CC/2016/08

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Recent developments in the Mode of Action and Human Relevance Framework

Introduction and background

1. The COC considered discussion papers on the WHO IPCS Mode of Action (MOA) and Human Relevance Framework (HRF) in 2005 (CC/05/2) and in 2008 (CC/08/3). A presentation was further made in November 2013 by a Committee member on more recent developments in the HRF. COC members agreed during horizon scanning sessions in 2013 and November 2015 that the Committee should be updated in more detail on how the concept and use of the framework has evolved since the last discussion of the topic in 2008.

2. The framework was originally developed through initiatives of the International Programme on Chemical Safety (IPCS) of the World Health Organisation (WHO), and the International Life Sciences Institute (ILSI) Risk Science Institute (RSI). The initial aim was to harmonise approaches to the development and communication of modes of action for chemical carcinogens (Sonich-Mullin et al, 2001), and to provide a conceptual framework for considering such data in the risk assessment of carcinogens (Boobis et al, 2006). Early work considered the MOA for a chemical established as a carcinogen in experimental animals as a means to evaluate the human relevance of the animal tumours. The framework has been developed and extended in its application in the years since 2006, and the WHO produced the latest update of their guidance in 2014 in a review (Meek et al., 2014a). Recently, the Organisation for Economic Co-operation and Development (OECD) has developed guidance on Adverse Outcome Pathways (AOPs), which share many characteristics of and build on the concepts of the MOA framework.

Development of the framework

3. The key papers describing the IPCS development of a framework for analysing the relevance of a cancer mode of action for humans are Sonich-Mullin et al. (2001) and Boobis et al. (2006). From the mid-1980s, it was increasingly recognised that not all chemicals causing cancer in animals do so by processes that directly involve interaction with DNA, and the relevance to humans of some of these was questionable. However, the use of this information in cancer risk assessment was not transparent or consistent. This led initially to the publication of a framework for clearly establishing the MOA for a carcinogen acting by a non-genotoxic MOA in animals (Sonich-Mullin et al, 2001).

4. Boobis et al. (2006) developed this further and provided a framework for determining the human relevance of the MOA. This is summarised in the scheme in Figure 1 in Annex 1. The refined aspects introduced in this scheme are the second step, which involves a qualitative analysis of the MOA to establish if animal data are

relevant to the pathway in humans, and the third step, which questions if human relevance can be excluded on quantitative differences in key events between animals and humans. This third step introduces consideration of the pharmacokinetics and pharmacodynamics of suspected carcinogenic agents in evaluating their relevance to carcinogenicity in humans.

5. A central concept in a MOA is that of key events, which are described as events that are critical to the induction of the response, as hypothesised in the postulated MOA. A key event must be measurable, and there has to be a body of experimental data in which it is characterised and consistently measured. Examples of the kind of information that might be considered when looking at key events would be toxicological response and relevant key events in the same cell type, sites of action logically related to the event(s), specific biochemical events, or changes in the expression or activity of enzymes.

6. Other aspects that require consideration when looking at human relevance are also discussed in this paper. Concordance of dose-response relationships is important – there is a description of how the dose dependency of increases in magnitude of a key event should ideally be correlated with increases in the severity (for example, lesion progression) of other key events occurring later in the process and with the ultimate response. Temporal relationships for each of the key events and for the response should also be characterised. The weight of evidence linking key events, any precursor lesions, and the response should be addressed. The biological plausibility of the postulated MOA has to be considered, and alternative MOAs that could be logical explanations also need consideration. Uncertainties should be explicitly stated, along with any gaps in the data that have been identified, and the level of confidence in the MOA should be clearly stated.

7. Boobis et al. (2008) describes the use of the IPCS framework to analyse the relevance of MOAs for non-cancer effects to humans. While this paper does not directly describe cancer MOAs, it does elaborate central ideas in the human relevance framework that apply equally in the evaluation of cancer MOAs. One important development was the ‘carry-over’ of information obtained during the application of the framework to the risk characterisation step, when it is not possible to dismiss the human relevance of the MOA.

Development of key events analysis

8. Chronologically, the next step in the human relevance approach was a more detailed, quantitative analysis of key events. Boobis et al (2009) describes a method of key events dose-response analysis for the risk assessment of chemical carcinogens and non-carcinogens; the approach to carcinogens is outlined here.

