The Carcinogenicity of Essential Oils, Flavors, and Spices: A Review

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Summary

Essential oils, flavors, and spices constitute a large and heterogeneous group of substances to which humans are exposed. The essential oils are defined as a group of odorous principles soluble in alcohol and only to a limited extent in water. They consist of a mixture of esters, aldehydes, ketones, and terpenes. Relatively few members of this group of substances have been shown to cause cancer in animals. This paper summarizes knowledge on the carcinogenic activity of safrole, citrus oils (d-limonene), turpentine oil (l-pinene), eucalyptus oil (phellandrene), bergamot oil, and red pepper. The possible role of polycyclic hydrocarbons biosynthesized in plants is also discussed.

Carcinogenic Activity of Safrole

Our interest in the carcinogenic activity of essential oils was initiated in the early sixties by the observation of B. Zeitlin (unpublished results), that safrole (4-allyl-1,2-methylenedioxybenzene, Compound I) causes hepatic adenomas in rats Homburger et al. (9-11). Safrole is a component of many essential oils, such as star anise oil, micranthum oil, camphor oil, and, especially, sassafras oil. It also occurs in oil of mace, nutmeg, Japanese wild ginger, California bay laurel, and cinnamon leaf oil. It has been widely used as a flavoring agent in root beer and in certain pharmaceutical preparations.

We have studied the effect of safrole at two levels and the nutritional factors that modify the hepatic adenomatosis induced by this substance. In concentrations of 1% of the diet, safrole is toxic, producing weight loss, testicular atrophy, and bone marrow depletion. It also induces hepatomas. Similar observations were made by Long et al. (13) at a 0.5% dietary level of safrole. In our studies on modifying factors of safrole carcinogenesis (9, 11), similar doses given to male Carworth Farms CFN rats on riboflavin, tocopherol, and protein-deficient diets resulted in much smaller hepatic adenomas in comparable periods of time. Casein supplements aggravated the safrole-induced adenomatosis, resulting in tumors larger than those observed on protein-deficient diets. Osborne-Mendel rats appeared to be more sensitive than Carworth Farms CFN rats (8). Biotin supplements inhibited hepatocarcinogenic activity of butter yellow as well as safrole, whereas pyridoxine deficiency reduced the carcinogenicity of butter yellow but not that of safrole.

At a 0.1% level safrole had no toxic effects. Several derivatives of safrole have been studied by Long and Jenner (12), who found that rats fed with dihydro safrole (Compound la) have developed tumors of the esophagus and none of the liver.

Carcinogenicity of Citrus Oils

The testing of citrus oils for carcinogenic and cocarcinogenic activity has also been carried out during the last decade. Roe and Pierce (18) reported that repeated applications of various citrus oils on the skin of mice pretreated with an initiating carcinogen benzpyrene (BP), 7,12 dimethylbenz(a)anthracene (DMBA), or urethan resulted in benign and malignant skin tumors. The initiators in acetone solution were applied to the skin in doses which were not sufficient to cause complete carcinogenesis. Three weeks later the citrus oil was applied once weekly either in undiluted form or diluted with acetone. Whereas in the control experiments (subcarcinogenic doses of BP, DMBA, or urethan) only occasional benign tumors appeared, a relatively large number of benign and malignant tumors resulted from the promotion effect of orange, lime, lemon, and grapefruit oils.

Among these, orange oil and its major constituent, d-limonene, have been the main target of investigation. The same author (15) reported that in control experiments in which the animals were treated with orange oil only, no carcinogenic effect could be observed. A year later, however, Roe published another article (16) from which it appears that the terpene fraction of the orange oil containing mainly d-limonene, when applied on the skin of mice, gives rise to epidermic hyperplasia and subsequently to tumors.

These apparently contradictory results can be explained by the fact that d-limonene (Compound II), an unsaturated...
hydrocarbon with two double bonds, is an unstable compound, and is transformed gradually, mainly upon exposure to air, into its hydroperoxide. By analogy to studies of some of the hydrocarbons which are closely related to d-limonene, it can be deduced that the species responsible for the carcinogenic or cocarcinogenic effect is actually the autoxidized form of d-limonene. The compound, 1-vinyl-cyclohex-3-ene (Compound III), which differs from limonene only by the absence of two methyl groups, was reported as a carcinogen (21), but later it was found that the same compound was devoid of any carcinogenic effect (20) when sufficiently purified and protected from oxygen. The actual carcinogen was found to be the hydroperoxide derivative, 1-hydroperoxy-1-vinyl-cyclohex-3-ene (Compound IV).

