Carcinogenicity of aspartame, methyleugenol, and isoeugenol

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Declaration of interests
All Working Group Members declare no competing interests

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In June, 2023, a Working Group of 25 scientists from 12 countries met at the International Agency for Research on Cancer (IARC) in Lyon, France, to finalise their evaluation of the carcinogenicity of aspartame, methyleugenol, and isoeugenol. Aspartame was classified as "possibly carcinogenic to humans" (Group 2B) based on "limited" evidence for cancer in humans. There was also "limited" evidence for cancer in experimental animals and "limited" mechanistic evidence. Methyleugenol was classified as "probably carcinogenic to humans" (Group 2A) based on "sufficient" evidence for cancer in experimental animals and "strong" mechanistic evidence, including studies in humanised mice and supported by mechanistic studies in exposed humans. Isoeugenol was classified as "possibly carcinogenic to humans" (Group 2B) based on "sufficient" evidence for cancer in experimental animals. For both methyleugenol and isoeugenol, the evidence regarding cancer in humans was "inadequate", as no epidemiological studies were available. These assessments will be published in Volume 134 of the *IARC Monographs*.[1] Immediately following IARC's meeting on cancer hazard identification, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted a risk assessment exercise, including a review of the acceptable daily intake of aspartame. A summary of these results has been published.

Aspartame is a low-calorie artificial sweetener widely used in foods and beverages since the 1980s. The highest concentrations are found in tabletop sweeteners, chewing gums, and food supplements; historically, artificially sweetened beverages have been the major source of aspartame exposure (>90% of total exposure in some populations). Currently, artificially sweetened beverages remain an important source of aspartame exposure, but aspartame is typically used in mixtures with other sweeteners. Other sources of aspartame exposure include cosmetics and medicines. Occupational exposure by inhalation during the production of aspartame-containing products has been reported, but data are sparse. Available information indicates that the metabolism of aspartame is similar in humans and experimental systems; aspartame is hydrolysed to aspartic acid, to the essential amino acid phenylalanine, and to methanol. In experimental systems (primates), aspartic acid and methanol are predominantly excreted as CO_2; however, most of the phenylalanine is retained.

For cancer in humans, there was "limited" evidence that aspartame causes hepatocellular carcinoma. Prospective cohort studies assessing consumption of artificially sweetened beverages in time periods and countries in which artificially sweetened beverages predominantly contained aspartame and were the main source of aspartame exposure were considered informative for the evaluation, because artificially sweetened beverage consumption was judged to be a reliable proxy for aspartame exposure. The NutriNet-Santé study is the only large prospective cohort study that comprehensively assessed aspartame exposure from all dietary sources.[2] Although this study reported an association of aspartame with increased breast, obesity-related, and overall cancer risk, such findings were not consistent across all available studies. The NutriNet-Santé study did not investigate the association of aspartame with liver cancer risk. The Working Group identified three studies, comprising four prospective cohorts, that assessed the association of artificially sweetened beverage consumption with liver cancer risk. These included a large cohort study, conducted within ten European countries, that assessed the association of artificially sweetened beverages with incidence of hepatocellular carcinoma;[3] a second study, pooling data from two large US cohorts, that investigated the association between artificially sweetened beverage consumption and liver cancer incidence by diabetes status;[4] and another large US prospective cohort study that evaluated the association between artificially sweetened beverages and liver cancer mortality.[5] Among all three studies, positive associations between artificially sweetened beverage consumption and cancer incidence or cancer mortality were reported in the overall study population[3] or in relevant subgroups.[4,5] All three studies were of high quality and controlled for many potential confounders. However, the Working Group concluded that chance, bias, or confounding could not be ruled out with reasonable confidence in this set of studies. Thus, the evidence for cancer in humans was deemed "limited" for hepatocellular carcinoma and "inadequate" for other cancer types.

The Working Group evaluated several carcinogenicity studies in multiple species (mouse, rat, dog, and hamster), including regulatory study reports made publicly available by the European Food Safety Authority, which reported negative findings after oral exposure to aspartame. It was noted that several of the negative studies were conducted before the advent of Good Laboratory Practice (GLP) guidelines and had some limitations—eg, lack of information on the test substance purity and selective histopathology. No significant increase in the incidence of tumours was observed in three well-conducted GLP studies in male and female transgenic mice.[6] The Working Group noted that these

new transgenic mouse models may not have been sufficiently sensitive to detect a carcinogenic effect of chronic aspartame exposure. In Swiss mice and Sprague-Dawley rats exposed perinatally followed by postnatal oral administration (feed), aspartame caused hepatocellular carcinoma, hepatocellular adenoma or hepatocellular carcinoma (combined), bronchioloalveolar carcinoma, bronchioloalveolar adenoma or carcinoma (combined), lymphoblastic leukaemia, monocytic leukaemia, and total myeloid tumours in male mice; lymphoblastic leukaemia and leukaemia (all types) in female mice; malignant schwannoma in male rats; and mammary gland carcinoma, renal pelvis papilloma, and leukaemia (all types) in female rats.[7–9] In Sprague-Dawley rats exposed by oral administration (feed), aspartame caused renal pelvis and ureter carcinoma, renal pelvis and ureter papilloma or carcinoma (combined), and mammary gland carcinoma in females; and monocytic leukaemia, histiocytic sarcoma, and total myeloid tumours in males.[9,10] Because of concerns regarding some of the diagnoses for lymphomas and related combinations and other lymphoid proliferations in these studies, the Working Group focused its evaluation on all the other neoplastic lesions. Although the data from the above studies[7–10] suggested that aspartame had carcinogenic activity, overall, the Working Group considered the evidence for cancer in experimental animals to be "limited" because of questions about adequacy of the design, conduct, interpretation, and reporting of each of the studies. For example, the lack of adjustment for litter effects[7–9] may have led to false positive results for incidence and trend. A minority of the Working Group did not share these concerns about this set of studies and considered the evidence for cancer in experimental animals to be "sufficient"; thus, they supported a Group 2A classification rather than Group 2B classification for aspartame.

Regarding the key characteristics of carcinogens, in experimental systems, aspartame induced oxidative stress, shown by the alteration of oxidative stress biomarkers, including lipid peroxidation, in several tissues, including the liver, in multiple rodent studies. In different experimental systems, several studies suggested that aspartame induced chronic inflammation, and a small set of studies suggested that angiogenesis was increased. Although there were some positive findings regarding genotoxicity in several studies, many had limitations in design, data analysis, and interpretation. Based on the above findings, the mechanistic evidence for aspartame was "limited" for the key characteristics of carcinogens. Additionally, relevant studies in rodents showed that exposure to aspartame increased insulin serum levels.[11] Although these findings indicate alterations in insulin sensitivity, the Working Group considered the relevance of the findings to mechanisms of carcinogenesis to be a notable research gap.

Methyleugenol is a flavour and fragrance compound occurring naturally in essential oils of various plants. It is used in cosmetics and personal care products and as an insect attractant. Its use as a flavouring agent has been prohibited in the EU and the USA, but it is still present in various foods and consumer products due to its natural occurrence in herbs and spices. No data on occupational exposure were available. The general population is ubiquitously exposed, mostly to low levels, through the ingestion of food or dermally through personal care products. Although data were scarce, in humans, methyleugenol appears to be absorbed after oral exposure and to permeate the derma. In rodents, methyleugenol forms active metabolites in the liver (eg, 1′-hydroxymethyleugenol) and is excreted in the urine as sulfate or glucuronide conjugates after oral exposure.

The "sufficient" evidence for cancer in experimental animals for methyleugenol was based on an increase in the incidence of malignant neoplasms and a combination of benign and malignant neoplasms in two species in two studies that complied with GLP. In B6C3F_1 mice and F344 rats exposed by oral administration (gavage), methyleugenol caused hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined), in male and female mice and rats; hepatoblastoma in male and female mice; hepatocholangioma, hepatocholangiocarcinoma, and benign and malignant neuroendocrine tumours of the glandular stomach in male and female rats; and renal tubule adenoma, mammary gland fibroadenoma, skin fibroma, skin fibroma or fibrosarcoma (combined), and mesothelioma in male rats.[12]

