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

G07- Alternatives to the 2-year Bioassay, Part c) Emerging technologies: toxicogenomics and high-throughput screening

This paper presents an overview of the potential applications of toxicogenomics and high-throughput screening technologies to carcinogenicity evaluation. It is intended to form the basis of initial discussions for part c of the G07 guidance statement "Alternatives to the 2-year Bioassay". The preliminary conclusions in Section 5 are based on the views and opinions expressed by the Committee during discussions relating to G07 part d (discussion paper CC/2016/07) at the July 2016 meeting.

Members are invited to comment on the content of the paper, the preliminary conclusions, and to consider the questions in Section 6 at the end of the document.

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Alternatives to the 2-year Bioassay

COC/G07: Part c) ***Emerging technologies: toxicogenomics and high-throughput screening***

Abbreviations

BMD	Benchmark dose
EPA	Environmental Protection Agency
HTS	High-throughput screening
PoD	Point of departure
SAR	Structure activity relationships
TGx	Toxicogenomics
Tox21	Toxicology in the 21st Century
ToxCast	Toxicity Forecaster
TT21C	Toxicity Testing in the 21st Century

1. Introduction

C1. Characterisation of the carcinogenic potential of the vast number of untested chemicals present in the human environment using conventional *in vivo* bioassays is not feasible and alternative methods are required for this purpose. Such methods would also be useful for early-stage evaluation of chemicals during development, such as pharmaceutical drugs and biocides (Benigni, 2014). Moreover, there is a limit to the amount of information about mechanisms of carcinogenicity in humans that can be obtained from studies in experimental animals. Whilst the large majority of genotoxic carcinogens can be detected using short-term *in vitro* and *in vivo* tests for genotoxicity, such tests are not available for non-genotoxic carcinogens. The need to address such issues has promoted attempts to develop alternative, faster and higher-throughput approaches for the identification and characterisation of chemical carcinogens. To this end, tiered approaches that incorporate structural alerts, *in vitro* mutagenicity assays and cell transformation assays have shown some promise (Benigni, 2014). However, newer approaches are being developed such as omics technologies or high-throughput screening (HTS) assays to address one or more of the above issues. The goal is to develop predictive methods that are rapid, cheaper than current bioassays and/or high-throughput. They should be based on human-relevant mechanisms of carcinogenesis. This document provides an introductory overview of these developing techniques and their potential applications to human cancer risk assessment.

2. Toxicogenomics

2.1 Omics technologies

C2. The collective term ‘omics’ refers to the genomic (DNA sequence analysis) and post-genomic (e.g. transcriptomics, proteomics, metabolomics, epigenomics) technologies that are used for the characterisation and quantitation of pools of biological molecules (e.g. mRNAs, proteins, metabolites), and the exploration of their roles, relationships and actions within an organism (Ward & Daston, 2014). ‘Toxicogenomics’ (TGx) describes the application of omics technologies to the study of adverse effects of toxicants or environmental stressors (Waters, 2016). The idea is that chemicals producing similar types and levels of toxicity will share similar gene, protein or metabolite expression profiles, and such patterns of toxicant-induced molecular changes (‘fingerprints’ or ‘signatures’, sometimes referred to as biomarkers) identified using TGx technologies can be used to assess toxicity in a number of ways. For example, they can be used to investigate aspects such as hazard identification, mechanism of action, exposure (e.g. gene-expression analysis to indicate exposure type; proteomic analysis of biofluids to develop biomarkers of exposure), dose-response (including qualitative as well as quantitative changes with dose), extrapolation between species (conservation of biologic response pathways; orthologous genes), individual variability and epigenetic effects. TGx methods may identify changes at much earlier time points than adverse effects observed at the tissue, organ or whole-organism level, and the post-genomic technologies can be used to follow toxicant-induced changes dynamically (Waters, 2016). Omics methods

produce large amounts of biological information that can be integrated and analysed using bioinformatics tools. Systems biology describes the integration of such data using advanced computational methods to create *in silico* models of function at levels from the sub-cellular through to the whole organism, which may be used as a basis for modelling toxic responses (Ward & Daston, 2014).

2.2 Toxicogenomics in carcinogenicity evaluation

C3. The aim of predictive TGx in carcinogenicity evaluation is to use molecular expression profile data to create high-resolution profiles of biological responses, to enable the mapping of causal events, processes and pathways that occur as a function of dose and time, reflecting carcinogenic modes of action (Waters, 2016). Although currently not yet suitable as high-throughput screening tests, TGx methods have shown great utility in determining mechanisms of action of chemical carcinogens, and as a prioritising and/or predictive tool for carcinogen identification.

