

## GENOTOXIC CARCINOGENS AND REACH - DNELS AND DMELS

### SUMMARY

This paper proposes an approach to developing “Derived No Effect Levels” (DNELs) and “Derived Minimal Effect Levels” (DMELs) for genotoxic carcinogens. The approach contains three options selected according to the type and extent of carcinogenicity evidence available. (i) For carcinogens that are genotoxic but for which there is robust evidence of a threshold, it is proposed that DNELs should be derived following already agreed methodology using standard toxicological uncertainty factors as judged appropriate.

(ii) For non-threshold genotoxic carcinogens with robust human exposure-response data, it is proposed that DMELs be derived on the basis of a quantitative risk assessment. From the risk estimates produced, DMELs would be selected so as to correspond to a low increased level of risk compared to existing background rates for that tumour type.

(iii) For other non-threshold genotoxic carcinogens it is proposed that DMELs be derived following the application of a large assessment factor (AF) to a suitable reference point (BMDL10 or T25). This AF-based approach is closely based on a position paper produced by the Scientific Committee to the European Food Safety Authority (EFSA)<sup>1</sup>. This approach has the advantage that it is not based on unverifiable assumptions concerning the shape of the dose-response relationship for cancer at low levels of exposure. Furthermore, for risk communication purposes the DMELs so derived would be interpreted simply as representing exposure levels judged to be of “very low concern”, rather than indicating a precise (but potentially misleading) numerical level of risk. In this regard, this approach differs from methods based on mathematical modelling and extensive extrapolation below the observed range of data.

### GENERAL CONSIDERATIONS

1. Under REACH, ‘derived-no-effect-levels’ (DNELs) are intended to represent a level of exposure to humans at which, according to current scientific knowledge, there would be no indication of risk to health. The purpose of DNELs is to act as a benchmark for determining adequate control of exposure.

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<sup>1</sup> Opinion of the Scientific Committee on a request from EFSA related to a Harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. ([http://www.efsa.europa.eu/etc/medialib/efsa/science/sc\\_committee/sc\\_opinions/1201.P ar.0002.File.dat/sc\\_op\\_ej282\\_gentox\\_en3.pdf](http://www.efsa.europa.eu/etc/medialib/efsa/science/sc_committee/sc_opinions/1201.P ar.0002.File.dat/sc_op_ej282_gentox_en3.pdf)).

2. The derivation of DNELs requires the identification or estimation of a 'no-observed adverse effect level' (NOAEL). The concept of NOAELs applies only to toxicological endpoints that exhibit a *threshold* of effect on the dose-response curve. Thresholds exist for most toxicological endpoints such as liver or kidney damage, cytotoxicity, developmental toxicity and sensory irritation. Such effects will not be elicited at sub-threshold levels of exposure.

3. It is generally accepted that thresholds also exist for cancer caused by non-genotoxic mechanisms (e.g. chloroform causes liver cancer as a result of chronic cytotoxicity and repeated episodes of cell proliferation). DNELs can be established for such substances according to agreed methods by application of assessment factors (AFs) to the NOAEL for the critical toxicological effect.

4. However, for genotoxicity, and therefore also for cancers that develop, or are assumed to develop from a genotoxic mechanism (i.e. carcinogens that are genotoxic even though it is unclear whether or not it is the genotoxicity that leads to cancer development— from now onwards termed “genotoxic carcinogens” for simplicity), the concept of thresholds is complex. For genotoxic carcinogens that react directly with DNA, there has been a tradition in regulatory work to assume that there is no threshold in the dose-response relationship for genotoxicity. The consequence of this thinking is that any exposure to such DNA-reactive genotoxic carcinogens, regardless of how low, could incur some increased risk of cancer.

5. However, the concept of the existence of thresholds<sup>2,3</sup> for genotoxic carcinogens is gaining increasing prominence. There is wide support for the view that thresholds are likely to exist for genotoxic substances that do not react directly with DNA. This would apply to aneugens that interfere with proteins of the spindle apparatus involved in cell division, and to substances that cause indirect DNA damage through the generation of reactive oxygen species. There is also a growing awareness that thresholds may exist even for some DNA-reactive genotoxic carcinogens<sup>4</sup>; this is supported by evidence that for some genotoxicants that interact directly with DNA, collateral effects such as cytotoxicity make important contributions to the carcinogenic mechanism. In addition, the existence of DNA-repair mechanisms and processes such as metabolic activation/deactivation give support to the possibility of thresholds<sup>5</sup>.

