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**Quantitative Methods in Toxicology for
Human Dose-Response Assessment**

An overview

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Quantitative Methods In Toxicology for Human Dose-Response Assessment

SUMMARY

The process of human risk assessment can be divided into hazard identification, dose-response assessment, exposure assessment and risk characterisation (NAS,1983). For human risk assessment quantitative methods and models are applied. Which model should be applied depends on the nature of the question to be answered. A simple model can be applied if a standard has to be established, while a more complex model is required in the case a standard is exceeded and the health impact on a population has to be quantified. In this report an overview is given of important dose-response assessment methods and models as well as their application area.

A distinction is made in methods and models for genotoxic compounds and non-genotoxic compounds. The non-genotoxic compounds are assumed to have a threshold below which no effect occurs. Methods to estimate the threshold dose and to derive a human reference dose are presented. Furthermore, extrapolation problems that arise when animal data have to be translated to human data are discussed briefly. PBPK modelling is discussed as a method to improve interspecies extrapolation.

For non-genotoxic as well as genotoxic compounds curve-fitting models are described. In addition a biologically based model for genotoxic agents is briefly discussed. Finally methods that are applied and investigated at RIVM are presented and recommendations are given.

SAMENVATTING

Het process van de humane risicoschatting kan verdeeld worden in risico-identificatie, vaststellen van dosis-respons relaties, vaststellen van blootstelling en risico-karakterisering (NAS, 1983). In de humane risicoschatting worden kwantitative methoden en modellen toegepast. Welk model dient te worden toegepast is afhankelijk van de vraagstelling. Voor het stellen van een norm kan een eenvoudig model gebruikt worden. Indien een norm overschreden wordt, en men wil een schatting van de gevolgen voor de gezondheid van de bevolking, dan is een complex model noodzakelijk. In dit rapport wordt een overzicht gegeven van een aantal belangrijke dosis-responsmodellen en methoden. Daarnaast wordt aangegeven waarvoor de verschillende methoden gebruikt kunnen worden.

Het rapport is onderverdeeld in methoden en modellen voor genotoxische stoffen en niet genotoxische stoffen. Aangenomen wordt dat niet-genotoxische stoffen een drempelwaarde hebben waaronder geen effect optreedt. Voor deze stoffen wordt een overzicht gegeven van methoden om de drempelwaarde te schatten en methoden om een toxiciteitsnorm voor mensen vast te stellen. Daarnaast worden enkele extrapolatieproblemen die ontstaan bij het vertalen van diergegevens naar de mens in het kort beschreven. Vervolgens wordt uitgelegd wat "physiologically-based pharmacokinetic" (PBPK) modellen zijn en welke extrapolatieproblemen ermee kunnen worden opgelost.

Voor non-genotoxische en genotoxische stoffen worden enkele dosis-responsmodellen beschreven. Zowel curve-fit procedures als biologische modellen worden gepresenteerd. Ten slotte wordt een overzicht gegeven van modellen die gebruikt en onderzocht worden binnen het RIVM en wordt aangegeven wat, met het oog op de toekomst, belangrijk is op dit gebied.

Quantitative Methods In Toxicology for Human Dose-Response Assessment

1 INTRODUCTION

Humans are exposed to many chemical substances which may cause adverse health effects. The adversity of effects is discussed in a report by Kramer and Jansen (in preparation, 1994) and will not be discussed here. The probability that adverse health effects arise depends on several factors such as concentration of the compound, time of exposure, route of exposure, sensitivity of the individual etc. The process of estimating this probability is called risk assessment.

According to the National Academy of Science (NAS) of the United States, risk assessment[1] can be divided into hazard identification, dose-response assessment, exposure assessment and risk characterisation (NAS, 1983). Hazard identification includes the identification of toxic compounds potentially causing adverse health effect. Dose-response assessment is necessary to demonstrate the relationship between the dose and the effect. Exposure assessment is necessary to determine the dose to which individuals are exposed. Risk can be characterised when these two factors are known (NAS, 1983).

In the process of dose-response assessment, exposure assessment and risk characterisation, the determination of the following issues are important:

- toxicity standards
- the population at risk if a toxicity standard is exceeded
- the risk in subpopulation; for example highly exposed people in a population or sensitive subgroups
- individual risk

The Directorate General of Environmental Protection is interested in quantitative risk assessment. Various methods and models have been developed for this purpose. In this report we make an overview of methods and models that have been developed for dose-response assessment. The overview is meant to make clear what methodologies are available and for which dose-response assessment problem or problems the methods can be used. Therefore assumptions and concepts underlying the methods as well as their application are described here.

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[1] Risk assessment: The total of hazard identification, dose-response assessment, exposure assessment and risk characterisation. Thus, risk assessment includes more than determination of a toxicity standard

Risk estimation: The total of dose-response assessment and exposure assessment

Risk characterisation: Integration of dose-response assessment and exposure assessment

2 DOSE-RESPONSE ASSESSMENT

2.1 Introduction

Dose-response relationships are important for risk assessment. They represent, at a fixed exposure time, the probability of the occurrence of an effect or the magnitude of an effect at a certain dose. The probability of an effect is indicated when the data are presented as quantal data e.g. the number of affected animals as a fraction of the total number. The magnitude of the effect is indicated when the effect is continuous, for example increase in enzyme activity. These data are called continuous data.

