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## Carcinogenicity of aspartame, methyleugenol, and isoeugenol

Elio Riboli, Frederick Beland, Dirk Lachenmeier, M Matilde Marques, David Phillips, Eva Schernhammer, Abdul Afghan, Ricardo Assunção, Giovanna Caderni, J Christopher Corton, et al.

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1 **Carcinogenicity of aspartame, methyleugenol, and isoeugenol**

2 In June 2023, a Working Group of 25 scientists from 12 countries met at the International  
3 Agency for Research on Cancer (IARC) in Lyon, France, to finalise their evaluation of the  
4 carcinogenicity of aspartame, methyleugenol, and isoeugenol. Aspartame was classified as  
5 “possibly carcinogenic to humans” (Group 2B) based on “limited” evidence for cancer in  
6 humans. There was also “limited” evidence for cancer in experimental animals and “limited”  
7 mechanistic evidence. Methyleugenol was classified as “probably carcinogenic to humans”  
8 (Group 2A) based on “sufficient” evidence for cancer in experimental animals and “strong”  
9 mechanistic evidence, including studies in humanised mice and supported by studies in  
10 exposed humans. Isoeugenol was classified as “possibly carcinogenic to humans” (Group 2B)  
11 based on “sufficient” evidence for cancer in experimental animals. For both methyleugenol  
12 and isoeugenol, the evidence regarding cancer in humans was “inadequate”, as no studies  
13 were available. These assessments will be published in Volume 134 of the *IARC*  
14 *Monographs*.<sup>1</sup>

15 Aspartame is a low-calorie artificial sweetener widely used in foods and beverages since  
16 the 1980s. Highest concentrations are found in tabletop sweeteners, chewing gums, and food  
17 supplements; artificially sweetened beverages (ASB) are an important source of aspartame  
18 exposure. Other sources include cosmetics, medicines, and tobacco products. Occupational  
19 exposure by inhalation during the production of aspartame-containing products has been  
20 reported, but data are sparse. Available information indicates that the metabolism of  
21 aspartame is similar in humans and experimental systems: aspartame is hydrolysed to aspartic  
22 acid, phenylalanine, and methanol. In experimental systems (primates), aspartic acid and  
23 methanol are predominantly excreted as CO<sub>2</sub>; however, most of the phenylalanine is retained.

24 For cancer in humans, there was “limited” evidence that aspartame causes liver cancer,  
25 specifically, hepatocellular carcinoma. Cohort studies assessing consumption of ASBs in time  
26 periods and countries in which aspartame was used as the main artificial sweetener were

27 considered informative for the evaluation, since this assessment was judged to be a reliable  
28 proxy for aspartame exposure. Only the NutriNet-Santé cohort comprehensively assessed  
29 aspartame exposure from all dietary sources<sup>2</sup>. Although this study reported an association  
30 with increased breast, obesity-related, and overall cancer risk, such findings were not  
31 consistent across all available studies. The study did not assess liver cancer risk. Among all  
32 three available studies assessing ASB consumption and risk of liver cancer, positive  
33 associations were reported in the overall study population<sup>3</sup> or in relevant subgroups<sup>4,5</sup>. In one  
34 large study across 10 European countries, each weekly serving (330 mL) of ASB was  
35 associated with a 6% increase in risk of hepatocellular carcinoma (95% CI 3–9%)<sup>3</sup>. Despite  
36 positive findings in high-quality studies that controlled for many potential confounders, the  
37 Working Group concluded that chance, bias, or confounding could not be ruled out with  
38 reasonable confidence.

39 In Swiss mice and Sprague-Dawley rats exposed by transplacental and perinatal followed  
40 by oral administration (feed), aspartame caused hepatocellular carcinoma, hepatocellular  
41 adenoma or hepatocellular carcinoma (combined), bronchioloalveolar carcinoma,  
42 bronchioloalveolar adenoma or carcinoma (combined), lymphoblastic leukaemia, monocytic  
43 leukaemia, and total myeloid tumours in male mice; lymphoblastic leukaemia and leukaemia  
44 (all types) in female mice; malignant schwannoma in male rats; and mammary gland  
45 carcinoma, renal pelvis papilloma, and leukaemia (all types) in female rats<sup>6,7</sup>. In Sprague-  
46 Dawley rats exposed by oral administration (feed), aspartame caused renal pelvis and ureter  
47 carcinoma, renal pelvis and ureter papilloma or carcinoma (combined), and mammary gland  
48 carcinoma in females; and monocytic leukaemia, histiocytic sarcoma, and total myeloid  
49 tumours in males<sup>8</sup>. Because of concerns regarding some of the diagnoses for lymphomas in  
50 these studies, the Working Group focused its evaluation on all neoplastic lesions other than  
51 lymphoid tumours and related combinations. Although the data indicated that aspartame had  
52 carcinogenic activity, overall, the Working Group had questions about the adequacy of the  
53 design, conduct, interpretation, and reporting of each of the studies. For example, the lack of

54 adjustment for litter effects may have led to false positive results in tests for increased  
55 incidence and trend. A minority of the Working Group considered the evidence for cancer in  
56 experimental animals to be “sufficient”, and therefore supported a Group 2A classification for  
57 aspartame.

