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Matters Arising CC/2022/04

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

COC Guidance Statement (G04): The Use of Biomarkers in Carcinogenic Risk Assessment – final draft – updated following COM comments

1. The COC has periodically published guidelines for the evaluation of chemicals for carcinogenicity. The first guidance was published in 1982 and has undergone several updates since then to reflect advances in development and validation of methods for assessing risk of chemical carcinogenicity. These updates included the separation of the overall guidance into individual documents to allow faster revisions to be made in the case of rapidly developing areas. This included a separate document addressing the use of biomarkers in carcinogenic risk assessment (G04).

2. During the latest round of updating G04, the COC considered it would be helpful to have COM comment on the DNA adducts and genotoxicity biomarkers sections, which had been extensive in the previous version. The COC suggested that COM might be better placed to produce guidance on these topics, which COC could then make reference to.

3. At the last discussion of this draft update to G04, the COC agreed that subject to any comments from COM, the document could be agreed by Chairs action.

4. The current draft document, attached for final agreement by the Committee at Annex A, includes further amendments in light of comments received from the COM, and makes reference to the COM specific guidance on biomarkers, which the COM agreed would be taken forward.

Questions for the Committee

5. Members are asked to consider the COC draft guidance statement and, in particular, whether the guidance document can now be published as COC guidance.

IEH Consulting under contract supporting the PHE COC and COM Secretariat
July 2022

DRAFT

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CC/2022/04 – Annex A

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

COC Guidance Statement (G04): The Use of Biomarkers in Carcinogenic Risk Assessment – final draft – updated following COM comments

Final draft COC guidance statement

IEH Consulting under contract supporting the PHE COC and COM Secretariat
July 2022

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Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC)

COC Guidance Statement G04 – version 1.2 draft 0.d

The Use of Biomarkers in Carcinogenic Risk Assessment

DRAFT

https://www.gov.uk/government/groups/committee-on-carcinogenicity-of-chemicals-in-food-consumer-products-and-the-environment-coc

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COC Guidance Statement G04 v1.2 draft 0.d

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

The Use of Biomarkers in Carcinogenic Risk Assessment

Introduction

1. The focus of this guidance statement are biomarkers of toxicological relevance, defined as “any substance, structure or process that can be measured in an organism, related to a specific exposure or effect or which can influence the incidence of the effect" (Choi et al., 2015). Biomarkers can provide valuable information to aid chemical risk assessment processes and are used as investigative tools in both animal and human studies which aim to evaluate carcinogenic hazards and risk. Readers are also referred to the Committee on Mutagenicity (COM) guidance document that provides more in-depth information on biomarkers of exposure and effect for genotoxic carcinogens, which is currently in preparation.

2. For the purposes of this document, biomarkers will be broadly characterised as those of *exposure* and those of *effect*, although the distinction between these two is not always clear-cut. Biomarkers in the context of carcinogenicity can mean proof of exposure to a carcinogen, detection of a reaction product or an indication that a preliminary genotoxic event or actual DNA damage has occurred. Other types of biomarkers exist, for example biomarkers of susceptibility, which were initially introduced as interpretative aids to epidemiological investigations of chemically-induced carcinogenesis.

- *Biomarkers of exposure* - “an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism” (Laideira and Viegas, 2016). Biomarkers of exposure are further divided into those reflecting ‘internal dose’ and those reflecting ‘effective dose’. The concentration of a chemical (or metabolite) in blood following exposure is a basic measure of the internal dose, indicating the likely level of chemical (or metabolite) at the target site. The effective dose is a more accurate measurement of the exposure levels associated with the target molecule, structure or cell itself (Laideira and Viegas, 2016) (refer to paragraphs 16 - 26).

- *Biomarkers of effect* - "a measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognised as associated with an established or possible health impairment or disease" (IPCS, 1993; Jeddi et al., 2021), for example measures of chromosome damage, related to carcinogenicity (refer to paragraphs 27 to 36).

- *Biomarkers of susceptibility* - A biomarker of susceptibility may be defined as an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a chemical (Manno et al., 2010) (refer to paragraphs 37 and 38).

3. The over-arching summary Guidance Statement (G01) provides the Committee's views on the general principles relating to carcinogenic hazard and risk assessment and a background to the individual components of the risk assessment process and how these are integrated. This statement aims to provide detail of how biomarkers are utilised within the individual components of the risk assessment process.

