

**www.cropwatch.org**



THE FIRST TRULY INDEPENDENT WATCHDOG FOR THOSE  
WORKING WITH NATURAL AROMATIC MATERIALS

E: [info@cropwatch.org](mailto:info@cropwatch.org) T: ++44 (0)7771 872 521

---

## **Safrole & Safrole-Containing Essential oils - a Bibliography.**

Compiled by Cropwatch v1.04 Nov. 2009.  
[To be continually updated].

### **Contents**

**Safrole: General**  
**Safrole: Oral Carcinogenicity – Betel & Areca Quid Chewing etc.**  
**Safrole: Carcinogenicity: General**  
***Cinnamomum* spp. & Safrole**  
**Mango Ginger (*Curcuma amada* Roxb.) & Safrole**  
**Nutmeg (*Myristica fragrans* Houtt.) oil & Safrole**  
***Piper* spp. & Safrole**  
**Sassafras oils (*Sassafras albidum* (Nutt.) Nees & *Ocotea pretosia*) & Safrole.**  
**Isosafrole**  
**Dihydrosafrole.**

[N.B. Certain publications may appear in more than one section].

### **Cropwatch Comments:**

Safrole, a constituent of a number of essential oils, is currently classified as a carcinogen category 2 and mutagen category 3 in the IFRA-IOFI labelling manual 2009/ EFTA CoP 2009 (Safrole: Hazard symbol: T; Risk phrases R45-22-68). A review of the political & scientific situation surrounding safrole's ingredient status in food, herbal products, essential oils and flavourings, is available in the *Cropwatch Files* section of the Cropwatch website under the working title "Safrole: Human Carcinogenicity Risk Over-Stated?".

### **Safrole General.**

Abbott D. D. *et al.* (1961) "Chronic oral toxicity of oil of sassafras and safrole." *Pharmacologist* **3**, 62.

Association Francaise de Normalisation, France (1986). "Essential oils of sassafras and nutmeg. Determination of contents of safrole and cis- and trans-isosafrole. Packed column and capillary column gas chromatographic methods." *French-Standard NF T 75-407*.

Choong Y.-M. & Lin H.-J. (2001) "A Rapid and Simple Gas Chromatographic Method for Direct Determination of Safrole in Soft Drinks." *Journal of Food and Drug Analysis* **9**(1), 27-32. [Abstract](#). A simple and rapid method was developed to determine the safrole and isosafrole in soft drink using a megapore semi-polar column (CP-SIL 8CB, 30 m × 0.53 mm, 1.5 µm) with direct injection gas chromatography. Direct quantitative analysis of safrole and isosafrole in soft drinks was carried out without any sample pretreatment procedure. The water soluble compound 1,4-dihydroxybenzene (DHB) was used as the internal standard. The detection limit for safrole and isosafrole was 0.25 µg/mL. A recovery study was performed using one of the soft drinks by spiking 1 mL with safrole and isosafrole at 5.0 and 10.0 µg, respectively. The recovery was found in the range of 98-108% with coefficients of variation being less than 8.7%. Twenty-five commercial soft drinks were analyzed by the current method, and results indicated that 20 out of 25 soft drink samples contained safrole and/or isosafrole, and the amount of safrole was 3-5 fold over the regulated amount, 1 µg/mL. .

Fritsch, P., de Saint Blanquat, G. & Derache, R. (1975) "Absorption gastro-intestinale, chez le: Rat, de l'anisole, du transanethole, du butylhydroxyanisole et du safrole." *Fd Cosmet. Toxicol.* **13**, 359-363.

Fritsch P., Lamboeuf Y. & de Saint Blanquat G. (1975) "Effet de l'anisole, de l'anethole, du butylhydroxyanisole et du safrole sur l'absorption intestinale chez le rat." *Toxicology* **4**, 341-346.

Gimeno P, Besacier F, Bottex M, Dujourdy L, Chaudron-Thozet H. (2005) "A study of impurities in intermediates and 3,4-methylenedioxymethamphetamine (MDMA) samples produced via reductive amination routes." *Forensic Sci International* **155**(2-3), 141-57. [Abstract](#). Impurities found in various sources of precursors (sassafras oil, safrol, isosafrol, piperonal), intermediates (beta-nitroisosafrol, piperonylmethylketone (PMK)) and final product (3,4-methylenedioxymethamphetamine (MDMA)) are presented and discussed. Particular attention is paid to the chemical origin of each impurity found in the prepared samples. Impurity profiles of isosafrol, piperonal, and PMK samples obtained from industrial sources or from sassafras oil were first compared. Then PMK samples produced from isosafrol through isosafrol glycol or through beta-nitroisosafrol were compared. At last, attention was paid to the reductive amination of PMK to MDMA using different reductive agents. Possible use of this profiling method to determine the synthesis route is discussed for all products.

Gold L.S., Slone T.H., Stern B.R., Manley N.B., & Ames BN (1992) "Rodent carcinogens: setting priorities." *Science* **258**(5080),261-265. [Abstract](#). The human diet contains an enormous background of natural chemicals, such as plant pesticides and the products of cooking, that have not been a focus of carcinogenicity testing. A broadened perspective that includes these natural chemicals is necessary. A comparison of possible hazards for 80 daily exposures to rodent carcinogens from a variety of sources is presented, using an index (HERP) that relates human exposure to carcinogenic potency in rodents. A

similar ordering would be expected with the use of standard risk assessment methodology for the same human exposure values. Results indicate that, when viewed against the large background of naturally occurring carcinogens in typical portions of common foods, the residues of synthetic pesticides or environmental pollutants rank low. A similar result is obtained in a separate comparison of 32 average daily exposures to natural pesticides and synthetic pesticide residues in the diet. Although the findings do not indicate that these natural dietary carcinogens are important in human cancer, they cast doubt on the relative importance for human cancer of low-dose exposures to synthetic chemicals.

ISO 7355 (1985) (Now withdrawn). "Oils of sassafras and nutmeg -- Determination of safrole and cis- and trans-isosafrole content -- Gas chromatographic method on packed columns." [Abstract](#). A small quantity of the oils is analyzed by gas chromatography under specified conditions on a packed column. The safrole and cis- and trans-isosafrole contents are determined by using the internal standard method. As stationary phase are used polydimethylsiloxane, or polyethylene glycol 20000. Operating temperatures: Oven: isotherm about 100 °C when using polydimethylsiloxane; isotherm about 125 °C when using polyethylene glycol 20000. Injection system and detector: about 170 °C.

Jelen H. & Kaminski E. (1994). "Determination of safrole and myristicin in essential oils by GC/MS-SIM." *Bromatologia Chemia Toksykologiczna*. **27**, 269-274.

Maia J.G., Green C.L. & Milchard M.J. (1993) "New sources of natural safrole." *Perfumer & Flavorist* **18**(2), 19-22.

MAFF (1996) Food Surveillance Paper No. 48, Flavourings in Food, London, HMSO.

Opdyke, D. L. J. (1974) "Fragrance raw materials monographs: Safrole." *Fd. Cosmet. Toxicol.*, **12** (Suppl.), 983-986.

SCF (1979). Report of the Scientific Committee for Food on Flavourings (opinion expressed on 21 September 1979). *Reports of the Scientific Committee for Food (9th Series)*, Office for Official Publications of the European Communities, Luxembourg, October 1979.

SCF (2001). 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, 12<sup>th</sup> December 2001.

### **Safrole Oral Carcinogenicity: Betel & Areca Quid Chewing etc.**

Chang M.J.W., Ko C.Y., Lin R.F. & Hsieh L.L. (2002) "Biological monitoring of environment exposure to safrole and the Taiwanese betel quid chewing." *Arch. Environ. Contam. Toxicol.* **43**, 432–437. [Abstract](#). A rapid and sensitive biological monitoring (BM) method for assessing exposure to the environmental carcinogen safrole has been developed. The method is an isocratic high performance liquid

chromatographic (HPLC) analysis of urinary dihydroxychavicol (DHAB) and eugenol, the urinary metabolites of safrole. Good linearity, precision, and accuracy were demonstrated. A recovery of  $98.8 \pm 5.4\%$  (SD,  $n = 3$ ) was found for DHAB and  $84.1 \pm 3.4\%$  ( $n = 3$ ) for eugenol. The quantitation limits of the method were 8 ng for DHAB and 10 ng for eugenol. The validity of the method was demonstrated by a linear dose-response relationship observed in rats given oral doses of safrole at 30, 75, and 150 mg/kg body weight. The method was also used to monitor the environmental exposure to the Taiwanese betel quid (TBQ) chewing, because TBQ used in Taiwan not only contains areca (betel) nut, slaked lime, and catechu but also Piper betle inflorescence or its leaves. Both of the latter have a high content of safrole. The feasibility of the method to monitor TBQ chewing was demonstrated by an analysis of 153 spot human urine samples. The results showed that the p value of the nonparametric group comparison was  $< 0.001$  for DHAB and 0.832 for eugenol. The TBQ chewers also exhibited a significantly higher rate of urinary DHAB (but not eugenol) than the nonchewers with an odd ratio of 3.47 (95% CI, 1.61–7.51). However, when only the eugenol-positive subjects were taken into analysis, the ratio rose to 24.38 (95% CI, 3.00–197.90).

Chen C.-L., Chi C.-W., Chang K.-W., & Liu T.-Y. (1999) "Safrole-like DNA adducts in oral tissue from oral cancer patients with a betel quid chewing history." *Carcinogenesis* **20**(12), 2331 – 2334.

Chung Y.T., Chen C.L., Wu C.C., Chan S.A., Chi C.W. & Liu T.Y. (2008) "Safrole-DNA adduct in hepatocellular carcinoma associated with betel quid chewing." *Toxicol Lett.* **183**(1-3), 21-7. [Abstract](#). Betel quid chewing, which contributes high concentration of safrole in saliva, is a popular oral habit in Taiwan. Safrole is a documented rodent hepatocarcinogen, yet its hepatocarcinogenic potential in human is not known. Here, we used LC/ESI-ITMS(n) and LC/QTOF-MS confirmed safrole-dGMP as reference standard to detect the safrole-DNA adduct in hepatic tissues from HBsAg-/HCV-seronegative hepatocellular carcinoma patients by (32)P-postlabeling. We first synthesized and confirmed safrole-dGMP by LC/MS. Two isomeric safrole-dGMPs were characterized as N(2)-(trans-isosafrol-3'-yl) deoxyguanosine and N(2)-(safrol-1'-yl) deoxyguanosine. This technique was able to detect hepatic safrole-DNA adduct in mice that were treated with safrole but not sensitive enough to detect safrole-DNA adduct in human samples. Using the nuclease P1 version of the (32)P-postlabeling technique, we detected the presence of safrole-DNA adduct in two out of 28 hepatic tissues from hepatocellular carcinoma patients, and only these two patients had a history of betel quid chewing lasting more than 10 years. From co-chromatography with the mass confirmed safrole-dGMPs, this safrole-DNA adduct was identified as N(2)-(trans-isosafrol-3'-yl) deoxyguanosine. These results suggest that betel quid-containing safrole might be involved in the pathogenesis of hepatocellular carcinoma in human beings and LC/MS has the potential to identify DNA adducts in clinical samples.

Chung Y.T., Hsieh L.L., Chen I.H., Liao C.T., Liou S.H., Chi C.W., Ueng Y.F. & Liu T.Y. (2009) "Sulfotransferase 1A1 haplotypes associated with oral squamous cell carcinoma susceptibility in male Taiwanese." *Carcinogenesis*. **30**(2), 286-94. [Abstract](#). We have previously demonstrated that betel quid containing safrole induced DNA adducts are highly associated with the development of oral squamous cell carcinoma (OSCC) in Taiwan. Sulfotransferase (SULT) is essential for the formation of these adducts. To elucidate the effects of SULT1A1 haplotypes on OSCC susceptibility, 160 male OSCC cases and 218 age- and sex-matched controls were screened for single-nucleotide polymorphisms within the coding region of SULT1A1 by sequencing. We found that 445C>T (His149Tyr) and 507C>T polymorphisms were significantly associated with increased risk of OSCC. Based on the genotype analysis, haplotypes were constructed for 445C>T (His149Tyr), 507C>T, 600G>C and 638G>A (Arg213His) using GENECOUNTING software. After adjustment for age, cigarette smoking and betel quid chewing, we found that haplotype c containing 445C>T (His149Tyr), 507C>T or 600G>C but not 638G>A (Arg213His) variant was significantly associated with increased risk of OSCC (odds ratio, 3.24; 95% confidence interval, 1.57-6.68) when compared with the haplotype a (wild-type). We analyzed the activity in sulfonation of 2-naphthol and 1'-hydroxysafrole of recombinant His149Tyr (445C>T) variant, which led to 51 and 33% reduced activity, respectively; Arg213His (638G>A) variant led to 72 and 54% reduced activity, respectively, when compared with the wild-type. Taken together, haplotype analysis provides a novel evaluation of the SULT1A1 gene as a risk modifier on environmental carcinogen in OSCC and the association of SULT1A1 haplotypes with the risk of OSCC might be modified by betel quid chewing.

Dunham L.J., Herrold K. M. (1962) "Failure to produce tumors in the hamster cheek pouch by exposure to ingredients of betel quid; histopathologic changes in the pouch and other organs by exposure to known carcinogens." *J. Natl. Cancer Inst.* **29**, 1047

Hsieh L.L., Wang P.F., Chen I.H., Liao C.T., Wang H.M., Chen M.C., Chang J.T. & Cheng A.J. (2001) "Characteristics of mutations in the p53 gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese." *Carcinogenesis*. **22**(9):1497-503. [Abstract](#) p53 mutations are etiologically associated with the development of oral squamous cell carcinomas (OSCCs) or are associated with exposure to specific carcinogens. In this study, we used PCR-single strand conformation polymorphism and DNA sequencing to analyze the conserved regions of the p53 gene (exons 5-9) in OSCC tumor specimens from 187 patients with varied histories of betel quid, tobacco and alcohol use. Ninety-one of the 187 OSCCs (48.66%) showed p53 gene mutations at exons 5-9. The incidence of p53 mutations was not associated with age, sex, TNM stage, status of cigarette smoking or betel quid chewing. However, alcohol drinkers exhibited a significantly higher incidence (57/101, 56.44%) of p53 mutations than non-users (39/86) (P = 0.02). The effect of alcohol on the incidence of p53 mutations was still statistically significant (RR = 2.24; 95% CI, 1.21-4.15) after adjustment for cigarette smoking and betel quid

(BQ) chewing. G:C to A:T transitions were the predominant mutations observed and associated with BQ and tobacco use. Alcohol drinking could enhance these transitions. After adjustment for cigarette smoking and BQ chewing, alcohol drinking still showed an independent effect on G:C to A:T transitions (RR = 2.41; 95% CI, 1.01-5.74). These findings strongly suggest an important contributive role of tobacco carcinogens to p53 mutation in this series of Taiwanese OSCCs and alcohol might enhance these mutagenic effects. As safrole-DNA adducts have been detected in 77% (23/30) of the OSCC tissues from Taiwanese oral cancer patients with a BQ chewing history, we cannot rule out the possibility that safrole or other carcinogens present in the BQ may cause a similar pattern of mutagenesis. Determination of the role of safrole and other carcinogens present in BQ on the pattern of p53 gene mutation in OSCC will require further study.

Huang J.K., Huang C.J., Chen W.C., Liu S.I., Hsu S.S., Chang H.T., Tseng L.L., Chou C.T., Chang C.H. & Jan CR. (2005) "Independent  $[Ca^{2+}]_i$  increases and cell proliferation induced by the carcinogen safrole in human oral cancer cells." *Naunyn Schmiedebergs Arch Pharmacol.* **372**(1), 88-94. [Abstract](#). The effect of the carcinogen safrole on intracellular  $Ca^{2+}$  movement and cell proliferation has not been explored previously. The present study examined whether safrole could alter  $Ca^{2+}$  handling and growth in human oral cancer OC2 cells. Cytosolic free  $Ca^{2+}$  levels ( $[Ca^{2+}]_i$ ) in populations of cells were measured using fura-2 as a fluorescent  $Ca^{2+}$  probe. Safrole at a concentration of 325 microM started to increase  $[Ca^{2+}]_i$  in a concentration-dependent manner. The  $Ca^{2+}$  signal was reduced by 40% by removing extracellular  $Ca^{2+}$ , and was decreased by 39% by nifedipine but not by verapamil or diltiazem. In  $Ca^{2+}$ -free medium, after pretreatment with 650 microM safrole, 1 microM thapsigargin (an endoplasmic reticulum  $Ca^{2+}$  pump inhibitor) barely induced a  $[Ca^{2+}]_i$  rise; in contrast, addition of safrole after thapsigargin treatment induced a small  $[Ca^{2+}]_i$  rise. Neither inhibition of phospholipase C with 2 microM U73122 nor modulation of protein kinase C activity affected safrole-induced  $Ca^{2+}$  release. Overnight incubation with 1 microM safrole did not alter cell proliferation, but incubation with 10-1000 microM safrole increased cell proliferation by 60+/-10%. This increase was not reversed by pre-chelating  $Ca^{2+}$  with 10 microM of the  $Ca^{2+}$  chelator BAPTA. Collectively, the data suggest that in human oral cancer cells, safrole induced a  $[Ca^{2+}]_i$  rise by causing release of stored  $Ca^{2+}$  from the endoplasmic reticulum in a phospholipase C- and protein kinase C-independent fashion and by inducing  $Ca^{2+}$  influx via nifedipine-sensitive  $Ca^{2+}$  entry. Furthermore, safrole can enhance cell growth in a  $Ca^{2+}$ -independent manner.

