RESPONSE TO REVIEWERS

Response to Reviewer 1

We thank the reviewer for their comments and agree that the manuscript provides a framework by which mechanistic data on chemical carcinogens can be used to inform human health risk assessment and that such a framework is urgently needed by agencies that are tasked with carcinogenic hazard identification. The reviewer suggests we respond to a number of issues as follows:

1. It is not clear if the 10 key-characteristics will be equally weighted in an assessment; and whether one particular key-characteristic will have its weight changed if there are a significant number of literature citations to support that characteristic.

Response: The key characteristics have been employed in two IARC monographs to date. The strength of the evidence for each characteristic is weighed on the basis of the available scientific findings rather than the size of the literature. This is now discussed in the revised paper as follows:

"All of these factors make assignment of descriptors such as 'strong' to the mechanistic evidence challenging, but recent experience with two monograph meetings suggest that the weighing of the evidence on the basis of the 10 key characteristics focuses the group discussion on the available science and allows rapid consensus to be reached."

2. With ten key characteristics listed do the authors see replacement of the weak", "moderate" or "strong" ranking with a numerical one.

Response: This is an interesting idea to consider. However, as agents may exhibit one or several key characteristics of carcinogens, a numerical rank may not accurately reflect the overall strength of mechanistic evidence. Instead, we envision continuation of "weak", "moderate" or "strong" ranking, ensuring that it encompasses consideration of the strength of evidence for the key characteristics. No change has been made to the revision.

3. Characteristic 1: "Is Electrophilic or can be Metabolically Activated to Electrophiles." The framework should provide some accommodation for endogenously generated electrophiles that can adduct DNA, e.g. lipid peroxidation break down products, formaldehyde and acetaldehyde.

Response: We agree although lipid peroxidation products would be categorized under oxidative stress and the formation of endogenous adducts could be seen as background genotoxicity. Neither are really indications of metabolic activation or electrophilicity but we have added a sentence that should accommodate the Reviewer's suggestion under Characteristic 2. We now state "In some cases the exogenous agents may also be generated endogenously, such as formaldehyde and acetaldehyde, producing a background level of DNA damage."

4. Characteristic 8: "Modulates Receptor Mediated Events" could be developed further: the statement "Both classes of receptors can be involved in carcinogenic mechanisms, but not necessarily through activation of the receptor" seems unclear. Also, the role or tumor promoters and compounds that might act through PKC seem underplayed.

Response: We appreciate this comment and have re-phrased this section extensively. Because of the space limitations in EHP, we do not have room to discuss in detail the specific signaling cascades through various kinases, including PKC. We do include a reference to an excellent review on the role of PKC-mediated signaling in cancer (Greiner and Kazaniets 2007).

5. In conducting human risk assessment based on these characteristics there should be some statement made as to how this is a population based approach but could be informed more strongly by measurement of intermediate cancer biomarkers that might determine individual risk. Similarly, inherited mutations or SNPs that pre-dispose individuals are usually included in the mode-of-action sections of IARC monographs. Will this be retained?

Response: In short, yes. Indeed, inherited mutations or SNPs that pre-dispose individuals to cancer will continue to be addressed in Section 4 of the IARC Monographs. Additionally, the population-based focus of the approach, and the value of intermediate cancer biomarkers in understanding individual risk, will be retained. The sentence in the revised manuscript has been revised to now say "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," so that specific reference to intermediate biomarkers is made.

Response to Reviewer 2

We have attempted to fix the imbalance in referring less to benzene and more to PCBs. We have also included statements and reference to recent examples of the use of the characteristics in evaluations of 4 pesticides. Other specific responses follow:

1. On page 5 the authors state: "Herein, we demonstrate the applicability of this proposed systematic strategy for searching and organizing the literature using benzene and polychlorinated biphenyls as examples. The mechanistic study database for benzene is complex, comprising over 1,800 studies, many with multiple mechanistic endpoints. We conducted systematic literature searches for endpoints pertinent to the 10 key characteristics of human carcinogens, utilizing literature trees to indicate the human and experimental animal studies that reported endpoints relevant to each characteristic. To further indicate their contribution to benzene carcinogenesis,characteristics, exemplar endpoints and any linkages with evidential support were then organized into a graphical network representative of an overall mechanistic pathway."
Add here the parallel statement for the PCBs. No doubt there are many more that 1,800 studies.

Response: The reviewer is correct. We have added a parallel statement indicating that there are almost 3,900 studies on PCBs.

2. Minor issues:
a. On page 6, under electrophiles, targets mentioned do not include lipids. Is this because binding to lipids is thought not to contribute to carcinogenesis?

Response: Lipids have been added.

b. On page 7, first paragraph under Characteristic 3, sentence should read "The nature of the error, the flanking sequence, the presence of DNA damage and the ability to correct errors, all impact the outcome of this process…"

Response: It now reads as the reviewer suggests although Word shows a grammatical error by adding the additional comma.

c. On page 10, include PCBs among the examples in the last line of the top paragraph.

Response: Change has been made in substantially revised paragraph.

Response to Reviewer 3

This manuscript is a report by a group of participants in two 2012 IARC workshops on the mechanisms of carcinogenicity. The authors outline 10 key characteristics of human carcinogens, which they hope can help conduct a more systematic review of literature during carcinogenicity assessment by regulatory agencies. The 10 listed characteristics do not include anything new to somebody working in the field of carcinogenicity, which is perhaps appropriate considering that the main effort was focused on improving regulatory evaluations that have to rely on reasonably well recognized principles.

