This is a background paper for discussion.
It does not reflect the view of the Committee and must not be quoted, cited or reproduced

CC/2021/02

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

First draft revised Guidance Statement (G04): The Use of Biomarkers in Carcinogenic Risk Assessment

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.

2. These updates included the separation of the overall guidance into individual documents during 2012 – 2014 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).

3. Guidance statement G04 was last revised in 2018 and, as part of the rolling review process for COC documents, is being considered for update again. An updated version of G04 containing proposed amendments is presented here.

Questions for the Committee

4. Members are asked to:

   i. Comment on the proposed updates presented in this document.

   ii. Highlight any updates or new areas that are not currently covered.

   iii. Comment on whether the Committee summary is still appropriate.

IEH Consulting under contract supporting the PHE COC Secretariat
March 2021

This is a background paper for discussion.
It does not reflect the view of the Committee and must not be quoted, cited or reproduced

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

COC Guidance Statement G04 – version 1.2 draft 0.a

The Use of Biomarkers in Carcinogenic Risk Assessment

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

COC Secretariat
c/o Public Health England
Centre for Radiation, Chemical and Environmental Hazards
Chilton, Didcot, Oxfordshire OX11 0RQ
© Crown copyright 2018

You may re-use this information (excluding logos) free of charge in any format or medium, under the terms of the Open Government Licence v3.0. To view this licence, visit OGL or email psi@nationalarchives.gsi.gov.uk. Where we have identified any third party copyright information you will need to obtain permission from the copyright holders concerned. Any enquiries regarding this publication should be sent to COC@phe.gov.uk.

This is a background paper for discussion.
It does not reflect the view of the Committee and must not be quoted, cited or reproduced

COC Guidance Statement G04 v1.2 draft 0.a

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

The Use of Biomarkers in Carcinogenic Risk Assessment[RB1][RB2]

Introduction

1. A biomarker is 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. 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. 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.

2. The Committee recommends a four-stage approach to the risk assessment [RB3]of chemical carcinogens 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:

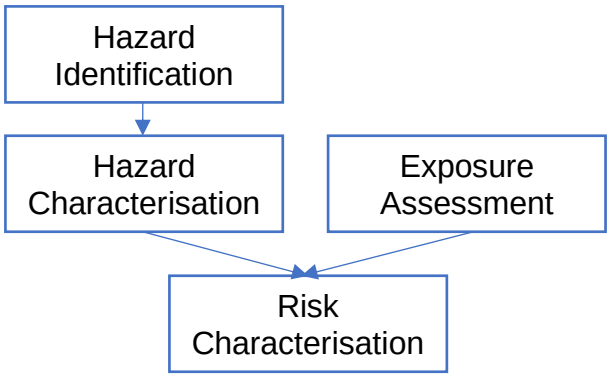

Figure 1: Four stage evaluation strategy for the risk assessment process of carcinogenic hazard

3. 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.

4. 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 are increasingly being introduced as interpretative aids to epidemiological investigations of chemical-induced carcinogenesis.

5. When utilising biomarker data, it is important to consider that there is usually a long latency period between 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. In the future permanent changes in gene expression and epigenetic changes may provide new biomarkers of exposure and effect that will have utility in longer term epidemiological studies.[RB4]

6. Biomarkers are powerful tools for investigating the mode of carcinogenic action (MOA) and can be incorporated into animal studies for this purpose[LL5]. 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.

7. 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.

Biomarker characterisation and validation

8. 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 (IPCS, 2001; Albertini et al., 2000; Angerer et al., 2007). These include:

- selection of a suitable biological matrix
- ability to reflect internal exposure and/or biological/biochemical effects with a clear relationship between dose, exposure and biomarker level

- suitable and reliable analytical method with adequate evaluation of the sensitivity and specificity (limit of detection, precision and accuracy)
- knowledge of the half-life and kinetics of the biomarker including an understanding of biomarker stability post-collection
- investigation of intra- and inter-individual variation in a non-exposed population (i.e. background), and the reference and limit values enabling interpretation of results.

8. Biomarkers used in animal studies must also be suitably characterised and validated and this should be based on the principles detailed for human biomarkers. The validation of biomarkers in epidemiological studies should also utilise ACCE criteria (analytical validity, clinical validity, clinical utility and ethical, legal and social implications) (Gallo et al., 2008). In relation to biomarkers, the STrengthening the Reporting of OBservational studies in Epidemiology – Molecular Epidemiology (STROBE-ME) initiative provides guidance on reporting of factors such as collection, handling and storage of biological samples and aspects such as method reliability, biomarker validation and study design (Gallo et al., 2011). The STROBE-ME initiative provides standardised guidelines and a 'checklist' for the reporting of [RB6]biomarker and molecular epidemiology studies (see http://www.equator-network.org/reporting-guidelines/strobe-me/, accessed May 2020).

Biomarkers of exposure

10. 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 (Angerer et al., 2007). However, epidemiological studies ~~often~~ can have major limitations related to measurement of exposure to carcinogens over long periods, for example inaccuracies as a result of recall bias in certain study designs. Consequently, in these studies, 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~~s~~ to measure exposure directly, assumptions need to be made about the relationship between results from short-term sampling and predicted long-term exposure. To help overcome these limitations, biomarkers of exposure were developed (Angerer et al., 2007). 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; Angerer et al., 2007, IPCS, 1999).

