Committee on MUTAGENICITY

Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM)

Statement 2018/S1

Statement on the quantitative approaches to the assessment of genotoxicity data

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

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Introduction and background to the current review

1) Genetic toxicology has traditionally been based on the development and implementation of *in vitro* and *in vivo* assays designed to identify substances which cause damage to DNA and/or other cellular components which regulate the fidelity of the genome. The information derived from these testing strategies is used in a qualitative manner, to establish whether or not the chemical is a genotoxic or mutagenic hazard. Accordingly, risk management approaches are based on this dichotomous (yes/no) decision, which helps protect against public exposure to potentially genotoxic [and therefore potentially carcinogenic] agents (COM 2011; EFSA 2011a). These assays are also useful during product development to 'design out' genotoxic liability. However, this is a conservative approach that can result in potentially valuable chemicals being screened out and discarded unnecessarily, or the implementation of strategies to remove agents from the environment or food, despite the fact that exposure, and risk, may be very low (Kirkland et al 2007; Pottenger and Gollapudi 2010).

2) Conventional approaches to assessing the risk of chemicals which are toxic /non-genotoxic are generally based on establishing a non-toxic level in *in vivo* studies (the reference dose (RfD), derived from the point of departure (POD) and applying uncertainty factors to estimate an exposure which represents a Health-based Guidance Value (HBGV) such as a maximum acceptable daily intake (ADI)) (IPCS 2009). In general, for genotoxic carcinogens, the view is that there is no threshold. A margin of exposure (MOE) approach based on a POD derived from a carcinogenicity study can be utilised for carcinogens that are genotoxic and for which there is unavoidable exposure (EFSA 2005; Barlow et al 2006; Benford et al 2010; COC 2012). Currently, there is considerable interest in the development and evaluation of methodologies which would enable the analysis of genotoxicity dose-response data to be carried out in a quantitative manner.

3) Modification of the traditional [yes/no] approach to genotoxicity is a substantial departure from current practices. Development of a strategy based more on quantitative analyses would require extensive evaluations of the dose response methodologies employed and a more detailed understanding of the relationship of the genotoxicity endpoint to a human health effect, before it would be possible to

establish the appropriateness and/or usefulness of quantitative assessments of genotoxicity data. Reports from the International Workshops on Genotoxicity Testing (IWGT) working group in quantitative approaches to genetic toxicology risk assessment (the QWG) (MacGregor et al 2015a,b) and publications arising from a workshop organised by the Health and Environmental Sciences Institute (HESI) Genetic Toxicology Technical Committee (GGTC) (summarised in White and Johnson 2016) provide insight into how international groups are addressing this changing risk assessment environment.

4) It is suggested that refining approaches for the assessment of genotoxicity data could contribute to reductions and improvements in the use of animals in toxicity testing (reduction, refinement, replacement; 3R's) (Johnson et al 2014; Soeterman-Hernández et al 2016).

5) The Committee on Mutagenicity (COM) first considered quantitative approaches for assessing genotoxicity data, and how they may be used in chemical risk assessment, at its Horizon Scanning exercise in June 2013. Members were aware of the work being conducted by IWGT and HESI on quantifying genotoxic responses and assessing non-linear dose-response relationships, and agreed that the implications of this work should be considered. The possibility of developing quantitative (or semi-quantitative) methods for the analysis of dose-response data from *in vivo* genotoxicity studies for chemicals present in the environment, which have not been tested for carcinogenicity, similar to that utilised for an MOE approach using carcinogenicity data, was raised.

6) The COM were given a presentation by Dr George Johnson (Swansea University), a member of key working groups, and considered papers summarizing the key research in the field (MUT/2016/07; MUT/2017/02; MUT/2017/03).The following key themes and questions were considered pivotal to the evaluation of this topic:

- What dose response modelling methods are available, and which are most appropriate for evaluating genotoxicity data?
- Which POD metric is best for assessing genotoxicity data and how can appropriate benchmark responses (BMR) be established?
- How do factors such as endpoint, tissue, sampling time and study design impact on assessing data quantitatively?
- Can quantitative information from genotoxicity data be used in risk assessment, and if so, how?
- Is it possible to characterise carcinogenic risk from genotoxicity data alone?

This statement is a summary of the information considered by the COM and the resultant discussions and opinions.

Current hazard and risk assessment approaches

7) The genotoxicity testing strategy currently recommended by COM (COM 2011) for the detection of mutagenic hazard is based upon a core set of *in vitro* tests, chosen to provide information on three types of genomic damage; gene mutation, clastogenicity and aneuploidy. These are followed, if necessary, by appropriate *in vivo* tests designed to investigate whether *in vitro* genotoxic activity, including the specific mutagenic effect identified, also occurs *in vivo* (i.e. in the whole animal). The testing strategy may also include assays for specific target organs (e.g. site of contact tissues or site of rodent tumours detected in carcinogenicity bioassays) or germ cells. If a chemical is considered to be genotoxic it is generally assumed that there is no exposure level below which there is no effect. For chemicals for which potential exposure cannot be eliminated, the ALARA (as-low-as-reasonably-achievable) or ALARP (as-low-as-reasonably-practicable) approach is advised. This suggests that levels of the chemical must be controlled to ensure that intake is minimised to be as low as reasonably, or technically, possible (Barlow et al 2006) and is a widely adopted principle used by regulatory authorities in Europe and many other regions. It is a purely qualitative (hazard-based) risk management approach, and there is no consideration of the genotoxicity or carcinogenicity data in a quantitative manner.