4. The Committee recommends a four-stage approach to the risk assessment of chemical carcinogenicity which is based on the widely adopted paradigm proposed by the National Academy of Sciences (US National Academy of Sciences, 1983). This is summarised as follows:

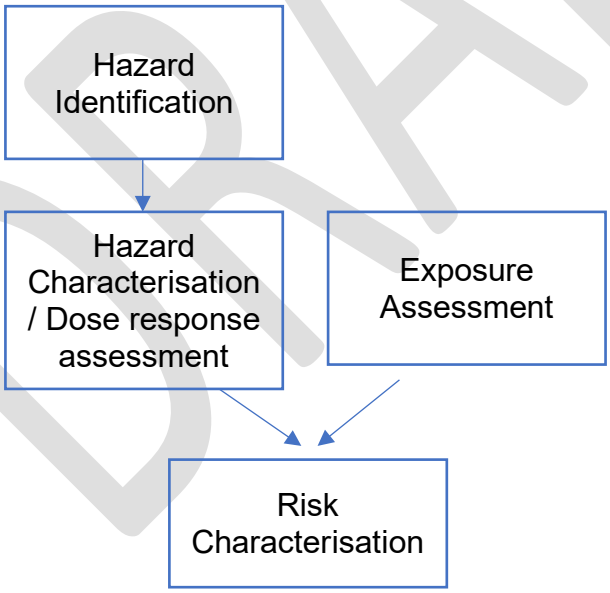

Figure 1: Four stage evaluation strategy for the risk assessment process of carcinogenic hazard

5. 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 (Harrison and Doe, 2021).

6. Within exposure assessment, biomarkers can be used (usually) to establish recent exposures to, and uptake of, actual or putative carcinogens in human populations or experimental animals. Within hazard assessment, biomarkers may be used to quantitatively associate a dose or exposure with either a precursor carcinogenic effect or the probability of a disease outcome. In this context, biomarkers can provide specific evidence that a chemical has the potential to cause a carcinogenic effect and may also provide information on mode of action. Therefore, biomarkers provide a range of possible measurements from systemic exposure through to resulting causal events in the process of carcinogenesis.

7. When utilising biomarker data, it is important to consider that there is usually a long latency period between first exposure to the carcinogen and the clinical onset of cancer. Currently, established biomarkers of exposure often represent recent exposure but some which show organ or tissue retention can be used to assess long-term exposure.

8. Biomarkers are powerful tools for investigating the mode of action (MOA) and can be incorporated into animal studies for this purpose. Indeed, biomarkers, where a clear rationale for the alteration of the level of biomarker with the underlying latent variable, can be useful to discern mechanisms of action. Conversely, knowledge of MOA may help in the development of better biomarkers for use in human exposure scenarios. It should be noted however, that any biomarker used in animal studies must have relevance to humans and not represent an animal (species)-specific response (see for example, Bartsch et al., 2018).

Biomarker characterisation and validation

9. The Committee has a role in evaluating the entire spectrum of biomarkers including the development, validation and practicality of new techniques and the applicability and interpretation of well-established methods.

10. Biomarkers must be appropriately characterised and validated before conclusions are drawn from their use. There are a number of criteria that should be considered when selecting and validating suitable biomarkers for use in human biomonitoring studies (Vorkamp et al., 2021). For example, the general criteria used for the evaluation of the most suitable exposure biomarkers (EB) and matrix (M) for the current European initiative, HBM4EU for carcinogens and non-carcinogens, include:

- Specificity – concentration of the exposure biomarker in the selected matrix should exclusively reflect exogenous exposure and is a consequence of environmental and/or occupational exposure. It is noted that for chemicals with very limited data, exclusivity may not be achievable, however concentrations should be a correct indication of exposure.
- Biological sensitivity - the measured concentration of the EB/M correlates strongly with the substance intake dose. Variations of EB/M concentration reflect the variation of exposure to the substance of interest. For chemicals with very limited data, the measured

concentration of the EB/M is an acceptable indication of the substance intake dose.

