Section 4. Mechanistic and other data

Section 4 provides a concise synthesis of the data on mechanisms of carcinogenesis for the agent under consideration, drawn from representative studies in humans, experimental animals, and in vitro, and judged to be important by the Working Group.

Rationale

Mechanistic data may provide evidence of carcinogenicity, and can play a role in up- or downgrading an evaluation based on cancer findings in animals or humans (see Appendix 1). Important determinations in the evaluation of mechanistic data are:

- Is there strong evidence of an operative carcinogenic mechanism(s)?

- Is the evidence from exposed humans, human in-vitro systems, or animals?

- Does the mechanism only operate in animals?

- Does the agent belong to a class of agents evaluated as Group 1 or Group 2A (e.g. as in *Monograph* Volume 100F)?

In a style of a review article, Section 4 of the *Monograph* summarizes human and animal evidence and data gaps informative for addressing these questions, covering:

- Toxicokinetics (metabolites, enzymes involved, kinetic factors, etc.)

- Carcinogenic mechanisms, based on 10 key characteristics of carcinogens (see Table 7; Smith et al., 2016)

- Other relevant evidence (e.g. structure–activity relationships, susceptibility, target-organ toxicity).

The approach to identifying, screening, organizing and summarizing these data is presented below. Two online tools facilitate this approach: the HAWC Literature Search (https://hawcproject.org/), and the IARC Table Builder.

Identifying the relevant information

Step 1. Searching the literature: With support from the IARC secretariat, the Working Group identifies relevant studies through comprehensive searches of peer-reviewed literature, supplemented by manual searching (e.g. of past *IARC Monographs* or other authoritative reviews). Databases (e.g. PubChem) and peer-reviewed government reports can also be searched. The HAWC Literature Search tool (https://hawcproject.org/) documents the search terms (covering the agent, metabolites, toxicokinetics, and mechanisms), sources, and results.

Step 2. Screening and organizing the results: The Working Group screens the retrieved literature for relevance, with assistance from the IARC secretariat.

The Working Group organizes the relevant literature by mechanistic topic, noting if human or animal, in vivo or in vitro, using literature tags in the HAWC Literature Search tool. Authoritative, balanced review articles are identified. Literature trees document the number of studies identified, excluded and categorized per mechanistic topic and species.

Step 3. Summarizing the evidence: The Working Group develops a synthesis reflecting the extent of data available, addressing the range of study designs and doses tested, whether effects are observed at the physiological, cellular, or molecular level, and any consistencies or differences in results within and across experimental paradigms. Emphasis is given to data in humans, where such data exist. Gaps in evidence are identified.

Although based on comprehensive searches and review, the *Monograph* includes only information relevant to making the evaluation and does not necessarily cite all retrieved studies. **The length is similar to a review article, i.e. 25–50 pages of combined text, tables, figures and references in Section 4**. Authoritative, balanced reviews may be cited in lieu of numerous supporting references.

Section outline

Text is submitted in Word electronically via the IARC Online Publications System (IOPS).

4.1 Toxicokinetic data: Evidence on absorption, distribution, metabolism and elimination is described in subsections **4.1.1 Humans** and **4.1.2 Experimental systems**, totalling 3–5 pages. Evidence for the metabolic fate, including metabolites and the enzymes involved in the activation and detoxification, are presented. A metabolic schema may indicate the relevant pathways, products and whether supporting evidence is from humans and/or experimental animals. Kinetic factors that may affect dose–response relationships or cross-species comparisons (e.g. uptake, deposition, half-life in tissues, protein binding, metabolic activation) are presented. Evidence for modulation of metabolic enzymes is presented.

4.2 Mechanisms of carcinogenesis: Representative data on the 10 key characteristics are presented in Table 7. An expert Working Group convened by IARC concluded that Group 1 carcinogens commonly show one or more of these 10 key characteristics (Smith et al., 2016). These characteristics can provide a basis for systematically collating and analysing mechanistic information.