C4. TGx methods are being developed for evaluation of the effects of exposures to genotoxic and non-genotoxic carcinogens, *in vivo* and *in vitro*. *In vitro* to *in vivo* extrapolation is being addressed by parallel studies *in vitro* and in rodents, with the incorporation of findings from epidemiological studies where available.

TGx *in vivo*

C5. To date, TGx *in vivo* has been most used for identifying mechanisms of carcinogenicity in rodents (e.g. Guyton et al., 2009; Fielden et al., 2011; Uehara et al., 2011) and for the classification and prioritisation of compounds for further evaluation (e.g. Ellinger-Ziegelbauer et al. 2008; Melis et al. 2014; Thomas et al. 2009; Watanabe et al. 2012; Yamada et al. 2012) (details of these original citations are listed in Schaap et al., 2015; Luijten et al., 2016) .

C6. Several groups have reported studies to predict the outcomes of 2-year rodent bioassays by applying TGx methods to short-term (from single- to 90-day exposures) studies *in vivo*. The majority of these studies have focussed on mRNA profiling in rat liver, with bioinformatics procedures (e.g. statistical or machine-learning algorithms) applied to identify signatures that may predict carcinogenic compound class. Gene signatures have been identified to discriminate between direct- and indirect-acting genotoxic carcinogens, non-genotoxic carcinogens and non-carcinogens. Progress in this field was summarised in a review by Waters et al. (2010), updated by Auerbach (2016).

C7. Ellinger-Ziegelbauer and colleagues have described gene-expression changes that can discriminate between genotoxic and non-genotoxic hepatocarcinogens (Ellinger-Ziegelbauer et al., 2005, 2008, 2009). Genotoxic carcinogens acting by direct DNA modification induced changes indicative of DNA damage response at the gene expression level while non-genotoxic carcinogens induced a profile indicative of increased cell-cycle progression.

C8. The identification of non-genotoxic carcinogens to distinguish them from non-carcinogens using TGx biomarkers is more complex due to the large variety of

modes of action involved, many of which are tissue-specific, requiring large numbers of gene signatures to indicate the various specific molecular changes that can occur during the process of carcinogenesis (discussed by Luijten et al., 2016). Some modes of non-genotoxic carcinogenicity, for example oxidative stress, may show very early signature gene expression changes after a single exposure. Repeat-dose studies can then be useful to determine 'false positives' among these early changes. Auerbach et al. (2010) performed a cross-evaluation of 14-, 28- and 90-day studies to classify compounds for non-genotoxic hepatocarcinogenicity in rats and concluded that exposure duration is an important factor, with TGx signatures in 90-day studies more predictive for carcinogenicity than those from shorter-term studies. These authors hypothesised that longer term studies better allow the identification of gene expression markers downstream of the mode of action of the initial toxicity and proposed the concept of a 'shared cancer biology', whereby a common pre-cancerous biology may be identified by common gene expression markers that are to some degree independent of the specific exposure. 'Profiling to the phenotype' extends this concept, taking as the starting point a transcriptional profiling of tissue samples corresponding to cancer pathologies identified in 2-year bioassays and 'working backwards' using these profiles as markers for earlier prediction based on the shared pre-cancer biology concept. Such data could be cross-referenced to archived human tissue samples to improve human relevance (Waters, 2016).

Phenotypic anchoring

C9. A huge catalogue of (*in vivo* and *in vitro*) datasets is now available in TGx databases (e.g. DrugMatrix, TG-GATEs), based on a large set of compounds, consistent study designs and standardised experimental protocols (Chen et al., 2012). These databases contain dynamic gene expression data over multiple doses/concentrations and also companion data (e.g. compound pharmacology, toxicology, clinical chemistry and histopathology). This information can be used for 'phenotypic anchoring' – relating specific changes in gene-expression profiles to adverse effects observed in conventional toxicity tests, to allow the identification of gene-expression changes that are causally related to the development of the toxicity phenotype (Paules, 2003).