### **Genotoxic carcinogens for which a threshold can be determined**

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<sup>2</sup> Bolt HM. Position paper on OELs for carcinogens. SCOEL/INF/739 (2006)

<sup>3</sup> Tsuda H et al (2003) Value of GST-P positive preneoplastic hepatic foci in dose-response studies of hepatocarcinogenesis; evidence for practical thresholds with both genotoxic and non-genotoxic carcinogens. A review of recent work. Toxicologic Pathology. Vol 31 (1): 80-86

<sup>4</sup> Jenkins et al (2005) Do dose-response thresholds exist for genotoxic alkylating agents? Mutagenesis 20 (no 6) : 389-398

<sup>5</sup> Jenkins et al (2005) Do dose-response thresholds exist for genotoxic alkylating agents? Mutagenesis 20 (no 6) : 389-398

6. Currently, there are very few genotoxic carcinogens for which there are suitable and sufficient experimental data to allow the reliable identification of thresholds. It is notable that for such substances (formaldehyde and vinyl acetate being the main exemplars) there are very rich toxicological databases including mechanistic studies. Such a wealth of data is able to provide a convincing basis to support the existence of thresholds of relevance to human health. In the case of formaldehyde, it is understood that its genotoxicity is due to its ability to produce DNA-protein cross-links (DPX). However, there is convincing evidence to indicate that an increased rate of DPX formation is not significant at exposures below the threshold for cytotoxicity. Hence, exposures below this threshold would not be predicted to lead to any increased risk of cancer or to genotoxic damage. For genotoxic carcinogens such as formaldehyde, DNELs can be derived following already agreed methodology involving the application of toxicological uncertainty factors to the NOAEL for the critical toxicological effect.

7. As noted by the EU Scientific Committee on Occupational Exposure Limits (SCOEL) (SCOEL/SUM 86 2003), in general, epidemiological data cannot be used to support the existence of a threshold for genotoxic carcinogenicity. Any apparent threshold at low levels of exposure will be associated with statistical confidence intervals that will include a positive risk estimate value. Overall, it would not be possible to “prove” the existence of a threshold from epidemiological data due to statistical considerations. Hence, evidence concerning the existence of thresholds will need to be based on mechanistic and experimental rather than epidemiological data.

8. The Appendix to this paper briefly summarises the experimental evidence and the scientific rationale supporting the existence of a threshold for formaldehyde and for vinyl acetate, and illustrates how DNELs could be derived for such substances that would represent “safe” levels of exposure for humans.

### **Non-threshold genotoxic carcinogens**

9. For most genotoxic carcinogens, although the existence of a threshold could be postulated on theoretical grounds, based on the experimental data that are usually available it is not possible to verify the existence of a threshold, or if so, to identify the location of the threshold on a dose-response curve. Therefore, for such substances, it is difficult to specify with confidence, a level of exposure in the range relevant to workers or the general public, at which there would be no increased risk of cancer development. Overall, it is not possible to apply the DNEL methodology to such substances. However, for the purposes of REACH, there is a need to assess the risks to human health arising from the use of such substances (Art 59.4). Hence, in analogy to the DNEL concept, it has been proposed that reference levels should be determined for such substances to aid in the risk characterisation process. It has been suggested that such levels could be referred to as “Derived Minimal Effects Levels” (DMELs). This paper proposes two approaches to the development of DMELs for “non-threshold” genotoxic carcinogens, depending on the type of data available.

(i) Derivation of DMELs for non-threshold genotoxic carcinogens with robust human exposure-response data

10. For substances in this group, assuming that the available experimental evidence is insufficient to identify a threshold, it is proposed that DMELs be derived on the basis of a quantitative risk assessment (QRA) using epidemiological data. The calculation of cancer risk estimates from epidemiological data will require experience and expertise in this subject and it is beyond the scope of this document to give detailed guidance, so that only a few key points are noted here.

11. For some substances, quantitative risk estimates for cancer may be based on a single good quality epidemiology study, and in other cases they may be based on pooled data from a number of studies or on a formal meta-analysis<sup>6</sup>. This will depend on the number of studies, their homogeneity, and their relative quality. There are two main study designs of relevance, cohort and case-control. Where cases are drawn from the general population (such as cancer registries) it is unlikely that they will contain any quantitative exposure data hence they will not be able to usefully contribute to a QRA. Cohort studies and nested case-control studies (nested within a workplace cohort study) are more likely to contain quantitative exposure data.

12. Cohort studies of cancer incidence or mortality need to be large-scale, and given that cancer is a disease of long latency the exposed subjects need a long period of follow-up (at least ~ 20 years). Adequate account needs to be taken of possible confounding factors and the appropriateness of study design and subject selection criteria.

13. The quality and reliability of the exposure data are of the utmost importance. The assessment of the quality of the exposure data will need to take account of whether or not the data were measured or modelled; if measured, consideration needs to be given to the methods of sampling, whether sampling was personal or area sampling, the timing and frequency of sampling (full shift TWA or peak exposures), the representativeness of the sample measurements to the various subjects in the cohort, and whether or not there were any changes to the measurement methods over the time period of the study. A statistical analysis of the exposure sampling data may be helpful in order to help illustrate the confidence intervals around mean values as well as to ensure the most reliable exposure estimates are used in the cancer risk models.

