CC/2021/03

Committee on CARCINOGENICITY

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC).

Scope of New Guidance Statement – Weight of Evidence Approach to Assessing Modification of Cancer Risk (v2)

Background

1. In recent discussions, COC has expressed the aspiration to move away from traditional risk assessment approaches for potential carcinogens, to a more holistic approach encompassing consideration of the effects of chemicals on all stages of cancer development. This has been reinforced by increasing concern over the reliability and applicability of the rodent two-year bioassay in predicting chemical carcinogenicity.

2. As part of an ongoing review of the current guidance statement series, the Committee agreed at the July 2020 meeting that the critical components of two of the current COC guidance documents – Hazard Identification and Characterisation (G03) and Alternatives to the 2-year Bioassay (G07), could be reworked into a new guidance document on use of a weight of evidence approach to assessing modification of cancer risk. This would include existing aspects from the current risk assessment process that work well, and build in new conceptual ways of considering information on the cancer process and how chemicals might influence it.

3. A draft scope of the new guidance statement on using a 'weight of evidence' approach to assessing modification of cancer risk was discussed by COC at the November 2020 meeting (CC/2020/12). The updated scope presented here in Annex A provides more detail against the original scope as requested and addresses other aspects discussed by Committee in November 2020.

4. This proposed guidance document will, necessarily, be used by risk assessors in making assessments of chemicals as to the potential for carcinogenicity. The intended new document will replace the documents in the current guidance series on hazard identification and characterisation, but will not in the immediate future replace the documents on identifying points of departure or risk characterisation. As such aspects around quantification and risk communication are

not covered in the draft scope. In considering this new 'weight of evidence' approach, COC may wish to consider whether there is sufficient information currently available on all aspects of cancer development and the potential modification of these events by chemicals to facilitate its use by risk assessors, to determine whether the draft scope can be worked up to a guidance statement.

5. Alternatively, if COC conclude that this proposed guidance cannot be produced at present, it may be prudent to actively develop the document as a watching brief while reviewing the information sources and the systems being developed by other authorities such as the OECD Integrated Approach to Testing and Assessment (IATA) for non-genotoxic carcinogens, for combining knowledge on multiple events in cancer development. Although the IATA is developing strategies for non-genotoxins specifically, the methods appear to be applicable for the assessment of all chemicals that have may potentially modify cancer development. If COC decided on an active watching brief, this could include reviews of evidence of the knowledge presently available for consideration of events in the development of cancer and the modifying effects of chemicals (such as that recently outlined in the Watching Brief on the Tumour Microenvironment) and the methods available for observing the modifying effects of chemicals on these events.

Questions for the Committee

6. Members are asked to:
   i. Comment and discuss the scope of the revised guidance document (draft v2) outlined in this paper.
   ii. Inform the Secretariat of any relevant areas and/or publications to reference in the new document.
   iii. Consider whether the proposed guidance document using a weight of evidence approach will provide risk assessors with sufficient guidance to facilitate a carcinogenicity risk assessment, or whether an alternative watching brief should be developed initially.

IEH Consulting under contract supporting the PHE COC Secretariat, March 2021

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Introduction

1. The main traditional sources of evidence used in the current risk assessment of potential carcinogens are human epidemiology studies and rodent long-term bioassays, with additional evidence from further studies being seen as supportive. The overall approach currently recommended by the COC for assessing carcinogenic risk of chemicals is outlined in guidance statement G01 (A strategy for the risk assessment of chemical carcinogens).

2. This general strategy has proved to be a successful one when the substance under consideration has sufficient available information for evaluation. However, the approach can be limited as good epidemiology data is only available for a relatively limited number of chemicals, usually where there is measurable occupational exposure over a long duration. In addition, the two-year rodent bioassay is primarily used to identify hazard rather than risk and the applicability of the findings in experimental species to humans is being increasingly called into question (Doe et al., 2019). Other pressures on the use of the rodent bioassay, which may make it unsustainable in the future, include ethical and financial considerations.