Toxicological experiments
The dose-response relationship for legislative purposes is determined under experimental conditions according to OECD guidelines; the dose varies while duration of exposure is constant for a dose-response curve. The dose-response curve must be extrapolated[2] to the human situation to determine the possible effect in humans at a certain dose. In general this extrapolation consists of several steps:

- interspecies variation; difference in sensitivity to toxic agents between animals and humans
- intraspecies variation; difference in sensitivity to toxic agents between humans
- high-to-low dose extrapolation: humans are usually exposed to relatively low doses as compared to the experimental doses applied to animals.
- different exposure routes between humans and experimental animals.
- exposure scenario: difference in exposure period and exposure concentration

Dose and Response
A point of attention concerning the dose in toxicology is that the dose administered to animals is usually extrapolated to humans directly, which implies that dose distribution in animals and humans is similar. A technique to adjust the animal dose to a human dose is allometric scaling (Hertzberg and Miller, 1985). This technique will be discussed in chapter 2.3.3.
The administered dose is usually expressed as mass of the compound per unit body mass per time unit. This assumes a homogeneous distribution of the compound over the body. However, due to differences in kinetics, the administered dose (external dose) is not usually homogeneously distributed. Between species differences in kinetics exist too. This indicates that the distribution of a chemical in different species is not necessarily similar.

Genotoxic and non-genotoxic compounds are approached differently in risk estimation. For genotoxic compounds it is assumed that no threshold dose exists, which implies that no dose, which cause no effect can be established. Therefore a dose corresponding to an acceptable risk is determined, which usually is the occurrence of tumours in one in a

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[2]Extrapolation: Estimation of data outside the tested dose range
    Interpolation: Estimation of data within the tested dose range, under the condition
    that the intervals are relatively small

million people after lifelong exposure. For non-genotoxic compounds it is assumed that a threshold dose exists, which implies that a safe dose can be determined.

In addition, the response induced in animals by genotoxic compounds is extrapolated without an adjustment to compensate for interspecies differences. This assumes a similar response between animals and humans as well as a similarity in sensitivity in the test animal population and the human population. For non-genotoxic compounds factors are applied to compensate for differences between animals and humans.

2.2 Estimation threshold value for non-genotoxic compounds

Dose-response relationships are used to determine a safe dose for non-genotoxic compounds, which is a dose equal or smaller than the threshold dose. In this chapter methods to estimate the threshold dose are discussed.

Dose-response relationships of genotoxic compounds are discussed in chapter 2.4.

2.2.1 No-Observed-Adverse-Effect-Level (NOAEL)

The NOAEL is the highest experimental dose which does not cause a statistically significant different adverse effect from the control value. The NOAEL is used for the setting of human toxicity standards, such as the Acceptable Daily Intake (=ADI), and is determined from a dose-response relationship of a semi-chronic or chronic toxicity study.

Advantage
The NOAEL can be determined even if limited dose-response data are available. A statistical method to find a statistically significant difference between the effect in different dose groups and the control is the only requirement.

Disadvantage
The main disadvantage of the NOAEL is that it depends on the choice of the doses and the number of animals tested. The NOAEL is therefore a poor estimate of the threshold dose. Another disadvantage of the NOAEL is that the dose-response curve is not taken into account. This means that the toxicity cannot be quantified if a toxicity standard is exceeded.

2.2.2 Benchmark dose, Gaylor's linear extrapolation method and Bounded Effect Dose

The disadvantages that are described for the NOAEL method motivated Gaylor, Crump and Hoekstra to develop alternative methods without these disadvantages; a method which is independent of the tested dose range, and a method that take the dose-response curve into account.

The benchmark (BM) dose was defined by Crump (1984): "the lower statistical confidence limit for the dose corresponding to a specified increase in the level of health effect over the background". Figure 1A presents Crump's method. First the benchmark effect level is determined on the dose-response curve. After interpolation on the dose axis the confidence limits on the dose are calculated. The Lowest Confidence Limit (=LCL) is the benchmark dose.

Gaylor (1988) developed a linear extrapolation method after determining a dose that induces a specified effect. Figure 1B illustrates the method by Gaylor. When the 10% effect at the Upper Confidence Limits (=UCL) on the effect is determined, the corresponding Lowest Effective Dose at a 10% effect level (LED_{10}) is retrieved subsequently by interpolation. This dose is the lowest confidence limit for the dose: the lowest dose that can induce a 10% effect within certain confidence limits. Gaylor used the LED_{10} as the safe dose for animals and the LED_{10}/safety factor to derive a human toxicity standard. The safe dose level corresponding to this accepted effect can be considered safe. He also used the LED_{10} to start linear extrapolation to zero to determine a safe dose for carcinogens.

Hoekstra (1993) proposed a method to extrapolate linearly to lower toxicity levels. As a starting point she defined the Bounded-Effect-Dose (BED). The concept behind this method is that linear extrapolation from a point on the curve in the convex part to zero results in a conservative ($dose_{linear} \leq dose_{convex}$) estimate of a dose corresponding to a certain effect.
Figure 1C illustrates the determination of the BED. The BED is the highest dose (of the tested doses) where the confidence limits for excess risk do not exceed 25%. The 25% effect level was chosen to be sure the 25% effect level would fall within the experimental dose range, and to be sure that the effect level would fall in the convex part of the curve. This criterion is needed to avoid unreliable extrapolation outside the tested dose range. The effect level is rather high, but it should be kept in mind that the method has been developed for eco-toxicological data.

Both Crump and Gaylor apply a dose-response model to fit the data and calculate confidence limits (CL); Crump calculates a Lower Confidence Limit (LCL) for the <u>dose</u> and Gaylor calculates the Upper Confidence Limit (UCL) on the <u>effect</u>. Hoekstra does not apply a dose-response model but she does calculate confidence intervals on the effect. Application of confidence limits on the effect or dose helps defining a dose range that may induce a certain effect. Crump calculates this dose range, while Hoekstra and Gaylor determine the dose range by interpolation.
Crump illustrated his method with an effect level of 1%, 5% and 10 %. Gaylor (1989) took into account the severity of the effect: in the case of a severe effect he used the 1% effect level on the UCL as a safe dose. In other cases Gaylor defined the range for a 10% effect level. Hoekstra started the linear extrapolation at a 25% effect level to avoid unreliable extrapolation to the low dose range.