- Half-life - the EB/M should preferably have a half-life sufficiently long to avoid an excessive intra-individual variability in EB/M concentration measurement.
- Stability after sample collection - cryo-preservability of EB/M is sufficient to guarantee a high stability during storage in the biobank or analyse the sample as soon as possible.
- Matrix availability and sample collection - the sample collection of the relevant matrix is not considered too invasive. Easy collection and transportation of the required amount of sample with a validated sampling protocol is beneficial. It is advantageous if it is possible to determine more than one EB in the same matrix where it is relatively easy to obtain a sufficient sample volume for a required number of samples.
- Measurement validity - the EB/M concentration in the sample is not likely to be altered by contamination with a ubiquitous parent substance from the environment preceding and during the analysis. Variations in matrix composition can be easily corrected for (e.g. creatinine in urine, lipids in serum). Sample contamination by a ubiquitous parent substance might occur, but the level of contamination is low compared to expected levels and special precautions can be applied to minimize the amount of contamination.

11. Biomarkers used in animal studies must also be suitably characterised and validated and this should be based on the principles detailed for human biomarkers. In relation to biomarkers, the STrengthening the Reporting of OBservational studies in Epidemiology – Molecular Epidemiology (STROBE-ME) initiative (Gallo et al., 2011) provides standardised guidelines and a 'checklist' for the reporting of biomarker and molecular epidemiology studies (see http://www.equator-network.org/reporting-guidelines/strobe-me/, accessed June 2021).

Strategic uses of human biomonitoring (HBM)

12. Probably the most well-developed use of HBM has been in occupational settings where exposures to a chemical of particular concern might be relatively high. Here, routine HBM might be more informative about risk than air monitoring and, various types of reference values used for risk management might exist for the chemical of concern. In the general population, HBM is often used to inform on exposure to chemical of particular concern and also, for changes over time (increase or decrease) for substances of interest related to industrial or consumer usage to existing or newly introduced substances (Bevan et al., 2017).

13. Ongoing national and international surveillance programmes such as the US National Health and Nutrition Examination Survey (NHANES), the Canada Health Measures Survey (CHMS) and the German Environmental Survey (GerES) typically use well-established biomonitoring techniques (e.g. biomarkers which are known to reflect exposure to the chemical of interest, standardized sampling methods and

verified analytical techniques) to collect information on population exposures to environmental hazards that are known to be significant to public health. As biomonitoring does not generally determine exposure sources and routes of exposure, environmental monitoring remains crucial (Ladeira and Viegas, 2016).

14. A Biomonitoring Equivalent (BE) is an estimated concentration or range of concentrations of an environmental chemical in humans that is consistent with existing health-based guidance values such as the Tolerable Daily Intake (TDI) or reference dose or concentration (RfD, RfC). It provides a way of interpreting biomonitoring data in the context of these values (Hays et al., 2008; LaKind et al., 2008). It is envisaged that they will be useful for understanding and prioritising risk management practices and will enable the available biomonitoring data to be utilised more fully. However, to date, there is limited information on the use of BEs for estimating chemical exposure in the context of carcinogenesis (Faure et al., 2020).

15. Human biomonitoring guidance values (HBM-GVs) are being derived by the European Human Biomonitoring Initiative referred to as HBM4EU. There is currently a diversity in the derivation of health-based guidance values for both the general population and for occupational exposure. The HBM4EU initiative aims to increase confidence in HBM-GVs derived using a harmonised, systematic and generally accepted strategy for the derivation of HBM-GVs at the European level (Vorkamp et al., 2021).

Biomarkers of exposure

16. The objective of human exposure assessment is to estimate probable exposure by determining exposure routes, source, magnitude and duration of contact with the chemical of concern. However, epidemiological studies can have limitations related to measurement of exposure to carcinogens over long periods, and exposure assessment is frequently identified as the main area of uncertainty in the overall risk assessment process. Although the alternative approach of personal monitoring (e.g. dermal patch studies) provides a way to measure exposure directly, assumptions need to be made about the relationship between results from short-term sampling and predicted long-term exposure. Approaches used in exposure assessment and the characterisation of uncertainties and variability in the resulting estimates have been extensively reviewed elsewhere (Ladeira and Viegas, 2016).

17. Biomarkers of exposure can indicate the presence of a carcinogenic compound or its biological interactions. This is achieved by assaying levels of the chemical, a metabolite or a reaction product in blood, urine, saliva, cerebrospinal fluid, or other biological samples. In this way, exposure biomarkers can provide direct evidence of human exposure to a carcinogen through internal dose. In addition, any factors that may impact on target organ concentrations, such as individual phenotype should be taken into consideration.