8) A few exceptions to the 'no safe level' assumption have previously been established. These are based on the demonstration of a non-linear dose response and a mode of action that is biologically relevant and exhibits a threshold. COM generated a Guidance Statement on thresholds for *in vivo* mutagens in April 2010 (COM 2010). A number of different threshold terms were defined in this document (i.e. true threshold, threshold dose, practical threshold, biologically meaningful threshold, threshold mode of action).

9) An example of a threshold in a mutagenic response is that demonstrated by some low molecular weight alkylating agents, a consequence of the repair of DNA adducts. An extensive investigation and human risk assessment were undertaken following the discovery of ethyl methanesulfonate (EMS), a known genotoxic carcinogen, as an impurity in tablets of Viracept (nelfinavir mesilate), an HIV protease inhibitor (Walker et al 2009; Muller and Gocke 2009). It was estimated that consumption of contaminated drug batches at the maximal daily dose resulted in patients ingesting EMS at up to 0.045 mg/kg/day (daily Viracept dosage of 2.92 g/day). The responsible pharmaceutical company (Roche) went on to perform a comprehensive quantitative risk assessment of EMS, agreed with European regulatory agencies (Muller and Singer 2009), and determined a 'safe level'. The disparity between the frequency of DNA adducts and mutations suggested that a DNA repair factor was involved in the conversion of adducts to mutations, and that this mechanism exhibits a threshold (Jenkins et al 2005; Doak et al 2007). Therefore, it is possible that an organism could be subjected to a low level of DNA damage without deleterious effects because the damage is effectively and efficiently repaired,

and it is only when repair mechanisms are exhausted or overwhelmed that a mutation occurs. The risk assessment was based entirely on establishing a mode of genotoxic action which had a clear threshold from which a POD was established.

10) The COC has defined approaches for risk characterisation of carcinogens and these are described in Guidance Statements COC/G-05 and COC/G-06 (COC 2012; 2014). These are broadly in accordance with those proposed by European Food Safety Authority (EFSA) (EFSA 2005). These include the MOE approach and the threshold of toxicological concern (TTC). The TTC is a *de minimis* approach developed to facilitate the risk management of substances, primarily contaminants in food, for which good (or at least conservative) exposure estimates are possible but when chemical-specific toxicity data, including genotoxicity data, are insufficient for normal risk characterisation (Kroes et al 2004; Dewhurst and Renwick 2013). Exposure levels below which safety concerns are not anticipated are given for different classes of chemicals including genotoxic carcinogens[1].

11) When applied to chemicals shown to be genotoxic and carcinogenic, the MOE approach takes into account carcinogenic potency and estimated exposure (EFSA 2005; Barlow et al 2006). The MOE is calculated using a POD derived from suitable rodent bioassay data or human epidemiology information, which is divided by the measured or estimated exposure. The resulting value, which is a ratio, has been classified by the COC (based on MOEs calculated using animal carcinogenicity data) as follows:

- may be a concern (MOE<10,000);
- unlikely to be a concern (MOE 10,000-1,000,000) or
- highly unlikely to be a concern (MOE >1,000,000)

12) This method has gained acceptance by some regulatory bodies (including EFSA, European Medicines Agency (EMA) and World Health Organisation (WHO)) for managing genotoxic carcinogens that cannot be avoided (e.g. contaminants). EFSA recommend using a benchmark dose (BMD) as the POD for MOE calculations. The approach uses mathematical modelling to calculate the lower one-sided 95% confidence limit of a dose BMD i.e. the BMDL causing a defined response (Benchmark Response (BMR) or Critical Effect Size (CES)), typically a 10% increase in tumours in a cancer bioassay, i.e. the $BMDL_{10}$ (EFSA 2009; 2016). This is also replacing the 'traditional' no observed adverse effect level (NOAEL) approach for non-cancer endpoints. Furthermore, because the models use all the dose–response

[1] A TTC approach has been developed for DNA reactive (mutagenic) impurities in pharmaceuticals (ICH M7R1A).
(http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_R1_Addendum_Step_4_31Mar2017.pdf)

data, confidence intervals provide a quantitative estimate of the uncertainties and the quality of the data. To date this approach is only useful when good quality carcinogenicity studies are available.

General publications on quantitative risk assessment of genotoxicity data

13) The COM considered a number of publications which examined the application of a range of dose response modelling methods using data from a variety of genotoxicity studies and the quantitative analyses resulting from them (Gollapudi et al 2013; Johnson et al 2014; MacGregor et al 2015a,b). Three principle POD metrics were compared; these were:

i) The no observable genotoxic effect level (NOGEL). This is the highest experimental dose level where there is no statistically significant increase in the genotoxic effect measured in the study.

ii) The threshold effect, lower confidence limit (TdL). This was used in the EMS/Viracept analysis and is based on the assumption of a 'hockey stick' dose-response (Lutz and Lutz 2009; Gocke and Wall 2009). It involves fitting a mathematical model which assumes that the dose response is bi-linear with a region where there is no effect (it is similar to breakpoint dose (BPD) and Slope Transition dose (STD) models). It has been argued that the assumptions made with the use of this model need to be supported by mechanistic data.

iii) The BMD approach. This is determined by mathematical modelling of the dose –response curve and has been widely used in other branches of toxicology. The approach involves, firstly, fitting a mathematical model to experimental dose response data and, secondly, determining the BMD which is estimated to produce a defined increase in the response over the control/background level (BMR or CES). For example, using data from a carcinogenicity study a 10% increase in tumours over the control incidence is considered the BMR and the estimated dose is termed the BMD_{10}. The lower one-sided 95% confidence limit (or bound) on the dose, termed the $BMDL_{10}$ is then used as the POD in further considerations related to risk assessment such as the derivation of a MOE.