Ko Y.C., Huang Y.L., Lee C.H, Chen M.J, Lin L.M., Tsai C.C. (1995) "Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan." *J Oral Pathol Med* **24**, 450-3.

Lee J.M., Liu T.Y., Wu D.C., Tang H.C., Leh J., Wu M.T., Hsu H.H., Huang P.M., Chen J.S., Lee C.J. & Lee Y.C. (2005). "Safrole-DNA adducts in tissues from esophageal cancer patients: clues to areca-related esophageal carcinogenesis." *Mutat Res.* **565**(2), 121-8. [Abstract](#). Epidemiological studies have demonstrated

that areca quid chewing can be an independent risk factor for developing esophageal cancer. However, no studies are available to elucidate the mechanisms of how areca induces carcinogenesis in the esophagus. Since the areca nut in Taiwan contains a high concentration of safrole, a well-known carcinogenic agent, we analyzed safrole-DNA adducts by the <sup>32</sup>P-postlabelling method in tissue specimens from esophageal cancer patients. In total, we evaluated 47 patients with esophageal cancer (16 areca chewers and 31 non-chewers) who underwent esophagectomy at the National Taiwan University Hospital between 1996 and 2002. Of the individuals with a history of habitual areca chewing (14 cigarette smokers and two non-smokers), one of the tumor tissue samples and five of the normal esophageal mucosa samples were positive for safrole-DNA adducts. All patients positive for safrole-DNA adducts were also cigarette smokers. Such adducts could not be found in patients who did not chew areca, irrespective of their habits of alcohol consumption or cigarette smoking ( $p < 0.001$ , comparing the areca chewers with non-chewers). The genotoxicity of safrole was also tested in vitro in three esophageal cell lines and four cultures of primary esophageal keratinocytes. In two of the esophageal keratinocyte cultures, adduct formation was increased by treatment with safrole after induction of cytochrome P450 by 3-methyl-cholanthrene. This paper provides the first observation of how areca induces esophageal carcinogenesis, i.e., through the genotoxicity of safrole, a component of the areca juice.

Liu T.Y., Chung Y.T., Wang P.F., Chi C.W. & Hsieh LL. (2004) "Safrole-DNA adducts in human peripheral blood--an association with areca quid chewing and CYP2E1 polymorphisms." *Mutat Res.* **559**(1-2), 59-66. [Abstract](#). It has been recently demonstrated that safrole (4-allyl-1,2-methylenedioxybenzene)-DNA adducts are present in oral cancer tissue from patients who have chewed areca quid (AQ) containing high concentration of safrole. In this study, the presence of safrole-DNA adducts in peripheral white blood cells from 88 subjects with a known AQ chewing history and 161 matched controls were studied with the aim of identifying the adducts as a biomarker for safrole exposure. This study also analyzed the correlation between the level of safrole-DNA adducts and polymorphism of the CYP2E1 gene, alone and in combination with the GST M1 and GST T1-deletion polymorphisms. The results demonstrated the presence of safrole-DNA adducts in 83 (94.32%) of the DNA samples from subjects with current AQ chewing history and 21 (13.04%) of the control samples without known AQ chewing habit ( [Formula: see text] ). Individuals with at least one CYP2E1 c2 allele had a significant higher frequency of safrole-DNA adducts (odds ratio (OR), 4.00; 95% confidence interval (CI), 1.03-15.53) than those with the CYP2E1 c1c1 genotype while chewing less than 20 areca quids per day. In conclusion, this study demonstrates the presence of safrole-DNA adducts in peripheral blood lymphocytes (PBL), and the presence of these safrole-DNA adducts is correlated with AQ chewing. In addition, the CYP2E1 would seem to play an important role in the modulation of safrole-DNA adduct formation.

Liu C.J., Chen C.L., Chang K.W., Chu C.H. & Liu T.Y. (2000) "Safrole in betel quid may be a risk factor for hepatocellular carcinoma: case report." *CMAJ*

162(3): 359–360. [Abstract](#). Chewing betel quid or the combination of chewing betel quid and smoking cigarettes is associated with an increased risk of oral squamous cell carcinoma. 1 The composition of betel quid varies with geographic location. In Taiwan betel quid is composed of areca nut (*Areca catechu*, an Asian tropical palm), slaked lime, and the inflorescence or leaf of Piper betle (an Asian climbing plant). The inflorescence of Piper betle contains high concentrations (15 mg/g fresh weight) of safrole, an essential oil used in cosmetics and as a food flavouring. Safrole is classified as a rodent hepatocarcinogen,<sup>2</sup> and chewing betel quid may contribute to human exposure to this compound. The saliva of a person chewing betel quid contains on average 420 µmol/L of safrole. 3 We describe a case of hepatocellular carcinoma in a Taiwanese man who had chewed betel quid for over 32 years; safrole-DNA adducts, a likely cause of liver carcinogenesis, were found in liver biopsy specimens.

Mori H., Matsubara N., Ushimaru Y. & Hirono I. (1979) "Carcinogenicity examination of betel nuts and piper betel leaves." *Experientia* **35**, 384.

Muir C. S. & Kirk R. (1960) "Betel, tobacco, and cancer of the mouth." *Br. J. Cancer* **14**, 597.

Ranadive K. J., Gothoskar S. V., Rao A. R., Tezabwalla B. U., Ambaye R. Y. (1976) "Experimental studies on betel nut and tobacco carcinogenicity. *Int. J. Cancer* **17**, 469.

Reddy D. G. & Anguli V. C. (1967) "Experimental production of cancer with betel nut, tobacco and slaked lime mixture." *J. Indian Med. Assoc.* **49**, 315.

Suri K., Goldman H. M. & Herbert W. (1971) "Carcinogenic effect of a dimethyl sulphoxide extract of betel nut on the mucosa of the hamster buccal pouch." *Nature* **230**, 383.

### **Safrole Carcinogenicity: General.**

Adam L. & Postel W. (1992) "Bestimmung von alpha- und beta-Thujon, Safrol, Isosafrol, beta-Asaron und Cumarin in weinhaltigen Getränken und Spirituosen." *Die Brantweinwirtschaft*, **132**, 202-206.

Benedetti M.S., Malnoë A. & Broillet A.L. (1977) "Absorption, metabolism and excretion of safrole in the rat and man." *Toxicology* **7**(1), 69-83. [Abstract](#). The metabolic disposition of different doses of [<sup>14</sup>C] safrole were studied in rat and man. In both species, small amounts of orally administered safrole were absorbed rapidly and then excreted almost entirely within 24 h in the urine. In the rat, when the dose was raised from 0.6 to 750 mg/kg, a marked decrease in the rate of elimination occurred as only 25% of the dose was excreted in the urine in 24 h. Furthermore, at the high dose level, plasma and tissue concentrations of both unchanged safrole and its metabolites remained elevated for 48 h probably indicating impairment of the degradation/excretion pathways. The main urinary metabolite in both species was 1,2-dihydroxy-4-allylbenzene which was excreted in a conjugated form. Small amounts of eugenol or its isomer 1-methoxy-2-hydroxy-4-allylbenzene were also detected in rat and man. 1'-Hydroxysafrole, a

proximate carcinogen of safrole, and 3'-hydroxyisosafrole were detected as conjugates in the urine of the rat. However, in these investigations we were unable to demonstrate the presence of the latter metabolites in man.

Boberg E.W., Liem A., Miller E.C. & Miller J.A. (1987) "Inhibition by pentachlorophenol of the initiating and promoting activities of 1'-hydroxysafrole for the formation of enzyme-altered foci and tumors in rat liver." *Carcinogenesis*. **8**(4), 531-9. [Abstract](#). The hepatocarcinogen 1'-hydroxysafrole (HOS) exhibited weak initiating activity and strong promoting activity for the induction of enzyme-altered foci and tumors in rat liver. Thus, administration of a single dose of HOS to rats 18 h after a 70% hepatectomy, followed by administration of phenobarbital (PB) in the diet for 6 months, induced a low, but statistically significant, number of foci of enzyme-altered cells. This treatment did not result in gross liver tumors, even when the PB treatment was continued for 16 months. Large numbers of enzyme-altered foci developed when HOS was administered in the diet at levels of 0.05-0.25% to rats previously administered a single dose of N,N-diethylnitrosamine (DEN) 24 h after a 70% hepatectomy. Similarly, rats given a single dose of DEN 24 h after a partial hepatectomy and then fed 0.10 or 0.25% of HOS in the diet for 10 months developed a high incidence of hepatocellular carcinomas. In the absence of pretreatment with DEN, dietary administration for at least 4 months of 0.10 or 0.25% of HOS induced significant numbers of enzyme-altered foci; these data and liver tumor induction by continuous feeding of HOS, in the absence of pretreatment with DEN, provide additional evidence for an initiating, as well as a promoting, activity of HOS in rat liver. Concurrent administration of the hepatic sulfotransferase inhibitor pentachlorophenol with HOS in each of the above assays almost completely inhibited the initiating and promoting activities of HOS for the formation of enzyme-altered foci and tumors; these data strongly suggest that both the initiating and promoting activities are mediated by the sulfuric acid ester, 1'-sulfooxysafrole. HOS also exhibited initiating activity in adult mouse liver. Thus, dietary administration of 0.25% of HOS for only 1 month, followed by administration of the hepatic tumor promoter 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene resulted in a high incidence and multiplicity of hepatomas by 10 months. In the absence of the promoter, administration of HOS for only 1 month induced no hepatomas; 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene alone induced only a low incidence. In mice not given the promoter, continuous administration of HOS for 3-6 months was required for hepatoma development by 16 months.

Boberg E.W., Miller E.C., Miller J.A., Poland A. & Liem A. (1983) "Strong evidence from studies with brachymorphic mice and pentachlorophenol that 1'-sulfooxysafrole is the major ultimate electrophilic and carcinogenic metabolite of 1'-hydroxysafrole in mouse liver." *Cancer Res*. **43**, 5163-5173.

Boberg E.W., Miller E.C. & Miller J.A. (1986) "The metabolic sulfonation and side-chain oxidation of 3'-hydroxyisosafrole in the mouse and its inactivity as a hepatocarcinogen relative to 1'-hydroxysafrole." *Chem Biol Interact*. **59**(1), 73-97. [Abstract](#). The chemically synthesized sulfuric acid esters of 1'-hydroxysafrole and

3'-hydroxyisosafrrole, 1'-sulfooxysafrrole and 3'-sulfooxyisosafrrole, respectively, are both strong electrophiles. Each ester reacted with deoxyguanosine (dGuo) in aqueous solution to form both safrol-1'-yl- and isosafrol-3'-yl-deoxyguanosine adducts. Both 1'-hydroxysafrrole and 3'-hydroxyisosafrrole were also formed from each ester in the presence of water. When either 1'-[3H]hydroxysafrrole or 3'-[3H]hydroxyisosafrrole was incubated with mouse liver cytosols fortified with 3'-phosphoadenosine-5'-phosphosulfate (PAPS) and RNA, similar levels of RNA- and protein-bound adducts were formed; thus, the hepatic sulfotransferase activities for these two substrates appear to be similar. In contrast, the levels of hepatic nucleic acid and protein adducts formed after administration of 3'-[3H]hydroxyisosafrrole to mice were only 2-4% and 8-14%, respectively, of those obtained after an equimolar dose of 1'-[3H]hydroxysafrrole. Likewise, when 3'-hydroxyisosafrrole was injected into 12-day-old male B6C3F1 mice at a level of 0.1 or 2.5  $\mu\text{mol/g}$  body wt., the average numbers of hepatomas per mouse (0.2 and 0.4, respectively) were not significantly increased over the average number for mice treated only with the solvent (0.2). By contrast, mice that received 0.1  $\mu\text{mol}$  of 1'-hydroxysafrrole/g body wt. developed about 2 hepatomas per mouse. The metabolism of 3'-hydroxyisosafrrole in the rat and mouse differed markedly from that of 1'-hydroxysafrrole. 3'-Hydroxyisosafrrole rapidly underwent side-chain oxidation to yield 3,4-methylenedioxybenzoic acid and 3,4-methylenedioxybenzoyl glycine, the major urinary metabolites, together accounted for 39% and 63% of the dose administered to rats and mice, respectively. The glucuronide of 3'-hydroxyisosafrrole was not detected in the urine, whereas urinary excretion of the glucuronide of 1'-hydroxysafrrole at 2 h accounted for approx. 40% of a dose of 1'-hydroxysafrrole.

Bolton J.L., Acay N.M. & Vukomanovic V. (1994) "Evidence that 4-allyl-o-quinones spontaneously rearrange to their more electrophilic quinone methides: potential bioactivation mechanism for the hepatocarcinogen safrole." *Chem. Res. Toxicol.* **7**, 443–450.

Borchert P., Miller J.A., Miller E.C. & Shires T.K. (1973) "1'-hydroxysafrrole, a proximate carcinogenic metabolite of safrole in rat and mouse." *Cancer Research* **33**, 590-600. [Abstract](#). When fed as 0.5% of the diet for 8 to 10 months, 1'-hydroxysafrrole induced a high incidence of hepatocellular carcinomas in male rats; safrole induced only a low incidence under these conditions. 1'-Acetoxysafrrole, because of its toxicity, was fed to rats at only 0.6 the molar level of the above compounds. These rats did not develop hepatic tumors, but all of those that survived at least 6 months developed multiple papillomas of the forestomach; squamous cell carcinomas were found in the forestomachs of two of these rats. A few isolated papillomas of the forestomach were observed in rats fed 1'-hydroxysafrrole. Eighty-four and 82%, respectively, of male mice given injections of 1'-hydroxy- or 1'-acetoxysafrrole at 1 to 21 days of age (total dose, 9.5  $\mu\text{moles}$  and killed at 12 to 14 months of age had liver tumors; the incidences were 40% for male mice given injections of safrole and 8% for the controls. Of the adult male mice fed 0.4 or 0.5% of safrole for 13 months and killed at 16

months, 30% had liver tumors; the incidence was 11% in the controls. Some liver tumors also developed in adult male mice fed 1'-hydroxysafrole, but the number of survivors was low. All of these liver tumors were diagnosed as highly differentiated hepatic carcinomas. Between the 14th and 16th months, 46% of the mice that were fed 1'-hydroxysafrole and that survived at least 12 months developed sarcomas, most of which were diagnosed as angiosarcomas, in the interscapular region; only two safrole-fed mice and one control mouse developed tumors at this site. Twenty s.c. injections of 18.6 umoles each of 1'-acetoxysafrole or 1'-hydroxysafrole induced sarcomas in 30 and 8%, respectively, of adult male rats. These sarcomas were observed 12 to 18 months after the 1st injection. Skin tumors did not develop in mice treated topically 14 times with 1.8 umoles of safrole; its 1'-hydroxy, 1'-methoxy, or 1'-acetoxy derivatives; or the corresponding 2',3'-dihydrosafrole derivatives and then given twice weekly applications of phorbol-12,13-didecanoate. On the basis of the above data and our finding that a conjugate of 1'-hydroxysafrole is excreted in the urine of rats and mice given p.o. or i.p. safrole, we conclude that 1'-hydroxysafrole is a proximate carcinogenic metabolite of safrole.

Borchert P., Wislocki P.G., Miller J.A., & Miller E.C. (1973) "The metabolism of the naturally occurring hepatocarcinogen safrole to 1'-hydroxysafrole and the electrophilic reactivity of 1'-acetoxysafrole." *Cancer Res.* **33**, 575-589. [Abstract](#). A conjugated form of 1'-hydroxysafrole was identified as a urinary metabolite of safrole; the conjugate was cleaved by commercial  $\beta$ -glucuronidase preparations. The conjugated 1'-hydroxysafrole accounted for 1 to 3% of an i.p. dose of safrole administered to rats, hamsters, or guinea pigs. Pretreatment of rats with phenobarbital or 3-methylcholanthrene increased about 10-fold the excretion of 1'-hydroxysafrole conjugate(s) after a dose of safrole; these treatments had little effect on the urinary excretion of conjugated 1'-hydroxysafrole after injection of the 1'-hydroxy derivative. Pretreatment with phenobarbital did not increase the excretion of conjugated 1'-hydroxysafrole by guinea pigs or hamsters given safrole injections. Male mice excreted 30% or more of a dose of safrole or 1'-hydroxysafrole as a conjugate of the latter compound. The 1'-hydroxysafrole released by  $\beta$ -glucuronidase treatment of urine from rats given safrole injections was isolated and characterized by comparison of its ultraviolet, nuclear magnetic resonance, and mass spectra with those of the synthetic compound. The synthetic model ester 1'-acetoxysafrole is an electrophilic reactant. It reacts at neutrality with methionine to yield 3'-methylmercaptoisosafrole, which was characterized by the identity of its ultraviolet, nuclear magnetic resonance, and mass spectra with those of the compound prepared from 3'-bromoisosafrole and methylmercaptan. 1'-Acetoxysafrole also reacts with guanosine and adenosine and, probably to a small extent, with cytidine as evidenced by the formation in reaction mixtures containing  $^{14}\text{C}$ -labeled nucleosides of  $^{14}\text{C}$ -containing products which are less polar than the parent nucleosides. These products did not contain a significant quantity of  $^3\text{H}$  from 1'-acetoxysafrole-acetoxy- $^3\text{H}$ . A major nucleotide product was isolated from a large-scale reaction of 1'-acetoxysafrole with guanosine monophosphate. This nucleotide was degraded to the nucleoside, which was acetylated. The ultraviolet, nuclear magnetic resonance,

and mass spectra of the acetylated nucleoside are consistent with its characterization as O-6-(isosafrol-3'-yl)-N-2-acetylguanosine-2',3',5'-triacetate. The syntheses of the following new compounds are reported: 1'-acetoxysafrole, 3'-acetoxyisosafrole, 3'-bromoisosafrole, 3'-methylmercaptosisosafrole, and 1'-methoxysafrole.