Advantages
The techniques by Crump, Gaylor and Hoekstra have the following advantages relative to the NOAEL. All three methods use confidence intervals which indicate the confidence of the threshold or safe dose. In addition, an effect level is used to determine a safe dose or a threshold in stead of a no effect level for the NOAEL.
The method of Crump and Gaylor can be used to determine a safe dose which is independent of the experimental doses. These methods help the estimation of toxicity when a BM or LED_{10} or safe dose level is exceeded. However, this application is limited; the risk can only be estimated properly in the case the BM or LED_{10} is exceeded for a period comparable to the exposure period on which the dose-response curve is based and assuming that no interspecies differences exist.

The technique to derive a bounded-effect dose does not use a dose-response model to fit the data. Therefore, this technique can be applied in the case insufficient dose-response data are available to determine a safe dose.

Disadvantages

A disadvantage of the BED to determine a safe dose is its conservatism. Another disadvantage is that the Bounded Effect Dose depends on the choice of the experimental dosages.

Crump and Gaylor apply a dose-response model to the data. The choice of the dose-response model to fit the data is arbitrary, as most models fit the data equally well. In the low-dose region, however, the calculated curves become distinctive. This implies that the dose, corresponding the effect level, varies between models (see chapter 2.4). Gaylor sometimes determines the LED_{01} (= 1% effect level) usually in the low dose region of the dose-response curve. The estimation of the curve in this part of the curve depends on the choice of the mathematical model to fit the data. Under these circumstances the choice of the model influences the outcome of the method.
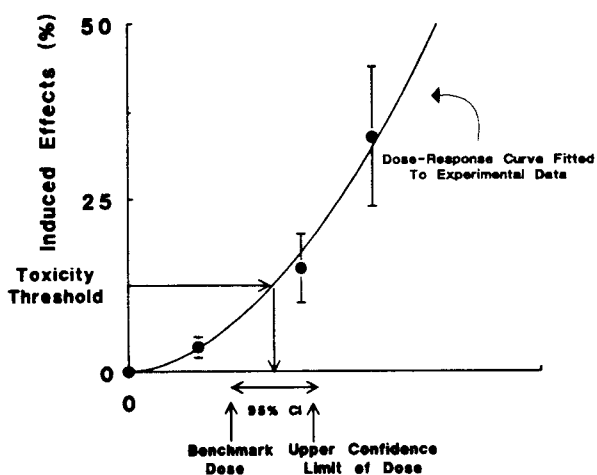
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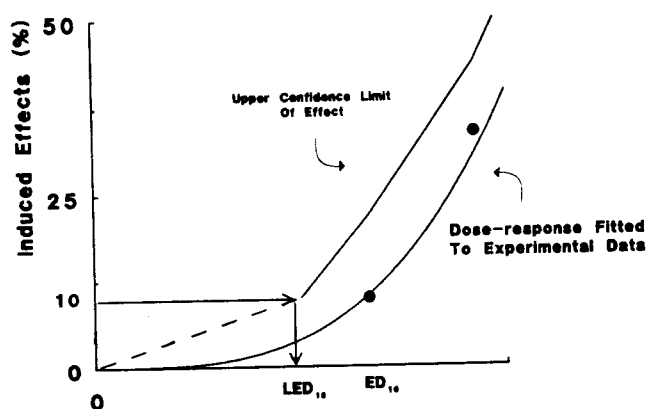
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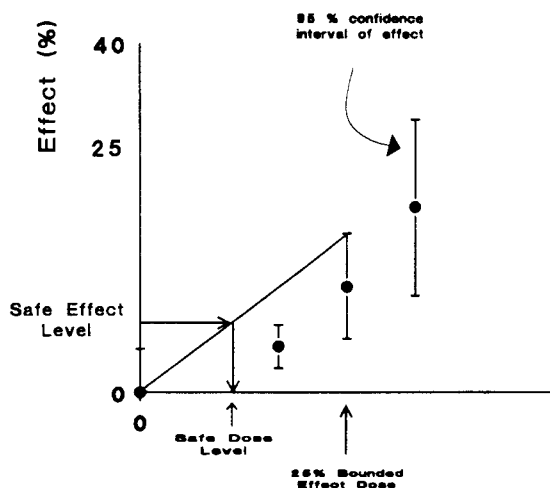
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Fig. 1 Illustration of Crump's Benchmark Dose (A) Gaylor's linear extrapolation method (B) and Hoekstra's Bounded-effect dose (C).
(A) The effect level is interpolated on the dose-response curve. The dose interval corresponding the effect level is calculated. (B) The effect level (10%) is interpolated on the upper confidence limit on the effect. The corresponding dose is the LED_{10}. There linear extrapolation to the control level starts. (C) The linear extrapolation to zero starts from the upper confidence limit of the 95% confidence interval of the dose i.e. the BED, inducing a 25% effect level

