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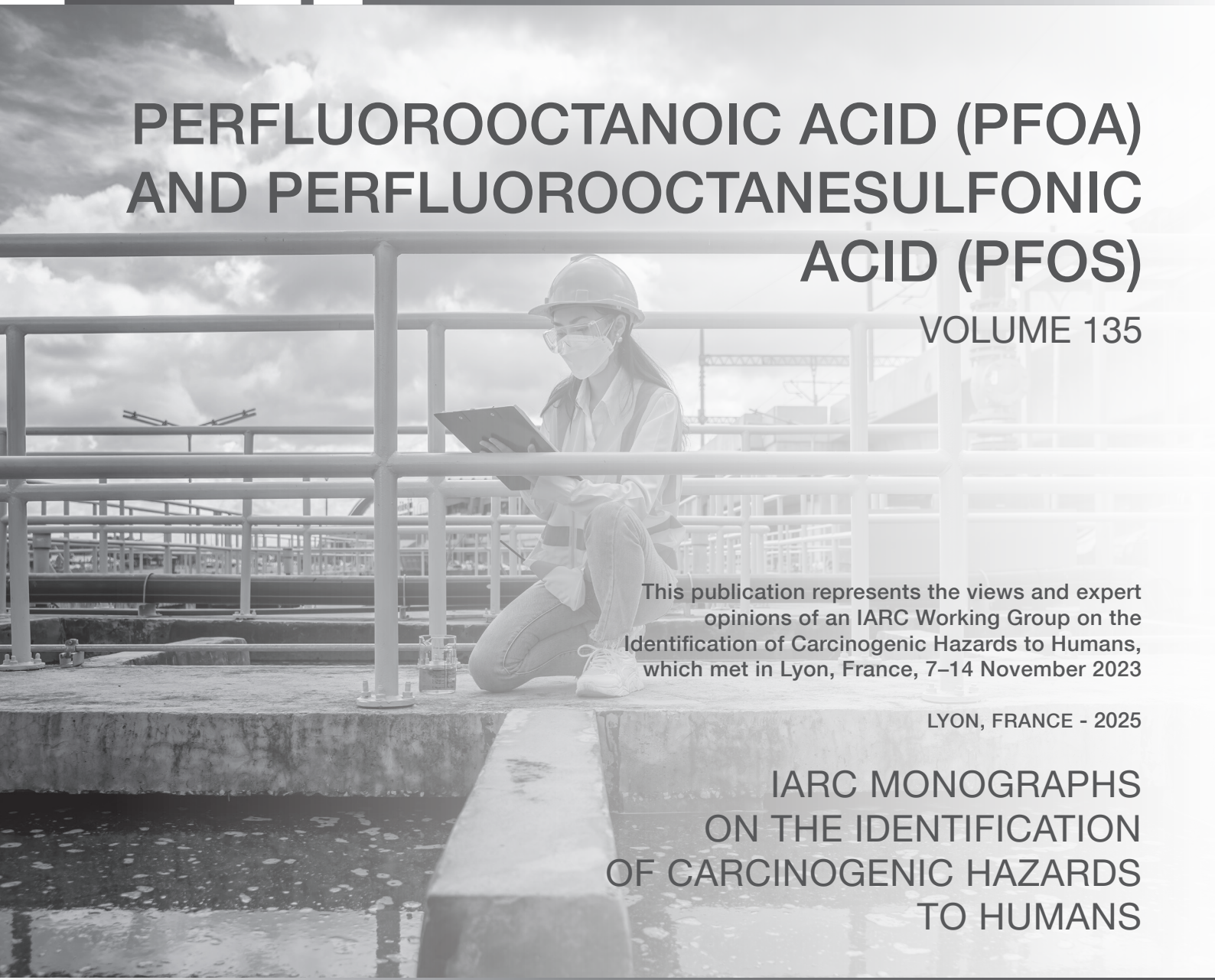

PERFLUOROOCTANOIC ACID (PFOA) AND PERFLUOROOCTANESULFONIC ACID (PFOS)

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IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

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GENERAL REMARKS

This one-hundred-and-thirty-fifth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), and their corresponding isomers and salts.

PFOA was considered previously by the *IARC Monographs* programme in 2014 (IARC, 2016), when it was evaluated as *possibly carcinogenic to humans* (Group 2B). PFOS has 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 some perfluorinated compounds, such as PFOA, be evaluated with high priority (IARC, 2019a; Marques et al., 2019), largely on the basis of emerging evidence of carcinogenicity in experimental animals and of mechanistic evidence related to the key characteristics of carcinogens (KCs). A summary of the findings of this volume appears in *The Lancet Oncology* (Zahm et al., 2023).

PFOA and PFOS in the context of the broader class of PFAS

PFOA and PFOS are part of a class of thousands of synthetic per- and polyfluoroalkyl substances (PFAS) that are used widely throughout the world. The Working Group noted that the carbon–fluorine bond is one of the strongest in organic chemistry and is responsible for the environmental and biological persistence, long-range environmental transport, as well as bioaccumulation and biomagnification of this class of chemicals. In the present monograph, the Working Group assessed the carcinogenic hazard of only two PFAS, the uses of both of which have restricted or eliminated under the Stockholm Convention to which more than 180 countries are parties (UNEP, 2023). Information is limited regarding exposure to precursors of PFOA and PFOS and to PFAS used to replace PFOA and PFOS, and few studies in humans have examined these substances as the primary exposure metric when evaluating health outcomes.

Lack of comprehensive exposure data for PFOA and PFOS

The Working Group noted major gaps in the existing literature that hampered the understanding of PFOA and PFOS exposure worldwide. When stratified by location or exposure source, including country (e.g. within the USA and Europe versus outside, as well as in communities with a known source of contamination versus those without), this gap in knowledge was exacerbated by the absence of surveillance initiatives.

Although many countries have phased-out the production and/or use of PFOA or PFOS, the Working Group identified studies indicating that certain precursor PFAS are known to break down or transform into PFOA and PFOS in the environment and biological systems, including in humans. This suggests that ongoing exposure may be expected, even if production and use of PFOA and PFOS compounds were to cease entirely around the world.

Although the workplace is often the source of highest exposure to PFAS, characterization of occupational exposure to PFOA and PFOS was limited to only a few occupations. The majority of studies focused on biomonitoring of fluorochemical-production workers (including perfluoroalkyl polymer-production workers) and first responders (especially firefighters), and other occupations that produce, use, or dispose of products that have been treated with or contain PFAS have been examined to a lesser degree (if at all). Female workers are largely absent in the available literature, limiting potential epidemiological analyses of occupational exposure sources among women. Additionally, the relative contribution of different exposure routes, namely dermal absorption versus inhalation, in these settings is poorly understood.

New evidence on cancer in humans published since the previous *IARC Monographs* evaluation

When PFOA was evaluated by the *IARC Monographs* programme in 2014, the epidemiological evidence consisted of studies on three occupationally exposed populations, one population exposed to drinking-water that was highly contaminated via a nearby industrial facility, and three case–control studies of members of the general population in communities without a PFOA pollution point source (this ambient exposure is referred to in the present monograph as "background" exposure). The present evaluations of PFOA and PFOS are based on 36 epidemiological studies, including further reports on the same three occupationally exposed populations, two additional populations in highly contaminated areas, and many case–control studies in the general population. The latter included nested case–control studies using prospectively collected biospecimens and less-informative, non-nested case–control studies using biospecimens collected after diagnosis of cancer and, in some instances, after treatment for cancer. Ecological studies, with the exception of one with an extremely high contrast in environmental exposure to PFOA relative to exposure to other PFAS, were excluded from the review. Despite these additional studies, data gaps and limitations remain, including low exposure contrasts in the studies of "background" exposure, potential healthy-worker survivor bias in most of the occupational studies, and, in case–control studies, uncertainties surrounding the measurement of PFOA and PFOS after diagnosis and, possibly, treatment for cancer. Additionally, there were few studies that addressed cancer subtypes defined by histology, genotype, receptor status, and other characteristics. Another data gap was the lack of studies among additional populations known to have occupational or substantial environmental exposure, such as workers in fluorochemical production or residents in communities with substantial pollution, e.g. in Italy, France, or Australia. Such studies might help address the data gaps noted above related to the carcinogenicity of PFOS and to the specific cancer types linked to PFOA and PFOS exposure in populations with high exposure contrast.

One challenge in the epidemiological literature is the difficulty in evaluating the effects of individual PFAS compounds, because there is widespread co-exposure to many highly correlated PFAS compounds. The evaluation of the cancer hazard resulting from exposure to

mixtures of PFAS compounds, although important, was beyond the scope of the present volume and may require the development of new statistical analytical approaches.

The Working Group conducted three new analyses of existing epidemiological data, which assisted in their evaluation (see Annex 3, Supplementary analyses used in reviewing evidence on cancer in humans): (i) an analysis based on summary statistics for repeated serological measurements of PFOA that were available from subsets of participants in two nested case–control studies, which were used to evaluate the representativeness of serum PFOA measurements from a single time point as a surrogate for longer term levels; (ii) a meta-analysis of PFOA exposure and kidney cancer; and (iii) an ecological analysis of the correlation between serum concentration measurements of PFOA and the rates of orchiectomies (a strong correlate of testicular cancer incidence in this region) within 21 municipalities in the Veneto region of Italy where there had been industrial contamination of drinking-water with PFOA.

Extensive mechanistic evidence

Since the previous *IARC Monographs* evaluation of PFOA in 2014, by far the greatest increase in the amount of research available has occurred with respect to toxicokinetic data and mechanistic evidence, including data relevant to the KCs (see Section 4). Particularly noteworthy is the extent of evidence related to epigenetic alterations (Section 4.2.4) and immunosuppression (Section 4.2.7) in exposed humans. The Working Group noted that there are only a few agents evaluated by the *IARC Monographs* programme (e.g. occupational exposure as a firefighter) for which there are such extensive data from multiple studies in multiple populations supporting these KCs. These data, combined with the data from cancer bioassays in experimental animals, underpin the rationale for the evaluation of PFOA as *carcinogenic to humans*, Group 1. Moreover, this is the first time that mechanistic evidence from a variety of test systems specifically for these two KCs has supported a Group 1 evaluation, particularly in the absence of strong evidence in exposed humans for either genotoxicity (KC2) or modulation of receptor-mediated effects (KC8).

It should also be noted that the contribution of mechanistic evidence in exposed humans to a Group 1 evaluation does not require a PFOA-specific mechanism of carcinogenicity to be identified. Thus, although empirical data directly linking PFOA-specific effects on the epigenome and immune system to increased cancer risk in humans were not available, it was the judgement of the Working Group that the observed effects in exposed humans, supported by evidence in human primary cells and in experimental systems, were sufficiently linked to carcinogenic processes to support a Group 1 evaluation, in combination with the positive results in cancer bioassays in animals.

Challenges in using PFOA and PFOS to define a mechanistic class of carcinogens

For the present volume, the Working Group identified overall similar mechanistic evidence for PFOA and PFOS across the KCs on the basis of data obtained in exposed humans, in human primary cells, and in experimental systems; as reported above, this included consistent and coherent mechanistic evidence for the KCs "induces epigenetic alterations" (KC4) and "is immunosuppressive" (KC7). In addition, it was reported that both agents have long half-lives in humans and both bind to multiple relevant protein targets, including nuclear receptors,

membrane transporters, and carrier proteins. However, it remained unclear whether PFOA might represent a mechanistic class of carcinogens to which PFOS (or other PFAS compounds) may belong. Despite a rich mechanistic database (as reviewed in Section 4 of the present monograph), the Working Group could not identify a common specific mechanism by which exposure to PFOA and PFOS leads to carcinogenesis; it is possible or even likely that multiple mechanisms are in play.

It is worth noting that additional data streams that could be helpful in establishing a chemical class, including studies on the non-cancer-related toxicity of PFOA and PFOS independent of the KCs, relative potency considerations, and mixture effects, were beyond the scope of an *IARC Monographs* evaluation. Although both PFOA and PFOS appear to activate a similar suite of nuclear receptors in human primary cells and experimental systems in vivo (e.g. there was consistent and coherent evidence for activation of peroxisome proliferator-activated receptor alpha (PPARα), constitutive androstane receptor (CAR), and pregnane X receptor (PXR) and suggestive evidence for activation of PPARγ in human primary cells), the degree of activation differs between the receptors, potentially influencing the strength of receptor-driven carcinogenic effects. Additionally, technical challenges hinder the ascertainment of whether there is modulation of these receptor pathways in exposed humans, since accessible and specific biomarkers of these pathways are not readily available in humans. This data gap is compounded by the fact that there are species differences in the events associated with modulation of these pathways by PFOA and PFOS. As yet, no studies have been conducted in mice expressing human genes for these receptors.

Overall, the Working Group was not able to conclude whether PFOA could represent a mechanistic class to which PFOS (or other PFAS) belong, based on considerations described in the Preamble to the *IARC Monographs* (see present volume; IARC, 2019b).

Relevance to humans of PFOA and PFOS effects on altered lipid metabolism in rodents

The Working Group noted that nuclear receptor activation and deregulation of lipid metabolism are relevant KC-related end-points and mechanisms that might contribute to the hepatocarcinogenicity of PFOA and PFOS in rodents.