C10. Eichner et al. (2014), for example, used two bioinformatics evaluation protocols to test the predictivity of short-term mRNA expression profiles for chronic effects *in vivo* for non-genotoxic hepatocarcinogen datasets in the TG-GATEs database, and compared their results with the outcomes of several previously published evaluations. Non-genotoxic carcinogen 'training compounds' were selected based on classification by experimental evidence from previously published studies. The signals that were often identified as early indicators of non-genotoxic carcinogens included genes involved in pathways of DNA damage response via the p53 signalling pathway, energy metabolism and anabolic processes that are typically observed in tumour cells, and drug metabolism. The same group also reported that the predictive power of short-term TGx data to predict non-genotoxic hepatocarcinogenesis in the rat would be increased by the integration of expression data obtained across multiple omics platforms (mRNA, miRNA and protein

expression), plus the abstraction from individual signature genes to higher-order levels, such as pathway enrichments and molecular interactions (Römer et al., 2014).

C11. Commentaries note that it is now important to extend studies to target organs/cell populations other than liver and to perform studies simultaneously in several different organs. Waters (2016) suggested that for the prioritisation of chemicals for further carcinogenicity testing it would be useful to develop gene expression biomarkers for the top five tumour sites in rats and mice (liver, lung, mammary gland, kidney, haematopoietic system). Thomas and colleagues (cited by Waters, 2016) suggested that biomarkers should be developed for all of the 24 main target tissues and that this may eventually be useful in replacement of the rodent bioassay.

TGx *in vitro*

C12. Gene expression studies in cultured cells exposed to toxicants have also focussed mainly on liver, using either primary hepatocytes or cell lines (see the review by Doktorova et al., 2012 for references). These studies have shown utility in identifying genotoxic carcinogens, for which the importance of using p53-competent cell types is emphasised. Buick and Yauk (2016) reviewed the development of *in vitro* predictive TGx genotoxicity biomarkers, noting that the field is currently at the proof-of-principle stage, with large-scale collaborative efforts (international and interdisciplinary) required for validation.

C13. *In vitro* studies have as yet proven less useful for discriminating non-genotoxic carcinogens, in large part due to the wide diversity of modes of action involved. Indeed, the feasibility of using *in vitro* models based on only one cell type for predicting the development of cancer *in vivo*, which requires interaction between different cell types in tissues/an organism, has been questioned. However, these methods are considered to be useful in characterising toxicity pathways to elucidate modes of action (Doktorova et al., 2012; Luijten et al., 2016).

C14. Schaap and colleagues recently described a ‘comparison approach’ to the identification of non-genotoxic carcinogens using *in vitro* TGx studies. In this strategy, a limited set comprising the 30+30 most significantly up- and down- regulated genes is compared for overlap across different chemical exposures, in order to identify the best match for a chemical of interest. This method was used to categorise chemicals by their mode of action using primary mouse hepatocytes (e.g. peroxisome proliferators, aryl hydrocarbon receptor agonists, metalloids and skin tumour promotors) or mouse embryonic stem cells (immunosuppression) (Schaap et al., 2015). The authors noted the requirement for a combination of different *in vitro* systems. These studies are being extended to incorporate tests over chemical concentration ranges (Schaap et al., 2016). Further developments of *in vitro* methods for use in cancer hazard assessment will require the integration of toxicokinetic data, and the establishment of standardised approaches to test relevant human concentrations (Luijten et al., 2016).

C15. The EU-funded CarcinoGENOMICS project was set up to develop mechanism-based *in vitro* TGx tests for carcinogenicity screening relating to major

target organs (liver, kidney, lung), using a well-defined set of model compounds (genotoxic carcinogens, non-genotoxic carcinogens, non-carcinogens). Progress achieved during this collaboration was summarised in the AXLR8 Consortium (2012) report.

Human relevance and biological significance: the parallelogram approach and concordance model

C16. The parallelogram approach, initially proposed by Sobels (1977) and further developed by Sutter (1995) can be used in the assessment of risk to humans by extrapolating findings from two different *in vitro* model systems, one of which should be human (e.g. rodent *in vitro* and human *in vitro*) and from *in vivo* studies in the non-human species (e.g. rodent *in vivo*) (Figure 1).