2.2.3 Dose-severity diagrams

DeRosa et al. (1985) developed a method in which response data are categorised into effect categories: No-Observed-Effect-Level (NOEL), No-Observed-Adverse-Effect-Level (NOAEL), Lowest-Observed-adverse-Effect-Level (LOAEL), Frank-Effect-Level (FEL) (DeRosa, 1985). The criteria are described in appendix A. The US EPA developed a rating value scale for effects (RV_e). This scale gives a rank-ordered progression of adverse effects from mild to severe (see appendix B). This scale can be seen as a sophistication of the categorisation by DeRosa. Beside a special effect scale also a special dose scale was developed which are called rating values for the dose (RV_d). Once RV_e and RV_d values are available a scatter plot is made of the RV_e vs. RV_d, as is illustrated in Figure 3. Then at the right side of the data a line is drawn to determine the NOEL. The NOEL is the RV_d where the line intersects the x-axis. The line is called apparent severity slope and indicates the toxic potency of the compound.
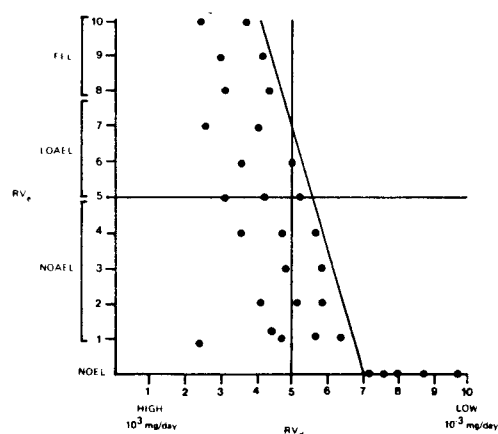

Fig. 3 Dose-severity plot.
An illustration of the method: effects are categorised according to EPA criteria and scaled against the log transformed dose.

Advantages
All available dose-response data are used to estimate the overall NOAEL of toxicity. In the case an ADI is exceeded, this method can give an indication of the toxic potency of the toxic compound.

Disadvantages
Difficulties arise when an effect has to be classified. Although a list of criteria exists for the various categories, it still remains difficult to classify, because the criteria are not well defined. Another problem is the scaling of the categories: the effects are ordinal and scaled on an interval scale. For example, it is not clear whether the difference between category 2 and 1 is equal to the difference between category 5 and 6.

2.3 Extrapolation methods for non-genotoxic compounds

If a safe dose was established in animals it must be extrapolated to a safe dose for humans. Several methods are available to do this, such as the Safety Factor method,

Renwick's method, Allometric scaling, comparison of Area-Under-Curve (AUC), and PBPK modelling. Besides these models that take into account most extrapolation differences, other methods are developed for specific extrapolation differences: Haber's Law for extrapolation in exposure duration and a method for route-to-route extrapolation. The safety factor method, Renwick's method, specific extrapolation methods, and more sophisticated methods are presented below.

2.3.1 Safety factor method

The safety factor method (safety factor is sometimes referred to as uncertainty factor) is only applied to non-genotoxic compounds, because it is assumed that a toxicity threshold value exists for these compounds. The safety factor method is applied to the estimate of the toxicity threshold, to translate experimental toxicological data to a human toxicity standard. The threshold estimate (see 2.2.), usually the NOAEL, is divided by a safety factor which is usually 100, reflecting interspecies differences (factor 10) multiplied by intraspecies differences (factor 10). This calculation results in a Acceptable Daily Intake or a Reference Dose (R_fD). The R_fD is value comparable with the ADI, but only used in the United States. Zeilmaker et al. (1994) described the method in more detail.

Other extrapolations such as exposure duration, dosing route, and in the estimation of the threshold by the Lowest-Observed-Adverse-Effect-Level (LOAEL) can also be taken into account by the Safety Factor method: each extrapolation step maximally has the value of 10. The safety factor to be applied then is the product of all relevant extrapolation steps.

Advantages
The method is very simple and can always be applied if suitable dose-response data are available. In addition, the method also can take various extrapolation problems into account.

Disadvantages
The difference between human and animal response is not exactly a factor 100. This factor is arbitrarily chosen and is intended to be on the safe side and therefore not the best estimate. A specification of the differences between experimental data and the human situation would allow the risk assessor to apply a more reasonable safety factor to get a more reliable toxicity standard.

2.3.2 Renwick's safety factor method

This method is a refinement of the Safety Factor method, as both generalised and specific information about kinetics and dynamics are implemented (Renwick, 1993). Renwick compared kinetic parameters like enzyme activities, clearance rates, absorption, and half-life times in humans and experimental animals to qualify the interspecies and intraspecies differences. The ratios of the parameters were calculated and used to indicate the interspecies and intraspecies differences in kinetics.

Renwick divides the standard safety factor of 100 into several factors that represent intraspecies and interspecies differences in kinetics and dynamics. Figure 4 demonstrates Renwick's proposed safety factors.

In the case the ratio of an enzyme activity in humans and animals is known, it can be used as a factor to adjust the standard safety factor of 100. For example the ratio for some elimination rate (animal/human) is 2; the kinetic value for interspecies differences therefore becomes 2, thus, the total safety factor is: 2 * 2.5 (dynamic human) * 2.5 (dynamic animal) * 4 (kinetic human)=50.

toxicodynamics toxicokinetics

	toxicodynamics	toxicokinetics
inter-species difference	2.5	4
intra-species difference	2.5	4

Fig. 4 Subdivision of a safety factor of 100 (Renwick, 1993)

Advantage
Intraspecies and interspecies differences can be specified by Renwick's method. In the case no information about differences in kinetics or dynamics is available a standard safety factor of 100 can be applied.

Disadvantages
It is not clear which factors should be selected to define e.g. the interspecies or intraspecies difference for kinetics. Selection of factors to define interspecies for dynamics is difficult because human dynamic as well as animal dynamic data are scarcely available. In addition Renwick considers the external dose instead of the internal dose. Besides the premise still is a safety factor 100. As long as no ratios can be determined this factor is applied.