11. Biomarkers of exposure [RB7]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 as well as the internal dose[RB8]. It is important to take into consideration any factors that may impact on target organ concentrations, such as individual phenotype. Unless a relationship can be established between biomarker levels and external and internal dose, data from

exposure biomarkers cannot be used to back-calculate the initial dose[LL9]. This is because there may be interfering factors to be taken into account, for example, that the presence of a biomarker associated with one chemical may also be attributable to unrelated chemicals, or the background exposure to the chemical in question may be at a level where the metabolism is saturated and as a result the biomarker does not reflect exposure.

12. 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. In general, biomarkers of exposure are short-lived and provide only short- to medium-term indications of internal exposure.

13. Biomonitoring, the direct measurement of a chemical~~putative carcinogen~~ or its metabolites in biological samples, has been widely used within the risk assessment process, including for putative carcinogens. ~~Some examples of~~Validated b~~ b~~iomarkers 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) ~~are provided in~~ (Angerer et al., 2007). 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 utilise DNA and haemoglobin (Hb) adduct data (Hogervorst et al., 2010; Klaunig and Kamendulis, 2005).

DNA adducts

14. DNA adducts, where DNA is covalently bound to a chemical moiety, 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. Their measurement can be used in human biomonitoring studies investigating environmental exposures to chemicals. DNA adducts can be measured in peripheral blood lymphocytes (PBLs), exfoliated cells, such as from the urothelium or buccal mucosa, and in tissue biopsy samples.

15. DNA adducts are commonly used as biomarkers of exposure when investigating exposure to ~~polycyclic aromatic hydrocarbons (~~PAHs~~)~~ from sources such as tobacco smoking (Phillips, 2005; Veglia et al., 2003), environmental pollution (Farmer et al., 2003; Singh et al., 2007) or occupational exposure (Lee et al., 2003; Taioli et al., 2007). The epidemiological link between aflatoxin B1 exposure and hepatocellular carcinoma development is strongly supported by investigations using DNA adducts as biomarkers of exposure (Rundle, 2006; Wogan et al., 2011). In addition, aflatoxin biomarkers have sufficient predictive value for cancer outcome to be used as short-term indicators for intervention trials (Kensler et al., 2003). Exposure to acrylamide is strongly associated with the production of DNA adducts *in vitro* and in experimental animals but the correlation is less clear in humans (Xu et al., 2014, Li et al., 2016). The mode of action of aristolochic acid, a naturally

occurring component of plants of the *Aristolochia* ~~species~~ family associated with nephropathy and urothelial cancer, has been investigated using DNA adducts and specific DNA adducts have been identified as a biomarker in an exposed population (Jadot et al., 2017).

16. The biological significance of DNA adducts has been considered by ECETOC and 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. However, adducts vary greatly in their mutagenic potency and it is not possible to establish a generic level below which there is no adverse biological response. Understanding the role of processes such as DNA repair, cell turnover and death is critical to establishing the significance of adducts in the generation of mutagenic lesions and the subsequent development of a tumour. Accordingly, association of an adduct with a disease does not automatically indicate causality, although there is considerable evidence indicating that they can inform epidemiological investigations with regard to causation. It has also been proposed that DNA adducts can be useful biomarkers of cumulative exposure, representing cumulative unrepaired DNA damage (Vineis and Perera, 2000).

17. Frameworks and guidance have been developed by ILSI-HESI workgroups with a view to standardising methodological approaches and for data presentation and interpretation. An organisational approach for the assessment of DNA adduct data outlines how information which defines and ~~characterizes~~ characterises the DNA adduct (e.g. type of adduct, frequency, persistence, type of repair process) should be integrated with other relevant data, such as dosimetry, toxicity, toxicokinetics, genotoxicity, and tumour incidence, to inform on the chemical MOA. DNA adducts are considered 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 (Jarabek et al., 2009). DNA adduct data are most effectively utilised when viewed in the context of other information within the risk assessment framework.

18. Methods of identification and quantitation of DNA damage include ^{32}P-postlabelling, mass spectrometry, immunoassay and fluorescence detection (Himmelstein et al., 2009). Himmelstein et al (2009) provide comprehensive discussions of the collection, processing and storage of biological samples for subsequent analysis of biomarkers of DNA damage. Attention should be given to validation at all stages of development, and this should address analytical and biological aspects of the methods including the half-life of the adduct under investigation.

Protein (Haemoglobin or Albumin) adducts

19. Adducts of chemicals with proteins such as Hb and albumin can also be used as biomarkers of exposure to carcinogens. 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[RB10][BG11]

20. 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). It is important to recognise that, in some instances, these biomarkers of effect may only be indicative of immediate alterations and may not represent injury resulting in actual impairment of health or disease. Biomarkers of effect are frequently not specific to a given exposure or a specific agent. The relationship between exposure (acute, subacute, or chronic), the biomarker of effect, and carcinogenic event must be established in order to determine validity. For non-genotoxic carcinogens, biomarkers measuring key events in the respective mode of action can be of value.[LL12] Examples include changes in hormone levels, such as elevated thyroid stimulating hormone seen in rats given thiazopyr (Dellarco et al., 2006) and evidence of cell-specific toxicity, such as the peroxisome proliferation induced by a variety of chemicals such as phthalate esters which are carcinogenic in the rodent liver (Holsapple et al., 2006; Klaunig et al., 2003).