14. The results of the study(ies) are subject to statistical analysis involving the application of various mathematical models. A number of different models may be applied to the data, testing a variety of assumptions, with the aim of identifying the model that provides the best fit to the data points. The exposure data may be expressed in terms of cumulative exposure, average

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<sup>6</sup> Goldblohm RA et al (2006). Risk estimation for carcinogens based on epidemiological data; a structured approach, illustrated by an example on hexavalent chromium. Available on line at [www.sciencedirect.com](http://www.sciencedirect.com) Regulatory Toxicology and Applied Pharmacology xxx (2006)

exposure, or some other function of exposure in order to determine which exposure metric gives the best fit to the data.

15. The choice of mathematical model used for cancer risk assessment may require assumptions to be made concerning the shape of the dose-response curve at low levels of exposure below the range of observed data, and the usual default is to employ a model that assumes low-dose linearity without a threshold. **For this reason, it is important that risk estimates based on epidemiological data do not involve extensive extrapolation orders of magnitude below the range of observed data; this would lead to risk estimates that would be of very uncertain credibility.** Risk estimates that refer to exposure levels close to the observed range of data will be associated with a much higher degree of credibility, and are more likely to represent a “true” level of risk. A balance needs to be struck between the desire to predict exposures that would incur a very low increased risk of cancer, and the desire to maintain scientific credibility and not to stray too far from the observed data.

16. The consequence of the above paragraph means that it is not possible to advocate that DMELs derived on the basis of human data should all indicate a consistent level of risk (eg increased lifetime risk of  $10^{-6}$ ). Clearly, the value of the risk levels that can be derived with credibility will depend on the extent of epidemiological data available.

17. In practice, examples of genotoxic carcinogens with suitable and sufficient epidemiological data to permit a reliable quantitative risk assessment are very limited, and include 1,3-butadiene (SCOEL/SUM/75 2005), hexavalent chromium compounds (SCOEL/SUM/86 2003) and polycyclic aromatic hydrocarbons<sup>7</sup>.

18. The Appendix to this document explains the approach taken by SCOEL in deriving quantitative risk estimates for hexavalent chromium, and indicates how DMEL values for a genotoxic carcinogen could be selected based on a quantitative risk assessment of human data. In essence, based on an evaluation of epidemiological data to which a linear no-threshold model was applied, SCOEL suggested that consideration be given to setting an occupational exposure level (OEL) for hexavalent chromium compounds at either 10 or 25  $\mu\text{g}\cdot\text{m}^{-3}$  (Cr VI). These exposure levels were predicted to lead to lifetime increased risks of lung cancer per 1000 males of between 1-6 and 2-14 respectively. Given that the current lifetime risk of lung cancer in males is 8% (based on data for Great Britain), the risk estimates associated with these values represent a low increased risk for human health.

(ii) Assessment Factor (AF) approach for deriving DMELs

19. For the remaining genotoxic carcinogens, those for which a threshold cannot be identified from experimental data and those for which there are insufficient human data to permit a quantitative risk assessment for cancer, it

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<sup>7</sup> Lung Cancer Risk after Exposure to Polycyclic Aromatic Hydrocarbons: A Review and Meta-Analysis. Armstrong et al. Environmental Health Perspectives: Annual Review Issue Volume 112, Number 9, June 2004

is proposed that DMELs be derived by application of a large arbitrary uncertainty factor to a toxicological reference point or dose descriptor such as a BMDL10 or TD25 (discussed further below). This method would not lead to any specified numerical level of risk associated with the DMEL. However, a DMEL derived by this method would represent a low level of exposure at which there would be “very low concern” for an increased risk of cancer. This proposed approach is closely based on a position paper produced by the Scientific Committee of the European Food Safety Authority (EFSA). It should be noted that the starting point (BMDL10 or TD25) might have to be modified first to account for differences in routes of exposure between experimental animals and humans (in case of route-to-route extrapolation), for differences in bioavailability between routes (in case of route-to-route extrapolation) and between experimental animals and humans, for differences in respiratory volumes between experimental animals (usually at rest) and humans (light activity in case of workers) and for differences between occupational and lifetime exposure conditions (7/5x52/48x75/40) in accordance with the methodology already agreed for threshold effects.

20. It is recognised that some authorities advocate an approach to deriving exposure standards for genotoxic carcinogens based on mathematical modelling of animal carcinogenicity data to predict exposure levels associated with a predetermined level of risk in humans (e.g. excess lifetime risk of developing cancer of  $10^{-5}$  or  $10^{-6}$ ). It is possible that in practice, DMELs derived by such approaches may differ little numerically from the DMELs derived from the AF approach. However, DMELs derived from the AF approach would not be linked to a specified level of cancer risk, but would be interpreted in a qualitative sense only, as representing an exposure level of judged to be of “very low concern”. From a scientific perspective (as discussed below), and also from a risk communication perspective, the AF-based approach is strongly preferred.

21. The Appendix to this document gives an example (acrylamide) on how a DMEL can be derived by applying a large AF to the toxicological starting point.

