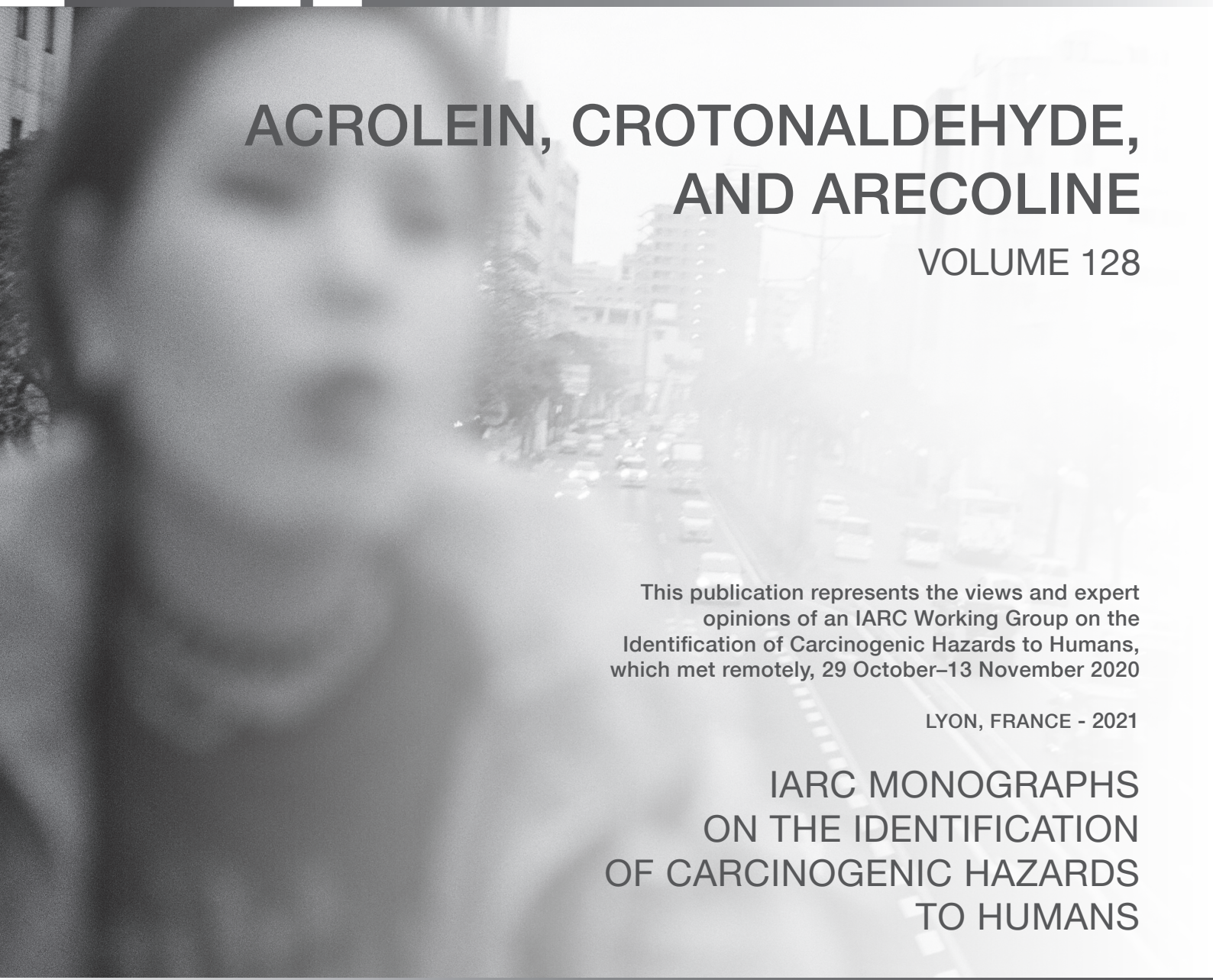
IARC MONOGRAPHS



ACROLEIN, CROTONALDEHYDE, AND ARECOLINE

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This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met remotely, 29 October–13 November 2020

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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

This one-hundred-and-twenty-eighth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of acrolein, crotonaldehyde, and arecoline. Due to the coronavirus disease (COVID-19) pandemic, this meeting, which was scheduled to be held in Lyon, France, was held remotely.