Genotoxicity Biomarkers

21. Cytogenetic endpoints such as micronuclei (MN) and chromosome aberrations (CA) are considered to be biomarkers of early carcinogenic (genotoxic) effect and are thought to be predictive for cancer risk in humans (Bonassi et al., 2011; Fenech et al., 1999). Sampling of blood and the preparation and analysis of peripheral blood lymphocytes (PBLs) for MN or CA are techniques often used in occupational and environmental biomonitoring studies (Bonassi and Au, 2002; Bonassi et al., 2005).

22. An example of the use of genotoxicity biomarkers in risk assessment is the detailed assessment that was undertaken by the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) of studies measuring MN and CA in workers exposed to pesticides (Bull et al., 2006). Factors such as age, gender, vitamin B12 and folate status were found to impact strongly on background levels of these biomarkers and, because of this inherent variability, it was difficult to evaluate the significance of the findings (Battershill et al., 2008; Fenech and Bonassi, 2011). It was concluded that these factors need to be accounted for when designing biomonitoring studies and similar conclusions are documented in a COM statement (COM, 2006).

23. The comet assay, an assay which detects single strand breaks and alkali-labile lesions in DNA using PBLs, can also be used in investigations evaluating populations potentially exposed to genotoxicants and has shown some promise as a biomonitoring tool (Collins et al, 2014). Although not all types of carcinogenic

exposures will cause lesions in PBLs detectable as comets, the assay is considered to be a valuable method for detection of genotoxic exposure in humans. However, its value for predicting cancer is not yet known because it has not been investigated in prospective cohort studies. An understanding of the factors influencing background levels is also critical in the design of such studies and a role of genotype is also implicated in this variability (Koppen et al., 2017).

24. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a marker of oxidative damage to DNA developed as a biomarker of biochemical effect. 8-OHdG levels can be assessed using PBLs and, as oxidised DNA repair products are excreted, they can also be assayed in biofluids such as urine (Loft et al., 2012a). 8-OHdG levels have been widely used in studies examining workers occupationally exposed to PAHs (Angerer et al., 2007; Marczynski et al., 2002). There is good evidence that increases in this biomarker correlate with exposure to potential mutagens and these increases are broadly in accordance with comet results (Loft et al., 2012b). Whilst there is good experimental evidence that 8-OH-dG has potential as a biomarker of effect, its reliability is still being evaluated and is the subject of extensive research.[RB13][LL14]

25. Sister chromatid exchanges (SCE) reflect an interchange between DNA molecules at homologous loci within a replicating chromosome. The *in vitro* sister chromatid exchange (SCE) test in mammalian cells has been used to investigate chemicals with the potential to damage DNA. However, not all test substances that induce chromosome aberrations induce SCEs. The significance to human health is unclear as SCEs are a reflection of DNA repair by homologous recombination. The test is no longer recommended for the routine evaluation of test materials and has been superseded by other methodologies, such as the comet assay for this purpose (OECD, 2017).[BG15]

26. An increased incidence of MN and DNA damage has been demonstrated in hospital personnel exposed to antineoplastic drugs (Mahmoodi et al., 2017) and a meta-analysis showed that frequencies of MN and CA in PBLs may be indicators of early genetic change in populations occupationally exposed to PAHs (Wang et al., 2012). However, evidence that genotoxicity biomarkers are indicative of cancer risk in humans is not extensive. Furthermore, the presence of genotoxicity biomarkers does not inform on the precise nature of the chemical exposure which has occurred to give rise to the measured endpoint.

Molecular Epidemiology in Cancer Risk Assessment

27. Molecular epidemiology is a term which encompasses the use of biomarkers to investigate the events and potential mechanisms which occur during the process of carcinogenicity, from initial exposure to disease (Vineis and Perera, 2007). The methods used can potentially represent biomarkers of exposure and biomarkers of effect. There have been significant developments in this field, 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).

28. Studies designed to investigate the relationship between chemical exposures and genetic changes, the 'meet in the middle approach', are considered a plausible and increasingly necessary progression to predict more accurately the impact of environmental exposures on cancer aetiology (Vineis and Chadeau-Hyam, 2011; Vineis and Perera, 2007). There is an expectation that an improvement of exposure assessment will greatly enhance understanding of early changes in the carcinogenic process. However, it is noteworthy that many of the techniques are still experimental and although they are useful for qualitative measurements and/or MOA investigations, it is not currently possible to provide specific guidance on their usefulness in a quantitative capacity.[RB16]

Biomarkers of Susceptibility[RB17]

29. The role of genetic polymorphisms and other factors that determine an individual's susceptibility to cancer is becoming an increasingly widespread topic in cancer risk assessment. Individual gene polymorphisms, which may be considered to be biomarkers of susceptibility, differ for different tumour types. For example, associations between polymorphisms in genes coding for xenobiotic biotransforming enzymes such as *N*-acetyltransferase 2 (*NAT2*) and glutathione *S*-transferase M1 (*GSTM1*) and individual susceptibility to a number of different bladder, lung and colorectal carcinogens have been made, although the evidence is not conclusive [RB18](Agundez, 2008; Dong et al., 2008; Garcia-Closas et al., 2005; Sanderson et al., 2007). Polymorphisms in DNA repair enzymes have also been implicated as biomarkers of cancer susceptibility (Karahalil et al., 2012).