14) Recent interest in the development of quantitative analysis of genotoxicity data has focused on developing a similar BMD approach to that used elsewhere in toxicology. The QWG and HESI groups agreed that BMD modelling is the preferred approach for deriving a POD for genotoxicity data (Gollapudi et al 2013; Johnson et al 2014; MacGregor et al 2015a). It was also noted that the BMDL usually produces a lower and, hence, more conservative value for the POD than the other metrics (NOGEL, BPD, STD) considered. The BMDL takes account of the amount of variability in the data by considering the width of the confidence interval of the BMD; i.e. the ratio of the BMDU (the upper one-sided 95% confidence limit (or bound) of

the BMD) to the BMDL has been proposed as a useful metric for the assessment of the uncertainty in the BMD estimate (EFSA 2017). The COM agreed that the BMDL: BMDU ratio reflects the overall quality of the data and will be a useful metric for use in risk management scenarios (e.g. choice of uncertainty factor).

15) The COM acknowledges that developments in dose response modelling have been made which make it possible for genotoxicity data, of acceptable quality, to be analysed quantitatively rather than only qualitatively and that the authors of these publications have provided essential contributions to these developments. The COM broadly agreed with the conclusion that the BMD approach provides the best representation of the dose response. However, it was agreed that these publications present an overly optimistic view of the ease with which dose response modelling can be applied. It was considered that more comprehensive discussion is required, in particular the biological relevance of each endpoint and the choice of BMR and CES, before the utility of the quantitative approaches can be realised. A lack of consensus amongst users of the approach was also highlighted.

Benchmark dose approach

16) A number of areas were identified which were considered important for the COM to address in more detail when evaluating the potential of using genotoxicity data in a quantitative manner. In particular, there appear to be substantial differences in the use of the dose response modelling and in the derivation of BMD metrics. These differences include; choice of software package, the dose response models, the statistical evaluation of model fit, the use of constraints/options, the choice of BMR and methods for selecting or combining multiple BMDs. COM noted that these areas are highly technical and require further clarification. It is important that the rationales for the choices made are transparent and can be understood by the toxicologists and risk assessors who will be working with the results or the modelling processes.

Software, dose response modelling and BMD metrics

17) There are two principle software packages for the derivation of BMDs (Davis et al 2011; EFSA 2016). The BenchMark Dose Software (BMDS) package was developed by the US Environmental Protection Agency (EPA) in order to standardize approaches to evaluating dose response assessments. The software has over 30 different mathematical models or model variants which can be used for the analysis of quantal data, continuous data, nested developmental toxicology data, multiple tumour analysis, and concentration-time data. The software is freely available on the EPA website https://www.epa.gov/bmds. There are also extensive documentation guides and training webinars on its use. New versions of the software are released from time to time.

18) The PROAST software package has been developed by the Dutch National Institute for Public Health and Environment (RIVM), and is freely available from their

website http://www.rivm.nl/en/Documents_and_publications/Scientific/Models/PROAST. A comprehensive discussion of the software is available in the EFSA scientific opinion (2009 – appendix p47-72*).* Various guides to its use are also provided with the instructions for its installation.

19) COM noted that the PROAST software is frequently updated but that these changes were not documented so that users could understand the impact of the changes. COM also highlighted some uncertainty with regard to how the versions are numbered. For example, the current version available at the RIVM website is version 38.9. (July 2017) but the examples in EFSA (2016) use version 61.6.[2]

20) Both packages provide methods for fitting similar mathematical models to dose-response data. However, there are some differences in the methodologies used which are the subject of debate. Two major differences in the default approaches have been described:

i) In the transformation of response data. PROAST (RIVM) uses the default assumption of a log-normal distribution and transforms the data using logs whereas BMDS (EPA) recommends choosing the most appropriate transformation of the response data for the analyses (which may or may not be a log transformation) based on an assessment of how well the models describe the data, with the default being no transformation.

ii) Choice of BMR or CES: BMDS uses 1 standard deviation (1SD) above the background as the default BMR for continuous data, whereas PROAST uses a percentage increase e.g. 5%, 10% or some other percentage which may be appropriate for a particular endpoint, above the background for the CES. However, recent versions of BMDS can also be used in this way.

21) The COM also discussed the various dose response modelling methods used in BMD analysis. The IWGT consider, for risk assessment, that it should be possible to relate the POD to an acceptable exposure level by extrapolating from data which includes mode of action (MOA) and mechanistic information if available (i.e. so that a threshold mechanism, if demonstrated, can be taken into account). It was also noted that BMD_{10} for quantal and continuous data will be substantially different. For continuous genotoxicity data this represents a percent increase above a spontaneous incidence as opposed to an absolute increase of a quantal parameter; i.e. a 10% increase in micronuclei (MN) formation (from say 2 to 2.2 micronuclei (MN)/1000) compared to a 10% increase in tumour incidence relative to the

[2] In February 2018 PROAST released a new version 65.5 which allows for model averaging for quantal data and two web applications of PROAST which avoid R and the installation of software. These web applications do not, however, include all the options available in the R version of PROAST,

unaffected control population (from, say 5% to 15% in the number of animals with tumours in a carcinogenicity study) (MacGregor et al 2015a).

