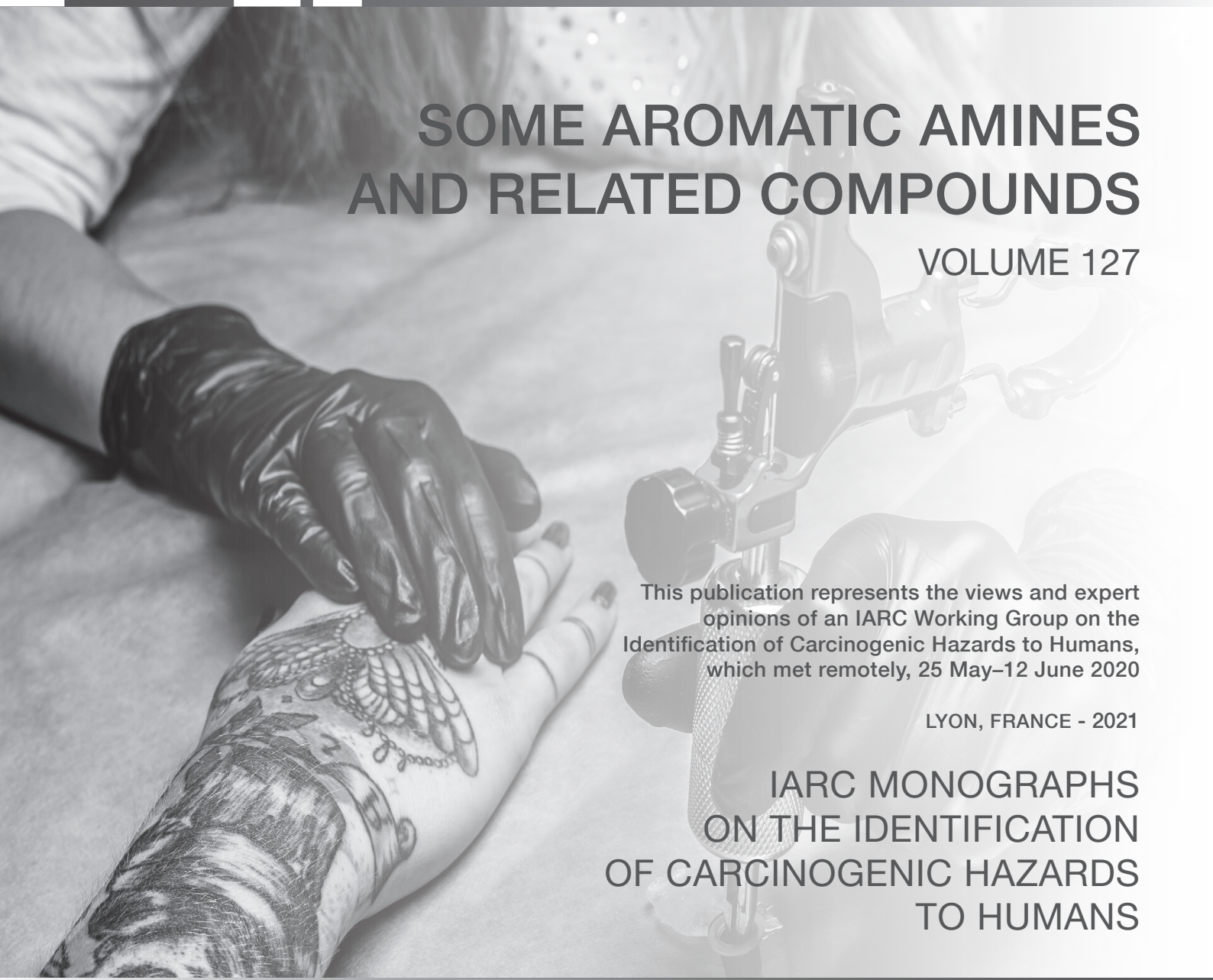
IARC MONOGRAPHS



SOME AROMATIC AMINES AND RELATED COMPOUNDS

VOLUME 127

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met remotely, 25 May–12 June 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-seventh volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of some aromatic amines and related compounds, including *ortho*-nitroanisole, *ortho*-anisidine (and its salt, *ortho*-anisidine hydrochloride), aniline (and its salt, aniline hydrochloride), and cupferron. Due to the coronavirus disease (COVID-19) pandemic, this meeting, which was scheduled to be held in Lyon, France, was held remotely.

ortho-Nitroanisole was considered previously by the *IARC Monographs* programme in Volume 65 of the *IARC Monographs* (IARC, 1996), when it was evaluated as *possibly carcinogenic to humans (Group 2B)* because of *sufficient evidence* in experimental animals. *ortho*-Anisidine, a High Production Volume chemical and metabolite of *ortho*-nitroanisole, was considered previously in Supplement 7 (IARC, 1987) and Volume 73 of the *IARC Monographs* (IARC, 1999), when it was evaluated as *Group 2B* because of *sufficient evidence* in experimental animals. Aniline is a High Production Volume chemical that was considered previously in Volume 27 (IARC, 1982) and Supplement 7 of the *IARC Monographs* (IARC, 1987), when it was evaluated as *not classifiable as to its carcinogenicity to humans (Group 3)* because of *limited evidence* in experimental animals and *inadequate evidence* in humans. Cupferron has not been previously evaluated by the *IARC Monographs* programme.

The Advisory Group to Recommend Priorities for the *IARC Monographs* recommended that *ortho*-anisidine (together with the structurally similar *ortho*-nitroanisole), aniline, and cupferron 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.[1]

A summary of the findings of this volume appears in *The Lancet Oncology* (DeMarini et al., 2020).

[1] Standardized searches of the PubMed database were conducted for each agent and for each outcome (cancer in humans, cancer in experimental animals, and mechanistic evidence, including the key characteristics of carcinogens). The literature trees, including the full set of search terms for the agent name and each outcome type, are available at: https://hawcproject.iarc.fr/assessment/623/ (*ortho*-anisidine and its hydrochloride salt), https://hawcproject.iarc.fr/assessment/624/ (*ortho*-nitroanisole), https://hawcproject.iarc.fr/assessment/625/ (aniline and its hydrochloride salt), and https://hawcproject.iarc.fr/assessment/627/ (cupferron).

Information gaps in exposure characterization

For all the agents in this volume, the Working Group noted substantial data gaps regarding production and use, as well as environmental and occupational exposure levels. Data gaps exist for high-income countries but are particularly notable in low- and middle-income countries. The Working Group has noted in the monograph on aniline that these data gaps were especially surprising given that aniline is a High Production Volume chemical with widespread occupational use, and for which there is long-standing concern about toxicity and potential carcinogenicity.

Considerations relating to studies of cancer in humans

The body of literature related to cancer in humans exposed to these agents was sparse. Exceptionally, case reports or case series were considered for *ortho*-anisidine and aniline as potentially providing information on rare cancers among exposed workers, as well as historical context. However, the paucity of exposure information and co-exposures to other carcinogenic agents limited the informativeness of these reports. The few available case–control or cohort studies of occupational exposure to aniline mostly investigated cancer of the urinary bladder. Most of these studies could not distinguish the effects of aniline from those of co-exposures to known bladder carcinogens, or had other important limitations.

Mechanistic class considerations

Three of the agents evaluated (*ortho*-nitroanisole, *ortho*-anisidine, and aniline) were classified in IARC Group 2A on the basis of *strong* mechanistic evidence. In view of the metabolism of *ortho*-nitroanisole to the aromatic amine *ortho*-anisidine, and mechanistic considerations for all three agents, *ortho*-nitroanisole, *ortho*-anisidine, and aniline were classified on the basis of belonging to a class of aromatic amines for which several members (i.e. 4-aminobiphenyl, 2-naphthylamine, and *ortho*-toluidine) have been classified as *carcinogenic to humans* (IARC Group 1). *ortho*-Nitroanisole, *ortho*-anisidine, and aniline are similar to this class of aromatic amines with respect to the formation of common DNA-reactive moieties, genotoxicity, and target organs of carcinogenicity in animal bioassays for chronic toxicity. No data on DNA adducts in humans were available for *ortho*-nitroanisole, *ortho*-anisidine, or aniline. However, data on metabolism and DNA-adduct formation from human in vitro systems and studies of exposed rodents supported the view that *ortho*-nitroanisole, *ortho*-anisidine, and aniline are metabolically activated to reactive electrophiles and undergo binding to DNA in a manner that parallels the established paradigm for carcinogenic aromatic amines. *ortho*-Nitroanisole, *ortho*-anisidine, and aniline are genotoxic. The urinary bladder is a common target organ of carcinogenicity for several of these aromatic amines in experimental animals. In the rat urinary bladder, *ortho*-nitroanisole and *ortho*-anisidine form DNA adducts, induce DNA damage, and cause malignant tumours when administered orally. The Group 1 agents *ortho*-toluidine and 2-naphthylamine similarly cause malignant bladder tumours in the rat, and 4-aminobiphenyl causes malignant tumours of the urinary bladder when administered orally to dogs and mice (IARC, 2012). Other common target organs

of carcinogenicity in experimental animals are the spleen and testis. DNA binding and malignant tumours are seen in the spleen after oral administration of aniline to male Fischer 344 rats. The Group 1 agent *ortho*-toluidine similarly induces malignant splenic tumours in these rats, and an increased incidence of mesothelioma of the tunica vaginalis of the testis is seen with both aniline and *ortho*-toluidine (IARC, 2012). Overall, the mechanistic considerations that strongly support classification of *ortho*-nitroanisole, *ortho*-anisidine, and aniline in Group 2A go beyond chemical structural similarity to encompass biological and biochemical similarities relevant to common key characteristics of carcinogens.

Data gaps regarding cupferron

Cupferron (*N*-nitroso-*N*-phenylhydroxylamine) is a *N*-nitroso hydroxylamine that has a unique chemical structure (Hrabie & Keefer, 2002). Such chemicals have attracted research interest for pharmaceutical applications because of their capacity to release nitric oxide (NO) under physiological conditions. Several experimental studies in acellular and non-mammalian systems support the view that cupferron can generate NO. For instance, in an acellular system, Alston et al. (1985) showed that cupferron is an excellent substrate for horseradish peroxidase (with $k > 10^7$ M^{-1} s^{-1}) and generated NO and nitrosobenzene. Similarly, Hou et al. (1999) demonstrated that the *O*-alkyl derivatives of cupferron could function as NO photoreleasing donor compounds, as also supported by Thomsen et al. (2018). The exposure of *Vicia faba* roots to cupferron and visible light in the absence of oxygen caused the induction of chromosomal aberrations, as did NO in this test system (Kihlman, 1959). Overall, these results indicate that cupferron and its derivatives can be oxidized to the unstable oxy radical, which then spontaneously decomposes to nitrosobenzene. Considering that many mammalian peroxidases (e.g. thyroid peroxidase, lactoperoxidase, eosinophil peroxidase) have a similar function to that of plant horseradish peroxidase (Josephy, 1986; Vlasova, 2018), it is reasonable to assume that in mammalian species cupferron is likely to be oxidized to nitrosobenzene and produce NO by certain peroxidases, especially in the presence of oxygen. Interestingly, nitrosobenzene is a reactive metabolite of aniline, supporting mechanistic commonalities between aniline and cupferron. However, no data on absorption, distribution, metabolism, or excretion in humans or in other mammalian systems (in vivo or in vitro) were available for the main metabolites formed from cupferron to inform conclusions about any commonalities with aniline or other aromatic amines (e.g. *ortho*-toluidine).

The available information from tests for genotoxicity with cupferron in experimental systems indicated that cupferron is mutagenic and clastogenic. Quantitative structure–activity relationship (QSAR) modelling predicted the mutagenic potential of cupferron. The findings in animal cancer bioassays that cupferron induces tumours at multiple sites in both sexes of rats and mice are consistent with expectations for chemicals that are mutagenic and clastogenic. However, there are significant gaps in evidence for cupferron including a lack of information in humans and other in vivo mammalian systems relevant to key characteristics of carcinogens. As noted above, studies on absorption, distribution, metabolism, or excretion of cupferron in mammalian species or cells were not available, although chemistry information and acellular data support the inference that cupferron can be metabolized to nitrosobenzene, especially in the presence of oxygen. Overall, there is strong evidence from experimental systems that cupferron exhibits key characteristics of carcinogens; cupferron is genotoxic. Cupferron was

carcinogenic in both rats and mice, inducing tumours of the forestomach and liver. Even in the absence of the observed *sufficient evidence* for carcinogenicity in experimental animals, the available *strong* mechanistic evidence alone supports the classification of cupferron in Group 2B.