2.3.3 Allometric scaling

A technique to adjust the animal dose in units of mass per body weight to a human dose in mass per body weight is allometric scaling (reviewed by Rauws and Groen, 1994). Allometry empirically relates the magnitude of a particular physiological characteristic to body weight, for example log-clearance of an administered compound versus log-body weight. When this relationship is generalised from one animal species to another (or to man), it is referred to as allometric scaling. Extrapolation from animal data to man should be based upon a solid interpolation and correlation between several test animal species. Allometric scaling can be particularly useful when trying to adjust for species-related differences in internal dose with the same external dose. In small animals especially clearance of administered compounds tends to be higher compared to that in larger animals including man. Interspecies interpolation produces the fewest problems if the compound administered is excreted unchanged via some elimination pathway. In contrast, greater problems reside in the qualitative interspecies differences in metabolism. In

addition, allometric scaling per se does not take into account species differences in absorption, bioavailability and protein binding (a characteristic influencing the extent of tissue distribution). However, when these characteristics are known, one may fine-tune allometry. In toxicology, allometric scaling is rarely applied.

An example may clarify this former statement. Allometric scaling formulas are usually of the form

$$X = k . W^{n-1}$$

X:	physiological characteristic
k:	constant
W:	body weight
n:	constant, usually in the range 0.7-0.8

When looking at the physiological characteristic clearance per unit body weight (Cl/W), assuming n=0.75, and comparing man (65 kg) and rat (0.2 kg) the following formula applies:

$$Cl/W = k . W^{n-1}$$

For the 0.2 kg rat:

$$Cl/W = k . 1.50$$

For the 65 kg man:

$$Cl/W = k . 0.35$$

This leads to the conclusion that per unit body weight, clearance can be expected to be (1.50 / 0.35 =) 4.3 times higher in the rat than in man, based on allometric scaling.

2.3.4 Specific extrapolation methods

Haber's Law

To extrapolate exposure duration, Haber's Law is used (Calabrese and Kenyon, 1991): C*T=K, i.e. the product of the administered dose (C) and the exposure time (T) till the effect appears is a constant. This implies that if the product is constant the toxic effect is the same (Haber, 1924).

Sometimes, for example in inhalatory experiments, animals are exposed intermittently to toxic agents. These results must be extrapolated to the human situation, where exposure often is continuous. Here Haber's Law is also applied. In general this equation is applied when differences in exposure scenarios occur, for all exposure routes, and risk should be determined under such exposures. Pieters and Kramer (1994) evaluated this method and concluded that in general this method is not valid and that the internal dose at the target should be determined.

Route-to-Route extrapolation

Route-to-Route extrapolation is necessary in the case humans are exposed via a different route of entry than the test animals from which dose-response characteristics were obtained. A bioavailability factor is included to correct for differences in blood serum levels related to the exposure route. To extrapolate oral toxicity data to inhalatory data (intraspecies) the following equation is applied (Van de Meent and Toet, 1992):

$$NOAEL_{oral} * Body\ Weight * B_{oral} = NOAEC_{inh} * IR * B_{inh} * 24$$

$NOAEC_{inh}$ = No-Observed-Adverse-Effect-Concentration (mg/m³/day)
$NOAEL_{oral}$ = No-Observed-Adverse-Effect-Level (mg/kg/day)
B_{oral} = oral bioavailability (set at 100%)
B_{inh} = inhalatatory bioavailability (set at 75%)
IR = inhalation rate

Such a correction is only necessary in the case systemic toxicity is expected. If one chemical induces different toxic effects by different routes of exposure, this extrapolation is not allowed. As long as no appropriate data are available this method can be used as a first approach.

Advantage
These methods are very simple to apply to all chemicals, if dose-response data are available.

Disadvantage
In general the methods are not valid. For example bioavailability factors cannot be taken as a constant.

2.3.5 Other extrapolation models, AUC and PBPK modelling

All models that have been discussed here relate the administered dose (=external dose) to the effect. However, the dose at the target organ or target tissue (the effective dose or internal dose concept) should be considered. The internal dose is the resultant of kinetic processes, physiological processes, and physico-chemical interactions. Thus, to estimate the internal dose knowledge of kinetics and physiology and physico-chemical parameters is necessary. Comparison of the internal dose in animals and humans may partly explain interspecies difference in sensitivity. A method to estimate the internal dose is determination of the Area-Under-the-Curve (AUC) or Physiologically Based PharmacoKinetic modelling (PBPK).

Extrapolation based on AUC (a method used in pharmacology)
According to pharmacokinetic theory, internal exposure can be quantitated by calculating the area under the plasma (or blood) concentration-time curve, or AUC. Following the same external dose in units of mass per body weight, the AUC is lower with lower body weight, as a rule. This can largely be ascribed to the relatively higher clearance value with smaller body size.
Upon a single administration of the compound of interest, an accurate value for the AUC and hence a valid approximation of the internal exposure can only be obtained when following the changes in concentrations appropriately. For example following an oral dose both the initial increase in circulating levels (absorption) and the final decrease in circulating levels (distribution, elimination) are to be described completely by the data. This implies that a complete pharmacokinetic study is required.
When the compound of interest is administered chronically, AUC can be approached by multiplying the average concentration at steady state by time (usually 24 h). Furthermore, the average concentration approach can also be used in case of a compound that is dosed chronically but intermittently (for example once daily dosing by gavage) that shows a low

elimination rate. In this case pharmacokinetic theory shows that fluctuation in concentration levels within a dosing interval at steady state is relatively small. Finally, this approach might be used with compounds that are administered to experimental animals as a feed admixture. As absorption largely occurs in the intestines, not in the stomach, for most compounds, absorption of the compound of interest may be spread over a more prolonged period of time compared to after an oral dose by gavage. This way, a better approximation of a continuous constant dose is attained. However, food consumption of small experimental animals is not spread evenly over 24 h, therefore approximation of the AUC on the basis of only one concentration value can largely underestimate the actual 24-h AUC.

Advantages

This method takes into account interspecies differences in absorption, metabolism and elimination. It can also take into account differences in distribution provided protein binding in plasma (or blood) is corrected for. Changes in exposure duration can be easily incorporated by multiplication. Furthermore, no additional correction is necessary for exposure route, provided that for both exposure routes AUC data are available. If necessary active or toxic metabolites can be taken into account (of course this implies that these have to be measured as well).