22) A direct comparison between PROAST and BMDS, based on $BMDL_{10}$ and BMD_{1SD} values (respectively), from different *in vivo* and *in vitro* genotoxicity studies on methylnitrosourea (MNU) was undertaken by Johnson et al (2014). From this limited analysis, the authors concluded that the two approaches produce comparable results and that both can be recommended for defining POD's for continuous data. In a study examining the correlation between genotoxicity and carcinogenicity, the BMD_{05}, calculated from bone marrow micronucleus (BMMN) data, was selected for comparison of PODs with the $BMDL_{10}$ values derived from carcinogenicity studies (Soeteman-Hernández et al 2016). No rationale was given for selecting a 5% increase as the BMR for calculating the BMMN POD, but the authors stated that the choice of BMR was not crucial for their analyses. COM commented that the choice of BMD_{05} as a BMR in this study was not transparent, which meant these results were difficult to interpret. EFSA (2009) concluded that a default BMR value of 10% be used for quantal and 5% for continuous toxicological data from animal studies in the absence of specific information on what constitutes a biologically relevant change. Both EFSA and EPA noted that, where specific information is available, the BMR should be based on statistical or toxicological considerations. However, no specific considerations of genetic toxicity data are given.

23) COM established that further explanations of the basic assumptions used and the uncertainties that are applied to each model were required before they would be able to come to any conclusions or make any recommendations on which software model should be used. COM highlighted the current lack of concordance with regard to choice of BMR for genotoxicity endpoints and what represents biologically relevant responses. Furthermore, COM agreed that it was not obvious at present that the BMD modelling could be transposed directly from its use with other toxicological endpoints to use in genetic toxicology.

Endpoints and tissues

24) How the most accurate and/or conservative risk estimations should be derived when using genotoxicity data has not yet been broadly addressed. For example, the relative increase in DNA damage measured by the comet assay is likely to differ appreciably from the relative increase in BMMN induced by the same chemical in the same animals, since each response will be influenced by the chemical's MOA, and the ability to detect a response of a defined magnitude will be determined by the dynamic range. Furthermore, it is not certain what the background levels of damage or the induced increases of each genotoxicity biomarker represent in a risk assessment scenario. The COM considered how results from the different types of genotoxicity studies (and therefore endpoints) or different tissues will impact on the derivation of POD values for use in potency estimations or risk assessment scenarios. The importance of sampling time in the development and detection of

damage measured in genotoxicity assays (i.e. that sampling tissues at a single point in time may not represent the peak response for different chemicals) was highlighted, and may be important when PODs are being used to compare potency. A number of publications were examined with the aim of addressing the importance of differences. Many of these studies investigating the differences in genotoxicity endpoints have focused on the alkylating agents ethylmethanesulphonate (EMS); methylmethanesulphonate (MMS); 1-methyl-1-nitrosourea (MNU); and 1-ethyl-1-nitrosourea (ENU), although some publications also examined polycyclic aromatic hydrocarbons (PAH) as model genotoxicants.

25) A comprehensive evaluation of the dose-responses generated i*n vivo* following MNU and ENU exposure for a variety of endpoints including BMMN, gene mutations in *lacZ* transgenic mice, or in *Pig-A* (in mice), was undertaken as part of a programme of work developing POD-based evaluations of genotoxicity data (Gollapudi et al 2013; Johnson et al 2014). The lowest BMDL value for each chemical was derived from the *in vivo* gene mutation studies. These values were conservative (lower) when compared with the values derived from the cancer bioassay. However, COM commented that the generalisation that a value derived from genotoxicity data will always be conservative compared to cancer bioassay, cannot be made based only on data from this class of chemical**.** It is noted that whilst potent *in vivo* genotoxins are likely to be potent carcinogens, dependent on MOA, some weak genotoxins may also be potent carcinogens.

26) Zeller et al (2016) used MMS to examine the relationship of an endpoint with the chemical MOA and to address the effect this has on the choice of CES/BMR. The results showed that MMS acts primarily as a clastogen and its potency as a gene mutagen is lower. Accordingly, it would not be appropriate to apply the same CES to both chromosomal damage and gene mutation endpoints for this chemical. The authors concluded that a 'one-size-fits-all' CES for genotoxicity data may be sub-optimal because of the variability in baseline values, scoring systems and the inherent differences in the characteristics of each end-point.

27) Detailed comparisons of endpoints and dose responses following administration of a number of PAH's including benzo[*a*]pyrene (BaP) and dibenz(a,h)anthracene (DBahA) to MutaMouse were undertaken with a view to improving the interpretation of genotoxicity dose response data (Wills et al 2016a). BMMN, *Pig-A* and *lacZ* gene mutations were examined from a variety of tissues. Covariate analyses (e.g. combining data from sexes or different tissues) were used and the BMR was chosen as a 100% increase relative to control (i.e. doubling). Confidence interval data indicated that tissue specific differences in BMD values spanned an order of magnitude. Such large increases could have a significant impact if they were used in a risk assessment or MOE evaluation to establish acceptable human exposure limits**.** It is noted that sampling time was not considered as a variable and it is known that mutation patterns vary with expression time and show tissue specific responses.

