-hydroxysafrole (total dose 2000 mg/kg bw) and 1'-hydroxysafrole-2',3'-oxide at two doses (total doses 2100 mg/kg and 4200 mg/kg bw).

The injections were given twice weekly for 12 weeks. The experiment was terminated at 8 months. Lung adenomas were found in 1/19 (5%) mice treated with safrole, in 2/21 (10%) mice treated with 1'-hydroxysafrole, and in 5/18 (28%) and 9/20 (45%) respectively at the low and high doses in mice treated with 1'-hydroxysafrole-2',3'-oxide. In uninjected controls the lung adenomas were 1/25 (4%).

Safrole was administered by gavage to pregnant B6C3F<sub>1</sub> mice starting at the 12th day of gestation by 4 doses (total dose 500 mg/kg bw) and to lactating mice from the delivery day (total dose 1500 mg/kg bw). The offspring groups were of 70-100 animals for each sex. The experiment was terminated at 92 weeks.

The mice bearing hepatoma in transplacentally treated mice were 2/63 (3.2%) in males and zero in females. In lactation treated mice the animals bearing hepatoma were 28/85 (34%) in males and 2/80 (2.5%) in females. In controls the hepatomas were 3/100 (3%) in males and absent in females. In the combined transplacental female groups 14/199 (7%) kidney epithelial tumours were found. These tumours were absent in treated males and in both male and female controls (Vesselinovitch *et al.*, 1979).

### *Rats*

In the same large study by Miller *et al.* (1983) groups of 20 male Fischer rats were administered by s.c. injection 1'-hydroxysafrole, safrole-2',3'-oxide and 1'-hydroxysafrole-2',3'-oxide (total doses about 1000, 1100 and 1200 mg/kg bw) twice weekly for 10 weeks. The experiment was terminated at 24 months.

Rats treated with 1'-hydroxysafrole developed 11/20 (55%) hepatic carcinomas, whereas no hepatic carcinomas occurred in rats injected with safrole-2',3'-oxide or 1'-hydroxysafrole-2',3'-oxide. No tumour was seen in controls injected with trioctanoin only. Whereas 1'-hydroxysafrole did not induce sarcomas at the injection site, one sarcoma was induced by safrole-2',3'-oxide and four by 1'-hydroxysafrole-2',3'-oxide.

Groups of 15 male CD rats were fed with 5000 mg/kg safrole for 18 months. One group was administered phenobarbital in drinking water (0,1%). The experiment was terminated at 22 months. The rats bearing hepatomas were 3/15 (20%) in safrole treated rats, and 12/15 (80%) in rats treated with safrole and phenobarbital. In the controls no hepatomas were found (Wislocki *et al.*, 1977).

### Genotoxicity

#### *In vitro*

Safrole was generally negative or weakly positive in the *Salmonella* reverse mutation assay (Ames test) (Green and Savage, 1978; Swanson *et al.*, 1979; Baker and Bonin, 1985). 1'-hydroxysafrole was directly mutagenic for strain TA100; its mutagenicity was increased by supplementation with NADPH-fortified rat liver microsomes and cytosol (Swanson *et al.*, 1979). Other possible metabolites such as 1'-acetoxysafrole, safrole-2',3'-oxide, 1'-acetoxysafrole and 1'-oxosafrole are directly mutagenic in strain TA100 and also, except 1'-acetoxysafrole, in strain TA1535 (Wislocki *et al.*, 1977). Safrole was positive in *Escherichia coli* and *Saccharomyces cerevisiae* (Poirier and de Serres, 1979) and in a cell transformation assay (Purchase, 1978). Safrole was positive in various *in vitro* mammalian



cell genotoxicity assays such as chromosomal aberrations (Ishidate & Sofuni, 1985), gene mutations (Mihir *et al.*, 1985) and sister chromatid exchanges (SCEs) (Bradley, 1985). It induced unscheduled DNA synthesis (UDS) in cultured rat hepatocytes (Howes *et al.*, 1990), but not in HeLa cells (Martin *et al.*, 1978). It induced DNA damage (single-strand breaks) in cultured rat hepatocytes (Bradley, 1985).

#### *In vivo*

Safrole was positive in the *in vivo* i.p. host-mediated assay with *S. typhimurium* strain TA1535 or *S. cerevisiae* (Poirier and de Serres, 1979; Green & Savage, 1978).