3. COC has been considering a new approach to the assessment of chemicals for potential carcinogenicity using a framework based on an increasing understanding of the carcinogenic process and the development of cancer in humans. It incorporates new sources of emerging evidence regarding the influence of a number of different physiological and biochemical processes, such as those outlined in ‘The Hallmarks of Cancer’ (Hanahan and Weinberg, 2000, 2011), on a dynamic carcinogenic process. It is hoped that such an approach will assist risk assessors when answering questions relating to potential impact on cancer risk of a specific chemical exposure.

4. Consideration would be given to the evidence of a chemical’s ability to modify cancer risk rather than simply the classification of a substance or industrial process/exposure as carcinogenic/non-carcinogenic.

New concept formulation

5. COC has considered the use of 10 hallmarks of cancer (as outlined below) in risk assessment (Hanahan and Weinberg, 2000, 2011). IARC also suggested similar

possible mechanisms by which agents may cause cancer in humans and identified 10 characteristics (Smith et., 2016).

The Ten Hallmarks of Cancer

- *Genetic instability and mutation* – allowing changes in one cell to pass to daughter cells through mutation or epigenetic changes in the parent cell DNA.
- *Tumour-promoting inflammation* – helping cancer cells grow using the same growth signals which normal cells provide to each other during wound healing and embryonic growth; inflammation further contributes to the survival of malignant cells, angiogenesis, metastasis and the subversion of adaptive immunity.
- *Sustained proliferative signalling* – cancer cells appear to grow at an unlimited rate.
- *Insensitivity to anti-growth signals* – cancer cells are insensitive to anti-growth signals or withdrawal of normal growth signals.
- *Resistance to cell death* – cancer cells avoid the processes by which abnormal or redundant cells trigger apoptosis.
- *Replicative immortality* – cancer cells do not senesce or die after a limited number of cell divisions.
- *Dysregulated metabolism* – disrupting metabolism is needed to support the increased demands of rapid proliferation, thus enabling the development of cancer.
- *Angiogenesis* – eliciting new blood vessels to sustain growth.
- *Tissue invasion and metastasis* – invasive tumours creating a space to expand into normal tissue, while in situ or non-invasive cancers (e.g. breast ductal carcinoma in situ; carcinoma in situ in colon polyps) grow into pre-existing spaces.
- *Avoiding immune destruction* – tumour cells avoiding immune surveillance that would otherwise mark them out for destruction.

6. The Halifax Project was a large-scale project with the aim of examining the challenge of assessing the carcinogenic potential of low-dose exposure to chemical mixtures in the environment (Goodson et al., 2015). The underlying concept of this project suggests that if individual chemicals can induce some, but not all, of the Hallmarks of Cancer, then combinations of chemicals at low doses may be able to act through different modes of action in concert to induce carcinogenesis. The Halifax Project reviewed toxicological data on 85 environmental chemicals considered to be non-carcinogens, including pesticides, metals, plasticisers, etc. (Goodson et al., 2015). These chemicals were all judged to have 'hallmark'-inducing actions for key pathways and mechanisms relating to carcinogenesis and were divided into groups according to their hallmark effect, with some chemicals appearing in more than one group. Of these, 15% showed evidence of a dose-response threshold and 59% had evidence of effects at low dose, with the remaining 26% having no dose-response data. The authors concluded that there are a significant number of environmental chemicals exerting non-genotoxic, low-dose