9. The authors begin by observing that current risk assessment methods are based on the assumption that, in the absence of sufficient data, carcinogenesis does not have a dose-threshold. For humans, extrapolation is performed either on the assumption of a linear dose-response curve, or utilising a margin of exposure (MOE). In the former, the point of departure for the observable range of responses in a cancer bioassay, which is typically the dose associated with a 10% response, is

extrapolated to a "virtually safe dose" [1], usually associated with a risk of 1 in 10^5 or 1 in 10^6. In the latter, the ratio between the point of departure (for a 10% response) in a cancer bioassay and human exposure is determined. Values above 10,000 are considered of low concern. This ratio is equivalent to a risk of 1 in 10^5 for a linear dose-response. However, the authors argue that, if it is possible to identify key events for a specific toxicological effect, these may be more amenable to the experimental demonstration of a threshold than the effect itself. It would be possible to use information on specific interventions, which would be quantifiable, to obtain evidence for thresholds in a key event. It might then be possible to identify a rate limiting key event for a specific MOA, and whether any one key event is critical for the toxicological response to occur. Thus, if non-linearity of the dose-response curve could be established for any given chemical, regulators would be able to use reference doses and concentrations that incorporated the information on non-linearity for each specific chemical, instead of general default values for uncertainty factors.

10. The scheme presented in Figure 2, Annex 1 represents a mode of action for a toxicological effect as a series of key events, from the external dose, through absorption, target tissue exposure, biological perturbation and pathological change, to the toxicological effect of concern. Each key event has its own dose-response curve, and possibly also a threshold. The threshold would be determined by the factors such as whether absorption and distribution occur or if there is homeostatic compensation or adaptation and repair, and the threshold at each point would dictate whether there is progression to the next key event at a given dose. The toxicological effect of concern would only occur if all the key events operate.

The case study of chloroform

11. Chloroform was used as a case study by the ILSI working group because it exhibits both target organ toxicity, and carcinogenicity by a non-genotoxic mode of action. A review by Komulainen (2004) established that the compound had been intensively evaluated for carcinogenicity in laboratory animals in a number of separate studies, and that IARC, the International Agency for Research on Cancer, had considered the animal data as sufficient to classify chloroform as possibly carcinogenic to humans (2B). The MOA of chloroform can be broken down into a series of key events which lead to carcinogenesis.

12. Chloroform is metabolised by the liver enzyme CYP2E1 to phosgene, a cytotoxic product which can bind covalently to tissue macromolecules and generate reactive oxygen species. In tissues with high levels of CYP2E1, such as the liver, the local formation of cytotoxic products leads to sustained cell death, and resulting organ toxicity. Persistent necrosis leads to regenerative hyperplasia, which eventually results in the formation of tumours.

13. To describe the key events in this MOA, Boobis et al. (2009) used the generic scheme described above, and detailed specific events in the action of chloroform

[1] The COC does not recommend the use of this approach because the resultant cancer risk estimate has a degree of precision which does not reflect the uncertainties about the shape of the dose response curve orders of magnitude below the doses administered in animal studies. Instead, the Committee recommends using a margin of exposure approach to characterise the risk of such compounds. – See Guidance Statement G06: Risk Characterisation Methods

(illustrated in Figure 3, Annex 1). The key event of generation of phosgene is rate limiting, as the magnitude of the overall response depends upon the levels of this cytotoxic metabolite that are produced. The levels of phosgene produced themselves depend on the levels of CYP2E1 that are expressed, and these depend upon both genetic and environmental factors. Following the key event of generation of phosgene, the next key event is sustained cytotoxicity. This only occurs above certain levels of phosgene, as repair and adaptive processes enable cells to recover from a certain degree of damage while maintaining their viability. These processes are not well defined for chloroform, and there is a data gap here. For the next key event of cell proliferation, it is clear that the preceding cytotoxicity must be of a certain magnitude and sustained for a certain period of time before proliferation occurs. At low doses, or with shorter periods of exposure, adaptive and/or repair processes enable recovery of the organ without an increase in cell proliferation.

14. Despite some gaps in knowledge, particularly those relating to the influence of host factors on key events, the general conclusion is that the mode of action for chloroform is such that the dose-response would be nonlinear. This has allowed the regulatory body, the US EPA, for instance, to establish maximum contaminant levels for chloroform in drinking water based on the fact that the chloroform dose-response is non-linear, and that chloroform is likely to be carcinogenic to humans only at high exposure levels. The EPA has thus been able to move from a no-threshold, low-dose extrapolation approach to one based on the concept of a biological threshold with a non-linear dose-response relationship.

