Committee on CARCINOGENICITY

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

COC Guidance Statement G05 – version 2-0

Defining a Point of Departure and Potency Estimates in Carcinogenic Dose Response

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COC Secretariat

c/o Public Health England

Centre for Radiation, Chemical and Environmental Hazards

Chilton, Didcot, Oxfordshire OX11 0RQ

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

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Contents

1.0 Introduction

1. This guidance statement (G05) forms part of a series by the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC), and should be read in conjunction with these. The Guidance Statement series aims to provide users with an accessible overview of the various stages of the risk assessment process for chemical carcinogenicity, including for regulatory submissions, as advised by the COC.

2. Points of departure (PODs) and potency estimates (PEs) describe different points on the dose response curve for a chemical. The POD aims to define the point where the dose response curve moves away from background and can be used as a basis for the setting of health-based exposure limits. PEs aim to define a point higher up the dose response curve and they can be used to compare the relative potency of chemicals to enable risk assessments for mixtures or multiple exposures.

3. The overall strategy of risk assessment of chemical carcinogenicity is detailed in guidance statement G01 and is illustrated in the overview framework shown below (Figure 1). A key step in the framework is the review of available toxicological data to determine whether there is evidence of carcinogenicity, mutagenicity or other toxicity relevant to those endpoints. The framework then utilises two different approaches for risk assessment based on whether there is evidence to support a genotoxic or non-genotoxic mode of action. For genotoxic carcinogens, it is recommended that exposures should be as low as reasonably practical (ALARP). However, for non-genotoxic carcinogens it is recommended that a health-based guidance value (HBGV) be calculated and used for comparison to exposure levels, which should be below the HGBV.

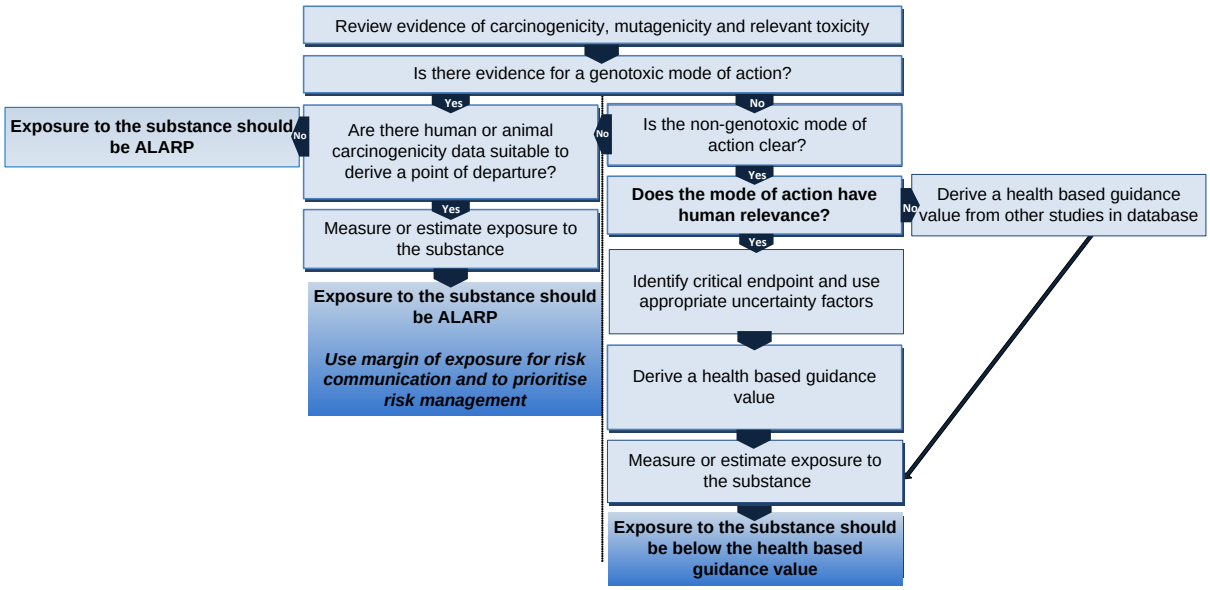

Figure 1: An overview framework for risk assessment of substances possessing evidence of carcinogenic or mutagenic activity
This framework is under continual review by the COC to reflect and incorporate updated understanding of the carcinogenic process as data becomes available.

