Committee on Hazardous Substances

Guide for the quantification of cancer risk figures after exposure to carcinogenic hazardous substances for establishing limit values at the workplace

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Committee on Hazardous Substances (Ausschuss für Gefahrstoffe - AGS)

Guide for the quantification of cancer risk figures after exposure to carcinogenic hazardous substances for establishing limit values at the workplace

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Committee on Hazardous Substances:

Comprehensive risk borders and quantification of cancer risk figures for activities with carcinogenic substances

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1 Scope of risk quantification

1.1 Preliminary remarks: Principles of risk quantification where data are limited

The present Guide is intended to provide the basis for describing exposure-risk relationships for carcinogenic substances according to harmonised regulations including the option of a scientific rationale for occupational exposure limits for these substances. For this purpose, criteria are established to assess the suitability of available data on a substance and procedures are recommended to determine exposure-risk relationships from these data in the best possible way.

The protection of workers from the risks related to exposure to carcinogenic chemicals (carcinogens) at the workplace is in particular regulated by EU Directive 2004/37/EC (Carcinogens Directive; EU, 2004) and the German Hazardous Substances Ordinance (GefStoffV; Bundesministerium für Arbeit und Soziales, 2005). Under the Carcinogens Directive, "carcinogen" is defined as a substance which meets the criteria for classification as a Category 1 or 2 carcinogen set out in Annex VI to Directive 67/548/EEC (EU, 2007). The same risk management is to be applied to substances of Categories 1 and 2 for carcinogenic substances ("carcinogens") both within the meaning of the Carcinogens Directive and according to the GefStoffV. According to these provisions, it is thus irrelevant whether a substance was identified and classified as carcinogenic on the basis of epidemiological findings (Category 1) or of animal studies (Category 2)[1]. Since cancer is considered to be a particularly severe disease and the Carcinogens Directive assumes that an exposure level below which no health hazard occurs cannot be determined, legislation provides for particularly far-reaching preventive measures for these substances.

Because of their direct relationship to humans, data from epidemiological studies or human studies are of special relevance for describing exposure-risk relationships especially when compared with data from animal studies. However, even though the quality of data may be better, such human data remain a non-desirable exception (since effects on humans must have occurred in this case); therefore, the higher uncertainty resulting from a use of data from animal studies generally needs to be deliberately accepted for an ultimate assessment. Uncertainties in epidemiology are involved in assessing exposure since no measured values are generally available for historical exposures and person-related exposure assessments are inaccurate. Moreover, the possible impact of uncontrolled confounders must always be discussed in epidemiological observation studies (non-interventional studies). In contrast, animal studies can be carried out under controlled conditions and well-defined exposure conditions, but have the disadvantage that animal studies are designed with a smaller number of animals compared with the number of subjects in epidemiological studies. The resulting restrictions in the statistical power of the dose-response relationship established should be taken into account cor-

[1] The GHS (Globally Harmonised System of Classification and Labelling of Chemicals) categories for carcinogens are adopted by REACH (EU, 2006). The current draft provides for two categories: Category 1 for known or presumed carcinogens and Category 2 for suspected carcinogens. Category 1 has two subcategories, 1A and 1B. The classification criteria for the three categories, 1A, 1B and 2, are substantially the same as those of Category 1, 2 and 3 carcinogens in the current EU system.

respondingly. When transferring findings from animal studies, the species differences must also be considered with regard to dose equivalents and modes of action.

The question of the regulation for carcinogenic hazardous substances arises irrespective of the suitability of the database. Risk management involves establishing a limit value using the available, often not sufficiently reliable exposure-risk relationships. Therefore, uncertainties should be determined and specified for every decision that has been taken. Even the conclusion that the available data are not sufficient to establish a quantitative exposure-risk relationship may be drawn. Findings on the modes of action can be included in the selected exposure metrics and in the assessment of the form of the observed exposure-risk relationship. The possible modes of action should be considered in risk extrapolation. This results in a number of assessment standards with different reliabilities of extrapolation.

The scientific community has recently also been discussing minimum doses (known as threshold levels) for carcinogenic substances, *i.e.* exposure ranges below which a hazard is considered to be unlikely contrary to previous conviction – for example because of effective biological protective and repair mechanisms. However, this is controversial, and the methods applied to provide evidence and define such thresholds are problematical (Lutz, 2000; Neumann, 2006a,b,c). Such findings can currently be used for regulatory purposes only if they are adequately verified. This involves the definition of quantitative limits specifying the exposure level for these thresholds in addition to plausibility considerations (for example on the assumed mechanism of action). Quantitative risk assessment together with conventions on risk acceptance are therefore of special importance when establishing limit values for carcinogenic substances. “Risk” is understood to mean the absolute lifetime risk exceeding the background risk after a given exposure (for a more accurate definition see Section 1.4 and Glossary).

To understand risk assessments based on the present Guide it is important to know the general conditions and scientific limits, specify them and accept the assessment made on the basis of the specific data until better data are available. Whereas neither a “real” risk nor a “real” limit value can currently be established by the scientific community, risk managers must accept the scientific assessment as the currently best possible derivation and thus as “presumably real” in order to be able to take action. Since exposure-risk relationships and limit values are derived as anticipated expert opinions and as a precaution, this assumption is possible not least from a legal point of view.

The present Guide deals with the scientific-methodological conventions to be used to bridge knowledge gaps in the area of acceptable and tolerable exposures to carcinogenic substances. The purpose of this Guide is not to weigh economic interests and a social benefit of technology up against health risks to workers (*e.g.* no cost-benefit considerations). The members of the working group “Risk derivation” are however aware that the selection of many standards (*e.g.* definition of adverse effects, confidence interval used as a basis, inclusion or exclusion of specific extrapolation models and interpretation of the term of precaution) implies that judgements are formed from a scientific understanding that is not only based on scientific rationale.

1.2 Validity

(1) The regulations of this Guide only refer to risk quantification for carcinogenic substances within the meaning of the implementation of the Hazardous Substances Ordinance. The risk of developing cancer that is quantified on the basis of this Guide is also to be used for deriving an occupational exposure limit (OEL) for carcinogenic substances under Section 3(6) of the GefStoffV.

(2) For this purpose, this Guide is to help assess exposure-risk relationships according to uniform and transparent methods. The main focus is on the extrapolation of risks into the low dose range where data are limited. Risk management measures can be based on the risk determined in this way.

It is thus possible that the result of risk quantification is not only a point estimate of the risk, but also shows the exposure-risk relationship over a wide range. The Guide can thus also be used for a three-range "traffic light model" (two evaluation points instead of one limit value)[2] and the exposure-risk relationships can help to establish "process- and substance-related criteria" (VSK; verfahrens- und stoffspezifische Kriterien) pursuant to Section 9 (4) GefStoffV (Bundesministerium für Arbeit und Soziales, 2005).

(3) This Guide does not cover other aspects of methods for deriving an OEL for carcinogenic substances nor does it specify the risk level of developing cancer on which the OEL is based, in particular the level of a tolerable and/or acceptable risk.

This Guide will thus not answer the question of a risk level for an OEL. In a separate step, it will however be possible to include regulatorily relevant evaluation points in the established exposure-risk relationship (e.g. conditions for exemptions associated with a given risk level).

All risk assessments are based on cancer incidences from animal studies that recorded both the animals that developed cancer and those that died, as well as from human data, where preference was also given to cancer incidences over mortality data. Questions of the curability of tumour diseases are not considered.

(4) The method of this Guide is not designed to predict actual cancer incidence rates for a real workplace situation or to make projections as to the frequencies of developing cancer in the exposed population.

The misuse of risk quantifications for other purposes (e.g. to project the number of exposure-related deaths) must be avoided. Exposure-risk modelling, extrapolation to low risks and the assumed exposure scenario are subject to specific conventions that are required for a harmonised procedure under the given regulatory conditions, but are not necessarily adequate for other purposes. Thus, this approach does not need to be suitable for example for calculating a compensation claim according to the Occupational Disease Ordinance.

(5) Exposure assessments for individual workplaces are not covered by this Guide. A standard exposure scenario for the workplace is assumed only ("nominal risk") (see Section 4.4).

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[2] Cf. BAuA, 2005

1.3 Importance of default assumptions

(1) The methods proposed in this Guide often have a default character, *i.e.* they are to be applied if no substance-specific information justifies deviation from the default. However, if more qualified, substance-specific data are available, deviation from the default is possible, but needs a scientific rationale and documentation (see Section 8).

Findings of low relevance are not always adequate to justify deviation from the default. Additional findings may also be misused for a risk quantification carried out according to a deviating method: The margin of discretion left open here ("deviation is possible") allows the maintenance of the default and is limited by the required scientific rationale.

(2) In general, assessments with the relatively highest probability (for example: geometrical mean and maximum likelihood estimate) are used to establish the default.

Defining (reasonable) worst case assumptions for all parameters has been expressly avoided. The selection involves a difficult deliberation process, which has to be transparent. The differentiated procedure was selected against the background of the relatively high uncertainty resulting from the extrapolation steps that need to be taken here. At present, there is no procedure (e.g. probability calculation) that is suited to reduce this uncertainty. The combination of numerous worst case assumptions would lead to a risk quantification with a very conservative character. The result cannot be validated and increasingly becomes a matter of speculation. The specified convention is selected in the present Guide to focus the discussion of a scientific rationale on actual risk assessment rather than on a suitable estimate of the range of uncertainty that cannot objectively be defined in more detail.

(3) Assessment of the data for individual substances and the resulting conclusions (for example about the mode of action to be assumed and degree of deviation from the default value in the individual case) are not covered by this method.

The substance-specific procedure – if it deviates from the default procedure formulated here – is based on standards that must be substantiated for each individual substance.

1.4 Definition and classification of the risk figure

(1) This Guide deals with the methods of calculating a risk figure. The risk figure calculated under specific assumptions for the purposes defined in the introduction is a value for the exposure-related lifetime risk in the scenario of exposure over the entire working lifetime (for defined exposure scenario see Section 4.4). The lifetime risk refers to the likelihood that a person will develop a specific type of tumour or cancer if mortality from other causes is about equally high as in a non-exposed population. The risk figure can also be referred to as a (statistical-mathematical) estimate of the excess risk or as additional risk or extra risk since the background incidence was specifically taken into account here (see Section 3.5).

A number of scientists believe that the validity of the excess risk determined in animal studies for an excess risk in humans is so low that they reject a risk quantification made on this basis because of too high uncertainties. However, with one exception,

the authors of this Guide support using the risk figure with an interpretation as excess risk. They expressly refer to the definition (explicit specification of the boundary conditions of the calculated risk and uncertainty) and distinction from risk that can actually be observed in humans.

The term lifetime risk indicates the inclusion of the total period up to old age is considered, the same distribution of lifetimes being used as a basis as in a general population or in the control group of a carcinogenicity study (Becher and Steindorf, 1993). In the practice of quantitative risk assessment, however, derivation of the risk generally refers to a specific age, i.e. about 2 to 2.5 years in animal studies and 70 to 90 years for epidemiological data (e.g. 89 y.: Goldbohm et al., 2006; 85 y.: Attfield and Costello, 2004; Rice et al., 2001; SCOEL, 2003; Sorahan et al., 1998; Stayner et al., 1998, 2000; 80 y.: HEI-AR, 1991; 75 y.: Stayner et al., 1995; Steenland et al., 2001). The 2006 Statistical Yearbook of Germany (Statistisches Bundesamt Deutschland, 2006) includes average life expectancies that were calculated by means of the age-specific mortality rates of 2002/2004. Accordingly, the statistical life expectancy (from an age of 20 years) is up to 76 years for men and up to 82 years for women. The cancer risk based on the life table method should therefore be calculated at least up to an age of 80 years.

Risk management can be based not only on risk figures, but also on the ALARA principle (as low as reasonably achievable). The ALARA principle on its own is considered to be inadequate to establish priorities in handling carcinogenic substances in a differentiated way. In principle, the ALARA can be followed in parallel. This Guide does not specify this risk management instrument.

(2) The present concept identifies a risk figure defined in (1) rather than a margin of exposure (MoE; see Glossary; cf. *e.g.* EC, 2006); in this way, the nominal risk can be quantified for a wide range of the exposure-risk relationship.

The procedure of identifying a risk figure (quantified risk) instead of a MoE is also used because it is desirable that OELs that are to be calculated later can regularly be based on the same (assumed) nominal risk (defined level of protection). It is not sufficient to determine a MoE for this classification.

As a final step of risk characterisation in the chemicals assessment with a MoE,

- *a quantification is made (margin between a prevalence – for example as a benchmark dose (10%) – and the exposure level is calculated)*
- *this margin is assessed, i.e. it is interpreted as "sufficient" or "not sufficient". There have been no regulations to date as to how non-linearity in the dose-response relationship assumed via the mode of action should be reflected in the interpretation of this measure of the margin.*

(3) This approach based on the selection of the risk figure as an assessment criterion differs from the concept of the European Food Safety Authority (EFSA). The EFSA approach results in a point estimate (sufficiently safe dose or concentration specified), whereas the present concept defines the exposure-risk relationship over a broad possible exposure range.

While the risk figure is based on the average risk (sensitive persons are protected if the risk for moderately sensitive persons is sufficiently low), the EFSA concept tries to explicitly consider the protection of sensitive groups of persons by means of safety factors. If the safety factors are sufficiently high, no residual risk is quantified, which is similar to assuming a threshold (see EFSA, 2005).

The guides for compiling a Chemical Safety Report (CSR) under the chemicals policy (REACH) propose using the risk figure for specifying a DMEL (derived minimal effect level) (here intended procedure) or, alternatively, the method according to EFSA (modified). The EFSA procedure was originally designed to describe a margin required between prevalence in the experimental scenario and exposure level after ingestion rather than for the workplace (different safety factors), but it can be adjusted accordingly. There are currently no supportive statistical data or regulations for the levels of the safety factors used in the modified EFSA procedure (interspecies variability, intraspecies variability and further individual differences in cancer defence mechanisms). Society would have to agree on conventions used (1% risk for sensitive persons notified). Standards would have to be established for deviating from the default procedure for a specific substance when more qualified information is available (differentiated procedure for different modes of action). The result for a DMEL routinely calculated according to the modified EFSA procedure can however be identical with that for a DMEL calculated according to the concept of the risk figure. There is currently no social consensus on a tolerable and/or acceptable (nominal) risk level for the risk figure being used and transformed into a DMEL under REACH (this risk level must be specified if the present Guide is also to be used on a national level, for example for establishing an occupational exposure limit for carcinogenic substances).

1.5 Database

(1) If human data are available for risk quantification, these must primarily be reviewed for their suitability for risk quantification and used, if appropriate, but the data quality (incidence data; course of exposure) is to be considered. Risk quantifications on the basis of animal studies and human epidemiological data must be compared with each other (plausibility check with human data).

Epidemiological studies can be used only if effects (tumours) occurred in humans. Negative epidemiology can generally not be used for the plausibility check of a positive finding from an animal study. For the classification of the relevance of human data compared with animal studies see also Goldbohm et al., 2006.

(2) The procedure of this Guide takes into account that only data from animal studies can be used as a basis for risk quantification in most cases; the definitions used in this Guide thus apply to data from animal studies, although human data are treated in the same way unless another procedure is described in the specific quantification step.

(3) Non-positive epidemiological study results are generally not evidence of the absence of a potential risk. They must be interpreted with due caution and their suitability for the question concerned taken into account (statistical power, exposure level and quality of exposure classification).

Literature:

Ahlbom et al., 1990

Doll and Wald, 1994

1.6 Data quality

(1) If a minimum quality is guaranteed (see Section 7 of this Guide), risk quantifications can generally be made. Limited quality and a resulting uncertainty must however be documented in the particular step of risk quantification.

Studies with a quality that is possible or desirable today cannot always be assumed as a basis for risk quantification. There is no clear-cut dividing line between the lack of data quality and uncertainties inherent in the process of risk quantification if knowledge is incomplete. Therefore, only a cut-off criterion can be defined when the total uncertainty (from poor data plus risk quantification with extrapolation steps) is so great that the resulting statement is to be considered speculative and can thus no longer be used (see Section 7). The particular individual step of risk quantification and Section 1.3 of this Guide – additionally – establish how uncertainties should be handled.

2 Discussion of the predominant mode of action

2.1 Mode of action as a guidance parameter for risk quantification

(1) Information on the predominant mode of action or the predominant modes of action of the observed carcinogenic effect of a substance is useful both for determining the point of departure (Section 3) and for extrapolation into the low risk range (Section 5). For this purpose, the following factors must be characterised: a) the type of possible genotoxic effects, b) the type of non-genotoxic events as impact parameters on the multifactorial process of carcinogenicity, and c) the respective importance of these factors for the mode of action of carcinogenicity and the uncertainty of the relevant conclusion. The results must be documented in an appropriate way (Section 8).

2.2 Primary and secondary genotoxicity

(1) It must be examined whether direct interaction of the substance with the genetic material is substantiated or to be assumed based on other information. Secondary genotoxicity (*e.g.* via oxidative stress, interference with the mitotic process, inhibition of topoisomerase, inhibition of the DNA repair enzymes, etc.) is to be distinguished from primary genotoxicity (direct/indirect: DNA interaction, adduct formation and mutations caused by the parent substance or metabolites).

(2) The quality and verification of the assessment of genotoxic properties must be characterised (differentiation according to *in vivo/in vitro* findings, compatibility of the available study results, impact of the dose range in the available test and information about gaps).

(3) Information on genotoxicity (type of genotoxicity and quality and verification of the findings) can be essential for the specificity on the target organ in which tumourigenicity was observed. For some forms of genotoxicity (*e.g.* aneuploidies), minimum concentrations of dangerous substances that are required to cause cancer can be assumed.

In the assessment of genotoxicity tests, it must be considered that up to 80% of the substances that are negative in carcinogenicity tests in rodents are positive in one or several in vitro tests. This applies particularly to chromosome aberration tests, micronucleus tests and the mouse lymphoma test. Depending on the test system used and the class of substances, there are numerous reasons why in vitro results cannot be transferred to the in vivo situation; some of them are listed below by way of example:

- the use of high concentrations that overload metabolic detoxification mechanisms,

- absence of phase II enzymes and their cofactors in the test system,

- test system with DNA repair deficiency (all Salmonella strains and E. coli),

- test system without or with abnormal expression of p53 protein (CHO cells, L5178Y cells and V79 cells), and

- effects with a threshold that is not reached in vivo: aneuploidy, inhibition of DNA polymerase, of topoisomerases or kinases, cytotoxicity or pH change.

Transferability to humans is furthermore restricted through a use of rat-specific metabolic activation that does not reflect the pattern of activating enzymes metabolising xenobiotics in humans (Kirkland et al. 2007a). However, it is possible that activation in the organism is not reproduced in standard in vitro tests, e.g. if the substance is activated via sulfotransferases and false negative results are therefore obtained (Kirkland et al. 2007b).

The relevance of in vitro genotoxicity test results must therefore be examined on the basis of the conditions used in the tests (e.g. comparison of the dose-response relationships of genotoxicity and cytotoxicity and high dose effects) and of the structure of the tested substance to decide whether a carcinogenic substance is primarily genotoxic. If necessary, structure-effect relationships should be included. In unclear cases, the results of valid in vivo tests are decisive for systemically acting carcinogens. For locally acting carcinogens, negative in vivo tests are conclusive only if it has been demonstrated that the target organ was reached.

2.3 Non-genotoxic events

(1) Information on non-genotoxic effects with a potentially causal impact on the process of carcinogenicity must be recorded and described and the dose range determined must be compared with the carcinogenic doses. This mainly includes cytotoxicity (*e.g.* irritation, inflammation and necrosis), induced cell proliferation, toxicokinetic information (*e.g.* enzyme induction, saturation or new metabolites typical of high doses), receptor-mediated processes, protein binding, direct hormonal effect, indirect impact on hormonal feedback systems, organ specificity and sex specificity.