The (quantitative) key events dose-response framework, (Q-)KEDRF

15. Key events analysis has been refined further in the latest work on this aspect of MOAs. The paper of Simon et al. (2014) describes how to incorporate information about timing of occurrence of events, and quantitative aspects of dose-response, into the KEDRF. Two further concepts for understanding MOA are elaborated, those of Associative Events (AEs) and Modulating Factors (ModFs).

16. Simon et al. (2014) begin by proposing a series of organising questions for the evaluation of a MOA, starting with the framework proposed by several earlier papers (Boobis et al. 2006, Meek et al. 2003). Once a MOA has been outlined, the following questions would be considered in the Q-KEDRF approach:

- Which events are necessary (causal), and thus truly key events (KEs)?
- Which events are associative events (AEs)?
- What are the modulating factors (ModFs)?
- Is the proposed MOA likely to be relevant to humans?

17. A definition of a key event (KE) is an empirically observable causal precursor step to the adverse outcome, and is a necessary element of the MOA. It does not have to be sufficient for the adverse outcome to occur, as single KEs by themselves are not usually sufficient for the adverse outcome. To consider whether a KE is essential, it is suggested to consider if removal or blockade of its occurrence could be accomplished. If it could, with no effect on the outcome, then the event is not a KE in the mode of action of the chemical concerned.

18. Associative events are biological processes that are themselves not KEs for the MOA, but reliable indicators or biomarkers for KEs. They can be used as surrogates

or biomarkers for a KE in a MOA evaluation. AEs may reflect exposure, the resulting effect, or both. The relationship of AEs to KEs may need to be explored in the evaluation, especially if an AE is needed to measure the KE.

19. Modulating Factors (ModFs) are biological and individual factors that can modulate the dose-response relationship of one or more KEs, thereby altering the probability or magnitude of the adverse outcome. ModFs fall into 3 categories: host factors, lifestyle, and environment. Host factors include sub-categories such as genetic variation, and the specific aspect of polymorphisms; disease of the host, and whether it is acute or chronic; defence mechanisms, such as immune responsiveness or the capacity of DNA repair processes; and physiology, such as life stage or hormonal status. Lifestyle includes such categories as diet, use of dietary supplements, or use of tobacco, alcohol, or illegal drugs. Environment involves a consideration of co-exposures of the host, such as exposures from air, water, food or through occupation.

20. Besides the identification of KEs, the development of a proposed MOA also requires understanding of their dose-responses and temporal relationships. Equally, dose-responses and temporal relationships between the proposed KEs and the adverse outcome have to be understood. Thus, Dose-Time Concordance tables can be constructed to visualise specific dose and time points where the KE has occurred. The name of the framework thus includes dose-response as well as key events analysis, and becomes (quantitative) key events/dose-response framework, Q-KEDRF. A scheme representing the use of the MOA human relevance framework, alongside the Q-KEDRF, is shown in Figure 4 in Annex 1. The MOA HRF scheme used in the illustration is modified from that given in the Meek et al. (2003) paper.

Case study illustrating use of the Q-KEDRF

21. To illustrate the quantitative nature and the various aspects of the Q-KEDRF, Simon et al. (2014) used dimethylarsinic acid (DMA) as a case study. The MOA was that proposed by the US Environmental Protection Agency (USEPA, 2005), and the animal experiments and results of the 2-year bioassay are described by Cohen (2006). Briefly, four KEs have been identified in the MOA for bladder tumours in rats from DMA. These are: 1) generation of the reactive metabolite trivalent DMA (DMA^{III}); 2) cytotoxicity occurring in the superficial epithelial layer of the urinary bladder; 3) consequent regenerative proliferation; and 4) hyperplasia of the urothelium. A Dose-Response Species Concordance Table for the four KEs is drawn up, with qualitative concordance of the events between animals and humans and the plausibility of such concordance; quantitative dose-response for animals is included. Table 1 below is based on the Dose-Response Species Concordance table in Simon et al (2014), page 25:

Table 1 Dose-response species concordance table for Key Events in the MOA of dimethylarsinic acid (DMA^V), based on information in Simon et al. 2014