58 In experimental systems aspartame induced oxidative stress, as oxidative stress biomarkers,  
59 including lipid peroxidation, were altered in several tissues, including liver, in multiple rodent  
60 studies. In different experimental systems, several studies suggested that aspartame induced  
61 chronic inflammation, and a small set of studies suggested angiogenesis. Additionally, aspartame  
62 increased insulin serum levels in rodents. Although there were some positive findings in several  
63 studies available for genotoxicity, many had limitations in design, data analysis, and interpretation.  
64 Based on the above findings, the mechanistic evidence for aspartame was “limited” for the key  
65 characteristics of carcinogens.

66 Methyleugenol is a flavour and fragrance compound occurring naturally in essential oils of  
67 various plants. It is used in cosmetics and personal care products and as an insect attractant. Its use  
68 as a flavouring agent has been prohibited in the EU and the USA, but it is still present in various  
69 foods and consumer products due to its natural occurrence in herbs and spices. No data on  
70 occupational exposure were available. The general population is ubiquitously exposed, mostly to  
71 low levels through the ingestion of food or dermally using personal care products. Although data  
72 were sparse, in humans methyleugenol appears to be absorbed after oral exposure and to permeate  
73 the derma. In rodents, methyleugenol forms active metabolites in the liver, e.g., 1'-  
74 hydroxymethyleugenol, and is excreted in the urine as sulfate or glucuronide conjugates after oral  
75 exposure. In B6C3F<sub>1</sub> mice and F344 rats exposed by oral administration (gavage), methyleugenol  
76 caused hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or  
77 carcinoma (combined), in male and female mice and rats; hepatoblastoma in male and female  
78 mice; hepatocholangioma, hepatocholangiocarcinoma, and benign and malignant neuroendocrine  
79 tumours of the glandular stomach in male and female rats; and renal tubule adenoma, mammary  
80 gland fibroadenoma, skin fibroma, skin fibroma or fibrosarcoma (combined), and mesothelioma in

81 male rats<sup>9</sup>. Methyleugenol exhibits multiple key characteristics of carcinogens in experimental  
82 systems including humanised mice, supported by human studies. Pro-mutagenic methyleugenol  
83 DNA adducts were detected in human liver and lung samples, in the livers of mice transgenic for  
84 human sulfotransferase (SULT1A1/2), and in other experimental systems. Methyleugenol RNA  
85 and protein adducts were found in rodents. Methyleugenol and 1'-hydroxymethyleugenol caused  
86 DNA strand breaks, but not micronuclei in human cells in vitro and in experimental systems.  
87 Methyleugenol induced unscheduled DNA synthesis, sister-chromatid exchange, and gene  
88 mutagenicity in rodents. It was mutagenic in bacteria strains expressing human SULT enzyme  
89 isoforms. There is suggestive evidence that methyleugenol induces cell proliferation, alters related  
90 biomarkers and hyperplasia in the liver and other tissues in rodents, and induces chronic  
91 inflammation in experimental systems in a small set of data.

92 Isoeugenol is a fragrance and flavour compound that occurs in many plant species and in  
93 wood smoke. It is used in food, cosmetics, household products, animal feed and veterinary  
94 medicines and allergen patch testing. Workers involved in the synthesis of the compound,  
95 handling products containing it, and firefighters may be exposed by dermal and/or inhalation  
96 routes. Exposure of the general population occurs through the diet and the use of household  
97 products and cosmetics. Evidence on absorption, distribution, metabolism, and excretion of  
98 isoeugenol in humans is sparse and is limited to dermal exposure. After oral and dermal exposure  
99 in rodents, isoeugenol is rapidly absorbed and excreted predominantly in the urine as glucuronide  
100 or sulfate conjugates, with little retention in tissues. In B6C3F<sub>1</sub> mice and F344 rats exposed by oral  
101 administration (gavage), isoeugenol caused hepatocellular adenoma, hepatocellular carcinoma,  
102 and hepatocellular adenoma or carcinoma (combined) in male mice; a significant increasing trend  
103 in histiocytic sarcoma (multiple sites) in female mice; and a significant increasing trend in  
104 mammary gland carcinoma and benign or malignant thymoma in male rats<sup>10</sup>. Although significant  
105 increasing trends were observed with dose, leading the Working Group overall to consider the  
106 evidence “sufficient”, the pairwise comparison to the controls did not reach statistical significance  
107 in any of the treated groups; therefore, a minority of the Working Group considered the evidence

108 in experimental animals to be “limited” and supported a Group 3 classification for isoeugenol.  
109 Isoeugenol is a skin sensitiser that can be converted photochemically to electrophiles that form  
110 protein adducts. However, isoeugenol–DNA adducts were not detected in experimental systems.  
111 Overall, the mechanistic evidence for isoeugenol was “inadequate”.

112

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143 Elio Riboli, Frederick A Beland, Dirk W Lachenmeier, Maria M Marques, David Phillips, Eva  
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