30. Genome-wide association studies (GWAS) examine common genetic variants in different individuals, principally single-nucleotide polymorphisms (SNPs), and attempt to identify variations associated with traits or diseases, including cancer. GWAS are used by epidemiologists to understand gene-environment interactions responsible for carcinogenesis (Boffetta et al., 2012; Vineis et al., 2008). Several large projects and consortia are now in progress, studying genetic variation in the aetiology of different cancers, e.g. under the International Agency for Research on Cancer (see [RB19]http://epic.iarc.fr/research/activitiesbyresearchfields/geneticepidemiology.php, accessed 10/10/20) and the US National Cancer Institute (see https://epi.grants.cancer.gov/gameon/, acc[RB20][LL21]essed 10/10/20). The Committee has considered GWAS previously and interactions between genotype and chemicals in the environment. A paper on how these data should be used in the risk assessment process was also considered. It was concluded that, whilst such data are useful, it would be difficult to use the derived information for the risk assessment of specific chemical carcinogens at the current stage of technique development without a clearer understanding of the functional links and biological relevance of each genotype (COC, 2011).

Omics technologies

31. The development of omics technologies (genomics, proteomics, metabolomics) to ~~, the~~ investigat~~ion of~~e 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). With reference to mutagenesis, the COM has examined the literature for studies using toxicogenomic techniques which provide evidence of specific patterns of gene alterations induced following exposure to mutagenic chemicals. It was concluded that there was insufficient information to identify clearly genotoxic responses *in vivo* and that there was a need for more research on the application of integrated toxicogenomic approaches to evaluating changes in response to exposure to mutagens and determining carcinogenic modes of action (COT, COC and COM, 2004). The specific use of omics technologies for biomarkers of exposure or the potential for their use in examining the outcome of chemical exposures in human populations is not yet validated. Understanding and differentiating between exposures to genotoxic and non-genotoxic carcinogens will likely be facilitated by the use of omics approaches (Hochstenbach et al., 2012).[RB22]

32. Metabolomics is the study of the biochemical composition of the outcome of metabolic pathways (metabolites) including those which occur after exposure to chemicals. The metabolome can be measured noninvasively by sampling body fluids such as urine. Profiles can inform on chemical exposures and the effects of those exposures and show promise in biomarker development [RB23](Chadeau-Hyam et al., 2011). Metabolomes have the potential to be used as biomarkers of exposure and effect, and to provide information on both genotoxic and non-genotoxic carcinogenic modes of action. Examples of the use of metabolomics in the assessment of cancer risk are starting to emerge (Harris et al., 2015[RB24]).

33. The application of omics technologies to carcinogenicity evaluation was considered by the COC as part of its discussions on alternatives to the use of the 2-year rodent bioassay for carcinogenicity risk assessment.[LL25] The Committee concluded that the further development of biomarkers is necessary, and while much information has been generated in this area, a better understanding of the key markers is required before this can progress (see COC Guidance Statement G07 Part C~~1~~).[BG26]

34. The conceptual term ‘exposome’ ~~has been coined~~is used to describe the totality of environmental exposures to chemicals and there is increasing discussion on how this can be utilised to understand disease (Peters et al., 2012; Rappaport and Smith, 2010; Wild, 2005, 2012[RB27]). Some examples of approaches include omics technologies, the use of large scale prospective cohorts such as Biobank UK, and improved monitoring, for example in occupational settings or dietary intakes (Wild, 2012, Athersuch, 2016). EXPOsOMICS [RB28]is a collaborative EU project using omics techniques and environmental exposure (air, water) data to study the role of the environment in human disease (Vineis et al., 2017). Exposome-Explorer (http://exposome-explorer.iarc.fr) is a database dedicated to the collation of biomarkers of exposure to environmental risk factors (Neveu et al., 2017).

35. Epigenetics, heritable changes in gene expression which are independent of changes in DNA sequence, is another rapidly growing area of investigation which is

implicated in 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). In the near future, permanent changes in gene expression and epigenetic changes may provide new biomarkers of exposure and of effect that will have utility in longer term epidemiological studies. [RB29]The possibility of use of epigenetic change as a biomarker of exposure has been explored in an ECETOC workshop on markers for improved retrospective exposure assessment. (ECETOC, 2009) and 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).

36. miRNA species are another promising area for biomarker development. These short RNA species are non-protein-coding RNAs, which have a role in the regulation of translation of protein from mRNA. These species 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). This gives them much utility as biomarkers of effect. 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 [RB30](Vrijens et al., 2015). The use of non-coding RNAs as potential biomarkers in regulatory toxicology was discussed at an ECETOC workshop, ~~during~~ the summary of which ~~it was~~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).

37. A Biomonitoring Equivalent (BE) is an estimated concentration or range of concentrations of an environmental chemical in humans which 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.[RB31]

Summary[LL32]

38. A biomarker, in the context of chemical carcinogenesis, is defined as an observable change related to the carcinogenic process following a specific exposure or effect.

39. In cancer risk assessment, biomarkers can be utilised for hazard identification and characterisation and for exposure assessment.

40. The relationship between the biomarker and the carcinogenic response should be established.

41. Biomonitoring studies should fulfil pre-defined criteria and 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.

42. Biomarkers of exposure include DNA and protein adducts, MN and CA. Biomarkers of effect include genotoxicity biomarkers such as MN and CA, and the indicator of oxidative damage, 8-OHdG.

43. 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.

44. 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.a
Date TBC

References

Agundez, J.A. (2008). Polymorphisms of human N-acetyltransferases and cancer risk. Current drug metabolism *9*, 520-531.

Albertini, R.J., Anderson, D., Douglas, G.R., Hagmar, L., Hemminki, K., Merlo, F., Natarajan, A.T., Norppa, H., Shuker, D.E., Tice, R.*, et al.* (2000). IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. Mutat Res *463*, 111-172.