Event or factor	Animals	Humans	Concordance
Key Event 1 Metabolism to DMA^{III}	DMA^{III} detected in urine following 26 weeks treatment with 100 ppm DMA^V	Evidence following DMA^V exposure too limited to draw conclusions, but DMA^{III} shown to be present in humans	Plausible
Key Event 2 Urothelial Cytotoxicity	Urothelial toxicity observed *in vivo* in rats at 2 ppm but not enough for successive key events	Potential to occur in humans but unknown if sufficient DMA^{III} formed	Plausible
Key Event 3 Urothelial Proliferation	observed at 0.5 mg/kg/d DMA^V	Potential to occur in humans but unknown if sufficient DMA^{III} formed	Plausible
Key Event 4 Hyperplasia	observed at 2 mg/kg/d or 0.3 to 2 mmol DMA^{III} in urine	Potential to occur in humans but unknown if sufficient DMA^{III} formed	Plausible
Apical Event - Tumours	observed at 5 mg/kg/d DMA^V or 0.8 to 5.05 µmol DMA^{III} in urine	No data in humans	Concordance cannot be made because there is no human data

22. Dose-response information for humans is not available, but toxicokinetic interspecies extrapolation could be based on differences in the metabolism and kinetics of DMA^V in rats and humans. Evidence shows that DMA^V is a poor substrate for the methylating enzyme for arsenicals in humans (As^{III}-methyltransferase), whereas in rats this enzyme can readily methylate DMA^V to trimethyl arsenic oxide (Thomas 2007). Furthermore, *in vitro* cytotoxicity assays using rat urothelial cells have shown effects occur at concentrations of approximately 0.2 µM or higher, whereas *in vitro* human urothelial cells were less sensitive, and cytotoxicity occurred at concentrations of 0.5 µM and higher (Cohen et al. 2006). Thus overall, humans would be less susceptible than rats based on both kinetics and dynamics. The quantitative differences could potentially be used to develop a data-derived species extrapolation factor or chemical-specific adjustment factor for DMA^V.

23. A particular modifying factor (ModF) may also be applicable to the response to DMA^V. Low protein or vegetarian diets are known to decrease the availability of S-adenosyl-methionine (SAM), which is used as a methyl donor in arsenic methylation. Hence, diet may be a ModF that needs consideration (Gamble and Hall, 2012).

Update on the evolution and application of the MOA/HRF

24. Meek et al. (2014a) provides a description of the most recent developments in the MOA/HRF, and includes examples of its application. There are currently around 30 case studies that have been used to illustrate the usefulness of the MOA

approach in evaluating human relevance and in guiding dose-response assessment. The paper presents a “mode of action roadmap”, which is a development of the kind of schemes presented in Meek et al.(2003) and Boobis et al. (2006), with new elements for consideration, together with how MOA information can be applied. It is shown in Figure 5, Annex 1.

25. The roadmap presents a fresh approach to the formulation and use of the MOA framework. The extent and depth of the MOA analysis are tailored to the issue under consideration, and therefore the first step is one of problem formulation, which involves considering the scope and goals of risk management, in consultation with risk managers. In relation to any given exposure scenario, resources, the urgency of the assessment and the level of uncertainty acceptable all need to be considered, and then appropriate methods and MOA analysis can be carried out. There is a difference, for example, between decisions concerning prioritisation of chemicals for testing, and those relating to setting regulatory standards – in the first instance, higher levels of uncertainly are likely to be acceptable. Thus the MOA is tailored to the context in which it is to be used.

26. The second step in the framework is to consider information on the MOA in a modified framework, which is explained in paragraph 26 below. The process involves hypothesis-based analysis of the weight of evidence for operative key events, and qualitative and quantitative concordance of the key events within and between species. Data from different sources can be used, and the amount of detail can vary, depending on the toxicity studied and the needs of the risk assessment. If additional data are needed, the assessment enters the “research” part of the roadmap. Data are then generated that are relevant and focussed on the particular MOA being considered – this is “assessment-specific data generation”. Finally a risk assessment is performed.

27. In the roadmap, there are two different applications that are possible. One is the application of the MOA for observed adverse effects, as in the previous concept of the MOA. Now a second way in which the framework can be applied is considered, and that is to predict likely adverse effects. The outcome of such an analysis would be the development of a case to predict an effect based on knowledge of putative key events. It would be relevant where the adverse effect has not been demonstrated, but could be presumed based on putative early key events, in an established MOA. Thus, there might be previous knowledge of the involvement of certain key events in a MOA, for example, for related chemicals on which there are more data. Or a plausible case could be made, based on existing biological understanding, that the key events may reasonably lead to the adverse outcomes under certain time- and dose-dependent conditions. Extrapolation of quantitative dose-response effects from *in vitro* experiments would be important, and physiologically based toxicokinetic modelling might also be used.

