Section 4. Mechanistic and Other Data

Section 4 summarizes data on mechanisms of carcinogenesis for the agent under consideration drawn from representative studies in humans, 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 1)
- 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 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 (https://hawcproject.org/). 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 summarizes 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 human data, where they 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

4.1 Toxicokinetic data: Evidence on absorption, distribution, metabolism and elimination is described in sub-sections 4.1.1 Humans and 4.1.2 Experimental systems. 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.

4.2 Mechanisms of carcinogenesis: Representative data on the 10 key characteristics in Table 1 is presented. An expert Working Group convened by IARC concluded that Group 1 carcinogens commonly show one or more of these 10 key characteristics. These characteristics can provide a basis for systematically collating and analysing mechanistic information. The evidence for these characteristics may be grouped into mechanistic categories, using the colours in Table 1 as one example. Within each group, subsections summarize evidence from (1) humans and (2) experimental systems. Evidence is further organized (as appropriate) by species, sex, strain, target organ, and end-point. Tabular presentations capture the experimental system, dose (exposure), and result. Separate tables may depict evidence for each mechanistic category and metabolites. 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.
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. Any targets of toxicity not addressed in sections 4.1–4.4, including reproduction or development, can be identified in a paragraph, if relevant to cancer hazard identification.

4.6 **Summary of mechanistic evidence.** A tabular summary developed in the IARC Table Builder gives key conclusions, identifying references and if human and/or animal, in vivo and/or in vitro.

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:

- Is there strong/moderate/weak evidence (i.e. from human or animal data) of an operative carcinogenic mechanism(s)?
- Does the agent belong to a class of agents evaluated in Group 1 or Group 2A? and
- 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 1).
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
• Clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1

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 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>
<thead>
<tr>
<th>Characteristic</th>
<th>Example of relevant evidence</th>
<th>Commonly linked characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Electrophilic or ability to undergo metabolic activation</strong></td>
<td>Parent compound or metabolite with an electrophilic structure (e.g. epoxide, quinone, etc.), formation of DNA and protein adducts</td>
<td>2, 3, 4, 7, 8, 9</td>
</tr>
<tr>
<td><strong>2. Genotoxic</strong></td>
<td>DNA damage (DNA strand breaks, DNA–protein crosslinks, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g. chromosome aberrations, micronucleus formation)</td>
<td>1, 3, 4, 5, 10</td>
</tr>
<tr>
<td><strong>3. Alter DNA repair or cause genomic instability</strong></td>
<td>Alterations of DNA replication or repair (e.g. topoisomerase II, base-excision or double-strand break repair)</td>
<td>1, 2, 4, 6, 7, 9, 10</td>
</tr>
<tr>
<td><strong>4. Epigenetic alterations</strong></td>
<td>DNA methylation, histone modification, microRNAs</td>
<td>1, 6, 10</td>
</tr>
<tr>
<td><strong>5. Oxidative stressor</strong></td>
<td>Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g. DNA, lipids)</td>
<td>2, 6, 8, 10</td>
</tr>
<tr>
<td><strong>6. Induce chronic inflammation</strong></td>
<td>Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production</td>
<td>3, 4, 5, 7, 8, 10</td>
</tr>
<tr>
<td><strong>7. Immunosuppressant</strong></td>
<td>Decreased immunosurveillance, immune system dysfunction</td>
<td>1, 3, 6, 8, 9</td>
</tr>
<tr>
<td><strong>8. Modulate receptor-mediated effects</strong></td>
<td>Receptor in/activation (e.g. ER, PPAR, AhR) or modulation of exogenous ligands (including hormones)</td>
<td>1, 5, 6, 7, 10</td>
</tr>
<tr>
<td><strong>9. Immortalization</strong></td>
<td>Inhibition of senescence, cell transformation</td>
<td>1, 3, 7, 10</td>
</tr>
<tr>
<td><strong>10. Alter cell proliferation, cell death and nutrient supply</strong></td>
<td>Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signalling pathways related to cellular replication or cell-cycle control, angiogenesis</td>
<td>2, 3, 4, 5, 6, 8, 9</td>
</tr>
</tbody>
</table>

*aColours in this column indicate characteristics for which an individual Working Group or Committee member or group of members work together to identify data and draft the initial language.

*bSome mechanisms can cause cancer without other mechanisms, but generally it is the combined effect of multiple mechanisms that will be the root cause. Any of the 10 characteristics in this table could interact with any other, but there are some common interactions that are highlighted in the column (e.g. oxidative stress, DNA damage and chronic inflammation when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone*