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# DOSE-DEPENDENT VARIATION IN THE DISPOSITION OF EUGENOL IN THE RAT

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The allylbenzenes are an important group of food flavours which occur naturally in the essential oils of many herbs and spices having extensive culinary use. One of the most important of these, safrole (3,4-methylenedioxyallylbenzene) was shown some 20 years ago to be a rodent hepatocarcinogen and was subsequently withdrawn from human use (1). Since then, various other allylbenzenes have also been shown to be rodent carcinogens. These compounds are chemically unreactive per se, but have been shown to undergo metabolic activation by hydroxylation at the 1'-position of the side chain (2), a reaction which increases greatly in importance with increasing dose size (3,4).

Eugenol (4-hydroxy-3-methoxyallylbenzene) is the major component of Oil of Cloves, and is used as a food flavour and fragrance agent, and is widely employed in dental therapeutics. Human exposure from the diet has been estimated to be 0.6mg/capita/day. It is apparently not a carcinogen at dose levels at which both safrole and estragole induce liver tumours in rodents (5).

A knowledge of metabolic fate is often of importance in explaining differences in the toxicity of structurally closely related compounds, and this is particularly relevant with compounds such as the allylbenzenes, whose toxicity apparently arises through metabolites. To further our knowledge of the relationships between chemical structure, dose size, metabolic fate and toxic effects amongst the allylbenzenes, we now present a preliminary account of studies on the fate of eugenol in rat at a range of doses, from levels close to human dietary exposure to doses resembling those used in toxicity tests.

[ring-$^{14}$C]Eugenol (custom synthesis by Midwest Research Institute, Kansas City) dissolved in trioctanoin was administered to female Wistar albino rats (b.wt. 200g) by stomach tube at dose levels of 0.5,5,50 and 1000mg/kg (10-15μCi/animal in a total volume of 0.8ml/animal). The rats were kept in metabolism cages and their urine and faeces collected daily for 3 days. The excretion of $^{14}$C was monitored by liquid scintillation counting. Urinary metabolites were separated by selective solvent extraction, TLC and HPLC before and after treatment of the urine with β-glucuronidase or sulfatase, and characterised by GC-MS as such and as methylated or TMS derivatives.

After the administration of $^{14}$C-eugenol to rats, there occurred rapid and extensive excretion of $^{14}$C, predominantly in the urine (75-80% of dose) with some 10% being found in the faeces. The rate and route of excretion was independent of dose size. At all dose levels, the major excretion product was conjugated eugenol (ca.50% of dose), but the nature of the

conjugate was dependent upon dose. Thus, at low doses eugenol sulfate predominated, but at 1000mg/kg, the glucuronide was the major metabolite. These conjugates were accompanied by metabolites in which the C=C bond of the allyl side chain had been reduced, namely 4-hydroxy-3-methoxypropylbenzene and 3,4-dihydroxypropylbenzene. These compounds were excreted conjugated with either glucuronic acid or sulfate, the relative proportions of these conjugates varying with dose in the same way as the conjugates of eugenol. The variation in the excretion of these metabolites with dose is presented in Table 1. In addition to these compounds, some 10% of the dose at each dose level is excreted in the form of a number of as yet uncharacterized acidic metabolites.

TABLE 1 Variation in the excretion of eugenol and its principal metabolites following the administration of $^{14}$C-eugenol at different dose levels

| Compound | Dose(mg/kg) | %0-24hr urinary $^{14}$C excreted in that form 0.5 | 5 | 50 | 1000 |
|---|---|---|---|---|---|
| Eugenol | | 50 | 60 | 55 | 60 |
| 3,4-Dihydroxy-propylbenzene | | 15 | 5 | 15 | n.d. |
| 3-Methoxy-4-hydroxy-propylbenzene | | 3 | 1 | 1 | n.d. |

n.d. = not detected (<0.5% urinary $^{14}$C)

Incubation of $^{14}$C-eugenol with rat caecal contents under anaerobic, but not aerobic conditions indicates the ability of the gut flora to effect both the reduction and O-demethylation of this compound.

In summary, therefore, we have shown that $^{14}$C-eugenol is rapidly eliminated, principally in the urine, by rats following the administration of a wide range of doses. The major route of metabolism involves conjugation of the free hydroxyl group of eugenol with glucuronic acid or sulfate, the relative importance of which changes with dose. Other routes of metabolism include O-demethylation and reduction of the allylic double bond.

The metabolism of the structurally related rodent hepatocarcinogens safrole and estragole changes significantly with increasing dose size. In particular, increasing dose throws greater emphasis upon oxidation of the side chains to the presumed proximate carcinogenic metabolites. By contrast, the metabolism of eugenol in the rat involves conjugation and C=C bond reduction, reactions generally associated with a reduction in chemical reactivity, and this perhaps accounts for the lack of carcinogenicity of this particular allylbenzene.

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