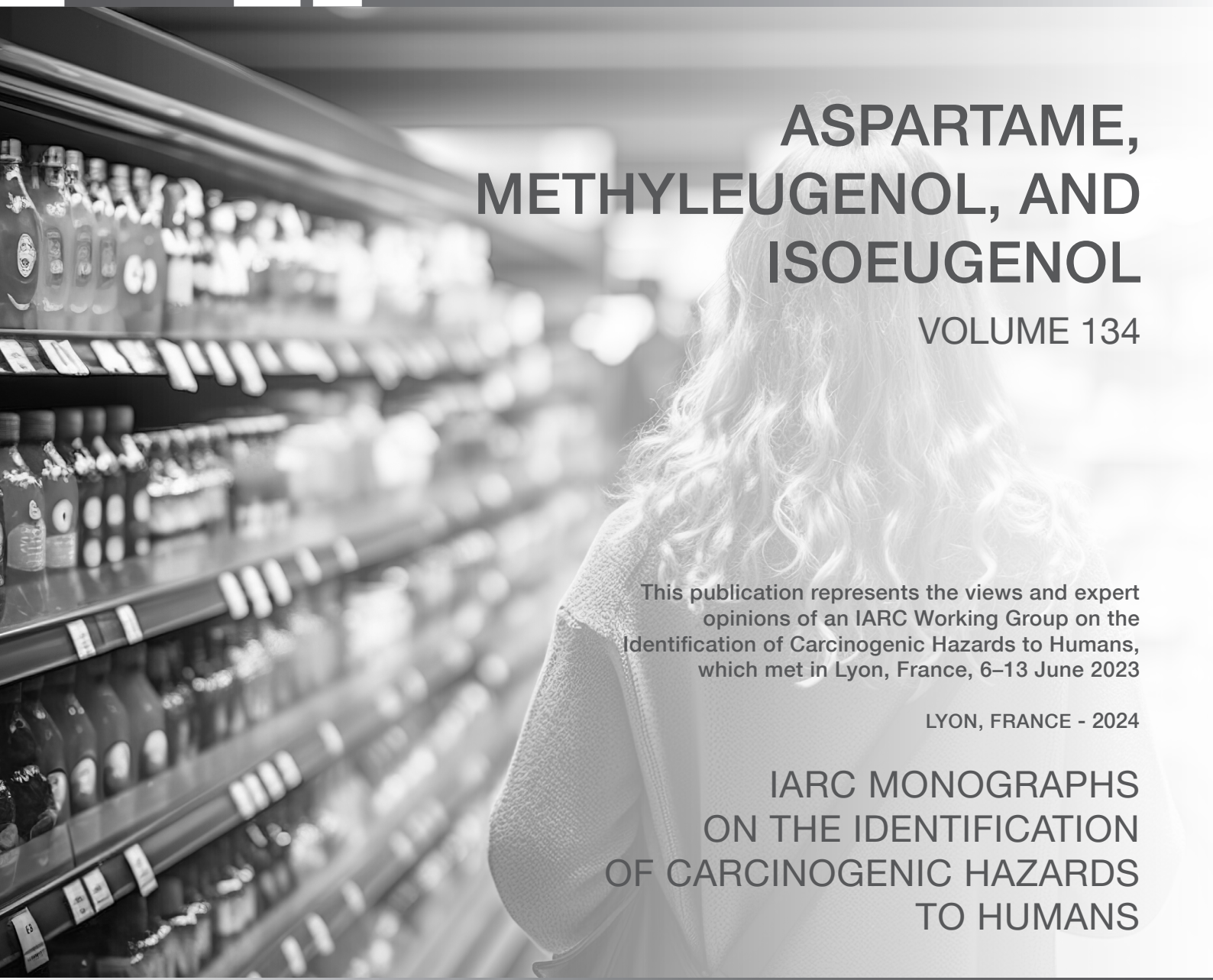
IARC MONOGRAPHS



ASPARTAME, METHYLEUGENOL, AND ISOEUGENOL

VOLUME 134

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met in Lyon, France, 6–13 June 2023

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IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

GENERAL REMARKS[a]

This one-hundred-and-thirty-fourth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of aspartame, methyleugenol, and isoeugenol.

Methyleugenol was considered previously by the *IARC Monographs* programme in 2011 (IARC, 2013), when it was evaluated as *possibly carcinogenic to humans* (Group 2B). Aspartame and isoeugenol have not been evaluated previously by the *IARC Monographs* programme.

The Advisory Group to Recommend Priorities for the *IARC Monographs* that met in 2019 recommended that all three agents be evaluated with high priority (IARC, 2019a; Marques et al., 2019). A summary of the findings of this volume appears in *The Lancet Oncology* (Riboli et al., 2023).

Coordination between the *IARC Monographs* programme and JECFA for the evaluation of aspartame

The monograph on aspartame is the result of a highly coordinated effort undertaken within WHO. First, IARC evaluated the carcinogenic hazard of aspartame. Subsequently, JECFA, the Joint FAO/WHO Expert Committee on Food Additives, conducted a risk assessment for cancer and other noncommunicable diseases, including reviewing and updating the acceptable daily intake (ADI) and dietary exposure assessment for aspartame. The monograph reports the results of the IARC evaluation of aspartame for cancer hazard identification; the results of the JECFA review of aspartame for dietary exposure and risk assessment have been published separately (WHO, 2023, 2024).