4. The approach underpinning the framework comprises hazard identification, hazard characterisation, exposure assessment and risk characterisation. The term ‘hazard’ describes the intrinsic capacity of a chemical to cause an adverse effect on human health, such as cancer. ‘Risk’ is the probability that the adverse health effect will occur. When a carcinogenic hazard is identified, the level of risk will depend on circumstances such as the nature and degree of exposure to the chemical in question.

5. Hazard identification involves a qualitative description of the nature of the hazard, and hazard characterisation provides a quantitative description of the change in effect caused by differing doses of a chemical substance after a certain exposure time, i.e. the dose-response relationship. The purpose of analysing the dose-response relationship is to estimate the response and, ultimately, the risk from the levels of exposure to the chemical in the environment, food etc.

6. The relationship between dose and response may be used to aid hazard characterisation by allowing a comparison of carcinogenic potency. These estimates give an indication of the dose of a substance (administered over a standard animal lifespan) that results in a fixed incidence (e.g. 5, 25 or 50%) of tumours, after correction for the spontaneous background incidence of tumours among controls (Barlow et al., 2006). The possible impact of human-specific factors on the dose-response relationship established in experimental species, should always be considered; these include species differences in absorption, distribution, metabolism and excretion (ADME), mode of action and variability in susceptibility between species (inter-species) and within humans (intra-individual).

7. There are a number of methods for the characterisation of hazard based on whether a carcinogen acts via a genotoxic or non-genotoxic mechanism. However, both types of carcinogen can be classified with regard to tumourigenicity on the basis of potency. Although potency is ideally represented by the overall position and shape of the dose-effect or dose-response curve, the value (dose) at a particular point on the curve is most often used as a surrogate. This point, also known as the POD or reference point is the starting point for risk characterisation, whether using a margin of exposure approach or deriving a health-based guidance value (see G06 for more information).

8. This Guidance Statement (G05) provides an overview of the various methods used for deriving PODs and for potency estimates associated with exposures to chemical carcinogens, including the Committee’s views of their utility.

9. The tools outlined are those that are available to use *when considered appropriate by the risk assessor* and include: derivation of a POD using the No Observed Adverse Effect Level/Lowest Observed Adverse Effect Level approach (NOAEL/LOAEL; section 2.1); derivation of a POD using the Benchmark Dose approach (BMD; section 2.2); derivation of a POD using the T25 (section 2.3). In addition, relative carcinogenicity potency estimations using the T25 and TD50 are described (section 3).

10. This guidance document also details the Threshold of Toxicological Concern (TTC) approach which the Committee views as a 'pragmatic screening and prioritisation tool' that can help the assessment of chemicals for which there is a known structure but a lack of chemical-specific toxicity data, and for which exposure can be estimated.

11. It should be noted that there is no difference in the methodology used for determining PODs for genotoxic and non-genotoxic carcinogens. It is how the dose-response relationship and the POD are used in the final assessment of risk that varies, depending on whether the carcinogenic response occurs through a thresholded or non-thresholded mode of action (see G06 for further detail).

2.0 Overview of the approach recommended by COC

12. The Committee recommends the use of the BMDL as the POD for all carcinogens (see also G03). For genotoxic carcinogens, the likeliest use of the BMDL would be to calculate a MOE. For non-genotoxic carcinogens, the BMDL can be used to establish guideline values such as TDI/ADI using appropriate uncertainty factors, if carcinogenicity is the critical endpoint (see G06).

13. If a BMDL cannot be set for a chemical, the Committee agrees that, although it might be possible to derive a T25 from the dataset, this is not recommended. Instead a NOAEL can be adopted for non-genotoxic compounds, and even for genotoxic compounds, noting that this should be used in a way that does not imply the existence of a threshold for effect.

14. Potency estimates can be of pragmatic use in the risk assessment of carcinogenicity as an aid to prioritising carcinogenic substances (e.g. for risk re-evaluation) but the Committee considers that such potency estimates do not provide a quantitative estimate of risk. Although potency/toxicity estimates can be used to rank chemicals within a particular group (such as structurally related groups of putative genotoxic chemicals), extrapolating from high to low dose and from animals to humans introduces sources of uncertainty.