Disadvantage

This method requires pharmacokinetic data, not only of experimental animal species, but also of man. This is not feasible for most compounds undergoing risk assessment. However, for medicinal compounds, pharmacokinetic data in animal species used in toxicity studies and in patients (or volunteers) are required for the registration by the regulatory authorities. With medicinal compounds, exposure based on AUC values are preferred by the (Dutch) authorities to compare doses administered to animals in toxicity studies to doses prescribed to patients. Difference in pharmacodynamics are not taken into account.

Physiologically-Based Pharmacokinetic (PBPK) modelling

The basis of a PBPK model is a representation of an organism in a schematic physiological way. Organs or tissues with a function in kinetic process relevant to toxicity, are represented as compartments arranged in correct anatomical configuration and connected by the cardiovascular system. Distribution, elimination, absorption and metabolism of the compound are described as dynamic, i.e. time-dependent, processes.

PBPK models describe the kinetic processes that determine chemical disposition, within a physiological context. Parameters like tissue partitioning, organ volumes, and blood flow rates as well as biochemical constants for metabolism and protein binding are incorporated in PBPK models. PBPK models are partly generic and partly compound specific. Most physiological parameters such as blood flow, cardiac output etc. can be used for most PBPK models. This is the generic part of the model. The compound specific part includes parameters that are relevant for the induced toxic effect, e.g. metabolic rate, elimination rate.

Physiological parameters such as blood flow rate and cardiac output used in the PBPK models are average values for an average animal or average human. Monte Carlo simulation can incorporate intraspecies variation (if known) of the various parameters to simulate the distribution of the internal doses in a population. After a complete description

of the animal PBPK model including its validation, the animal model is extrapolated to the human body. Human model parameters are obtained from literature or by allometric scaling or from *in vitro* or *in vivo* studies. Validation of the human PBPK model requires toxicokinetic data of the compound of interest in human tissue.

Poorly defined PBPK models may be resilient in their estimations. It has been demonstrated that relatively wide ranges of parameter values in a PBPK model fitted data equally well (Bois et al.,1991). This indicates the need for more precise values for model parameters and precisely defined models.

Advantages

Validated PBPK models can estimate the internal dose, i.e. the dose at a target organ or tissue under all kinds of exposure conditions. Several uncertainties relating to extrapolation problems mentioned can be quantified by comparing the human internal dose and the animal internal dose: route-to-route, high-to-low dose, different exposure scenarios.

Disadvantages

The models can be used to estimate the internal dose which is associated with a toxic effect. This implies that PBPK models do not describe the effect (no dynamic modelling). However some models are available that can describe the formation of metabolites that are causally related to cytotoxicity, for example the methylene chloride model by Andersen et al. (1987).

2.4 Dose-response modelling

Dose-response modelling is a mathematical technique to describe the dose-response data of toxicological experiments. With a mathematical description of a dose-response curve it is tried to estimate toxicity in the low-dose range. In fact, still unreliable extrapolation of the data occurs, because uncertainty exists about induced toxicity in the low-dose range.

The first dose-response models were developed to fit tumour incidence data and were based on limited mechanistic concepts. As the knowledge of the mechanism of carcinogenesis advanced, more mechanistically models have arisen.

In this section first empirical models will be described for both non-genotoxic and genotoxic compounds, then biologically based models will be presented.

2.4.1 Empirical models for non-genotoxic and genotoxic compounds

Non-genotoxic compounds

Much experience in effect modelling has been gained in pharmacology. Pharmacodynamic modelling is a mathematical technique to describe the correlation between the concentration at the effect site with the pharmacodynamic effect observed. The same models used in pharmacodynamic research, however, can also be used to model toxic effects. Generally, pharmacodynamic models are empirical only, and neither include nor offer an explanation for the mechanism of the effect.

Sigmoid E_{max} model

In pharmacology, the sigmoid E_{max} model is the most common and most versatile model used to characterize *in vivo* concentration effect relationships. In order to obtain an accurate description of the actual concentration-effect relationship, it is essential to choose

concentrations examined high enough to be sure to include the maximal effect that can be reached.

Fixed effect or logistic model

This model can be used for quantal concentration effect relationships. It describes the relation between concentration and the probability of response in the population.

$$probability\ of\ response = C^n\ /\ (\ C^n + EC_{50}\ )$$

C	concentration of the compound of interest
n	a constant expressing the steepness of the concentration-effect relationship
EC_{50}	concentration that will produce 50% probability of response, in other words the concentration that will elicit the examined effect in 50% of the population

Genotoxic compounds

At present no single mathematical procedure is recognised as the most appropriate for low- dose extrapolation in carcinogenesis. The mathematical models that have been used to describe the relation between the administered dose, time and tumour incidence are based on either tolerance-distribution, mechanistic assumptions, or sometimes on both assumptions. A summary of the most frequently cited models may be listed as follows:

Tolerance distribution models	Mechanistic models	
	Hit-models	*Biologically based models*
Logit	One-hit	Moolgavkar(MVK)[1]
Probit	Multihit	Cohen and Ellwein
Mantel-Bryan	Weibull (Pike)[1]	
Weibull	Multistage (Armitage-Doll)[1]	
Gamma-Multihit	Linearised Multistage	

([1] these models also exist in a time-to-tumour mathematical model)

These dose-response models are usually applied to tumour-incidence data corresponding to only a limited number of experimental doses, which is due to the standard design of the bioassay. Instead of determining the complete dose-response curve, a carcinogenicity study is in general limited to three (or two) relatively high doses, using the MTD as highest dose (MTD= maximum tolerated dose). These high doses are used to overcome the inherent low statistical sensitivity (10-15 % over background) of such bioassays, which is due to fact that (a.o. for practical reasons) a relatively low number of animals is tested. Because data for the low dose region are not available (i.e. cannot be determined

experimentally), extrapolation outside the range of observation is required. For almost all data sets, most of the above listed models fit equally well in the observed dose range, due to the limited number of doses and animals. However, in the low-dose region these models diverge several orders of magnitude, thereby introducing large uncertainties to the risk estimated for these exposure levels.

Because the actual form of the dose-response curve in the low-dose range can not experimentally be generated, mechanistic insight in the carcinogenic process is crucial to be able to discriminate on this aspect between the various models. In the remaining part of this section some major characteristics of the above listed dose-response models will be briefly discussed. In addition, remarks on the usefulness of some models and the relevance of their assumptions will be made in view of our current knowledge of the molecular mechanisms that underlie the process of carcinogenesis.

Tolerance distribution models

Tolerance distribution models were reviewed by Johannsen (1990), Carlborg (1981a), Park and Hawkins (1993) and EPA (1987). Tolerance distribution models assume that each member of a given population has a threshold or tolerance level below which that individual will not respond to the exposure in question and that the variability among individuals can be expressed as a probability distribution. Thus the tolerance distribution models describe the distribution of these threshold values in a given population and models such as probit, logit, gamma-multihit, Weibull and Mantel-Bryan can all be generated by using different probability distributions.

The parameters in the model are used to improve the fit but have no biological meaning and, therefore, cannot be validated (Johannsen, 1990; Park and Hawkins, 1993). This class of models is nonlinear at low doses and their estimates very rapidly decline to zero response. Therefore they have declined in use with the development of the hypothesis of a non-threshold mechanism of action of genotoxic carcinogens.

Hit models

These models, which were extensively reviewed (Johannsen, 1990; Carlborg, 1981a,b; Park and Hawkins, 1993; Munro and Krewski, 1981), are based on the assumption that a tumour originates from a single cell that has been damaged by one or more successive 'hits' (one-hit, multihit, multistage). With the exception of the Linearised multistage model (LMS), only the major characteristics of the other models will be briefly discussed in this section.

In general Hit models calculate the tumour incidence in a population as a function of dose after lifetime exposure. Time is not taken into account as a variable factor. It is assumed, especially in the low-dose region, that the relationship between dose and risk is linear. Because the one-hit model has only one parameter (other than background), it usually does not fit experimental data well. On the other hand, the low dose estimates, which are relatively conservative, are rather insensitive to minor changes in the observed tumour incidence. The multi-hit model assumes that the target cell must absorb at least "k" number of hits before a tumour can be observed. The probability of a hit is proportional to the dose. However, the parameters in the one-hit and multi-hit model, although referring to a mechanism of carcinogenesis, cannot be biologically interpreted.

The multistage model as developed by Armitage and Doll (1954) was developed to explain the observation that the age-specific incidence of many human adult carcinomas increases roughly with the power of age. This model, therefore, has been used frequently

for the analysis and low-dose extrapolation of epidemiological data. It reflects the most prevalent theory of carcinogenesis in the 1980s. That is, a normal cell must progress through a series of irreversible genetic changes or stages before it can become malignant. The extension of the one-hit model is that the transition rates between the successive stages are not required to be equal and at least one of the stages is assumed to be rate-limiting and linearly related to dose.

Another version of the multistage model is the Linearised Multistage Model (LMS) developed by Crump et al. (1976). A mathematical adaptation was incorporated in the multistage model; the upper confidence limit (UCL) instead of the maximum likelihood estimate (MLE) was included. The MLE i.e. the "best" estimate of the multistage model is rather sensitive to small changes in the observed tumour incidence. Because of this instability and to be sure that risk is not underestimated some regulatory organisations use the more stable linearised 95% UCL.

The Linearity at low doses is based on the argument that the ever present background tumour incidence is only linearly enhanced by the carcinogenic agent (Crump, 1984b). The LMS model is still very popular and applied by organisations like US-EPA, although its mechanistic basis is still a crude oversimplification of the real biological processes. Because of the low-dose linearity all "hit" models are fairly conservative in their estimations.

Time-to-Tumour models

In general, these models attempt to relate dose, tumour latency (median time until a tumour or death by cancer occurs), and cancer risk. These models (e.g. the empirical models of Druckrey, Armitage-Doll, Weibull in time), have not been validated extensively. One of the complications is the fact that actual response times are often difficult to determine: some tumours may only be seen at sacrifice.

2.4.2 Biologically based models

Biologically based modelling is the process by which the specific mechanistic steps that are involved in toxic action of chemicals, are expressed in quantitative terms by a set of equations leading to prediction of the outcome of specific toxicological experiments (Andersen et al., 1992). These models improve risk assessment because toxicity can be estimated for all possible exposure conditions instead of only under the experimental conditions, i.e. they are able to extrapolate toxicity across dose, route of entry and exposure time. However, the most important improvement is the extrapolation to the low-dose region. An example of a biologically based model is the model by Moolgavkar, Venzon and Knudson.

Moolgavkar-Venzon-Knudson (MVK)

Recent advances in molecular genetics and experimental carcinogenesis revealed many factors that play an important role in the control of cell proliferation. An attempt to incorporate this parameter into a mathematical model for cancer risk assessment was pioneered by Moolgavkar and coworkers (Moolgavkar, 1989; Moolgavkar et al., 1989; Moolgavkar and Luebeck, 1990). They proposed an alternative multistage model based on the concept of recessive oncogenesis that also accounts for proliferation of intermediate cells. In this model two transitions are required for the transformation of a normal into a malignant cell: one from a normal to a intermediate cell, a second one from a intermediate

into a transformed cell. The model allows for normal and intermediate cells to proliferate, differentiate, or die.