Acrolein was considered previously by the *IARC Monographs* programme in 1978 (IARC, 1979), 1984 (IARC, 1985), 1987 (IARC, 1987), and most recently in 1995 (IARC, 1995), when it was evaluated as *not classifiable as to its carcinogenicity to humans (Group 3).* Crotonaldehyde was also evaluated by the *IARC Monographs* programme in 1995, when the Working Group concluded that it was *not classifiable as to its carcinogenicity to humans (Group 3)* (IARC, 1995).

Arecoline itself has not been previously evaluated by the *IARC Monographs* programme. However, arecoline is the most abundant alkaloid considered in the context of betel-quid and areca-nut chewing in *IARC Monographs* Volume 85 (IARC, 2004) and Volume 100E (IARC, 2012a). It is the primary active ingredient of the areca nut, which is classified as *carcinogenic to humans (Group 1)* (IARC, 2012a).

The Advisory Group to Recommend Priorities for the *IARC Monographs* that met in 2019 recommended that all three agents be evaluated with high priority (Marques et al., 2019). New data have become available, primarily bioassay and mechanistic evidence, and these data have been included and considered in the present volume. A summary of the findings of this volume appears in *The Lancet Oncology* (Marques et al, 2021).

Electrophilicity as a key characteristic of carcinogens

A characteristic feature of many carcinogens is that they are either direct-acting electrophiles or are metabolized to an electrophile. Electrophiles can bind to nucleophilic sites in DNA, forming adducts with DNA and potentially leading to DNA damage, including mutations, DNA strand breaks, and chromosomal aberrations. As described in a recent IARC Scientific Publication, multiple factors and mechanistic processes may play a role in determining whether electrophiles will result in carcinogenicity (IARC, 2019). A key factor is the extent of DNA binding of the electrophile, which can be affected by physiochemical properties (i.e. binding affinity for DNA or protein), time-dependent tissue concentrations, and the presence of alternative molecular targets (e.g. glutathione). In addition, the fate of the induced DNA lesion(s) may be influenced by various other molecular, cellular, and physiological factors, including the physical

properties of the lesion (e.g. persistence and mutagenic potential), the activities and effectiveness of relevant DNA repair processes, and the rates of cell division and cell death. Importantly, related mechanistic effects, including inhibition of topoisomerase II and inhibition of DNA repair, may also be triggered by some electrophiles, which can amplify the resulting DNA damage. Therefore, when considering whether a compound "is electrophilic" in a toxicologically meaningful way, its reactivity with biologically relevant nucleophiles, the nature of the resulting lesion(s), and the physiological context should be taken into account.

Evidence that an agent "is electrophilic" may come from a range of computational analyses, molecular and cellular experiments, and other types of studies. Relevant findings may predict inherent DNA reactivity of the parent compound or its metabolite(s), characterize the structure of the DNA adduct, and illustrate adduct formation during controlled experiments in experimental animals or after occupational or other exposures in humans. Studies on DNA-adduct formation may provide an essential part of this evidence, depending on the specificity of the adduct for the exposure and the outcome. As highlighted in the Preamble to the *IARC Monographs* and relevant for this key characteristic, data from studies investigating susceptibility to cancer in experimental animals or in humans can provide important support for mechanistic conclusions. For example, the Group 1 evaluations of trichloroethylene, *N*′-nitrosonornicotine, and nitrosomethylamino)-1-(3-pyridyl)-1-butanone (IARC, 2012a; 2014) were supported by studies showing that polymorphisms in metabolic enzymes affecting the formation or detoxification of electrophiles can influence cancer risk. The strength of these molecular epidemiology studies in supporting the conclusions of the Working Group relies on the evaluation of study quality, as elaborated in the Preamble, and is the focus of the collaborative review undertaken with Working Group members reviewing exposure characterization, studies of cancer in humans, and mechanistic data.