15. The TTC approach is acknowledged as providing a pragmatic means of assessing whether exposure to a chemical is of low concern or whether further testing is required. However, the Committee reiterates that the TTC is not a replacement for data on any chemical under consideration but could be used where data are lacking or insufficient, to help in reaching a decision on prioritisation.

3.0 Points of Departure and Potency Estimates

3.1 The NOAEL (No Observe Adverse Effect Level) approach

16. For the majority of toxicological effects, with the exception of most genotoxic effects or where extensive testing has failed to identify a threshold (e.g. in the case of neurotoxicity for lead), it is generally assumed that there is an exposure threshold below which no adverse effects occur. The highest administered dose at which no

statistically significant or biologically relevant adverse difference from the concurrent control group or appropriate historical control is observed is designated the No Observed Adverse Effect Level (NOAEL) and is often used as a POD in risk assessments. Use of a NOAEL, instead of a No Observed Effect Level (NOEL) in risk estimates ensures that the assessment is based on adverse effects rather than on minor or adaptive effects.

17. If a statistically significant adverse effect, compared to the control group, is observed at all tested dose levels, however, the lowest dose used in the study, i.e. the LOAEL (Lowest Observed Adverse Effect Level), may be used as the POD.

18. Typically, the NOAEL (or if one is not available, the LOAEL) is determined for the most sensitive, human relevant effect identified in epidemiological studies or from sub-chronic or chronic studies in experimental species.

19. Although the NOAEL has been widely used as a POD for many years by risk assessors, a number of limitations have been identified (WHO, 2019). One major limitation is the constraint that the NOAEL has to be one of the applied experimental doses. As a result, dose spacing, the shape of the dose-response curve, the number of animals per group, the statistical test used or the statistical variation in the response and its measurement, are not considered. A consequence of this is that studies with low power (e.g. small group sizes) and/or insensitive methods may only detect relatively large effects resulting in higher NOAELs than a better designed study with appropriate power and/or sensitivity to detect effects (WHO, 2009). This may then impact on the risk characterisation process.

3.2 Benchmark Dose (BMD) approach

20. The BMD_x is defined as the dose that corresponds to a specific change (x%) in response[1] compared to the (modelled) response in control animals, the benchmark response (BMR) (Crump et al., 1995). The BMD is determined by fitting a range of "best fit" mathematical curves to the dose-response data over the range of observable responses from animal studies or human studies (if available), using a selection of different mathematical models. From each 'statistically acceptable'[2] modelled dose–response curve, values for the BMD and the lower and upper bound 95% confidence limits (BMDL and BMDU) are obtained. To provide an estimate of the experimental uncertainty, the lower 95% confidence bound on the benchmark dose ($BMDL_x$)[3] is used as the POD.

21. EFSA notes (EFSA, 2017) that 'the BMR is not defined as a change with regard to the observed mean background response, but with regard to the

[1] In the case of quantal data e.g. tumours the percentage refers to the increase over the control level i.e. a 10% increase over a 5% background is a incidence of 15%; in the case of continuous data (e.g. body weight the percentage refers to the increase over the negative control mean i.e. a 10% increase over a mean body weight of 200g relates to a mean of 220g (but see also para 21).

[2] The precise definition of 'statistically acceptable' has not currently been agreed by the software developers.

[3] Where x refers to the specific change in the response (see above).

background response predicted by the fitted [mathematical] model'. This means that a response of, for example 10%, is identified from the predicted data and not the measured data and generally, the fitted curve does not match the observed background response exactly. There are a number of steps involved in applying the BMD approach which include:

a. Specification of an endpoint(s) and selection of the data type, e.g. individual data points (preferred) or summary data (mean; SD; sample size).

b. Specification of a BMR - predetermined level of change in response relative to controls; typically set at the lower end of the range of responses that can be detected experimentally, or the observations in epidemiological studies.

c. Fitting of a set of dose-response models and calculation of the BMD and its lower confidence bound (BMDL – 95% lower confidence bound) for each to give a set of BMDLs. The selection of the models depends on the endpoint (quantal or continuous) and the experimental design used to generate the data (e.g. number of dose groups). The upper bound of the BMD confidence interval (BMDU) is also calculated as the BMDU/BMDL ratio can be used to reflect the uncertainty in the BMD estimate.

d. Derivation of a single BMDL from the set available, preferably derived by model averaging (see para 24). Where a range of endpoints have been identified, an overall study BMDL is selected based on the choice of what is considered the most critical endpoint.