18. Where a relationship can be established between biomarker levels and external and internal dose, data from exposure biomarkers can be used to calculate the initial external exposure. It is important that a relationship can be established to counter the potential impact of interfering factors. Physiologically Based

Pharmacokinetic (PBPK) models are valuable tools to help define safe external levels of chemicals based on internal doses at target organs in experimental animals, humans and organisms used in environmental risk assessment (Paini et al., 2021).

19. Biomarkers such as adducts (DNA or protein) are important in understanding the kinetics and potential biological interactions of a chemical, for example if it is capable of interacting with DNA. Many biomarkers of exposure are short-lived and provide short- to medium-term indications of internal exposure. Adipose tissue (AT) is also recognised as a significant site of lipophilic toxicant bioaccumulation, thereby reflecting cumulative exposure. It has been suggested that AT plays a major role in the storage and overall toxicokinetics of hydrophobic xenobiotic persistent organic pollutants (POPs), which has both positive and negative consequences. The sequestration of POPs can be both protective, i.e. by removing them from the blood stream but are potentially harmful if released from AT, for example during rapid periods of weight loss (Jackson et al., 2017).

20. Biomonitoring, the direct measurement of a chemical or its metabolites in biological samples, has been widely used within the risk assessment process, for both carcinogens and non-carcinogens. Validated biomarkers of internal exposure have been identified for a wide range of environmental chemicals and metabolites, including: metals; polyaromatic hydrocarbons (PAHs); polychlorinated dibenzodioxins (PCDD), polychlorinated biphenyls (PCB); phthalates; pesticides; aromatic amines; perfluorinated substances; tobacco smoke components; and volatile organic compounds (VOCs) (Jeddi et al., 2021). Biomarkers of exposure can be used in animal studies to provide important information which can contribute to carcinogenic MOA investigations. For example, investigations of the carcinogenic potential of acrylamide utilising DNA and haemoglobin (Hb) adduct data (Virk-Baker et al., 2014; Xu et al., 2014).

DNA adducts

21. DNA adducts, where a chemical moiety is covalently bound to DNA , characterise the first stage of DNA damage and provide a marker of exposure to reactive chemicals or their metabolites. The presence of DNA adducts may demonstrate systemic exposure to specific target tissues and can be measured in peripheral blood lymphocytes (PBLs), exfoliated cells, such as from the urothelium or buccal mucosa, and in tissue biopsy samples (see for example: Farmer et al., 2003; Lee et al., 2003; Veglia et al., 2003; Phillips, 2005; Singh et al., 2007; Taioli et al., 2007; Ewa and Danuta, 2017; Ma et al., 2019; Totsuka et al., 2021).

22. The biological significance of DNA adducts has been considered by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) and International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI) workshops (Pottenger et al., 2009; Sander et al., 2005). Both reached the general consensus that DNA adducts had an important role in the risk assessment process and in establishing mode of carcinogenic action, although the association of an adduct with a disease does not automatically indicate causality. It has also been proposed that where the relative timing of exposure and sampling are

known, DNA adducts can be useful biomarkers of cumulative exposure, representing cumulative unrepaired DNA damage (Kwack et al., 2014). The COM considers DNA adducts to be biomarkers of exposure, whereas gene mutations and chromosomal alterations represent biomarkers of early biological effects but are also potential bio-indicators of the carcinogenic process (COM 2021).

23. It has been estimated that endogenous DNA adducts are present in animal and human cells at levels of approximately 50,000 adducts per cell, formed predominantly through oxidative stress. Many of these can result in mutations if DNA replication occurs prior to repair and are considered a major contributor of cancer and other diseases (Swenberg et al., 2011). It is important to consider this in risk assessments.

24. The comet assay detects single strand breaks and alkali-labile lesions in DNA using PBLs and is well established as a biomonitoring tool for occupational and environmental exposures (Azqueta et al., 2020). However, its value for predicting cancer is not yet known because it has not been investigated in prospective cohort studies. An understanding of the covariates influencing inter-individual background levels is also critical in the design of such studies (Azqueta et al., 2020) and a role of genotype is also implicated in this variability (Koppen et al., 2017). The advantages and limitations of using the *in vivo* comet assay in a regulatory context have been reviewed (Cordelli et al., 2021).

25. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a non-specific marker of oxidative damage to DNA that can be assessed using PBLs or assayed in biofluids such as urine (Loft et al., 2012). There is good evidence, for example from studies examining workers exposed to PAHs and air pollutants, that increases in this biomarker correlate with exposure to potential mutagens and these increases are broadly in accordance with comet assay results (Brucker et al., 2020).

Protein (haemoglobin or albumin) adducts

26. Adducts of chemicals with proteins such as haemoglobin (Hb) and albumin can also be used as biomarkers of exposure to carcinogens (Hartwig et al., 2020). Occupational exposure to 1,3-butadiene and styrene have been effectively investigated using Hb-adduct methodology (Vacek et al., 2010; Boysen et al., 2012; Ogawa et al., 2006). Acrylamide exposure in humans has been successfully monitored by measuring Hb adducts of acrylamide itself or its metabolite glycidamide (Vikstrom et al., 2012). Similarly, albumin adducts of aflatoxin have been detected in exposed populations (McCoy et al., 2008) and biomonitoring of arylamines and nitroarenes utilises albumin adducts (Sabbionu and Turesky, 2017).

Biomarkers of Effect

27. Biomarkers of a key event implicated in a carcinogenic mode of action may be used to characterise the hazard. With regard to carcinogenicity, the most commonly studied biomarkers of effect measure genotoxicity endpoints such as chromosomal changes (Albertini et al., 2000; Bonassi et al., 2005 and 2011). It is important to recognise that, in some instances, these biomarkers of effect may only be indicative

of immediate, and not fixed, alterations and therefore may not represent injury resulting in impairment of health or disease. Biomarkers of effect are frequently not specific to a given exposure or a specific agent and are influenced by sources of inter- and intra-individual variability, including species, sex and age. The relationship between exposure (acute, subacute, or chronic), the biomarker of effect, and carcinogenic event must be established in order to determine validity (Jeddi et al., 2021).

Genotoxicity Biomarkers

28. Some of the currently used biomarkers of genotoxicity are briefly summarised here. However, it should be noted that evidence that genotoxicity biomarkers are indicative of cancer risk in humans is not extensive, and that the presence of genotoxicity biomarkers does not inform on the precise nature of the chemical exposure that has given rise to the measured endpoint. Further information on genotoxicity biomarkers is expected to be published by COM in due course.

29. Cytogenetic endpoints such as micronuclei (MN) and fixed chromosome aberrations (CA) are considered to be biomarkers of early carcinogenic effect and are thought to be predictive for cancer risk in humans (Bonassi et al., 2011; Wang et al., 2012; Bonassi et al., 2016; Barth et al., 2017; Vodenkova et al., 2015; Hopf et al., 2019). are They are often used in occupational and environmental biomonitoring studies (Mahmoodi et al., 2017; Brucker et al., 2019). Aneuploidy, a change in chromosome number, is being increasingly used as a biomarker of effect as its mechanisms and adverse effects are well defined. It is considered a 'hallmark' of cancer which is present in cancerous and precancerous lesions caused by both genotoxic and non-genotoxic carcinogens (Mandrioli et al., 2016).

30. Epigenetic features are increasingly considered sensitive to environmental exposures, and therefore could serve as an important biomarker of effect (Sanchez-Guerra et al., 2015; Shukla et al., 2019). Epigenetic modifications are flexible genomic parameters that can change genome function under exogenous influences. To date, epigenetic changes include DNA methylation, histone modifications, and miRNAs that affect post-transcriptional regulation, without changes to nucleotide sequences. It has been proposed that environmental factors could influence epigenetic processes, leading to epigenetic reprogramming and contributing to the development of several diseases (Ji et al., 2016).

31. Despite progress in the identification of biomarkers, gene mutation-based approaches still face considerable challenges as cancer evolves from a complex interplay between environment and host. Therefore, identification of an epigenomic signature might be useful for early diagnosis with the potential to reduce the environmental-associated disease burden (Ceccaroli et al., 2015).

Other biomarkers of carcinogenic effect

32. Extracellular vesicles such as exosomes contain proteins, lipids, RNAs and microRNAs and mediate cell-to-cell communication. They can originate from both

healthy and tumour cells and are ejected into the bloodstream. Functional protein biomarkers representing the tumour proteome and carried in the extracellular vesicle can be used for early detection of cancers, including prostate and pancreatic cancers (Yu et al., 2021).