*Approach recommended by the Scientific Committee of EFSA*

22. The Scientific Committee of EFSA (European Food Safety Authority)<sup>8</sup> was recently asked for an opinion on how to judge the cancer risk from carcinogens that are also genotoxic when they are present as unavoidable contaminants in food. Although the Scientific Committee endorsed the As Low As is Reasonably Achievable (ALARA) principle, it recognised the need for a basis for decision-making that would be of more practical use to risk managers and thus advised a Margin of Exposure (MOE) approach; if the genotoxic contaminant is present in food at concentrations 10,000-fold lower than a specified dose-level, this would correspond to a dose estimate that

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<sup>8</sup> Opinion of the Scientific Committee on a request from EFSA related to a Harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. ([http://www.efsa.europa.eu/etc/medialib/efsa/science/sc\\_committee/sc\\_opinions/1201.P ar.0002.File.dat/sc\\_op\\_ej282\\_gentox\\_en3.pdf](http://www.efsa.europa.eu/etc/medialib/efsa/science/sc_committee/sc_opinions/1201.P ar.0002.File.dat/sc_op_ej282_gentox_en3.pdf)).

would be of low concern from a public health point of view and might be considered as a low priority for risk management actions.

23. It should be noted that this approach was not intended to provide “safety standards” as there was no wish to appear to condone the presence of genotoxic contaminants in food. Rather, it represents a MOE approach to risk assessment. Although the approach was advocated in the context of food safety, it is proposed here that similar principles could apply to the derivation of DMELs for genotoxic carcinogens in the context of REACH, such that the 10,000-fold factor for the MOE be adopted as a default AF.

*Basis for the 10,000-fold MOE factor*

24. The position paper from the Scientific Committee of EFSA explains that the MOE needs to reflect a consideration of the various uncertainties in cancer risk assessment. The uncertainties were categorised into three areas:

(a) Those relating to interspecies and interindividual variability in toxicokinetics and toxicodynamics. In the absence of clarifying information, by default an overall assessment factor (AF) of 100 is normally used for such sources of variability for non-genotoxic substances, and it was considered that similar uncertainties are likely to apply to carcinogens that are genotoxic.

(b) Additional uncertainties for substances that are genotoxic and carcinogenic relating to differences in cell cycle control and DNA repair, which affect the carcinogenic process.

(c) Further uncertainties because the starting point is not equivalent to a NOAEL, and the dose-response relationship below the starting point is not known.

25. Taking into account the usual default AF of 100 for intraspecies and inter-individual differences, together with the uncertainties surrounding points (b) and (c) an overall AF of 10,000 was judged appropriate as a generic default.

26. It needs to be recognised that the selection of AFs is not a purely scientific process. The selection of AF values has an arbitrary element that reflects a subjective and societal (non-scientific) opinion concerning an appropriate degree of reassurance of health protection. It reflects concerns relating to the severity of the health effect, the need to protect the target population, and in specific cases may also reflect a balance between the societal benefits of the substances against the potential human health risks.

27. In the case of the 10,000-fold MOE proposed by the EFSA Scientific Committee for genotoxic contaminants, this may be judged to provide a high and appropriate degree of reassurance for the general public in the context of food safety. It might be possible to argue that, for workers, the 10,000 default AF could be reduced to a default value of 5,000 by applying the default intraspecies factor of 5 (rather than 10) as already proposed for threshold effects.

28. Clearly the selection of the value of the default AF for DMELs is of crucial importance and it warrants careful consideration. However, it may not be possible to provide a better or more persuasive rationale than that suggested by the Scientific Committee of EFSA for a default value of 10,000. This would have the advantages of consistency and transparency. It may be possible to develop arguments for a lower default AF; for example, one argument could be that processes such as cell cycle control and DNA repair are physiological processes that will proceed at rates that correlate with basal rates of metabolism in different species (i.e. will relate to oxygen consumption). Hence, the default AF of 100 for inter-species and intra-individual differences might be considered adequate to also cover differences in cell cycle control and DNA repair. This might argue for a reduction in the default AF of 10,000. Alternatively, this argument may be regarded as speculative. Overall, the optimum solution seems to be that in general, a default AF of 10,000 should be used in the derivation of DMELs for genotoxic carcinogens based on animal data. However, deviations from the default AF of 10,000 should nevertheless be acceptable on the basis of chemical specific data in the same way as the standard 10 x 10 AFs for non-carcinogenic endpoints may be replaced by chemical specific assessment factors.

#### *Selection of starting point*

29. The Scientific Committee of EFSA presents two options for the starting point dose-level. The preferred option is based on the use of benchmark dose methodology (BMD). BMD methods require the application of a mathematical model that is fitted to the carcinogenicity data points in order to identify a dose giving a specified level of response. The dose estimated to cause a 10% increased response is known as the BMD10. The lower 95th confidence interval of the BMD10 is referred to as the BMDL10. In general, the BMD10 should be based on the most sensitive tumour type (i.e. the tumour that develops at the lowest dose levels).