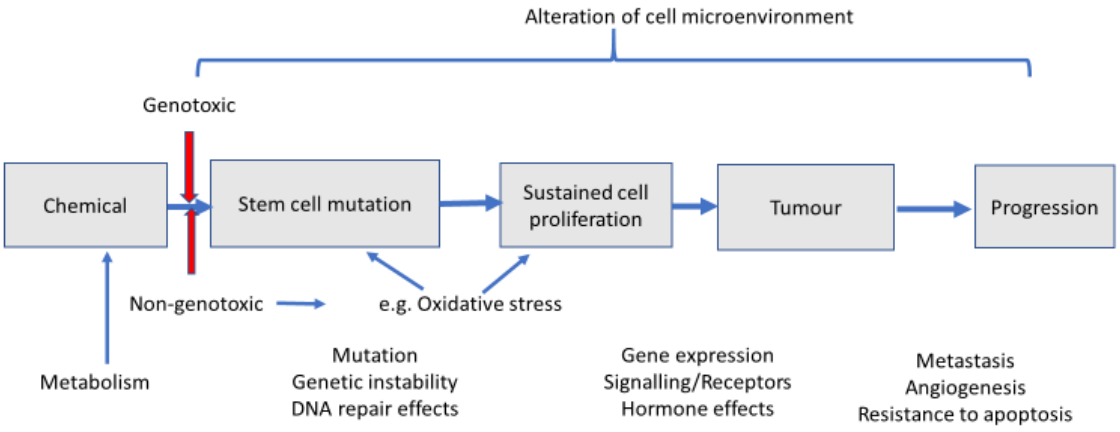
effects through hallmark mechanisms for which there is evidence for a contributory or modifying role in carcinogenesis.

7. There have been a number of other recent papers on the development of AOPs for carcinogens. Two papers have assessed a possible AOP for Aflatoxin B1 as a data-rich human, genotoxic carcinogen (Moore et al., 2018; Kang et al., 2018). In Moore et al. (2018), early key events were identified as early induced mutations in cancer critical genes (such as the tumour suppressor gene, p53), cell proliferation, clonal expansion of mutant cells and progression, with hepatocellular carcinoma as the adverse outcome. However, the authors concluded that assessment of this known human carcinogen, for which there is ample data, showed that definitive evidence was not available for all key events.

8. The most developed of the 'new approaches' to risk assessment for carcinogenicity, based on AOP, has been conducted by an OECD expert group - the integrated approach for testing and assessment (IATA) of non-genotoxic carcinogens (Jacobs et al., 2020). This group identified models with a number of modes and mechanisms of action on the pathway to cancer, with cell proliferation as a basic element. They further suggested that a panel of tests would be needed to assess the effect of chemicals on common hallmarks of cancer, many of which would require further development and assessment.

9. The new approach being considered by COC would be based on the use of a Dynamic Cancer Risk (DCR) model (Figure 1; modified from a presentation to COC by Dr John Doe) which considers the stages of cancer development, based on mutation, sustained cell proliferation, tumour progression and alteration of the cell microenvironment leading to tumour formation, as a basic framework. In addition, such a model would allow the impact of modifying factors on this process, such as those indicated in the hallmarks of cancer model, to be included and evaluated.

Figure 1. Dynamic Cancer Risk Model – Potential Risk Modification Points



10. A DCR model would be developed for the chemical being evaluated, incorporating a potential carcinogenic pathway for both biochemical (e.g. metabolism) and biological events (similar to MoA and AOP) [drafting note: some details of these approaches are outlined in G03 (Hazard identification and characterisation: conduct and interpretation of animal carcinogenicity studies) and would be carried across]. This may be driven by a chemical directly reacting with DNA (i.e. genotoxic) or via non-genotoxic mechanisms, such as the induction of oxidative stress.

Detection of 'Primary events'

11. A tiered approach might be appropriate based on the DCR, with data on mutation and proliferation being considered primary effects in cancer and subsequent modifying effects on cancer development being considered secondary. Evidence would be assessed according to "modification points" identified in Figure1. Many of the assays for non-genotoxic events in the development of cancer have been assessed in the IATA process and are summarised below (Jacobs et al, 2020).

12. Consideration of mutagenic potential using validated *in vitro* and *in vivo* assays, including genotoxicity assays such as the Comet assay and transgenic studies, might give some indication as to a target organ when used in animal studies. Identification of mutations in specific genes such as oncogenes, tumour suppressor genes and DNA mismatch repair genes (e.g. mutations in APC, Ki-ras, p53, mmr detected in colon cancer) may also suggest a potential target organ. Recent research has further identified mutations in key genes in a range of cancers in different organs and tissues (see review by Cieslik and Chinnalyan, 2020).