28) An examination of the response of *gpt*-delta transgenic mice, which have a lower spontaneous mutation frequency than in the earlier Muta^{TM}Mouse studies, to EMS indicated substantially lower POD's in the *gpt*-delta mice (Cao et al 2014) although the NOGELs were similar. The COM commented that it was not clear whether the results were a consequence of the lower baseline, a different strain of mouse or a particularly sensitive endpoint, but the lower POD's may also reflect more heterogeneous data (larger BMDL-BMDU range) than in MutaMouse. Accordingly, it demonstrated the importance of understanding a chemical MOA, the appropriateness of the endpoint, the sensitivity of the genetic target and the quality of the data in interpreting genotoxicity data quantitatively.

29) Establishing genotoxic MOA information was highlighted as vital in deciding on the most relevant endpoints to use for POD determination (Gollapudi et al 2013; MacGregor et al 2015b; Johnson et al 2014). Furthermore, it was suggested that the selection of appropriate tissues for a quantitative analysis should be based on the following: site-specific toxicity; mechanisms of toxicity; distribution and metabolism; any chemical accumulation; cell proliferation; the ability for DNA repair capacity to be induced by the chemical. Sensitivity of each endpoint and background mutation or micronucleus frequency, will also affect the outcome of the analysis. COM noted that, to date, there has been no discussion of the importance of sampling time when deriving BMD's despite the knowledge that time to maximum mutation frequency is tissue specific in transgenic mice (Wang et al 2005) and time to maximum MN frequency will depend on chemically-induced cell cycle delay.

30) The COM broadly agreed with the use of covariate analyses for combining data from different tissues where this was appropriate. The preliminary data available to them highlighted the importance of the selection of relevant endpoints and tissues if quantitative data were going to be used effectively. However, it was noted that it will be crucial for the developers of the software to provide clarity on how these factors are incorporated into the modelling and how the data are intended for use.

31) COM agreed that, whilst studies examining different endpoints and tissues contribute useful information to this area of research and the development of the quantitative analysis approaches, it was not possible to extrapolate findings from specific chemicals or chemical classes (e.g. alkylating agents) to generate broad assumptions. They considered that not enough is known about the quantitative relationships of different genotoxic or mutagenic effects, pre-neoplastic lesions and tumours to be able to interpret dose-response data accurately from a particular endpoint/tissue for each chemical. They suggested that more robust analyses of a larger number of more varied chemicals were required before any conclusions could be reached. An evaluation of the use of comet assay data in quantitative analyses has not been undertaken. COM recommends that a database which enables the comparison of BMDs across chemicals, endpoints and tissues would provide useful starting material for a more comprehensive evaluation of the utility of quantitative

assessment of genotoxicity data. The COM also pointed out that the applicability of the quantitative approaches to germ cell mutagenesis had not been addressed.

Use in carcinogenicity risk assessment

32) Proponents of these developments have argued that using quantitative methods for the analysis of genotoxicity data will provide the potential to move away from a 'hazard-only' approach towards a risk-based approach (Johnson et al 2013; MacGregor et al 2015a,b). To do this, a detailed evaluation of the biological relevance of the endpoints and BMR's is required. COM examined some publications from groups exploring the possibility of using POD's derived from *in vivo* genotoxicity studies in place of those generated from long term carcinogenicity studies, for example in MOE assessments (Sanner and Dybing 2005; Soeteman-Hernández et al 2015; Soeteman-Hernández et al 2016). It is understood that, with regard to potential exposure to chemicals that are (or could be) genotoxic carcinogens, there are a number of risk management needs. These range from determination of the potential level of concern for exposure to unavoidable contaminants or constituents of the diet, to market authorisation of new products (such as pesticides and human medicines). Hence, it is unlikely that a single approach would be suitable for all risk management situations. Indeed, as discussed above (para 11-12), the approach currently utilised by the COC varies depending on the risk management context.

33) The quantitative use of dose response data in MOE approaches for genotoxic chemicals in food was considered by Benford (2016). Attention was drawn to the importance of considering factors such as study design and quality, strain and species, and chemical MOA when using carcinogenicity data, and that these factors would also be critical if genotoxicity data are used. It is noted that a comparison of potency in carcinogenicity and genotoxicity assays is necessary using a broad range of carcinogen classes and MOAs. EFSA (2009) recommend the MOE approach for substances that are both genotoxic and carcinogenic, when risk assessment is necessary. They proposed the use of the $BMDL_{10}$ as the POD based upon tumour data from carcinogenicity studies. To date, EFSA has not expressed a view on the use of a POD derived from genotoxicity data in place of a carcinogenicity value.

34) The COM was provided with a number of publications detailing comparisons of mutagenic and carcinogenic potency using BMD dose response modeling. A preliminary evaluation was undertaken by Sanner and Dybing (2005) who concluded that there was a correlation between carcinogenic and mutagenic potencies. A framework, using the lowest effect dose (equivalent to the LOGEL) in a micronucleus study, was proposed as having the potential to be used in regulatory settings when a chemical was considered to be mutagenic, but for which carcinogenicity studies are either not available or of poor quality.