30. The Scientific Committee of EFSA recommends that where possible, the BMDL10 be used as the starting point for risk assessment of genotoxic contaminants in food. The BMD10 corresponds to the lowest statistically significant increased incidence of cancer that can be measured in most studies, and has the advantage that it requires little or no extrapolation outside of the observed experimental data points, and its estimation takes account of all data points in the dose-response curve. However, the disadvantage is that at least three data points are needed in order to apply BMD methods, and if tumours are only observed at the top dose in a study then BMD methodology cannot be applied. There are various models available for BMD modelling<sup>9</sup> (saturated model, one-stage model, two stage model, log logistic model, Weibull model, and Proast 2). The choice of the model may depend on the model that gives the best fit to the data, or on whichever model software is available and familiar to the assessor, or alternatively on whichever model gives the lowest (most stringent) BMDL10 value. There might be a concern that variation in options for model selection

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<sup>9</sup> O'Brien et al (2006) Approaches to the risk assessment of genotoxic carcinogens in food: a critical appraisal. Food and Chem Toxicol 44:1613-1635

could lead to inconsistency in BMDL10 values from different assessors. However, in practice, the outputs from different models may not necessarily differ substantially<sup>9</sup>.

31. For substances for which the dose-response data are inadequate for derivation of a BMD10 and BMDL10, the Scientific Committee of EFSA recommend that the T25 potency index should be used as an alternative. The T25 has been defined as “the chronic dose rate in mg/kg/bw per day which will give 25% of the animals tumours *at a specific tissue* site, after correction for spontaneous incidence, within the standard life time of that species.” One advantage of the T25 is its derivation does not require as many data points as the BMDL10. In fact the T25 approach can be applied in studies where only one dose shows a statistically significant difference from control data.

### **Linear extrapolation methods**

32. It is recognised that some authorities advocate approaches for deriving exposure standards for “non-threshold” genotoxic carcinogens based on mathematical modelling of animal carcinogenicity data to predict exposure levels associated with a predetermined level of risk in humans (e.g. excess lifetime risk of developing cancer of  $10^{-5}$  or  $10^{-6}$ ). There are also “model-free” linear extrapolation approaches<sup>10</sup>, but both methods involve extensive extrapolation below the observed range of data and both methods rest on the assumption of low dose linearity. The Scientific Committee of EFSA identified a number of concerns related to these methods that are elaborated below: -

- 1) These methods all rely on an assumption of low-dose linearity but at low exposures this assumption may not be correct. DNA repair mechanisms may lead to a sub-linear dose response at lower doses leading to overestimates of risk.
- 2) The risk estimates derived by mathematical modelling or linear extrapolation lie far below the observed experimental range of data and can never be validated by empirical observation (to validate a lifetime increased risk of  $10^{-5}$  or  $10^{-6}$  would require far more than the 50 animals per sex per dose group used in standard carcinogenicity bioassays).
- 3) These approaches do not adequately convey the uncertainties involved and so imply a level of precision that cannot be justified. They therefore convey a spurious impression of scientific accuracy.
- 4) The usual design of rodent carcinogenicity studies (low, medium and high dose groups of 50 per sex) cannot distinguish between the various mathematical models available in terms of identifying a model that provides the best fit to the data.
- 5) Although the various models can be shown to fit the observed data equally well, these models can lead to risk estimates at low doses that vary over several orders of magnitude, and there is no way of knowing which model, if any, leads to a true approximation of risk.

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<sup>10</sup> Bolt HM. Position paper on OELs for carcinogens. SCOEL/INF/739 (2006)

6) Risk estimates produced by different models often show a direct proportionality to the absolute value of the top dose used in a study, but the outcome should be more dependent on the slope of the dose-response curve. Overall, the risk estimates show a much higher dependence on the choice of model rather than on the actual data.

33. The use of an arbitrary assessment factor approach to derive DMELs for “non-threshold” genotoxic carcinogens has the advantage that it is not based on unverifiable assumptions concerning the dose-response relationship for cancer at low levels of exposure. Furthermore, for risk communication purposes, the DMELs so derived would be interpreted simply as being of “very low concern” rather than indicating a precise (but potentially misleading) level of risk.

### **Exceptions and the need for case-by case expert judgement**

34. It is conceivable that there could be examples of substances that are judged to be carcinogens, and that are also judged to be genotoxic, but for which the available data are insufficient to allow the identification of a threshold, or for a quantitative risk assessment based on epidemiological data, or to allow the identification of a BMD or a T25. How might a DMEL be derived for such substances?

35. An example that could be offered for consideration is that of arsenic, which is agreed to be carcinogenic in humans based on evidence for lung cancer, but for which the exposure-response information in humans is weak, and for which there are no useful animal carcinogenicity data. For substances such as arsenic and its compounds, there may be a need to develop a DMEL that takes account of the carcinogenic hazard in humans. It is proposed that in such cases where the quantitative evidence relating to cancer potency is very limited, a DMEL would need to be derived on a case-by-case basis using expert judgment, leading to a well-argued case to support a DMEL. Arguments would need to be presented stating why a robust quantitative risk assessment or a BMDL10/T25 could not be obtained.