22. Model selection and model constraints are important considerations in BMD estimation and should be clearly recorded and justified. For model selection, an important criterion is that the selected model should adequately describe the data, especially in the region of the BMR.

23. Once the selected models have been fitted to the data, a series of scientific judgements must be made to ensure that the fitted models describe the data adequately. Different statistical tests can be used to assess the adequacy of model fit. The EFSA guidance recommends using the Akaike information criterion (AIC) to assess the goodness of fit (EFSA, 2017). This is a test which assesses the degree of fit by accounting for the number of free parameters in the model; the intention is to balance between under-fitted and over-fitted models. The EFSA modelling approach is outlined in their guidance (EFSA, 2017).

24. Often a number of models will fit the data adequately, based on statistical considerations. In such cases EFSA recommends using Model Averaging (Wheeler and Bailer, 2007) as the preferred approach of dealing with model uncertainty. This weights the results (on the basis of the AICs) from each of the plausible fitted models to derive a definitive BMD confidence interval. However, in situations where Model Averaging tools are not available, selection/rejection of models based on AIC value

or lowest BMDL can be considered as a sub-optimal alternative (EFSA, 2017). The most recent version of the US EPA's BMDS (V 3.1) software has an option which grades model fit into three categories: viable, questionable and unsuitable. The criteria for this categorisation are based upon the BMDL or AIC criteria defined in the EPA Benchmark Dose Technical Guidance (U.S. EPA, 2012) and are shown as a flow chart (Figure 7; US EPA, 2019). The decision logic option can be modified or turned off by the modeller. The EPA's recommended approach for selecting models is detailed in its technical guide (EPA, 2012; section 2.3.9). BMDS (V 3.1) also includes a Bayesian Modelling Average option.

25. It should also be noted that the WHO (2019) has a chapter on dose-response modelling that is in preparation for publication after completion of a public consultation, which draws up recommended approaches for modelling.

26. Although the current International guidelines for experimental study design (e.g. OECD Test Guidelines) have been developed with the NOAEL approach in mind, they can also be used with the BMD approach. The current guidelines may not, however, be optimal for the BMD approach which allows for more freedom to balance the number of dose groups and group sizes (Slob, 2014); although data from the NOAEL approach can be used, the confidence intervals may be higher than with a larger number of dose groups, which will be reflected in greater uncertainty. The opportunity to recommend study designs that could result in better dose–response information (e.g. more dose levels with the same total number of animals may be possible when guidelines (e.g. within the OECD Test Guidelines Programme) are revised,

3.3 Comparing NOAEL and BMD methodologies for use in risk assessment

27. Once the BMDL is derived and chosen as the POD, the assessment moves to the risk characterisation stage which brings together hazard identification and hazard characterisation and the exposure assessment process (see Risk Characterisation Guidance Statement G06).

28. The BMD approach has a number of advantages over the NOAEL approach in that it makes more complete use of all the available dose–response data, takes into account the shape of the dose-response curve more explicitly and, is less dependent on dose spacing. BMD also enables quantification of the uncertainties in the dose-response data using statistical methodology (EFSA, 2017). The inclusion, however, of the top dose in the modelling should be considered as there remains debate about the relevance of results from doses around the MTD to low level human exposures; conversely, by not including the top dose in the modelling important information may be omitted (Sewell et al, 2017; Heringa et al., 2020; Woutersen et al., 2020).

29. Different software programmes are currently available for BMD analysis. The US EPA developed the Benchmark Dose Software (BMDS[4]) and PROAST[5] was developed by the Dutch National Institute for Public Health and the Environment and is available from the RIVM website[6]. EFSA provides a web-based platform[7] for performing BMD analysis based on the PROAST software.

30. Despite the adoption of the BMD approach as an alternative to the NOAEL in determining a POD, there continues to be a need for the NOAEL/LOAEL approach. Not all data sets are amenable to BMD modelling, such as those resulting from incomplete data availability or, from a lack of models that can describe a dataset adequately (US EPA, 2012). The NOAEL approach is recommended to be used in this instance, subject to suitability of the data set, as it is feasible that a data set may not be appropriate for derivation of any POD.

