15-09912-REV
Separate Comments

We have attempted below to respond to all your editorial comments. We thank you for your careful review of our manuscript. It is now much improved and we hope ready for publication. Responses are shown in tracked format.

Requests from the EHP Science Editor

Please add citations for the following statements as appropriate. In some cases citations provided earlier or later in the same paragraph may apply, and if so a revision is not needed. Some general statements may represent commonly accepted concepts, but still should indicate a source or sources for supporting information (e.g., a review). If a statement reflects the consensus of the authors, revise the text to clearly indicate that it represents a conclusion of the authors. Websites may be appropriate sources of additional information in some cases- see additional instructions for citing websites below.

- Page 7: "A large proportion of Group 1 carcinogens are genotoxic, as documented in IARC Monograph Volume 100 A-F." Please provide an appropriate url here.
- A URL to the monographs has been added.
- Page 8: "However, evidence for a causal role of epigenetic changes in cancer caused by Group 1 agents was considered to be limited in Volume 100, and for many agents, their impact on the epigenome was considered to be a secondary mechanism of carcinogenesis."
- Herceg et al reference added
- Page 8: "However, oxidative stress is not unique to cancer induction and is associated with a number of chronic diseases and pathological conditions, e.g., cardiovascular disease, neurodegenerative disease, and chronic inflammation."
- 3 recent references have been added to this sentence.
- Page 8: "Oxidative stress is also a common occurrence in neoplastic tissue and can be part of the tumor environment."
- A reference has been added
- Page 9: "Numerous carcinogens act as ligands to receptor proteins, including menopausal hormone therapy, 2,3,7,8-tetrachlorodibenzo-para-dioxin and PCBs."
- Reference added
- Page 9: "Molecular pathways that are regulated through ligand-receptor interaction and are most relevant to carcinogenesis include cell proliferation (e.g., stimulation of the normal proliferative pathways as is the case for estrogen-dependent tissues and hormone therapy), xenobiotic metabolism, apoptosis, as well as modulation of the bioavailability of endogenous ligands by affecting biosynthesis, bioactivation, and degradation." Is there a review that you can cite here? Sources elsewhere in this paragraph are all for specific mechanisms.
- Review cited.
- Page 9: "Several human DNA and RNA viruses, including various human papillomaviruses, Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus, hepatitis B virus, hepatitis C virus, and human immunodeficiency virus, are

carcinogenic to humans." Cite appropriate IARC monograph(s)?

- Cite to Lancet Oncology summary of IARC Monograph 100B added
- Page 10: "The result of these viral effects is to immortalize the target tissue cells such that they are not subject to the Hayflick limit, the point at which cells can no longer divide due to DNA damage or shortened telomeres."
- Reference added
- Page 10: "For example, the Human Papillomavirus type-16 (HPV-16) *E6* and *E7* oncogenes are selectively retained and expressed in cervical carcinomas, and expression of *E6* and *E7* is sufficient to immortalize human cervical epithelial cells"
- Reference added to review
- Page 12: "Using the 10 key characteristics as a basis, the collected information can be organized to form hypotheses and evaluate the evidentiary support for mechanistic events as a function of relevant aspects (e.g. dose, species, temporality, etc)." Please cite a source for more information on "relevant aspects" and how they are used to assess support.
- Citations have been added that discuss relevant aspects to this sentence and the one before.

Minor requests for clarification

Page 10: "Neoangiogenesis, in which new blood vessels grow into a tumor, is key to providing this supply of nutrients. Thus, agents that promote or inhibit angiogenesis, such as arsenic, will promote or delay tumor growth (Wang et al. 2013; Yang et al. 2014)." Arsenic is an interesting example given that it does both, but it is also confusing as written since it is not clear whether you are using arsenic as an example of one mechanism and not providing an example for the other. Unless a reader already knows that arsenic can do both, or they go to the cited sources for clarification, it will not be clear. Please consider providing separate examples of chemicals that promote vs. inhibit angiogenesis and/or revise to make it clearer that arsenic has been shown to operate in both ways.
We have removed the references to arsenic as it is conflicting and have cited instead a recent review of this topic by Hu et al. that specifically discusses chemical exposure and angiogenesis.

Page 11: "…and exposure and comparator (the agent and relevant metabolites compared to unexposed)…" Please consider splitting up the parenthetical explanation, e.g., "The population (humans and any relevant experimental systems), exposure (the agent and relevant metabolites), and comparator (the unexposed comparison group or condition) …"

We have made this revision

Page 11: "may be sufficiently broad to identify a range of available mechanistic data informative of the overall evaluation of carcinogenic hazard." Should "may be" be "should be"?
Changed to should.

Page 11: "if no toxicological endpoints are reported" This may seem too obvious to be

necessary, but if the absence of toxicological endpoints is a formal exclusion criterion, then "toxicological endpoints" should be explicitly defined. For example, would chromosomal translocations in human peripheral lymphocytes be considered a toxicological endpoint, but not translocation-positive lymphomas?
We have clarified this as follows:
Based on title and abstract review, studies identified initially are excluded if no data on the chemical or a metabolite are reported, or if no data on toxicological or other cancer-related effects of the chemical is provided. For example, a study on levels of a chemical, but not effects of the chemical, would be excluded.

Page 12: "based on the mechanistic endpoints and species evaluated" Please clarify—are in vitro studies classified as human species if existing/established human cell lines are used, or do "human studies" only involve outcomes measured in individual humans or biological samples collected from individual humans?