36. In the case of arsenic, there are epidemiological studies with quantitative exposure data, although the quality of the exposure data is limited. An expert assessment of the data would be needed to derive a reference level of exposure, based on a weight of evidence approach to the data, and it is proposed that a large AF be applied to this reference point. The reference point might be an exposure level at which there was no detectable increased risk of cancer, or an exposure range over which there was a definite increased incidence of cancer. This example illustrates a case where it is preferable to apply a large AF to an uncertain data point, than to attempt to perform a quantitative risk assessment leading to numerical risk levels of very dubious credibility.

37. There will undoubtedly be other examples where there could be difficulties in deriving a DMEL due to limitations in the available data. It is proposed that emphasis needs to be placed on the need for expert judgment taking into account all aspects of the data on the substance in question, including

mechanistic data, read-across to similar substances if relevant, and including dose-response evidence on precursor lesions relevant to tumour development.

## APPENDIX

### EXAMPLES

#### 1. Genotoxic carcinogens for which a threshold can be determined

##### **Example 1. Formaldehyde**

1.1 Formaldehyde is a substance that is carcinogenic in animals, producing nasal tumours in rats, and is now also recognised to be carcinogenic in humans based on evidence for nasopharyngeal cancer. Formaldehyde is also genotoxic, producing DNA-protein cross-links (DPX) in tissues at the site of contact following inhalation exposure. The German Federal Institute for Risk Assessment (BfR) has undertaken an assessment of formaldehyde<sup>11</sup> and has derived a “safe” level of exposure (analogous to a DNEL) for the general public. SCOEL (SCOEL/SUM/125 F) has also assessed formaldehyde and has recommended a health-based OEL.

1.2 These authorities concluded that the carcinogenic mechanism of formaldehyde is based on a combination of repeated episodes of cell proliferation due to the cytotoxicity (irritancy) of inhaled formaldehyde, and also to the formation of DPX. Hence, the mechanism is a combination of non-genotoxic and genotoxic events. The animal evidence indicated a NOAEL for cytotoxicity of 2 ppm; a clear threshold for the induction of DPX could not be identified but dose-response data showed that a steep increase in DPX formation only occurred at concentrations above 2 ppm. Increases in cell proliferation only occurred at concentrations above 2.7 ppm. Taking other observations into account it was considered that there would be no increase in genotoxic changes at exposures below the NOAEL for cytotoxicity.

1.3 A clear threshold for respiratory tract cytotoxicity could not be identified in humans, and both authorities considered that the NOAEL for sensory irritation in humans could be used as a surrogate. A weight of evidence approach to the analysis of human data from a number of volunteer studies indicated a NOAEL for sensory irritation of 0.1 ppm. In the BfR assessment it was noted that the NOAEL for sensory irritation was not contraindicated by animal data indicating a threshold for cytotoxicity at 2 ppm. Overall, 0.1 ppm was proposed as a “safe-level” for exposure to formaldehyde in the general public.

1.4 The approach taken by BfR is similar to that taken by SCOEL in their recommendation for an occupational exposure limit (SCOEL/SUM/125F March 2006). Formaldehyde illustrates an example of an approach that could be adopted in developing a DNEL for a carcinogen that is genotoxic, but for which a non-genotoxic mechanism makes an essential contribution to the carcinogenic process. However, formaldehyde is a rare example of where this could be done with confidence, due to the considerable amount of information required.

##### **Example 2. Vinyl acetate**

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<sup>11</sup> [http://www.bfr.bund.de/cm/290/toxicological\\_assessment\\_of\\_formaldehyde.pdf](http://www.bfr.bund.de/cm/290/toxicological_assessment_of_formaldehyde.pdf)

1.5 Vinyl acetate is another example of a carcinogen that is genotoxic, for which a threshold can be identified and a DNEL could be derived representing a level of exposure at which there would be no risk to human health. SCOEL have recommended a health-based OEL for vinyl acetate (SCOEL/SUM 122 October 2005) although did not apply a systematic formal consideration of AFs.

1.6 Vinyl acetate has a large toxicological database including mechanistic studies and PBPK with combined computation fluid dynamic (CFD) modelling. Its metabolism and toxicology has been extensively investigated. Vinyl acetate produces nasal tumours in rats following inhalation exposure, and tumours of the digestive tract in rats and mice following oral exposures. Vinyl acetate is rapidly metabolised to acetic acid and acetaldehyde, which are both endogenously occurring metabolites. Vinyl acetate is genotoxic in vitro and in vivo. The genotoxicity of vinyl acetate is attributed to the formation of DPX due to its metabolism to acetaldehyde. Comparative studies with acetaldehyde indicated that vinyl acetate was more effective at causing DPX than equimolar concentrations of acetaldehyde. There was evidence to indicate that this was due to the drop in pH caused by the formation of acetic acid from vinyl acetate. This acidity was judged to be responsible for the observed irritant cytotoxicity and regenerative hyperplasia in tissues at the site of contact. The existence of physiological pH buffering systems was noted to be consistent with the non-linear dose-response for carcinogenicity, such that it was considered that exposures below the threshold for local cytotoxicity would not lead to an increased risk of cancer. The NOAEL for cytotoxicity in the rat nasal epithelium following repeated inhalation exposure was 50 ppm, and SCOEL recommended a health-based OEL of 5 ppm (8-hr TWA) and a short-term exposure limit (STEL) of 10 ppm for workers.

1.7 The recommended OEL is 10-fold lower than the NOAEL for cytotoxicity. It would be interesting to see what DNEL value would be derived following a systematic consideration of toxicological uncertainties. However, it is recognised that the wealth of mechanistic data significantly alleviates the uncertainties regarding potential inter-species or intra-individual differences in response. It may be concluded that there are unlikely to be substantive inter-species or intra-individual differences in toxicokinetics given that the toxicity of vinyl acetate is dependent on its metabolism by ubiquitously occurring carboxylesterase enzymes; also the carcinogenic effects occur in tissues only at the site of contact, such that systemic distribution is not an issue. There are also unlikely to be substantive interspecies differences in toxicodynamics (oral dosing studies in rats and mice show similar effects suggesting that inhalation exposures would also produce similar effects). Overall, the 10-fold difference between the OEL and the rat NOAEL seems adequate to cover potential intra-individual differences in susceptibility to the inhalation effects of vinyl acetate.

## **2. Non-threshold genotoxic carcinogens with robust human exposure-response data**

**Example. Hexavalent chromium**

2.1 A risk assessment on hexavalent chromium (Cr VI) compounds was published in 2003 (SCOEL/SUM/86 final). The assessment is based on the application of a linear no-threshold model to the results from 10 epidemiological studies. It was considered reasonable to apply a linear no-threshold approach to such “comprehensively genotoxic compounds”. Three different sets of assumptions were made concerning the duration and levels of exposure among the study subjects, and concerning the extent of possible confounding by cigarette smoking. A number of sources of uncertainty in the data were identified, including the fact that the possible modifying effect of the period of follow-up was not estimated. Risk estimates were derived for each set of the three sets of assumptions leading to a range of risk estimates for each specified exposure level.

2.2 Notwithstanding that SCOEL considered the use of a linear no-threshold approach to be appropriate, the SCOEL SUM noted that the assumption of low-dose linearity without a threshold might not be correct, leading to an overestimate in the risk estimates. This is because the cytotoxic and irritant properties of Cr (VI) at higher exposures may enhance the carcinogenic mechanism. Also, it was noted that the lungs have the capacity to reduce Cr (VI) to the non-carcinogenic Cr (III) form, and that there would be no increased risk of cancer until this detoxification mechanism is overwhelmed, this again might lead to an overestimate of risk at low levels of exposure. Overall therefore, the risk estimates for hexavalent chromium may be interpreted as possibly overestimating human health risk at low levels of exposure.

2.3 For males exposed over a working lifetime to Cr (VI) at 10 or 25  $\mu\text{g}\cdot\text{m}^{-3}$  (8-hr TWA), the risk estimates presented in the SCOEL SUM indicated that there would be between 1-6 and 2-14 additional lung cancer deaths per 1000 male workers respectively. The range covers the risk estimates produced from the three different sets of assumptions made about exposures and the role of smoking. The SCOEL SUM suggested that consideration be given to setting an occupational exposure level at either of these values of 10 or 25  $\mu\text{g}\cdot\text{m}^{-3}$  (Cr VI). Given that the current lifetime risk of lung cancer in males is 8% (based on data for Great Britain), the risk estimates associated with these values represent a relatively low increased risk for human health, particularly considering that the risk estimates are probably higher than the true level of risk at such exposures.

2.4 Although the SCOEL SUM on hexavalent chromium sets out in a transparent way how a quantitative risk assessment can be conducted based on epidemiological data, and clearly lays out the sources of uncertainty associated with the data, it should be noted that there are very few chemicals with suitable and sufficient data to support a quantitative risk assessment for cancer based on human data. Furthermore, to carry out such a quantitative risk assessment would require specialised expertise in epidemiology and statistics.

### **3. Non-threshold genotoxic carcinogens with no suitable human exposure-response data but with robust animal data**

#### **Example. Acrylamide**

3.1 The potential carcinogenicity of acrylamide has not been thoroughly investigated in humans, and no firm conclusions can be drawn from the available human cohort mortality studies.

3.2 Acrylamide is carcinogenic in animals, producing increased incidences in a number of benign and malignant tumours identified in a variety of organs (for example the thyroid, mammary gland, adrenals, testes). The tumour types observed show a possible relationship with disturbed endocrine function and raise the possibility of a hormonal mechanism. There is also some inconclusive evidence that acrylamide may induce neoplastic neural lesions (tumours in brain and spinal cord). Given the genotoxicity profile of acrylamide (positive in vivo in both somatic and germ cells), genotoxic activity cannot be discounted from contributing to tumour formation. There are no mechanistic arguments to indicate that these findings would be restricted to animals and not relevant to humans. The BMDL10 and the T25 for the most sensitive tumour type (mammary tumours) identified in the available drinking water rat cancer bioassays are 0.3 and 0.9 mg/kg/day, respectively.