Methyleugenol exhibits multiple key characteristics of carcinogens in experimental systems including humanised mice, supported by human studies. Pro-mutagenic methyleugenol DNA adducts were detected in human liver and lung samples, in the livers of mice transgenic for human sulfotransferase (*SULT1A1/2*), and in other experimental systems. Methyleugenol RNA and protein adducts were found in rodents. Methyleugenol and 1′-hydroxymethyleugenol caused DNA strand breaks, but not micronuclei, in human cells in vitro and in experimental systems. Methyleugenol induced unscheduled DNA synthesis, sister-chromatid exchange, and gene mutagenicity in rodents. It was mutagenic in bacteria strains expressing human SULT isoforms. There is suggestive evidence that methyleugenol induces cell proliferation, alters related

Declaration of interests
SB is employed by ToxStrategies LLC, a consulting firm that has provided research services to The American Beverage Association and The Calorie Control Council. The International Council of Beverages Associations has sponsored her travel to the IARC Meeting, June 2023. SE is a consultant for The American Beverage Association. TG has issued written comments on the safety of aspartame in response to regulatory dockets issued by the US Department of Health and Human Services and Department of Agriculture. BM is employed by Health Science Consultants, which has received consulting fees by The Calorie Control Council for critical review of studies on aspartame. The Calorie Control Council also sponsored BM's attendance to the IARC Meeting. All other Observers declare no competing interests.

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Declaration of interests
All Secretariat declare no competing interests

Upcoming meetings

July 25–28, 2023: Scientific workshop on key characteristics-associated end-points for evaluating mechanistic evidence of carcinogenic hazards

Nov 7–14, 2023: Volume 135: Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS)

March 19–22, 2024: Advisory group to recommend priorities for the *IARC Monographs* during 2025–29

June 11–18, 2024: Volume 136: Talc and acrylonitrile

For the **risk assessment for aspartame carried out from June 27 to July 6, 2023, by the FAO/WHO Joint Expert Committee on Food Additives** see https://www.who.int/groups/joint-fao-who-expert-committee-on-food-additives-(jecfa)

For more on the **IARC Monographs evaluation of aspartame** see https://monographs.iarc.who.int/iarc-monographs-volume-134/

For the **Preamble to the IARC Monographs** see https://monographs.iarc.who.int/wp-content/uploads/2019/07/Preamble-2019.pdf

For **IARC declarations of interests** see https://monographs.iarc.who.int/wp-content/uploads/2022/06/Vol134-List-of-participants-final.pdf

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biomarkers and hyperplasia in the liver and other tissues in rodents, and induces chronic inflammation in experimental systems in a small set of data. Based on the above findings, the Working Group considered that there is "strong" mechanistic evidence for methyleugenol.

Isoeugenol is a fragrance and flavour compound that occurs in many plant species and in wood smoke. It is used in food, cosmetics, household products, animal feed, and veterinary medicines. Workers involved in isoeugenol synthesis or handling isoeugenol-containing products and firefighters may be exposed by dermal and inhalation routes. Exposure of the general population occurs through the diet and use of household products and cosmetics. Evidence on absorption, distribution, metabolism, and excretion of isoeugenol in humans is sparse and limited to dermal exposure. In rodents, after oral and dermal exposure, isoeugenol is rapidly absorbed and excreted predominantly in the urine as glucuronide or sulfate conjugates, with little retention in tissues.

In B6C3F$_1$ mice and F344 rats exposed by oral administration (gavage), isoeugenol caused hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in male mice; a significant increasing trend in histiocytic sarcoma (multiple sites) in female mice; and a significant increasing trend in mammary gland carcinoma and benign or malignant thymoma in male rats.[13] Significant increasing trends were observed with dose in the male rats and the female mice, leading the Working Group overall to conclude that the evidence for cancer in experimental animals was "sufficient" when considered alongside the findings in male mice. However, a minority of the Working Group considered the evidence to be "limited" and supported a Group 3 classification for isoeugenol because the pairwise comparison with the controls for male rats and female mice did not reach statistical significance in any of the treated groups.

Isoeugenol is a skin sensitiser that can be converted photochemically to electrophiles that form protein adducts. However, isoeugenol–DNA adducts were not detected in experimental systems. Overall, the mechanistic evidence for isoeugenol was "inadequate".

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