Caldwell J., Chan V.S.W., Marshall A.D., Hasheminejad G. & Bounds S.V.J. (1992) "1'-Hydroxylation is the only metabolic pathway of simple alkylbenzenes involved in their genotoxicity." *Toxicologist* **12**, 56.

Chang H.C., Cheng H.H., Huang C.J., Chen W.C., Chen I.S., Liu S.I., Hsu S.S., Chang H.T., Wang J.K., Lu Y.C., Chou C.T. & Jan C.R. (2006) "Safrole-induced Ca<sup>2+</sup> mobilization and cytotoxicity in human PC3 prostate cancer cells." *J Recept Signal Transduct Res.* **26**(3), 199-212 [Abstract](#). The effect of the carcinogen safrole on intracellular Ca<sup>2+</sup> mobilization and on viability of human PC3 prostate cancer cells was examined. Cytosolic free Ca<sup>2+</sup> levels ([Ca<sup>2+</sup>]<sub>i</sub>) were measured by using fura-2 as a probe. Safrole at concentrations above 10 microM increased [Ca<sup>2+</sup>]<sub>i</sub> in a concentration-dependent manner with an EC<sub>50</sub> value of 350 microM. The Ca<sup>2+</sup> signal was reduced by more than half after removing extracellular Ca<sup>2+</sup> but was unaffected by nifedipine, nicardipine, nimodipine, diltiazem, or verapamil. In Ca<sup>2+</sup>-free medium, after treatment with 650 microM safrole, 1 microM thapsigargin (an endoplasmic reticulum Ca<sup>2+</sup> pump inhibitor) failed to release Ca<sup>2+</sup>. Neither inhibition of phospholipase C with U73122 nor modulation of protein kinase C activity affected safrole-induced Ca<sup>2+</sup> release. Overnight incubation with 0.65-65 microM safrole did not affect cell viability, but incubation with 325-625 microM safrole decreased viability. Collectively, the data suggest that in PC3 cells, safrole induced a [Ca<sup>2+</sup>]<sub>i</sub> increase by causing Ca<sup>2+</sup> release from the endoplasmic reticulum in a phospholipase C- and protein kinase C-independent fashion, and by inducing Ca<sup>2+</sup> influx. Safrole can decrease cell viability in a concentration-dependent manner.

Chen W.C., Cheng H.H., Huang C.J., Lu Y.C., Chen I.S., Liu S.I., Hsu S.S., Chang H.T., Huang J.K., Chen J.S. & Jan C.R.. (2007) "The carcinogen safrole increases intracellular free Ca<sup>2+</sup> levels and causes death in MDCK cells." *Chin J Physiol.* **50**(1), 34-40. [Abstract](#). The effect of the carcinogen safrole on intracellular Ca<sup>2+</sup> movement in renal tubular cells has not been explored previously. The present study examined whether safrole could alter Ca<sup>2+</sup> handling in Madin-Darby canine kidney (MDCK) cells. Cytosolic free Ca<sup>2+</sup> levels ([Ca<sup>2+</sup>]<sub>i</sub>) in populations of cells were measured using fura-2 as a fluorescent Ca<sup>2+</sup> probe. Safrole at concentrations above 33 microM increased [Ca<sup>2+</sup>]<sub>i</sub> in a concentration-dependent manner with an EC<sub>50</sub> value of 400 microM. The Ca<sup>2+</sup> signal was reduced by 90% by removing extracellular Ca<sup>2+</sup>, but was not affected by nifedipine, verapamil, or diltiazem. Addition of Ca<sup>2+</sup> after safrole had depleted intracellular Ca<sup>2+</sup>-induced dramatic Ca<sup>2+</sup> influx, suggesting that safrole caused store-operated Ca<sup>2+</sup> entry. In Ca<sup>2+</sup>-free medium, after pretreatment with 650 microM safrole, 1 microM thapsigargin (an endoplasmic reticulum Ca<sup>2+</sup> pump

inhibitor) failed to release more Ca<sup>2+</sup>. Inhibition of phospholipase C with 2 microM U73122 did not affect safrole-induced Ca<sup>2+</sup> release. Trypan blue exclusion assays revealed that incubation with 650 microM safrole for 30 min did not kill cells, but killed 70% of cells after incubation for 60 min. Collectively, the data suggest that in MDCK cells, safrole induced a [Ca<sup>2+</sup>] increase by causing Ca<sup>2+</sup> release from the endoplasmic reticulum in a phospholipase C-independent fashion, and by inducing Ca<sup>2+</sup> influx via store-operated Ca<sup>2+</sup> entry. Furthermore, safrole can cause acute toxicity to MDCK cells.

Crampton R. F. *et al.* (1977) "Long-term studies on chemically induced liver enlargement in the rat. II. Transient induction of microsomal enzymes leading to liver damage and nodular hyperplasia produced by safrole and Ponceau MX." *Toxicology* **7**, 307-326.

Daimon H., Sawada S., Asakura S. & Sagami F. (1997) "Analysis of cytogenetic effects and DNA adduct formation induced by safrole in Chinese hamster lung cells." *Teratog Carcinog Mutagen.* **17**(1), 7-18. [Abstract](#). Safrole (1-allyl-3,4-methylenedioxybenzene) was tested for its ability to induce sister chromatid exchanges (SCEs) and chromosomal aberrations (CAs) and to form DNA adducts in Chinese hamster lung (CHL) cells, in order to investigate the relationship between cytogenetic effects and DNA adduct formation under the same treatment conditions. The cells were treated with 0.025-0.2 mg/ml safrole in the presence or absence of rat liver postmitochondrial supernatant fraction (S9). Safrole induced significant SCEs and CAs dose-dependently in the presence of S9. SCEs ranged in number from 15.6 to 21.1 SCEs/cell and CAs were observed in 4-37% of cells. Using the <sup>32</sup>P-postlabeling assay, two major and two minor safrole-DNA adducts were detected in DNA digests obtained from CHL cells in the presence of S9. The levels of total DNA adducts ranged from 1.3 to 22.8 adducts/10(7) nucleotides. The two major adducts were shown to be guanine derivatives since these adducts comigrated on polyethylenimine plates with the adducts produced by the reaction of safrole with 2'-deoxyguanosine 3'-monophosphate. A correlation was seen between DNA adducts and SCEs or CAs. Neither induction of SCEs and CAs nor formation of DNA adducts was observed in the absence of S9. These findings suggest that SCEs and CAs induced by safrole result from covalent DNA modification metabolically activated by S9 in cultured cells.

Daimon H., Sawada S., Asakura S., & Sagami F. (1997-1998) "Inhibition of sulfotransferase affecting in vivo genotoxicity and DNA adducts induced by safrole in rat liver." *Teratog Carcinog Mutagen.* **17**(6), 327-337. [Abstract](#). The effect of pretreatment with pentachlorophenol (PCP), a known inhibitor of sulfotransferases, on the induction of chromosomal aberrations, sister chromatid exchanges (SCEs), replicative DNA synthesis (RDS), and the formation of DNA adducts was studied in the liver of rats treated with safrole (1-allyl-3,4-methylenedioxy-benzene). Rats were given a single oral dose (1,000 mg/kg body weight) or 5 repeated doses (500 mg/kg body weight) of safrole, with or without intraperitoneal pretreatment with PCP (10 mg/kg body weight). Hepatocytes were

isolated 24 hr after administration of safrole and allowed to proliferate in Williams' medium E supplemented with epidermal growth factor to test for chromosomal aberrations and SCEs. For examination of RDS, hepatocytes were incubated in Williams' medium E containing 5-bromo-2'-deoxyuridine. Safrole-DNA adducts were detected by a nuclease P1-enhanced <sup>32</sup>P-postlabeling assay. A single dose of safrole induced significant SCEs and RDS, while chromosomal aberrations were induced by 5 repeated doses. Two major and 2 minor DNA adducts were detected by both a single dose and 5 repeated doses. PCP significantly decreased safrole-induced cytogenetic effects and RDS, and caused a decrease in DNA adducts formed by safrole. These results suggest that safrole is capable of inducing SCEs, chromosomal aberrations, and RDS in the rat liver in vivo and that these effects may be induced by the sulfuric acid ester metabolite that can bind DNA.

Daimon H., Sawada S., Asakura S. & Sagami F. (1998) "In vivo genotoxicity and DNA adduct levels in the liver of rats treated with safrole." *Carcinogenesis*. **19**(1), 141-6. [Abstract](#). The induction of chromosome aberrations, sister chromatid exchanges (SCEs), and the formation of DNA adducts was studied in hepatocytes of F344 rats exposed in vivo to safrole. Hepatocytes were isolated 24 h after a single dose of safrole or five repeated doses (once a day) by gastric intubation and allowed to proliferate in Williams' medium E supplemented with epidermal growth factor. Cells were fixed after 48 h in culture. Safrole-DNA adducts were detected by a nuclease P1-enhanced <sup>32</sup>P-post-labeling assay in isolated hepatocytes from the rats. While a single dose was not sufficient to induce detectable levels of chromosome aberrations at the time of assay, five repeated doses induced these changes with a maximum frequency of 13.4%, compared with the control value of 1.8%. Both a single dose and five repeated doses induced significant SCEs, to a maximum frequency of 0.81 SCEs per chromosome, while the control value was 0.59 SCEs per chromosome. Two major and two minor DNA adducts were detected after treatment with either a single dose or five repeated doses. The maximum amount of total DNA adducts was 89.8 DNA adducts/10(7) nucleotides. These results show that safrole is a genotoxic carcinogen in the rat liver in vivo and suggest that the cytogenetic effects of this compound may result from covalent DNA modification in the rat liver. This in vivo cytogenetic assay should provide a useful means of evaluation of the genotoxicity of hepatocarcinogens.

Delaforge M., Ioannides C. & Parke D.V. (1980) "Ligand binding of safrole to cytochrome P-450." *Arch Toxicol Suppl*. 1980(4), 45-8. [Abstract](#). Safrole, a hepatocarcinogen, is converted by the microsomal mono-oxygenase system to a reactive intermediate which interacts with cytochrome P-450 to form a ligand complex. The formation of this complex is accompanied by loss of mono-oxygenase activity. The present study describes the interaction of the safrole reactive intermediate with microsomes from phenobarbital, 3-methylcholanthrene and safrole pretreated animals.

Delaforge, M. *et al.* (1977). "Direct evidence of epoxide pathway for natural allylbenzene compounds in adult rat liver cell culture." In: A. Frigerio, ed., *Recent development in mass spectrometry in biochemistry and medicine*, NY and London, Plenum Press, Vol. 1, 521-539.

Dietz B. & Bolton J.L. (2007) "Botanical dietary supplements gone bad." *Chem Res Toxicol.* **20**(4), 586–590.

Drinkwater, N. R. *et al.* (1976) "Hepatocarcinogenicity of estragole (1 allyl-4-methoxybenzene) and 1'-hydroxyestragole in the mouse and mutagenicity of 1'-acetoxyestragole in bacteria," *J. Natl. Cancer Inst.* **57**, 1323-1331.

Dorange, J. L. *et al.* (1978) "Comparative survey of microsomal activation systems for mutagenic studies of safrole." *Mutation Research* **53**, 179-180

Dorange, J. L. *et al.* (1977) "Mutagenicity of metabolic derivatives of safrole." *Mutation Research* **46**(3), 217.

Dorange, J. L. *et al.* (1977) "Pouvoir mutagène de métabolites de la voie epoxyde-diol du safrol et d'analogues. Etude sur *Salmonella typhimurium*." *C.R. Séances Soc. Biol.* **171**, 1041-1048

Doumas J. & Maume B. F. (1977) "Activation métabolique par le tissu surrénalien du rat d'un carcinogène du foie: le safrole." *C.R. Séances Soc. Biol.*, **171**, 108-114,

Elcombe, C. R. *et al.* (1975) "Studies on the interaction of safrole with rat hepatic microsomes." *Biochem. Pharmacol.* **24**, 1427-1433.

Enomoto M. (1987) "Naturally occurring carcinogens of plant origin: Safrole." *Bioactive Mol* **2**, 139–59.

Fennell T.R., Miller J.A. & Miller E.C. (1984) "Characterization of the biliary and urinary glutathione and N-acetylcysteine metabolites of the hepatic carcinogen 1'-hydroxysafrole and its 1'-oxo metabolite in rats and mice." *Cancer Res.* ;**44**, 3231–3240. [Abstract](#). The hepatocarcinogen safrole is metabolized both to 1'-hydroxysafrole, a proximate hepatocarcinogen, and to 1'-oxosafrole, which is electrophilic but has little or no carcinogenic activity in rats and mice. As a part of a study on the metabolic interrelationships of these metabolites, their biliary and urinary conjugates were investigated. Administration of a single i.p. dose of [2',3'-<sup>3</sup>H]-1'-oxosafrole to male Sprague-Dawley rats or female CD-1 mice with cannulated bile ducts resulted in the excretion of 2 major biliary metabolites. These metabolites were isolated by high-performance liquid chromatography and characterized by <sup>1</sup>H-nuclear magnetic resonance spectroscopy as 3'-(glutathion-S-yl)-1'-oxo-2',3'-dihydrosafrole and 3'-(N-acetylcystein-S-yl)-1'-oxo-2',3'-dihydrosafrole. The latter conjugate was also found in the urine. These conjugates were synthesized by non-enzymatic reaction of 1'-oxosafrole with glutathione and N-acetylcysteine at pH 8. After a single i.p. dose of [2',3'-<sup>3</sup>H]-1'-hydroxysafrole, the major biliary and urinary metabolite in rats was the

glucuronide of this alcohol. Lower levels of the glutathione and N-acetylcysteine conjugates of 1'-oxosafrole appeared in the bile, and the latter conjugate was also found in the urine. Similar findings were made on the biliary metabolites of 1'-hydroxysafrole in mice. Although the sulfuric acid ester of 1'-hydroxysafrole is the major metabolite leading to the formation of DNA adducts in the liver, it was, at most, of minor importance in the formation of glutathione adducts. Only a very small percentage of a dose of 1'-hydroxysafrole was excreted in the bile of rats or mice as products that cochromatographed with 1'-(glutathion-S-yl)-safrole and 3'-(glutathion-S-yl)-isosafrole; no 3'-(N-acetylcystein-S-yl)-isosafrole was detected. These latter conjugates were synthesized by nonenzymatic reactions at pH 8.5 of the model electrophilic ester 1'-acetoxysafrole with glutathione or N-acetylcysteine.

Fennell T.R. & Bridges J.W. (1979) "Structure--activity relationship for 'safrole-type' cytochrome P-450 induction [proceedings]" *Biochem Soc Trans* **7**(5), 1104-6.

Fishbein L. *et al.* (1967) "Thin-layer chromatography of rat bile and urine following intravenous administration of safrole, isosafrole, and dihydrosafrole," *J. Chromatog.* **29**, 267-273

Gleason M.N. *et al.* (1984) *Clinical toxicology of commercial products*. 5<sup>th</sup> edn. Williams & Williams, Baltimore.

Gold *et al.*- see Carcinogenic Potency Project @ [www.potency.berkeley.edu/](http://www.potency.berkeley.edu/). ("through Levy D.D. (undated) below.

Gray T.J., Parke D.V., Grasso P. & Crampton R.F. (1972) "Biochemical and pathological differences in hepatic response to chronic feeding of safrole and butylated hydroxytoluene to rats." *Biochem J.* **130**(2), 91.