33. Many chemicals cause cancer through a broader variety of mechanisms than solely genotoxicity, for example by affecting epigenetics (see paragraph 30), the endocrine system, apoptotic signalling, cell proliferation, and/or gap-junctional intercellular communication. In addition, simultaneous alteration of multiple pathways is often required to result in carcinogenicity (Wilde et al., 2018). Examples of non-genotoxic carcinogens include 1,4-dichlorobenze that acts as a tumour promoter, 17β-oestradiol that is an endocrine-modifier, 2,3,7,8-tetrachlorodibenzo-p-dioxin that is a receptor-mediator, cyclosporine which is an immunosuppressant or metals such as arsenic and beryllium that induce tissue-specific toxicity and inflammatory responses (Hernández, et al., 2009).

Molecular Epidemiology in Cancer Risk Assessment

34. Epidemiologists are currently challenged with measuring the biological effect of an exposure occurring at low doses, whilst recognising the effect of a single component in a mixture and highlighting the link between genetic and environment factors in the aetiology of cancer. Population-based studies involving environmental and occupational exposure, infectious agents, personal susceptibility factors, and acquired genetic factors may identify high-risk populations that are likely to develop cancer; additionally, such studies are very informative and significant in designing future community-based health initiatives.

35. Molecular epidemiology is simply defined as the application of the techniques of molecular biology to the study of populations, with a particular focus on the investigation of disease prevalence (Vineis et al., 2005). The term encompasses the use of biomarkers to investigate the events and potential mechanisms which occur during the process of carcinogenicity, from initial exposure to disease (also called the 'meet in the middle approach'; Vineis and Perera, 2007; Vineis and Chadeau-Hyam, 2011).

36. Developments in this field have been underpinned by the improvement of genetic and molecular techniques identifying environmental and genetic risk factors in the aetiology of cancer. There is a large body of literature which describes the development of potential new biomarkers of exposure and effect and discusses the usefulness and limitations of biomarker measurement (e.g. Ceccaroli et al, 2015).

Biomarkers of Susceptibility

37. Evidence suggests that genetic susceptibility plays a role in an individual's response to exogenous and environmental exposures. Consequently, a number of studies have explored the interactions between genetics and exposures in the aetiology of disease (for example, Kelly and Vineis, 2014).

38. The use of detailed PBPK models for interpreting biomonitoring data also allows for the modelling of different sources of interindividual variability of the absorption, distribution, metabolism and excretion processes, such as body weight, age, genetic polymorphisms in xenobiotic metabolic pathways, excretion and elimination rates and others. The previously so-called confounders or uncertainty factors can be treated as analysable variables which reflect variations in the susceptibility within a population that is exposed to environmental pollutants (Ladeira and Viegas, 2016).

Omics technologies

39. The development of omics technologies (genomics, proteomics, metabolomics) to investigate gene and protein changes following chemical exposure, and its use in toxicological risk assessment has previously been reviewed in detail by the COT, COC and COM (COT, COC and COM, 2004; COT, 2012). In addition, COM is currently preparing an updated evaluation of the application of transcriptomics and next generation sequencing to genotoxicity and carcinogenicity assessment.

40. The conceptual term 'exposome' has been developed as a means to consider an individual's exposures over the entire life course from conception until death (Wild, 2005; Vineis et al., 2020; Huhn et al., 2021). It has been described as "the totality of internal human exposure with regards to exogenous chemicals, their biotransformation products, and endogenous chemicals sensitive to various environmental exposures and potentially involved in signaling pathways" (Escher et al., 2017).

41. Understanding the relation of external to internal exposures is a central aspect of an exposome assessment, however at present there is no consensus on how to assess the exposome. A number of major ongoing projects investigating approaches to implement the exposome concept with a focus on environmental chemicals have been developed, including: Human Early Life Exposure (HELIX); Health and Environment-wide Associations based on Large Population Surveys (HEALS); EXPOsOMICS; and HBM4EU. All of these projects related their research to existing infrastructures and data available in different European cohorts with the aim of comparing health outcomes and exposure information and to generate cohort-related biosamples and exposome data (Huhn et al., 2021).