This section describes evidence for those key characteristics of carcinogens for which there are adequate data for evaluation. The evidence for these characteristics may be organized into mechanistic categories, using the colours in Table 7 as one example. Within each group, subsections summarize evidence from (1) humans and (2) experimental systems, with further organization (as appropriate) by

species, sex, strain, target organ, and end-point. Following the Working Group's discussion, the subsections can be reordered as needed to reflect the conclusions on strength of evidence.

For genetic and related effects, tables are prepared using the IARC Table Builder online tool and capture the experimental system, end-point (e.g. mutation or chromosomal damage), test (comet assay), dose (exposure), and result (see Tables 1-5; Table 6 shows a completed sample table summarizing genetic and related effects for *ortho*-toluidine; see also the *Monograph* on 2,4-D). As needed, these tables can be adapted for other mechanistic effects. Information in tables is not repeated in text, although essential design details and key results of influential studies can be highlighted. When considered supportive of evidence in humans or higher organisms, non-mammalian studies (e.g. data from plants, lower eukaryotes) can be synthetically summarized, and representative studies cited, but results not detailed in tables.

4.3 Data relevant to comparisons across agents and end-points: If important to the evaluation, structure–activity relationships, any additional high-throughput/output data, etc., are summarized. This information is also organized and analysed by the 10 key characteristics of carcinogens (see the *Monograph* on 2,4-D as an example of the approach).

4.4 Cancer susceptibility: Cancer studies addressing differential susceptibility due to toxicokinetic or mechanistic factors (e.g. genetic polymorphisms, metabolic differences, etc.) are summarized.

4.5 Other adverse effects: If data on other topics are limited, relevant evidence confirming distribution to, or effects at, tumour sites is briefly summarized (up to one page of text). This discussion will typically identify important non-cancer endpoints not addressed in Sections 4.1–4.4 that have been observed in human studies or in rodent bioassays.

5.4 Mechanistic and other relevant data. Summary statements on the entirety of Section 4 covering toxicokinetics, major mechanisms, and other relevant data highlight key supporting evidence and gaps, and address:

(a) Is there strong evidence (i.e. from human or animal data) of an operative carcinogenic mechanism(s), based on the 10 key characteristics of human carcinogens?

(b) Does the agent belong to a class of agents evaluated in Group 1 or Group 2A? and

(c) Does the mechanism(s) of carcinogenicity *only* operate in animals?

6. Evaluation and rationale. Mechanistic and other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is highlighted (see Table 7).

Appendix 1. Role of mechanistic data in the overall evaluation

Group 1

- Sufficient evidence in humans OR

- Sufficient evidence in animals AND ***strong evidence in exposed humans*** *that the agent acts through a relevant mechanism*

Group 2A

- Limited in humans AND sufficient in animals

- Inadequate in humans AND sufficient in animals AND ***strong evidence*** *that the carcinogenesis is mediated by a mechanism that also* ***operates in humans***

- *Clearly belongs, based on mechanistic considerations, to a* ***class of agents*** *for which one or more members have been classified in Group 1 or Group 2A*

Group 2B

- Limited in humans AND less than sufficient in animals

- Inadequate in humans BUT sufficient in animals

- *Inadequate in humans AND less than sufficient in animals AND* ***supporting evidence*** *from mechanistic and other relevant data*

Group 3

- Inadequate in humans AND inadequate/limited in animals

- Inadequate in humans AND sufficient in animals AND *strong evidence that the mechanism of carcinogenicity in animals* ***does not operate in humans***

Table 7. Key characteristics of carcinogens	
Characteristic[a]	**Example of relevant evidence**
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g. epoxide, quinone, etc.), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein crosslinks, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g. chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g. topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g. DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g. ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death, or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signalling pathways related to cellular replication or cell-cycle control, angiogenesis

[a] Colours in this column indicate characteristics for which an individual Working Group Member or group of Members identify data and draft the initial language.

Any of the 10 characteristics in this table could interact with any other (e.g. oxidative stress, DNA damage and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor

From Smith et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*, 124(6):713–721. Available from: http://ehp.niehs.nih.gov/wp-content/uploads/124/6/ehp.1509912.alt.pdf.