FLAVOR AND EXTRACT MANUFACTURERS ASSOCIATION OF THE UNITED STATES
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Dr. C.W. Jameson
National Toxicology Program
Report on Carcinogens
MD EC-14
P.O. Box 12233
Research Triangle Park, North Carolina 27709

21 April 2001

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RE: National Toxicology Program; Request for Comments on Substances Nominated to 10th List of Human Carcinogens. 67 Fed. Reg. 75727 (9 March 2001)

Dear Dr. Jameson:

On behalf of the members of the Flavor and Extract Manufacturers Association (FEMA), we submit these comments on the nomination of methyl eugenol for inclusion in the 10th List of Human Carcinogens, a program administered by the National Toxicology Program (NTP). 67 Fed. Reg. 75727 (4 December 2000). FEMA believes that the inclusion of methyl eugenol on the list is premature given that

1) the studies used to characterize methyleugenol as an animal carcinogen were inadequate by NTP's own standards and,
2) the NTP choose to disregard relevant data in making its conclusions regarding carcinogenicity.

FEMA is the national association of flavor and extract manufacturers. FEMA's members manufacture and market the vast majority of flavoring substances that are incorporated into food and beverages in the United States. These flavoring substances are regulated by the U.S. Food and Drug Administration (FDA) as described at 21 C.F.R. Part 172, and have been thoroughly evaluated and are generally recognized as safe (GRAS) for inclusion in foods.

For more than two decades, FEMA has actively supported research into the safety of allylalkoxybenzene derivatives, methyl eugenol, estragole and other structurally substances. FEMA is currently engaged in ongoing research in this area especially with regard to methyl eugenol. It is with this experience that we offer the following comments on the decision of the recommendation to list methyl eugenol on the 10th List of Human Carcinogens.

Standards of Performance for the 2-Year Bioassay with Methyl eugenol

The 2-yr bioassay performed at NTP was severely compromised, in that, all dose levels (37.5, 75, or 150 mg/kg bw per day) of methyl eugenol administered by gavage caused significant liver toxicity resulting in hepatic dysfunction, gastric damage, and malnutrition in both mice and rats. Hepatic tumors occurred in severely damaged livers while neuroendocrine tumors of the glandular stomach were likely the result of chronic endocrine stimulation, leading to gastronemia and then secondarily, to chronic gastric toxicity. Clearly, the extensive toxicity to the alimentary system existed prior to or during carcinogenesis in these organs.

Dose levels selected for the 2-year study were too high to properly evaluate the carcinogenic potential of methyl eugenol. These dose levels were selected on the basis of a 14-week study that showed evidence of liver toxicity at levels of 30 and 100 mg/kg bw per day. Significant increases in relative liver weight of male rats and male mice (relative and absolute) at 30 and 100 mg/kg bw and increases in liver enzyme activities at 100mg/kg bw provided evidence that liver toxicity was present at 14 weeks at 30 mg/kg bw per day. Therefore, it is not unexpected that a daily dose of 37.5 mg/kg bw/day given over 2 years would produce hepatotoxicity.

In addition to the inappropriately high dose levels, an infection was detected in the livers of treated mice. The presence of *Heliobacter hepaticus* also compromised the interpretation of the findings. In the final analysis, the 2-year study did not permit one to evaluate the animals for carcinogenicity in the absence of systemic toxicity.

The NTP choose to disregard relevant data in making its conclusions regarding carcinogenicity

The NTP regards both methyl eugenol and saffrole as members of the class of allylalkoxybenzene derivatives. They have emphasized that these structural relatives exhibit remarkably similar toxicologic profiles. However, as early as 1985, metabolic data on another member of the same class p-

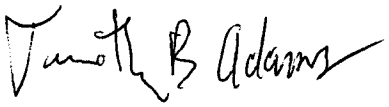
methoxyallylbenzene (estragole) clearly demonstrated that a dose dependent changeover to an intoxication pathway was occurring in the dose range of 10-30 mg/kg bw. Hepatic enzymatic studies with methyl eugenol and other allylalkoxybenzene derivatives indicate that the coenzyme (CYP2E1) primarily responsible for the formation of the proximate hepatotoxic metabolite is not induced below 10 mg/kg bw/day. Evidence of dose-dependent hepatic protein adduct formation and DNA adduct formation indicates the existence of non-linear dose-response relationship between the dose of methyl eugenol and toxicity including carcinogenicity. There is significant evidence that all dose levels in the NTP study were severely toxic to the liver.

Conclusions

The available biochemical and toxicologic information demonstrate that methyl eugenol was administered at dose levels that were toxic to both mice and rats. The mode of administration was a compounding factor in the toxic effect of the dose on the animals. We respectfully request that that the NTP repeat the study at lower dose levels using a more appropriate mode of administration, microencapsulated in the diet. With this additional data, the NTP would be able to more realistically relate the hazard identified in laboratory rodents to the risk to human health through exposure to methyl eugenol.

Thank you for your consideration of our comments.

Sincerely,



Timothy B. Adams, Ph.D.
The Flavor and Extract Manufacturers Association