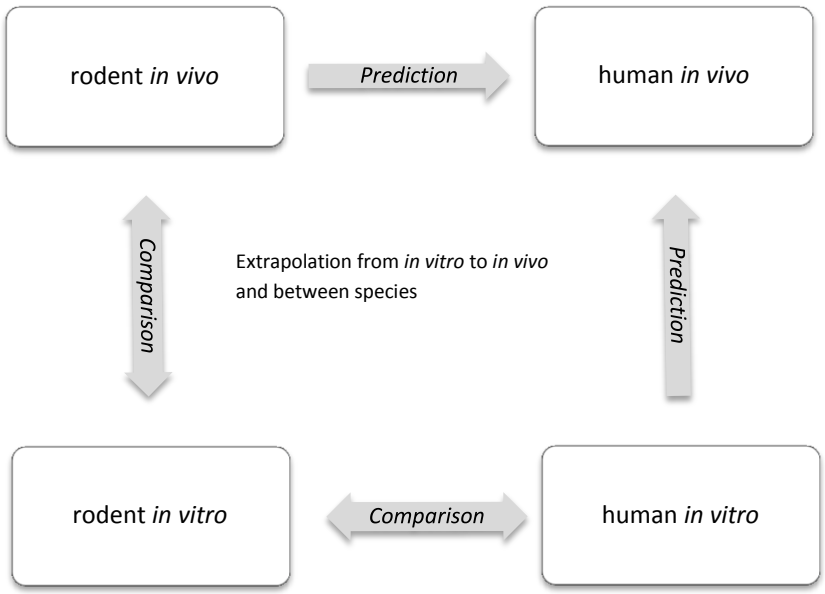

Figure 1: The parallelogram approach to toxicity prediction.

C17. It has been suggested that this approach could be useful to compare early key events and toxicity pathways indicated by TGx studies performed using sets of chemicals with well-established apical endpoints, to evaluate the likelihood of a similar mode of action in humans. This approach has been applied to TGx studies in hepatotoxicity, but may also be applicable to carcinogenicity evaluations.

C18. Kienhuis and colleagues studied model compounds (e.g. acetaminophen, cyclosporin A) to identify human-relevant modes of action for specific liver non-cancer endpoints (hepatotoxicity, cholestasis, steatosis, necrosis), integrating phenotypic and TGx data from rodent studies *in vivo* with data obtained using rodent and human hepatocytes *in vitro* (see the review by Kienhuis et al., 2016 for more details). Some limitations of the method were noted; the lack of correction for rodent-specific effects and *in vitro* artefacts, and the inability to detect *in vivo*-only and human-specific effects.

C19. To address this, Kienhuis and colleagues extended the parallelogram concept (see Figure 1) to develop the 'concordance model', in which TGx data from several animal species plus several *in vitro* (human) assays are included. This model facilitates the identification of *in vivo*-only (concordance between multiple species) and human-only (concordance between multiple *in vitro* findings) effects, which should lead to a greater level of confidence in the biological significance of the common toxicity pathways identified. Studies using model hepatotoxins are being extended using this approach to include data from a wider range of species, to assemble databases (compound, time, dose) of regulated gene clusters relating to specific aspects of hepatotoxicity. Some investigators are assessing the use of alternative test species (e.g. zebrafish embryo, Driessen et al., 2015).

Application of TGx to quantitative cancer risk assessment

C20. As described in the sections above, TGx has been used for hazard identification and to inform on mode of action, generally using data obtained from single- (high) dose chemical exposures. Progress is now being made to integrate TGx data into quantitative cancer risk assessments. These studies apply dose-response assessments to derive points of departure (PoDs) for TGx endpoints (usually benchmark doses (BMDs); often the lowest TGx-derived BMD), which are compared with PoDs from conventional/apical endpoints, allowing biological effects across a full dose-response range and time course to be investigated.

C21. Case studies for several model compounds have been described, and the standardisation of study protocols as well as methods to derive BMD values have been discussed (Thomas et al., 2011, 2012b, 2013; Chepelev et al., 2015) (reviewed by Thomas and Waters, 2016). A mode of action-based context is preferential in the application of transcriptomic dose-response in the derivation of the BMD. Gene expression changes can be correlated with key pathways related to adverse response as a function of dose, and this can be used in a weight of evidence evaluation. Dose-response studies performed over time can relate BMD value changes with adverse responses to identify transcriptional changes that are progressive or resolve. Transcriptomic dose-response analysis can also be applied to the assessment of cross-species extrapolation in mode of action and potency (National Research Council, 2007a; Thomas and Waters, 2016). Chepelev et al. (2015) noted a requirement for more information on quantitative *in vitro* to *in vivo* extrapolation.

C22. Use of the 'most-sensitive BMD' derived from TGx data may lead to an over-conservative risk assessment as the most sensitive changes in gene expression/pathway alterations may represent adaptive rather than toxicity endpoints. At present, this issue is addressed by phenotypic anchoring to traditional apical endpoints, with the intention that there will eventually be sufficient well-validated data that apical endpoints *in vivo* will no longer be required. The parallelogram approach and concordance model can be integrated to select pathways of human biological significance (Kienhuis et al., 2016).

C23. Thomas et al. (2013) outlined a framework for applying transcriptomic data to (non-cancer and cancer) risk assessment, as follows: 1) perform transcriptomic dose-response studies at a single time point between 5 days – 13 weeks, in rats and mice (M+F), incorporating eight tissues (liver, lung, mammary gland, stomach, vascular system, kidney, haematopoietic system, urinary bladder). 2) estimate PoD from lowest transcriptomic BMD across all tissues. 3) perform a genotoxicity evaluation. 4) carry out a weight of evidence analysis to determine genotoxic potential and to inform a decision on the appropriate (non-cancer- and cancer-related) extrapolation factor – working on the assumption that basing the PoD on the most sensitive pathway is generally protective until key adverse effect pathways are identified. This approach might be applicable to obtaining margins of exposure when cancer data are not available, but advice on relative risk is required. Thomas and Waters (2016) pointed out that although there may be issues of concern in using such an approach (for example whether the pathway-based transcriptomic BMD values are adequately protective for all chemicals; the use of transcriptional perturbation with no knowledge of risk), pragmatically, a PoD based on such information may be preferable to no PoD, which is currently the case for the vast majority of chemicals.

3. High-throughput screening

C24. Although individual TGx assays can provide information about multiple changes (e.g. expression levels of large numbers of genes) in response to a chemical exposure, they currently have limited applicability for use in high-throughput screening (HTS). Conversely, HTS methods, which evaluate only one or a small number of genes or processes per assay, are adapted to screen large numbers of chemicals over a wide range of assay conditions. A number of these methods were initially developed in the pharmaceutical industry for the rapid screening of libraries of candidate drugs or small molecules for specific types of biological activity or disease processes (Pereira and Williams, 2007) and are now being applied robotically to study chemical perturbations of biological pathways in relation to toxicity.

3.1 Assays

C25. HTS assays comprise two general categories. 1) Biochemical (cell-free) assays are usually homogenous reactions that measure effects on specific molecular targets (e.g. enzyme activity, receptor binding, ion-channel activity, nuclear receptor activity, protein-protein interactions) and can be easily miniaturised. 2) Cell-based assays can determine perturbations at different points in cellular pathways (e.g. functional assays, reporter gene assays, phenotypic assays for cell migration or cell division) and are often run in multiwell formats (from Waters, 2016).

3.2 HTS in toxicity/carcinogenicity evaluation

C26. HTS approaches are being used to try to predict carcinogenicity *in vivo.* They have the advantage of providing rapid, high-throughput, standardised testing of chemicals. A wide range of doses can be tested in each individual assay allowing the description of dose-response curves at low (human-relevant) doses, which can be

useful for comparison with low-dose TGx and *in vivo* study data. HTS is of particular value for hazard identification and prioritisation for further testing, and can be run in parallel with structure activity relationships (SARs) to predict potential targets prior to screening. A major challenge is how to incorporate the toxicokinetic and toxicodynamic parameters of *in vivo* studies, and some authors have questioned whether such methods can actually be useful in risk assessment to support regulatory decision-making.

C27. In 2007, the US National Research Council published the landmark report 'Toxicity Testing in the 21st Century: A Vision and a Strategy'. This report proposed a paradigm shift in toxicity testing from high-dose studies *in vivo* to an approach based on *in vitro* assays using human-relevant cells or tissues using a mode of action approach based on the evaluation of dynamic pathways underlying biological response (National Research Council, 2007b; Battacharya et al., 2011), a concept that has been generally labelled 'TT21C' (see https://chemicalwatch.com/11254/tt21c-what-does-it-take-to-become-a-new-paradigm, accessed 17/10/16). The aim stated is to test whether chemical compounds have the potential to disrupt processes in the human body that may lead to negative health effects. The two central aspects of the TT21C approach are: 1. The evaluation of 'toxicity pathways'[1] (innate cellular pathways that may be perturbed by chemicals, including stress-response, activation of specific endogenous receptors, regulatory network motifs underlying cellular homeostasis, decision making and phenotypic transitions) (for which omics techniques may be useful to characterise molecular signatures). 2. The determination of chemical concentration ranges in which these perturbations are likely to lead to adverse health effects. *In vitro* assays to evaluate these toxicity pathways would be combined with computational biology pathway models that allow a probabilistic risk assessment with flexible adaptation to exposure scenarios and individual risk factors. A third aspect in their application to risk assessment would then be pharmacokinetic modelling to extrapolate expected human exposures to equivalent tissue concentrations in exposed individuals.