13. Although the conduct of two-year bioassays for chemicals may become increasingly less frequent due to ethical and financial considerations, any information from such bioassays will continue to be important. For the conduct and statistical analysis of these (non-pharmaceutical) chemical carcinogenicity studies, readers are referred to the OECD test guidelines 451 and 453 and the accompanying guidance document as a source of information [drafting note: some of this information is available in the current guidance statement G07].

14. Shorter-term animal studies can now provide important information on target organs and modes of action: for example, Perkins et al. (2015) investigated an AOP for 1,4-dioxane and used targeted gene arrays after short-term animal studies. Gene expression of growth factors, signalling pathways and transcription supported regenerative cell proliferation and proliferation in the absence of cytotoxicity, while other gene expressions suggested a role for the 'inflammation-fibrosis-cancer axis' as a mode of action. Although there was no direct evidence for epigenetic effects, metabolism by P4502E1 in rat liver suggests that prolonged exposure could generate free radical species.

15. Thomas et al. (2013) outlined a framework for applying transcriptomic data to risk assessment. The proposed weight of evidence analysis incorporated an estimation of genotoxic potential and an extrapolation factor based on a Point of Departure (PoD) estimated from the lowest BMD determined from transcriptomic dose-response studies in 8 specified tissues at a single time point between 5 days and 13 weeks in rats and mice. The assumption is that basing the PoD on the most sensitive pathway is generally protective until such time as key adverse effect pathways are identified. This approach might be applicable to obtaining margins of exposure when cancer data are not available, but information on relative risk is required. Thomas and Waters (2016) commented that although there may be issues of concern in using such an approach, a PoD based on such information might be preferable to no PoD, which is currently the case for the vast majority of chemicals. This approach provides an example of problems that might occur with the new approach to risk assessment and how they might begin to be addressed.

16. A number of genetically-modified mouse strains have been developed with the aim of providing models for the quick and accurate detection of chemical carcinogens. These strains develop tumours more rapidly than wild-type mice as they contain transgenes which are critical to the carcinogenic process: the ras oncogene (rasH2, Tg:AC skin model) and the tumour suppressor gene ($p53^{+/-}$ hemizygous knockout mouse). These models may suggest target organs for mutation by the test chemical and some possible modes of action.

17. Evidence from human studies (such as occupational epidemiology, clinical studies and measurement of biomarkers such as DNA and protein adduct formation) providing information on target organs and MoA [see G04 and Synthesising Epidemiology Evidence Subgroup (SEES) Report.] can also be incorporated where available. Any known, or modelled, measure of exposure to the chemical would need to be considered, and improved methodology for the measurement of such exposure, including biomarkers (both route of exposure and biological detection) to both occupational and environmental chemicals, is needed.

18. Many of the newer approaches to carcinogen risk assessment are based on the use of ‘omics’ techniques. This refers to genomic (DNA sequence analysis) and post-genomic (e.g. transcriptomics, proteomics, metabolomics, epigenomics) methods used for the characterisation and quantitation of pools of biological molecules and their roles, relationships and action within an organism (Ward and Daston, 2014). Datasets are now available for results of *in vitro* and *in vivo* studies on a large set of compounds using consistent study design and standardised experimental protocols. These contain data on dynamic gene expression over multiple doses/concentrations plus other data (e.g. compound pharmacology, toxicology, clinical chemistry and histopathology). This information can be used for ‘phenotypic anchoring' – relating specific changes in gene-expression profiles to adverse effects observed in conventional toxicity tests, to allow the identification of changes in gene-expression that are causally related to the development of the

toxicity phenotype (Paules, 2003). Currently, much of this data is based on liver toxicity, but is now being expanded to cover other organs.

19. *In vitro* cell assays, such as organ-specific gene arrays, may give evidence as to the target organ and genes involved in the mechanism of cancer development. It should be noted that many of these assays have been developed to rapidly reproduce the results of a two-year bioassay and so cell lines involved may often be derived from rodent organs, usually the liver, which may be of limited use in newer approaches when the principal object is to assess the effects of chemicals on human carcinogenesis.

20. Measurement of cell proliferation can be assessed in shorter-term repeat-dose rodent assays, cell proliferation Ki-67 and hepatic DNA synthesis and bromodeoxyuridine uptake assays.

21. Cell transformation can be detected in short-term rodent studies, and a number of *in vitro* assays have been developed such as Bhas 42, Syrian Hamster Embryo (SHE), and BALB/c cell transformation assays. However, these assays are becoming less commonly used.

22. *In silico* assessment of the chemical structure, compared with chemicals of similar structure and MoA, may give further indication of carcinogenic potential.

Detection of 'Secondary events'

23. Onto the DCR, other important evidence would be superimposed of effects and factors known to influence the development of cancer, such as inflammation, immunosuppression, gene expression and cell signalling, hormonal influence and the tumour microenvironment. [drafting note: this will link to the Committee discussion in the Watching Brief on the possible role of the tumour microenvironment in carcinogenicity]. These can be measured in short-term animal studies or in *in vitro* cell experiments.

24. Consideration can also be given to other potential modifying events in the later stages of tumour progression. The methodology available for assessing the effects of exposure to chemicals on these events are currently under development. COC may wish to conduct a parallel review of the tests being developed to measure such effects and their potential use in risk assessment for carcinogenicity.

25. Assays detecting changes in gene expression and signalling pathways are being developed for the detection of effects of chemicals on different events in the development of cancer associated with the tumour microenvironment.

26. Oxidative stress *in vivo* and *in vitro* could lead to indirect and epigenetic effects on DNA leading to cell injury, modification of the immune response and inflammation. The involvement of metabolic enzymes such as P4502E1 may indicate the production of reactive oxygen species.

27. Changes in receptor binding and receptor agonism/antagonism have also been identified as potential hallmarks of cancer, and there are a number of validated oestrogen and androgen assays and an arylhydrocarbon transactivation assay, as well as assays detecting changes in steroidogenesis and aromatase.

28. Although not fully developed as validated *in vitro* assays, expression of vascular endothelial growth factor (VEGF) has been widely shown to be involved in angiogenesis. Methods involving the detection of metastatic markers may also need to be developed.

29. The cytokines IL-6, IL-17 and TNF, and the involvement of T-cells, NK cells and host resistance, are markers for inflammation and immune response in the development of cancer and assays detecting these markers are being developed.

30. Increased resistance to apoptotic cell death has also been identified as a hallmark of cancer; this can be identified using histopathological techniques in short-term rodent studies as well as in *in vitro* assays using caspase activation and DNA fragmentation.

31. Preclinical models of cancer using *in vitro/in vivo* xenografts derived from cancer patients are being developed to investigate the role of the tumour microenvironment in cancer development in humans.

32. Assessment of the influence of factors/modifiers, such as different patterns of exposure (discussed in G09,), interactions with other chemicals either simultaneously or in the future (discussed in G08), or lifestyle factors such as obesity can also be superimposed, as appropropriate, on the DCR model.

Evidence sources

33. It is envisaged that the following sources of evidence could be utilised. and that carefulconsideration would need to be given to the priority of information and, whether a tiered approach is possible, giving some indication of the weight of evidence derived from the following types of data sources:

- Epidemiology – precancer, cancer, other relevant effects. Further information on this is discussed in the Synthesising Epidemiology Evidence Subgroup (SEES) Report.

- *In silico* models – structural knowledge and structural alerts.

- *In vitro* studies – genotoxicity assays, mode of action studies, relevance and validation. IATA has begun to develop methods for the validation of new approaches and assays for the detection of non-genotoxic events in cancer aetiology (Jacobs et al., 2020).

- Animal studies – shorter-term (less than lifetime) studies with relevant endpoints and mode of action. This has been considered for pharmaceuticals by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (Van der Laan et al., 2016).

- Animal studies – 2-year bioassay and other chronic studies.

Future steps

34. Following the formulation of such a new approach and production of associated guidance, it is recognised that some additional points of clarification will likely be required across a number of other COC guidance documents.

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