Safrole was found negative in a bone-marrow micronucleus assay (Gocke *et al.*, 1981), in an *in vivo* rat liver UDS (Mirsalis *et al.*, 1982) and in a mouse dominant lethal assay (Epstein *et al.*, 1972). More recently (Daimon *et al.*, 1998) it has been shown that safrole is able to induce chromosome aberrations, SCEs and DNA adducts in hepatocytes of F344 rats exposed *in vivo*. Five repeated doses of 125 and 250 mg/kg bw induced dose-dependent increase of aberrant cells in the liver, with a maximum frequency of 13.4 % compared with the control value of 1.8%. A dose-dependent induction of SCEs in the liver was observed after a single dose of safrole at doses of 10-500 mg/kg bw.

#### *DNA adduct formation*

DNA adducts (two major and two minor) were detected in rat liver DNA after single doses of safrole at 1 or 100 mg/kg bw. These results suggest that the cytogenetic effects may result from covalent DNA modification in the rat liver (Daimon *et al.*, 1998).

Previous studies (Phillips *et al.*, 1981, 1984; Randerath *et al.*, 1984; Wiseman *et al.*, 1985; Lu *et al.*, 1986b) have shown that two major DNA adducts are formed in N<sup>2</sup> position of guanine after administration of safrole or 1'-hydroxysafrole to mice; minor reaction occurs with N<sup>6</sup> of adenine. The covalent binding index (CBI) value was about 30 for safrole, consistent with its weak hepatocarcinogenic activity. However, it has been shown (Gupta *et al.*, 1993) that DNA adduct formation in mouse liver was linear over a dose span of 10000-fold down to the lowest dose range, i.e. 1-10 µg (6-60 nmol) safrole/mouse, with no indication of a threshold; moreover the persistence of DNA adducts was rather long (up to 140 days in the liver of treated mice) and independent from their levels. For the high dose (10 mg/mouse), a slight deviation from linearity was observed (hepatotoxic effect with regenerative hyperplasia in liver tissue).

In another study safrole-DNA adducts, and to a greater extent, myristicine-DNA adducts were identified in livers of mice given cola beverages instead of drinking water (Randerath *et al.*, 1993).

Inhibition of both DNA adduct formation and carcinogenicity of 1'-hydroxysafrole was shown in the liver of mice deficient in the synthesis of PAPS or treated with pentachlorophenol, a strong inhibitor of sulfotransferase and PAPS formation (Boberg *et al.*, 1983).

In rodents, transplacental passage of the reactive metabolites of safrole was demonstrated by the presence of liver DNA adducts in the fetuses of mothers dosed with safrole (Randerath *et al.*, 1989).

The effects of pregnancy on the covalent binding of several carcinogens to DNA were investigated in ICR mice. Pregnancy lowered the binding of the ultimate carcinogenic

metabolite of B(a)P and increased 2.3 and 2.5 fold the binding of safrole and 1'-hydroxysafrole to liver and kidney DNA (Lu *et al.*, 1986a).

#### Reproductive and Developmental toxicity

No information was found.

#### Human data

No information was found.

#### Summary of the hazard identification/characterisation

Safrole produces liver tumours in mice and rats by oral administration; safrole also produces liver and lung tumours in male infant mice following its subcutaneous injection. The carcinogenic potency appears to be relatively low and dependent on the metabolism. Mice appear to be more susceptible than rats to the carcinogenic effect of safrole. Safrole is metabolically activated through the formation of intermediates able to directly react with DNA. Safrole is genotoxic in various *in vitro* mammalian cell systems causing induction of gene mutations, chromosomal aberrations, UDS and SCE. Several metabolites of safrole are directly mutagenic in *Salmonella*. *In vivo*, safrole was able to induce chromosome aberrations, SCE and DNA adducts in the liver of rats.

#### **Conclusion**

Safrole has been demonstrated to be genotoxic and carcinogenic. Therefore the existence of a threshold cannot be assumed and the Committee could not establish a safe exposure limit. Consequently, reductions in the exposure and restrictions in the use levels are indicated.

#### **References**

- Baker, R.S.U. and Bonin, A.M., 1985. Tests with *Salmonella* plate incorporation assay. In: Ashby and de Serres (eds.) Evaluation of Short-term Tests for Carcinogens. *Elsevier*, N.Y., 177-180.
- Benedetti, M.S., Malnoe, A. and Broillet, A.L., 1977. Absorption, metabolism and excretion of safrole in the rat and man. *Toxicology*, **7**, 69-83.
- Bolton, J.L., Acay, N.M. and Vukomanovic, V., 1994. Evidence that 4-allyl-O-quinones spontaneously rearrange to their more electrophilic quinone methides: potential bio-activation mechanism for the hepatocarcinogen safrole. *Chem Res. Toxicol.*, **7**, 443-450.
- Boberg, E.W., Miller, E.C., Miller, J.A., Poland, A. and Liem, A., 1983. Strong evidence from studies with brachimorphic mice and pentachlorophenol that 1'-sulphoöxysafrole is

the major ultimate electrophilic and carcinogenic metabolite of 1'-hydroxysafrole in mouse liver. *Cancer Res.*, **43**, 5163-5173.

Borchest, P., Miller, J.A., P.G., Miller, E.C. and Shires, T.K., 1973 a. 1'-hydroxysafrole, a proximate carcinogenic metabolite of safrole in the rat and mouse. *Cancer Res.*, **33**, 590-600.

Borchest, P., Wislocki, P.G., Miller, J.A. and Miller, E.C., 1973 b. The metabolism of the naturally occurring hepatocarcinogen safrole to 1'-hydroxysafrole and the electrophilic reactivity of 1'-acetoxysafrole. *Cancer Res.*, **33**, 575-589.

Bradley, M.O, 1985. Measurement of DNA single-strand breaks by alkaline elution in rat hepatocytes. In: Ashby and de Serres (eds). Evaluation of Short-term Tests for Carcinogens. *Elsevier*, N.Y., 353-357.

Council of Europe - Committee of Experts on Flavouring Substances 1995. Revised overview on occurrence of Alkenylbenzenes. Document RD 2.1/2-37 submitted by the Swiss delegation for the 37th meeting in Strasbourg, October 1995.

Council of Europe 1997. Committee of Experts on Flavouring Substances 41st meeting – RD 4.2/10-41. Revised datasheet on safrole.

Council of Europe, Committee of Experts on Flavouring Substances, 2001. Note on method used to calculate intake estimates. Document RD 4.11/1-48 submitted by the UK delegation for the 48th meeting in Strasbourg, 2-6 April 2001.

Council of Europe, 1992. Flavouring Substances and Natural Source of Flavourings 4th Edition.

Daimon, H., Sawada, S., Asakura, S. and Sagani, F., 1998. *In vivo* genotoxicity and DNA adduct levels in the liver of rats treated with safrole. *Carcinogenesis*, **19**, 141-146.

EEC 1988. Council Directive 88/388/EEC of 21 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production. *Official Journal of the European Communities*, 15.7.1988, L184/61-67.

Epstein, S.S., Arnold, E., Andrea, J., Bass, W. and Bishop, Y., 1972. Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol Appl. Pharmacol.*, **23**, 288-325.

Fritsch, P., de Saint Blanquet, G. and Derache, R., 1975a. Gastrointestinal absorption in the rat of anisole, trans-anethole, butyl-hydroxyanisole and safrole. *Food Cosmet. Toxicol.*, **13**, 359-363.

Fritsch, p., Lamboeuf, Y. and de Saint Blanquet, G., 1975b. Effect of anisole, anethole, butyl-hydroxyanisole and safrole on intestinal absorption in the rat. *Toxicology*, **4**, 341-346.