(2) The quality and reliability of the assessment of non-genotoxic properties must be characterised (differentiation according to *in vivo/in vitro* findings, compatibility of the available study results, impact of the dose range in the available test or information about gaps).

(3) Information on non-genotoxic events (type of effect and quality and reliability of the findings) must be specified particularly for its relevance in the target organ in which tumourigenicity was observed.

2.4 Relevance of different impacts in a multifactorial process

(1) According to a weight-of-evidence approach, the relevance of primary and/or secondary genotoxicity (see Section 2.2) and of non-genotoxic events (see Section 2.3) to the process of carcinogenicity must be assessed. The central factor(s) of impact on cancer is (are) to be described and its (their) assumed relevance to humans substantiated.

(2) A distinction of the assumed modes of actions differentiated according to tumour localisation and/or dose range may also be a result. The existence of several (possible) modes of action must be identified.

(3) The occurrence of pre-malignant effects (like the formation of foci in the liver) must be examined and their dose-response relationship described, if possible.

(4) Background rates and the occurrence of spontaneous tumours in the control group are to be assigned to the discussion of the mode of action.

2.5 Targeted conclusion

(1) After all the information has been recorded, the following statements can be made:

- **Postulated mode of action**
- **Key events (observed; agreement with mode of action)**
- **Dose-response relationship**
- **Time-related association**
- **Intensity of the association; consistency of the data for this conclusion; specificity of the association**
- **Biological plausibility**
- **Other possible modes of action**
- **Confidence in the assessment**
- **Data gaps; uncertainties**

(2) The following questions must specifically be answered:

- **Is the weight of evidence sufficient to identify a mode of action in an animal study?**
- **Can human relevance of the mode of action be ruled out with sufficient likelihood on the basis of fundamental qualitative differences in key events between animals and humans?**
- **Can human relevance of the mode of action be ruled out with sufficient likelihood on the basis of quantitative toxicokinetics and/or toxicodynamic differences between animals and humans?**
- **What is the confidence of a generated assessment (relevance)?**

There may also be a threshold for genotoxic events. Genotoxic events must be differentiated from this point of view (see TGD, Risk Characterisation, Section 4.14.3.4; Butterworth, 2006).

Non-genotoxic events cannot regularly be associated with a threshold either; for example, such a threshold cannot always be identified for some receptor-mediated processes (see TGD, Risk Characterisation, Section 4.14.3.3; Butterworth, 2006).

As far as data for an exposure-risk relationship in the experimental range are required to determine the relevance of the different statements, there is an interdependence between tasks according to Section 3 and tasks according to Section 2 of this Guide (in particular 2.4 and 2.5: Exposure-risk relationship). Accordingly, the items of this Guide cannot be dealt with in a strict chronological order.

The items mentioned under 2.5 are based on considerations by WHO (IPCS) and are explained in detail in Boobis et al. (2006). Examples of the procedure in the discussion of the mode of action can be found in Kirman et al. (2004), Cohen et al. (2003) and Preston and Williams (2005). The basic method for recording the mode of action is explained in Meek et al. (2003) and Seed et al. (2005).

In various publications (e.g. Streffer et al., 2004, Hengstler et al., 2006, Bolt and Huici-Montagud, 2007 and Foth et al., 2005), the differentiations of the mode of action postulated were similar to those used as a basis for the procedure described here. They lead to a differentiation, as is shown in Section 5.1 of this Guide.

Neumann (2006a,b,c) substantiates why it is impossible to find a definite threshold for a carcinogenic effect and recommends avoiding the term completely. However, since there are no alternatives that can be communicated better, the term will continue to be used in the present Guide with the above restrictions of its meaning.

3 Risk quantification in the range of observed cancer incidences

3.1 Selection of animal species, sex and tumour localisation(s)

(1) If tumour data are available for several of the customarily used animal species, preference is to be given to those on the species reacting most sensitively.

(2) The extent to which quantitative transferability to humans can be assumed must be considered for the selection of the animal species and the types and localisations of tumours observed there. Transferability can be assumed in particular if a tumour localisation is identical in a species comparison and/or findings on the mode of action support the occurrence of a specific type of tumour (or a specific tumour localisation).

Animal studies are carried out against the background that qualitative and quantitative transferability to humans is possible in principle (if necessary, considering extrapolation or correction factors). Thus, preference must always be given to the animal model with the closest relationship to humans. If it is not known which animal model is closest to humans in a particular case, a conservative approach is a suitable standard. This basically applies even if discrepancies were demonstrated in the individual case: The human metabolism of 1,3-butadiene seems to be more like that of the less sensitive rat than that of the more sensitive mouse. If risk quantifications based on epidemiological data are compared with those based on animal studies, agreement of the cancer risk for mice and humans is higher for 1,3-butadiene (Roller et al., 2006). This possible contradiction in the case of 1,3-butadiene means that a) particular importance is to be attached to human data (see Section 1.5(1)), b) conservative extrapolation steps such as assuming linearity in the low risk range should not be aban-

doned too hastily because of supposed mechanistic evidence, and c) the relative sensitivity of test animals compared with humans must be examined further.

(3) A tumour localisation observed in an animal study and that deviates from observations based on human epidemiological studies does not generally militate against its human relevance (see references under 3.1 (6)). The resulting risk quantification must however be regarded as less reliable.

(4) If increased tumour incidences were obtained in both sexes, the data for the sex with the higher tumour rate must generally be used. If the tumour rates are about the same in both sexes, the data can be added for both sexes to increase the statistical validity.

(5) If tumours were found in several organs, the data on all organs for which a statistically and/or biologically increased tumour incidence was observed at a specific dose and/or a statistically significant dose-response relationship (possibly only as a trend) was evident are to be used.

There are numerous typical forms of tumours whose spontaneous incidence is high and sometimes considerably varies in specific rodent strains and whose relevance to humans is not known (see 3.1 (6)). If their frequency is increased as a function of the dose compared with the current and mean historical control, an exposure-related effect is generally assumed.

Initially, it must be examined whether other types of tumours that can definitely not be assigned to spontaneous pathology occurred, possibly at even lower doses and/or at a higher incidence, and whether preference should be given to them as a basis of calculation for this reason alone.

(6) Whether or not specific tumour localisations (if necessary, with a restriction to specific animal species or strains) are taken into account must be considered on a case-by-case basis. The following references may provide answers to the question of the (qualitative and/or quantitative) transferability to humans:

- **No (qualitative or quantitative) transferability can be assumed for alpha2u-globulin-induced renal tumours of male rats.**
- **In general, only qualitative (no quantitative) transferability can be assumed if there is concurrent genotoxicity (nor is there qualitative transferability in the absence of genotoxicity) and if the following tumours are observed: liver tumours after PPARα-receptor stimulation ("peroxisome proliferation"), leukaemia in Fischer 344 rats, phaeochromocytomas if these only occur in male F344 rats, forestomach tumours and tumours of Zymbal's and Harderian glands as well as clitoral and preputial glands, if no other than theses tumour localisations are found.**

  The strictly qualitative species comparison is relevant for classifications, but not for determining the exposure-risk relationship considered here or for establishing a concentration with regard to a defined risk figure.

- **In general, quantitative transferability can be assumed with concurrent genotoxicity, but with more uncertainty (i.e. qualitative transferability only in the absence of genotoxicity) and if the following tumours are observed: Leydig cell tumours in rodents, liver tumours in B6C3F1 mice,**

phaeochromocytomas in F344 rats if these occur in both sexes (differential diagnosis on – age-related – hyperplasias to be considered; female animal data more appropriate for quantification), thyroid tumours in rats, forestomach tumours and tumours of Zymbal's and Harderian glands as well as clitoral and preputial glands if, apart from these tumour localisations, other tumour localisations are found.

- **In general, quantitative transferability can be assumed even without genotoxicity, but with considerable uncertainties in some cases: all other localisations and types of tumours; tumours in animal species or strains except for those mentioned.**
- **The substance concentration (observed or to be assumed) in the target organ is to be included in the consideration of quantitative transferability.**
- **The mode of action to be assumed (see Section 2) is to be included in the consideration of a quantitative extrapolation.**
- **If tumour incidences were obtained both in a) localisations with questionable human relevance and/or questionable quantitative transferability and in b) localisations with definite quantitative transferability, preference is generally to be given to the latter ones for risk quantification.**

A more detailed discussion about the background of this differentiation can be found in Annex 10.3 to this Guide (with literature references).

(7) The tumour incidences in the various organs selected under (5) and (6) must generally be quantified separately and compared with each other. In the standard case, risk quantification is based on the tumour localisation with the lowest T25, *i.e.* a dose or concentration at which cancer occurs in 25% of the animals. The different background rate is taken into account in the T25 calculation. In some exceptional cases, however, different tumour localisations must be combined (example: asbestos – mesotheliomas and lung tumours). If such an aggregation is made, the relevance of the total incidence for risk quantification must be substantiated.

In T25 procedures, based on a concentration with a significantly increased tumour incidence, a dose at which the incidence for this tumour in an animal study is 25% after lifetime exposure is determined by linear interpolation (i) taking into account the background incidence, (ii) if applicable, with correction of a non-lifetime study period, and (iii) assuming complete absorption (see also Glossary).

Calculation of a T25 or BMD for several tumour localisations, sexes and with or without benign tumours in later steps allows extrapolations to be made into the low risk range based on several points of departure (see Section 3.2) in parallel and together with a differentiated mechanistic discussion. Aggregations of findings are useful particularly if the question of the differentiation of various dose-response relationships (e.g. because of the homogeneity of the reactions observed) is of minor importance. It may thus be appropriate to aggregate the findings over different tumour localisations if a carcinogen has a uniform mode of action. The EU TGD points out: "For a substance inducing more than one type of tumour, the determination of a dose-descriptor value is from each relevant tumour type rather than from the number of tumour bearing animals. If several relevant data sets on tumour incidences are available, dose descriptors values should be derived for all these." (EC 2006, Section 4.14.2.3). Sev-

eral tumour localisations should not be aggregated if there are different background rates of tumours in different organs.

McConnell et al. (1986) argue in favour of a differentiated consideration of the possibilities of aggregating tumours for cancer risk calculations. The EPA interprets this evaluation: "The incidence of benign and malignant lesions of the same cell type, usually within a single tissue or organ, are considered separately and are combined when scientifically defensible." (A list of cases in which aggregations can be made is included in McConnell et al.).

The principle of adding up the total number of tumour bearing animals irrespective of the tumour localisation concerned is thus not supported.

Some older studies were designed in such a way that only suspected target organs were evaluated. Such selective studies can nevertheless be used for risk quantification if they reveal carcinogenic effects. Multiple tumours (multiplicity) are usually reported additionally if they are observed.

(8) If several types of tumours were found in one organ/tissue, a combined consideration should generally be chosen. In certain substantiated cases (*e.g.* human relevance of only one type of tumour), an individual consideration is appropriate.

(9) If benign and malignant tumours are obtained in one organ, their incidences are generally added. Different types of tumours found in *one* animal are not added since the total incidence (related to the organ > 100%) may be exceeded. If there is evidence that, for example, the malignant degeneration of a benign tumour in humans is unlikely, no addition is required based on a scientific rationale.

3.2 Selection of a point of departure

(1) The point of departure (POD) for further steps of risk assessment is a defined exposure level with risk assignment to the concentration-risk function for a substance. The POD is at or close to the exposure level (concentration range) for which data on cancer incidences are available from epidemiological observations or animal studies. For the POD, the risk as cancer incidence in percent is compared with the relevant concentration (mg/m^3). The POD is a normalised value. "Normalisation" is to be regarded as the conversion to lifetime (occupational) exposure (see Section 4.3), route-to-route extrapolation to the route of inhalation (see Section 4.2) and consideration of the background incidence (see Section 3.4) in an appropriate way. The POD is a starting point for extrapolation or for comparison; depending on the level of comparison, the T25 is thus to be specified as a human equivalent (hT25) or to be applied at the level of animal studies. The boundary conditions for using a T25 must always be specified precisely.

(2) If data of sufficient quality are available from observations, the POD is to be identified as the benchmark concentration or benchmark dose. The central estimated value (BMD) rather than the 95-percent confidence interval (BMDL)[3] is to be used here.[4] The POD is used as a starting point

[3] For terminology on the benchmark procedure see EPA, 2000

for extrapolation or for comparison; depending on the level of comparison, the benchmark dose can thus already be specified as a human equivalent (HBMD; HBMDL)[5] or can be applied at the level of animal studies. The boundary conditions for using a benchmark dose must always be specified precisely.

The criteria of sufficient data quality for modelling according to the benchmark approach must be defined separately (see Section 3.3). The relation between BMD and BMDL also indicates the quality of the applied modelling (quality of adjustment of the model function to the available experimental data). For the calculation of the BMDL, this factor can thus be used (apart from other criteria) for assessing the question of whether the benchmark approach should be applied at all in a particular case.

Selecting the BMD instead of the BMDL may imply a certain error (since it cannot be ruled out that the exposure-risk relationship is more appropriately described by the BMDL). However, selection of the BMD seems to be justified 1) because of analogy to the T25 where data are not adequate (T25 is also a central estimated value without confidence interval), 2) because of the possible low error (if there is a major deviation between BMD and BMDL, the benchmark approach would not be appropriate), and 3) since a conservative extrapolation procedure is selected anyway in most cases because of linearisation in the range below the BMD as the POD.

For conversion of a benchmark dose to equivalent human exposure see Section 4.

(3) The benchmark response at the POD is to be established at 10% for reasons of comparability.

In many cases, there are only minor deviations for the assumed risk if the T25 is compared with the BMD10 after correction (linear conversion) of the risk level (see Annex to EC, Technical Guidance Document, 2006). There may however be deviations depending on the course of the concentration-risk relationship. Therefore, and because of the more complete description of the derived course of the concentration-risk relationship in the experimental range, preference is to be given to the application of the benchmark approach. For examples see Section 5.2.

The present Guide continues to use modelling between the BMD10 and BMD0.1 in cases in which there is mechanistically substantiated non-linearity together with a good database (see Section 5.2). If the reasons for non-linearity are not sufficient, modelling with the benchmark method is carried out only for the experimental range up to a BMD10 as the POD. Earlier, the U.S. EPA used the linearised multistage (LMS) model. This procedure is almost identical with modelling by means of the multistage model in the experimental range and a continuation of the modelled function into the low risk range (e.g. if there is a benchmark response of 1:1000). In the LMS model, the confidence interval of 95 percent is however included.

(4) If a sufficiently qualified benchmark concentration cannot be specified, the T25 is to be used as the POD for the calculation according to the method of Sanner et al. (2001)/Dybing et al. (1997).

In cases in which the benchmark approach cannot be used, preference is given to the T25 as the POD over similar other values because

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[4] BMD (benchmark dose) or BMDL are used below even if airborne concentrations are referred to in the specific case (BMC; BMCL).

[5] For relevance of the term human equivalent and for conversion see Section 4

– *this corresponds to the method of risk quantification in various EU provisions on risk assessment,*

– *the "Steinhoff" method discussed earlier in Germany is compatible with the T25 as the POD, although it is not related to a normalised percentage (25%), or*

– *the LED(10) in the United States (EPA, 2005) requires using the benchmark approach although this is not always adequately qualified.*

The U.S. EPA ED(10) approach is also based on benchmark modelling (without consideration of the confidence interval) and its method is identical with the derivation of the BMD10. Since the difference between the T25 and ED10 is linearly taken into account when calculating a reference MoE (see "Margin of exposure" in the Glossary) according to EU/TGD, the ED10 may be used as the POD in the EU MoE approach.

(5) Specification of a POD is not formally required for extrapolations into the range below the obtained incidences for which continuation of the concentration-response relationship is assumed, as it exists in the range of observation (continuous function; see Section 5.2). It should nevertheless be specified for comparison.

(6) BMD_{10} or T25 must be calculated for all tumour localisations relevant to humans (for selection of tumour localisations and species see Section 3.1)

(7) For benchmark modelling with poorer data quality (see Section 3.3), it is appropriate to calculate both the T25 and the BMD_{10} to identify the effects of the uncertainty of the specific decision: The PODs established according to the respective procedures may be close together or show clear discrepancies. The specific information must be documented.

For examples see Section 5.2 (Case B)

3.3 Minimum criteria of data quality for application of the benchmark approach

(1) In general, data for at least the control group and three dose groups should be available when the benchmark approach is selected.

In Annex XI to the EU TGD, there are some examples in which the T25 is compared with the BMD05. The mentioned criterion was underlined.

(2) If the tumour incidence is identical, or differs only slightly in all dose groups (plateau effect), application of the benchmark approach is not appropriate.

(3) If there is only 1 dose group except for the control in which the effect level is clearly above the BMR[6], application of the benchmark approach is not appropriate.

(4) If the tumour incidence is below 100% in only one dose group (except in the control), application of the benchmark approach is not appropriate.

(5) The benchmark approach cannot be applied if fitting is not adequate based on modelling with the available data (model fit: $p < 0.1$; chi square

[6] For abbreviations in the benchmark approach see Glossary

outside -2 to +2). The uncertainty of the assessment is also too great if the BMD/BMDL ratio is > 10 in the considered BMR.

The listed criteria (2)-(5) are discussed and substantiated in the final report of the FKZ 201 65 201/01 project ("Vergleich der Verfahren zur Ableitung gesundheitsbezogener Wirkungsschwellen (Benchmark – NOAEL)" (Comparison of the procedures for deriving health-related thresholds (benchmark – NOAEL)), German Federal Ministry of the Environment 2003).

(6) For unclear cases with limited data quality, the procedure according to Section 3.2 (7) is to be selected, *i.e.* T25 and the benchmark approach must be weighed up against each other. The scientific rationale for the procedure must be documented.

For an example see Section 5.2 (Case B)

3.4 Application of the benchmark approach

(1) The models to be selected for curve fitting should be consistent with the mechanistic considerations about carcinogenicity. Therefore, the multistage model, which corresponds to the multistage model of carcinogenicity, is often used. The gamma function also corresponds to a mechanistic understanding of the multihit model of chemical carcinogenicity. Multistage or gamma function are thus the preferred models for modelling with the benchmark approach in the experimental range. Other models should however also be considered if the data can be adjusted in a clearly better way. Preference is to be given to models that have a similar quality of adjustment, but require fewer parameters for modelling (discernible from the AIC value from the results reported by the specific U.S. EPA software). The quality of data adjustment is more important in the range of low experimental concentrations than in the range of high concentrations.

The listed criteria (2)-(5) are discussed and substantiated in the final report of the FKZ 201 65 201/01 project ("Vergleich der Verfahren zur Ableitung gesundheitsbezogener Wirkungsschwellen (Benchmark – NOAEL)" (Comparison of the procedures for deriving health-related thresholds (benchmark – NOAEL)), German Federal Ministry of the Environment 2003).

3.5 Handling of background incidences

(1) In compliance with the standard procedure in the T25 and benchmark approaches (according to U.S. EPA software), the extra risk approach is generally to be used.

From a toxicological point of view, there is no well-founded scientific rationale for the convention of selecting the extra risk, although it is accepted as a standard procedure, since (i) the deviations are generally slight if there is a low background rate, (ii) there is agreement with many older unit risk calculations, (iii) there is guaranteed agreement with the T25 approach, and (iv) there is guaranteed agreement with the traditional procedure in the multistage approach.

(2) If very high incidences are observed in the control group or when comparing them with human data, the additional risk is to be used and a scientific rationale is to be given for this procedure.

3.6 Risk quantification by specifying the T25

(1) If a POD is established by specifying the T25 value according to the method of Sanner et al. (2001) and Dybing et al. (1997), no modelling of the dose-response relationship in the experimental range is required. The T25 is determined by linear interpolation. This procedure is to be used regularly if a qualified benchmark calculation cannot be made.

For a detailed definition of the T25 see Glossary

(2) If only the route of inhalation is relevant (applies to occupational exposure limits), the T25 value is expressed as airborne concentration (mg/m³ or ppm).