3.4 The T25 approach

31. Although primarily used for carcinogenic potency estimates, the T25 approach can also be used to derive a POD. For example, in deriving excess cancer risk estimates, the European Chemicals Agency (ECHA) recommends use of $BMDL_{10}$ as a POD, or the T25 can be used (ECHA, 2019). This may be particularly applicable to older data sets which may have minimal dose-response data.

32. The T25 is defined as the dose eliciting a 25% increase in the incidence of a specific tumour at a selected site above the background level within the standard lifespan of that species (Dybing et al., 1997; Sanner et al., 2001). The methodology does not require the application of complex statistical methods and is determined by simple linear interpolation of data and, in some cases extrapolation, beyond the experimental dosing points, preferentially from long-term carcinogenicity bioassays. The minimum data requirements to calculate a T25 are one incidence level significantly greater than the controls (Gillespie et al., 2011). The T25 is influenced by the quality of the bioassay information (e.g. design and evaluation of studies) and factors such as time to first tumour, the influence of toxicity on tumour induction and mortality, and the approach taken regarding statistical analysis of tumour data.

33. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has evaluated the use of T25 estimates for regulatory risk assessment of non-threshold carcinogens (ECETOC, 2002). They report that there may be uncertainties regarding the application of the T25 for potency ranking, particularly with regard to selection of the most sensitive site relevant for humans, the relevance of rodent tumours for humans, and different cancer susceptibilities between rodent species (ECETOC, 2002). The T25 is also the method used by the EU to assess relative potency for the setting of specific concentration limits of preparations and mixtures (EC, 1999). Using the T25 method, Sanner and Dybing (2005) reported a

[4] https://www.epa.gov/bmds
[5] https://www.rivm.nl/en/proast
[6] https://proastweb.rivm.nl/
[7] https://www.openanalytics.eu/

good correlation between the values obtained based on human epidemiological data and those based on experimental data in animals, although the data available for comparison was limited. Previously, the T25 approach has been used in risk assessment for regulation of non-food, genotoxic carcinogenic chemicals in the EU (EFSA, 2005).

3.5 Comparing BMD and T25 methodology for use in risk assessment

34. T25 and the BMD methodology differ in that the T25 is calculated from one data point on the dose-response curve whereas the BMD is derived from dose-response modelling of all the available data on the dose-response curve (EFSA, 2005).

35. Dybing et al. (2008) compared the Margin of Exposure (MOE), the numerical value obtained by dividing a POD on the dose-response curve by estimated human exposure to the chemical, for 6 substances obtained using either the $BMDL_{10}$ or the T25. They found that MOEs obtained using the T25 as the POD were, on average, around 2.35 times higher than those derived using the $BMDL_{10}$ as the POD (Dybing et al., 2008). Benford et al. (2010) compared MOEs for 12 substances in food that are genotoxic and carcinogenic (5 of which were the same as those examined by Dybing et al., 2008) and found that the ratio of MOEs derived from a T25 value varied from those using a $BMDL_{10}$ value by between 0.9 and 4.6, with a mean of 2.9 and a median of 2.6. These results were in line with the expected ratio of 2.5 to account for the 25% vs. 10% risk, assuming linearity in the dose-response relationship, when comparing the T25 with the $BMDL_{10}$ (Benford et al., 2010).

3.6 COC opinion on methods for deriving POD

36. The Committee recommends that, where possible, the BMD approach should be used for deriving a POD, as a starting point for human health risk assessment. This applies to most endpoints, including carcinogenicity by a genotoxic or non-genotoxic mode of action. This view is also supported by other bodies including the EFSA and the US EPA.

37. The BMDL can be used for setting regulatory levels such as acceptable daily intake (ADI) or tolerable daily intake (TDI) or reference doses/concentrations (RfD/RfCs) for effects for which it is assumed there is a threshold.

38. When data sets are not amenable to BMD modelling, the Committee recommends the NOAEL/LOAEL approach is adopted.

39. In the Committee's discussion of the MOE approach for G06, the guidance document on cancer risk characterisation methods, the Committee considered the use of the BMD approach as a means of deriving a POD to be superior to that of the T25. Therefore, where it is not possible to derive a $BMDL_X$, the Committee does not recommend the routine use of the T25 for risk characterisation.