The activation of hepatic PPARα and CAR/PXR in rodents has been reported to: transiently increase the activity of liver enzymes such as acyl coenzyme A (CoA) oxidase, and cytochrome P450s CYP4A, CYP2B, and CYP3A; increase the liver proliferative index and decrease the liver apoptotic index; decrease the frequency of hepatocellular glycogen-induced vacuoles; increase the frequency of centrilobular hepatocellular hypertrophy (Elcombe et al., 2012a, b), but also cause alterations in plasma cholesterol level, and increase centrilobular hepatocellular hypertrophy. Also, induction of hepatic steatosis has been observed in mice after dietary exposure to PFOS (Bagley et al., 2017).

The molecular mechanisms by which PFOA or PFOS can cause hepatotoxicity (e.g. fatty liver disease and other hepatotoxic effects) have not been fully described either in experimental animals or in humans. However, an accumulation of fatty acids and triglycerides and deregulation of the expression of genes related to the metabolism of fatty acids and triglycerides has been reported in a series of in vitro studies (as well as several epidemiological studies) (e.g. Wan et al., 2012; Louisse et al., 2020). These and other effects independent of the PPARα receptor that cause deregulation of gene expression, resulting in a substantial shift from carbohydrate metabolism

to fatty acid oxidation and hepatic triglyceride accumulation, have been also observed in human and rat primary liver cells (Vanden Heuvel et al., 2006; Bjork et al., 2011; Das et al., 2017; Rosen et al., 2017; Behr et al., 2018).

Other mechanisms that could be related to PFOA/PFOS-induced hepatocarcinogenicity through alterations in lipid metabolism, identified in studies in human hepatoma cells, include the activation of specific endoplasmic reticulum stress (ERS)-response genes (e.g. ATF4, DDIT3, ATF3) and enzymes involved in lipid metabolism, e.g. cholesterol (HMGCR), upon PFOS exposure (Louisse et al., 2023). After exposure to PFOA, activation of the unfolded protein response (UPR) pathway, induction of steatosis and fibrosis and expression of TNFα and IL6 inflammatory markers, increased production of endogenous reactive oxygen species in liver cells (Qi et al., 2023), and deregulation of the genes controlling lipid homeostasis (Das et al., 2017) were observed. In addition, ERS/UPR stress was also induced by PFOA in pancreatic acinar cells (Hocevar et al., 2020); and induction of cell proliferation and migration and invasion upon exposure to PFOA or PFOS were reported for various in vitro models (Matkowskyj et al., 2014; Pierozan et al., 2020; Hu et al., 2022).

Consistent with a potential involvement of both PFOA and PFOS in metabolic alterations, the Working Group identified data from metabolomic analyses in exposed humans suggesting increased activities of glycolytic pathways. Transcriptomic analyses have also indicated alterations in cell proliferation and in lipid metabolism pathways in human primary and experimental systems, respectively (see Section 4.2.11).

Data gaps for PFOS and other PFAS

The evaluation of the carcinogenicity of PFOS was hampered by a relative paucity of studies of cancer in humans and also by the existence of only one study in experimental animals that complied with Good Laboratory Practice and gave positive results. However, the strength of the mechanistic evidence for PFOS, together with its relatively potent toxic effects, suggest that additional carcinogenicity studies may fill this data gap.

Many "novel" or emerging PFAS are currently used, but the toxic and carcinogenic characteristics of most of these have not been tested systematically. Some emerging PFAS have a chemical structure similar to that of PFOA and PFOS, and a similar pattern of effects has been reported for PFOA, PFOS, and several other PFAS, e.g. perfluorononanoic acid (PFNA). In view of the large number of PFAS in past and present use, it has been suggested that grouping these compounds on the basis of chemical structure or exposure levels would facilitate the choice of suitable candidates for future research on their impact on human health. However, there is a gap in data to support such potential groupings of PFAS. Similarly, the effects of typical mixtures of PFAS have not been characterized systematically and remain a subject of ongoing research.

Scope of the systematic review

Standardized searches of the PubMed database (NCBI, 2023) were conducted for PFOA and PFOS for each outcome (cancer in humans, cancer in experimental animals, and mechanistic evidence, including the KCs). For cancer in humans, searches were also conducted in the Web of Science (Clarivate, 2023) and Embase (Elsevier, 2023) databases. The literature tree for PFOA and PFOS, including the full set of search

terms for the agent name and each outcome type, is available online.[a]

As described in the current Preamble to the *IARC Monographs* (last revised in 2019; IARC, 2019b; see present volume), 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. A public Call for Data was opened on the *IARC Monographs* website 1 year ahead of the meeting for Volume 135. Eligible studies were 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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[a] The literature tree for the monograph in the present volume is available at: https://hawcproject.iarc.who.int/assessment/664.

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