Angerer, J., Ewers, U., and Wilhelm, M. (2007). Human biomonitoring: state of the art. Int J Hyg Environ Health *210*, 201-228.

Athersuch, T. (2016). Metabolome analyses in exposome studies: Profiling methods for a vast chemical space. Arch Biochem Biophys *589*, 177-86.

Barrow, T.M., Michels, K.B. (2014) Epigenetic epidemiology of cancer. Biochem Biophys Res Commun *455*, 70-83.

Battershill, J.M., Burnett, K., and Bull, S. (2008). Factors affecting the incidence of genotoxicity biomarkers in peripheral blood lymphocytes: impact on design of biomonitoring studies. Mutagenesis *23*, 423-437.

Boffetta, P., Winn, D.M., Ioannidis, J.P., Thomas, D.C., Little, J., Smith, G.D., Cogliano, V.J., Hecht, S.S., Seminara, D., Vineis, P.*, et al.* (2012). Recommendations and proposed guidelines for assessing the cumulative evidence on joint effects of genes and environments on cancer occurrence in humans. International journal of epidemiology *41*, 686-704.

Bonassi, S., and Au, W.W. (2002). Biomarkers in molecular epidemiology studies for health risk prediction. Mutat Res *511*, 73-86.

Bonassi, S., El-Zein, R., Bolognesi, C., and Fenech, M. (2011). Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. Mutagenesis *26*, 93-100.

Bonassi, S., Ugolini, D., Kirsch-Volders, M., Stromberg, U., Vermeulen, R., and Tucker, J.D. (2005). Human population studies with cytogenetic biomarkers: review of the literature and future prospectives. Environ Mol Mutagen *45*, 258-270.

Boysen, G., Georgieva, N.I., Bordeerat, N.K., Sram, R.J., Vacek, P., Albertini, R.J., and Swenberg, J.A. (2012). Formation of 1,2:3,4-diepoxybutane-specific hemoglobin adducts in 1,3-butadiene exposed workers. Toxicol Sci *125*, 30-40.

Brase, J.C., Wuttig, D., Kuner, R., and Sultmann, H. (2010). Serum microRNAs as non-invasive biomarkers for cancer. Molecular cancer *9*, 306.

Bull, S., Fletcher, K., Boobis, A.R., and Battershill, J.M. (2006). Evidence for genotoxicity of pesticides in pesticide applicators: a review. Mutagenesis *21*, 93-103.

Calin, G.A., and Croce, C.M. (2006). MicroRNA signatures in human cancers. Nat Rev Cancer *6*, 857-866.

Ceccaroli, C., Pulliero, A., Geretto, M. and Izzotti, A. (2015) Molecular fingerprints of environmental carcinogens in human cancer. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev *33*, 188-228.

Chadeau-Hyam, M., Athersuch, T.J., Keun, H.C., De Iorio, M., Ebbels, T.M., Jenab, M., Sacerdote, C., Bruce, S.J., Holmes, E., and Vineis, P. (2011). Meeting-in-the-middle using metabolic profiling - a strategy for the identification of intermediate biomarkers in cohort studies. Biomarkers *16*, 83-88.

Chuang, J.C., and Jones, P.A. (2007). Epigenetics and microRNAs. Pediatric research *61*, 24R-29R.

COC (2011) Interaction between genotype and chemicals in the environment on the induction of cancer in risk assessment. In COT, COM and COC Joint Annual Report 2011, p53-54.

COC, COM, COT (2019) Statement from a joint Committee workshop on the use of epigenetics in chemical risk assessment. Available from: https://www.gov.uk/government/publications/epigenetics-in-chemical-risk-assessment-joint-committee-statement

Collins, A., Koppen, G., Valdiglesias, V., Dusinska, M., Kruszewski, M., Møller, P., Rojas, E., Dhawan, A., Benzie, I., Coskun, E., Moretti, M., Speit, G., Bonassi, S., ComNet project (2014). The comet assay as a tool for human biomonitoring studies: the ComNet project. Mutat Res Rev Mutat Res *759*, 27-39.

COM (2006). Statement on risk factors affecting the formation of chromosomal aberrations and micronuclei in peripheral blood lymphocytes. COM/06/S3-December 2006. Link: http://webarchive.nationalarchives.gov.uk/20140506144244/http://www.iacom.org.uk/statements/documents/pbl0603.pdf (accessed 27/04/17)

COT, COC and COM (2004) Joint statement on the use of toxicogenomics in toxicology. Available from: https://cot.food.gov.uk/cotstatements/cotstatementsyrs/cotstatements2004/toxicogenomics (accessed 13/07/2017)

COT (2012) COT statement on the use of toxicogenomics data in risk assessment. COT/2012/02. Available from: https://cot.food.gov.uk/cotstatements/cotstatementsyrs/cotstatements2012/646694 (accessed 13/07/2017)

Dellarco, V.L., McGregor, D., Berry, S.C., Cohen, S.M., and Boobis, A.R. (2006). Thiazopyr and thyroid disruption: case study within the context of the 2006 IPCS Human Relevance Framework for analysis of a cancer mode of action. Crit Rev Toxicol *36*, 793-801.

Dong, L.M., Potter, J.D., White, E., Ulrich, C.M., Cardon, L.R., and Peters, U. (2008). Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. JAMA : the journal of the American Medical Association *299*, 2423-2436.

