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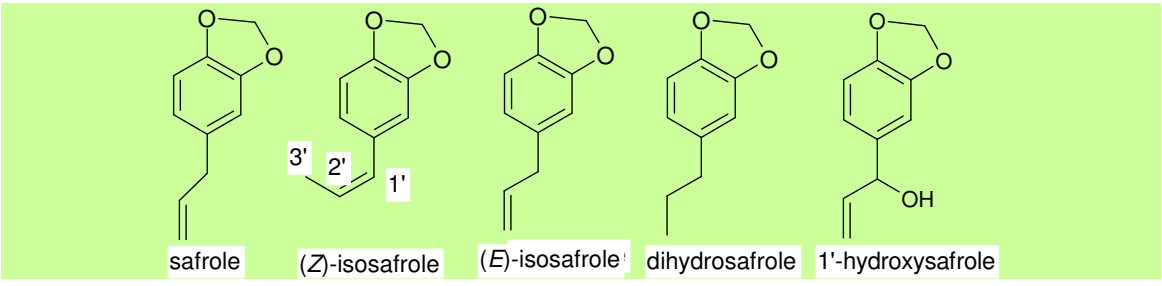
Safrole: Human Carcinogenicity Risk Over-Stated?

Pre-amble.

It almost borders on the heretical, perhaps, to suggest that the risk of human carcinogenicity from exposure to dietary safrole has been over-estimated over the years by some toxicologists, and that the existing national & international restrictions on safrole-containing ingredients & end-products can be seen as over-precautious. Weighing the evidence, a convincing case can be made that the human carcinogenic potential of safrole, if not quite negligible at low doses, is considerably less than that of ethanol (Duke 2002). As it is, the existing evidence for the carcinogenicity and genotoxicity of safrole mainly rests on a battery of experiments performed 30-40 years ago, on laboratory rodents dosed with high levels of safrole, where electrophilic metabolites generated by P450 enzymes and sulphurotransferases are identifiable as being responsible for the genotoxicity (see Cropwatch's extensive *Safrole Bibliography* at http://www.cropwatch.org/Safrole%20Bibliography.pdf). Different expert judgments have been made about the risk to humans from alkylbenzenes such as safrole, methyleugenol & estragole, and indeed on the relative importance for human cancer of low-dose exposures to synthetic chemicals generally (Gold *et al*. 1992). More insight into bioactivation of these (alkylbenzene) compounds in humans has been said to be required to interpret animal data to the human situation (Jeurissen 2007).



Safrole (4-allyl-1,2-methylenedioxybenzene; CAS No. 94-59-7) is known to occurs in the following natural products:

Chinese Angelica (*Angelica sinensis* L.)
Betel oil (*Piper betle* L.)
Brown & yellow camphor oil (fractions of *Cinnamomum camphora* L.) Yellow oil to 20%; brown oil to 80%.
Cangerana oil (*Cabralea cangerana* Saldanha)
Cinnamon leaf oil & bark oils (*Cinnamomum zeylanicum* Blume) both to 2%
Kuromoji oil (*Lindera* spp.) to 12%
Mace oil (*Myristica fragrans* Houtt.) to 2%
Mango ginger oil (*Curcuma amada* Roxb.) to 9.5%
Nutmeg oils [E.I. & W.I.], butter & oleoresins (*Myristica fragrans* Houtt.) E.I, oil to 2%; W.I. oil to 0.3%.
Pepper oil, black (*Piper nigrum* L.)
Piper auritum HBK oil to 90%
Sassafras oils, bark of roots, infusions of roots (*Sassafras albidum* (Nutt.) Nees to 95%.
Sassafras oil Brazilian: *Ocotea pretosia* (Nees) Mez, to 92%
Star Anise oil (*Illicium verum* Hook f.)
Ylang-ylang oils, absolutes (*Cananga odorata* (DC) Hook. f et Thoms subsp. *genuine*)

…as well as in several other *Cinnamomum* essential oils (*C. burmanni*; C. *porrectum*; *C. rigidissum* etc.). It also in occurs in witch-hazel (*Hamamelis viginiana* L.), hoja santa leaves (*Piper auritum* HBK) and in other natural herbal & spice products & preparations.

Safrole is currently classified as a carcinogen category 2 and mutagen category 3 in the IFRA-IOFI labelling manual 2009. Since out of the three alleged human carcinogens: safrole, estragole and methyl eugenol, safrole is arguably the weakest (see below), these classifications seem somewhat arbitrary.