3.3 For a genotoxic carcinogen it is not possible to identify a threshold level of exposure below which there would be no risk to human health and hence it is not possible to derive a DNEL. It is proposed that DMELs are derived for acrylamide by applying a large default assessment factor to the corrected starting points (route-specific and population-specific BMDL10 values).

3.4 The available BMDL10 value (0.3 mg/kg/day) for acrylamide was identified from a rat cancer bioassay performed by the oral route. The two most relevant routes of exposures in the workplace are inhalation and dermal. For acrylamide consumer use (cosmetics and soil conditioners) the relevant route of exposure is dermal. In order to derive DMELs for these two routes of exposure from the oral BMDL10, route-to-route extrapolation needs to be applied. There is no evidence of a clear first-pass effect by the oral route for acrylamide; furthermore, evidence from oral and dermal acute toxicity studies shows that toxicity by the oral route is more pronounced than by the dermal route. This implies that using oral data to predict toxicity by the dermal and inhalation routes is unlikely to underestimate toxicity. Overall, therefore, it is considered that route-to-route extrapolation is justified and does not introduce significant uncertainties. For indirect exposure of humans via the environment, drinking water represents the only significant intake of acrylamide. Therefore, only an oral DMEL is derived for this population.

3.5 Corrected dermal and inhalation BMDL10 values have been calculated to account for differences in routes of exposure between experimental animals and humans (r-t-r extrapolation) and for differences in respiratory volumes between experimental animals (usually at rest) and humans (light activity in case of workers). A further adjustment of the dermal and inhalation BMDL10 values for workers has been performed to account for differences between

occupational and lifetime exposure conditions ( $x7/5x52/48x75/40=x2.8$ ). This is shown below.

*Derivation of the inhalation BMDL10 for workers*

Oral BMDL10	Inhalation 8h-BMDL10	Inhalation 8h-BMDL10 adjusted for light activity	Inhalation 8h-BMDL10 adjusted for light activity and occupational exposure conditions
0.3 mg/kg/day	$:0.384 \text{ m}^3/8\text{h}/\text{kg}=0.78 \text{ mg}/\text{m}^3$	$x0.67=0.5 \text{ mg}/\text{m}^3$	$x2.8=1.4 \text{ mg}/\text{m}^3$

*Derivation of the dermal BMDL10 for workers*

Oral BMDL10	Dermal BMDL10	Dermal BMDL10 adjusted for occupational exposure conditions
0.3 mg/kg/day	0.3 mg/kg/day	$x2.8=0.8 \text{ mg}/\text{kg}/\text{day}$

*Derivation of the dermal BMDL10 for consumers*

Oral BMDL10	Dermal BMDL10	Dermal BMDL10 adjusted for consumer exposure conditions
0.3 mg/kg/day	<b>0.3 mg/kg/day</b>	?

3.6 The following default assessment factors (AF) have been selected for the relevant combination of populations and routes.

*Workers, inhalation*

Interspecies	2.5 as 4 implicitly taken into account in the r-t-r extrapolation step
Intraspecies	5
Additional uncertainties for substances that are genotoxic and carcinogenic relating to differences in cell cycle control and DNA repair, which affect the carcinogenic process.	10
Further uncertainties because the starting point is not equivalent to a NOAEL, and the dose-response relationship below the starting point is not known	10
Overall	1,250

*Workers, dermal*

Interspecies	4x2.5
Intraspecies	5
Additional uncertainties for substances that are genotoxic and carcinogenic relating to differences in cell cycle control and DNA repair, which affect the carcinogenic process.	10
Further uncertainties because the starting point is not equivalent to a NOAEL, and the dose-response relationship below the starting point is not known.	10
Overall	5,000

*Consumers, dermal*

Interspecies	4x2.5
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Intraspecies	10
Additional uncertainties for substances that are genotoxic and carcinogenic relating to differences in cell cycle control and DNA repair, which affect the carcinogenic process.	10
Further uncertainties because the starting point is not equivalent to a NOAEL, and the dose-response relationship below the starting point is not known	10
Overall	10,000

*Humans via the environment, oral*

Interspecies	4x2.5
Intraspecies	10
Additional uncertainties for substances that are genotoxic and carcinogenic relating to differences in cell cycle control and DNA repair, which affect the carcinogenic process.	10
Further uncertainties because the starting point is not equivalent to a NOAEL, and the dose-response relationship below the starting point is not known	10
Overall	10,000

3.7 By applying the selected overall population- and route-specific AF to the corrected BMDL10 values, the following DMEL values are derived for acrylamide.

Inhalation DMEL for workers =  $1.4/1,250 = 1.1 \mu\text{g}/\text{m}^3$  (8h-TWA)

Dermal DMEL for workers =  $0.8/5,000 = 0.16 \mu\text{g}/\text{kg}/\text{day}$

Dermal DMEL for consumers =  $0.3/10,000 = 0.03 \mu\text{g}/\text{kg}/\text{day}$

Oral DMEL for humans via the environment =  $0.3/10,000 = 0.03 \mu\text{g}/\text{kg}/\text{day}$