ECETOC (2009). Use of Markers for Improved Retrospective Exposure Assessment in Epidemiology Studies. Workshop Report No. 14. Available from: http://www.ecetoc.org/publication/workshop-report-14-use-of-markers-for-improved-retrospective-exposure-assessment-in-epidemiology-studies/ (accessed 13/07/2017)

ECETOC (2016) Noncoding RNAs and Risk Assessment Science. Workshop Report No. 32. Available from: http://www.ecetoc.org/publication/workshop-report-no-32-noncoding-rnas-risk-assessment-science-3-4-march-2016-malaga/ (accessed 02/05/17)

Farmer, P.B., Singh, R., Kaur, B., Sram, R.J., Binkova, B., Kalina, I., Popov, T.A., Garte, S., Taioli, E., Gabelova, A.*, et al.* (2003). Molecular epidemiology studies of carcinogenic environmental pollutants. Effects of polycyclic aromatic hydrocarbons (PAHs) in environmental pollution on exogenous and oxidative DNA damage. Mutat Res *544*, 397-402.

Fenech, M., and Bonassi, S. (2011). The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. Mutagenesis *26*, 43-49.

Fenech, M., Holland, N., Chang, W.P., Zeiger, E., and Bonassi, S. (1999). The HUman MicroNucleus Project--An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. Mutat Res *428*, 271-283.

Gallo, V., Egger, M., McCormack, V., Farmer, P.B., Ioannidis, J.P., Kirsch-Volders, M., Matullo, G., Phillips, D.H., Schoket, B., Stromberg, U.*, et al.* (2011). STrengthening the Reporting of OBservational studies in Epidemiology - Molecular Epidemiology (STROBE-ME): An extension of the STROBE statement. Mutagenesis *27*, 17-29.

Gallo, V., Khan, A., Gonzales, C., Phillips, D.H., Schoket, B., Gyorffy, E., Anna, L., Kovacs, K., Moller, P., Loft, S.*, et al.* (2008). Validation of biomarkers for the study of environmental carcinogens: a review. Biomarkers *13*, 505-534.

Garcia-Closas, M., Malats, N., Silverman, D., Dosemeci, M., Kogevinas, M., Hein, D.W., Tardon, A., Serra, C., Carrato, A., Garcia-Closas, R.*, et al.* (2005). NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet *366*, 649-659.

Harris, R.M., Williams, T.D., Waring, R.H. and Hodges, N.J. (2015). Molecular basis of carcinogenicity of tungsten alloy particles. Toxicol Appl Pharmacol *283*, 223-233.

Hays, S.M., Aylward, L.L., LaKind, J.S., Bartels, M.J., Barton, H.A., Boogaard, P.J., Brunk, C., DiZio, S., Dourson, M., Goldstein, D.A.*, et al.* (2008). Guidelines for the derivation of Biomonitoring Equivalents: report from the Biomonitoring Equivalents Expert Workshop. Regul Toxicol Pharmacol *51*, S4-15.

Himmelstein, M.W., Boogaard, P.J., Cadet, J., Farmer, P.B., Kim, J.H., Martin, E.A., Persaud, R., and Shuker, D.E. (2009). Creating context for the use of DNA adduct data in cancer risk assessment: II. Overview of methods of identification and quantitation of DNA damage. Crit Rev Toxicol *39*, 679-694.

Hochstenbach, K., van Leeuwen, D.M., Gottschalk, R.W., Gmuender, H., Stolevik, S.B., Nygaard, U.C., Lovik, M., Granum, B., Namork, E., van Loveren, H.*, et al.* (2012). Transcriptomic fingerprints in human peripheral blood mononuclear cells indicative of genotoxic and non-genotoxic carcinogenic exposure. Mutat Res.

Hogervorst, J.G., Baars, B.J., Schouten, L.J., Konings, E.J., Goldbohm, R.A., and van den Brandt, P.A. (2010). The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. Crit Rev Toxicol *40*, 485-512.

Holsapple, M.P., Pitot, H.C., Cohen, S.M., Boobis, A.R., Klaunig, J.E., Pastoor, T., Dellarco, V.L., and Dragan, Y.P. (2006). Mode of action in relevance of rodent liver tumors to human cancer risk. Toxicol Sci *89*, 51-56.

IPCS (1999). Principles for the assessment of risks to human health from exposure to chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 210).

IPCS (2001). Biomarkers In Risk Assessment: Validity and Validation. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 222).

Jadot, I., Decleves, A.-E., Nortier, J. and Caron, N. (2017). An integrated view of aristolochic acid nephropathy: update of the literature. Int J Mol Sci *18*, 297

Jarabek, A.M., Pottenger, L.H., Andrews, L.S., Casciano, D., Embry, M.R., Kim, J.H., Preston, R.J., Reddy, M.V., Schoeny, R., Shuker, D.*, et al.* (2009). Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization. Crit Rev Toxicol *39*, 659-678.

Karahalil, B., Bohr, V., and Wilson, D., 3rd (2012). Impact of DNA polymorphisms in key DNA base excision repair proteins on cancer risk. Human & experimental toxicology *31*, 981-1005.

Kensler, T.W., Qian, G.S., Chen, J.G. and Groopman, J.D. (2003) Translational strategies for cancer prevention in liver. Nat Rev Cancer *3*, 321-329.

Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M.*, et al.* (2003). PPARalpha agonist-induced rodent tumors: modes of action and human relevance. Crit Rev Toxicol *33*, 655-780.

Klaunig, J.E., and Kamendulis, L.M. (2005). Mechanisms of acrylamide induced rodent carcinogenesis. Advances in experimental medicine and biology *561*, 49-62.