Substance	Hazard symbol	Risk phrases	Carcinogen category	Mutagen category
Safrole	T	R45-22-68	2	3
Estragole	Xn	R22-40-43-68*	3	3
Methyl eugenol	Xn	R22-40-68*	3	3

Classification of some Carcinogens & Mutagens according to the IFRA-IOFI Labelling Manual 2009.

[*Thanks to Penny Williams of Formpak Ltd. for drawing our attention to this labelling issue; further implications over R68 status for estragole & methyl eugenol affecting common essential oils such as Aniseed, Bay, Basil, Fennel and Pine Oil Yarmor, are discussed at http://www.formpak-software.com/active/2009/09/estragol-methyl-eugenol-r68/**]**.

Previously the IARC had surmised that safrole was “Reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals” (IARC 1976); but that “No adequate human studies of the

relationship between exposure to safrole and human cancer have been reported" (- IARC 1976). The weak potency of safrole as a carcinogen is illustrated by the fact that level of safrole in the diet of rats necessary to elicit liver tumors ranges from 0.5% to 5.0% (Patri *et al.* 2002). The TD_{50} for safrole in rats was found to be 440mg/Kg/d (Gold *et al*.) compared with 51mg/Kg/d for mice. This compares with a TD_{50} value for methyl eugenol of 20mg/Kg/d for rats and 19mg/Kg/d for mice. However the TD_{50} for the proximate carcinogen 1'-hydroxysafrole was found to be 18mg/Kg/d for rats compared with 71 mg/Kg/d for mice.

The hazardous dose of sassafras oil for humans (which typically contains 80% safrole) has been put at 0.66 mg/Kg, based on experimental animal data, and a safety factor of x100; this is claimed to be way- exceeded by imbibing a standard portion of sassafras tea which has been estimated to give a dose of 3mg/Kg for a 60Kg man (Bisset 1994; Segelaman 1976). By comparison, Levy (Levy undated) gives a figure of 20 ppm safrole content of root beer before the sassafras FDA prohibition, approximating to a 5mg dose for an 8oz serving. Safrole-free extracts of sassafras have been approved by the FDA for food flavouring use, but apart from being organoleptically inferior, It is also of note that safrole-free extracts of sassafras have produced malignant mesenchymal tumors in laboratory rats (Benedetti *et al.* 1977).

Safrole & sassafras oil were banned as food & flavouring additives by the FDA on 3rd Dec 1960 (FDA Ban 21 CFR 189.180; revised April 1 2008), the ban now includes isosafrole & dihydrosafrole (the latter not being known in nature), & sassafras root bark, but in practice both sassafras oil and bark are still widely available in the US, from health food stores and internet suppliers. Safrole appears in Annex II/360 of the EU Cosmetics Directive EU 76/768, and its concentration is limited to 100ppm in finished cosmetic products (50 ppm for oral/dental use; zero for children's toothpaste). IFRA prohibits the addition of safrole to fragrances as such, and limits the safrole content of perfumes formulated with safrole-containing essential oils (basil, nutmeg, sassafras, cinnamon leaf etc.) to 0.01% (100ppm) for both skin contact & non-skin contact fragrances. These restrictions have caused a significant problem with certain fragrance styles entering the market place – for example in the deployment of cinnamon & nutmeg ingredients in masculine fougères and spicy masculine notes.

The restriction of safrole to low levels in foodstuffs was originally considered to be a threat to the economic welfare of the nutmeg trade, and so exceptions were made (note that curiously, no such exceptions are ever made for natural ingredients in the cosmetics area, presumably because academic 'expert' committees in this field are unable to accurately predict the socio-economic effects of their policies). European Council's Directive on food flavourings 88/388/EEC, amended by 91/71/EEC and implemented into UK national law in the Flavourings in Food Regulations 1992, limits safrole in foodstuffs to 1ppm, except for foodstuffs containing nutmeg (15ppm) or alcoholic drinks >25% volume alcohol (5ppm) and other alcoholic drinks (2ppm). It is of interest to note

that Choong & Lin (2001) analysed 25 soft drinks, including Coca-cola and Pepsi, from supermarkets & convenience stores in Tainan and Pingtung, for safrole and isosafrole contents in 1998, finding 20 out of 25 soft drink samples contained safrole and/or cis-isosafrole and the contents of safrole were up to 3-5 times the use limit of 1*μ*g/mL according to the food additive regulations.