Green N.R. & 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. & Randerath K. (1993) "Formation and persistence of safrole-DNA adducts over a 10,000-fold dose range in mouse liver." *Carcinogenesis* **14**, 1517–1521. [Abstract](#). The spice constituent safrole (l-allyl-3, 4-methylenedioxy-benzene) and related allylbenzenes form DNA adducts and are rodent carcinogens. This study examined both dose and time dependence of hepatic safrole-DNA adduct formation over a 10 000-fold dose range up to 30 days after single administration. Female CD-1 mice were treated with safrole i.p. at 0.001, 0.01, 0.1, 1.0, and 10.0 mg/mouse in 0.2 ml tricapylin or with vehicle alone. Liver DNA was analyzed at 0.5, 1, 2, 3, 7, 15 and 30 days via the dinucleotide/mono-phosphate version of the <sup>32</sup>P-postlabeling assay. An 10-fold increase in total safrole adduct levels with each successive 10-fold increase in dose was observed, giving relative adduct labeling (RAL) values of 10-9-10-5. Each dose elicited identical kinetics of adduct formation, showing

peak levels at 2 days and only slight decreases thereafter. The time course of adduct persistence was independent of the dose (0.01 –10 mg/mouse). An in vitro experiment established that the assay responded in strictly linear fashion to adduct concentration over a 10 000-fold range, and thus was suitable for in vivo dosimetry. DNA synthesis, as measured by [3H]thymidine incorporation, was enhanced only for the 10.0 mg dose at 2, 3 and 7 days. These results indicate a linear response of safrole-DNA adduct formation and persistence in mouse liver following administration of minute (0.001 mg/mouse) to high (10.0 mg/mouse) doses of the carcinogen.

Hagen E.C., Jenner, P.M., Jones, W.I., Fitzhugh, O.G., Long E.L., Brouwer J.G. & Webb W.K. (1965). "Toxic properties of compounds related to safrole." *Toxicol. Appl. Pharmacol.* **7**, 18-24.

Homburger, F., Bogdonoff, P. D. & Kelley, T. F. (1965) "Influence of diet on chronic oral toxicity of safrole and butter yellow in rats." *Proc. Soc. Exptl. Biol. Med.* **119**, 1106-1110

Homburger F. *et al.* (1962) "Sex effect on hepatic pathology from deficient diet and safrole in rats." *Arch. Pathol.* **73**,118-125

Homburger F., Kelley T. Jr., Friedler G. & Russfield A. B. (1961) "Toxic and possible carcinogenic effects of 4-allyl-1,2-methylenedioxybenzene (safrole) in rats on deficient diets." *Med. Exptl.* **4**, 1-11.

Howes A.J., Chan V.S.W. & Caldwell J. (1990) "Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis assay in rat hepatocytes." *Food & Chemical Toxicology* **28**(8), 537-542.

Hung S.L, Chen Y.L. & Chen Y.T. (2003) "Effects of safrole on the defensive functions of human neutrophils." *J Periodontal Res.* **38**(2), 130-4. [Abstract](#). The effects of safrole on the defensive functions of human neutrophils were examined. At the concentrations employed in this study, safrole did not significantly affect the viability of peripheral blood neutrophils as verified by their ability to exclude trypan blue dye. However, exposure of neutrophils to safrole inhibited their bactericidal activity against oral pathogens, including *Actinobacillus actinomycetemcomitans* and *Streptococcus mutans*, in a dose dependent manner. In addition, safrole inhibited the production of bactericidal superoxide anion by neutrophils as measured by cytochrome c reduction. In conclusion, the results demonstrated that safrole reduced the antibacterial activity and the superoxide anion production of neutrophils. Inhibition of the defensive functions of neutrophils may be one possible mechanism by which safrole compromises the oral health.

IARC (1976). "Some Naturally Occurring Substances." *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans*, Vol. **10**, 231-244. Lyon, France: International Agency for Research on Cancer.

IARC (1987). IARC Monographs on the Evaluation of the Carcinogenic Risk of chemicals to Humans: Overall Evaluations of Carcinogenicity: An Updating of *IARC Monographs Volumes 1-42*, Supplement 7, 51, 65.

Ioannides, C, Delaforge, M & Parke, DV (1981). "Safrole: Its metabolism, carcinogenicity and interactions with cytochrome p-450." *Fd Cosmet Toxicol*, **19**, 657–666.

Ishidate M. & 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.

Janiaud P. & Padiou, P. (1977) "Adult rat liver epithelial cells for studies of carcinogenic activity of safrole," *Mutation Research* **46**, 221-222.

Janiaud P. *et al.* (1976) "Etude comparative en culture cellulaire de foie de rat du métabolisme de différents analogues et métabolites d'un hépatocarcinogène naturel: le safrole." *C.R. Séances Soc. Biol.* **170**, 1035-1041

Jeng J.H., Wang Y.J., Chang W.H., Wu H.L., Li C.H., Uang B.J., Kang J.J., Lee J.J., Hahn L.J., Lin B.R. & Chang M.C. (2004) "Reactive oxygen species are crucial for hydroxychavicol toxicity toward KB epithelial cells." *Cell. Mol. Life Sci.* **61**, 83–96.

Jeurissen S.M.F. (2007) *Bioactivation and genotoxicity of the herbal constituents safrole, estragole & methyleugenol*. Thesis Wageningen University, The Netherlands 2007. [Abstract](#). The herbal constituents safrole, estragole & methyleugenol belonging to the chemical class of the alkenylbenzenes are genotoxic & carcinogenic compounds. The genotoxicity of these alkenylbenzenes proceeds via electrophilic metabolites generated by cytochrome P450 enzymes (P450) and sulfotransferases (SULT). Carcinogenicity of these compounds was demonstrated in animal experiments using relatively high doses of single compounds. Human exposure to these compounds is much lower than the doses used in the animal experiments and humans exposed to alkenylbenzenes in a complex food matrix. The first aim of this thesis was to identify the human P450 enzymes that are responsible for the bioactivation of the alkenylbenzenes into their proximate carcinogenic 1'-hydroxymetabolites. Several *in vitro* studies using recombinant P450 enzymes and human liver microsomes were undertaken to identify the main enzymes involved in the 1'-hydroxylation of the alkenylbenzenes and to determine their kinetics. These studies showed that at low substrate concentrations P450 1A2 is the major enzyme in the bioactivation of methyleugenol, P450 1A2 and P450 2A6 are the main enzymes in the bioactivation of estragole, and P450 2A6 is the main enzyme in the bioactivation of safrole. The second objective of this thesis was to study the influence of other herbal constituents on the bioactivation and the genotoxicity of herb-based alkenylbenzenes. An on-line high-performance liquid chromatography detection system was developed for the detection of P450 inhibitors in herbal extracts. The

presence of P450 1A2 inhibitors in basil, a herb that contains methyleugenol & estragole, was demonstrated using this on-line system. In addition to these P450 1A2 inhibitors, also the alkenylbenzenes themselves may act as inhibitors competing for the active site of P450 1A2 (estragole and methyleugenol) or P450 2A6 (estragole and safrole). Furthermore it was demonstrated that basil extract is able to strongly inhibit sulfonation and subsequent DNA adduct formation of 1'-hydroxyestragole in incubation with rat and human S9 homogenates and in the human hepatoma HepG2 cell line. These *in vitro* results suggest that P450- and SULT-catalysed bioactivation of methyleugenol and/or estragole and subsequent adverse effects may be lower in a matrix of other herbal components than would be expected on the basis of experiments using single compounds. *In vivo* experiments have to point out whether the protective effects that are found in these *in vitro* studies can be extrapolated to the *in vivo* situation. It may turn out that rodent carcinogenicity data on estragole and methyleugenol considerably overestimate the risks posed when humans are exposed to these compounds in a herbal matrix.

Jeurissen S.M., Punt A., Boersma M.G., Bogaards J.J., Fiamegos Y.C., Schilter B., van Bladeren P.J., Cnubben N.H. & Rietjens I.M. (2007) "Human cytochrome P450 enzyme specificity for the bioactivation of estragole and related alkenylbenzenes." *Chem Res Toxicol.* **20**(5), 798-806. [Abstract](#). Human cytochrome P450 enzymes involved in the bioactivation of estragole to its proximate carcinogen 1'-hydroxyestragole were identified and compared to the enzymes of importance for 1'-hydroxylation of the related alkenylbenzenes methyleugenol and safrole. Incubations with Supersomes revealed that all enzymes tested, except P450 2C8, are intrinsically able to 1'-hydroxylate estragole. Experiments with Gentest microsomes, expressing P450 enzymes to roughly average liver levels, indicated that P450 1A2, 2A6, 2C19, 2D6, and 2E1 might contribute to estragole 1'-hydroxylation in the human liver. Especially P450 1A2 is an important enzyme based on the correlation between P450 1A2 activity and estragole 1'-hydroxylation in human liver microsomal samples and inhibition of estragole 1'-hydroxylation by the P450 1A2 inhibitor alpha-naphthoflavone. Kinetic studies revealed that, at physiologically relevant concentrations of estragole, P450 1A2 and 2A6 are the most important enzymes for bioactivation in the human liver showing enzyme efficiencies (kcat/Km) of, respectively, 59 and 341 min<sup>-1</sup> mM<sup>-1</sup>. Only at relatively high estragole concentrations, P450 2C19, 2D6, and 2E1 might contribute to some extent. Comparison to results from similar studies for safrole and methyleugenol revealed that competitive interactions between estragole and methyleugenol 1'-hydroxylation and between estragole and safrole 1'-hydroxylation are to be expected because of the involvement of, respectively, P450 1A2 and P450 2A6 in the bioactivation of these compounds. Furthermore, poor metabolizer phenotypes in P450 2A6 might diminish the chances on bioactivation of estragole and safrole, whereas lifestyle factors increasing P450 1A2 activities such as cigarette smoking and consumption of charbroiled food might increase those chances for estragole and methyleugenol.

Jeurissen S.M.F., Bogaards J.J.P., Awad H.M., Boersma M.G., Brand W., Fiamegos Y.C., van Beek T.A., Alink G.M., Sudholter E.J.R., Cnubben N.H.P. & Rietjens IMCM (2004) "Human cytochrome P450 enzyme specificity for bioactivation of safrole to the proximate carcinogen 1'-hydroxysafrole." *Chem. Res. Toxicol.* **17**, 1245-1250. [Abstract](#). In the present study, the cytochrome P450 mediated bioactivation of safrole to its proximate carcinogenic metabolite, 1'-hydroxysafrole, has been investigated for the purpose of identifying the human P450 enzymes involved. The 1'-hydroxylation of safrole was characterized in a variety of in vitro test systems, including Supersomes, expressing individual human P450 enzymes to a high level, and microsomes derived from cell lines expressing individual human P450 enzymes to a lower, average human liver level. Additionally, a correlation study was performed, in which safrole was incubated with a series of 15 human liver microsomes, and the 1'-hydroxylation rates obtained were correlated with the activities of these microsomes toward specific substrates for nine different isoenzymes. To complete the study, a final experiment was performed in which pooled human liver microsomes were incubated with safrole in the presence and absence of coumarin, a selective P450 2A6 substrate. On the basis of the results of these experiments, important roles for P450 2C9\*1, P450 2A6, P450 2D6\*1, and P450 2E1 were elucidated. The possible consequences of these results for the effects of genetic polymorphisms and life style factors on the bioactivation of safrole are discussed. Polymorphisms in P450 2C9, P450 2A6, and P450 2D6, leading to poor metabolizer phenotypes, may reduce the relative risk on the harmful effects of safrole, whereas life style factors, such as the use of alcohol, an inducer of P450 2E1, and barbiturates, inducers of P450 2C9, and polymorphisms in P450 2D6 and P450 2A6, leading to ultraextensive metabolizer phenotypes, may increase the relative risk.

Kamienski F.X. & 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.

Lake B.G. & Parke D.V. (1972) "Interaction of safrole and isosafrole with hepatic microsomal haemoproteins." *Biochem J.* **127**(2), 9-10.

Lake B. G. & Parke D. V. (1972) "Induction of aryl hydrocarbon hydroxylase in various tissues of the rat by methylenedioxyphenyl compounds." *Biochem. J.*, **130**, 86.

Levi P., Janiaud P., Delaforge M., Morizot J.P., Maume B.F. & Padieu P. (1977). "The occurrence of glucuroconjugated metabolites of safrole in the urine of treated rats." *C.R. Séances Soc. Biol.* **171**, 1034-1040.

Levy D.D. (undated) "Eugenol & the allylbenzenes: a case study on genotoxic risk." – see <http://www.gta-us.org/2008Presentations/Levy.pdf>.

Lin H.C., Cheng H.H., Huang C.J., Chen W.C., Chen I.S., Liu S.I., Hsu S.S., Chang H.T., Huang J.K., Chen J.S., Lu Y.C. & Jan C.R. (2006) "Safrole-induced

cellular Ca<sup>2+</sup> increases and death in human osteosarcoma cells." *Pharmacol Res.* **54**(2):103-10. [Abstract](#). The effect of the carcinogen safrole on intracellular Ca<sup>2+</sup> movement has not been explored in osteoblast-like cells. This study examined whether safrole could alter Ca<sup>2+</sup> handling and viability in MG63 human osteosarcoma cells. Cytosolic free Ca<sup>2+</sup> levels ([Ca<sup>2+</sup>]<sub>i</sub>) in populations of cells were measured using fura-2 as a fluorescent Ca<sup>2+</sup> probe. Safrole at concentrations above 130 microM increased [Ca<sup>2+</sup>]<sub>i</sub> in a concentration-dependent manner with an EC<sub>50</sub> value of 450 microM. The Ca<sup>2+</sup> signal was reduced by 30% by removing extracellular Ca<sup>2+</sup>. Addition of Ca<sup>2+</sup> after safrole had depleted intracellular Ca<sup>2+</sup> induced Ca<sup>2+</sup> influx, suggesting that safrole caused Ca<sup>2+</sup> entry. In Ca<sup>2+</sup>-free medium, after pretreatment with 650 microM safrole, 1 microM thapsigargin (an endoplasmic reticulum Ca<sup>2+</sup> pump inhibitor) failed to release more Ca<sup>2+</sup>; and pretreatment with thapsigargin inhibited most of the safrole-induced [Ca<sup>2+</sup>]<sub>i</sub> increases. Inhibition of phospholipase C with U73122 did not affect safrole-induced Ca<sup>2+</sup> release; whereas activation of protein kinase C with phorbol ester enhanced safrole-induced [Ca<sup>2+</sup>]<sub>i</sub> increase. Trypan exclusion assays revealed that incubation with 65 microM safrole for 30 min did not kill cells, but incubation with 650 microM safrole for 10-30 min nearly killed all cells. Flow cytometry demonstrated that safrole evoked apoptosis in a concentration-dependent manner. Safrole-induced cytotoxicity was not reversed by chelation of Ca<sup>2+</sup> with BAPTA. Collectively, the data suggest that in MG63 cells, safrole induced a [Ca<sup>2+</sup>]<sub>i</sub> increase by causing Ca<sup>2+</sup> release mainly from the endoplasmic reticulum in a phospholipase C-independent manner. The safrole response involved Ca<sup>2+</sup> influx and is modulated by protein kinase C. Furthermore, safrole can cause apoptosis in a Ca<sup>2+</sup>-independent manner.

Lipsky M.M., Hinton D.E., Klaunig J.E., Trump B.F. (1981) "Biology of hepatocellular neoplasia in the mouse. I. Histogenesis of safrole-induced hepatocellular carcinoma." *J Natl Cancer Inst.* **67**(2), 365-76. [Abstract](#). A sequential, histologic analysis of the livers of male BALB/c mice chronically fed the hepatocarcinogen safrole (4,000 ppm) was performed at 2, 4, 8, 16, 24, 36, 52, and 75 weeks. The transplantability of selected lesions to syngeneic hosts was also assessed. Histopathologic liver alterations at 2, 4, 8, and 16 weeks induced hypertrophy of centrilobular hepatocytes, oval cell proliferation, fatty change in periportal hepatocytes, including basophilic, acidophilic, and clear cell, were noted. At 36 and 52 weeks, hepatocellular adenomas occurred in 4 of 10 and 7 of 10 mice, respectively. At 75 weeks they occurred in 5 of 5 mice. Adenomas were larger than a lobule in diameter compressed the adjacent parenchyma, and distorted the hepatic architecture. Individual adenomas were composed of a mixture of basophilic, acidophilic, clear, and lipid-laden cells, arranged in disorganized cords, one to three cells in thickness. None of the 10 adenomas tested grew upon subcutaneous transplantation into syngeneic hosts. Hepatocellular carcinomas (HPC) developed in 2 of 10 safrole-exposed mice at 52 weeks and 3 of 5 mice at 75 weeks. These lesions were large, multilobed and, unlike adenomas, seemed to invade adjacent parenchyma. The HPC were heterogeneous in cell composition. Their architecture was disorganized with trabeculae of 1-10 or more cells in thickness. No central veins or portal tracts

were seen. All HPC proliferated when transplanted into syngeneic hosts. The results of this study demonstrated a sequential development of altered hepatocyte populations leading to HPC in safrole-treated mice. The transplantability of HPC indicated their malignant nature.