35) A comprehensive evaluation of potency estimates was undertaken by Hernández et al (2011) using 18 chemicals listed as either IARC class 1 or 2A carcinogens. BMD_{10} values for carcinogenicity and genotoxicity were derived using the PROAST dose-response modelling current at the time. Those from genotoxicity data were based on a range of endpoints (BMMN, comet, mutations in transgenic mice) from various tissues and from multiple studies. Some of the carcinogenicity studies, however, used only two treatment dose levels and different exposure routes were used in some cases. The authors concluded that there was some degree of association and a correlation between the BMDs for mutagenicity and carcinogenicity, despite the differences in study designs and routes of exposure.

36) An extension of this study, using similar methodologies, evaluated 48 chemicals, (Soeteman-Hernández et al 2016) and calculated BMD_{05} values from MN studies. The log10 of these BMD_{05} values were plotted against the log10 of the cancer BMD_{10}values. The BMD_{05} values were calculated for individual datasets (i.e. if there were several studies available, the data were not pooled) and a version of PROAST current at that time was used for dose-response modelling of both sets of data. The plot of the data showed a wide scatter but the authors concluded that there was a positive correlation between the BMMN and carcinogenic potencies (as measured by the BMDs), although prediction of carcinogenic potency from the genotoxicity data had an uncertainty of two orders of magnitude (i.e. factor of 100). As stated previously (para 22), it is understood that choice of BMD_{05} as the BMR was based on data comparisons rather than a value that represents a biologically meaningful effect and is not proposed for use in risk assessments.

37) The COM considered that the causal relationship implied by the association of the mutagenicity and carcinogenicity potency was problematic and were unconvinced by the 1:1 associations that seemed to be inferred in the publications. They felt that the complex relationships between adducts, mutations, pre-neoplastic lesions and tumours make it unlikely that evidence for a simple ratio/association is robust and that pursuit of a simple correlation is overly ambitious. It was considered possible that the dose inducing a biologically relevant genotoxic effect in an appropriate tissue would be lower and therefore be a more conservative POD for protecting health than a $BMDL_{10}$ for cancer. However, before this could be substantiated, COM felt that analysis of more datasets using a much broader range of chemicals and chemical classes is essential before any assumptions could be made. In particular, they pointed out that much less is known about the pattern of responses for weak genotoxins: for example, styrene, which causes tumours in nasal turbinates, induces relatively weak responses in genotoxicity assays.

Study designs, data quality and use of uncertainty factors

38) The COM considered it important to evaluate the impact of study design, and to consider the quality of the available data before conducting or interpreting quantitative analysis of genotoxicity data in order to generate PODs. It was noted

that for an optimal statistical design for BMD modelling it is preferable to distribute a fixed number of animals in a study into more dose groups with fewer animals per group. However, this may not accord with current OECD guidelines for *in vivo* genotoxicity tests. Nevertheless, it was agreed that there is some flexibility within these study designs and that the two designs (i.e. for OECD and for BMD estimation) were not necessarily incompatible Current OECD guideline designs of genotoxicity studies were suitable for quantitative analysis for chemicals for which there are sufficient data to determine a dose-response relationship. For chemicals where a dose response has not been established it will be difficult to determine a POD and carryout a BMD assessment from OECD guideline study designs which typically use no more than three dose levels.

39) Data quality is partly reflected in the width of confidence intervals, which is also dependent upon the number of dose groups and animals per group. The COM commented that guidance should be provided on what level of uncertainty in the data and what ratio of BMDU: BMDL would be considered unacceptable.

40) One important aspect of fitting mathematical models to dose-response data is testing whether the model is a good fit to the data. Models which are not a good fit should not be used. The COM noted that the choice of model based solely upon the results of 'goodness of fit' tests is a contentious area when a number of models provide a satisfactory fit. EFSA (2016) recommend that a model averaging approach is used, rather than a single default model. However, it is not clear if there is currently suitable software, readily available, to use the method with quantitative data**.** COM note that there are ongoing discussions with regards to optimising study designs, especially with regard to what is considered to be generation of the most suitable dose response. These factors require clarification before any definitive conclusions can be drawn.

41) Whilst the use of uncertainty factors was introduced by some authors, it was noted that more precise attempts at quantification has not been undertaken (Johnson et al 2014; MacGregor 2015b). COM commented that the uncertainty factors should be a reflection of the data quality, species differences, the endpoint measured, and presence or absence of a threshold mechanism, but until further examples are available, no conclusion can be reached.

Use of *in vitro* genotoxicity data for deriving POD's

42) There are a number of recent publications that have examined the use of BMD assessments of *in vitro* genotoxicity studies for potency comparisons, or for comparisons of *in vitro* with *in vivo* BMD's (Soeterman-Hernández et al 2015; Bemis et al 2016; Wills et al 2016b). COM commented that, whilst an interesting innovation, these approaches are at an early stage of development and currently cannot be considered for risk assessment scenarios or for potency ranking. COM decided not to consider this *in vitro* use of quantitative models further at this time.

Overall discussion and conclusions

43) COM considered the current literature on quantitative analyses of dose-response data from genotoxicity studies, including the reports from IWGT and ILSI/HESI, and discussed the recent developments of the approaches in this area.

44) It is noted that a move towards quantitative assessment of data is a significant departure from the current practices which are based principally on establishing only whether a chemical represents a mutagenic hazard. Discussions such as this should enable exploration of concepts which underpin the use of genotoxicity data in risk assessment; for example, would such an approach imply that all genotoxic chemicals have an exposure level below which the risk is considered tolerable and how might this level be identified?