42. Tools such as Exposome-Explorer (http://exposome-explorer.iarc.fr; accessed September 2021) are also available. This is the first database dedicated to the collation of biomarkers of exposure to environmental risk factors for diseases and aims to provide comprehensive data on all known biomarkers of exposure to dietary factors, pollutants, and contaminants measured in population studies (Neveu et al., 2017).

43. The application of omics technologies to carcinogenicity evaluation was previously considered by the COC as part of its discussions on alternatives to the use of the 2-year rodent bioassay for carcinogenicity risk assessment (COC Guidance Statement G07, Part C). However, as a result of discussions to move

away from traditional risk assessment approaches for potential carcinogens, G07 is undergoing substantial revision which will include an updated evaluation of the use of omics technologies.

44. Epigenetics, heritable changes in gene expression which are independent of changes in DNA sequence are being increasingly recognised as part of the process of carcinogenesis (Barrow and Michels, 2014). Epigenetic mechanisms include changes in DNA methylation. There is evidence that some chemical exposures result in epigenetic modifications which could impact on the induction of cancer and may act as historical biomarkers of exposure (Verma, 2015). The possibility of use of epigenetic change as a biomarker of exposure has been discussed at the joint COC, COM and COT meeting in October 2017 where use of epigenetics in chemical risk assessment was discussed (COC, COM, COT, 2019).

45. Non-coding RNAs (ncRNAs) constitute the majority of the human transcribed genome and have been implicated in many pathological conditions, especially cancer (Chan and Tay, 2018). One subclass of ncRNAs, miRNAs, which have a role in the regulation of translation of protein from mRNA, have been explored for development as a biomarker of effect. These miRNAs are differentially expressed in many cancer types and found in the circulation (Brase et al., 2010; Calin and Croce, 2006; Mitchell et al., 2008; Mo et al., 2012). miRNA species are coded from regions of the genome that can be under epigenetic control and can be differentially methylated in cancer (Chuang and Jones, 2007; Li et al., 2012; Lujambio et al., 2008). This raises the possibility that epigenetic change resulting from carcinogen exposure may lead to altered miRNA expression via differential methylation and that this could be a biomarker of historical carcinogen exposure and arbiter of potential future effect (Vrijens et al., 2015; Krewski et al., 2020).

46. The use of ncRNAs as potential biomarkers in regulatory toxicology was discussed at an ECETOC workshop, the summary of which noted
"To make available ncRNAs as biomarkers for regulatory toxicology and RA, normal and adverse ncRNA profiles and dose-response relationships of effects should be determined, and ncRNA expression profiles should be linked to phenotypic alterations. Further, it should be determined whether ncRNA levels in specific body fluids reflect levels in specific target tissues. Even though a number of research projects demonstrated a lack of toxicologically relevant uptake and activity of ingested RNAs, bioavailability of ingested ncRNAs and potential impacts to the consuming organism may merit further investigation" (ECETOC, 2016).

Summary

47. A biomarker, in the context of chemicals and carcinogenicity, is defined as an observable change related to the carcinogenic process following a specific exposure or effect.

48. In cancer risk assessment, biomarkers can be utilised for hazard identification and characterisation, for exposure assessment and in some instances to inform the causal mechanism. The causal relationship between

the biomarker and the carcinogenic response should be established where possible.

49. Biomonitoring studies should fulfil pre-defined criteria and human-specific biomarkers should be appropriately characterised and validated. Particular attention should be given to ascertaining the stability and half-life of the biomarker and how these impact on the interpretation of epidemiological data.

50. Biomarkers of exposure include DNA and protein adducts. Biomarkers of effect include genotoxicity biomarkers such as MN and fixed CA. Non-genotoxic carcinogens demonstrate a broader mechanistic variety than genotoxic carcinogens, which is reflected in the potentially greater number of system effects. In addition, simultaneous alteration of multiple pathways is often required to prompt non-genotoxic carcinogenesis (Wilde et al., 2018).

51. The Committee maintains an on-going awareness of the development of newer techniques including molecular epidemiology methods, omics technologies and the emergence of the exposome. However, many of the techniques are still experimental and are useful only for deriving qualitative measurements or information contributing to MOA investigations. It is not currently possible to provide specific guidance on their use in a quantitative capacity.

52. The Committee continues to evaluate the usefulness of the entire spectrum of biomarker techniques including the applicability and interpretation of well-established methods.

COC Guidance Statement G04 v1.2 draft v0.d
Date TBC

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