Koppen, G., Azqueta, A., Pourrut, B., Brunborg, G., Collins, A.R. and Langie, S.A. (2017). The next three decades of the comet assay: a report of the 11th International Comet Assay Workshop. Mutagenesis *32*, 397-408.

LaKind, J.S., Aylward, L.L., Brunk, C., DiZio, S., Dourson, M., Goldstein, D.A., Kilpatrick, M.E., Krewski, D., Bartels, M.J., Barton, H.A.*, et al.* (2008). Guidelines for the communication of Biomonitoring Equivalents: report from the Biomonitoring Equivalents Expert Workshop. Regul Toxicol Pharmacol *51*, S16-26.

Lee, K.H., Ichiba, M., Zhang, J., Tomokuni, K., Hong, Y.C., Ha, M., Kwon, H.J., Koh, S.B., Choi, H.R., Park, C.G.*, et al.* (2003). Multiple biomarkers study in painters in a shipyard in Korea. Mutat Res *540*, 89-98.

Li, D., Wang, P., Liu, Y., Hu, X., Chen, F. (2016) Metabolism of Acrylamide: Interindividual and Interspecies Differences as Well as the Application as Biomarkers. Curr Drug Metab *17,* 317-26.

Li, X.Q., Guo, Y.Y., and De, W. (2012). DNA methylation and microRNAs in cancer. World journal of gastroenterology : WJG *18*, 882-888.

Loft, S., Danielsen, P., Løhr, M., Jantzen, K., Hemmingsen, J.G., Roursgaard, M., Karotki, D.G., Møller, P. (2012a) Urinary excretion of 8-oxo-7,8-dihydroguanine as biomarker of oxidative damage to DNA. Arch Biochem Biophys *518*, 142-50.

Loft, S., Svoboda, P., Kawai, K., Kasai, H., Sorensen, M., Tjonneland, A., Vogel, U., Moller, P., Overvad, K., and Raaschou-Nielsen, O. (2012b). Association between 8-oxo-7,8-dihydroguanine excretion and risk of lung cancer in a prospective study. Free Radic Biol Med *52*, 167-172.

Lujambio, A., Calin, G.A., Villanueva, A., Ropero, S., Sanchez-Cespedes, M., Blanco, D., Montuenga, L.M., Rossi, S., Nicoloso, M.S., Faller, W.J.*, et al.* (2008). A microRNA DNA methylation signature for human cancer metastasis. Proc Natl Acad Sci U S A *105*, 13556-13561.

Mahmoodi, M., Soleyman-Jahi, S., Zendehdel, K., *et al.* (2017). Chromosomal aberrations, sister chromatid exchanges, and micronuclei in lymphocytes of oncology

department personnel handling anti-neoplastic drugs. Drug Chem. Toxicol. *40*, 235-240.

Marczynski, B., Rihs, H.P., Rossbach, B., Holzer, J., Angerer, J., Scherenberg, M., Hoffmann, G., Bruning, T., and Wilhelm, M. (2002). Analysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine and DNA strand breaks in white blood cells of occupationally exposed workers: comparison with ambient monitoring, urinary metabolites and enzyme polymorphisms. Carcinogenesis *23*, 273-281.

McCoy, L.F., Scholl, P.F., Sutcliffe, A.E., Kieszak, S.M., Powers, C.D., Rogers, H.S., Gong, Y.Y., Groopman, J.D., Wild, C.P., and Schleicher, R.L. (2008). Human aflatoxin albumin adducts quantitatively compared by ELISA, HPLC with fluorescence detection, and HPLC with isotope dilution mass spectrometry. Cancer Epidemiol Biomarkers Prev *17*, 1653-1657.

Mitchell, P.S., Parkin, R.K., Kroh, E.M., Fritz, B.R., Wyman, S.K., Pogosova-Agadjanyan, E.L., Peterson, A., Noteboom, J., O'Briant, K.C., Allen, A.*, et al.* (2008). Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A *105*, 10513-10518.

Mo, M.H., Chen, L., Fu, Y., Wang, W., and Fu, S.W. (2012). Cell-free Circulating miRNA Biomarkers in Cancer. Journal of Cancer *3*, 432-448.

Neveu, V., Moussy, A., Rouaix, H., Wedekind, R., Pon, A., Knox, C., Wishart, D.S., Scalbert, A. (2017) Exposome-Explorer: a manually-curated database on biomarkers of exposure to dietary and environmental factors. Nucleic Acids Res *45*, D979–D984.

OECD (2017). Overview on genetic toxicology TGs. OECD Series on Testing and Assessment *238*. Available from: https://www.oecd-ilibrary.org/environment/overview-on-genetic-toxicology-tgs_9789264274761-en

Ogawa, M., Oyama, T., Isse, T., Yamaguchi, T., Murakami, T., Endo, Y., and Kawamoto, T. (2006). Hemoglobin adducts as a marker of exposure to chemical substances, especially PRTR class I designated chemical substances. J Occup Health *48*, 314-328.

Peters, A., Hoek, G., and Katsouyanni, K. (2012). Understanding the link between environmental exposures and health: does the exposome promise too much? J Epidemiol Community Health *66*, 103-105.

Phillips, D.H. (2005). DNA adducts as markers of exposure and risk. Mutat Res *577*, 284-292.