For further standardisation of the T25 to the exposure pattern at the workplace see Section 4.2

(3) Details on the procedure used in this T25 approach are available in the cited literature (*e.g.* EC, 1999, or REACH RIP 3.2-1B preliminary Technical Guidance Document). The most important items are:

- **The lowest dose group showing a significantly increased tumour incidence is selected as the point of departure.**

  The criterion of significance is to be established either on a statistical (Fisher's exact test to compare the dose group with the control group) or on a biological basis. In analogy to the FDA (2001), levels of significance of $p < 0.05$ are to be used for rare tumours or tumours with a spontaneous incidence ≤ 10%, and $p < 0.01$ for tumours with a spontaneous incidence higher than 10%. If necessary, both the experimental control group and the historical control data are to be employed for comparison (for historical control incidences see e.g. Derelanko and Hollinger, 2002).

- **The spontaneous incidence in the control group is subtracted from the tumour incidence in the treated group.**

  If there is high mortality in the considered dose group, the resulting greater uncertainty of the T25 value must be discussed, or the next lower dose group must be selected, since mortality rates are generally not corrected. High mortality may also mean that the study can no longer be used for risk quantification (see Section 7, minimum criteria).

- **T25 values are generally calculated separately for species, sex and organ/type of tumour (see Section 3.1 (6)).**

  The types of tumours/organs/sexes can be combined if this is scientifically substantiated (see Section 3.1(6)).

- **A shorter exposure period compared with the standard lifespan of the test species and a reduced single-day exposure period are corrected.**

  The shorter exposure period (w1 in weeks) compared with the standard lifespan (w in weeks) of the test species and a reduced single-day exposure period (w2 in weeks) is corrected by multiplication with the factor (w1/w)x(w2/w) (see Section 4.4).

- **Exposure patterns deviating from the selected standard values are considered.**

  Linear correction factors are used for this, for example for doses/day, exposure days/week and exposure period/day in the case of inhalation.

- **The lowest T25 value regarded as relevant to humans (with regard to species/organ/type of tumour) is used for risk quantification (see also Section 3.1).**

  This does not fully agree with the usual procedure according to EU. The T25 value was originally designed as a dose of the substance related to body weight and was thus specified in mg/kg body weight/day. If several studies are available in which gavage was not used in every case, but animals were for example exposed via the drinking water, diet or inhaled air, conversion of exposure to the body weight-related dose has been suggested to be used as the common basis of comparison (EC, 1999). However, in the present case, a concentration must be specified (mg/m^3).

  If no route-to-route extrapolation is permitted (see Section 4.3), the specific (oral or dermal) point of departure may be used for an inhalative T25.

(4) The T25 is converted to a human equivalent (hT25) by means of the factors specified in Section 4.

3.7 Procedure in the case of available human data

The relevance attached to epidemiological observation studies in the quantification of occupational cancer risks as compared with animal studies has already been discussed in Section 1.1 and in the explanations of the data to be used as a basis (Section 1.5 (1)). For the risk term applied here see Section 1.4 (Risk figure)

The following references on the procedure require an adequate epidemiological database (for minimum criteria see Section 7.6 of this Guide).

(1) The selection of epidemiological studies should be based on the following procedure:

- **Evidence from available studies should be identified by means of a well-structured, systematic literature search and reviewed for its quality and suitability for risk assessment. Principles established for the selection of occupational epidemiological studies for carrying out a meta-analysis should be considered here. It must be decided in each individual case whether several studies are combined to a pooled estimator for an assessment in a meta-analysis or whether individual studies are assessed separately to be able to specify a range of potential risk scenarios.**

  Literature: Blair et al., 1995; Roller et al., 2006, Chapter 5.2

- **In general, analytical study designs with an individual exposure estimate are to be selected for risk assessment. Both cohort and case control studies can be used for risk assessment.**

  Study designs used in occupational epidemiology can be classified in the following descending order of evidence: (1) cohort study; (2) case-control study (CCS); (3) cross-sectional study (CS); (4) ecological or correlation study (see also Glossary).

  Quantitative exposure data are more often available from cohort studies, whereas case-control studies generally guarantee a better consideration of confounding (for further details on the special strengths and weaknesses of study designs see Ahrens et al. 2008). When justified, in exceptional cases, e.g. in the case of a case-control

study embedded in a cohort with more specific or detailed information on exposure and/or the end point, a CCS can be more appropriate for an assessment of occupational exposure limits than the underlying cohort study.

(2) The consideration of target parameters should be based on the following procedure:

- **In general, preference is to be given to measures with reference to cancer incidence over those to cancer mortality unless incidence and mortality are regarded as identical because high lethality is involved in a specific type of cancer (as e.g. in the case of lung carcinoma).**
- **The information density of the strata decreases, the more finely the considered end points are classified. It must thus be considered in each individual case whether different end points can be combined in an appropriate way to increase the statistical power (i.e. combination of various related tumour entities into one group) even if causal factors may differ in detail, e.g. in the case of leukaemias and lymphomas, head-neck tumours, etc.**
- **It must be decided in each individual case whether early end points such as biological markers, which are regarded as necessary precursors on the causal chain to an examined target disease, may be included in the assessment of the available studies as a surrogate parameter. It is appropriate to include them if evidence of an early clinical effect is to be regarded as a warning signal.**

  (Warning signals can justify the introduction of preventive measures.)

(3) The following procedure may be used for the calculation of the risk figure:

- **A point estimator for every exposure category (e.g. median and geometric mean) is the preferred specification for risk derivation.**
- **If merely an exposure range was reported (e.g. 1-9 ppm-years), the class mean (here 5 ppm-years) can be used alternatively as a basis for the calculation. Concentrations specified in mg/m^3 should be converted to substance-specific ppm. This calculation is based on 240 working days/year and an inhaled volume of e.g. 10 m^3 per working day, which is estimated to be 8 hours (the inhaled volume depends on the workload; 10 m^3 refers to slight to moderate physical activity).**

  (See van Wijngaarden and Hertz-Picciotto, 2004 and Section 4.5 of this Guide)

- **Subsequently, the cumulative concentrations specified in ppm-years must be converted to the long-term mean after 40 years.**
- **Depending on the database, direct measures of absolute risk (e.g. cumulative risk) or – if these were not reported – measures of the relative risk must be related to exposure. Measures such as SMR, SIR, RR or OR will generally be available. For the calculation of the lifetime risk of the exposed persons, these relative risk increases can be multiplied by an estimated value for the lifetime risk of the reference group, e.g. the general population, unless the detailed life table method is used.**

- **The risk measure reported for the exposure range (RR/SIR, etc.) can be correlated with the cumulative exposure value in a regression analysis, which allows extrapolation into the high or low risk range and statements to be made about the risk per unit increase (1 ppm) of exposure. In this way, the lifetime risk can be assessed in relation to a specific exposure level or an assumed occupational exposure limit.**
- **After subtraction of the risk of the non-exposed persons (e.g. general population), an estimated value of the exposure-related excess risk is obtained.**
- **Restrictions of the validity of the results are to be discussed.**

A procedure in analogy to Roller et al., 2006 and Goldbohm et al., 2006 has therefore been suggested.

Bias, possible residual confounding and misclassification, for example, may restrict the validity of the results. Risk estimators that were adjusted for confounder effects should be used. Calculations of adjusted vs. non-adjusted risks should be compared with each other, if possible, since adjustment depends on the model and this allows for an assessment of the intensity of possible confounding.

Inconsistent or non-existing dose-response relationships can often be observed in epidemiological studies. However, the data can also be considered in cases in which the test results only suggest the existence of a cause-effect relationship. Deviations from an expected dose-response relationship and their possible causes and consequences for risk extrapolation are to be discussed.

It must be considered that the described procedure ignores variations of the risk among individuals due to different susceptibility. The transferability of the results to other populations must be evaluated in each individual case. Possible restrictions of transferability, e.g. if there is a healthy worker effect, must be considered. However, these considerations are of subordinate relevance against the background of assessing the risk of occupational exposure and establishment of limit values to improve occupational safety.

If semiquantitative exposure specifications and no other epidemiological data are available, the authors of the original publications may be contacted to be able to establish classification criteria for exposure levels and thus make a quantitative exposure assessment.

(4) Deviations from the default are possible in the following cases:

- **In order to be able to check the consistency of the results under different conditions, exposure models deviating from cumulative exposure (intensity, duration, exposure peaks or threshold) may also be considered depending on the mode of action if specific estimators were documented in the assessed literature.**
- **If no adequate data from studies are available, the results of cross-sectional or correlation studies may be used as an exception. The validity of such study results must be discussed with considerable reservations and must include a detailed description of the limitations. In general, cross-sectional studies and ecological studies should at best be used to supplement data from animal studies.**

(5) For extrapolation into the low-risk range, see procedure for toxicological data from animal studies (Section 5). Human data should, if possible, be

used to check the plausibility of the extrapolation factors in transferring animal studies to humans.

4 Transferring data from animal studies to humans

4.1 Consideration of species differences

(1) In the derivation of risk figures, this Guide generally assumes the same sensitivity of test animals and humans for carcinogenic effects after inhalation exposure. There is no reliable verification of this assumption. Since it has only limited scientific validation, it has the character of a convention.

Roller et al. (2006) demonstrated for many carcinogens that the sensitivity of humans in inhalation studies is usually higher than that of test animals. The authors thus concluded: "The results suggest that species extrapolation based on equivalent exposure without taking toxicokinetic or toxicodynamic species differences into special account generally does not lead to an overestimation of the risk for humans." This finding supports the statement made in Section 4.1 (1). Roller et al. even go further on the basis of their findings and propose that identical sensitivity should also be assumed "if mechanistic data, for example, suggest lower human sensitivity."

(2) Substance-specific data showing a clear deviation from the average (*e.g.* from pharmacokinetic models) can be used for substantiating a risk quantification deviating from the default.

This procedure allows deviation from the default if there is a "clear deviation from the average." What importance is attached to mechanistic or kinetic findings suggesting lower human sensitivity with sufficient likelihood is a matter of consideration or decision in the individual case (expert judgement).

4.2 Procedure based on an animal inhalation study

(1) For substances with a blood/air partition coefficient > 10 and systemically occurring tumours, the airborne concentration (6-hour exposure/day; resting conditions) used in animal studies must be adjusted to the workplace scenario (8-hour exposure/day; light activity) as the human equivalent exposure level by means of a correction factor of 2.

The background data for this conversion are explained in the draft of the REACH implementation project (REACH RIP 3.2-1B preliminary Technical Guidance Document):

	Rat	**Human**
Body weight	250 g	70 kg
Respiratory volume (standard; sRV)	0.2 l/min/rat => allometric scaling* 0.8 l/min/kg body weight (bw) →	0.2 l/min/kg bw
For various exposure periods		
6-h exposure	0.29 m^3/kg bw	**5 m^3**/person
8-h exposure	0.38 m^3/kg bw	6.7 m^3/person
24-h exposure	1.15 m^3/kg bw	20 m^3/person
Respiratory volume during light activity at work (wRV)		
8-h exposure		**10 m^3**/person

** scaling factor 4 for rats - humans*

For example, a T25 (rat) of 10 mg/m^3 after 6-h exposure/d corresponds to a hT25 (humans; 8h/day) of 5 mg/m^3 for systemic effects.

Since the blood/air partition coefficient is not known for all substances, water solubility (> 1g/l; readily water soluble substances) can be used as an approximate value.

(2) If there are species differences in absorption, these must be considered in the interspecies extrapolation.

4.3 Procedure based on an animal study with oral administration

If there are no study-specific data on the dose related to body weight, and only concentrations in the diet or water have been reported, the following default values can be used for conversion (according to REACH RIP 3.2-1B preliminary Technical Guidance Document).

Default values for body weights, food and water intake for the calculation of doses in lifetime studies						
Test animal	**Sex**	**Body weight (kg)**	**Food consumption per day[a] (g)**		**Water consumption per day[a] (ml)**	
Mouse	Male	0.03	3.6	(120)	5	(167)
	Female	0.025	3.25	(130)	5	(200)
Rat	Male	0.5	20	(40)	25	(50)
	Female	0.35	17.5	(50)	20	(57)
Hamster	Male	0.125	11.5	(92)	15	(120)
	Female	0.110	11.5	(105)	15	(136)

a) The daily food or water consumption is given in brackets in g or ml per kg body weight per day, as appropriate.

(2) A dose administered in an animal study (unit: mg/kg body weight x day) is transformed into a human equivalent dose by applying an allometric scaling factor. As a default, conversion is carried out via allometric scaling based on the basal metabolic rate ($(\text{body weight}_{human}/\text{body weight}_{animal})^{0.25}$). The following rounded factors are obtained:

- **dog and monkey 2**
- **rat 4**
- **mouse 7**

If an oral study is used as a basis, consideration of a scaling factor is no conservative extrapolation step, but instead represents biologically substantiated data adjustment in the default case (see TGD, Section 4.14.2.4, and Table 11 (scaling factors based on default weights); EPA, 2005; Kalberlah and Schneider, 1998).

(3) In the next step, the human equivalent dose is to be transformed into an airborne concentration unless specific reasons militate against route-to-route extrapolation, in particular:

- **pronounced first pass effect;**
- **local tumours in the respiratory tract are expected (especially relevant to locally acting, but also persistent substances such as metal compounds);**
- **local tumours after oral administration play a role relevant to assessment (e.g. forestomach tumours in rodents);**
- **organ concentrations deviating considerably in the critical target organ are expected after inhalation and relevant to assessment (e.g. often decisive in studies with administration by gavage).**

Differing route-specific absorption rates must be corrected in a route-to-route extrapolation.

The limits of route-to-route extrapolation were specified, for example, when the German ARW concept was developed by the Committee on Hazardous Substances. See ARW-Konzept (no author, 1998)

(4) If no route-to-route extrapolation can be made based on a study with oral administration and if no inhalation studies or findings from inhalation of the carcinogen by humans are available, risk quantification is generally not possible (see Section 7).

4.4 Procedure for studies with a shorter exposure and/or observation period

(1) If exposure was stopped before the end of the study (longer observation period), a correction calculation must be carried out. Assuming an experimental period of 100 weeks, this means for example:

actual exposure: 50 ppm in the diet for 70 weeks and observation period for 30 weeks;

calculated exposure: 50 ppm x 70 / (30 + 70) = 35 ppm throughout the entire experimental period.

If all animals of a dose group die prematurely, the exposure period and lifespan of the animal showing the greatest longevity is used as a basis for conversion.

(Source: Swirsky Gold et al., http://potency.berkeley.edu/)

If an exposure period of about 100 weeks in an animal study is converted to a human equivalent, this equivalent exceeds the proportion of a working lifetime of about 40 years. Even if lifetime exposure is back-calculated to exposure over a working lifetime in further steps, it is a conservative approach to use the observations after this longer exposure period as a basis for the quantifications.

(2) If the experimental period is shorter than the lifespan, another correction of the experimental period to the lifespan is generally carried out using the correction factor f^2 with f = experimental period/standard lifespan (*e.g.* experiment stopped after 100 and standard lifespan is 104 w: correction factor = $(100/104)^2$ = 0.92). The following standard lifespans are assumed: mouse, rat and hamster: 2 years; dog: 11 years; monkey (Macaca): 20 years.

Dybing et al. (1997) select a corresponding approach for their T25 concept (see also Section 3.6 (3) of this Guide):

Shorter exposure (w_1) compared with the total study period (w_2 weeks): correction factor $f = w_1/w_2$

Shorter experiment (w_1) compared with the total lifespan (w_2 weeks): correction factor $f = (w_1/w_2)^2$

This "standard lifespan" is not a very conservative convention. In divergence from this standard, it may be necessary to assume a prolonged lifespan especially for lung tumours (rats). In rats, exposure-related lung tumours occur especially at the age of more than 2 years. The spontaneous rate for lung tumours is low in rats; it is about 1 to 2% after 2.5 years, somewhat higher in one strain and even lower in the other one. The observation period should definitely be more than 2 years for quantitative risk as-

sessment. McConnell and Swenberg (1994) state, e.g.: "Following the 24-mo exposure period, the animals were held for lifetime observation (until ~20% survived)." This implies that 24 months are not a lifetime observation, but that a specific criterion (here 20% survival rate) can be used for the definition of "lifetime" (longer than 24 months) for pragmatic reasons.

(3) If the exposure concentration is reduced during the study, the time-weighted mean is generally used for the exposure level.

The simple approach of a cumulative dose metric over the entire lifespan (according to Druckrey; see below) does not consider that a carcinogenic substance is specifically able to induce one or several stages of carcinogenicity. If an early stage of carcinogenicity is affected, exposures at the beginning of life are especially critical. Persisting substances can maintain a persistent systemic load even after early discontinuation of treatment.

The Guidelines for Carcinogen Risk Assessment (2005) of the U.S. EPA point out (http://www.epa.gov/IRIS/cancer032505.pdf): "For chronic exposure studies, the cumulative exposure or dose administered often is expressed as an average over the duration of the study, as one consistent dose metric. This approach implies that a higher dose administered over a short duration is equivalent to a commensurately lower dose administered over a longer duration. Uncertainty usually increases as the duration becomes shorter relative to the averaging duration or the intermittent doses become more intense than the averaged dose. Moreover, doses during any specific susceptible or refractory period would not be equivalent to doses at other times. For these reasons, cumulative exposure or potential dose may be replaced by more appropriate dose metric when indicated by the data."

For the multistage and Moolgavkar models, there are for example mathematical proposals of adjustment for intermittent and short-term exposures occurring in arbitrary periods of life (Crump & Howe, 1983; Chen et al., 1988; Yamasaki, 1988). However, these seem to be too complex for routine use.

According to Druckrey's rule, tumourigenicity of a total dose effective over the entire lifetime is constant (d x t = const.). This description applies to many genotoxic substances. However, it does not consider depot effects, i.e. constant effects of poorly soluble or otherwise biopersistent substances after inhalation or injection (such as metal compounds, asbestos and wood dust). Druckrey's rule may also underestimate the late sequelae of high, tissue-damaging doses acting for a short period because, for example, increased proliferation rates increase the sensitivity of target tissues, establish genotoxic lesions and promote the migration of stem cells into target tissue. However, Druckrey's rule is the primary basis of linear dose extrapolation and also of usual time extrapolation.

Literature:

Chen et al., 1988

Yamasaki, 1988

Crump and Howe, 1984

Dybing et al., 1997

(4) Studies in which the exposure period is less than half of the standard lifespan are not suitable for risk quantification. The observation period should generally not be below 18 months in a study with mice and not below 2 years in a study with rats.

In a rough estimate, half of the standard lifespan approximately corresponds to the ratio between lifespan and working lifetime in humans. For example, an exposure pe-

riod of 1 year (rat) is generally sufficient for a quantitative application of the specific tumour findings. However, if the observation period is short, it is likely that the risk is underestimated to a relevant degree.

4.5 Standardisation of the daily exposure period

(1) The following standard assumptions apply to occupational exposure: exposure period during working lifetime: 40 years; duration of workday: 8 hours; weekly working days: 5 d/week; working weeks/year: 48 weeks; body weight: 70 kg; inhaled volume: 10 m^3/workday (8 h). Deviating exposure patterns are generally converted linearly to the standard assumptions referred to here. If information from the general population is available, the following exposure parameters are assumed (unless otherwise specified): exposure period: 75 years; body weight: 70 kg; food intake/day 1.4 kg; water intake: 2 litres/day; inhaled volume: 20 m^3/day (24 h).

For conversion on the basis of an animal study, care must be taken to avoid duplicate calculation: According to Section 4.2 (1), conversion from 6 h/d (resting conditions; animal study) to 8 h/d (light activity; workplace) is carried out via a factor of 2 for water-soluble substances.

(2) If an animal study is extrapolated to humans, the experimental exposure period (per day/per week) is generally specified and is converted linearly to the above-mentioned duration (occupational exposure).