28. The paper gives three case examples of how this type of MOA analysis might be used. In the first instance, a MOA can be hypothesised based on reference pharmaceuticals or other chemicals, if the key events leading to a specific effect are known in sufficient detail. Then *in vitro* systems and *in silico* models could be used to predict early and subsequent rate-limiting key events. The toxicity of other chemicals acting through the same MOA could in theory be characterised and predicted based on responses obtained in the *in vitro* systems and *in silico* models.

A research initiative known as SEURAT-1, Safety Evaluation Ultimately Replacing Animal Testing, is based on this premise (Gocht et al., 2013), and includes research projects combining research from over 70 European universities, public research institutes and companies.

29. If data are only available on one or a limited number of key events, and the link to an effect is not sufficiently demonstrated, such data may be used to rank and prioritise chemicals for additional testing, based on likely relative hazard. The second case example discussed in the paper is that of prioritising chemicals for evaluation of their endocrine disruptive potential. A QSAR (Quantitative Structure-Activity Relationship model) system has been developed to predict oestrogen receptor binding affinity using MOA knowledge (OECD, 2009). A third example of the use of MOA analysis in hypothesising adverse effects is in the creation of chemical categories, specifically the class of pyrethroids. The MOA for this group of pesticides has been established with confidence, and therefore the risk assessment of a new pyrethroid could be based on the assumption that it will share the MOA of other pyrethroids, and read-across can be used to determine the likely relative hazard of the chemical. Many of the concepts elaborated in this paper for the 'bottom-up' application of MOA information are shared by the Adverse Outcome Pathway (AOP) approach described below.

Comparative analysis of weight of evidence in MOA/HRF

30. A second paper by Meek et al. (2014b) discusses the aspect of considering relative weight of evidence (WOE) among different cases and hypothesised MOAs. The purpose of a comparative WOE analysis is to contribute to the transparency of indicating the relative confidence or uncertainty in the MOA/HR analysis. Comparative WOE analyses are given for two compounds, carbon tetrachloride and 1,2,3-trichloropropane (TCP). The approach involves constructing WOE summary tables, based on consideration and evaluation of data in existing assessments. For each MOA, supporting data, inconsistent data and missing information are tabulated. The tabulated information describes what has been observed, not what might be possible if more experiments were performed. Blank cells indicate where data either do not exist or are inadequate for evaluation, and a discussion on whether the missing information is critical and would detract from conclusions on the proposed MOA should ideally accompany the comparative WOE table. In the two examples used in the paper, the analysis for carbon tetrachloride indicated inconsistencies in the database and concluded that the carcinogenic MOA for the compound is not known. In contrast, it was possible to conclude that TCP is likely to be carcinogenic to humans through a mutagenic MOA, with two key events occurring – metabolism to a DNA-reactive compound, and early induction of mutations. The comparative WOE uses assessments which have already been performed, highlights inconsistencies and knowledge gaps, and explains transparently how conclusions about compounds are reached.

A recent initiative – the Halifax Project

31. A recent initiative involving cancer researchers from around the world was started in August 2013, when the organisation Getting to Know Cancer (www.gettingtoknowcancer.org) held workshops in Halifax, Nova Scotia. Researchers organised two task forces, one of which was focused on the

carcinogenic potential of low dose exposures to mixtures of chemicals in the environment. The task force looked at the possibility that exposures to mixtures of disruptive chemicals at low doses in people's everyday lives might be contributing to the current high rates of cancer incidence. They chose 85 chemicals that were not considered to be carcinogenic to humans, and reviewed their effects against a list of mechanisms that are important for cancer development. Separate teams focused on various hallmarks of cancer, and the group found overall that 50 of the chemicals supported key cancer-related mechanisms at environmentally-relevant levels of exposure. The idea is that chemicals may be capable of acting with one another to cause cancer, even if low level exposures to those chemicals individually may not be carcinogenic. The results of the work of the task force have been published in a special edition of the journal *Carcinogenesis*, with separate review papers for each hallmark (Halifax Project, 2015).

Adverse Outcome Pathways (AOPs)

32. The term Adverse Outcome Pathway (AOP) was first defined by Ankley et al (2010) in relation to ecological risk assessment, but it has similarities to the concept of a MOA. An AOP is a means of portraying existing knowledge showing a link between a molecular initiating event (an early key event), and an adverse outcome at a biological level that is relevant to risk assessment. Thus, the initiating event may be a receptor-ligand interaction, or a molecule binding to DNA. Cellular responses occur as a result, such as gene activation, or altered signalling. The cellular responses lead to organ responses, such as altered physiology. Organ responses lead to responses of the whole organism, one of which may be cancer. The adverse outcome may also be expressed at the population level, particularly for ecotoxicological endpoints. An AOP may contain gaps, where details of the exact chain of events leading to the adverse outcome are not known, but overall there is sufficient information about the pathway to help improve risk assessment decisions.