Lipsky M.M., Hinton D.E., Klaunig J.E., Goldblatt P.J. & Trump B.F. (1981) "Biology of hepatocellular neoplasia in the mouse. II. Sequential enzyme histochemical analysis of BALB/c mouse liver during safrole-induced carcinogenesis." *J Natl Cancer Inst.* **67**(2), 377-92. [Abstract](#). Sequential alterations in enzyme histochemical profiles and reaction of hepatocytes to rapid iron overload were examined in male BALB/c mice during chronic, safrole exposure. At 24 weeks after initiation of safrole treatment, foci of enzyme-altered hepatocytes were noted. These foci were composed of cells showing a decrease in reactivity for glucose-6-phosphatase (Glc-6-Pase) and succinate dehydrogenase (SDH) and an increase for gamma-glutamyl transpeptidase (gamma-Glu-T). In control, iron-loaded mice, the livers were intensely siderotic. In safrole-exposed, iron-loaded mice, foci of iron-negative hepatocytes, varying from a few cells to a lobule in diameter, were initially noted at 24 weeks. Both enzyme-altered and iron-negative foci occurred in the livers of exposed mice at all time periods after 24 weeks. After 36, 52, and 75 weeks of safrole treatment, hepatocellular adenomas were noted with altered enzyme histochemical profiles. Hepatocytes from adenomas were characterized by a decreased staining for Glc-6-pase and SDH and increased staining for gamma-Glu-T and glucose-6-phosphate dehydrogenase (Glc-6-PD). In addition, a few nodules showed a decrease in staining for 5'nucleotidase. In iron-loaded mice, hepatocytes of adenomas showed a decreased to negative reaction for iron when the surrounding parenchyma was siderotic. Hepatocellular carcinomas (HPC) occurred in livers of mice exposed to safrole for 52-75 weeks. The cells of HPC displayed similar enzyme histochemical reactions as cells of adenomas. They were decreased for Glc-6-Pase and SDH activity and increased for gamma-Glu-T and Glc-6-PD. In iron-loaded mice, the HPC cells were negative for stainable iron. Foci, adenomas, and HPC displayed some variability in enzyme histochemical reactions. Variability existed between lesions as well as between cells of the same lesion.

Liu T.Y., Chen C.C., Chen C.L. & Chi C.W.. (1999) "Safrole-induced oxidative damage in the liver of Sprague-Dawley rats." *Food Chem Toxicol.* **37**(7), 697-702. [Abstract](#). Safrole is a weak hepatocarcinogen, and its carcinogenic effect has been linked to the formation of stable safrole DNA adducts. In this study, we tested whether safrole also induces oxidative damages in Sprague-Dawley rats. By single i.p. injection, safrole dose-dependently induced the formation of hepatic lipid hydroperoxides (LHP) and 8-hydroxy-2'-deoxyguanosine (8-OH-dG). The safrole-induced LHP reached peak level on day 3 and gradually returned to the basal level on day 15. On the other hand, 8-OH-dG levels from the similarly treated rats peaked on day 5 and returned to basal level on day 15. Safrole also dose-dependently induced serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. We also examined the protective effect of

vitamin E, deferoxamine and N-acetylcysteine against the safrole-induced oxidative damage. N-Acetylcysteine, the precursor of glutathione, exerted the greatest protective effect among the three antioxidants tested. In contrast, buthionine sulfoximine, the glutathione synthesis inhibitor, enhanced the safrole-induced oxidative damage, as evidenced by the elevation of LHP and 8-OH-dG levels on day 3 ( $P < 0.05$ ). These findings demonstrate that safrole treatment induces oxidative damage in rat hepatic tissue, and glutathione plays an important protective role. This oxidative damage may be involved in the hepatocarcinogenic effect of safrole.

Lotlikar P. D. & Wasserman M. B. (1972) "Effects of safrole and isosafrole pretreatment on N- and ring-hydroxylation of 2-acetamidofluorene by the rat and hamster." *Biochem. J.*, **129**,937-943. [Abstract](#). The effects of safrole and isosafrole pretreatment on both N- and ring-hydroxylation of 2-acetamidofluorene were studied in male rats and hamsters. 2. Isosafrole (100mg/day per kg body wt.) pretreatment of rats for 3 days did not have any effect on urinary excretion of hydroxy metabolites of 2-acetamidofluorene. However, similar pretreatment with safrole produced increased urinary excretion of N-, 3- and 5-hydroxy derivatives. 3. Similar treatment with these two chemicals for 3 days increased ring-hydroxylation activity by rat liver microsomal material. Increases in N-hydroxylation were much less than those in ring-hydroxylation. Isosafrole was twice as effective as safrole. 4. Increases in hydroxylating activity due to safrole or isosafrole treatment were inhibited by simultaneous administration of ethionine. Similarly, ethionine inhibition was almost completely reversed by the simultaneous administration of methionine. 5. Safrole or isosafrole (0.1mm and 1mm) inhibited 7-hydroxylation activity by liver microsomal material from control rats. At 1mm these two chemicals inhibited both 5- and 7-hydroxylation activity by liver microsomal material from 3-methylcholanthrene-pretreated rats. 3-Hydroxylation activity was not inhibited by 1mm concentrations of these two chemicals. 6. A single injection of safrole (50100 or 200mg/kg body wt.) 24h before assay had no appreciable effect on either N- or ring-hydroxylation activity by hamster liver microsomal material. However, isosafrole (200mg/kg body wt.) treatment inhibited N-, 3- and 5-hydroxylation activities by hamster liver microsomal material; it had no effect on 7-hydroxylation activity.

Long E.L., Nelson A.A., Fitzhugh O.G. & Hansen W. H. (1963) "Liver tumors produced in rats by feeding safrole." *Archives of Pathology* **75**, 395-604.

Long E.L. & Jenner P.M. (1963) "Esophageal tumors produced in rats by the feeding of dihydrosafrole." *Federation Proceedings of the Federation of American Societies for Experimental Biology*, **2**, 275.

Lu L.J., Disher R.M. & Randerath K. (1986). "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. & Randerath K. (1986). "<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.

Luo G. & Guenther T.M. (1996). "Covalent binding to DNA in vitro of 2',3'-oxides derived from allylbenzene analogs." *Drug Metab. Dispos.* **24**, 1020–1027. [N.B. Erratum appears in *Drug Metab Dispos* **25**(1), 131].

McKinney, J. D. *et al.* (1972) "On the mechanism of formation of Mannich bases as safrole metabolites," *Bull. Environ. Contamin. Toxicol.* **7**, 305-310.

McPherson F.J., Bridges J.W. & Parke D.V. (1976) "The effects of benzopyrene and safrole on biphenyl 2-hydroxylase and other drug-metabolizing enzymes." *Biochem J.* **154**(3), 73–780. **Abstract.** A study was made of the nature and specificity of the increase in biphenyl 2-hydroxylase activity after preincubation of liver microsomal preparations with various carcinogens in vitro. This enhancement of enzyme activity in vitro was investigated in mouse, hamster and rat, and although the rat appears to be atypical in the variation of the pattern of 2- and 4-hydroxylation with age, similar enhancements were detectable in each species examined. An increase in biphenyl 2-hydroxylase activity was apparent 2h after intraperitoneal administration of safrole or benzopyrene to mature Wistar albino rats and appeared to be similar in nature to that observed after preincubation of liver microsomal preparations with the same chemical in vitro. Investigation of other drug-metabolizing enzyme systems suggests that the enhancing effects of carcinogens in vitro are specific for biphenyl 2-hydroxylase. No correlation between the enhancement of biphenyl 2-hydroxylase and inhibition of biphenyl 4-hydroxylase was apparent.

Miller E.C., Miller J.A., Boberg E.W., Delclos K.B., Lai C.C., Fennell T.R., Wiseman R.W. & Liem A. (1985) "Sulfuric acid esters as ultimate electrophilic and carcinogenic metabolites of some alkenylbenzenes and aromatic amines in mouse liver." *Carcinog. Compr. Surv.* **10**, 93–107.

Miller J.A. & Miller E.C. (1983) "The metabolic activation and nucleic acid adducts of naturally-occurring carcinogens: recent results with ethyl carbamate and the spice flavors safrole and estragole." *Br J Cancer* **48**(1), 1-15. **Abstract.** A small (approximately 30) but varied group of organic and inorganic compounds appear to be carcinogenic in both humans and experimental animals. A much larger number and wider variety of chemical carcinogens, primarily synthetic organic compounds, are known for experimental animals. These agents include a small (approximately 30) and varied group of metabolites of green plants and fungi. Many more of these carcinogens must exist in the living world. As with the synthetic carcinogens, the majority of these naturally occurring carcinogens are procarcinogens that require metabolic conversion into reactive electrophilic and mutagenic ultimate carcinogens. These strong electrophiles combine covalently and non-enzymatically with nucleophilic sites in DNAs, RNAs, proteins, and small molecules in target tissues. One or more of the DNA adducts appear to initiate carcinogenesis in an irreversible manner. The subsequent promotion step leading to gross tumours may be completed by further administration of

carcinogen or by treatment with non-carcinogenic promoters. Roles for the RNA and protein adducts in the carcinogenic process have not been excluded. Recent data on the metabolic activation and reactivity in vivo of the naturally occurring carcinogens ethyl carbamate and certain of the alkenylbenzene spice flavours are illustrative of these principles. These agents can initiate the carcinogenic process in male mouse liver with small doses given prior to weaning. Subsequent growth of the liver and male hormonal factors appear to function as promoters leading to gross hepatic tumors after one year. Reactive electrophilic metabolites of ethyl carbamate and of safrole and estragole and their nucleic acid adducts formed during initiation in mouse liver have been characterized.

Miller E.C., Swanson A.B., Phillips D.H., Fletcher T.L., Liem A. & 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**(3), 1124-34. [Abstract](#). Twenty-three naturally occurring and synthetic alkenylbenzene derivatives structurally related to the hepatocarcinogen safrole (1-allyl-3,4-methylenedioxybenzene) were assayed for their hepatocarcinogenicity in mice. Some of these compounds (safrole, estragole, eugenol, anethole, methyleugenol, myristicin, elemicin, and dill and parsley apiols) may be ingested in very small amounts by human as natural components of certain spices, essential oils, or vegetables. Estragole (1-allyl-4-methoxybenzene) and its proximate carcinogenic metabolite 1'-hydroxyestragole, previously shown to induce hepatic tumors when administered to male CD-1 mice only during the preweaning period, also induced hepatic tumors on administration for 12 months in the diet of female CD-1 mice. Eugenol (1-allyl-4-hydroxy-3-methoxybenzene) and anethole (trans-4-methoxy-1-propenylbenzene) were inactive in this assay; they were also inactive when administered i.p. during the preweaning period at total doses of up to 9.45  $\mu\text{mol}/\text{mouse}$  to male CD-1 or C57BL/6 x C3H F1 (hereafter called B6C3F1) mice. Methyleugenol (1-allyl-3,4-dimethoxybenzene) and its 1'-hydroxy metabolite had activities similar to those of estragole and its 1'-hydroxy metabolite for the induction of hepatic tumors in male B6C3F1 mice treated prior to weaning; 1-allyl-1'-hydroxy-4-methoxynaphthalene was somewhat less active. At the levels tested, myristicin (1-allyl-5-methoxy-3,4-methylenedioxybenzene), elemicin (1-allyl-3,4,5-trimethoxybenzene) and its 1'-hydroxy metabolite, dill apiol (1-allyl-2,3-dimethoxy-4,5-methylenedioxybenzene), parsley apiol (1-allyl-2,5-dimethoxy-3,4-methylenedioxybenzene), 1'-hydroxyallylbenzene, 3'-hydroxyanethole, and benzyl and anisyl alcohols had no detectable activity for the initiation of hepatic tumors on administration to male mice prior to weaning. The acetylenic derivative 1'-hydroxy-2',3'-dehydroestragole was much more active than either 1'-hydroxysafrole or 1'-hydroxyestragole when administered to preweanling mice. The 2',3'-oxides of safrole, estragole, eugenol, and 1'-hydroxysafrole, which are metabolites of these alkenylbenzenes, had little or no activity in this test. The 2',3'-oxides of safrole and estragole and their 1'-hydroxy derivatives likewise had little or no activity for the induction of lung adenomas in female A/J mice or for the induction of tumors on repetitive injections s.c. in male Fischer rats. However, the 2',3'-oxides of safrole, estragole, eugenol, 1'-hydroxysafrole, and 1'-

hydroxyestragole, when administered topically to female CD-1 mice at relatively high doses, initiated benign skin tumors that could be promoted with croton oil.

Miller E.C. *et al.* (1979) "The metabolic activation of safrole and related naturally occurring alkylbenzenes in relation to carcinogenesis by these agents." In: Naturally occurring carcinogens-mutagens and modulators of carcinogenesis. *Proceedings of 9<sup>th</sup> International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo University, Park Press, Baltimore.*

Munerato M.C., Sinigaglia M., Reguly M.L. & de Andrade H.H. (2005) "Genotoxic effects of eugenol, isoeugenol and safrole in the wing spot test of *Drosophila melanogaster*." *Mutat Res.* **582**(1-2), 87-94. [Abstract](#). In the present study, the phenolic compounds eugenol, isoeugenol and safrole were investigated for genotoxicity in the wing spot test of *Drosophila melanogaster*. The *Drosophila* wing somatic mutation and recombination test (SMART) provides a rapid means to evaluate agents able to induce gene mutations and chromosome aberrations, as well as rearrangements related to mitotic recombination. We applied the SMART in its standard version with normal bioactivation and in its variant with increased cytochrome P450-dependent biotransformation capacity. Eugenol and safrole produced a positive recombinagenic response only in the improved assay, which was related to a high CYP450-dependent activation capacity. This suggests, as previously reported, the involvement of this family of enzymes in the activation of eugenol and safrole rather than in its detoxification. On the contrary, isoeugenol was clearly non-genotoxic at the same millimolar concentrations as used for eugenol in both the crosses. The responsiveness of SMART assays to recombinagenic compounds, as well as the reactive metabolites from eugenol and safrole were considered responsible for the genotoxicity observed.

National Toxicology Program (2002) "Safrole". *Rep Carcinog.* **10**, 213-4.

Oswald E.O., Fishbein L., Corbet B. J., & Walker M. P. (1971) "Identification of Tertiary Aminomethylenedioxypropiophenones as urinary metabolites of safrole in the rat and guinea pig." *Biochim. Biophys. Acta* **230**, 237-247.

Parke D. V. & Rahman, H. (1970) "The induction of hepatic microsomal enzymes by safrole," *Biochem. J.*, **119**, 53.

Parke D. V. & Rahman, H. (1971) "Induction of a new hepatic microsomal haemoprotein by safrole and isosafrole" *Biochem. J.* **123**, 9.

Peele J.D. & Oswald E.O. (1978). "Metabolism of the proximate carcinogen 1'-hydroxysafrole and the isomer 3'-hydroxyisosafrole." *Bull. Environ. Contam. Toxicol.* **19**, 396-402.

Phillips D.H. (1994) "DNA adducts derived from safrole, estragole and related compounds, and from benzene and its metabolites." *IARC Sci Publ.* 1994 (125), 131-40.

Phillips D.H. *et al.* (1998) “<sup>32</sup>P post-labelling analysis of DNA adducts found in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes II. Newborn male B6C3F1 mice.” *Carcinogenesis* **5**(12), 1623-1628.

Phillips D.H., Miller J.A., Miller E.C. & Adams B. (1981) "N2 atom of guanine and N6 atom of adenine residues as sites for covalent binding of metabolically activated 1'-hydroxysafrole to mouse liver DNA in vivo." *Cancer Res.* **41**(7), 2664-71. [Abstract](#). Administration of 1'-[2'-3'-3H]hydroxysafrole to adult female mice resulted in the formation of DNA-, ribosomal RNA-, and protein-bound adducts in the liver that reached maximum levels within 24 hr. The levels of all three macromolecule-bound adducts decreased rapidly between 1 and 3 days after injection, at which time the amounts of the DNA-bound adducts essentially plateaued at approximately 15% of the maximum level. The amounts of the protein and ribosomal RNA adducts were very low by 20 days. Comparison by high-performance liquid chromatography of the deoxyribonucleoside adducts obtained from the hepatic DNA with those formed by reaction of deoxyguanosine and deoxyadenosine with 1'-acetoxysafrole, 1'-hydroxysafrole-2',3'-oxide, and 1'-oxosafrole indicated that the four in vivo adducts studied were derived from an ester of 1'-hydroxysafrole. Three of the four in vivo adducts comigrated with adducts formed by reaction of 1'-acetoxysafrole with deoxyguanosine; the fourth adduct comigrated with the major product of the reaction of this ester with deoxyadenosine. Adduct formation in vivo at low levels by the other two electrophilic metabolites was not excluded. The three adducts obtained by reaction of 1'-acetoxysafrole with deoxyguanosine appeared to be substituted on the 2-amino group of the guanine residue on the basis of their partitions between aqueous buffer solutions and 1-butanol:ethyl ether as a function of pH and their retention of 3H from [8-3H]deoxyguanosine. The corresponding three adducts derived from the hepatic DNA of mice given 1'-[2',3'-3H]hydroxysafrole had pH partition patterns not significantly different from the three adducts formed in vitro. Adduct II was further characterized from its nuclear magnetic resonance spectrum as N2-(trans-isosafrol-3'-yl)deoxyguanosine. Adduct IV, derived from the reaction of 1'-acetoxysafrole with deoxyadenosine 5'-phosphate, was characterized in the same manner as N6-(trans-isosafrol-3'-yl)deoxyadenosine.