- Gocke, E., King, M.T., Eckardt, K. and Wild, D., 1981. Mutagenicity of cosmetics ingredients licensed by the European Community. *Mutat. Res.*, **90**, 91-109.
- Green, N.R. and Savage, J.R., 1978. Screening of safrole, eugenol, their ninhydrin positive metabolites and selected secondary amines for potential mutagenicity. *Mutat. Res.*, **57**, 115-121.
- Gupta, K.P., Van Golen, K.L., Putman, K.L. and Randerath, K., 1993. Formation and persistence of safrole-DNA adducts over a 10000-fold dose range in mouse liver. *Carcinogenesis*, **14**, 1517-1521.
- Hagan, E.C., Hansen, W.H., Fitzhugh, O.G., Jenner, P.M., Jones, W.I., Taylor, J.M., Long, E.L., Nelson, A.A. and Brouwer, J.B., 1967. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. *Food Cosm. Toxicol.*, **5**, 141-157.
- Hagan, E.C., Jenner, P.M., Jones, W.I., Fitzhugh, O.G., Brouwer, J.G. and Webb, W.K., 1965. Toxic properties of compounds related to safrole. *Toxicol. Appl. Pharmacol.*, **7**, 18-24.
- Howes, J.A., Chan, V.S.W. and Caldwell, J., 1990. Structure-specificity of the genotoxicity of some naturally occurring alkenyl-benzenes determined by the unscheduled DNA synthesis assays in rat hepatocytes. *Food Chem. Toxicol.*, **28**, 537-542.
- Innes, J.R., Ulland, B.M., Valerio, M.G., Petrucelli, L., Fishbein, L., Hast, E.R., Pallota, A.J., Bates, R.R., Falk, H.L., Gart, L.L., Klein, M., Mitchell, I. and Peter, J., 1969. Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. *J. Natl. Cancer Inst.*, **42**, 1101-1114.
- IARC Monographs, Vol. 10, 1976
- IARC Monographs, Suppl. 7, 1987
- Ishidate, M. and Sofuni, T. 1985. The *in vitro* chromosomal aberration test using Chinese hamster lung (CHL) fibroblast cells in culture. In: Ashby and de Serres (eds). Evaluation of Short-term Tests for carcinogens. *Elsevier*, N.Y., 427-432.
- Jenner, P.M., Hagan, E.C., Taylor, J.M., Cook, E.L. and Fitzhugh, O.G., 1964. Food flavourings and compounds of related structure. I. Acute oral toxicity. *Food Cosm. Toxicol.*, **2**, 327-343.
- Kamienski, F.X. and Casida, J.E., 1970. Importance of demethylenation in the metabolism *in vivo* and *in vitro* of methylene-dioxyphenyl synergists and related compounds in mammals. *Biochem. Pharmacol.*, **19**, 91-112.
- Levi, P., Janiaud, P., Delaforge, M., Morizot, J.P., Maume, B.F. and Padieu, P., 1977. The occurrence of glucuroconjugated metabolites of safrole in the urine of treated rats. *C.R. Séances Soc. Biol.*, **171**, 1034-1040.

- Long, E.L., Nelson, A.A., Fitzhugh, O.G. and Hansen, W.H., 1963. Liver tumours produced in rats by feeding safrole. *Arch. Pathol.*, **75**, 595-604.
- Lu, L.J., Disher, R.M. and Randerath, K., 1986a. Differences in the covalent binding of benzo(a)pyrene, safrole, 1'-hydroxysafrole and 4-aminobiphenyl to DNA of pregnant and non-pregnant mice. *Cancer Letters*, **31**, 43-52.
- Lu, L.J., Disher, R.M., Reddy, M.V. and Randerath, K., 1986b. <sup>32</sup>P-Post-labelling assay in mice of transplacental DNA damage induced by the environmental carcinogens safrole, 4-aminobiphenyl, and benzo(a)pyrene. *Cancer Res.*, **46**, 3046-3054.
- Martin, C.N., Mc Dermid, A.C. and Garner, R.L., 1978. Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells. *Cancer Res.*, **38**, 2621-2627.
- Mihr, B., Bowers, L. and Caspary, W.J., 1985. Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture. In: Ashby and de Serres (eds.). Evaluation of Short-term Tests for Carcinogens. *Elsevier*, N.Y., 555-568.
- Miller, C., Swanson, A.B., Phillips, D.H., Fletcher, T.L., Liem, A. and Miller, J.A., 1983. Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. *Cancer Res.*, **43**, 1124-1134.
- Mirsalis, J.C., Tyson, K., and Butterworth, B.E., 1982. Detection of genotoxic carcinogens in the *in vivo* – *in vitro* hepatocyte DNA repair assay. *Environ. Mutagen*, **4**, 553-562.
- Observatoire des Consommations Alimentaires, 1998. Estimation des niveaux d'ingestion de substances aromatisantes Safrole, Estragole, Coumarine et Capsaïcine. Note technique N° 98.25, Paris
- Oswald, E.O., Fishbein, L., Corbett, B. and Walker, M.P., 1971. Identification of tertiary aminomethylenedioxypropiofenones as urinary metabolites of safrole in the rat and guinea pig. *Biochim. Biophys. Acta*, **230**, 237-247.
- Phillips, D., Reddy, M.V. and Randerath, K. 1984. <sup>32</sup>P- Postlabelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. II. Newborn male B6C3F<sub>1</sub> mice. *Carcinogenesis*, **5**, 1623-1628.
- Phillips, D.H., Miller, J.A., Miller, E.C. and Adams, B., 1981. N<sup>2</sup> atom of guanine and N<sup>6</sup> of adenine residues at sites for covalent binding of metabolically activated 1'-hydroxysafrole to mouse liver DNA *in vivo*. *Cancer Res.*, **41**, 2624-2671.
- Poirier, L.A. and de Serres, F. J., 1979. Initial National Cancer Institute studies on mutagenesis as a pre-screen for chemical carcinogens: an appraisal. *J. Natl. Cancer Inst.*, **62**, 919-926.