33. In 2012 the OECD (Organisation for Economic Co-operation and Development) issued a guidance document on how to develop and assess AOPs (OECD 2012). This document further outlines the necessary steps to establish an AOP. In general, three main blocks of information are necessary: the molecular initiating event (MIE), intermediate events and the final, or apical, adverse outcome. Each of the three main information blocks has to be clearly identified. It is possible to develop the AOP starting from any of the three blocks, depending on what knowledge is available at the outset, but ultimately the start and end points have to be clear, that is, the MIE and the apical endpoint of interest, as the AOP is "anchored" at its two ends by a chemical and biological interaction at one end, and an outcome of interest to risk assessment at the other. The apical endpoint is often associated with an *in vivo* OECD Test Guideline. The apical effect determines the most relevant mechanistic information and the intermediate effects related to this endpoint.

34. In the OECD approach, it is important to be able to gauge the reliability and robustness of an AOP. This should be done by evaluating the experimental support for the pathway, using a very similar approach to that developed for establishing a MOA. Thus the key steps should be scientifically proven, both qualitatively and if possible quantitatively. Usually, the assessment of the experimental evidence and the empirical data clearly support the qualitative understanding of the AOP, although the prediction of the relative potency of the inducer may be difficult due to lack of

data. Therefore the quantitative data is not always as complete as the qualitative understanding. The OECD has created a web-based repository for AOPs at https://aopwiki.org/wiki/index.php . AOPs are separated into different categories, most importantly those that have been approved by OECD and those under development. The latter can be at very different stages.

35. The usefulness of the AOP concept is its flexibility, as it can include linking relationships that are: "causal, mechanistic, inferential, or correlation based, and the information on which they are based may derive from *in vitro*, *in vivo*, or computational systems" (Ankley et al, 2010). It can include both mechanism and mode of action. In the short term, AOPs can inform chemical grouping or categories, help to increase certainty of interpretation of information, and be used in setting up testing strategies (Willett, 2012). In the longer term, it is hoped that AOPs can be used to identify key events for which non-animal tests can be developed, and to help provide predictive toxicological assessments which have high human relevance, ultimately without the use of animals.

36. The development of a carcinogenicity AOP has been proposed by Veith (2010). The process starts by outlining information that is already known, and then by identifying whether the parent chemical acts by direct binding to DNA or by indirect DNA damage, or by non-genotoxic mechanisms. Conclusions are drawn, based on the quality of the data, on the cancer-causing potential of the chemical.

37. The AOP approach fits well with the integrated strategy that REACH guidance requires; however, within REACH, assessment of genotoxicity is performed *in vivo* by default if positive results are seen *in vitro* (Willett, 2012). Nevertheless, if all indications from lower-tier information are negative with respect to genotoxicity and neoplasia, such as information available from repeat-dose studies, then the REACH strategy recommends *in vitro* cell transformation assays or further characterisation to improve grouping and read-across, and only short-term *in vivo* studies that could indicate carcinogenic potential. Although limited, there is some possibility here of making a reduction in the use of animals.

Summary

38. Overall, the development of mode of action and human relevance frameworks has provided approaches that can be used by risk assessors which build on previous knowledge of chemicals, and increase the possibility of using read-across and other information without automatically resorting to animal studies. Information on MOA is now in regular use, for both classification and risk characterisation, by organisations such as EFSA (the European Food Safety Authority) and the US EPA (United States Environmental Protection Agency). The development of AOPs is another technique for drawing together and assessing the significance of previous knowledge. Databases of chemicals assessed in these ways continue to grow.

Questions for the Committee

1) Does the Committee wish for any further analysis or discussion of this topic?

2) Does the Committee want to make any changes to Guidance Statement G03: Hazard identification and characterisation, in which the MOA and HRF are discussed?

3) Is any update needed to the discussion in the overarching guidance statement G01: A strategy for the risk assessment of chemical carcinogens?

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COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Recent developments in the Mode of Action and Human Relevance Framework

This annex contains the published figures and tables referred to in the discussion document.

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The figures and tables are attached. They are not being made publicly available for copyright reasons.

COC Secretariat
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