Another chemically related hydroperoxide 1-hydroperoxy-cyclohex-3-ene (Compound V), which differs from Compound IV only by the absence of a vinyl group, also exhibited carcinogenicity.

To prove this assumption, we are now testing the carcinogenic and cocarcinogenic effects of a highly purified sample of d-limonene, and of a sample of partially autoxidized d-limonene containing 6% of the hydroperoxide. This sample of purified limonene, which displays no other peaks except that of d-limonene itself when assayed by gas chromatography, was supplied to us by the Givaudan Corporation, Clifton, New Jersey.

Another support for the assumption that the hydroperoxide is the active form of d-limonene is found in an observation reported by I. Zukerman (22). This author has studied the inhibitive action of both purified d-limonene and its autoxidized form on various microorganisms by adding them to the nutrient medium. He has found that the purified (freshly distilled over sodium) d-limonene has no inhibitive action, but that it reveals the inhibitive properties when exposed to air and this increases with the duration of exposure. When l-ascorbic acid was added to the nutrient medium, a decrease of inhibition was observed.

Roe and Pierce (18) have also studied the induction of tumors at the urethral orifice of female mice during treatment with orange oil, with or without DMBA pretreatment. In this case the number of tumors induced (all of which were benign papillomas) was relatively low.

A more recent paper by Field and Roe (3) deals with the effect of oral administration of citrus oils on the tumor promotion in the forestomach epithelium of mice. The initiation was carried out by a single treatment with 50 mg of DMBA or BP in polyethylene glycol given by stomach tube after food had been withheld overnight.

The initiation alone (as a control experiment) resulted in a very small number of tumors, but when this was followed by forty once-a-week treatments of 0.05 ml undiluted lime oil, the tumor incidence in the forestomach was markedly increased. Orange oil and d-limonene also induced a few tumors when applied in the same way.

**Other Essential Oils**

In addition to the oils mentioned above, various others have been tested for cocarcinogenic effect but with less significant results.

Turpentine oil and l-pinene, its major constituent, were shown to promote skin tumor development in the rabbit (14) but not in the mouse (1, 19). It is possible that the different results obtained were also due to different compositions of the oils used in these experiments.

Eucalyptus oil and phellandrene, one of its constituents, have also been tested as promoting agents on mouse skin (17). About 10% of the mice treated developed tumors.

Oils with less terpenes, such as bergamot oil which consists mainly of alcohols and esters, prove less active as tumor-promoting agents. Bergamot oil was completely inactive when tested on the skin of mice, although linalool, one of its principal alcohols, elicited a weak tumor-producing response (17).

**Flavors and Spices**

Although there are many types of different flavoring agents and spices, very little is known of their possible tumorigenic effect. The case of saffrole, which served for many years as a flavoring agent of soft drinks, and is a minor constituent of several spices, was discussed previously.

The use of lemon oil and similar potential cocarcinogens as food additives should also be considered. Special attention should be given to the prevention of their autoxidation which apparently produces the biologically active species. Even if the active species is present in foodstuffs in very small amounts, this does not exclude the possibility of cancer hazards, since prolonged exposures to minute amounts of carcinogens may result after a long latent period in the development of cancers in the exposed animals.

The formation of liver tumors in rats fed red pepper (Capsicum) has been reported by Hoch-Ligeti (7). When rats were fed 10% chili (red pepper) in their diet, a higher incidence of neoplastic changes occurred in their livers than in the control experiment.

**Natural Occurrence of Polycyclic Aromatic Hydrocarbons (PAH) in Plants**

In the previous chapters, it was shown that some components of certain essential oils, flavors, and spices of
plant origin play a role in the carcinogenic effect of these foodstuffs when tested in animals. In most of the cases, these components are only promoting agents and have no effect unless pretreatment with an initiator takes place. It is, therefore, of great importance to determine whether these initiators (such as BP, etc.) also occur in plants.

As a result of Wieland and Dane's classical work on the synthesis of methylcholanthrene from bile acids, many attempts have been made to detect endogenous formation of carcinogenic PAH in animal tissues, especially in tumors and body fluids of cancerous subjects, but these attempts have so far been unsuccessful (2). In plants, however, the natural occurrence of PAH has recently been demonstrated by Graef (4), who has extracted plants, grains, and fruits and examined the fluorescent fractions of their extracts. Benzpyrene was one of the eight hydrocarbons identified in these extracts. "These PAH's show a growth-promoting effect in plants and they seem to function as hormonal foreign bodies and elicit neoplasms," (5). Since plants grown in artificial media and those from distant sites had the same content of hydrocarbons, the substances are believed to be endogenous. Graef also believes that this finding may explain why stomach and intestinal tumors occur more often among vegetarians.

REFERENCES

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