We have clarified this by adding the categories. Human on the figure is both in vivo and in vitro. We have clarified this in the Figure legend to Fig. 2

Page 12: "Figure 4 presents a similar overview for PCBs based on data from IARC Monograph Volume 107 (IARC 2015). In summarizing the mechanistic evidence, this Monograph Working Group indicated that PCBs may induce up to 7 of the 10 key characteristics…" Figure 4 includes a box labeled "xenobiotic metabolism induction"—this is distinct from metabolic activation of the compound, and does not seem to correspond to any of the 10 key characteristics, unlike all of the other mechanisms indicated in the figure. I may be misinterpreting the information, and even if I am not I am not sure that is it necessary for each box in the figure to correspond to one of the key characteristics, but at least a brief note of clarification in the figure caption might be helpful to avoid confusion. Note: This may be why the box for xenobiotic metabolism induction is brown, but since there is no explanation of the different colors, there is no way to know what this means. Likewise, it is not obvious why some boxes are darker blue than others, and if someone is looking at a black and white version (e.g., if they print out a copy) none of the variations in color are clear except for the white background in the PCB Exposure box. Finally, please cite IARC 2015 in the figure caption, as well as in the text.

We have added the following sentence to the Figure legend for clarification:
Receptor binding to CAR and AhR (a key characteristic) leads xenobiotic metabolism induction (not a key characteristic, brown not blue box) that in turn leads to genotoxicity and other key characteristics.

We have added the appropriate citation to the text and the figure legend.

Page 12: "strong mechanistic evidence exists for 5 key characteristics being involved in malathion carcinogenicity, 3 in DDT carcinogenicity and 2 each for diazinon and glyphosate" Please list the characteristics for each chemical for completeness.

The characteristics have been added in the text.

Page 13: "In general, the strongest indications that a particular mechanism operates in humans derive from data on humans or by measuring intermediate biomarkers in biospecimens obtained from exposed humans." Please clarify what you mean by "data on humans" other than intermediates measured in biospecimens—as noted previously, it is not clear what outcomes would be included as toxicological endpoints, and the distinction between data on humans and data on biomarkers in humans suggests that disease endpoints might be included. If so, cancers only? What about diseases that might also be a consequence of one or more of the 10 key characteristics? (Since almost every disease is related to inflammation in some way, this seems too broad—but if you are defining a specific approach to evaluation, these distinctions need to be clear and unambiguous.)

We have simplified this sentence to clarify it and make it unambiguous.

Figure 1
You do not discuss splitting the different characteristics up among different working groups in the text, and do not identify the different working groups in any way. Given that the need for or utility of using different working groups is not discussed, the use of different colors to indicate them in the figure is more distracting than informative. Please consider adding a brief discussion of how different characteristics are divided up among groups, or consider dropping the use of different colors in the figure. If you include more about this, please consider adding a column to the figure to identify the working groups for each characteristic, which also would help make the information more meaningful. Finally, if you still want to use colors in the figure, please use colors that can be distinguished if the figure is printed in black and white (which would also be helpful to readers with impaired color vision). See additional instructions for figure titles and captions below.

We have removed the colors and the subtext.

Figure 2
Please cite a source or url for Section 4.
A url for this section of the preamble has been added -
http://monographs.iarc.fr/ENG/Preamble/currentb4studiesother0706.php

Figure 4
See comments above.
Revised.

Title
Would it be possible to add something to the title to indicate that the paper is the product of an IARC Working Group? If the title comes up on a literature search and someone is not familiar with the authors the paper may be overlooked—the current title is very generic. For example, would something along the following lines be possible?
Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis: A Summary of IARC Working Group Recommendations

This is not solely the product of an IARC working group. He process began in a working group at IARC but has been expanded to involove other EPA and NTP scientists and all three organizations are now using some form of the the 10 key characteristics in their hazard evaluations. This point is made in the Abstract. Thus we would like to keep the title as is. It was arrived at after much discussion.

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Information for PubMed indexing

The information below will be submitted to PubMed for indexing. Please carefully review the information and confirm that it is correct, including the first author's affiliation(s) (department, institution, city, state/province if applicable, country) and the order in which the authors are listed.

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Author order, author names and initials:

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, DeMarini DM, Caldwell JC, Kavlock RJ, Lambert P, Hecht SS, Bucher JR, Stewart BW, Baan R, Cogliano VJ, Straif K

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Main Manuscript

Manuscript formatting

Please double-space all text except for the References.

Revised as requested.

Figures

- Please delete the figures from the main manuscript file, and submit each figure as a separate file in EPS or PDF format instead
- Do include the figure titles and captions as text on the last page of the manuscript (after the references.) For example, the title and caption for Figure 1 might be:

  Figure 1: Key Characteristics of Carcinogens. Colors indicate characteristics for which an individual working group or group of members work together to 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).

Revised as requested.

Author affiliations

Author affiliations should include the following: department (if appropriate), institution or company, city, state/province (if appropriate for your country), and country (including affiliations for US authors. All state and province names in author affiliations must be spelled out in full. Delete postal codes and street addresses from author affiliations.

See author Portier: add a symbol next to his name in the author list, instead of the word [retired] inserted into his affiliation. Add a note marked with the same symbol below the list of affiliations to indicate that this author is retired.

Revised as requested

Correct format for citations in text

Please use *EHP* formatting for all citations. Examples:

(Smith 2010)
(Jones and Martin 2013)
(Carroll et al. 1989)

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Updated and corrected

Journal abbreviations

All journal names must be abbreviated according to PubMed conventions (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals).