IARC MONOGRAPHS

GENTIAN VIOLET, LEUCOGENTIAN VIOLET, MALACHITE GREEN, LEUCOMALACHITE GREEN, AND CI DIRECT BLUE 218

VOLUME 129

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met remotely, 22 February to 5 March 2021

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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-ninth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of gentian violet, leucogentian violet, malachite green, leucomalachite green, and CI Direct Blue 218. Due to the coronavirus disease (COVID-19) pandemic, this meeting, which was scheduled to be held in Lyon, France, was held remotely.

None of these agents have been evaluated previously by the *IARC Monographs* programme.

The Advisory Group to Recommend Priorities for the *IARC Monographs* that met in 2019 recommended that gentian violet, malachite green, and leucomalachite green be evaluated with high priority, and CI Direct Blue 218 with medium priority (Marques et al., 2019). A summary of the findings of this volume appears in *The Lancet Oncology* (LeCurieux et al., 2021).

Paucity of exposure data

For all the five dyes in this volume, the Working Group observed substantial data gaps regarding production and use, as well as environmental and occupational exposure levels. These data gaps are particularly notable in low- and middle-income countries but also exist in high-income countries. The Working Group has noted in the monograph on CI Direct Blue 218 that these data gaps were especially surprising given that CI Direct Blue 218 is listed by the Organisation for Economic Co-operation and Development (for the year 2007) as a High Production Volume chemical (OECD, 2009), and has widespread potential for occupational exposure during the manufacturing process (synthesis, processing, packaging, transportation, or maintenance and clean-up), during the application of the dye on products, and also during any additional processing of dyed products that results in particle formation (NIOSH, 1983).

Dye purity

The Working Group noted that the poor purity of all the dyes considered in the present volume, but especially CI Direct Blue 218, has been shown to be an important drawback to interpretation of the results of the available studies. If more experiments were performed in the future, dyes of a high purity (> 95%, if possible) should be tested to ensure that any effect observed can be attributed to the dye itself and not to other compounds (i.e. impurities) present in the sample.

Metabolism and mutagenicity of CI Direct Blue 218

Among the evidence gaps identified in this volume was whether CI Direct Blue 218 is metabolized to benzidine (classified as *carcinogenic to humans*, Group 1), or the benzidine congeners 3,3′-dihydroxybenzidine or 3,3′-dimethoxybenzidine. This gap was noted previously in *IARC Monographs* Volume 99, when CI Direct Blue 218 was not included in the classification of the agent "Dyes metabolized to benzidine" (as *carcinogenic to humans*, Group 1), in contrast to other dyes such as Direct Black 38, Direct Blue 6, and Direct Brown 95 (IARC, 2010). The Working Group at that time suggested that future mechanistic studies should determine whether enzymatic reduction of CI Direct Blue 218 would generate the benzidine congener 3,3′-dimethoxybenzidine. Another evidence gap was the lack of informative studies elucidating the mutagenicity of CI Direct Blue 218. The Working Group considered that mechanistic studies are also warranted to test CI Direct Blue 218 in assays for gene mutation in the presence of endogenous metabolic activation with *Salmonella typhimurium* strains YG1041 or YG1024 that are particularly sensitive to aromatic amines.

Distinguishing between various salts of malachite green in exposure characterization data

The dye malachite green occurs as a chloride but is also available as an oxalate and as other salts, which are each used in various amounts for different and common applications. While some information was available to the Working Group regarding specific applications for the different chemical forms of malachite green, all are often referred to interchangeably by the general term "malachite green". This is particularly true in the literature on exposure characterization (e.g. reports of concentrations of malachite green residue measured in various matrices), in which the distinction between the different chemical forms of malachite green is often not made.

Environmental transformation of gentian violet and malachite green to and from their leucometabolites

In the environment, malachite green is transformed via a reversible reaction (reduction↔oxidation) under anaerobic conditions into leucomalachite green. In the atmosphere and in water, malachite green and gentian violet may undergo photodegradation into leucomalachite green and leucogentian violet, respectively. In vivo, enzymatic transformation of malachite green and gentian violet into their corresponding leucometabolites is well documented, although data on humans are scarce. Consequently, co-occurrence of each dye with its leucometabolite is likely.

Data from high-throughput screening assays

The analysis of the in vitro bioactivity of gentian violet, malachite green chloride, malachite green oxalate, and leucomalachite green 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). All compounds were considered active in a variety of the assay end-points mapped to the following key characteristics of carcinogens: induces oxidative stress, modulates receptor-mediated effects, and

alters cell proliferation, cell death, or nutrient supply. Specifically, gentian violet and malachite green oxalate were considered active in most of the "is genotoxic" assay end-points, and malachite green chloride was considered active in all the "induces epigenetic alterations" assay end-points. The mapping of assay end-points to each key characteristic follows that described in *IARC Monographs* Volume 123 (IARC, 2019). All ToxCast/Tox21 data were downloaded from the United States Environmental Protection Agency CompTox Chemicals Dashboard 10th Release (US EPA, 2021) on 2–19 October 2020 or on 24 February 2021 (malachite green oxalate). These programmes are constantly being improved and new assays are added over time. However, at present, the general lack of metabolic activation and the small number of genotoxicity assays in these high-throughput screening programmes restrict their value in determining whether a chemical is genotoxic as part of an assessment of carcinogenicity.

Scope of the systematic review

Standardized searches of the PubMed database (NCBI, 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]

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[1] The literature trees for the present volume are available at: https://hawcproject.iarc.who.int/assessment/626/ (gentian violet), https://hawcproject.iarc.who.int/assessment/655/ (leucogentian violet), https://hawcproject.iarc.who.int/assessment/648/ (malachite green), https://hawcproject.iarc.who.int/assessment/649/(leucomalachite green), and https://hawcproject.iarc.who.int/assessment/652/ (CI Direct Blue 218).

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