45) Broadly, COM is in agreement with the principle of evaluating genetic toxicology data quantitatively. It is hoped that such approaches have the potential to improve the interpretation of genotoxicity data, potentially reducing the need for long-term carcinogenicity studies, and, hence, reduce the number of animals used in chemical risk assessment (with 3R's benefit). As these approaches are developed and their utility demonstrated, there may be scope for them to be incorporated into regulatory frameworks.

46) COM recognised the importance of the developments in the software and use of BMD methodologies to evaluate genotoxicity quantitatively. However, it was noted that, to date, much of the analyses have been performed by a small number of specialists and that the continual modifications to versions of the software made it difficult for those less well acquainted with the models and approaches to understand the significance of the changes. Many of the analyses are complex, and will require explanation and clarification before they can be considered by a broader audience. Some aspects of the dose-response modelling continue to evolve whilst other aspects vary between the developers of the methods. Therefore COM could not conclude on the appropriateness of the different models for use with genotoxicity data. It was concluded that changes to software should be documented and if software comparisons are undertaken, that it is made clear which aspects of the modelling are being compared.

47) With regards to the usefulness of POD's from genotoxicity data in risk assessment, COM recommend that a detailed evaluation of the different software methodologies is undertaken before any conclusions can be reached. Furthermore, the COM felt that a clarification of the outstanding issues in the use of the methodology was needed (e.g. choice of dose-response models, use of constraints) so that non-experts in the field were aware of the implications (if any) of the use of

the different software packages and options that have been proposed. COM suggested that precise descriptions of the methodologies and underlying assumptions (explicit and implicit) are developed so that a detailed and informed evaluation can be undertaken by potential users of the methods.

48) Guidance is needed on how to assess data quality and goodness of fit of the models to help decide on the suitability of a dataset for modelling. Clarification is needed on the level of uncertainty in the estimates in terms of the upper to lower confidence limit ratios which are considered acceptable and the factors which drive these uncertainties.

49) COM noted that there is a lack of consensus with regards to the selection of an appropriate CES/BMR for specific genotoxicity endpoints and that this was a complex area which requires more extensive discussion and evaluation. COM felt that it was unlikely that a similar size response (e.g. 10% increase over the negative control value) would be suitable for different genotoxicity endpoints such as, for instance, micronucleus induction and gene mutations. Selecting a BMR will require an understanding of the biological relevance of each endpoint and characterisation of the relative magnitude of response over background. Further investigations of what constitutes an appropriate BMR/CES for determining BMDs using a variety of genotoxicity study types is needed, with emphasis placed on the biological relevance of the choice of BMR/CES. Overall, it was difficult to conclude on selection of BMR/CES given the limited datasets available.

50) COM concluded that it was not possible to make any broad assumptions based on data from limited chemical classes. COM remain to be convinced of the close associations in comparisons of genotoxicity and carcinogenicity data reported by some investigators and highlighted the need for a more extensive evaluation of suitable datasets including a broader assessment of different chemicals classes, genotoxicity endpoints, tissues and timepoints. It was considered that BMD's from genotoxicity studies would, generally, be lower than those from carcinogenicity studies. However, at present, there are insufficient examples and a lack of understanding of the appropriate BMRs for the various endpoints for COM to draw any definitive conclusions. Consequently, the COM, at present, was unable to make any recommendations for the inclusion of quantitative genotoxicity data in MOE calculations.

COM

March 2018

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Glossary

Acceptable daily intake (ADI): The estimate of the amount of a chemical in food or drinking-water, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk to the consumer. It is derived on the basis of all the known facts at the time of the evaluation. The ADI is expressed in milligrams of the chemical per kilogram of body weight (a standard adult person weighs 60 kg).

Adduct: A chemical grouping which is covalently bound to a large molecule such as DNA or protein.

ALARA/ALARP: As Low As is Reasonably Achievable/ As Low As is Reasonably Practicable: A risk management approach under which exposure to a substances or mixture is reduced to the lowest level that it is deemed to be reasonably practicable to achieve in particular circumstances.

Alkylating agents: Chemicals which leave an alkyl group covalently bound to biologically important molecules such as proteins and nucleic acids (see adduct). Many alkylating agents are mutagenic, carcinogenic and immunosuppressive.

Aneugen: A chemical which induces aneuploidy (qv).

Aneuploidy: The occurrence of an abnormal number of chromosomes in a cell, such that the total number of chromosomes within the cell is not an exact multiple of the normal (haploid) number. Chromosomes may be lost (monosomy) or gained (trisomy) during cell division. An extra or missing chromosome is a common cause of genetic disorders (birth defects or spontaneous abortions). Some cancer cells also have abnormal numbers of chromosomes. (Chemical induction of aneuploidy is aneugenicity).

Benchmark dose (BMD): A dose of a substance associated with a specified low incidence of risk, generally in the range of 1–10%, of a health effect; the dose associated with a specified measure or change of a biological effect.

Benchmark dose (BMD) modelling: A mathematical modelling approach to dose-response assessment that aims to be more quantitative than the NOAEL process. An estimate of the dose that corresponds to a particular level of response (a benchmark response), often 10% is derived and a measure of uncertainty is also calculated. The lower one-sided 95% confidence limit (or bound) on the benchmark dose is called the BMDL and the upper one-sided 95% confidence limit (or bound) is the BMDU. The BMDL can be used as the point of departure for derivation of a health-based guidance value or a margin of exposure.