As further noted in the Preamble, evidence for a group of key characteristics of carcinogens described by Smith et al. (2016) can provide additional context and can strengthen mechanistic conclusions overall. For many Group 1 agents that are electrophilic (e.g. ethylene oxide; IARC, 2012b), or that can be metabolized to electrophiles (e.g. aflatoxins, vinyl chloride, aristolochic acid; IARC, 2012b, c), the mechanistic conclusions were strengthened by evidence that these agents are genotoxic. Studies of micronucleus induction in exposed workers or studies of molecular signatures in the DNA (e.g. the *TP53* mutation signature for aristolochic acid; IARC, 2012c) have been especially influential in this regard. For the agents evaluated in the present volume, all of which are electrophilic without requiring metabolic activation, there was evidence from a range of studies conducted in different systems and supporting multiple key characteristics of carcinogens. For two of the agents, acrolein and crotonaldehyde, there was supporting evidence for these mechanistic conclusions from studies on DNA adducts in humans; while providing important support, human studies on DNA adducts did not alone provide "strong" evidence from exposed humans of key characteristics of carcinogens. Acrolein is a strongly electrophilic α,β-unsaturated aldehyde (enal) that readily reacts with DNA bases and proteins. Acrolein is genotoxic; it alters DNA repair or causes genomic instability; it induces oxidative stress; it is immunosuppressive; it induces chronic inflammation; and it alters cell proliferation, cell death, or nutrient supply. Crotonaldehyde is an electrophilic bifunctional α,β-unsaturated aldehyde (enal) that can form cyclic adducts in DNA, DNA interstrand crosslinks, and DNA–protein crosslinks. Crotonaldehyde is genotoxic; it induces oxidative stress; and it induces chronic inflammation. Arecoline is an electrophilic

α,β-unsaturated ester that can undergo Michael addition with cellular nucleophiles. It is genotoxic; it alters DNA repair or causes genomic instability; and it induces oxidative stress.

Differentiating endogenous from exogenous exposures

For two of the agents considered in the present volume, acrolein and crotonaldehyde, human exposure may derive from both endogenous processes and exogenous sources. Findings were similar for some other aldehydes previously evaluated by the *IARC Monographs* programme, such as acetaldehyde and formaldehyde (IARC, 2012a, b).

As discussed in the monographs on acrolein and crotonaldehyde, many studies considered only tobacco smoking as an external exposure source to these chemicals and attributed findings in non-smokers (such as DNA adduct formation) to endogenous exposures. However, a diversity of exogenous exposures, including tobacco smoke as well as air pollution, diesel engine exhaust, inflammatory conditions, and a high fat diet, have been demonstrated in well-controlled experimental studies to increase the levels of aldehyde DNA adducts derived from acrolein and crotonaldehyde. Appropriate attribution of the effects of these diverse exogenous exposures is important in consideration of endogenous formation of acrolein and crotonaldehyde.

Endogenous formation of acrolein and crotonaldehyde is mechanistically plausible. For acrolein, endogenous formation may occur by several pathways, including the reaction of myeloperoxidase with hydroxyl-amino acids such as threonine; the oxidation of spermine and spermidine by amine oxidase; and peroxidation of polyunsaturated fatty acids (see the monograph on acrolein). Lipid peroxidation and metabolism, specifically from ω-3 polyunsaturated fatty acids, including docosahexaenoic acid, linoleic acid, and eicosapentaenoic acid, are also suggested endogenous sources of crotonaldehyde. As a result, aldehyde-derived DNA adducts are constantly formed in cellular DNA as endogenous background lesions.

The question remains as to how informative these endogenous processes are for cancer hazard assessment. Firstly, endogenous background levels of an agent pose a challenge in untangling external from internal exposures, especially if the external exposure can only be indirectly assessed from metabolites or biomarkers. The interpretation of data on such biomarkers is not always straightforward and it may be difficult to separate the contribution of endogenous formation of metabolites (e.g. originating from lipid peroxidation or inflammation) from exogenous sources. Secondly, decreased or increased adduct levels may result from alterations in endogenous processes, including during the toxic response to the agent or during the course of cancer development. For example, endogenous formation of formaldehyde may be important in the development of leukaemia in patients with Fanconi anaemia (IARC, 2012b). For these reasons, DNA adducts in human cancer cells cannot be considered as a marker of external exposure, since cells have undergone several cycles of molecular changes and selection that affect the internal concentration of adducts.

Data from high-throughput screening assays

The analysis of the in vitro bioactivity of acrolein and arecoline was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). The results from

these assays were uninformative regarding the carcinogenicity of these agents. Although neither programme includes assays for mutagenicity or DNA-adduct formation, they do include a few assays to detect end-points encompassed by the key characteristics of carcinogens (Smith et al., 2016), such as DNA repair, altered gene expression, oxidative stress, and modulated receptor-mediated effects. Nonetheless, a recent analysis of data from five such assays in Tox21 showed < 40% sensitivity for agents that are direct-acting genotoxicants in standard assays (i.e. Ames test, chromosomal aberrations in vitro, micronucleus formation in vivo) (Hsieh et al., 2019). These programmes are constantly being improved and new assays are included over time; however, at present, the general lack of metabolic capacity and the small number of genotoxicity assays limits the value of these high-throughput screening programmes for carcinogenicity assessments of genotoxic and other chemicals.