In line with the procedures established for communication and collaboration between the *IARC Monographs* programme and other WHO programmes, the *IARC Monographs* Meeting 134 on 6–13 June was followed closely by the JECFA Ninety-sixth Meeting on 27 June to 6 July. Aspartame was evaluated for the first time by IARC and for the third time by JECFA. The two bodies conducted independent but complementary reviews of all the available scientific literature. To ensure continuity and exchange of relevant information, three WHO scientists from the JECFA programme (Drs Sanaa and Montez and Mr Petersen) joined the IARC/WHO Secretariat for the *IARC Monographs* meeting, and two scientists from the *IARC Monographs* programme (Drs Madia and Benbrahim-Tallaa) joined the WHO Secretariat for the JECFA meeting.

[a] The previously posted "Preliminary General Remarks" relevant to the monograph on aspartame (published in advance in April 2024) were updated to include general remarks relevant to the full volume.

Furthermore, three of the seven Observers attending the IARC meeting (Drs Agudo, Barlow, and Wu) also served as members of the expert committee at the JECFA Ninety-sixth Meeting, and relevant literature search results were shared between the two programmes as permitted by any confidentiality requirements.

Exposure data for aspartame

The occurrence of aspartame in food, beverages, and consumer products and human exposure levels have been poorly documented over the years, despite the fact that this sweetener has been a commonly used food additive for several decades. The Working Group noted that few databases were available (see Sections 1.2 and 1.4 of the monograph on aspartame in the present volume) that reported comprehensive information on the presence of aspartame in various food categories, including beverages. In several databases, the Working Group also noted the lack of information on maximum permitted levels. Furthermore, information on dietary exposures in populations from low- and middle-income countries was lacking, as were data on occupational exposure during the manufacture or use of aspartame. Likewise, it was observed that precise quantification of aspartame exposure across various dietary sources in large-scale prospective cohorts has been performed only rarely (e.g. in the NutriNet-Santé cohort study by Debras et al., 2022).

Evaluation of aspartame metabolites

In the available literature investigating the absorption, distribution, metabolism, and excretion of aspartame, it was reported that once absorbed, this sweetener undergoes hydrolysis to form mainly its constituents: aspartic acid, phenylalanine, and methanol (see Section 4.1 of the monograph on aspartame in the present volume). The three hydrolytes undergo absorption from the intestinal lumen and reach the systemic circulation, in a similar manner to endogenous and exogenous amino acids and methanol obtained from other dietary sources. The homeostasis of the amino acids and methanol seems not to be influenced by the consumption of aspartame. In primates, the amino acid phenylalanine was reported to be retained in the body at higher levels than those of aspartic acid or methanol. Regarding specifically methanol, which can enter the portal circulation and is oxidized by hepatic alcohol dehydrogenase to formaldehyde (classified by IARC as *carcinogenic to humans*, Group 1; IARC, 2012) and finally to formic acid and then carbon dioxide, the Working Group noted that there was no evidence that the overall amount of formaldehyde formed as a result of aspartame consumption (up to the levels of the ADI of 40 mg/kg per day) would significantly alter normal endogenous formaldehyde concentrations. This is also valid for endogenous levels of aspartic acid and phenylalanine. For this reason, in the evaluation of the carcinogenic hazard of aspartame, the Working Group did not assess each individual metabolite separately with regard to evidence of cancer in experimental animals and mechanistic evidence.

Research gaps identified during the evaluation of aspartame

Glucose imbalance, insulin resistance, and altered lipid metabolism have been associated with increased risk of obesity, diabetes, and cancer. The Working Group reported on an increasing number of studies published over the past two decades that have investigated the effects of various non-nutritive sweeteners,

including aspartame, after single or repeated dosing in experimental systems in vivo and showed consistent alterations in insulin levels (see Section 4.3 of the monograph on aspartame in the present volume). Additionally, emerging literature has suggested associations with microbiome alterations and potential effects of aspartame on metabolism and cell growth mediated by sweet taste receptors, including a potential role of its metabolite phenylalanine. The interactions of aspartame with sweet taste receptors, which have been implicated in the signalling cascade that activates metabolism in the body and with the gut microbiota, were identified by the Working Group as notable research gaps. Likewise, studies of effects on end-points related to alterations of metabolism in humans, both those who are healthy and those with various health conditions (e.g. people with obesity or diabetes or who are pregnant), provided unclear results. A number of interventional or cross-sectional epidemiological studies presented several limitations associated with the small size of the study populations, with inadequate control for confounding variables in observational studies, or with the high complexity of the different study designs and protocols. In many studies, aspartame exposure was not precisely assessed, and the sweetener was considered as the reference positive control to be compared with other sweeteners, thus missing information on an appropriate background (unexposed) control.