Data from high-throughput screening assays

The analysis of the in vitro bioactivity of several of the agents reviewed in the present volume 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 all 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 activation and the small number of genotoxicity assays restricts the value of these high-throughput screening programmes for carcinogenicity assessments of genotoxic and other chemicals.

Methaemoglobinaemia

Methaemoglobinaemia is an adverse outcome commonly seen after exposure to many toxicants, including *ortho*-nitroanisole, *ortho*-anisidine, aniline, and various other aromatic amino- and nitroaromatic compounds classified by the *IARC Monographs* programme (e.g. the Group 2B agents 2-chloronitrobenzene, 4-chloronitrobenzene, 2-amino-4-chlorophenol, and *N*,*N*-dimethyl-*p*-toluidine). For several such agents, methaemoglobin formation has been attributed to the formation of *N*-hydroxyarylamine and other metabolites that engage in Kiese redox cycling yielding methaemoglobin and increasing cellular oxidative stress (Sabbioni, 2017). In methaemoglobinaemia, oxidation of the haem iron reduces the oxygen-carrying capacity of the blood. As a biological marker of exposure, methaemoglobin in blood is used as a basis for exposure indices, such as those set by the American Conference of Governmental Industrial Hygienists (ACGIH, 2008).

References

ACGIH (2008). American Conference of Governmental Industrial Hygienists TLVs and BEIs. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati (OH), USA: American Conference of Governmental Industrial Hygienists.

Alston TA, Porter DJ, Bright HJ (1985). Generation of nitric oxide by enzymatic oxidation of *N*-hydroxy-*N*-nitrosamines. *J Biol Chem*. 260(7):4069–74. doi:10.1016/S0021-9258(18)89232-8 PMID:3980468

DeMarini DM, Carreón-Valencia T, Gwinn WM, Hopf NB, Sandy MS, Bahadori T, et al.; IARC Monographs Vol 127 group (2020). Carcinogenicity of some aromatic amines and related compounds. *Lancet Oncol*. 21(8):1017–8. doi:10.1016/S1470-2045(20)30375-2 PMID:32593317

Hou YC, Janczuk A, Wang PG (1999). Current trends in the development of nitric oxide donors. *Curr Pharm Des*. 5(6):417–41. PMID:10390607

Hrabie JA, Keefer LK (2002). Chemistry of the nitric oxide-releasing diazeniumdiolate ("nitrosohydroxylamine") functional group and its oxygen-substituted derivatives. *Chem Rev.* 102(4):1135–54. doi:10.1021/cr000028t PMID:11942789

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 (1982). Some aromatic amines, anthraquinones and nitroso compounds, and inorganic fluorides used in drinking-water and dental preparations. *IARC Monogr Eval Carcinog Risk Chem Hum*, 27:1–341. Available from: https://publications.iarc.fr/45 PMID:6955259

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 (1996). Printing processes and printing inks, carbon black and some nitro compounds. *IARC Monogr Eval Carcinog Risks Hum*, 65:1–578. Available from: https://publications.iarc.fr/83 PMID:9148039

IARC (1999). Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. *IARC Monogr Eval Carcinog Risks Hum*, 73:1–674. Available from: https://publications.iarc.fr/91.

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

Josephy PD (1986). Benzidine: mechanisms of oxidative activation and mutagenesis. *Fed Proc.* 45(10):2465–70. PMID:3091404

Kihlman BA (1959). Studies on the production of chromosomal aberrations by visible light: the effects of cupferron, nitric oxide and wavelength. *Exp Cell Res.* 17(3):590–3. doi:10.1016/0014-4827(59)90096-5 PMID:13672232

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

Sabbioni G (2017). Hemoglobin adducts and urinary metabolites of arylamines and nitroarenes. *Chem Res Toxicol.* 30(10):1733–66. doi:10.1021/acs.chemrestox.7b00111 PMID:28933159

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

Thomsen H, Marino N, Conoci S, Sortino S, Ericson MB (2018). Confined photo-release of nitric oxide with simultaneous two-photon fluorescence tracking in a cellular system. *Sci Rep.* 8(1):9753. doi:10.1038/s41598-018-27939-4 PMID:29950654

Vlasova II (2018). Peroxidase activity of human hemoproteins: keeping the fire under control. *Molecules.* 23(10):2561. doi:10.3390/molecules23102561 PMID:30297621