This approach is based on the biological model assumption that the cumulative dose (c x t) of an effect is the dose metric determining the risk. This procedure is selected (for the default case) although it is known that this is a conservative step of simplification in most cases. The levels of the parameters have been adopted from the EU Technical Guidance Document (see Section 4.14.2.5 and Table 12 there).

5 Extrapolation to lower risk levels

5.1 Definition of the procedure according to the mode of action

(1) If, based on the information in Section 2, a mode of action determined essentially by direct genotoxicity was established for carcinogenicity, linear extrapolation is carried out in the default case.

(2) If, based on the information in Section 2, it was demonstrated that the mode of action is only characterised by non-genotoxic events and if a dose-response relationship with a threshold can be identified for the parameter(s) to be determined, this threshold must be calculated.

(3) If no mode of action is known or sufficiently reliable, linear extrapolation is also carried out in the default case.

(4) In cases in which the mode of action is essentially known, but a) direct genotoxicity is of no predominant importance, b) there is no definite threshold for carcinogenicity, or c) a threshold cannot be quantified on the basis of the available data, a sublinear dose-response course into the low-risk range is generally assumed.

The explanations in Section 2.5 must be considered for the term "threshold". In principle, a NOAEL for carcinogenic effects (no observed significantly increased incidence above background) is not considered quantitatively equivalent to a threshold.

(5) If assignment to (1) to (4) is unclear, it must be examined via various methods of parallel risk quantification (see Section 5.2) whether differences are obtained and how relevant the establishment of a mode of action is. If the dose-risk courses are close together, it may not be necessary to establish the predominant mode of action in order to quantify the risk without any relevant errors. The uncertainty in risk quantification must be documented. If parallel risk quantifications still lead to comparable risk figures for exposures with an increased risk (*e.g.* in the case of additional lifetime risks down to the per mille range), although considerable deviations occur at lower risks, the range in which specific dose-risk courses are valid must be defined.

(6) Risk extrapolation into the low risk range using the model function showing the best adjustment to the data for the experimental range is generally not the suitable procedure. For example, supralinearity may be found in the experimental range, but sublinearity in the low risk range.

The convention to use the benchmark method instead of the linearised multistage model as a mechanistically substantiated basis for the experimental range and the low risk range (see Section 3.2 (3) and Section 5.2 (2)) if sublinearity has been proven is inconsistent with this statement (6). This modelling is used for extrapolation because it offers a simple convention for describing sublinearity. However, it cannot be concluded that the "correct" slope was found in the low risk range by means of this model.

5.2 Extrapolation to lower risk levels for non-linear courses

(1) Based on information corresponding to case (4) in Section 5.1, a non-linear dose-response course is assumed with sufficient likelihood. In this case, plausibility is established for this non-linear function.

(2) If the data are sufficiently qualified to use the benchmark approach, it is assumed that non-linearity can also be reproduced in a risk range ≥ 1:1000 using benchmark modelling even if the experimental range only covers risks for example from 1% or 5%. Linear extrapolation is carried out between the $BMD_{0.1}$ (1:1000) and the origin or background.

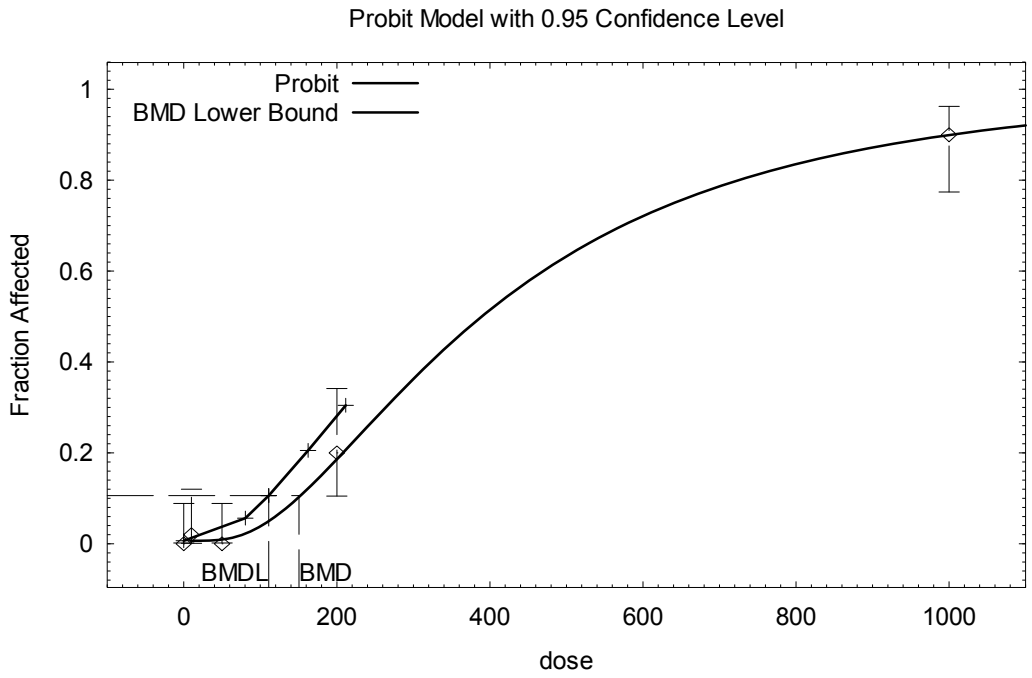
Reference to the BMD instead of the BMDL is justified, a) because orientation to the BMD is the maximum likelihood estimate, b) because according to case (4), Section 5.1, there must be additional reasons supporting a non-linear course, which means that modelling that ought to be regarded as mathematically possible using the BMDL is considered unlikely for these, for example mechanistic reasons, and c) because benchmark modelling is regarded as adequate only if the differences between the BMD and BMDL are so small that the risk is not expected to be underestimated if reference is made to the BMD (even if "in reality" the BMDL should reflect the risk more correctly). The procedure also results from the continuity in the method of the T25, which does not include a confidence interval either.

The following examples (Cases A and B) show a distinction between a case with non-linearity (Case A) and linearity (Case B). In Case A, additional mechanistic evidence supporting non-linearity would be necessary. If this cannot be provided, the BMD_{10} is the POD, below which there would be linear extrapolation.

<u>CASE A:</u> Good database refers to non-linear relations.

Concentration (mg/m³)	*Number of animals*	*Number of tumours*	Comment
0	*50*	*0*	Course indicates clear non-linearity; good database; *e.g.* mechanistic evidence of non-linearity
10	*50*	*1*	
50	*50*	*0*	
200	*50*	*10*	
1000	*50*	*45*	

Result; graphically:



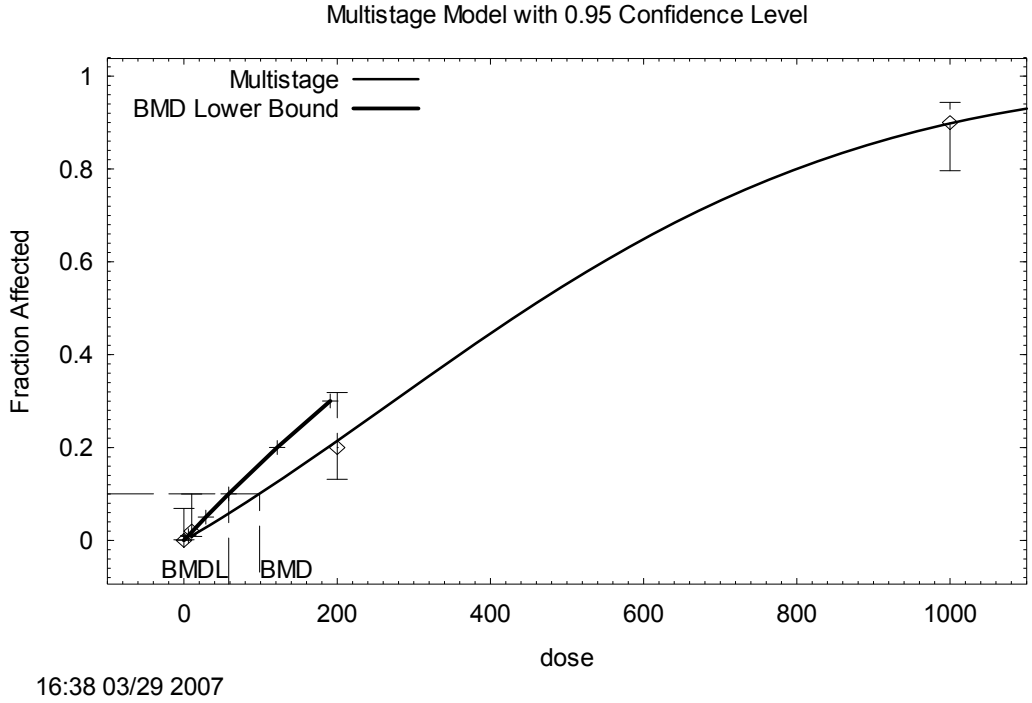
Result in figures; explanation:

Model	BMD_{10}	$BMDL_{10}$	$BMDL_{0.1}$ = 1 per mille	$BMDL_{0.1}$ = 1 per mille	T25	T25/250 = 1 per mille
	150	110	22	40	235	0.94
Comment 1:	*Difference 40/0.94 shows that the BMD (1 per mille) indicates a clearly lower risk than the T25 approach, which would not be suitable for this database.*			**40**	←→	**0.94**
Comment 2:	*The slight difference between 150 and 110 (or 40 and 22) shows that there is no relevant difference between the BMD and BMDL if there is a good database.*					
Comment 3:	*The log probit model was used. Because AIC = 100.87, p value = 0.34 and chi square = 2.15, this is justified compared with multistage. There: AIC: 103; p value: 0.19; chi square: 3.3 and thus poorer adjustment ➔ multistage would hardly reveal non-linearity.*					

<u>CASE B:</u> Moderate database refers to non-linear or linear relations.

Concentration (mg/m^3)	Number of animals	Number of tumours	Comment
0	50	0	Course does not rule out linearity, although linearity also possible; moderate database (criteria met according to Guide 3.1)
10	50	1	
200	50	10	
1000	50	45	

Result; graphically:



Result in figures; explanation:

Model	BMD_{10}	$BMDL_{10}$	$BMDL_{0.1}$ = 1 per mille	$BMDL_{0.1}$ = 1 per mille	T25	<u>Linear:</u> T25/250 = 1 per mille
	99	58	0.56	1.1	231	0.92
Comment 1:	Difference 0.92/1.1 shows that BMDL (1 per mille) indicates almost the same risk as the T25 approach since linearity is possible (see graph).			**1.1**	←→	**0.92**
Comment 2:	There is no substantial difference between the BMD and BMDL.					
Comment 3:	The multistage model (2 degrees of freedom) was used. Because AIC = 98.86, p value = 0.43 and chi square = 0.63, this is justified compared with log probit. There: AIC: 99.74; p value: 0.31; chi square: 1.01 and thus poorer adjustment ➔ similar extrapolation linear/benchmark approach					

(3) If the T25 was used as the POD for cancer, it is assumed for the case of substantiated non-linearity that a non-carcinogenic effect which at higher doses decisively contributes to cancer can be described quantitatively as an enhancing mechanism (*e.g.* irritation to the respiratory tract or cytotoxicity in the kidneys). There are four steps to determine the assumed exposure-risk course.

- **<u>Step 1:</u> A human equivalent threshold (TC*; as airborne concentration) is determined for this (non-carcinogenic per se) enhancing effect by considering usual extrapolation factors.**

  Extrapolation procedures for non-carcinogenic effects are carried out according to the EU approach (DNEL; RIP 3.2.2).

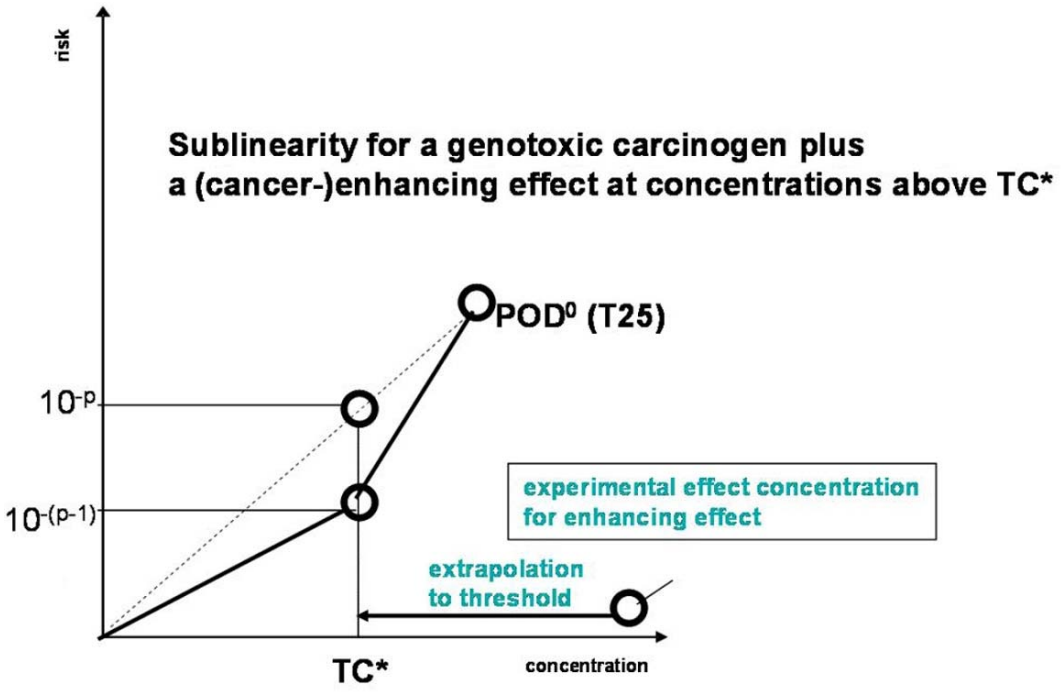
- **<u>Step 2:</u> Based on a T25 normalised and converted to the human equivalent (hT25), the cancer risk (10-p) is calculated as an interim step after linear extrapolation between the T25 and the origin or background at the point TC*.**

- **<u>Step 3:</u> Pragmatically, a cancer risk (1 order of magnitude: 10-(p-1)) that is ten times lower than that after linear extrapolation is assigned to the point TC*.**

- **<u>Step 4:</u> Finally, linear extrapolation is carried out from the point TC* to the T25 and to the origin (or to the background). The nominal risk can thus be identified for every point between zero and the T25 with a break point of the function at the extrapolated threshold (TC*) for the enhancing mechanism.**

  This "hockey stick" approach takes into account that the assumption of a non-linear course for the concentration-risk relationship is generally known, although no other parameters quantitatively describing the non-linearity of cancer are known. The unknown degree of "sagging" of the sublinear function is replaced by a reduction factor at the extrapolated threshold for the enhancing effect.

  The following figure basically shows the above-mentioned steps for a case in which there is a T25 for cancer and additionally sufficient data are available to determine a threshold (TC) for an enhancing effect (for explanation see text):*



The standardisations required in Sections 3.6, 4.2 and 4.4 must be carried out before the T25 is calculated.

An example of calculation has been included in the Annex (Section 10.2).

5.3 Extrapolation with an assumed threshold phenomenon

(1) If a minimum dose or threshold is assumed for carcinogenicity (Case (2) in Section 5.1), this threshold must be quantified on the basis of available experimental data including specific extrapolation factors. It is assumed that neither direct genotoxicity nor other modes of action without any threshold play a role in this case.

(2) To establish the threshold, special care must be taken to record particularly early evidence of the specifically relevant critical change is recorded. For example, in the case of nephrotoxicity relevant to cancer, initial early damage to the kidneys manifest in the form of specific proteinuria would have to be included. The dose-response relationship, LOAEL and NOAEL are to be established for this effect (although it is not carcinogenic itself, but) regarded as decisive for carcinogenicity.

(3) If no differentiated experimental findings are available on early lesions that are regarded as decisive for the carcinogenic effect, this is to be compensated for by conservative extrapolation factors. From this point of view, establishing an irritation threshold for a carcinogenic substance, for example, requires lower extrapolation factors than establishing an irritation threshold for a substance for which irritation is an important parameter for the mode of action in cancer.

(4) For this reason, the usual extrapolation factors are increased by a factor of 10. Against the background of cancer as the possible secondary effect, the threshold level (that must not be exceeded) is assessed particularly reliably. According to the terminology in Section 5.2, this conservative threshold is therefore at TC*/10, with TC* referring to carcinogenic rather than to cancer-enhancing effects.

Extrapolations to calculate the TC are based on the DNEL calculation (RIP 3.2.2).*

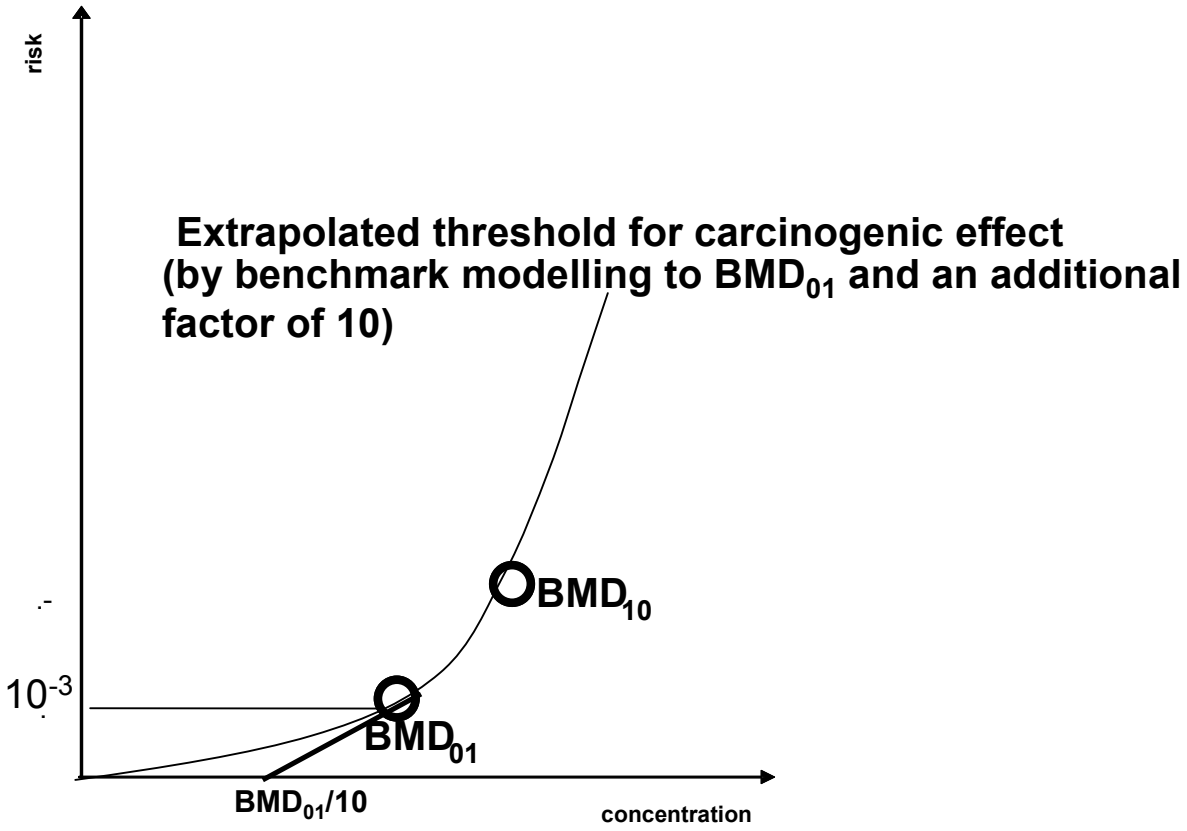
If the "usual" NOAEL is considered to be a value that may well be associated with an effect level of 5% (even if an effect was no longer observed in an experimental system), a definitely lower effect level will have to be associated with the resulting NAEL via this factor of 10 (e.g. effect level of 0.5%).