Randerath K., Putman K.L. & Randerath E. (1993) “Flavor constituents in cola drinks induce hepatic DNA adducts in adult and fetal mice.” *Biochem. Biophys. Res. Commun.* **192**, 61–68.

Randerath K., Haglund R.E., Phillips D.H. & Reddy M.V. (1984) “<sup>32</sup>P-post-labelling 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**(12), 1613-22 [Abstract](#). The binding of a series of alkenylbenzenes to liver DNA of adult female CD-1 mice, isolated 24 h after i.p. administration of non-radioactive test compound (2 or 10 mg/mouse), was investigated by a modified <sup>32</sup>P-post-labelling assay. The known hepatocarcinogens, safrole, estragole and methyleugenol, exhibited the

strongest binding to mouse-liver DNA (1 adduct in 10 000 - 15 000 DNA nucleotides or 200 - 300 pmol adduct/mg DNA after administration of a 10 mg dose), while several related compounds, which have not been shown thus far to be carcinogenic in rodent bioassays, bound to mouse-liver DNA at 3 - 200x lower levels. The latter compounds included allylbenzene, anethole, myristicin, parsley apiol, dill apiol and elemicin. Eugenol did not bind. Low binding to mouse-liver DNA was also observed for the weak hepatocarcinogen, isosafrole. Two main <sup>32</sup>P-labelled adducts, which appeared to be guanine derivatives, were detected for each of the binding chemicals on thin-layer chromatograms. The loss of safrole adducts from liver DNA was biphasic: a rapid loss during the first week (t<sub>1/2</sub> approximately 3 days) was followed by a much slower decline up to 20 weeks after treatment (t<sub>1/2</sub> approximately 2.5 months). Adducts formed by reaction of 1'-acetoxysafrole, a model ultimate carcinogen, with mouse-liver DNA *in vitro* were chromatographically identical to safrole-DNA adducts formed *in vivo*. Pretreatment with pentachlorophenol, a known inhibitor of sulphotransferases, inhibited the binding of safrole to mouse-liver DNA, providing further evidence that the metabolic activation of the allylbenzenes proceeds by the formation of 1'-hydroxy derivatives as proximate carcinogens and 1'-sulphoöxy derivatives as ultimate carcinogens.

Rietjens I.M., Boersma M.G., van der Woude H., Jeurissen S.M., Schutte M.E. & Alink G.M. (2005) "Flavonoids and alkenylbenzenes: mechanisms of mutagenic action and carcinogenic risk." *Mutat Res.* **574**(1-2), 124-38. [Abstract](#). The present review focuses on the mechanisms of mutagenic action and the carcinogenic risk of two categories of botanical ingredients, namely the flavonoids with quercetin as an important bioactive representative, and the alkenylbenzenes, namely safrole, methyleugenol and estragole. For quercetin a metabolic pathway for activation to DNA-reactive species may include enzymatic and/or chemical oxidation of quercetin to quercetin ortho-quinone, followed by isomerisation of the ortho-quinone to quinone methides. These quinone methides are suggested to be the active alkylating DNA-reactive intermediates. Recent results have demonstrated the formation of quercetin DNA adducts in exposed cells *in vitro*. The question that remains to be answered is why these genotoxic characteristics of quercetin are not reflected by carcinogenicity. This might in part be related to the transient nature of quercetin quinone methide adducts, and suggests that stability and/or repair of DNA adducts may need increased attention in *in vitro* genotoxicity studies. Thus, *in vitro* mutagenicity studies should put more emphasis on the transient nature of the DNA adducts responsible for the mutagenicity *in vitro*, since this transient nature of the formed DNA adducts may play an essential role in whether the genotoxicity observed *in vitro* will have any impact *in vivo*. For alkenylbenzenes the ultimate electrophilic and carcinogenic metabolites are the carbocations formed upon degradation of their 1'-sulfoöxy derivatives, so bioactivation of the alkenylbenzenes to their ultimate carcinogens requires the involvement of cytochromes P450 and sulfotransferases. Identification of the cytochrome P450 isoenzymes involved in bioactivation of the alkenylbenzenes identifies the groups within the population possibly at increased risk, due to life style factors or genetic polymorphisms leading to rapid

metaboliser phenotypes. Furthermore, toxicokinetics for conversion of the alkenylbenzenes to their carcinogenic metabolites and kinetics for repair of the DNA adducts formed provide other important aspects that have to be taken into account in the high to low dose risk extrapolation in the risk assessment on alkenylbenzenes. Altogether the present review stresses that species differences and mechanistic data have to be taken into account and that new mechanism- and toxicokinetic-based methods and models are required for cancer risk extrapolation from high dose experimental animal data to low dose carcinogenic risks for man.

Rietjens I.M., Martena M.J., Boersma M.G., Spiegelberg W. & Alink GM. (2005) "Molecular mechanisms of toxicity of important food-borne phytotoxins. *Mol. Nutr. Food Res.* 49, 131–158."

Schiestl R.H., Chan W.S., Gietz R.D., Mehta R.D. & Hastings P.J. (1989) "Safrole, eugenol and methyleugenol induce intrachromosomal recombination in yeast." *Mutat. Res.* **224**(4), 427-436. [Abstract](#). Deletion of an integrated plasmid, a specific type of intrachromosomal recombination, was evaluated for inducibility with the phenylpropenes safrole, eugenol and methyleugenol in the yeast *Saccharomyces cerevisiae*. These phenylpropenes are found in food products, spices, pharmaceuticals and clove cigarettes. Safrole and eugenol are known carcinogens in animals and methyleugenol is a suspected carcinogen. These phenylpropenes are not detectable by the Ames assay and most other short-term tests used currently in predictive carcinogenesis. Like safrole, which has been shown to be nonmutagenic with the Ames assay, eugenol and methyleugenol were found to be nonmutagenic with the Ames assay. In contrast, with the yeast assays which screen for intra- and inter-chromosomal recombination of logarithmic phase cultures, all 3 compounds gave a positive dose-related response. These results demonstrate further that the yeast system can be modified easily to detect various genetic endpoints and that it deserves serious consideration as a test system for predictive carcinogenesis.

Sekizawa J. & Shibamoto J. (1982). "Genotoxicity of safrole-related chemicals in microbial test systems." *Mutat. Res.*, **101**, 127-140. [Abstract](#). The genotoxicity of safrole, 9 compounds that are structurally similar to safrole (anethole, cinnamaldehyde, cinnamyl alcohol, estragole, methyl eugenol, eugenol, isoeugenol, isosafrole, piperonal), 5 essential oils, cassia oil, cinnamon bark oil, clove oil, fennel oil) which contain the chemicals tested, and 1 oleoresin was studied in 3 microbial test systems. Only anethole showed mutagenicity in the Ames Salmonella reversion assay. All chemicals except anethole, estragole and isosafrole were positive in the *Bacillus subtilis* DNA-repair test (Rec assay) without S9. All samples tested were negative in the *Escherichia coli* WP2 uvrA reversion test. The essential oils and pimenta oleoresin were positive in the DNA-repair test. The results obtained are discussed in relation to the nature of the problems encountered with each test method.

Seto T.A. & Kemp W. (1969) "Effects of alkylmethoxybenzene and alkylmethylenedioxybenzene essential oils on phenobarbital & ethanol sleeping time." *Arch Int Pharmacodyn* **180**, 232-240.

Stillwell W.G., Carman M.J., Bell L. & Horning, M.G., 1974. "The metabolism of safrole and 2',3'-epoxysafrole in the rat and guinea pig." *Drug Metabol. Dispos.*, **2**, 489-498. [Abstract](#). The metabolism of safrole (4-allyl-1,2-methylenedioxybenzene) and safrole epoxide [1,2-methylenedioxy-4-(2,3-epoxypropyl)benzene] was studied in the rat and guinea pig. After intraperitoneal administration of safrole, the major urinary metabolites identified by gas chromatography and mass spectrometry were 1,2-dihydroxy-4-allylbenzene, 1,2-methylenedioxy-4-(1-hydroxyallyl)benzene, 1,2-methylenedioxy-4-(2,3-dihydroxypropyl)benzene, 1,2-dihydroxy-4-(2,3-dihydroxypropyl)benzene, 2-hydroxy-3-(3,4-methylenedioxyphenyl)propanoic acid, and 3,4-methylenedioxybenzoylglycine. The conversion of the allyl side chain to a 2,3-dihydroxypropyl side chain probably involves safrole epoxide as an intermediate. After intraperitoneal administration of safrole epoxide, 1,2-methylenedioxy-4-(2,3-dihydroxypropyl)benzene, 1,2-dihydroxy-4-(2,3-dihydroxypropyl)benzene, 2-hydroxy-3-(3,4-methylenedioxyphenyl)propanoic acid, and 2-hydroxy-3-(3,4-dihydroxyphenyl)propanoic acid were identified in rat and guinea pig urine. A small amount of a triol [1,2-methylenedioxy-4-(1,2,3-trihydroxypropyl)benzene] was found only in rat urine. Unchanged safrole epoxide was also found in the urine of both species, indicating that the epoxide was sufficiently stable in vivo to circulate in the blood and to be excreted in urine.

Swanson A.B., Chambliss D.D., Blanquist J.C., Miller E.C. & Miller, J.A. (1979). "The mutagenicities of safrole, estragole, trans-anethole and some of their known or possible metabolites for *Salmonella typhimurium* mutants." *Mutat. Res.* **60**, 143-153. [Abstract](#). Safrole, estragole, anethole, and eugenol and some of their known or possible metabolites were tested for mutagenic activity for *S. typhimurium* TA1535, TA100, and TA98. Highly purified 1'-hydroxyestragole and 1'-hydroxysafrole were mutagenic (approximately 15 and 10 revertants/micromole, respectively) for strain TA100 in the absence of fortified liver microsomes; trans-anethole and estragole appeared to have very weak activity. 3'-Hydroxyanethole was too toxic for an adequate test. Supplementation with NADPH-fortified rat-liver microsomes and cytosol converted 3'-hydroxyanethole to a mutagen(s) and increased the mutagenic activities for strain TA100 of 1'-hydroxyestragole, 1'-hydroxysafrole, estragole, and anethole. No mutagenicity was detected for safrole or eugenol with or without added NADPH-fortified liver preparations. The electrophilic 2',3'-oxides of safrole, 1'-hydroxysafrole, 1'-acetoxysafrole, 1'-oxosafrole, estragole, 1'-hydroxyestragole, and eugenol showed dose-dependent mutagenic activities for strain TA1535 in the absence of fortified liver microsomes. These mutagenic activities ranged from about 330 revertants/micromole for 1'-oxosafrole-2',3'-oxide to about 7000 revertants/micromole for safrole-2',3'-oxide. The arylalkenes, their hydroxylated derivatives, or their epoxides did not show mutagenic activity for strain TA98, except for 1'-oxosafrole-2',3'-oxide, which had weak activity. Since the

arylalkenes are hydroxylated and/or epoxidized by hepatic microsomes, hydroxy and epoxide derivatives appear to be proximate and ultimate mutagenic metabolites, respectively, of the arylalkenes.

Swanson A.B., Miller EC & Miller J.A. (1971) "The side-chain epoxidation & hydroxylation of the hepatocarcinogens safrole and estragole and some related compounds by rat and mouse liver microsomes." *Biochim Biophys Acta* **673**, 504-516.

Tan D., Reiter R.J., Chen L., Poeggeler B., Manchester LC. & Barlow-Walden L.R. (1994) "Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by the carcinogen safrole." *Carcinogenesis* **15**(2), 215-218. [Abstract](#). Hepatic DNA adduct formation induced by the chemical carcinogen, safrole, was suppressed by both endogenous pineal melatonin release and by the exogenous administration of melatonin to rats. DNA damage after administration of 100 mg/kg safrole (i.p.) was measured by the PI enhanced 32P-postlabeling analysis method. The RAL (relative adduct labeling) x 107 of carcinogen modified DNA in the liver of untreated controls and in safrole treated animals killed during the day, at night, after pinealectomy and pinealectomy plus melatonin injection (0.15 mg/kg x 4 or a total of 0.6 mg/kg) was 0, 12.6 ± 0.75, 10.9 ± 0.72, 13.6 ± 1.12 and 5.7 ± 0.53 respectively. For the same groups of animals, circulating melatonin levels at the termination of the study were 31±3, 29 ± 2, 276 ±31, 24 ± 1 and 13 950 ± 1016 pg/ml serum respectively. The higher the melatonin concentration in the serum the lower was DNA adduct formation in the rat liver. Thus, high nocturnal levels of melatonin were protective against safrole-induced DNA damage. These findings indicate that the functional pineal gland plays an important role in oncostatic actions of carcinogens such as safrole. At physiological levels, melatonin seemed to prevent especially the formation of what was referred to as the NI DNA adduct. Melatonin's ability to suppress DNA adduct formation may relate to its inhibitory effect on a mixed function oxidase, cytochrome p-450, and on the recently identified hydroxyl radical scavenging capacity of the indole. The oncostatic action of melatonin is also suggested by its nuclear accumulation and DNA stabilization characteristics. At pharmacological levels melatonin is extremely potent in preventing DNA modification induced by the chemical carcinogen, safrole.

Tan D.X., Pöeggeler B., Reiter R.J., Chen L.D., Chen S., Manchester L.C., Barlow-Walden L.R. (1993) "The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole in vivo." *Cancer Lett.* **70**(1-2), 65-71. [Abstract](#). Melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole in a dose-dependent manner. Total DNA-adduct formation after in vivo administration of 300 mg/kg safrole measured by 32P-postlabeling analysis of carcinogen-modified DNA in rat liver was 36,751 +/- 2290 counts/min/10 micrograms DNA. Coadministration of 300 mg/kg safrole with either 0.2 mg/kg (low dose) or 0.4 mg/kg (high dose) melatonin reduced DNA-adduct formation induced by safrole to 22,182 +/- 987 counts/min/10 micrograms DNA and 462 +/- 283 counts/min/10 micrograms DNA, respectively. Circulating

melatonin concentrations at the termination of the study in safrole, low melatonin and high melatonin groups were 50 +/- 8, 3140 +/- 430 and 10,040 +/- 2610 pg/ml serum, respectively. The results suggest that melatonin protects against safrole associated DNA damage.

Taylor J.M., Jenner P.M. & Jones W.I. (1964). "A comparison of the toxicity of some allyl, propenyl, and propyl compounds in the rat." *Toxicol. Appl. Pharmacol.* **6**, 378-387.

To L.P., Hunt T.P. & Naderson M.E. (1982) "Mutagenicity of trans-anethole, estragole, eugenol, and safrole in the Ames *Salmonella typhimurium* assay." *Bull. Environm. Contain. Toxicol.* **28**, 647-654.

Ueng Y.F., Hsieh C.H., Don M.J., Chi C.W. & Ho L.K. (2004) "Identification of the main human cytochrome P450 enzymes involved in safrole 1'-hydroxylation." *Chem Res Toxicol.* **17**(8), 1151-6. [Abstract](#). Safrole is a natural plant constituent, found in sassafras oil and certain other essential oils. The carcinogenicity of safrole is mediated through 1'-hydroxysafrole formation, followed by sulfonation to an unstable sulfate that reacts to form DNA adducts. To identify the main cytochrome P450 (P450) involved in human hepatic safrole 1'-hydroxylation (SOH), we determined the SOH activities of human liver microsomes and *Escherichia coli* membranes expressing bicistronic human P450s. Human liver (n = 18) microsomal SOH activities were in the range of 3.5-16.9 nmol/min/mg protein with a mean value of 8.7 +/- 0.7 nmol/min/mg protein. In human liver (n = 3) microsomes, the mean K(m) and V(max) values of SOH were 5.7 +/- 1.2 mM and 0.14 +/- 0.03 micromol/min/nmol P450, respectively. The mean intrinsic clearance (V(max)/K(m)) was 25.3 +/- 2.3 microL/min/nmol P450. SOH was sensitive to the inhibition by a CYP2C9 inhibitor, sulfaphenazole, and CYP2E1 inhibitors, 4-methylpyrazole and diethyldithiocarbamate. The liver microsomal SOH activity showed significant correlations with tolbutamide hydroxylation (r = 0.569) and chlorzoxazone hydroxylation (r = 0.770) activities, which were the model reactions catalyzed by CYP2C9 and CYP2E1, respectively. Human CYP2C9 and CYP2E1 showed SOH activities at least 2-fold higher than the other P450s. CYP2E1 showed an intrinsic clearance 3-fold greater than CYP2C9. These results demonstrated that CYP2C9 and CYP2E1 were the main P450s involved in human hepatic SOH.