C28. The TT21C approach is being evaluated in proof-of-concept studies using well-studied prototype compounds whose toxicity has already been examined with *in vivo* and *in vitro* assays. A worked-example case study using quercetin (a known genotoxin that is not carcinogenic in rodent bioassays) was performed as a preliminary effort to address the question of applying the TT21C pathway-based risk assessment approach to human carcinogenicity risk assessment of a commercial chemical without using rodent 2-year bioassay data. The toxicity pathway considered was 'DNA damage mediated by the p53 response network'. The worked example aimed to develop exposure estimates, define pathway readouts for p53-mediated DNA damage responses, develop HTS assays and look at computational model development of the p53 pathway and the use of biokinetic models to perform *in vitro* to *in vivo* extrapolation. From this analysis, the authors highlighted the importance of understanding *in vitro* kinetics to the interpretation of *in vitro* assays (Adeleye et al., 2015).

[1] This is somewhat of a misnomer, as the pathways reflect normal biological processes, which result in toxicity only when they are sufficiently perturbed.

C29. To date, the major initiatives applying the TT21C approach have been based in the US, in projects such as Tox21 and ToxCast (https://www.epa.gov/chemical-research/toxicology-testing-21st-century-tox21, accessed 17/10/16) (see below). There are also various European projects moving to a toxicity pathway approach linked in with a reduction, replacement and refinement in the use of animals in toxicity testing. The AXLR8 consortium includes details of other EU funded research investigating these (http://axlr8.eu/, accessed 10/10/16).

Tox21

C30. Tox21 (Toxicology in the 21st Century) is a collaboration in the US between partners at the National Institutes of Health (NIH), Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) that began in 2008 in response to TT21C. The stated goals of Tox21 are 'to:

- Identify environmental chemicals that lead to biological responses and determine their mechanisms of action on biological systems.
- Prioritize specific compounds for more extensive toxicological evaluation.
- Develop models that predict chemicals' negative health effects in humans.
- Annotate all human biochemical pathways and design assays (tests) that can measure these pathways' responses to chemicals.'

C31. Tox21 utilise quantitative HTS *in vitro* assays and computational toxicology approaches to cover a range of cell responses and signalling pathways to rank and prioritise chemicals. The HTS assays target multiple genes, proteins, pathways and cancer-related processes. To date, over 10,000 chemicals have been screened in approximately 50 assays.

ToxCast

C32. The EPA ToxCast (Toxicity Forecaster) project is related to, but separate from, Tox21. The results from ToxCast form a contribution to Tox21. ToxCast uses a similar approach to Tox21, but includes a much wider range of assays and endpoints. To date, more than 1800 chemicals, including industrial and consumer products, and food additives, have been screened in the ToxCast program for over 700 endpoints. All of the resulting information is publicly available on a database, together with tools for visualising and analysing the data (https://www.epa.gov/chemical-research/toxicity-forecasting, accessed 29/09/16).

C33. In ToxCast Phase I, a set of around 300 chemicals with pre-existing toxicity data were run through >600 HTS assays. ToxCast HTS data relating to perturbation of carcinogenesis-related pathways were then used to develop a model for classifying carcinogens (mostly non-genotoxic) based on 2-year data in the EPA Toxicity Reference Database (ToxRefDB), comprising largely pesticides. This dataset was applied to an external test set of 33 pesticides. The model showed some (limited) capability to discriminate between possible/probable and negative/unlikely

carcinogens, but several known carcinogens were identified as false negatives (Kleinstreuer et al., 2013). Further, independent analyses using this data set have found that assay design and coverage are not yet adequate and need development to improve the accuracy of prediction of rodent carcinogenicity and of the relevance of predictions to humans (Benigni, 2013; Cox et al., 2016). Problems faced in developing and improving the ToxCast assays are discussed in the review article by Benigni (2014), who concluded that the next phase should focus on including exogenous metabolic activation in the HTS assay systems and developing a set of well-characterised, standard carcinogens.