4.0 Potency Ranking of Genotoxic Carcinogens

40. The relative potency estimates discussed below could have some pragmatic use in carcinogenic risk assessment as an aid in the prioritisation of genotoxic carcinogenic substances but are not considered adequate for quantifying cancer risks. The uncertainties inherent in potency ranking mean that relative potencies should not be over-interpreted. For example, it is unclear whether the relative ranking identified in the observed dose range would be maintained at low doses, and whether the relative potency in animal studies would be applicable to humans.

41. Data from animal bioassays can be used to rank carcinogenic potency without reference to human intake. Carcinogenic potency estimates make use of the available dose-response data, and the POD can be derived from TD50, T25 or BMD approaches for use in potency ranking. For example, in a series of publications Gold and colleagues tabulated data on a large number of compounds allowing their carcinogenic potencies to be expressed as the TD50 (Gold et al., 1984;1997). These values can be used to indicate the relative potencies of a series of compounds.

42. The TD50 (Peto et al., 1984) is defined as "For any particular sex, strain, species and set of experimental conditions, the TD50 is the dose rate (in mg/kg body weight/day) that, if administered chronically for a standard period-the "standard lifespan" of the species-will halve the mortality-corrected estimate of the probability of remaining tumor-less throughout that period". The TD50 concept is based on the assumption that there is linearity between dose and hazard until tumour onset, which may be complicated by premature deaths from causes other than tumour formation. The concept also depends on the assumption that tumour onset times are observable prior to mortality and, as a result, the approach relies heavily on careful observation of the animals. Tumours that are discovered after death within the study period may cause confounding between mortality and tumour onset and would ultimately result in a biased TD50 estimate. Alternatively, tumours that do not significantly alter survival and remain undiscovered until death would result in the TD50 value relating to the ‘rate of death with tumour’, rather than the tumour incidence rate. This undermines the objective of the carcinogenicity study, which is to evaluate tumour incidence.

43. When comparing the TD50 and T25 approaches for estimating potency, the TD50 has an advantage in that it takes account of effects of chemicals on survival, however it requires specific software to undertake its derivation. In contrast, the T25 is quick and easy to calculate. There is evidence of a good correlation between rank order produced by TD50 and T25 (Dybing, 1997). In 2006, the COC compared the TD50 with the T25 in an attempt to develop an approach for potency ranking of genotoxic carcinogens for single exposure. Very limited data were available for this purpose and little correlation was found among those substances for which it was possible to obtain chronic TD50 and T25 values, compared to acute T25 values (COC, 2006).

44. The use of Potency Equivalency Factors (PEFs) and Toxic Equivalency Factors (TEFs) have been suggested in circumstances where there is a good surrogate (i.e. share a common mode of action) compound for comparison. Examples of use include for the inhalation of polycyclic aromatic hydrocarbons (PAHs) (Collins, 1998; Pufulete et al., 2004). The US EPA (2010) also developed an approach for assessing cancer risk for PAH mixtures using relative potency factors (RPFs), which estimates the cancer risk of individual PAHs relative to that of benzo[a]pyrene (BaP). Although the US EPA suggests that RPFs are applicable to all routes of exposure they note that there is appreciable uncertainty in doing this.

45. It is noted by the Committee that PHE has adopted a surrogate marker approach rather than the use of PEF/TEFs for assessment of the public health risk of PAHs in contaminated land. This assumes that the cancer risk of a complex mixture of PAHs is proportional to the concentration of a surrogate marker PAH (BaP). The decision to use a surrogate marker approach was due to concerns regarding under-prediction of carcinogenic potency with the TEF approach for PAHs (PHE, 2017).

4.1 COC view on potency ranking tools

46. The Committee reiterates its previous position that the TD50 is a practical quantitative estimate of carcinogenic potency for the ranking of genotoxic carcinogens, but not for deriving a POD.

47. Whilst it is acknowledged that the T25 approach can be used in potency ranking of genotoxic carcinogens, the Committee is of the view that due to a number of inherent uncertainties, the estimates should not be over-interpreted. Currently, there is no need to use the T25 to rank non-genotoxic carcinogens, for which tolerable exposure levels can be derived using an approach based on knowledge of mode of action, identification of a NOAEL, and the use of uncertainty factors.