Ueng Y.F., Hsieh C.H. & Don M.J.. (2005) "Inhibition of human cytochrome P450 enzymes by the natural hepatotoxin safrole." *Food Chem Toxicol.* **43**(5), 707-12. [Abstract](#). The hepatotoxin, safrole is a methylenedioxy phenyl compound, found in sassafras oil and certain other essential oils. Recombinant cytochrome P450 (CYP, P450) and human liver microsomes were studied to investigate the selective inhibitory effects of safrole on human P450 enzymes and the mechanisms of action. Using *Escherichia coli*-expressed human P450, our results demonstrated that safrole was a non-selective inhibitor of CYP1A2, CYP2A6, CYP2D6, CYP2E1, and CYP3A4 in the IC(50) order CYP2E1 < CYP1A2 < CYP2A6 < CYP3A4 < CYP2D6. Safrole strongly inhibited CYP1A2, CYP2A6, and CYP2E1 activities with IC(50) values less than 20 microM. Safrole

caused competitive, non-competitive, and non-competitive inhibition of CYP1A2, CYP2A6 and CYP2E1 activities, respectively. The inhibitor constants were in the order CYP1A2 < CYP2E1 < CYP2A6. In human liver microsomes, 50 microM safrole strongly inhibited 7-ethoxyresorufin O-deethylation, coumarin hydroxylation, and chlorzoxazone hydroxylation activities. These results revealed that safrole was a potent inhibitor of human CYP1A2, CYP2A6, and CYP2E1. With relatively less potency, CYP2D6 and CYP3A4 were also inhibited.

Vesselinovitch KVN, *et al.* (1979). "Transplacental and lactational carcinogenesis by safrole." *Cancer Res.* **39**, 4378-4380.

Weinberg, M. S. & Sternberg, S. S. (1966) "Effect of chronic safrole administration on hepatic enzymes and functional activity in dogs," *Toxicol. Appl. Pharmacol.* **8**, 2. **Cropwatch comments:** No tumors seen in dogs fed diet containing safrole for 7 years.

WHO *Safrole: WHO Food Additives Series 16.*

Wiseman R.W., Miller E.C., Miller J.A. & Liem A. (1987). "Structure-activity studies of the hepatocarcinogenicities of alkenylbenzene derivatives related to estragole and safrole on administration to preweanling male C57BL/6J x C3H/HeJ F1 mice." *Cancer Res.*, **47**, 2275-2283. **Abstract.** Further information on the structure-activity relationships among the synthetic and naturally occurring alkenylbenzene derivatives was obtained by examining their hepatocarcinogenicities for mice following administration of one or a few doses prior to weaning. Under these conditions preweanling male C3H/HeJ mice were more susceptible than male C57BL/6J mice or females of either strain to liver tumor induction by 1'-hydroxyestragole (1'-hydroxy-1-allyl-4-methoxybenzene) and 1'-hydroxyestragole (1'-hydroxy-1-allyl-3,4-methylenedioxybenzene). Male C57BL/6J x C3H/HeJ F1 mice given a single dose of 1'-hydroxyestragole at 12 days of age developed approximately twice as many hepatomas per liver as did those given the same dose per g of body weight at 1 day of age. The acetylenic compounds 1'-hydroxy-2',3'-dehydroestragole and 1'-hydroxy-2',3'-dehydroestragole were the most potent derivatives studied; they were 5- and 10-fold more potent (based on the average numbers of hepatomas per liver) than the corresponding allylic benzene derivatives. 1'-Acetoxyestragole and 1'-acetoxy safrole had activities similar to those of their respective 1'-hydroxy derivatives; estragole derivatives were consistently 2- to 3-fold more potent than the related safrole derivatives. 1'-Hydroxyelemicin (1'-hydroxy-1-allyl-3,4,5-trimethoxybenzene), its acetic acid ester 1'-oxoestragole, and 3'-bromo-trans-anethole (3'-bromo-1-trans-propenyl-4-methoxybenzene) each had very weak, but statistically significant, hepatocarcinogenic activity. The propenylic derivatives cis-anethole, trans-isosafrole, 1:1 cis, trans-isosafrole, 3'-hydroxy-trans-anethole, piperine, and trans-cinnamaldehyde showed no hepatocarcinogenic activity at the levels examined. In contrast, the propenylic derivatives cis- and trans-asarone (1-propenyl-2,4,5-trimethoxybenzene) were each active; the hepatocarcinogenicities of the asarones were not inhibited by prior administration of pentachlorophenol, a sulfotransferase inhibitor that abolished the

hepatocarcinogenicity of estragole under the same conditions. Furthermore, precocene II (6,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran), a cyclic propenylic plant metabolite and asarone analogue, showed strong hepatocarcinogenic activity similar to that of 1'-hydroxy-2',3'-dehydroestragole and 1'-hydroxy-2',3'-dehydrosafrole; precocene I (the 7-methoxy analogue of precocene II) was less active than precocene II but more active than cis-asarone.

Wiseman R.W., Fennell T.R., Miller J.A. & 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 mouse liver in vivo, including new adducts at C-8 and N-7 of guanine residues." *Cancer Res.* 45(7), 3096-105. [Abstract](#). The identities of the adducts formed on reaction of the model electrophilic and carcinogenic esters 1'-acetoxysafrole or 1'-acetoxiestragole with deoxyguanosine in vitro and those formed in vivo in the hepatic DNA of 12-day-old male C57BL/6 X C3H/He F1 (hereafter called B6C3F1) mice treated with 1'-hydroxysafrole or 1'-acetoxysafrole were investigated further with more discriminating high-performance liquid chromatography systems than previously used. The adducts formed from the reactions of 1'-acetoxysafrole or 1'-acetoxiestragole are strictly analogous and are distinguished by the prefixes S and E, respectively. Five adducts, including S(E)-II identified by Phillips et al. (*Cancer Res.*, **41**: 176-186, 2664-2671, 1981) as N2-(trans-isosafrol-3'-yl)deoxyguanosine and the analogous isoestragole derivative, have been characterized from the reactions with each ester. Adducts S-I and E-I, tentatively identified by Phillips *et al.* as N2-(safrol-1'-yl)- or N2-(estragol-1'-yl)deoxyguanosine, were each resolved into a pair of diastereomers. The proposed structures for each diastereomer were confirmed by nuclear magnetic resonance and circular dichroism spectroscopy. Two new adducts, i.e., S(E)-V and S(E)-VI, were isolated from each reaction mixture. On the basis of their pKas, their loss of 3H from [8-3H]deoxyguanosine, their retention of 3H from [1',2'-3H]deoxyguanosine, and their nuclear magnetic resonance spectra, Adducts S-V and E-V were characterized as 8-(trans-isosafrol-3'-yl)- and 8-(trans-isoestragol-3'-yl)deoxyguanosine, respectively. Adducts S-VI and E-VI were characterized in a similar manner as 7-(trans-isosafrol-3'-yl)- and 7-(trans-isoestragol-3'-yl)guanine, respectively. Adducts S-III and E-III, minor components described in the earlier studies, were not observed in the present work. High-performance liquid chromatography of hydrolysates of the hepatic DNA of male 12-day-old B6C3F1 mice killed 9 h after a single dose (0.1  $\mu\text{mol/g}$  body weight) of [2',3'-3H]-1'-hydroxysafrole showed that Adducts S-Ia, S-Ib, S-II, S-IV (identified by Phillips et al. as N6-(trans-isosafrol-3'-yl)deoxyadenosine), S-V, and S-VI were present at average levels of 3.5, 7.0, 24.4, 2.9, 1.2, and 3.6 pmol/mg DNA, respectively. Similar levels of these adducts were found in the hepatic DNA after administration of the same dose of [2',3'-3H]-1'-acetoxysafrole under identical conditions.

Wislocki P.G., Miller E.C., Miller J.A., McCoy E.C. & Rosenkranz H.S. (1977) "Carcinogenic and mutagenic activities of safrole, 1'-hydroxysafrole, and some known or possible metabolites." *Cancer Research* **37**, 1863-1891. [Abstract](#). The

carcinogenic and mutagenic activities of metabolites and possible metabolites of safrole and 1'-hydroxysafrole were investigated as guides to their importance as possible proximate or ultimate carcinogens. 1'-Acetoxysafrole and the 2',3'-oxides of safrole, 1'-hydroxysafrole, 1'-acetoxysafrole, and 1'-oxosafrole, were directly mutagenic for *Salmonella typhimurium* strains TA1535 and TA100. No significant mutagenicity was detected with safrole, isosafrole, 2',3'-dihydrosafrole, 1'-hydroxysafrole, 3'-hydroxyisosafrole, 3'-acetoxylisosafrole, or 1'-oxosafrole either with or without the addition of reduced nicotinamide adenine dinucleotide phosphate-fortified hepatic microsomes plus cytosol. Multiple topical applications to mice of 1'-hydroxysafrole-2',3'-oxide, followed by repetitive doses of croton oil, caused the formation of skin papillomas. Under the same conditions significant numbers of papillomas were not initiated by safrole, 1'-acetoxysafrole, 1'-hydroxysafrole, 1'-oxosafrole, safrole-2',3'-oxide, or 1'-acetoxysafrole-2',3'-oxide. 1'-Oxosafrole had little or no carcinogenic activity on p.o. administration to rats or on s.c. administration to preweanling mice or adult rats. The incidence of hepatocellular carcinomas in rats fed safrole was markedly increased by simultaneous administration of phenobarbital. 1'-Hydroxysafrole p.o. was more hepatotoxic and hepatocarcinogenic for adult female mice than for adult male mice. Injection of [2',3'-<sup>3</sup>H]-1'-hydroxysafrole i.p. yielded at least 10-fold greater levels of hepatic DNA-, ribosomal RNA-, and protein-bound derivatives in preweanling male of female mice and in adult female mice than in adult male mice. The levels of hepatic macromolecule-bound tritiated derivatives in adult mice were produced by prefeeding of nonradioactive 1'-hydroxysafrole prior to the administration of [3H]-1'-hydroxysafrole.

Wislocki P.G. *et al.* (1976). "The metabolic activation of the carcinogen 1'-hydroxysafrole *in vivo* and *in vitro* and the electrophilic reactivities of possible ultimate carcinogens." *Cancer Research* **36**, 1686-1695.

Wislocki P. G., Bordiert P., Miller E. C. & Miller J. A. (1972) "1'-Hydroxysafrole: A Proximate Carcinogenic Metabolite of Safrole." *Proc. Am. Assoc. Cancer Res.*, **13**, 2.

Zhou G.D., Moorthy B., Bi J., Donnelly K.C. & Randerath K. (2007) "DNA adducts from alkoxyallylbenzene herb and spice constituents in cultured human (HepG2) cells." *Environ Mol Mutagen.* **48**(9), 715-21. [Abstract](#). Alkoxy derivatives of allylbenzene, including safrole, estragole, methyleugenol, myristicin, dill apiol, and parsley apiol, are important herb and spice constituents. Human exposure occurs mainly through consumption of food and drinks. Safrole, estragole, and methyleugenol are weak animal carcinogens. Experimental data reveal the genotoxicity and/or carcinogenicity of some allylbenzenes; however, except for safrole, the potential capacity of allylbenzenes for forming adducts in human cellular DNA has not been investigated. In the present study, we have exposed metabolically competent human hepatoma (HepG2) cells to three concentrations (50, 150, and 450  $\mu$ M) of each of the six aforementioned allylbenzenes and shown by the monophosphate (<sup>32</sup>P)-postlabeling assay that each compound formed DNA adducts. With the exception of methyleugenol, DNA adduction was

dose dependent, decreasing in the order, estragole > methyleugenol > safrole approximately myristicin > dill apiol > parsley apiol. These results demonstrate that safrole, estragole, methyleugenol, myristicin, dill apiol, and parsley apiol are capable of altering the DNA in these cells and thus may contribute to human carcinogenesis.

### **Cinnamomum spp. & Safrole.**

Cheng B Q, Xu Y, Ma X X, Yu X J, Ding J K. (1996) "Study on the new economic plant of safrole of *Cinnamomum heyneanum* (Nees) H.W. Li et B.Q. Cheng." *J. Pl. Res. Environ.* **5** (4), 33-37.

Dung N.X., Moi L.D., Hung N.D. & Leclercq P.A. (1995) "Constituents of the essential oils of *Cinnamomum parthenoxylon* (Jack) Nees from Vietnam ." *JOER* **7**(1), 53-56. [Abstract](#). The essential oils obtained by steam distillation of the root bark and wood of *Cinnamomum parthenoxylon* (Jack) Nees growing wild in Vietnam were investigated by a combination of GC and GC/MS. More than 30 compounds in the root bark oil, and about 20 components in the wood oil have been identified. The main constituent of the root bark oil was benzyl benzoate (52.0%), whereas the wood oil consisted mainly of safrole (90.3%). The oil yield and the safrole content of the wood oil from different geographic regions in Vietnam showed little variation.

Jantan I., Ayop N., Ali N.A.M., Ahmad A.S., Yalvema M.F., Muhammad K. & Azizi A.R. (2004) "The essential oils of *Cinnamomum rhyncophyllum* Miq. as natural sources of benzyl benzoate, safrole and methyl (E)-cinnamate." *Flav & Frag J.* **19**(3), 260-262. [Abstract](#). The leaf, bark and wood oils of *Cinnamomum rhyncophyllum* Miq. were investigated by gas chromatography on two columns of different polarity, retention indices and GC-MS. The oils were composed mainly of phenylpropanoids and benzylic compounds. The leaf oil may be a potential natural source of benzyl benzoate, since it constituted up to 77% of the oil. Other compounds present in appreciable amounts in the oil were -phellandrene (6.3%) and methyl (E)-cinnamate (4.2%). The bark and wood oils were found to possess compositional similarities with little variation in the levels of each component. Safrole (43.3-51.0%) and methyl (E)-cinnamate (40.5-43.1%) were the major constituents in these oils. However, the bark oil could be distinguished from the wood oil by containing a higher concentration of benzyl benzoate.

Jantan I. & Goh S.H. (1992) "Essential oils of *Cinnamomum* species from peninsular Malaysia" *JOER* **4**(2), 161-171. [Abstract](#). The chemical composition of the leaf, bark and wood oils of seven Malaysian *Cinnamomum* species (*C. pubescens*, *C. javanicum*, *C. iners*, *C. impressicostatum*, *C. mollissimum*, *C. porrectum* and *C. camphora*) was examined by co-chromatography with authentic samples on three columns of different polarity, capillary GC/MS and selective proton NMR. The major components of the leaf, bark and wood oils were identified. These species could be useful new natural sources for safrole, eugenol, linalool, camphor and benzyl benzoate, which are commercially important chemicals in the flavor, fragrance and pharmaceutical industries. The distribution and accumulation of the compounds in different parts of the plant

within the same species or among different species may be used for taxonomic purposes and for future use in the identification of possible varieties of cinnamomum species.

Jantan I. & Goh S.H. (1990) "The essential oils of *Cinnamomum mollissimum* as natural sources of safrole and benzyl benzoate." *J. Trop Forest Prod*, 1990

Reynertson K.A., Balick M.J., Lee R., Raynor W., Pelep Y. & Kennelly E.J. (2005) "A traditional method of *Cinnamomum carolinense* preparation eliminates safrole from a therapeutic Pohnpean tea." *Journal of Ethnopharmacology* **102**, 269–274. [Abstract](#). *Cinnamomum carolinense*, locally known as madeu, is a tree endemic to the volcanic mountains of the Island of Pohnpei in the Eastern Carolines of the South Pacific. The bark is harvested from trees and brewed to make a medicinal tea and hot beverage that is regularly consumed. Many species of *Cinnamomum* contain the known hepatocarcinogen safrole, sparking concern regarding habitual consumption of this beverage. HPLC-PDA analysis confirmed the presence of the carcinogen in alcoholic extracts of *Cinnamomum carolinense* bark shavings (0.435%, w/w), but safrole was not detected in the tea. The limit of detection and limit of quantitation of safrole were determined to be 1.25 and 3.75 µg/mL, respectively. The traditional preparation method, which boils the bark shavings, degrades the safrole.

#### **Mango ginger (*Curcuma amada* Roxb.) & Safrole.**

Shiva M.P. *et al.* (2002) *Aromatic & Medical Plants* Intl Book Distributors Dehra Dun 2002. [Cropwatch comments](#): The authors suggest that safrole occurs to 9.3% in the oil of *Curcuma amada*.

#### **Nutmeg (*Myristica fragrans* Houtt.) & Safrole.**

Baldry J., Dougan J., Matthews W. S., Nabney J., Pickering G. R. & Robinson F.V. (1976) "Composition and flavour of nutmeg oils." *International Flavours and Food Additives* 7 (1) 28-30. [Abstract](#). The composition of oils prepared by steam distillation of crushed nutmegs obtained from (i) Grenada and (ii) Indonesia was determined and results tabulated. The composition was also determined of oils from St. Vincent, Grenada (Malaysian seedlings), Indonesia, Papua, New Guinea and Penang, together with commercial oils from Indonesia and Singapore. Some 40 components were detected in (i) and (ii); 28 being identified by IR and 1 by retention time. 6 of the identified components had not been previously reported in nutmeg essential oil. Comparison of nutmeg oils of different geographical origin indicated that they were similar qualitatively with the same volatile components present in all the oils but that quantitative differences existed. Oils of W. Indian origin are low in α-pinene, safrole and myristicin, with higher amounts of sabinene. E. Indian oils have a characteristically high myristicin concn.