Response: We agree and confirm that the main goal of the paper is to simply describe categories into which the literature can be binned for easier analysis of the mechanistic data.

The descriptions of individual characteristics vary in their clarity and the quality of examples or discussion of caveats and can be improved with a relatively modest effort.

Response: We have attempted to make the descriptions of the different characteristics more consistent.

There are also two larger issues regarding the overall quality of this document.

Structural issues:
1) It would be very helpful to include an overview of cancer/carcinogenic process before the description of key characteristics. This overview can

be based on the classic and emerging hallmarks of cancer.

Response: We have revised the second paragraph of the Introduction to include this overview and have added an author (Prof. BW Stewart) who has written on this topic for IARC and was a member of the working group.

2) The second, larger issue is the absence of quantitative or other specific information regarding the frequency with which key characteristics are found among Group I carcinogens. From reading a current document, one cannot determine which of key characteristics are the most commonly associated with human carcinogens and the strength of these associations.

Response: We agree that such an analysis is needed and one is ongoing, but consider that this is beyond the scope of the current paper. Using the 10 key characteristics, other members of the Working Group led by Dan Krewski have developed a mechanistic database of Group 1 carcinogens that is currently being analyzed and will be published separately. This paper will document the frequency of the different characteristics among 96 Group 1 carcinogens.

Specific comments:
1) Characteristic 1: a) Cellular activation of procarcinogens does not always require enzymes. For example, a major human carcinogen chromium(VI) is activated via direct reduction by ascorbate and glutathione (Salnikow K. Chem Res Toxicol, 2008), b) Are carcinogens with characteristics 1 and 2 generally the same or there is only a partial overlap? What explains incomplete overlap if any? It is easy to see how DNA adducting chemical can be carcinogenic but what about protein-adducting only - What would be a mechanism for carcinogenicity via protein damage? For the last question, I can think of nickel and arsenic(III) as examples of protein binding but not DNA binding. For these metals, there is evidence that inactivation of genome maintenance components (arsenic) and the induction of hypoxia-like metabolic state via upregulation of HIF1 (nickel) can be important for carcinogenicity (Salnikow K. Chem Res Toxicol, 2008).

Response: We agree with the reviewer and have added the phrase "whereas others require chemical conversion within the body" including reference to (Salnikow K. Chem Res Toxicol, 2008).

2) Characteristic 3: a) DSB repair is error-prone, but not largely error-prone, b) there are no examples of carcinogens altering DNA repair – cadmium suppressing mismatch repair and arsenic inhibiting PARP could be potential examples. C) The example of genomic instability arising several generations post-IR is consistent with the presence of genetic alterations in these cells. Are there any examples of carcinogens that can induce genomic instability more directly?

Response: a) We have removed the paragraph containing the term 'largely'. B) We have added cadmium and formaldehyde as examples with appropriate references. C) We have edited this paragraph and added arsenic and cadmium as examples with appropriate

citations.

3) Characteristic 4: it would be helpful to explain how epigenetic changes can promote carcinogenic process: tumor suppressor silencing, oncogene activation, for example. Response: Sentence 2 under characteristic 4 has been rewritten as follows: 'Epigenetic phenomena, including changes to the DNA methylome and chromatin compaction states, along with histone modification can impact the carcinogenic process by affecting gene expression and DNA repair dynamics."

4) Characteristic 5, last line: clarify that it is applicable to agents causing chronic inflammation, not all infectious agents.

Response: This has been clarified in the text for both characteristics 5 and 6.

5) Characteristic 7: add reference(s) for azathioprine and a caveat that it also incorporates into DNA and may additionally act as a genotoxicant.

Response: All reference to azathioprine has been removed.

6) Characteristic 8: a) the overall description of the receptor-mediated mechanism is unclear with respect to the processes relevant to carcinogenesis. One mechanism that can be discussed is the stimulation of the normal proliferative pathways as it is the case for estrogen-dependent tissues and oral contraceptives. B) What are the examples of carcinogens and the nature of procarcinogenic process affected by receptor antagonists?

Response: This section was extensively revised to provide clarity while maintaining brevity due to space constraints of EHP. We now include discussion of both points brought up by the reviewer.

7) Characteristic 9: it is unclear how immortalization is achieved by HPV E6/7 proteins. There are only two known mechanisms: upregulation of telomerase and activation of ATL.

Response: The last sentence of the paragraph has been removed to save space and to simplify the statements made.

8) Characteristic 10: lacks good examples to support several general claims. A chemical hypoxia mimic nickel can be used as an example of carcinogen that establishes cancer-like glucose metabolism via stabilization of HIF1 (Salnikow K. Chem Res Toxicol. 2008).

Response: Many agents affect necrosis, apoptosis and/or autophagy and insufficient space is available to describe this in any detail.

9) Fig-3: needs 1-2 references unless it is a completely original mechanism.

Response: Figure 3 is original.

10) Table 1: the 3rd column has many problems for me. While some commonly linked characteristics are obvious (1-3, for example), others appear baseless. For example, nowhere in the manuscript we see evidence/examples that electrophilic compounds or their metabolites can cause immortalization. Perhaps the authors can group/identify the linked characteristics by the strength of the established or theoretically perceived linkage.

Response: We have removed the 3rd column that posed problems for the Reviewer.