This procedure is consistent with the concept of the individual extrapolation factors as a specific percentile of a distribution (e.g. 90th percentile for the intraspecies factor): Selecting an additional extrapolation factor is equivalent to increasing the intraspecies factor, for example, to include a higher percentile (e.g. 95th percentile) of different sensitivities, but it is generally included (not related to an individual factor such as intraspecies factor, interspecies variability factor or time factor, but to the total distribution, i.e. multiplied individual factors).

(5) Combined with the benchmark approach for cancer risks, the risk course is assumed up to the risk at 1% along the modelled function (as the BMD). This implies that the quality standards for using the benchmark approach are observed (see Section 3.3). Mechanistic findings must be consistent with the modelled course of the exposure-risk relationship. Pragmatically, a "zero" risk is assumed at a BMD_{01}/10.

Recommendations for quantification of the exposure-risk relationship in the range above the assumed threshold are made in this Guide only if benchmark modelling was applied. If no benchmark modelling is carried out, the threshold is calculated according to Section 5.3 (4), although no general statement is made about the course of the exposure-risk relationship above this threshold (an individual consideration may be required).

The following graph of the extrapolation procedure is obtained for a case in which benchmark modelling is able to reproduce cancer in a qualified way. The calculated threshold (BMD_{01}/10) must then be converted to a human equivalent (workplace scenario) before being used for regulatory purposes.



6 Intraspecies extrapolation

6.1 No application of intraspecies extrapolation

(1) No intraspecies extrapolation is carried out. Accordingly, the main focus is on the average individual risk as additional lifetime (occupational) risk. However, the protection of sensitive groups of persons is also considered indirectly using a lower average risk in risk management (which in turn lowers the risk for sensitive groups of persons). Whereas in the case of non-carcinogens, sensitive persons are (more or less) explicitly protected from health effects via an intraspecies factor (as a default factor for variabilities), this Guide suggests ensuring this protection in the case of carcinogens by selecting an appropriately lower average individual risk (considered to be acceptable or tolerable). If an appropriate intraspecies factor for a carcinogenic effect could be determined, direct conversion (to the risk for sensitive groups of persons) would be possible.

It is a frequently applied convention to disregard the intraspecies factor for carcinogens. There are only insufficient data that can adequately reproduce the wide range of sensitivities in this multifactorial process.

It is currently unforeseeable when adequate data about carcinogenic effects will be available for identifying a scientifically based default value for intraspecies variability. The level of a specific factor would thus be extremely uncertain. A provisional evaluation of data from animal studies did not reveal a clearly higher variability of outbred strains compared with inbred strains with reference to cancer. It is not possible to simply link enzyme activities and their variability to the variability in cancer.

A few approaches, for example EFSA (see also Section 1.4 (3)), specify an intraspecies factor of 10 for carcinogens, but this has no effect on the level of protection, i.e. level of the proposed limit value. EFSA proceeds from the assumption that the intraspecies variability for carcinogenic effects is identical with that for other effects.

The U.S. EPA also considers an intraspecies factor for cancer, but expressly only for infants, who have a special sensitivity that is generally not reproduced in animal carcinogenicity studies. As a specific object of protection, "child health" is not decisive for the workplace in this context.

However, sensitive groups of persons are explicitly taken into account for the quantification of non-carcinogenic effects that are considered as factors causing or enhancing carcinogenicity (see Section 5.2 (2) and 5.3 (4) of this Guide).

7 Minimum criteria for risk quantification

7.1 Classification of the substance to be assessed

(1) Exposure-risk relationships for carcinogens that are classified in carcinogen Categories 1 or 2 (EU) should generally be assessed quantitatively.

(2) Substances classified in carcinogen Category 3 can also be assessed if they are considered in each individual case, particularly if this classification is not based on the quality of the study or reporting or on questionable human relevance, but mechanistic uncertainties were decisive for classification (*e.g.* possible threshold mechanism and questionable genotoxicity in the case of otherwise definite findings of cancer).

(3) Carcinogens that were classified in Categories 4 or 5 according to the national assessment proposal of the MAK Commission (DFG, 2007) can generally be assessed quantitatively.

7.2 Information on carcinogenicity after inhalation

(1) Tumourigenicity data for the route of inhalation are required for deriving an exposure-risk relationship at the workplace or must be assessable via route-to-route extrapolations (see Section 4.3). For example, if cancer incidences are only available after oral or parenteral administration or dermal application without the possibility of qualified route-to-route exposure, no relevant quantification can be made.

7.3 Tumour localisations without quantitative transferability

(1) If specific tumour localisations occur in specific animal species (possibly also sex-linked or combined with other substance properties), these findings are regarded as not transferable or not transferable quantitatively. The specific restrictions must be considered when examining the minimum criteria (see Section 4.1).

7.4 Lack of studies

(1) If no long-term animal studies or qualified human studies are available for a substance, the nominal cancer risk can generally not be quantified. Quantification may be justified in individual cases on the basis of considerations by analogy and restricted substance-specific studies. The studies that are regularly required for an assessment include evidence of genotoxicity comparable with the reference substance. An appropriate scientific rationale must be submitted for this purpose.

7.5 Quality of the study and of reporting

(1) Publication with detailed reporting is generally assumed. The following information should be included: species, strain and sex of the exposed animals and control, number of exposed animals/exposure group/sex incl. control, doses or airborne concentration and analytical detection method for the specified exposure, weight of the animals at the beginning and end of exposure/comparison between exposure groups and control, exposure period and observation period, tumour incidences/group incl. control, detection method and scope of examinations to identify tumour incidences, mortality during and at the end of the study, concomitant non-malignant effects (control; dose groups) incl. effects related and not related to exposure, change in organ weights (relative and absolute), abnormalities in feed composition and feed consumption, identity of the substance incl. data on purity or impurities and additives.

Body weight gain should not be reduced by 10% or more and the life expectancy of the animals should not be markedly reduced for reasons other than tumourigenicity, *i.e.* the maximum tolerated dose (MTD) should not be exceeded.

If these quality criteria are clearly not met in a study or in reporting, the lifetime risk can generally not be assessed quantitatively in an individual case consideration.

Other substance toxicity must also be expected to occur in test groups with considerably increased tumour incidence. In general, the specific group can nevertheless be included in the analysis of the exposure-risk relationship.

7.6 Minimum criteria for considering epidemiological studies in risk derivation

(1) General requirements for epidemiological studies: If available epidemiological studies do not meet previously established minimum criteria, they should not be considered in the derivation of exposure-risk relationships and occupational exposure limits. There may be deviations from this rule if they are scientifically substantiated. Some central requirements for epidemiological studies are:

- **Study hypothesis/specific question formulated before the beginning of the study**

- **Number of persons studied appropriate for the question/the risk to be detected (statistical power)**
- **Consideration of confounders**
- **Avoiding selection effects (bias) or a critical discussion of possible impacts on study results**
- **Information allowing a critical assessment of the study results (consideration of consistency of dose-response relationships; robustness of the results (sensitivity analyses, e.g. after exclusion of specific subgroups, stratified according to duration of employment or according to exposure intensity, etc.)).**

Correlation studies should not be included in an assessment *a priori* because of their collective assignment of exposure. Nor are case studies without any reference group appropriate for risk derivation. Cross-sectional studies are only suitable for the assessment of acute effects since they are not time-related (monitoring studies with an individual classification of exposure). Cross-sectional studies are not suitable for assessing a cancer risk unless they are related to a relevant end point.

The German Society of Epidemiology developed Guidelines and Recommendations for Ensuring Good Epidemiological Practice (GEP) to ensure that a quality standard for epidemiological research is established in Germany, to help avoid dishonesty and scientific bias and to guarantee communication among scientists based on trust (http://www.dgepi.de/infoboard/stellungnahmen.htm). The central requirements for epidemiological studies have been taken from these Guidelines.

(2) Exposure assessment should include the following elements:

- **Description of the method and data sources for the exposure assessment**
- **Assessment rules formulated in advance for the determination of exposure**
- **Specification or at least possibility of the calculation of cumulative exposures, i.e. information about the duration and intensity of exposure**
- **Consideration of co-exposures: Unlike in a study, there are often mixed exposures which make it difficult to assign the risk of developing cancer to a specific agent. Possible co-exposures must therefore be considered.**

If these elements are not sufficiently taken into account, the exposure assessment does not meet the necessary minimum criteria for using human data for risk quantification.

Literature: Cordier and Stewart, 2005; Ahrens and Stewart, 2003; Kromhout, 1994

Particularly in cancer epidemiology, occupational exposures are often determined and assessed retrospectively (exposure assessment) with the risk of an incorrect classification of exposure. Various methods of exposure assessment have been developed to allow as valid an assessment of occupational exposure as possible. Irrespective of possible combinations and further sources of information, exposure determinations and assessments derived from occupational epidemiological studies are based on measured data, expert assessments, exposure classifications by means of job-

exposure matrices (JEMs) or information provided by study participants. All methods of exposure assessment have specific strengths and weaknesses. Regardless of this, all methods can basically be considered in the derivation of exposure-risk relationships if they allow an assessment of cumulative exposure.

For further details on the specific strengths and weaknesses of the study designs see Ahrens et al. 2008

8 Requirements for documentation

8.1 Rationale papers

(1) **A written, publicly accessible scientific rationale (rationale paper) is required when the derivation of substance-related exposure-risk relationships and risk figures requires is used for regulatory purposes (*e.g.* for limit values and conditions for risk management associated with risk levels).**

(2) **Rationale papers may refer to this Guide as regards their methodology, which means that default factors or individual steps do not have to be substantiated in each individual case if they comply with the procedure in this Guide. However, an explicit reference should be made (*e.g.* "the shorter exposure period was taken into account in accordance with the regulations of the Guide, Section 4.4").**

(3) **If rationale papers are based on published data and all the necessary data are included in the cited source (see also minimum criteria according to Section 7), unambiguous citation of the source is sufficient to describe the database of risk quantification.**

(4) **The main emphasis of a rationale paper should be on (a) rationales for the assumed predominant mode of action (see Section 2), (b) deviations from the default procedure proposed in this Guide, (c) selection of the tumour localisation (including species, sex, etc., see Section 3.1), and (d) description of the actual mathematical calculation.**

Moreover, a rationale is required whenever this is explicitly specified in the individual sections of this Guide.

(5) **Reference to third party risk quantifications and the rationale given there is sufficient only if the cited reference is consistent with the requirements of this Guide as regards methodology and the necessary transparency.**

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10 ANNEXES

10.1 Glossary

Acceptable/ tolerable risk:

According to an approach adopted by the Committee on Hazardous Substances (Ausschuss für Gefahrstoffe; AGS), risk to health (ibid.) posed by the impact of dangerous substances is a continuum, which can be divided into the following three ranges by means of two evaluation points:

- *If occurrence of damage is only possible, the risk involved is assessed as "acceptable". Basic worker protection measures are required for this risk (range below the acceptable risk).*
- *If occurrence of damage is not yet sufficiently likely but more than just possible, the risk involved is assessed as "undesirable". This risk indicates that there is concern about health damage.*
- *If occurrence of damage is sufficiently likely, the risk involved is assessed as "not tolerable". This risk refers to a health hazard (above the tolerable risk).*

The risk levels for the specified evaluation points (acceptable risk and tolerable risk) can only be socio-politically established rather than scientifically substantiated. Numerous criteria have to be taken into account. Apart from risk perception, these are, for example, severity of health damage, the possible extent of damage (type of damage and/or number of persons affected), relation to comparable other risks at the workplace, direct benefit and actual and possible risk reduction measures.

Additional risk:

Means of calculating the exposure-related lifetime risk as a difference between the risk of the exposed persons and the risk of the non-exposed control group:

$P_A(x) = P(x) - P(0)$

with $P_A(x)$: *additional risk during exposure x*

$P(x)$: *lifetime risk of the exposed persons*

$P(0)$: *"background risk" (lifetime risk of a non-exposed control group)*

The term additional risk is mainly used for data from animal studies, while the term excess risk (ibid.) is preferred for the analogous risk when discussing epidemiological data.

Adduct formation:

Here: binding of a xenobiotic or its metabolite to the DNA. DNA adducts in the nucleus may prevent cell division or induce mutations.

AIC (Akaike's information criterion):

Statistical method to describe the relative quality of adjustment of curve models. Curves that are better adjusted generally result in lower AIC values. Important test in the benchmark dose approach (ibid.).

Allometric scaling:

Element of interspecies extrapolation (ibid.) of small test animals to humans. Allometry is understood to mean the determination of the relation of various biological parameters to body size. In mathematical terms, allometric scaling takes into account that in mammals, for example, metabolic activity does not linearly increase with the body weight of the individual animal species. This means that humans seem to be more sensitive to comparable toxic effects than mice, for example, if the dose absorbed is related to body weight.

Alpha2u globulin:

Low-molecular protein, high amounts of which are produced in the liver of adult male rats. Specific light hydrocarbons (e.g. isophorone, 1,4-dichlorobenzene and limonene) bind to alpha2u globulin. The complexes formed in this way accumulate in kidney cells, which may result in cellular destruction with subsequent repair, regeneration and an increased occurrence of renal tumours. This non-genotoxic mechanism of tumourigenicity (see "Genotoxicity") is considered to be sex-specific and species-specific and of no relevance to humans.

Aneuploidy:

Deviation from the number of the normal (euploid) chromosome set by one or several chromosomes

Attributable risk:

Attributable risk or attributive risk refers to the proportion of persons affected by a disease that can be attributed to a specific risk factor (ibid.). Two factors must be known to calculate the attributive risk:

- *the incidence of the risk factor in the population, and*
- *the extent to which this risk factor increases the risk of developing cancer.*

For example, assuming that the risk of developing lung cancer is ten times higher in heavy smokers compared with non-smokers, and further assuming that the frequency of smoking for men in a population is 40%, this would result in an attributive risk of about 78%. Comparable estimates may be made for occupational exposure on the basis of data for exposure prevalence and by using risk estimates from studies available on a specific exposure.

The attributable risk among exposed persons (ARE) is distinguished from the attributable risk in the general population (population attributable risk; PAR). Whereas the ARE specifies the fraction of cases developing cancer in the exposed subpopulation, the PAR refers to the specific rate for the total population. Thus, although the PAR may be small for rare exposures, the specific fraction of the relative risk (RR) in the exposed subgroups, e.g. workers in a specific branch of production, may be very high if the level of the RR is correspondingly high, and may be more than 50% at a RR > 2.

Mathematical definitions of ARE and PAR:

$$ARE = \frac{RR - 1}{RR}$$
$$= \frac{incidence_{exposed} - incidence_{non-exposed}}{incidence_{exposed}}$$
$$PAR = \frac{P_{|E=1|} \times RR - 1}{P_{|E=1|} \times (RR - 1) + 1}$$
$$= \frac{incidence_{population} - incidence_{non-exposed}}{incidence_{population}}$$

Benchmark approach:

Adjustment of a mathematical model to the data obtained in a study for the dose-response relationship. Several model functions are available for these model functions.

*The benchmark approach is an instrument to determine a point of departure (ibid.) for quantitative risk assessments. The dose that leads to an effect with a certain likelihood can be estimated for a defined effect frequency or a defined effect measure, i.e. the **benchmark response (BMR)**. This dose is referred to as **benchmark dose (BMD)**. A BMD_{10} indicates the dose at which there is a 10% risk that the effect concerned would be likely to occur. The reliability of assessing a dose-response relationship is quantified by specifying a confidence interval. The value of the lower (generally 90 or 95%) confidence interval of the benchmark dose is referred to as the **benchmark dose lower bound (BMDL)**. The quality of adjustment of the results with different model functions can be checked by means of the AIC (ibid.).*

Bias:

In epidemiology, the term bias is understood to mean distortion attributable to a systematic error in obtaining the data. Unlike random errors, systematic errors lead to one-sided deviations.

BMD (benchmark dose):

See “Benchmark approach”

BMDL (benchmark dose lower bound):

See “Benchmark approach”

BMR (benchmark response):

See “Benchmark approach”

Calculation of the sample size:

The planning of every epidemiological study requires calculating the sample size that is necessary to verify or falsify the assumption used as a basis for the hypothesis to be investigated. Various parameters must be defined for calculating the sample size:

1. Significance level or probability of type I error: Identifies the statistical reliability that is used to calculate a possible difference (when comparing several groups) or an increase in risk. The significance level is usually established at maximally 5%. The smaller the significance level, the larger the sample size must be. If a significance level of 5% or below is calculated, this means that the difference calculated here will actually occur in at least 95% of all conceivable comparable studies.

2. Power or probability of type II error: Establishes in what percentage of all conceivable constellations an actual difference or an existing risk increase is not overlooked. A power of 90% would thus mean that the risk of ignoring a difference – although there is a difference – is not greater than 10%. It is of course desirable that the power of a study is as great as possible. The greater the power, the larger the sample size must be. In epidemiological studies, the power used should not be smaller than 80%.

3. Assumptions about the minimum size of a risk increase: The smaller the risk to be detected, the larger the sample size must be to detect the risk increase at a given power. Previous studies or plausible assumptions must be used as a basis for establishing this parameter. Risk increases of more than 100% relatively seldom occur under environmental exposure.

4. Assumptions about the frequency of a critical risk factor in the reference group or reference population: If several risk factors are to be analysed in a study at the same time, it is suitable to use the rarest risk factor as a basis for the calculation of the sample size. If no exact data are available for the frequency of risk factors, a pilot study should be carried out. Information from published studies may be used alternatively.

Case-control study:

The aim of case-control studies is to determine the importance of risk factors (ibid.) for the formation of diseases in quantitative terms. Case-control studies are based on the logical consideration that the incidence of a risk factor promoting the formation of a disease must have been higher in patients affected by this disease before its outbreak than in a reference group of persons not affected. Since searches in case-control studies only start after a disease has developed, i.e. they are directed towards the past, case-control studies are categorised as retrospective studies. A case-control study results in an odds ratio (ibid.), which specifies how many times more frequently the disease develops if the risk factor exists than without it. An odds ratio below 1.0 would indicate a reduced risk and a value above 1.0 would specify an increased risk. An odds ratio of 1.5 corresponds to a risk increase of 50%. However, the specific confidence interval (ibid.) must be calculated to assess the relevance of an odds ratio.

Case-control study nested in a cohort: *This design is a special case of the case-control study, which is often found in occupational epidemiology. All cases of a cohort are compared with a random sample of the control persons not affected at the time of the case diagnosis from the same cohort (incidence density sampling); in this way, the optimum conditions of incidental and complete case recruitment and the require-*

ment of random selection of non-affected persons from the same reference population are met.

Cell proliferation:

Rapid multiplication of cells in a tissue

Chi-square distribution:

A continuous probability distribution over the number of positive real figures

Clitoral gland:

See "Preputial gland"

Cohort study:

In epidemiology, a cohort is a group of persons with a common characteristic. This characteristic may be common exposure to a dangerous substance, living in a specific region, having an identical occupation or the like. In a cohort study, the members of a cohort are observed for the occurrence of end points over a defined period. These end points may be the occurrence of defined diseases or death from defined causes. Since the risk subsequent to developing a disease is examined in a cohort study, this is a prospective study design. In occupational medicine, the starting point of cohort studies is often shifted back. These studies are often referred to as historical cohort studies or studies with a historical-prospective design.

When epidemiological studies are planned, the required sizes of sample populations must be defined as in the case of animal studies.

Confidence interval:

A confidence interval allows assessment of the range of variation of an estimate (e.g. odds ratio, relative risk and standardised mortality ratio). The interval specifies the range into which 95 of 100 possible estimates would fall if the 95% confidence interval is calculated, or 99 of 100 if the 99% confidence interval is calculated. The 95% confidence interval is commonly used. If an odds ratio (ibid.) was estimated to be 1.41 and the confidence interval ranges from 0.95 to 1.67, no significant increase in the odds ratio is found because the 95% range also includes values below 1.0, i.e. the true risk may be slightly increased, unchanged or even slightly reduced.

Confounder:

A variable that distorts the association between the actually investigated impact (e.g. a specific substance at the workplace) and the investigated end point (e.g. carcinogenicity). Confounding is the mixing of confounder effects with the effect of the risk factor to be investigated.

Correlation studies:

See "Ecological studies"

Cross-sectional study:

In a cross-sectional study, a defined group of persons, in most cases a sample of the population, is examined at a defined time. Such a study allows the frequency of characteristics, patterns of behaviour and risk factors (ibid.) to be estimated. These frequencies are designated with the epidemiological term "prevalence" (ibid.). Apart from prevalences, means of measured values (e.g. systolic blood pressure and cholesterol level) can also be estimated. When a cross-sectional study is designed, the required size of the sample population must be calculated for both approaches; see calculation of the sample size.

Survey is a synonym for cross-sectional study. Cross-sectional studies are among the most important instruments for examining the health status of a population. According to the state of the art, surveys must be carried out as representative surveys, i.e. on the basis of a representative random sample from the population.

Cytotoxicity:

Damaging effect of a substance on tissue cells

Default:

Statistically supported standard value or assumption that is to be used in the absence of substance-specific or species-specific data. A default allows deviations and is a means to describe systems despite incomplete knowledge of their characteristics.

Dose-response relationship:

Functional relationship between dose and effect (effect level) of a pharmacologically or toxicologically active substance. Strictly speaking, dose-response relationships for the end point "cancer" are dose-incidence relationships and describe the tumour rate as a function of the dose (or concentration). These functions are continuous and in most cases asymptotically approach a maximum value for the tumour rate.

For the low dose range – generally accessible in animal studies – several courses of curves can be modelled, e.g. using the benchmark approach (ibid.):

- ***Linear dose-response relationship**: Curve section can be described by a straight line function.*
- ***Sublinear dose-response relationship**: The initially slow increase in the tumour rate, for example, accelerates more than proportionally with an increase in the dose ("sagging" curve).*
- ***Supralinear dose-response relationship**: Minor increases in the dose in the low dose range lead to a relatively steep increase in the tumour rate, for example, whereas dose increases exceeding the low range only lead to a slight increase in the tumour rate and thus to a flattened curve ("bulged" curve).*

These descriptions of the curves provide no information as to whether the functions pass through zero.

Ecological studies (or correlation studies):

These studies compare exposure and disease at the level of groups, i.e. no individual information is available on exposure or a disease (or both) (e.g. frequency of performing a specific production process and cancer mortality when two factories are compared). However, since exposure and disease statuses are not assigned individually, ecological studies should generally not be used for deriving exposure-risk relationships for the assessment of occupational exposure limits.

EFSA concept:

Strategy of the European Food Safety Authority (EFSA) for the risk assessment of genotoxic (see "Genotoxicity") and carcinogenic substances. The concept is based on the calculation of a margin of exposure (ibid.). The dose leading to a tumour rate of 10% in an animal study (calculated as the BMDL, ibid., if adequate data are available) is determined as a reference point on the dose-response curve. If the margin of exposure (i.e. the ratio between the dose absorbed via the digestive tract and the $BMDL_{10}$) is 10,000 or higher, the cancer risk for consumers of contaminated food is classified as low and it is suggested that these substances should be treated with low priority. The further the margin of exposure falls below 10,000, the more urgently minimising measures must be taken.

Enzyme induction:

Increase of the synthesis of specific enzymes in the cells of a tissue. If metabolic enzymes are induced, this may have an effect on the detoxification or toxification of absorbed xenobiotics.

Epidemiology:

Epidemiology is the study of the distribution and causes of health-related conditions or events in defined populations (ibid.) and the application of the results of such studies with the aim of avoiding health problems. "Study" refers to observation studies, surveys, hypothesis tests and analytical and experimental studies. "Distribution" involves the evaluation of specific data according to time, location and groups of persons. "Causes" are understood to mean all physical, biological, social, cultural and behaviour-related factors that may have an effect on health. "Health-related conditions or events" include diseases, causes of death, patterns of behaviour such as tobacco consumption, reactions to preventive measures and the provision and use of health services. "Defined populations" are understood to mean groups of humans with identifiable characteristics (age, sex, residence, etc.). "Application of the results ..." explicitly refers to the aim of epidemiology, i.e. to promote, protect and restore health (according to Last, 2001).

Estimate:

Unknown parameters of the population are approached by means of observational values from a sample. Various statistical methods are available for this purpose. Thus population means are estimated by sample means. The fuzziness of these point es-

timators is assessed by means of the variability of the relevant characteristic in the population, which was estimated by means of the sample. For a better assessment of point estimators such as estimated relative risks (RR), the point estimators and their variability estimators are combined in confidence intervals which, at a given confidence level of for example 95%, allow rough statements, such as "the RR is between 2.0 and 5.5 at a 95% probability", to be made.

Excess risk:

The term has several meanings:

(a) It is often defined as the additional risk of developing cancer among exposed persons in relation to the basic risk, also called risk difference (RD): RD = RR-1. It specifies the percentage of risk increase among exposed persons. For example, it is 50% at a relative risk (RR) of 1.5, 100% at a RR of 2.0 and, correspondingly, 900% at a RR of 10.

(b) In this Guide, it is understood to mean the exposure-related lifetime risk, which is generally defined as the difference between the risk of the exposed persons and the risk of a non-exposed reference group (e.g. general population):

$P_{excess}(x) = P(x) - P(0)$

with $P_{excess}(x)$: *excess risk during exposure x*

$P(x)$: *lifetime risk of the exposed persons*

$P(0)$: *"background risk" (lifetime risk of a non-exposed reference group)*

This definition of the term of excess risk is the one most prevalently used for epidemiological data; it is formally identical with additional risk (ibid.). In animal studies, the term "excess risk" may also be used if the exposure-related lifetime risk was calculated as an extra risk (ibid.), although this is not quite formally correct.

Extrapolation factor/ safety factor:

*An **extrapolation factor** is physiologically/empirically substantiated. Risk assessment is based on available toxicological data and extrapolations are made to an expected, not experimentally determined value (e.g. lowering of the effect concentration when extending the study period). This quantitative assessment must include a comprehensible interpretation of empirical data.*

*Additional, more qualitative aspects (data quality, severity of the effect or indicative facts) are considered to provide protection from unknown or scientifically/ empirically non-quantifiable risks in accordance with the precautionary principle. A factor used for this purpose is referred to as a **safety factor**.*

Extra risk:

Means of calculating the exposure-related lifetime risk by means of the risk of the exposed persons and the risk of a non-exposed control group according to the following formula:

$P_E(x) = [P(x) - P(0)] : [1 - P(0)]$

with $P_E(x)$: *extra risk during exposure x*

$P(x)$: *lifetime risk of the exposed persons*

$P(0)$: *"background risk" (lifetime risk of a non-exposed control group)*

It is thus the ratio of additional risk (ibid.) to the proportion of individuals who do not react in the absence of exposure. For mathematical reasons, the extra risk is calculated in specific dose-response models particularly for data from animal studies; in general, the result hardly differs from that of the additional risk.

First pass effect:

Substances that are absorbed by the digestive tract enter the liver after absorption via the hepatic portal vein. During their first pass through the liver, they may in some cases be metabolised to such a considerable extent that only a fraction of the substance itself reaches the remaining organs.

Forestomach:

Aglandular digestive organ in front of the main stomach of rodents. Forestomach tumours often develop after administration of genotoxic carcinogens (see "Genotoxicity") via the diet or by gavage to rodents. Humans have no forestomach.

Gamma function:

Special mathematical function from which a continuous probability distribution (gamma distribution) is derived

Gavage:

Administration of a substance by means of gavage

Genotoxic:

Toxic to the genome; damaging effect on the genetic material in cells. A broader term referring not only to the induction of gene, chromosome or genome mutations but also to effects that were detected in indicator tests (e.g. SOS chromotest and comet assay). These effects may be induced directly by the actual substance or indirectly by metabolites. Genotoxic substances may cause mutations and tumours.

They are classified into the following categories:

***primarily genotoxic** substances: the starting substance and/or metabolite(s) react directly with the DNA and can change the genetic information in this way*

***secondarily genotoxic** substances: induction of genetic lesions without direct interaction with the DNA. Examples are oxidative damage through the formation of reactive oxygen species or disturbance of DNA repair.*

Harderian gland:

Additional lacrimal gland of the nictitating membrane in the nasal canthus of many animal species. Humans have no nictitating membrane.

hT25:

Human equivalent T25 (ibid.); calculated from the T25 determined from animal data by extrapolation to humans

Incidence:

Refers to the incidence of new cases of a specific disease related to a defined period (generally one year) and a defined population. All patients newly affected in a defined region must be recorded to determine the incidence. This is possible on the basis of population-related epidemiological disease registries, e.g. the cancer registry and heart attack registry, or by carrying out specifically designed incidence studies. For Germany, incidence can be specified for only a few groups of diseases and for regionally very restricted areas. The Saarland Cancer Registry and the cancer registry of the former GDR until 1990 are the only epidemiological cancer registries that provide reliable incidence data for all age groups over prolonged periods. The cancer registries, which have been established on a Federal state level since the nineties under the Federal Cancer Registry Law, are not quite complete, but will in future increasingly provide usable data (see "Dachdokumentation Krebs" under www.rki.de). The German Childhood Cancer Registry, based in Mainz, provides data for malignant tumours during childhood (up to and including the age of 14) for the whole of Germany.

Cumulative incidence *(CI) specifies the proportion of newly affected persons for a specific disease at a defined time:*

$$CI = \frac{\text{number of persons developing a disease in a defined interval}}{\text{number of persons at risk of developing a disease in a defined interval}}$$

Interspecies extrapolation:

Here: conversion of results obtained from animal studies to the (average) conditions in humans

Intraspecies extrapolation:

Here: mathematical consideration of differences in sensitivity of the human population in risk assessment

Leydig cell tumour:

Neoplasm originating from the testosterone-producing Leydig cells of the testis. Whereas Leydig cell tumours very seldom occur in humans, a high spontaneous incidence is observed particularly in aging Fischer 344 laboratory rats.

Life table method:

Statistical method to calculate the lifetime risk of dying from a specific type of cancer. The age-specific mortality rates for a certain type of cancer and for all causes of death are used to calculate the lifetime risk in this method.

Margin of exposure (MoE):

Margin between the lowest concentration shown by experimental data to cause toxic effects (here: tumours) and the expected or measured concentration to which humans are exposed (at the workplace)

Maximum likelihood estimate:

Statistical method to estimate the highest probability as accurately as possible as a reference for the population on the basis of the available sample

Maximum tolerated dose (MTD):

Highest dose in an animal study at which no serious toxic effects of a general type occur. The MTD is generally determined on the basis of body weight gain. The MTD should be reached, but not exceeded in animal studies investigating the possible carcinogenic effect of a test substance.

Mesothelioma:

Malignant tumour of the peritoneum, pleura or pericardium. Human pleural mesotheliomas are mainly caused by inhaled biopersistent fibres (asbestos) of specific dimensions.

Mitosis:

Nuclear division in which one nucleus produces two daughter nuclei that are genetically identical

Mitotic process:

See "Mitosis"

Multihit model:

Dose-incidence model that can be used for the modelling of dose-response relationships (ibid.) of carcinogenic substances and is based on the assumption that several adverse events ("hits") are necessary for the formation of a tumour

Multistage approach, linearised:

*Risk estimate approach long propagated by the U.S. EPA. The underlying mathematical model function (**multistage model**) describes a multistage process that is assumed as a basis for the formation of clinically manifest tumours. It is used for modelling the dose-response relationship (ibid.) down to the low dose range by means of the available experimental data. The risks at low doses are then assessed by means of a straight line, which corresponds to the slope of the model function at zero.*

Necrosis:

Uncontrolled cell decay

Nephrotoxicity:

Specific toxic effect on the kidneys

Odds ratio:

The odds ratio (OR) is a measure of association between two odds. The odds is defined as the ratio of the probability of an event to the probability of no event (of developing a disease under a given exposure or of an exposure with a given disease). For rare diseases, the OR approximately specifies how many times more likely it will be for a disease to develop if a specific risk factor exists than in its absence. Odds ratios are obtained as the result of case-control studies (ibid.). An odds ratio below 1 indicates a reduced risk and an odds ratio above 1 specifies an increased risk. The specific confidence interval (ibid.) must be known to assess the relevance of an increase in the odds ratio. The odds ratio is interpreted as an estimator of the relative risk (ibid.) particularly in case-control studies, since the latter cannot be calculated in case-control studies. The rarer the disease, the better the RR is approximated by the OR.

OR:

Odds ratio (ibid.)

Parenteral administration:

Administration of a substance by bypassing the gastrointestinal tract (e.g. by inhalation or by injection into a vein)

Peroxisome proliferation:

Peroxisomes are cellular organelles that are of central importance in lipid metabolism, for example. Certain substances (peroxisome proliferators, e.g. fibrates and phthalates) are known to produce a marked proliferation of the liver peroxisomes of some vertebrates, particularly of rodents. This reaction is mediated by a specific receptor (PPARα receptor), which occurs much more frequently in the liver of rodents than in humans. As a result of peroxisome proliferation, tumours can be induced in the rodent liver. In most cases, there is no relevance to humans.

Pharmacokinetic model:

Physiologically based pharmacokinetic models (PBPK models) attempt to describe the behaviour of a substance in the organism and quantify tissue concentrations in test animals and humans.

Phaeochromocytoma:

Tumour of the adrenal medulla

Pituitary:

The pituitary gland produces numerous hormones.

Point of departure (POD):

Initial value for further steps of risk assessment (see "T25 approach")

Population:

In epidemiology, population is understood to mean every human group that can be defined by at least one characteristic. This may be the entire population of a country or region or a group of patients (patient population) characterised by a specific, defined disease.

Power, statistical:

See "Statistical Power"

PPARα receptor:

See "Peroxisome proliferation"

Pre-malignant effects:

Precursors of a malignant neoplasm in a tissue

Preputial gland:

Pheromone-producing gland located in front of the genitals of some mammals (e.g. rats and mice). It is commonly referred to as the clitoral gland in females. Humans do not have anatomical equivalents of the preputial/clitoral glands.

Prevalence:

The total number of patients with a defined disease related to a defined population at a given time or, cumulatively, within a specific observation period of a population. It defines a proportion, which is usually specified as a percentage with values between 0 and 1.

Primary genotoxicity:

See "Genotoxic"

REACH:

REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) is the fundamental regulation under the EU chemicals legislation that was introduced to

achieve harmonisation throughout Europe. It was finally adopted on Dec. 18, 2006 and entered into force on Jun. 1, 2007 (Regulation (EC) No. 1907/2006; Directive 2006/121/EC).

In REACH Implementation Projects (RIP), working groups are preparing the methods and guides for the implementation of the REACH regulation at a European level.

Relative risk:

Factor that specifies how many times more frequently (or less frequently) a specific event (disease or death) occurs in a population compared with a reference population. For example, the relative risk of dying from bronchial carcinoma is up to 25 for cigarette smokers depending on the number of cigarettes smoked daily and the lifetime number of packets of cigarettes smoked, i.e. a heavy smoker has a 25 times higher risk of dying from bronchial carcinoma than a non-smoker. For rare diseases, the relative risk can reliably be assessed on the basis of case-control studies by means of the odds ratio (ibid.). As a rule, this requirement is fulfilled for cancer.

The relative risk (RR) can be defined as the ratio of the incidence among exposed persons (I_1) to the incidence among non-exposed persons (I_0):

$RR = I_1/I_0$

RIP:

REACH Implementation Project, see "REACH"

Risk:

According to the socio-political and legal definition (see Section I Art. 2 of EU Directive 98/24/EC), in this connection, risk means the likelihood that cancer will develop under exposure to carcinogenic dangerous substances. The risk or likelihood of occurrence of damage increases with an increasing dose of the dangerous substance or exposure concentration of a carcinogenic substance.

Risk factor:

Characteristics of persons or external effects that may lead to a positive or negative impact on the risk of developing a disease/mortality risk. Thus cigarette smoking is a risk factor for the development of bronchial carcinomas, bronchitis, myocardial infarction, gastric and bladder carcinomas, leukaemia, etc. The LDL fraction of cholesterol is a risk factor for the development of arteriosclerotic changes, whereas the HDL fraction of cholesterol as a "positive" risk factor is apparently capable of preventing the development of myocardial infarctions. Some scientists also consider the sex and age of a person to be risk factors. Occupational exposure, environmental factors and socio-economical characteristics have been shown to be strong risk factors for a large number of diseases.

Risk figure:

In this connection, risk figure is a value calculated under specific assumptions for the exposure-related lifetime risk in the scenario of exposure over a whole working life-

time. The lifetime risk refers to the likelihood that a person will develop a specific type of cancer if mortality from other causes is about equally high as in a non-exposed population. The risk figure can also be referred to as an estimate of the excess risk (ibid.) or as additional risk (ibid.) or extra risk (ibid.) since the background incidence was taken into account correspondingly here.

Route-to-route extrapolation:

Extrapolation from one route of absorption to another. The main routes at the work-place are the absorption of substances via the respiratory tract (inhalation) and skin (dermal), whereas in animal studies test substances are often administered via the diet or drinking water (orally). Because of the first pass effect (ibid.), which can be pronounced in some cases, correction factors must sometimes be introduced for transferring the results from feeding, drinking water or gavage studies to workplace conditions.

RR:

Relative risk (ibid.)

Secondary genotoxicity:

See "Genotoxic"

Safety factor:

See "Extrapolation factor/safety factor"

SIR:

Standardised incidence ratio (ibid.)

SMR:

Standardised mortality ratio (ibid.)

Standardised incidence ratio (SIR):

Number of new cases of a disease observed in a study population in a specific period divided by the number of new cases of a disease that would be expected if the age-specific incidence rates (see "Incidence") of the study population were the same as the age-specific incidence rates of an external reference population.

Standardised mortality ratio (SMR):

Number of deaths (of a specific cause) observed in a study population in a specific period divided by the number of deaths that would be expected if the age-specific mortality rates of the study population were the same as the age-specific mortality rates of an external reference population.

Statistical power:

Probability at which a statistical test can detect (actually existing) differences (e.g. different tumour rates in exposed versus non-exposed test animals) and differentiate them from random variations. The statistical power for example depends on the sample size (number of test animals in a dose group). This parameter can thus be used to assess the size that a study population should have to verify established differences and exclude random effects (see also "Calculation of the sample size").

Stratum (plural: strata):

In epidemiology: subgroup of a cohort. The classification of a study population into subgroups (e.g. according to age, sex and smoking habits) is referred to as stratification.

Stratification:

See "Stratum"

Sublinearity:

See "Dose-response relationship"

Supralinearity:

See "Dose-response relationship"

T25:

Tumourigenic dose at which 25% additional incidence is expected. In the experimental system, the T25 is originally specified as a dose (mg/kg x d). In the present connection, transformations into an inhalation concentration are also referred to as T25 or hT25 (ibid.) (see also "T25 approach").

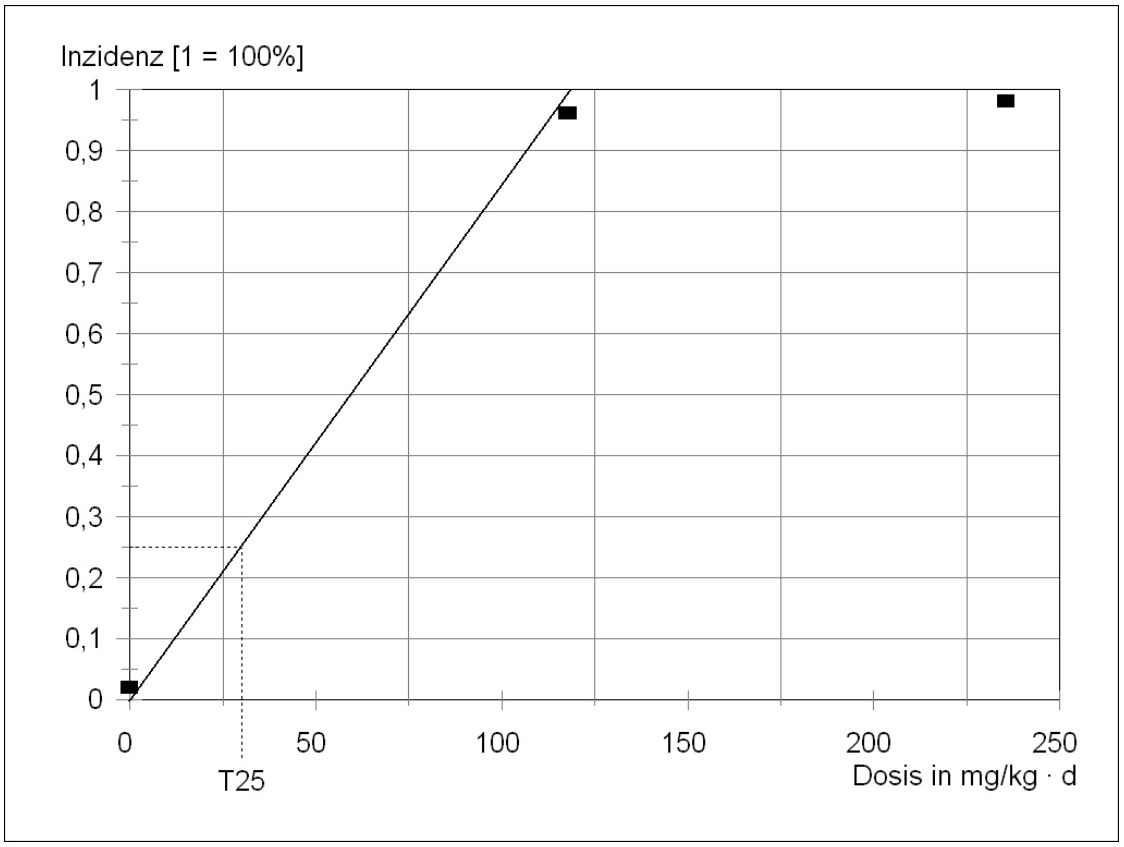
T25 approach:

Simple risk assessment method recommended by the European Commission for setting specific limits for preparations with carcinogens (EC, 2002; Dybing et al., 1997; Sanner et al., 1997). Based on a concentration with a significantly increased tumour incidence, a dose at which the incidence for this tumour in the animal study is 25% after lifetime exposure is determined by linear interpolation (i) taking into account the background incidence, (ii) if applicable, with correction of a non-lifetime study period, and (iii) assuming complete absorption.

$$T25 = C \cdot \frac{\text{reference incidence}}{([\text{incidence at C}] - [\text{incidence of the control group}])} \cdot \frac{(1 - [\text{incidence of the control group}])}{1}$$

with: C = lowest significant tumourigenic concentration or dose (mg/m³ or mg/kg · d)
reference incidence = 0.25 (25%)
incidence at C = tumour incidence in % divided by 100
incidence of the control group = tumours in % divided by 100

The T25 value can be used as a point of departure for estimating the risk for low doses by linear extrapolation into the low dose range (see Figure).



Graph of the T25 approach: Calculation of the T25 by means of the incidence of forestomach tumours in rats after exposure to styrene-7,8-oxide (data from Lijinsky, 1986)

The actual dose-response relationship and the variation of the experimental data are not considered in the T25 approach since only the background incidence and the incidence at an exposure concentration are used for the calculation of the tumourigenic dose 25%.

Threshold, toxicological:

A toxicological threshold level of a dose is generally understood to mean a dose or exposure concentration below which a specific effect does not occur. The term must not be confused with the "no observed effect level" (NOEL), which specifies a significant observed increase in effect compared with a "background" and depends on the relevant study design.

Just as there are many definitions for the toxicological threshold, it is controversial whether such threshold levels exist in individual steps of carcinogenicity induced by chemical carcinogens (Neumann 2006a,b,c). A threshold is generally assumed for "epigenetic", non-genotoxic carcinogens (e.g. cytotoxic [see "Cytotoxicity"], immune-damaging substances and hormone-like growth stimulators). However, it is also being

discussed whether the threshold model can be applied to specific secondarily genotoxic (ibid.) carcinogens (Hengstler et al., 2006). Although there are arguments in favour of such a view, the experimental detection of a threshold seems to be difficult in these cases.

Tolerable risk:

See "Acceptable/tolerable risk"

Topoisomerases:

Enzymes that are able to unwind the helically coiled DNA double strand and play an important role in cell division and protein synthesis

Toxicodynamics:

Study of the effect of toxic substances on the organism (see also "Toxicokinetics")

Toxicokinetics:

Study of the fate of toxic substances in the organism (absorption, distribution, metabolism and excretion) (see also "Toxicodynamics")

Zymbal's gland:

Sebaceous gland in the external auditory canal of rodents. Humans have no Zymbal's gland.

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10.2 Examples of calculation

Example 1: Trichloroethylene – Re. Section 5.2

Preliminary remark: Subcommittee III of the Committee on Hazardous Substances made an assessment of trichloroethylene including a mechanistic discussion and a quantitative discussion of the effect. For this reason, this example was selected by a German committee with reference to the regulatory assessment. However, the authors of this Guide are aware that, for most substances, inconsistent conclusions are available that have been derived from the data and their assessment (mode of action, validity of epidemiological or test animal data and quantitative conclusions). This also applies to trichloroethylene. The conclusions drawn by the Committee on Hazardous Substances and its subcommittees provide a basis that can be used for dealing with the calculation method of non-linear exposure-risk relationships, but the legitimacy of using this basis (i.e. the conclusions drawn for TRI and their scientific rationale) will not be a subject of discussion here.

In Germany, trichloroethylene (TRI) is classified as a human carcinogen, in particular because of the cases of kidney cancer observed after high occupational exposure, although it is assumed for reasons not to be explained in detail here that a cytotoxic effect on the kidney decisively contributes to cancer. Since local genotoxicity in the kidney cannot be ruled out, no definite threshold can be established for TRI. TRI is thus an appropriate example for Case (4), Section 5.1. The example will only explain the process of calculation using real data but not aspire to be a further documentation and discussion of the substance-specific information. In addition to the data referred to here, there are extensive studies on the mode of action, genotoxicity, nephrotoxicity, carcinogenicity in the kidney and carcinogenicity and toxicity in other organs, which will however not be addressed.

On the basis of the German studies on kidney cancer after occupational exposure to trichloroethylene, Roller (unpublished; CMR working group; March 2005) derived an excess risk of about 5% after exposure to 100 ppm (with peaks to 500 ppm) (18-year exposure; 2 h/d and 3 d/wk. peak exposure, otherwise about 100 ppm). A total of 3000 ppm-years of exposure are used as a basis for the calculation.

– *Exposure level:*

For the studies in which increased kidney cancer risks were found in Germany, very high exposures, which also led to pre-narcotic symptoms, were assumed for at least a substantial number of workplaces. It can be concluded from this that concentrations of 200 ppm were frequently exceeded. It is assumed that the concentrations may have been about 500 ppm for 2 or 3 hours on 2 or 3 days per week. The study of Henschler et al. (1995) is based on an average duration of employment of about 18 years. Moreover, assuming that TRI exposure continued in the period in which no peak exposures were reached, the following exposure can be estimated:

500 ppm, 2 h/d, 3 d/wk., 18 years

plus 100 ppm, 6 h/d, 3 d/wk., 18 years

plus 100 ppm, 8 h/d, 2 d/wk., 18 years

Altogether, this scenario corresponds to regular whole shift exposure to at least 100 ppm for 18 years, with exposure peaks of 500 ppm repeatedly occurring in every week for a prolonged period. Mathematically, a rounded value of 3000 ppm-years of cumulative exposure is obtained for this exposure scenario.

– *Risk assignment (excess risk)*

The increases in the kidney cancer risk that were observed in the German studies on TRI exposure slightly vary depending on the study period and the definition of the characteristic "exposed".

The odds ratios (OR) in the case-control studies are mainly statistically significant in a range of about 2 or 3, but higher values were also observed (e.g. "any exposure in metal degreasing"; OR = 5.57 in Brüning et al., 2003); the highest OR of 10.8 was observed in the study of Vamvakas et al. (1998). For risk assessment, the scores of the "relative risk" (particularly OR) mentioned in the studies must be converted to numerical values of the "absolute risk". Information on cancer mortality in the general population can be found in the WHO database (http://www.who.int/whosis/en/). The proportion of the cause of death "malignant neoplasm of kidney, except renal pelvis" (ICD/9 189.0) among all causes of death was specified to be 0.66% (2811/425093) in men and 0.42% (2085/496352) in women in 1990; in 1997, the proportions were 0.77 and 0.48% (WHO, 2003). Based on these figures, a lifetime mortality risk of about 0.7% must be assumed for kidney cancer in the general male population in Germany.

A doubling of this risk (RR, SMR or OR of 2.0) means an additional (excess) lifetime cancer risk at the same level.

The listed figures refer to mortality, while the incidence risk that is actually to be considered is higher. Precise data on kidney cancer incidence are not available for the whole of Germany, but the publication "Cancer in Germany" (2004) contains data-based estimates of the incidence rates. Here, the estimated incidence rates and the mortality rates for 2000 are compared with each other based on the official kidney cancer statistics. Accordingly, the rates are 22.0 (incidence) and 9.7 (mortality) per 100000 and year for men and 15.0 and 6.2 for women.

A value of about 2.3 is obtained for the ratio of incidence to mortality.

If this factor is applied to the mortality risk of 0.7%, a value of 1.6% results for the absolute basic incidence risk for kidney cancer for men in Germany in the nineties. Of course, odds ratio values of epidemiological studies on kidney cancer after exposure to TRI involve uncertainties, although it is unquestionable that a significant increase in the kidney cancer risk causally related to exposure is probable only if this significance is consistent with an excess incidence risk in the range of percent. At a basic risk of 1.6%, a relative risk of 2.0 means an excess risk of also 1.6%.

Therefore, it seems to be justified to assign an excess kidney cancer risk of 5% to the very high cumulative exposure of 3000 ppm-years.

– *Risk extrapolation (linear)*

Based on this calculation, we will use an excess risk of 5% after exposure for 3000 ppm-years as the point of departure below. Since an incidence lower than 25% is available for human data with specification of the risk, conversion to a T25 or HT25 is not suitable according to Section 3.7 (2). It is also possible to convert 3000 ppm-years over a whole working lifetime of 40 years to an average exposure of 75 ppm (x 40 years). A linear extrapolation based on this specification would lead to a risk of:

Average ppm	*ppm-years (40 years of exposure)*	*Excess risk*	*Remarks*
75 ppm	*3000*	*5%*	*POD; German epidemiological studies of kidney cancer*
15 ppm	*600*	*1%*	*Linear*
6 ppm	*240*	*0.4%*	*Linear; at threshold level for non-carcinogenic nephrotoxicity after exposure to TRI*
1.5 ppm	*60*	*0.1%*	*Linear*
60 ppb	*2.4*	*0.004%*	*Linear*

Assuming linearity, the excess risk can thus be described by the following equation:

Excess risk [%] = 0.067 x concentration [ppm]

for all ranges at and below 75 ppm

– *Risk extrapolation (non-linear)*

According to observations of Green et al. (2004), still significant subclinical kidney effects were found among workers exposed to TRI at a mean exposure level of 32 ppm. The biomarker for subclinical nephrotoxicity was no longer increased in 23 workers who had been exposed to 6 ppm TRI for several years (Seldén et al., 1993). In view of the only low effect level at 32 ppm, the NOAEL of 6 ppm can be used as a threshold for nephrotoxicity even for large cohorts without any further extrapolation steps. We therefore use the concentration of 6 ppm as the TC and assume that, at this point, the risk is lower by one order of magnitude than that determined by linear calculation (see Table above). For 6 ppm, this results in a risk (new) of 0.04% and an equation for the exposure risk of:*

Excess risk [%] = 0.072 x concentration [ppm] – 0.39

for the range between concentration [6 ppm; 75 ppm]

Excess risk [%] = 0.0067 x concentration [ppm]

for the range with concentrations [< 6 ppm]

Average ppm	*ppm-years*	*Excess risk*	*Remarks*
75 ppm	*3000*	*5%*	*POD; German epidemiological studies of kidney cancer*
19.3 ppm	*772*	*1%*	*Linearised ("steep" part)*
6.8 ppm	*272*	*0.1%*	*Linearised ("steep" part)*
6 ppm	*240*	*0.04%*	*"Break point"; at threshold level for non-carcinogenic nephrotoxicity after exposure to TRI*
1.5 ppm	*60*	*0.01%*	*Linearised ("flat" part)*
0.6 ppm	*24*	*0.004%*	*Linearised ("flat" part)*

For example, after linear extrapolation, the nominal risk of 1:1000 would be 1.5 ppm, while it would be about 7 ppm if there is a scientific rationale for assuming non-linearity. Below 6 ppm, there is a risk more or less reduced by one order of magnitude compared with the linear approach.

The result obtained in the low ppm range is presented graphically in the following figure:

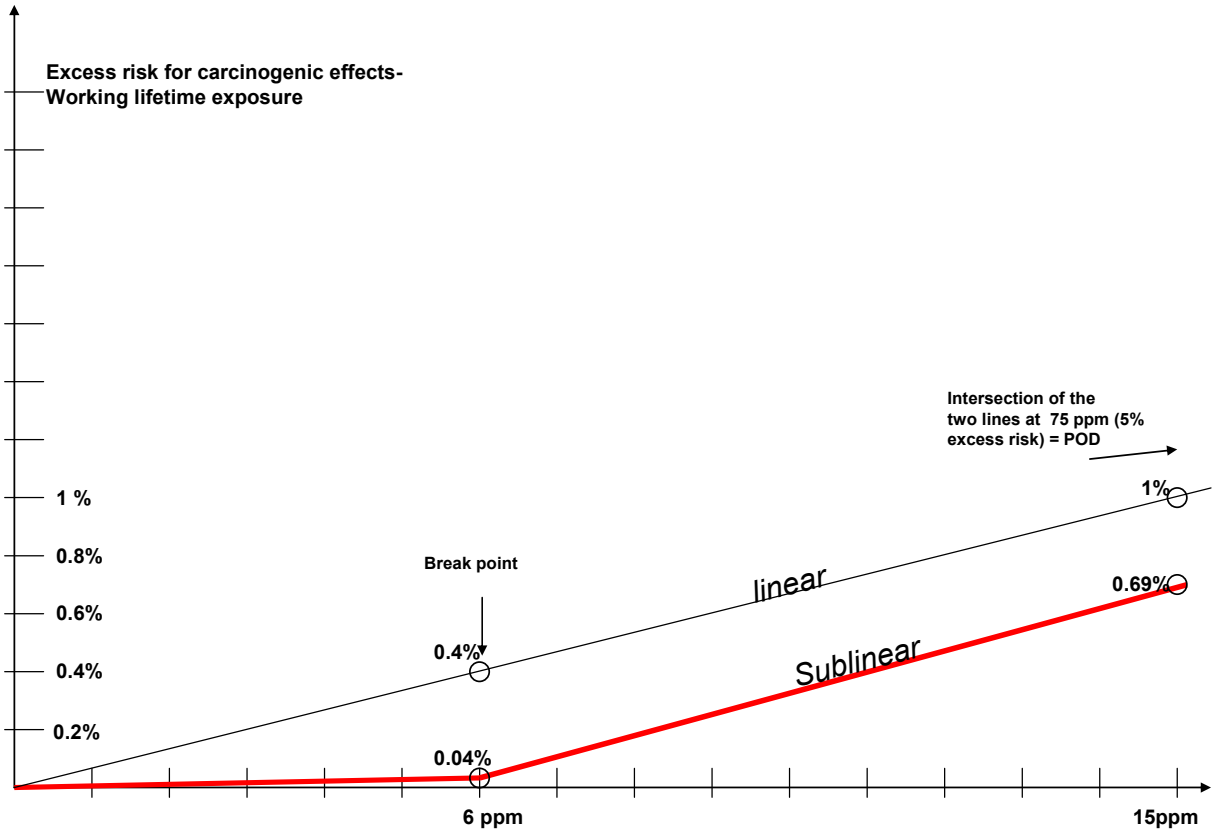

Figure: Exposure-risk relationship for trichloroethylene at an assumed threshold of 6 ppm (TC*) for a cancer-enhancing effect (nephrotoxicity) in humans in large cohorts and an excess kidney cancer risk of 5% derived from epidemiological studies at 75 ppm (working lifetime exposure)

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Example 2: Re. Section 5.3 (threshold assumption); theoretical example

Example:

Exposure to substance A leads to cancer in the respiratory tract in 3/50 animals (rat) at an airborne concentration of 200 mg/m^3, in 0/50 animals at 50 mg/m^3 and also in 0/50 animals in the control group (exposure pattern: 6 h/d; 5 d/wk.; 104 weeks; lifetime observation). It is assumed that a purely secondary reaction to an irritation to the respiratory tract with a NOAEL (90 days) of 100 mg/m^3 is sufficiently substantiated as the mechanism of carcinogenicity. According to the DNEL concept, the following extrapolation factors are to be applied (assuming that there is no rationale for any other corrections versus the default): time extrapolation: 2; interspecies extrapolation (variability): 2.5; intraspecies extrapolation: 5; additional factor because of the severity of the secondary tumourigenicity observed: 10. The total extrapolation factor is thus 25 or 250. The NOAEL corresponds to a human equivalent lifetime exposure of 50 mg/m^3 during light activity and 8-hour daily exposure (Section 4.2). After correction to working lifetime (x 75/40), the NOAEL is 93.75 mg/m^3. This results in a T of 93.75/25 = 3.75 mg/m^3 ~ 4 mg/m^3 or a T*/10 of 0.4 mg/m^3. A threshold of 0.4 mg/m^3 for the workplace would thus be indicated for regulatory purposes. If only irritation (no cancer) had been observed, 2 mg/m^3 would be calculated as a DNEL in the default (no correction for lifetime/working lifetime exposure in the DNEL concept for non-carcinogens). We assume a T25 of 833 mg/m^3 for this example. Compared with the T25, this assumed threshold is about 0.01 percent (1:10000) after linear extrapolation. (This (theoretical) example also demonstrates that there may be data sets which only lead to minor differences if a distinction is made between linear extrapolation, non-linear extrapolation and threshold assumption).*

Example of butadiene

(Based on the OEL documentation/position paper of the working group "Limit values and classifications for CM substances" (AK CM) of the Subcommittee UA III of the Committee on Hazardous Substances (AGS) on 1,3-butadiene)

1. Systematic literature search

The assessment was preceded by a structured, systematic literature search. The following studies on industrial exposure to BD and the risk of developing cancer were identified:

Numerous published results with detailed exposure estimates specifying the absolute butadiene concentration are available for a North American cohort of workers in the synthetic rubber industry. They refer to different follow-up times of the cohort or were calculated using different quantification concepts of exposure or different statistical methods. Mortality from specific tumours of the lymphohaematopoietic system is increased when handling butadiene.

Studies were also carried out in the production of the butadiene monomer, but no absolute data on exposure (*i.e.* ppm or mg/m^3) were published. These studies can therefore not be used for establishing exposure-risk relationships.

Two publications with a current follow-up of the cohort in the synthetic rubber industry, which moreover used an updated and improved job-exposure matrix (JEM) as a basis for exposure quantification, can be regarded as the most relevant evaluations of this cohort. They are therefore given preference in the assessment of exposure (Graff et al., 2007; Cheng et al., 2007). In one publication, the risk is calculated by means of a Poisson regression and in the other one, hazard rate ratios are calculated by means of Cox proportional hazards regression. Graff divides exposure categories into quartiles of exposure among the persons exposed and Cheng into deciles.

For the determination of limit values, all articles that describe different statistical methods or various exposure models should be evaluated separately and discussed critically. A meta-analysis will not be carried out.

2. Consideration of the target parameters

Mortality from specific tumours of the lymphohaematopoietic system was increased in the selected cohort studies. The most marked increases were evaluated when mortalities from the different forms of leukaemia were combined to "all leukaemia" or "leukaemia". Data on early end points based on biological markers were not published in the studies.

For the sake of simplicity, the following description of the calculation of the risk figure is only based on the study of Graff et al. (2005).

3. Calculation of the risk figure

Only two individual exposure scenarios will be described below: cumulative ppm-years and ppm-years based on exposure intensities of maximally 100 ppm.

- *Table 1 shows the exposure ranges and the relevant risk estimators calculated from Graff et al. Graff divides exposure categories into quartiles of expo-*

sure among the persons exposed. Since no medians or geometric mean are specified for the individual exposure categories, the class mean of the examined exposure categories is used as a basis.

- *Class mean divided by the duration of exposure of 35 years worked[7] provides the long-term mean of exposure in ppm. The class mean for the highest exposure category was estimated.*

- *The long-term means are plotted in a scatter plot against the relative risk, and a linear regression line is calculated, its slope expressing the increase in risk per exposure unit (ppm BD) (see Fig. 1 for the Graff study). Depending on the exposure model, there are slope coefficients for the relative risk of 0.16 or 0.31 per ppm after 35-year occupational exposure. The slope coefficients of the straight line in Fig. 1a suggest assigning a doubling of the risk (RR = 2) at a long-term mean of 5 ppm over a period of 35 to 40 years (which corresponds to a cumulative exposure of about 200 ppm-years). The slope coefficient of 0.31 per ppm for exposures smaller than or equal to 100 ppm is greater than when considering all exposure values (see Fig. 1b).*

- *Information on the basic risk (background risk) is required to transform this information into a statement about the absolute lifetime risk. A lifetime background risk of 1% for leukaemia is assumed on the basis of mortality from leukaemia and all causes in the general male population in the United States and other industrial countries (Roller et al., 2006). This means that the slope coefficients of the relative risk of 0.16 and 0.31 per ppm correspond to an increase in the absolute risk of 0.16 and 0.31% per ppm BD, respectively. The rounded lower of the two values means an excess lifetime risk of 0.2% (2 to 1000) after 35-year occupational exposure to a long-term mean of 1 ppm. Table 2 shows specific assignments of exposure and risk figure according to the linear model for various exposure scenarios.*

4. Deviating exposure models and potential bias

- *For risk assessment, various models were calculated in the original publications: the single agent model described here, which only considers exposure to BD (adjusted for age and time since the beginning of employment) or a multiple agent model), which considers possible confounding by other substances at the workplace and general confounders such as styrene and DMDTC. In BD production, exposure to styrene is however clearly lower than that to BD. Nor does styrene presumably have a higher leukaemogenic potency than BD. In the evaluation of Cheng et al., it was therefore not taken into account a priori as a possible confounder.*

- *Cheng et al. also examined whether considering different induction times of 5, 10, 15 or 20 years changes the results. Since this was not the case (Cheng et al., 2007), risk derivation – as above – can be carried out without considering an induction period.*

- *It should be pointed out that all exposure scenarios discussed in the vari-*

[7] The reference period of 35 years was selected in the evaluation of the AK CM, while 40 years should be used for future evaluations according to the Guide. In the case of butadiene, there is no essential deviation after rounding the result.

ous publications must be considered critically. For example, some of the results of the various scenarios described in the publication of Cheng differ considerably from each other. In the publication of Graff, the slope coefficient for exposure intensities <= 100 ppm is greater than when all exposure values are considered. This militates against a special relevance of exposure peaks greater than 100 ppm.

Table 1 Relative rate of leukaemia mortality depending on the category of butadiene exposure according to the study of GRAFF et al. (2005).

Cum. exposure; 1,3-butadiene (BD) [ppm-years]		**Long-term mean; 35 years[a] [ppm]**	**Person years**	**Leukaemia mortality**		
Range	**Class mean[a]**			**Observ. [N]**	**RR(1)[b] (95% CI)**	**RR(2)[c] (95% CI)**
0	0	0	116471	10	1 (ref. cat.)	1 (ref. cat.)
> 0 - < 33.7	16.85	0.48	154443	17	1.4 (0.7-3.1)	1.4 (0.5-3.9)
33.7 -< 184.7	109.2	3.12	144109	18	1.2 (0.6-2.7)	0.9 (0.3-2.6)
184.7 - < 425	304.9	8.71	49411	18	2.9 (1.4-6.4)	2.1 (0.7-6.2)
≥ 425.0	600	17.1	35741	18	3.7 (1.7-8.0)	3.0 (1.0-9.2)

[a] *Class mean calculated from the class limits of cumulative exposure (mean of cumulative exposure per category divided by 35 years); estimated mean for highest category*

[b] *Relative rate according to Poisson regression; multivariate model with the variables age, time since beginning of employment and butadiene exposure (CI = confidence interval)*

[c] *Relative rate according to Poisson regression; multivariate model with the variables age, time since beginning of employment, butadiene exposure, styrene exposure and DMDTC exposure (sodium dimethyldithiocarbamate)*

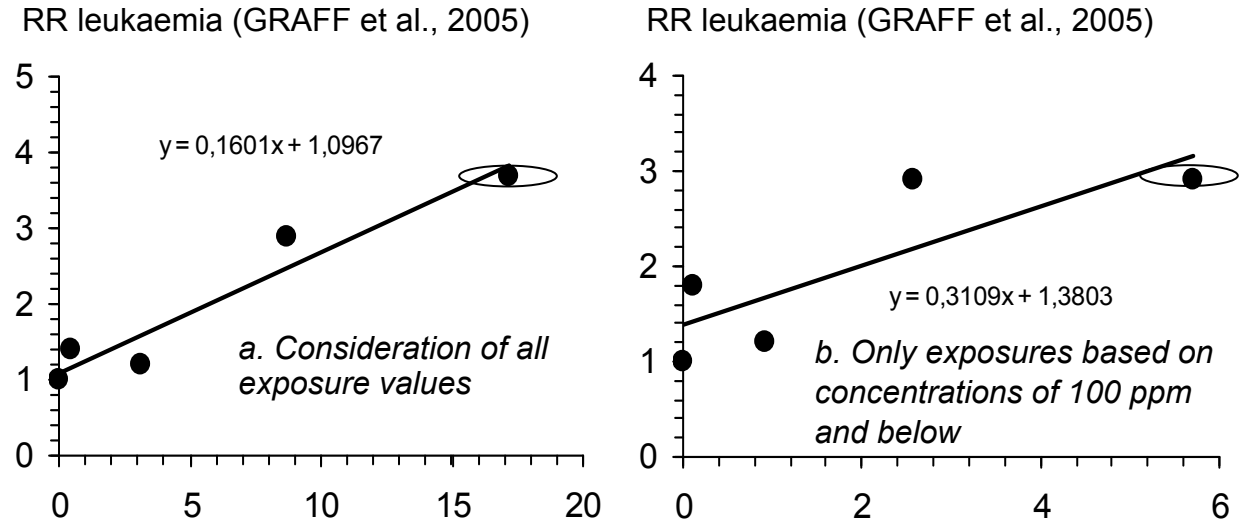

Fig. 1 Relative rates (RR) of leukaemia mortality depending on butadiene exposure, converted to a mean concentration over 35 years, according to data from the study of GRAFF et al. (2005). Ellipses indicate that specifying a mean exposure value for the highest – open ended – category involves uncertainties.

Table 2 Exposure-risk relationship for 1,3-butadiene according to the derivation of the AK CM for a scientific rationale of an occupational exposure limit (OEL)

Butadiene concentration; long-term mean; 35-40 years of occupational exposure		**Exposure-related lifetime leukaemia risk**
ppm	**µg/m³**	
15	33660	3%
5	11220	1%
2	4488	4 to 1000
1	2244	2 to 1000
0.5	1122	1 to 1000
0.05	112	1 to 10,000
0.005	11	1 to 100,000

5. Further aspects to be discussed

- *On the basis of the exposure-risk relationships found, no clear statement can be made about a course of the curve deviating from linearity. This is not a special feature of the data for butadiene. In general, no definite statements about specific courses of the curves of exposure-risk relationships can be made in the range below a lifetime risk of 1% based on epidemiological studies of possible associations between exposure to chemicals and cancer risks (Roller et al. 2006).*

- *In risk derivation, it must be decided which model or which scenario can be regarded as the "most realistic" or most appropriate one. These results must be used for risk derivation. Moreover, lifetime risks can be calculated for various scenarios and specified as a range.*

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10.3 Tumour localisations and their human relevance

There are numerous typical forms of tumours whose spontaneous incidence is high and sometimes also considerably varies in specific rodent strains and whose relevance to humans is not known (see 3.1 9). If their frequency is increased as a function of the dose compared with the current and mean historical control, an exposure-related effect is generally assumed. It should be explained in each individual case whether or not the tumour figures will be used as a basis for quantitative risk extrapolation.

Initially, it must be examined whether other types of tumours that can definitely not be assigned to spontaneous pathology occurred, possibly at even lower doses and/or at a higher incidence, and whether preference should be given to them as a basis of calculation for this reason alone.

It is also important whether it is a genotoxic substance. If a substance is genotoxic, human relevance can be ruled out for hardly any type of tumour. A default assumption would thus be based on a calculation using the type of tumour that would result in the worst-case risk figure. Alpha2u-globulin-induced kidney tumours of male rats would be the only exception from this (see below).

The concentration of the test substance (or the critical metabolites) in the target organ is a further aspect. Thus, for a substance that reaches its highest concentration at the portal of entry or in the kidneys, tumours of the respiratory tract or the kidneys would be considered more than an endocrine tumour with a high spontaneous incidence, for example.

Such mechanistic considerations would also be made for non-genotoxic substances and, if mathematical risk extrapolation were even carried out in such cases, ideally such tumours would be selected that match with the mode of action of the test substance (*e.g.* cytotoxic, mitogenic and endocrine) as regards the target organ and effective dose.

The following forms of tumours in rodents are examples of those having no or restricted quantitative transferability to humans:

– **Alpha2u-globulin kidney tumours of male rats** are a species- and sex-specific phenomenon and can be induced by a large number of non-genotoxic substances that bind to this protein.
This effect has no relevance to humans.

(IARC, 1999); see also **Annex 1**).

– **Liver tumours after PPARα-receptor stimulation ("peroxisome proliferation")**

These tumours are rodent-specific to a high degree. In most cases, there is no relevance to humans. (IARC, 2000; see also **Annex 2**).

– **Leukaemias of the Fischer 344 rat**

Mononuclear leukaemias very frequently occur in Fischer rats. Particularly in the case of non-genotoxic compounds, an increased incidence must initially be evaluated for its biological significance for the rat. In the case of a genotoxic substance, human relevance cannot be ruled out. However, in such cases, it would be examined whether this was really the only increased form of tumour which could be used for mathematical extrapolation (see also **Annex 3**).

– **Phaeochromocytomas of the Fischer 344 rat**
Mean historical rates and ranges of variation have to be taken into account as well as the differential diagnosis of the very frequent age-related hyperplasia. This tumour is apparently more likely to be formed in male rats than in female rats. Relevance to humans is restricted, particularly if there is a non-genotoxic mechanism and only the male sex is affected.

– **Thyroid tumours in rats**
Administration of substances that induce the glucuronidation route in the liver may also lead to a more rapid elimination of thyroid tumours from the blood and, as a result, to a stimulation of the thyroid tissue via the central feedback system (Goldstein and Tauroy, 1968; Hill et al., 1989; McClain, 1989). Liver hypertrophy or other signs of a general enzyme induction are not always observed, as the example of *tert*-butyl alcohol (NTP, 1995) shows, which led to thyroid hyperplasia in mice of both sexes and to an increased incidence of adenomas in females. Partial glucuronidation of this substance was detected in rabbits (Kamil et al., 1953).

In humans, the capacity of glucuronidation is generally affected less than in rats. Moreover, T_3 and T_4 are bound in the plasma with a high affinity and have a considerably longer half-life than in the rat (Döhler et al., 1979; Oppenheimer, 1979; Larsen, 1982). Thus, an increased concentration of glucuronidating enzymes is of less consequence for the T_3/T_4 metabolism of humans. Moreover, serum TSH is considerably higher in male rats than in female rats and many times higher than in humans, who do not reveal a species difference in the TSH levels (Chen, 1984). The male rat is typically disposed to benign and malignant thyroid tumours, whereas in humans thyroid carcinomas are not observed even after high TSH stimulation (Refetoff et al., 1993). Thus, there is obviously a low relevance of non-genotoxic thyroid carcinogens to humans. (IARC, 1999; *loc. cit.*).

– **Leydig cell tumours** occur with a considerably higher frequency in rodents than in humans. Their relevance to humans is low, particularly if a substance is not genotoxic (Cook et al., 1999).

– **Liver tumours of B6C3F1 mice**

  These tumours have a high background rate. According to Maronpot (1999), liver adenomas occur in about 30% of the males and in 15% of the females; hepatocellular carcinomas occur in 20% of the males and 10% of the females. There are doubts about whether quantitative transferability exists, particularly if the substance is not genotoxic and this type of tumour is the only one that occurs to an increased incidence (Gamer et al., 2002).

– **Forestomach tumours**

  Particularly in the case of non-genotoxic substances, relevance of these tumours to humans may be considerably restricted because of different anatomical conditions. For genotoxic substances, their suitability as a basis for a quantitative risk calculation must be decided on a case-to-case basis and depends on whether other target tissues are also affected.

Annex 1 to Annex 10.3:

α2u-Globulin nephropathy

is initiated by accumulation of α2u in the phagolysosomes of the proximal convoluted tissue with subsequent acceleration of apoptosis and replicative cell turn over (Alden and Frith, 1991; Caldwell et al., 1999).

A strong association between sustained α2u-globulin accumulation and renal neoplasia has been described by several groups of authors (Baetcke et al., 1991; Dietrich and Swenberg, 1991; IARC, 1999; Short et al., 1989; Swenberg and Lehmann-McKeeman, 1998). α2u was shown to cause morphological transformation in the pH 6.7 SHE cell transformation assay; this effect was not achieved by other proteins nor by typical α2u-inducing compounds such as d-limonene or 2.2.4-trimethylpentane (Oshiro et al.).

Annex 2 to Annex 10.3:

PPARα-receptor stimulation

In rats and mice, this form of enzyme induction is a metabolic situation that predisposes to the formation of liver tumours, although the actual carcinogenicity of the individual peroxisome proliferators varies very considerably. The threshold level and the extent of liver enlargement are of prognostic validity rather than the maximum peroxisome and enzyme activities in the high dose range.

Non-rodents are more or less resistant to the phenomenon of peroxisome proliferation (see below) and the associated effects such as enzyme induction, hepatomegaly and tumour induction. Hamsters, however, still show weak effects (Lake et al., 1984).

It is assumed today that the species differences are due to the density and functionality of a specific receptor type, the peroxisome-stimulating (PPARα) receptor, which is expressed to a particularly high degree and completely in rats and mice (Ashby et al., 1994; Bentley et al., 1993; Lee et al., 1995; Cattley et al., 1998; Maloney and Waxman, 1999). Stimulation of the receptors leads to a large number of transcriptions or gene expressions in the target cells and, morphologically, to a proliferation of cell organelles (peroxisomes, mitochondria and endoplasmic reticulum), to the suppression of apoptosis (Roberts et al., 1998) and to an at least initial, in some substances continuous increase of DNA synthesis (Marsman et al., 1988) and of the mitotic rate after activation of Kupffer cells (Rose et al., 1997); the liver is enlarged for a prolonged period at all active doses.

Transgenic mice that do not have the peroxisome-stimulating (PPARα) receptor did not show any peroxisome proliferation, hepatomegaly or increased DNA synthesis with DEHP (Ward et al., 1998). There was bioavailability, which was obvious from the testicular and renal lesions; these were however less pronounced than in the wild type. Moreover, even the highly active compound Wy-14,643 no longer led to any hepatocarcinogenicity in PPARα knock-out mice (Peters et al., 1997).

The human liver shows 1-10% of the functional PPARα receptor density of mice (Palmer et al., 1998). This might be the reason for the slighter toxicodynamic sensitivity of humans, as is also expressed *in vitro* in hepatocyte cultures.

<u>Annex 3 to Annex 10.3:</u> Fischer Rat leukaemias

Mononuclear cell leukaemia is a frequent finding in Fischer rats over 20 months old (Moloney et al., 1970; Moloney & King, 1973; Maita et al., 1987). Though rarely diagnosed up to the age of 18 months, this tumour may be the cause of up to 50% of all spontaneous early death cases in 2-year studies.

The tumour appears to originate from the spleen since splenectomized Fischer rats do not develop leukaemia (Moloney & King, 1973). Historical data show spontaneous incidences from ~ 10 to 50% depending on the size of groups and differential diagnostic measures (Moloney et al., 1970; Coleman et al., 1977; Goodman et al., 1973; Sacksteder, 1976; Sass et al., 1975).

The disease was sometimes erroneously called monocytic leukaemia or lymphoma and is correctly defined as large granular lymphocyte (LGL) leukaemia. On the basis of this more current definition, relatively recent reviews found the following incidences in control rats (Stromberg et al., 1983a,b; Stinson et al., 1990):

n = 1145	22.2%, male	20.5%, female
n = 2181	22.0%, male	15.6%, female

However, due to variation, the incidences in smaller groups (50 rats) may range up to 50% and in such cases represent a cluster.

The pattern of morphological, immunological, biochemical and functional characteristics of the LCL cells resembles those of normal large granular lymphocytes and in some respects also NK cells (Ward & Reynolds, 1983; Reynolds et al., 1981; Stromberg et al., 1983a,b). The tumour is transplantable (however, not with cell-free lysates) and, after transplantation, causes all clinical and immunological features observed also after spontaneous occurrence (Reynolds et al., 1984; Stromberg et al., 1985). So far, there has been no evidence of a viral aetiology.

A considerable number of genotoxic and non-genotoxic chemicals was associated with an increased incidence of this tumour.

Examples are:

NTP bioassay programme	Non-NTP studies
• 2-Amino-5-nitrothiazol • 3,3'-Dimethoxy-benzidine4.4-diisocyanate	• Ethylene oxide
• Arocolor 1254 • 2.4.6.-TCP • Phenol • Sulfisoxazole • Pyridine • Piperonylbutoxide • Lasiocarpine • Dimethylmorpholinophosphoramidate • Diazinone • Ally(l)thiocyanate • Ally(l)isovalerate	
• Diallylphthalate • Butylbenzylphthalate • (DEHP)	• Ethylene glycol (males) • DINP • Sanitizer 900

This shows that many compounds associated with increased LGL leukaemia were non-genotoxic.

Other compounds have shown reduced LGL leukaemia incidence, *e.g.*:

NTP bioassay programme:

- 1.1-Aminoundecanoic acid
- 2-Biphenylamine
- CI-Disperse yellow
- CI-Solvent yellow
- CI-Acid orange
- D & C red 9
- Propylgallate
- Monuron
- Ethoxyethanol

A review in 1983 described correlations between decreased incidence of leukaemia and elevated incidence of liver tumour (Haseman, 1983). Over the past decades, there is a general trend for an increase in leukaemia rates among male F344 rats in NCI/NTP studies. This is possibly related to the higher body weights in more recent studies (Haseman et al., 1989).

Conclusion:

LGL leukaemia is a typical and frequent tumour in aging Fischer rats. The aetiology has not been known so far. Many compounds that were associated with an increased occurrence of LCL cell leukaemia did not show genotoxicity. Quite frequently, it was the only increased tumour incidence that was observed in the course of a 2-year bioassay, either with or without dose relation. Furthermore, the spontaneous incidence within a 50 rat collective may be highly variable (➔ cluster formation). For these reasons, an increased incidence of LCL cell leukaemia is not regarded as a sufficient criterion to define a substance as carcinogenic. A more recent review by Caldwell (1999) comes to similar conclusions.

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