Scope of systematic review

Standardized searches of the PubMed database (National Library of Medicine, 2021) were conducted for the agent and for each outcome (cancer in humans, cancer in experimental animals, and mechanistic evidence, including the key characteristics of carcinogens). The literature trees for the agent, including the full set of search terms for the agent name and each outcome type, are available online.[1]

References

Hsieh JH, Smith-Roe SL, Huang R, Sedykh A, Shockley KR, Auerbach SS, et al. (2019). Identifying compounds with genotoxicity potential using Tox21 high-throughput screening assays. *Chem Res Toxicol*. 32(7):1384–401. doi:10.1021/acs.chemrestox.9b00053 PMID:31243984

IARC (1979). Some monomers, plastics and synthetic elastomers, and acrolein. *IARC Monogr Eval Carcinog Risk Chem Hum*. 19:1–513. Available from: https://publications.iarc.fr/37 PMID:285915

IARC (1985). Tobacco habits other than smoking; betel-quid and areca-nut chewing; and some related nitrosamines. *IARC Monogr Eval Carcinog Risk Chem Hum*. 37:1–291. Available from: https://publications.iarc.fr/55 PMID:3866741

IARC (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*. 7:1–440. Available from: https://publications.iarc.fr/139 PMID:3482203

IARC (1995). Dry cleaning, some chlorinated solvents and other industrial chemicals. *IARC Monogr Eval Carcinog Risks Hum*. 63:1–551. Available from: https://publications.iarc.fr/81 PMID:9139128

IARC (2004). Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines. *IARC Monogr Eval Carcinog Risks Hum*. 85:1–334. Available from: https://publications.iarc.fr/103 PMID:15635762

IARC (2012a). Personal habits and indoor combustions. *IARC Monogr Eval Carcinog Risks Hum*. 100E:1–575. Available from: https://publications.iarc.fr/122 PMID:23193840

IARC (2012b). Chemical agents and related occupations. *IARC Monogr Eval Carcinog Risks Hum*. 100F:1–599. Available from: https://publications.iarc.fr/123 PMID:23189753

IARC (2012c). Pharmaceuticals. *IARC Monogr Eval Carcinog Risks Hum*, 100A:1–435. Available from: https://publications.iarc.fr/118 PMID:23189749

IARC (2014). Trichloroethylene, tetrachloroethylene, and some other chlorinated agents. *IARC Monogr Eval Carcinog Risks Hum*. 106:1–514. Available from: https://publications.iarc.fr/130 PMID:26214861

IARC (2019). Tumour site concordance and mechanisms of carcinogenesis. IARC Sci Pub. 165. Available from: https://publications.iarc.fr/578.

[1] The literature trees for the present volume are available at: https://hawcproject.iarc.who.int/assessment/436/ (acrolein), https://hawcproject.iarc.who.int/assessment/646/ (crotonaldehyde), and https://hawcproject.iarc.who.int/assessment/638/ (arecoline).

Marques MM, Beland FA, Lachenmeier DW, Phillips DH, Chung F-L, Dorman DC, et al. (2021). Carcinogenicity of acrolein, crotonaldehyde, and arecoline. *Lancet Oncol*. 22(1):19–20. doi:10.1016/S1470-2045(20)30727-0 PMID:33248467

Marques MM, Berrington de Gonzalez A, Beland FA, Browne P, Demers PA, Lachenmeier DW, et al.; IARC Monographs Priorities Group (2019). Advisory Group recommendations on priorities for the IARC Monographs. *Lancet Oncol*. 20(6):763–4. doi:10.1016/S1470-2045(19)30246-3 PMID:31005580

National Library of Medicine (2021). PubMed [online database]. Bethesda (MD), USA: National Library of Medicine. Available from: https://pubmed.ncbi.nlm.nih.gov/

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*. 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Thomas RS, Paules RS, Simeonov A, Fitzpatrick SC, Crofton KM, Casey WM, et al. (2018). The US Federal Tox21 Program: A strategic and operational plan for continued leadership. *ALTEX*. 35(2):163–8. doi:10.14573/altex.1803011 PMID:29529324