Buchanan R.L., Goldstein S. & Budroe J.D. (2006) "Examination of chili pepper and nutmeg oleoresins using the Salmonella/mammalian microsome mutagenicity assay." *J Food Sci* **47**(1), 330-331. [Abstract](#). Past investigations have suggested that various arylalkene spice compounds warrant further study as possible naturally occurring mutagens and/or carcinogens. The present study

carried out a detailed examination of nutmeg oleoresin, myristicin, chili pepper oleoresin, capsaicin, and vanillylamine for in vitro mutagenicity using the Salmonella/mammalian microsome mutagenicity assay. None of the materials tested displayed significant mutagenicity over a wide range of concentrations.

Ehlers D., Kirchoff J., Gerard D. & Quirin K.-W. (2002). "High-performance liquid chromatography analysis of nutmeg and mace oils produced by supercritical CO<sub>2</sub> extraction comparison with steam-distilled oils comparison of East Indian, West Indian and Papuan oils." *Int J Food Sc & Tech* 33(3), 215-223. [Abstract](#). Nutmeg and mace oils, produced by supercritical CO<sub>2</sub> extraction, are now increasingly traded. Unlike the steam-distilled oils, no information about their composition is published. Supercritical CO<sub>2</sub> nutmeg and mace extracts from the East Indies, West Indies and Papua were analysed by high-performance liquid chromatography (HPLC) for myristicin, safrole, elemicin, eugenol, methyleugenol, isoeugenol, methylisoeugenol, methoxyeugenol and isoelemicin, and were very similar to steam-distilled oils from the same batch. East Indian, West Indian and Papuan oils displayed clear differences in their composition and could be clearly characterized by HPLC. The dominating aromatic ether in the East Indian oils was myristicin, in West Indian elemicin and in Papuan safrole.

### **Piper spp. & Safrole.**

**Cropwatch comments:** The essential oil of the Mexical Pepperleaf *Piper auritum* Kunth (safrole content to 90%) which grows in the Central & South Americas, has been suggested as an alternative potential source of safrole

Gupta M.P. & Arias T.D. (1985) "Safrole the main component of the essential oil from *Piper auritum* of Panama" **48**, 2, 330-343.

Maia, J.G. *et al.* (1987) "Especies de *Piper* da Amazonica ricas em safrol." *Quimica Nova*, 10(3), 200-204.

Rocha S.F.R. & Ming L.C. (1999) "*Piper hispidinervum*: A sustainable source of safrole." In: *Perspectives on new crops and new uses*. J. Janick (ed.), ASHS Press, Alexandria, VA. (1999) pp. 479–481. [Abstract](#). *Piper hispidinervum* (C. DC.), Piperaceae, is a promising source of sassafras oil, the source of safrol, currently derived from endangered plants of the Lauraceae such as *Ocotea pretiosa* Ness (Mez.), *Cinamomum petrophilum*, *C. mollissimum*, and *Sassafras albidum* Nutt. The essential oil of *P. hispidinervum* contains high levels (83–93%) of safrole in leaves which can be easily extracted by hydrodistillation.

Wang C. K. & Sun-Hwang L. (1993). "Separation of safrole from *Piper betle* flower." *J. Chin. Agri. Chem. Soc.* **31**, 566-569.

Wang C. K. & Sun-Hwang L. (1993). "Analysis of the phenolic compounds in betel quid." *J. Chin. Agri. Chem Soc.* **31**, 623-632.

### **Sassafras Oils (*Sassafras albidum* (Nutt.) Nees & Brazilian Sassafras: *Ocotea pretiosa*) & Safrole.**

Bazzaz F. A., Paape V. & Boggess W. R. (1972) " Notes: Photosynthetic and Respiratory Rates of *Sassafras albidum*." *Forest Science* **18**(3), 218-222. [Abstract](#). Net photosynthesis and transpiration rates indicate that sassafras may be adapted to tolerate the relatively dry and warm environment of old fields as well as to shaded pine plantations. Sassafras photosynthesizes efficiently under high plant water stress and at low light intensities.

Bonner F.T. & Maisenhelder L.C. (1974). "*Sassafras albidum* (Nutt.) Nees, sassafras." In: Schopmeyer C.S., tech. coord. *Seeds of woody plants in the United States*. Agric. Handbk. 450. Washington, DC: USDA Forest Service: 761B762.

Carlson M. & Thompson R.D. (1997) "Liquid chromatographic determination of safrole in sassafras-derived herbal products." *J.A.O.A.C. Intern.* **80**(5), 1023-1028. [Abstract](#). A liquid chromatographic (LC) method was developed for determining safrole in herbal products derived from sassafras (*Sassafras albidum*), as well as related compounds such as isosafrole and dihydrosafrole. The procedure involves solvent extraction and isolation of analyte by reversed-phase LC with UV detection at 235 nm. Safrole is resolved from related compounds and other sample constituents including thymol, a component of thyme. A linear concentration range of 0.003-0.200 mg/mL was obtained for safrole, isosafrole, and dihydrosafrole. Limits of detection (LOD) and quantitation (LOQ) were 0.0015 and 0.0051 µg/mL for safrole, 0.0018 and 0.0061 µg/mL for isosafrole, and 0.0038 and 0.0125 µg/mL for dihydrosafrole, respectively. Intraday relative standard deviations (RSDs) for safrole (n=5) from various samples ranged from 1.30 to 5.39% at analyte levels of 0.01-1.5%. Safrole contents of 26 samples including root bark powder, leaves, oils, tea concentrate, herbal extract tinctures, and herbal powder capsules ranged from <LOD for most leaf samples to 92.4% for an oil. Recoveries of safrole from fortified samples ranged from 83.6% for an oil to 117.2% for a tincture preparation. Safrole contents of 0.09-4.66 mg/cup were found for brewed teas prepared from sassafras root bark powders and tinctures.

Cohen R. (1955) ""Two new coccidioidal fungicides. I. Oil of sassafras. II. Sodium salt of cinnamic acid." *Ariz. Med.* **12**(3), 106.

Coppen J.J.W. (1995) "Sassafras oil." In: *Non-wood forest products 1: Flavors and fragrances of plant origin*. Food and Agriculture Organization of the United Nations, Rome, Italy pp. 19–25.

Duke J. (1989) "*Sassafras albidum*" (Nutt.) (1989/ In *CRC Book of Medicinal Herbs* CRC Press Boca Raton FL. Pp430-1.

Gottlieb O.R. & Magalhaes M.T. (1960) "Physiological varieties of *Ocotea pretiosa*. II." *Perfumery and Essential Oil Record* **51**, 18-21.

Grande G.A. & Dannewitz S.R. (1987) "Symptomatic sassafras oil ingestion." *Vet Hum Toxicol* **29**(6), 447.

Griggs MM. (1990). "*Sassafras albidum* (Nutt.) Nees, sassafras." In: Burns R.M., Honkala B.H., Tech. Coords. *Silvics of North America*. Volume 2, Hardwoods. Agric. Handbk. 654. Washington, DC: USDA Forest Service: 773B777.

Haines J.D., Jr. (1991) "Sassafras tea and diaphoresis." *Postgraduate Medicine* **90**(4), 75-76

Heikes D.L. (1994) "SFE with GC and MS determination of safrole and related allylbenzenes in sassafras teas." *J Chromatogr Sci.* **32**(7), 253-8 [Abstract](#). Safrole (4-allyl-1,2-methylenedioxybenzene), a natural plant component of the aromatic oil of sassafras root bark, possesses carcinogenic and mutagenic activity. Legal restrictions have been placed on safrole as a food additive. However, sassafras teas continue to be accessible from health food establishments in the United States. Supercritical fluid extraction (SFE) with gas chromatographic-mass spectrometric (GC-MS) determination is utilized in the formulation of a rapid, accurate, and specific method for the determination of safrole and related allylbenzenes in unbrewed sassafras teas. Samples are extracted in a static-dynamic mode with CO<sub>2</sub> at 690 bar and 80 degrees C with methanol as an extractor-added modifier. Levels of safrole exceeding 10,000 mg/kg (1.0%) are commonly encountered. Lesser amounts of other allylbenzenes, including eugenol and 4-allyl-1,2-dimethoxybenzene, are also reported. Recoveries of safrole and related compounds from previously extracted tea samples fortified at 100 and 1000 mg/kg ranged from 96 to 101%.

Hickey M.J. (1948) "Investigation of the chemical constituents of Brazilian sassafras oil." *J Org Chem.* **13**(3), 443-6.

Kaler K.M. & Setzer W.N. (2008) "Seasonal Variation in the Leaf Essential Oil Composition of *Sassafras albidum*." *Natural Product Communications* **3**(5), 829-832

Kamdem D.P. & Gage D.A. (1995) "Chemical composition of essential oil from the root bark of *Sassafras albidum*." *Planta Med* **61**, 574–5. [Abstract](#). The root bark of *Sassafras albidum* (Nuttall) Nees (Lauraceae) was extracted at room temperature with hexane and chloroform as solvents. The isolated essential oils were analyzed with GC and GC/MS. Thirty compounds were identified, nine of which have not been previously reported from this species. The major compounds were safrole (85%), camphor (3.25%), and methyleugenol (1.10%). Ten sesquiterpenes were also identified.

Kapadia G.J., Chung E.B., Ghosh B., Shukla Y.N., Basak S.P., Morton J.F. & Pradhan S.N. (1978) "Carcinogenicity of some folk medicinal herbs in rats." *Natl Cancer Inst.* **60**(3), 683-6. [Abstract](#). Twelve medicinal herbs were bioassayed to correlate a high incidence of esophageal carcinoma in natives of different places with their habitual consumption of these products. Outbred NIH Black rats were given 72 weekly sc injections of the total aqueous extracts of the plant materials. The tannin rich plant extracts from *Areca catechu* and *Rhus copallina* produced local tumors in 100 and 33%, respectively, of the experimental animals. Other

materials included *Diospyros virginiana* and extracts from plants not rich in tannins. *Diospyros* and extracts of *Sassafras albidum* and *Chenopodium ambrosioides* were tumorigenic in over 50% of the treated animals.

Lawrence B.M. (1985) "Progress in essential oils. *Ocotea* oil." *Perfumer and Flavorist*, **10**(4), 48-51.

Migliorini G. (2008) "Sassafras oil, a key raw material now largely banned from the market." *Chimica Oggi* **26**(5), 59-61

Mollan T.R.M. (1961) "The essential oils of the sassafras laurels. I. *Ocotea pretiosa*, Brazilian sassafras, safrole type." *Perfumery and Essential Oil Record*, **51**, 284-286.

Newall C. *et al.* (1996) "Sassafras" In *Herbal Medicine* Pharmaceutical Press London pp 235-6.

Segelman A.B., *et al* (1976). "Sassafras and herb tea: potential health hazards." *JAMA* **236**(5), 477.

Sethi M.L. *et al.* (1979) "Identification of volatile constituents of *Sassafras albidum* root oil." *Phytochemistry* **15**, 1773-1775. [Abstract](#). Sassafras root bark oil was examined by GC-MS for the possible natural occurrence of 1'-hydroxysafrole a potent hepatocarcinogenic mammalian metabolite of safrole. Six monoterpenes, 2 sesquiterpenes, 6 allylbenzenes, 2 propenylbenzenes, 2 acroleins and 1 benzaldehyde derivative were identified. Eleven out of these 19 sassafras constituents are reported for the first time. However, 1'-hydroxysafrole was not detected.

Sicuranza J. Castrataro N. Johnson B. & Maynard, B. (2005) "Softwood Cutting Propagation of Native Lauraceae (*Lindera benzoin* and *Sassafras albidum*) as Alternatives to Invasive Horticulture Plants." *Combined Proceedings - International Plant Propagators Society* **55**, 415-6.

Simić A, Soković MD, Ristić M, Grujić-Jovanović S, Vukojević J, Marin PD.(2004) "The chemical composition of some Lauraceae essential oils and their antifungal activities." *Phytother Res.* **18**(9), 713-7. [Abstract](#). The antifungal activity of *Aniba rosaeodora*, *Laurus nobilis*, *Sassafras albidum* and *Cinnamomum zeylanicum* essential oils were investigated against 17 micromycetes. Among the tested fungal species were food poisoning, spoilage fungi, plant and animal pathogens. In order to determine fungistatic and fungicidal concentrations (MIC and MFC) macrodilution and microdilution tests were used. Linalool was the main component in the essential oil of *A. rosaeodora*, while 1.8-cineole was dominant in *L. nobilis*. In sassafras essential oil safrole was the major component and in the oil of *C. zeylanicum* the main component was *trans*-cinnamaldehyde. The essential oil of cinnamon showed the strongest antifungal activity.

Tucker AO, Maciarello MJ, Broderick CE. (1994) "File and the essential oils of the leaves, twigs, and commercial root teas of *Sassafras albidum* (Nutt.) Nees

(Lauraceae)" In: Amsterdam: Elsevier Science B.V., 1994 xv, 764p. ISBN 444817611

Zwaving, J.H. (1966) "Composition of the essential oil from the root of *Sassafras albidum* (Nutt.) Nees." *JOER* **8**(2), 193-5. [Abstract](#). The essential oils of *Sassafras albidum*, obtained by hydrodistillation of the root bark and root wood, were analyzed by GC and GC/MS. The main component of the root bark and root wood oils was safrole (61.29-88.80%). Both oils contained the same constituents although there were some quantitative differences

### Isosafrole

Botelho L. H. *et al.* (1982) "Amino-terminal and carboxy-terminal sequence of hepatic microsomal cytochrome P-450d, a unique hemoprotein from rats treated with isosafrole." *Biochemistry* **21**, 1152-5.

Dickins M., Elcombe C.R., Moloney S.J., Netter K.J. & Bridges J.W. (1979) "Further studies on the dissociation of the isosafrole metabolite-cytochrome P-450 complex." *Biochem Pharmacol.* **28**(2), 231-8

Fennell T.R., Dickins M., Bridges J.W. (1979) "Interaction of isosafrole in vivo with rat hepatic microsomal cytochrome P-450 following treatment with phenobarbitone or 20-methylcholanthrene." *Biochem Pharmacol.* **28**(8),1427-9.

Howes A.J., Chan V.S.W. & Caldwell J. (1990) "Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis assay in rat hepatocytes." *Food & Chemical Toxicology* **28**(8), 537-542.

Klungsoyr J. & Scheline R.R. (1982). "Metabolism of isosafrole and dihydrosafrole in the rat." *Biomed. Mass Spectrom.* **9**, 323-329.

Murray M. & Reidy G.F. (1989). "In vitro formation of an inhibitory complex between an isosafrole metabolite and rat hepatic cytochrome P-450 PB-B." *Drug Metab. Dispos.* **17**, 449-454.

Naves Y.-R. & Ardizio P. (1957). "Études sur les matières végétales volatiles CXLVII(I). Sur les cis et trans-isosafroles." *Bull. Soc. Chim. France.* 1053-1057.

Ohyama, T *et al.* (1984) "Isosafrole-induced cytochrome P2-450 in DBA/2N mouse liver. Characterization and genetic control of induction." *J Biol Chem* **259**, 2675-82

Randerath K., Haglund R.E., Phillips D.H. & Reddy M.V. (1984) "<sup>32</sup>P-post-labelling 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**(12), 1613-22 [Abstract](#). The binding of a series of alkenylbenzenes to liver DNA of adult female CD-1 mice, isolated 24 h after i.p. administration of non-radioactive test compound (2 or 10 mg/mouse), was investigated by a modified <sup>32</sup>P-post-labelling assay. The known hepatocarcinogens, safrole, estragole and methyleugenol, exhibited the

strongest binding to mouse-liver DNA (1 adduct in 10 000 - 15 000 DNA nucleotides or 200 - 300 pmol adduct/mg DNA after administration of a 10 mg dose), while several related compounds, which have not been shown thus far to be carcinogenic in rodent bioassays, bound to mouse-liver DNA at 3 - 200x lower levels. The latter compounds included allylbenzene, anethole, myristicin, parsley apiol, dill apiol and elemicin. Eugenol did not bind. Low binding to mouse-liver DNA was also observed for the weak hepatocarcinogen, **isosafrole**. Two main <sup>32</sup>P-labelled adducts, which appeared to be guanine derivatives, were detected for each of the binding chemicals on thin-layer chromatograms. The loss of safrole adducts from liver DNA was biphasic: a rapid loss during the first week (t<sub>1/2</sub> approximately 3 days) was followed by a much slower decline up to 20 weeks after treatment (t<sub>1/2</sub> approximately 2.5 months). Adducts formed by reaction of 1'-acetoxysafrole, a model ultimate carcinogen, with mouse-liver DNA in vitro were chromatographically identical to safrole-DNA adducts formed in vivo. Pretreatment with pentachlorophenol, a known inhibitor of sulphotransferases, inhibited the binding of safrole to mouse-liver DNA, providing further evidence that the metabolic activation of the allylbenzenes proceeds by the formation of 1'-hydroxy derivatives as proximate carcinogens and 1'-sulphoöxy derivatives as ultimate carcinogens.

Ryan D.E., Thomas P.E. & Levin W. (1980). Hepatic microsomal cytochrome P-450 from rats treated with isosafrole. *J. Biol. Chem.*, **255**, 7941-7955.

### **Dihydrosafrole**

Reuber, M. D. (1979) "Neoplasms of the forestomach in mice ingesting dihydrosafrole," *Digestion* **19**, 42-47