BMDL: BMDU The ratio of the lower and upper one-sided 95% confidence limits of the benchmark dose which is a measure of the precision of the BMD. It accounts for the uncertainty in the estimate of the dose-response due to characteristics of the experimental design, such as sample size.

Benchmark response (BMR): An adverse effect, used to define a benchmark dose from which a reference dose can be developed. The change in response rate over background of the BMR is usually in the range of 5-10%, which is the limit of responses typically observed in well-conducted animal experiments. This term is often used synonymously with Critical Effect Size (CES).

Clastogen: An agent that produces chromosome breaks and other structural aberrations in chromosomes such as translocations. Clastogens may be viruses or physical agents as well as chemicals. Clastogenic events play an important part in the development of some tumours (clastogenicity).

Critical effect size (CES): The magnitude of the adverse effect seen at a lowest dose when a vulnerable population is exposed to a chemical. This term is often used synonymously with Benchmark Response (BMR).

Continuous Data: Continuous data is quantitative data that can be measured and has an infinite number of possible values within a selected range.

Gene mutation: A mutation resulting from a change in a single base pair in the DNA molecule (also called point mutation).

Genotoxicity: Genotoxicity refers to interaction with, or damage to, DNA and/or other cellular components which regulate the fidelity of the genome. It is a broad term that, as well as mutation includes damage to DNA such as the production of DNA adducts, by the chemical itself or its metabolites. Cells have the capacity to protect themselves from such potentially lethal or mutagenic genotoxic effects by many repair processes and therefore many genotoxic events do not become evident as mutations. However, the capacity to damage the genome (genotoxicity) is an indicator of potential mutagenicity. Thus, some methods that measure genotoxicity may not provide direct evidence of heritable mutation.

Genotoxic carcinogen: Carcinogen whose primary mode of action involves deoxyribonucleic acid or chromosomal alterations.

Health-based guidance value (HBGV): A numerical value derived by dividing a point of departure (a no-observed- adverse-effect level, benchmark dose or benchmark dose lower confidence limit) by a composite uncertainty factor to determine a level that can be ingested over a defined time period (e.g. lifetime or 24 h) without appreciable health risk.

***LacZ* gene mutations:** See transgenic gene mutation models.

Margin of exposure (MOE) approach: A methodology that allows the comparison of the risks posed by different genotoxic and carcinogenic substances. The MOE approach uses a reference point, often taken from an animal study and corresponding to a dose that causes a low but measurable response in animals. This

reference point is then compared with various dietary intake estimates in humans, taking into account differences in consumption patterns.

Micronuclei (MN) (including bone marrow micronuclei (BMMN)): Whole or broken chromosomes that fail to segregate normally during cell division and may be lost from the main nuclei, but remain in the body of the cell forming micronuclei. Centromere positive micronuclei contain DNA and/or protein material derived from the centromere. The presence of centromere positive micronuclei following exposure to chemicals *in vitro* or *in vivo* can be used to evaluate the aneugenic (qv) potential of chemicals.

Mode of action (MOA): A biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. A mode of action describes key cytological and biochemical events—that is, those that are both measurable and necessary to the observed effect—in a logical framework.

No Observed Adverse Effect Level (NOAEL): Greatest concentration or amount of a substance, found by experiment or observation, that causes no adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

No Observed Genotoxic Effect Level (NOGEL): This is the highest experimental dose level where there is no statistically significant increase in the genotoxic effect measured in the study.

***Pig-A* gene mutation assay:** An assay which utilises the *Pig-A* gene which codes for one subunit of a glycosylphosphatidyl inositol anchor protein. Loss of function arising from *Pig-A* mutations can readily be assessed using straightforward immunochemistry and flow cytometric methods, thus making it a useful to measure gene mutations induced by chemicals or radiation. The development of in vivo and in vitro models are ongoing but are not yet recognised as fully evaluated and there are no OECD guidelines.

Point of departure (POD): A reference point on a toxicological dose-response curve established from experimental data which corresponds to an estimated low or no effect level. Used for hazard characterisation (see also RfD).

Quantal Data: A quantal dose response is one in which the effect is designated to be an all or nothing response (i.e. an animal has a tumour or it does not).

Reference dose (RfD): An estimate of the daily exposure dose that is likely to be without deleterious effect even if continued exposure occurs over a lifetime.

Software: PROAST and BMDS – two software packages for benchmark dose modelling. The PROAST software package developed by the Dutch National Institute

for Public Health and Environment (RIVM). BenchMark Dose Software (BMDS) developed by the US Environmental Protection Agency (EPA).

Threshold: Dose or exposure concentration below which a biological effect is not expected.

Transgenic animal models: Animals which have extra (exogenous) fragments of DNA incorporated into their genomes. This may include reporter genes to assess in-vivo effects such as mutagenicity in transgenic mice containing a recoverable bacterial gene *(lacZ* or *lacI*). Other transgenic animals may have alterations of specific genes believed to be involved in disease processes (e.g. cancer). For example strains of mice have been bred which carry an inactivated copy of the p53 tumour suppressor gene, or an activated form of the *ras* oncogene which may enhance their susceptibility of the mice to certain types of carcinogenic chemicals.