4. Summary

C34. Conventional rodent bioassays cannot provide the high throughput and low cost required for screening the huge numbers of untested chemicals that are present in the human environment, and high-throughput, short-term tests are required for predictive carcinogenicity screening. In addition, rodent bioassays are poorly predictive of human carcinogenicity and a more mechanistically based approach is required. HTS techniques, based on appropriate endpoints, offer an opportunity to overcome these limitations, by providing a high-throughput, low-cost solution. They are well suited for preliminary hazard identification based on established pathways of toxicity, but at present are not adequately developed to be useful for risk assessment and regulatory decision-making. Omics technologies can be used to study molecular pathways of carcinogenicity, from cellular initiating events/pathways through to the formation of a histologically identifiable tumour. They are being used in parallel with conventional assays to establish mechanisms of toxicity and early markers of cancer, dose-response relationships, cross-species extrapolations, exposure assessment, individual variation and epigenetic effects, and are being developed towards use in predictive toxicology. Although TGx methods are currently not suitable for use in the high-throughput screening of thousands of chemicals over wide ranges of assay conditions, efforts are underway to address this (Thomas et al., 2016).

C35. The eventual integration of HTS and TGx data for predictive carcinogenicity, combining the individual strengths and utilities of these technologies in identifying pathway perturbations, investigating modes of action, and defining dose-response, will hopefully significantly increase their capability to predict toxicological outcomes and relevance to humans. These new methods will require some form of validation, to establish their fitness for purpose.

5. Draft COC conclusions on emerging technologies: toxicogenomics and high-throughput screening

To note these preliminary conclusions were discussed under G07 part d in July 2016.

C36. Use of the 2-year rodent bioassay to evaluate the carcinogenicity of the vast numbers of untested chemicals that are currently marketed is not practical and

alternative methods are required for this purpose. Genotoxicity tests can detect many, but not all, genotoxic carcinogens, and cannot detect non-genotoxic carcinogens.

C37. Structure activity relationships (SARs) to detect genotoxic effects are a well-accepted method, however these require good working knowledge of the underpinning evidence and correct interpretation of the results, which is not always the case. In addition, their use for non-genotoxic carcinogens is limited.

C38. Omics technologies may be useful as a part of new strategies based on human-relevant modes of action. Toxicogenomic (TGx) approaches may be used to extrapolate between animal *in vivo* and *in vitro* experiments and human *in vitro* experiments to predict likely outcomes for humans *in vivo*. This requires the development of biomarkers, and while a lot of information has been generated in this area, a better understanding of the key markers is required before this can progress. TGx approaches are not currently suitable for high-throughput screening.

C39. High-throughput screening (HTS) technologies using biochemical or cell-based assays that allow rapid screening of large numbers of chemicals over a wide range of concentrations may be useful for hazard identification and prioritisation, but are currently not useful for risk assessment.

Questions for the Committee

i. Do Members wish to base G07, part c on the material provided here or undertake a more thorough review of the area?

ii. Aspects could also be incorporated into the horizon scanning. Aspects to consider could be:

<u>Toxicogenomics</u>

- To date, most *in vivo* toxicogenomic studies of carcinogens have focused on liver – to what extent can the conclusions of such studies be extrapolated to other tissues? Similarly, many of the *in vitro* studies on toxicogenomics have been in hepatocytes or liver-derived cells – to what extent would to lack of metabolic activity in other cell systems limit the interpretation of such studies?

- TGx data can be used semi-empirically (profiles of expression changes) or mechanistically (pathway analysis) – what are the relative merits of these two approaches?

- What role could *in vivo* toxicogenomics play in assessing the carcinogenicity of chemicals, e.g. discriminating mode of action (genotoxic vs. non-genotoxic carcinogens; hazard identification; determination of a PoD for calculating a margin of exposure;

predictively or retrospectively? What role could *in vitro* toxicogenomics data play in assessing the carcinogenicity of chemicals?

<u>High-throughput screening</u>

- Do current HTS approaches adequately cover all of the biological processes relevant to the detection of chemical carcinogen? How important is it to map assays to key events in defined adverse outcome pathways for carcinogenesis?

- What are the limitations of HTS assays (e.g. types of cell line, poor or no metabolic activity, range of tissues covered) with respect to their possible application in screening for chemical carcinogens?

- HTS assays may enable screening for toxicity pathways, but how predictive is this likely to be of carcinogenic potential as opposed to more general toxicity?

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