5.0 The Threshold of Toxicological Concern (TTC)

48. The TTC approach is used to screen and prioritise the risk assessment of substances with a known chemical structure with little, or no, specific toxicity data (for example, pesticide residues). Application of the TTC approach has been most widely applied to the oral route of exposure and, as such, the following sections focus on that route. For the TTC approach to be applied, the estimated exposure of humans to the substance via the oral route should be low (EFSA, 2019).

49. Application of the TTC approach to inhalation and dermal exposure routes is not as widely applied but has been considered by the EU expert committees SCCS/SCHER/SCENIHR (2012) based on assessment of inhalation data (Carthew et al., 2009; Escher et al., 2010, Tluczkiewicz et al., 2016) and dermal data (Safford et al., 2008; Safford et al., 2011; Safford et al., 2015; Roberts et al., 2015). As the TTC approach is now widely used in human health risk assessment, this version of

G05 does not include contextual historical detail of the development of the TTC approach. This is summarised in CC/2012/18.

5.1 Initial considerations prior to applying the TTC decision tree

50. Prior to its use (section 4.3), it is important to confirm that the substance of interest is suitable for application of the TTC approach. Literature searches are required to evaluate the level of data available (including using read-across) to perform a risk-assessment. If the group of chemicals within which the substance sits has well-established toxicity data, then the TTC approach should not be used. In addition, substances falling under certain regulations, e.g. EU food/feed legislation, are excluded from use of the TTC where they require submission of toxicity data for approval.

51. Current exclusion categories are: groups of potent genotoxic carcinogens, aflatoxin-like, azoxy- or *N*-nitroso substances and benzidines, metals in elemental, ionic or organic form, metal-containing compounds, other inorganic compounds, substances known or predicted to bioaccumulate (for example, polyhalogenated-dibenzodioxins, -dibenzofurans and -biphenyls), proteins, substances with a steroid structure, nanomaterials, radioactive substances and organosilicons (EFSA, 2019).

52. The application of the TTC approach to mixtures requires evaluation on a case-by-case basis. Where all components are known, EFSA recommend a tiered approach to risk assessment, with the assumption of dose addition as a starting point. In the case of mixtures that are not fully defined, the TTC approach may be used, provided that analysis has shown that excluded compounds are not present. Under these circumstances, the unknown compounds are considered to be potentially DNA reactive carcinogens and the sum of the mixture components is evaluated against the lowest TTC value (0.0025 µg/kg bw/day). In circumstances where there are no excluded compounds, organophosphates or carbamates present and there is no concern for unknown components with regards to DNA reactivity the mixture is evaluated against a TTC value of 1.5 µg/kg bw/day.

5.2 Estimates of exposure

53. The TTC approach is driven by the exposure aspect which needs to be accurately measured and should be low. It is recommended that chronic exposure is estimated using the upper end of the distribution range from dietary exposure assessments; where this is unavailable, use of the maximum reported level is suggested (EFSA, 2019). Consideration should be given to subgroups of the population whose dietary exposure may be higher (for example infants and children).

54. In cases of acute exposure (i.e. < 24 h), where data is available, it is suggested to use the highest percentile levels in conjunction with high percentile food consumption. If data is unavailable then, as previously, the maximum reported level should be used.

55. EFSA recommends that TTC values should be expressed per kg body weight so that they are applicable to different age groups, differing in body weight (EFSA, 2019).

5.3 Application of the TTC decision tree

56. The latest version of the TTC decision tree is given as part of the most recent EFSA guidance (EFSA, 2019). For all chemicals of interest assessed using the framework, the estimated exposure is compared against an appropriate TTC value based on their estimated Cramer Class[8].

57. Of particular relevance to the risk assessment of chemical carcinogens is the assessment of the potential for DNA-reactive mutagenicity or carcinogenicity. For DNA-reactive mutagens or carcinogens, the TTC value is 0.0025 µg/kg bw/day.

58. Organophosphates and carbamates have been assigned a TTC value of 0.3 µg/kg bw/day and all other chemicals are grouped according to their Cramer Class, with TTC values of 30, 9 and 1.5 µg/kg bw/day for Classes I, II and III respectively.