Isosafrole (CAS No. 120-8-1), which occurs as (*E*)- & (*Z*)- geometric isomers, is a weak, non-genotoxic rodent hepatocarcinogen, classified as a carcinogen category 3 (IARC 1987) which has been alleged to occur in minor amounts in certain essential oils (such as Chinese angelica oil from *Angelica polymorpha* Max.), ylang-ylang & nutmeg oil & oleoresin, but Lawrence could not confirm its presence in nutmeg oils (Lawrence 1990), and MAFF have disputed its presence in ylang ylang & sassafras products (MAFF 1996a). However MAFF (1994) found 0.1% to 3.4% isosafrole (av. 0.3%) in 10 analysed samples of nutmeg oil and 0.1 to 2.7% (av. 0.9%) in 3 analysed nutmeg oleoresin samples (origins not disclosed). Since isosafrole usually co-occurs with safrole in certain natural products, at concentrations typically an order of magnitude lower than the safrole concentration (MAFF 1996), it was proposed by MAFF that isosafrole is an artefact formed during the processing of safrole-containing raw materials.

Safrole Metabolism.

Intraperitoneal dosing of rats and guinea pigs with safrole produces the following urinary metabolites; 1,2-dihydroxyl-4-allylbenzene, 1'-hydroxysafrole, 2-methylenedioxy-4-(2,3-dihyroxypropyl)benzene, 1,2-dihydroxy-4-(2,3-dihydroxypropyl)benzene, 2-hydroxy-3-(3,4-methylenedioxyphenyl) propanoic acid, and 3,4-methylenedioxybenzoylglycine (Stillwell *et al.* 1974). Two pathways have been proposed whereby hepatotoxic substances are produced from safrole (Dietz & Bolton 2007). The first proceeds via the P450 catalyzed hydroxylation of safrole to 1'-hydroxysafrole, and its subsequent conjugation with sulfate to produce a reactive sulfate ester, which creates a highly reactive carbocation via a SN1 displacement, which alkylates DNA. The second pathway involves the formation of hydroxychavicol via the P450 catalyzed hydroxylation of the methylenedioxy ring of safrole, which is subsequently oxidized to an o-quinone, which non-enzymically isomerizes p-quinone methide. Dietz & Bolton (2007) consider that these experiments by Bolton *et al*. (1994), Miller *et al.* (1985), Boberg *et al*. (1983), Daimon *et al.* (1997-1998) & Jeng *et al.* (2004) and the *in vitro* & *in vivo* experiments of Luo & Guenthner (1996), Gupta *et al.* (1993), Randerath *et al.* (1993), Daimon *et al.* (1998) & Daimon *et al*. (1997) prove the genotoxic effects of safrole and justify the regulatory action of the FDA & other authorities. Cropwatch takes issue with this conclusion; the mere existence of pathways in rodents fed high levels of dietary safrole which give rise to certain hepatotoxic substances does not, of itself, prove the potential for human carcinogenicity under normal living circumstances.

Although small amounts of safrole (0.63mg/Kg) have been shown to be cleared almost completely from the body within 24 hours in man & rats (Benedetti *et al*. 1977), the main urinary metabolite of safrole dosed in larger amounts is 1,2-

dihydroxy-4-allylbenzene in both rats & man; 1'-hydroxysafrole and 3'-hydroxyisosafrole were also detected in the urine of the rat, but not of man (Benedetti *et al.* 1977). Jeurissen (2007) has identified the human P450 enzymes involved in the 1'-hydroxylation of safrole, where important roles for a series of enzymes via a series of *in vitro* experiments were postulated. Lifestyles factors which may lead to poor or extensive metaboliser phenotypes, which either reduce or increase the relative carcinogenicity risk, were discussed.

Also compelling evidence for humans, perhaps, lies with studies made of habitual quid chewers of betel & areca nut, where a constant body-loading of safrole may give rise to tumors over an extended time period. In particular, inflorescences of betel have been shown to contain relatively high (15mg/Kg) concentrations of safrole (Liu *et al*. 2000).

Conclusion.

The classification of safrole as a Category 2 human carcinogen and the association of risk phrase R22-45-68 with the material seems disproportionate to the risks involved to humans from its traditional uses in spices, flavours, fragrances etc. Regulators appear to be forced by some unseen hand to deny the use of any traditional natural ingredients which have been shown to carry some health risks to susceptible animals at high doses, in an attempt to construct a clean, risk-free and largely synthetic-based world of their own. That is not the world that most of use wish to inhabit, and Cropwatch believes that many will ignore any restrictions which deny us the use of those familiar materials which we associate with our lives, our heritage & our traditions.

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