The Working Group identified several major gaps in the literature: robust investigations using up-to-date methodologies on associations between precisely quantified aspartame exposure across various dietary sources and end-points related to metabolic alterations, including gut microbiome composition and function in large-scale studies in humans, were missing. There were no high-quality studies investigating mechanistic end-points associated with the key characteristics of carcinogens "induces oxidative stress" and "induces chronic inflammation" (which were observed in experimental systems) in exposed humans. Additional gaps included elucidation of the potential effects of aspartame on metabolism and metabolic outcomes (e.g. metabolic syndrome, type 2 diabetes, obesity, etc.) and on cancer risk. New research would support a better understanding of positive signals for liver cancer observed in both experimental animals and in epidemiological studies (i.e. hepatocellular carcinomas seen in the three available cohorts; Stepien et al., 2016; Jones et al., 2022; McCullough et al., 2022) and isolated signals for cancer of the mammary gland or breast (NutriNet-Santé cohort) (Debras et al., 2022). Similar research gaps have been also identified by the JECFA Committee in its review of aspartame for dietary exposure and risk assessment (IARC and JECFA, 2023).

Relevance of DNA adduct formation induced by exposure to methyleugenol

The formation of agent-specific DNA adducts can be considered to be a relevant marker of exposure and effect. DNA adducts represent an important end-point for the key characteristic of carcinogens "is electrophilic or can be metabolically activated to an electrophile" (IARC, 2019b). The relevance of the end-point and the strength of the evidence are evaluated with consideration of the specificity of the adducts and information on the evidence for mutations (key characteristic of "is genotoxic"). In a previous monograph (Volume 128; IARC, 2021), the available evidence on DNA adduct formation in exposed humans after exposure to either acrolein or crotonaldehyde was not considered to provide *strong* evidence of the key characteristics of carcinogens in exposed humans; the Working Group for Volume 134 agreed that similar considerations would also apply to methyleugenol.

However, in its evaluation of the carcinogenicity of methyleugenol, the Working Group considered that the widespread presence of agent-specific adducts in the human liver, together with the knowledge that those adducts were mutagenic in experimental systems, was central to the rationale for the IARC Group 2A classification of methyleugenol, even in the absence of direct evidence of mutations in exposed humans. In addition, the suggestion that the same mechanism would occur in exposed humans was corroborated by the study of Auerbach et al. (2018), which revealed that the mutational signature of methyleugenol in mouse liver tumours (determined by exome sequencing) closely resembled that of COSMIC (Catalogue Of Somatic Mutations In Cancer) signatures 4 and 24. The former signature is very similar to that produced by benzo[*a*]pyrene and other dietary carcinogens (e.g. PhIP) and the latter is similar to that of aflatoxin B1. The Working Group identified the study of mutagenesis in humans as a research gap, which could have been addressed by measuring genotoxicity end-points in the urine. On the basis of information from the study by Schecter et al. (2004), which demonstrated an increase in serum concentrations of methyleugenol in humans after the consumption of gingersnap cookies, urine could be evaluated for individuals who have consumed a defined amount of methyleugenol in one of the many commonly consumed foods containing methyleugenol in significant amounts. There are well-characterized methods for biomonitoring of human exposure to mutagens by looking for micronuclei in the bladder epithelial cells normally found in urine samples. Measuring methyleugenol metabolites in urine would help to establish a link (or lack thereof) by providing more detailed information about the amount and timing of any formation of the presumptive pro-mutagen after routine dietary exposures.

Similar considerations would be appropriate for isoeugenol, for which there are almost no phamacokinetic data in any system; finding evidence of the formation of micronuclei, adducts, or perhaps even metabolites formed via the quinoline methide in human urine after dietary exposure might contribute to a reclassification of isoeugenol.

Scope of the systematic review

Standardized searches of the PubMed database (NCBI, 2023) were conducted for each agent for each outcome (cancer in humans, cancer in experimental animals, and mechanistic evidence, including the key characteristics of carcinogens). For cancer in humans, searches were also conducted in the Web of Science (Clarivate, 2023) and Embase (Elsevier, 2023) databases. The literature trees for aspartame, methyleugenol, and isoeugenol, including the full set of search terms for the agent name and each outcome type, are available online.[a]

As described in the current Preamble to the *IARC Monographs* (last revised in 2019; see pages 14–15 in the present volume; IARC, 2019b), the Working Group reviews publicly available scientific data, such as peer-reviewed papers in the scientific literature, and may also review unpublished reports, if made available in their final form by governmental agencies and if they contain enough detail for critical review. In the case of aspartame, the Working Group was able to consult and review literature derived from the Call for Data in 2011 for the European Food Safety Authority (EFSA) risk assessment, which was made available and accessible on the EFSA

[a] The literature trees for the monographs in the present volume are available at: https://hawcproject.iarc.who.int/assessment/680/ (aspartame); https://hawcproject.iarc.who.int/assessment/688/ (methyleugenol); and https://hawcproject.iarc.who.int/assessment/689/ (isoeugenol).

website (EFSA, 2011). In addition, IARC opened a public Call for Data on its website 1 year ahead of the meeting for Volume 134. Eligible studies are only those published or accepted for publication in the openly available scientific literature by the time of the Working Group meeting.

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