59. Where the estimated exposure to the chemical of interest is below the appropriate TTC value, it is considered that the probability to cause harm to humans is low. However, if the estimated exposure is higher than the TTC value, it is recommended that a non-TTC approach be adopted to reach a conclusion as to the potential for harm (EFSA, 2019).

5.4 Special considerations in applying the TTC decision tree

60. Exposure estimates in infants under the age of 16 weeks require additional considerations to be applied, as previously detailed (EFSA, 2017). In addition, differences in dietary exposure and reaction to certain substances in the diet between infants, children and adults are possible and these have also been discussed (EFSA, 2019).

5.5 Regulatory use of the TTC approach

61. In 2009, Felter et al. proposed refinements to the TTC decision tree, including consideration for chemicals that have structural alerts for genotoxicity but negative data from genotoxicity tests. They proposed using a threshold value of 1.5 µg/person/day (0.025 µg/kg bw/day) as an appropriate TTC exposure limit in such cases (Felter et al., 2009).

62. TTC values are used by EFSA and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for assessing flavouring substances in food (EFSA CEF Panel, 2010). Other uses by EFSA across their remit have included assessments of: impurities, metabolites and degradation products of food additives (EFSA ANS Panel, 2012); pharmacologically active substances present in food of animal origin

[8] widely used approach for classifying and ranking chemicals according to their expected level of oral systemic toxicity, for details see Cramer et al. 1978.

(EFSA CONTAM Panel, 2018); metabolites and degradation products of plant protection products in the context of residue definition for risk assessment (EFSA PPR Panel, 2016); derivation of 'maximum acceptable feed concentrations' for flavouring additives based on default values for feed consumption (EFSA FEEDAP Panel, 2017); development of the criteria for the safety evaluation of mechanical processes to produce recycled poly(ethylene terephthalate) (PET) intended to be used for manufacture of materials and articles in contact with food (EFSA CEF Panel, 2011).

63. The concept of a staged TTC was proposed by Müller et al. (2006) taking into account the duration of exposure as a key factor impacting on the probability of a carcinogenic response. In 2015, the European Medicines Agency (EMA) agreed to the use of a staged TTC approach during clinical development of medicines for a less than lifetime exposure and recommended limits for daily intake of genotoxic impurities of 1.5, 5, 10, 20 and 60 µg/day for greater than 12 months, 6-12 months, 3-6 months, 1-3 months and less than 1 month periods, respectively. For single doses, an intake of 120 µg/day was considered acceptable from a safety perspective (EMA, 2015).

64. A TTC of 1.5 µg/day is used as part of a staged assessment for the acceptability of known genotoxic impurities present in pharmaceuticals; this is considered appropriate as a risk of 1 in 10^5 (assuming linear extrapolation) is considered acceptable for human medicines (EMA, 2006). The use of a TTC of 1.5 µg/day by the EMA also applies to compounds that show evidence of genotoxicity in *in vitro* tests. A similar approach is used for genotoxic constituents of herbal medicinal products/preparations (EMA, 2008).

65. The TTC approach has also been proposed for use with assessing household and personal care products (Blackburn et al., 2005), skin sensitising substances (Safford, 2008) and for industrial chemicals assessed under REACH (ECHA, 2008).

5.6 COC view on the TTC approach

66. The TTC approach is acknowledged by the Committee to be a pragmatic means to assess the level of potential concern for exposure to chemicals with limited, or no, toxicity data.

6.0 Conclusions

67. This guidance statement (G05) outlines the current methodologies available for determining PODs and PEs associated with exposures to chemical carcinogens, including the Committee's views of their utility. The tools outlined are those that are available to use *when considered appropriate by the risk assessor.*

68. The BMDL is recommended as a POD for genotoxic carcinogens, where it can be used to calculate a MOE, and for non-genotoxic carcinogens where it can be used to establish a HBGV.

69. If a BMDL cannot be established, the NOAEL should be used for non-genotoxic compounds; for genotoxic compounds it is also possible to use a NOAEL but care needs to be taken that the existence of a threshold for effect is not implied by this.

70. PEs are considered a pragmatic, non-quantitative means to prioritise carcinogenic substances in the risk assessment process.

71. The COC acknowledges that the TTC approach is a pragmatic method for assessing the level of concern around exposure to a chemical when data are lacking or insufficient to allow prioritisation.

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7.0 References

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