Pottenger, L.H., Carmichael, N., Banton, M.I., Boogaard, P.J., Kim, J., Kirkland, D., Phillips, R.D., van Benthem, J., Williams, G.M., and Castrovinci, A. (2009). ECETOC workshop on the biological significance of DNA adducts: summary of follow-up from an expert panel meeting. Mutat Res *678*, 152-157.

Rappaport, S.M., and Smith, M.T. (2010). Epidemiology. Environment and disease risks. Science *330*, 460-461.

Rundle, A. (2006). Carcinogen-DNA adducts as a biomarker for cancer risk. Mutat Res *600*, 23-36.

Sabbioni, G., and Turesky, R.J. (2017). Biomonitoring Human Albumin Adducts: The Past, the Present, and the Future. Chem Res Toxicol 30, 332-366.

Sander, M., Cadet, J., Casciano, D.A., Galloway, S.M., Marnett, L.J., Novak, R.F., Pettit, S.D., Preston, R.J., Skare, J.A., Williams, G.M.*, et al.* (2005). Proceedings of a workshop on DNA adducts: biological significance and applications to risk assessment Washington, DC, April 13-14, 2004. Toxicol Appl Pharmacol *208*, 1-20.

Sanderson, S., Salanti, G., and Higgins, J. (2007). Joint effects of the N-acetyltransferase 1 and 2 (NAT1 and NAT2) genes and smoking on bladder carcinogenesis: a literature-based systematic HuGE review and evidence synthesis. American journal of epidemiology *166*, 741-751.

Singh, R., Sram, R.J., Binkova, B., Kalina, I., Popov, T.A., Georgieva, T., Garte, S., Taioli, E., and Farmer, P.B. (2007). The relationship between biomarkers of oxidative DNA damage, polycyclic aromatic hydrocarbon DNA adducts, antioxidant status and genetic susceptibility following exposure to environmental air pollution in humans. Mutat Res *620*, 83-92.

Taioli, E., Sram, R.J., Binkova, B., Kalina, I., Popov, T.A., Garte, S., and Farmer, P.B. (2007). Biomarkers of exposure to carcinogenic PAHs and their relationship with environmental factors. Mutat Res *620*, 16-21.

US NAS - National Academy of Science (1983). Risk assessment in the Federal Government: Managing the process. National Research Council, Committee on the Institutional Means for Assessment of Risks to Public Health. National Academy Press, Washington, DC.

Vacek, P.M., Albertini, R.J., Sram, R.J., Upton, P., and Swenberg, J.A. (2010). Hemoglobin adducts in 1,3-butadiene exposed Czech workers: female-male comparisons. Chem Biol Interact *188*, 668-676.

Veglia, F., Matullo, G., and Vineis, P. (2003). Bulky DNA adducts and risk of cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev *12*, 157-160.

Verma, M. (2015) The Role of Epigenomics in the Study of Cancer Biomarkers and in the Development of Diagnostic Tools. Adv Exp Med Biol *867*, 59-80.

Vikstrom, A.C., Warholm, M., Paulsson, B., Axmon, A., Wirfalt, E., and Tornqvist, M. (2012). Hemoglobin adducts as a measure of variations in exposure to acrylamide in food and comparison to questionnaire data. Food Chem Toxicol.

Vineis, P., Brennan, P., Canzian, F., Ioannidis, J.P., Matullo, G., Ritchie, M., Stromberg, U., Taioli, E., and Thompson, J. (2008). Expectations and challenges stemming from genome-wide association studies. Mutagenesis *23*, 439-444.

Vineis, P., and Chadeau-Hyam, M. (2011). Integrating biomarkers into molecular epidemiological studies. Curr Opin Oncol *23*, 100-105.

Vineis, P., Chadeau-Hyam, M., Gmuender, H., Gulliver, J., Herceg, Z., Kleinjans, J., Kogevinas, M., Kyrtopoulos, S., Nieuwenhuijsen, M., Phillips, D.H., Probst-Hensch, N., Scalbert, A., Vermeulen, R., Wild, C.P. EXPOsOMICS Consortium. (2017) The exposome in practice: Design of the EXPOsOMICS project. Int J Hyg Environ Health *220*, 142-151.

Vineis, P., and Perera, F. (2000). DNA adducts as markers of exposure to carcinogens and risk of cancer. Int J Cancer *88*, 325-328.

Vineis, P., and Perera, F. (2007). Molecular epidemiology and biomarkers in etiologic cancer research: the new in light of the old. Cancer Epidemiol Biomarkers Prev *16*, 1954-1965.

Vrijens, K., Bollati, V., Nawrot, T.S. (2015). MicroRNAs as potential signatures of environmental exposure or effect: a systematic review. Environ Health Perspect *123*, 399-411.

Wang, Y., Yang, H., Li, L., Wang, H., Xia, X., and Zhang, C. (2012). Biomarkers of chromosomal damage in peripheral blood lymphocytes induced by polycyclic aromatic hydrocarbons: a meta-analysis. International archives of occupational and environmental health *85*, 13-25.

Wild, C.P. (2005). Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev *14*, 1847-1850.

Wild, C.P. (2012). The exposome: from concept to utility. Int J Epidemiol. *41* 24-32

Wogan, G.N., Kensler, T.W., and Groopman, J.D. (2011). Present and future directions of translational research on aflatoxin and hepatocellular carcinoma. A review. Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 1-9.

Xu, Y., Cui, B., Ran, R., Liu, Y., Chen, H., Kai, G., Shi, J. (2014) Risk assessment, formation, and mitigation of dietary acrylamide: current status and future prospects. Food Chem Toxicol *69*, 1-12.