- Purchase, I.F.H., 1978. An evaluation of 6 Short-term tests for detecting organic chemical carcinogens. *Br. J. Cancer*, **37**, 873-903.
- Randerath, K., Haglund R.E., Phillips D.H. and Reddy, M.V., 1984. <sup>32</sup>P- Postlabelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. I. Adult female CD-1 mice. *Carcinogenesis*, **5**, 1613-1622.
- Randerath, K.P., Liehr, J.G. and Gladeck, A., 1989. Use of <sup>32</sup>P-Postlabelling technique assay to study transplacental carcinogens and transplacental carcinogenesis. In: *IARC Sci. Publ. Vol. 96*, 189-205.
- Randerath, K.P., Putman, K.L. and Randerath, E., 1993. Flavor constituents in cola drinks induce hepatic DNA adducts in adult and fetal mice. *Biochem. Biophys. Res. Commun.*, **192**(1), 61-68.
- Scientific Committee for Food, European Commission, 1979. Report of the Scientific Committee for Food on Flavourings (opinion expressed on 21 September 1979). *Reports of the Scientific Committee for Food (9<sup>th</sup> Series)*, Office for Official Publications of the European Communities, Luxembourg, October 1979.
- Stillwell, W.G., Carman, M.J., Bell, L. and Horning, M.G., 1974. The metabolism of safrole and 2',3'-epoxysafrole in the rat and guinea pig. *Drug Metabol. Dispos.*, **2**, 489-498.
- Swanson, A.B., Chambliss D.D., Blanquist J.C., Miller, E.C. and Miller, J.A., 1979. The mutagenicities of safrole, estragole, trans-anethole and some of their known or possible metabolites for *Salmonella thyphimurium* mutants. *Mutat. Res.*, **60**, 143-153.
- Taylor, J.M., Jenner, P.M. and Jones, W.I., 1964. A comparison of the toxicity of some allyl, propenyl and propyl compounds in the rats. *Toxicol. Appl. Pharmacol.*, **6**, 378-387.
- Vesselinovitch, S.D., Rao, K.V. and Mihailovich, N., 1979. Transplacental and lactational carcinogenesis by safrole. *Cancer Res.*, **39**, 4378-4380.
- WHO, 1981. Evaluation of certain food additives. Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No.16
- Wiseman, R.W., Fennell, T.R., Miller, J.A. and Miller, E.C., 1985. Further characterization of the DNA adducts formed by electrophilic esters of the hepatocarcinogens 1'-hydroxysafrole and 1'-hydroxyestragole *in vitro* and in the mouse liver *in vivo*, including new adducts at C-8 and N-7 of guanine residues. *Cancer Res.*, **45**, 3096-3105.
- Wislocki, P.G., Borchester, P., Miller, J.A. and Miller, E. C., 1976. The metabolic activation of the carcinogen 1'-hydroxysafrole *in vivo* and *in vitro* and the electrophilic reactivities of possible ultimate carcinogens. *Cancer Res.*, **36**, 1686-1695.

Wislocki, P.G. Miller, E.C., Miller, J.A., McCoy, E.C. and Rosenkranz, H.S., 1977. Carcinogenic and mutagenic activities of safrole, 1'-hydroxysafrole and some known or possible metabolites. *Cancer Res.*, **37**, 1883-1891.