The model provides a framework for the analysis of epidemiological and experimental data and accounts for the way in which (environmental) risk factors contribute to carcinogenesis by affecting transition rates, tissue growth and/or regeneration, and tissue differentiation. This approach has also been followed by others (Ellwein and Cohen, 1988; Chen and Farland, 1991), who developed similar models.

The biological principles of the model give the model parameters biological interpretation. Therefore, they can be measured, or if no measuring technique is available, the parameters can be estimated from the data. The experience of calibrating model parameters of the MVK model is still minimal. Therefore it is difficult to determine if the model describes the mechanism well. Another difficulty with this model is that Time-to-tumour data, necessary for calibration and validation, are not available in general.

The model offers also a possibility to model carcinogenesis induced by non-genotoxic agents. For such agents it is assumed that they cause an increase in cell division frequency as a result of induced cytotoxicity.

2.4.3 Dutch 'linear model'

In The Netherlands a 'simple' linear extrapolation method for quantitative cancer risk assessment for chemicals was adopted by the Health Council of The Netherlands in 1978 (Health Council, 1978). With this method a cancer risk estimation directly based on the bioassay dose-response curve (obtained from animal experiments) is performed: the excess cancer incidence at actual exposure levels is estimated by linear extrapolation through the origin (background tumour incidence subtracted) using dose-response data of the lowest (daily) carcinogenic 'lifetime' dose. Two considerations were fundamental to this approach, first the recognition of the multistage nature of carcinogenesis and second the assumption of linearity in the dose-response relationship at low dose levels, i.e. the carcinogen is expected to affect only one transition (one-hit kinetics) in the carcinogenic process, due to the relative abundance of endogenous background hits (Crump, 1984b; Lutz, 1990).

Recently, the Health Council has re-evaluated this method of quantitative risk assessment in view of the remarkable scientific progress after 1978, i.e. the identification of cellular oncogenes and tumour suppressor genes. The Council concluded that the linear approach is still considered appropriate for quantitative cancer risk estimations (Health Council, 1994).

3 CONCLUSIONS

3.1 Dose-response assessment

Table 1 gives an overview of methods and models that are presented in chapter 2. Table 1 indicates that in defining an acceptable dose corresponding to an acceptable risk and defining a safe dose protecting the human population, one or more methods and models are available for human dose-response assessment. However, due to differences in assumptions underlying the methods, the estimated safe dose or acceptable risk may differ considerably with the method that is used.

Human dose-response assessment implies determination of an ADI or R$_f$D (a US value) as well as determination of toxic effects after exceeding an ADI. Thus, a dose-response relationship is a necessity. Mathematical models exist to describe such a relationship. Most dose-response curves are based on animal data and, in general, only one dose (NOAEL) is extrapolated by a safety factor to the human situation. Therefore, it is hard to estimate toxic effects in humans when a ADI is exceeded. The other dose-response data are not taken into account.

The toxicity calculated by each method varies because each method treats uncertainties introduced by the extrapolation differently. The extrapolations mentioned here are: interspecies variation, intraspecies variation, route-to-route, high-to-low dose, exposure scenario.

Unfortunately, exceeding an ADI is usually neither continuous nor constant in time and dose. To estimate the toxicity in animals under variable exposure conditions, sometimes above the ADI, in principle all methods and models mentioned in Table 1 could be used. However, many assumptions are required for the adjustment of the dose-response relationship to the variable exposure conditions, thereby introducing additional uncertainty. Therefore Table 1 presents MVK as a model to estimate toxicity under variable exposure conditions. The MVK model estimates the tumour incidence as a function of time and dose. Thus, under exposure conditions which are variable in dose and time, tumour incidences can still be estimated.

The MVK model is based on biological concepts and mechanisms. Therefore extrapolation of the data beyond the experimental conditions, on which the model is based, can be done more reliably than with empirically based models. Interspecies extrapolation must be taken into account before applying the MVK model.

Methods to extrapolate animal data to the human situation are: the safety factor method, Renwick's safety factor method, Allometry, AUC method, PBPK modelling, Haber's Law and Route-to-Route extrapolation. The safety factor method and Renwick's safety factor method extrapolate animal toxicity data to human data by an arbitrarily chosen safety factor. The toxicity standard that is determined by application of these methods is therefore also arbitrary. The method for route-to-route extrapolation and Haber's Law are questionable and therefore do not improve the extrapolation from animal data to man. PBPK models estimate the internal dose in animals and in humans. Comparison of these internal doses indicates the difference in sensitivity between these species, under the assumption that the same toxic effect is induced. This assumption still leaves uncertainty about the interspecies extrapolation. To resolve this problem, it should be verified whether the same toxic mechanism is induced in animals and humans.

Table 1b gives an overview of the requirements for methods and models for dose-response assessment. The models are more or less presented in order of complexity. MVK modelling and PBPK modelling require more data and time before they can be applied. The more sophisticated models starting with Benchmark (BM) require an extended dose range to apply the model properly. In addition most methods need quantitative data.

In some cases it might not be advisable to apply a sophisticated model because of the time required to develop such a model while in other cases it is advisable. For example if a very small population is exposed to very low doses of a certain chemical, far below the

NOAEL in animals, it is not interesting for risk management to invest in the development of a sophisticated model. The risk of this population can be considered very small. In such a case the Safety Factor method can be used to establish a toxicity standard. A PBPK model might be required in the case a population risks to exceed a toxicity standard. The internal dose in humans at the exposure level can be estimated. With help of the animal dose-response curve an estimation of the risk in the human population can be given.

These examples are used to indicate that choices should be made before application of a certain model or method. The examples also make clear that some methods (Safety factor) can be used to establish a standard below which almost a complete population is protected, while others (PBPK) can be used to indicate how many people are at risk or how many individuals are protected.

3.2 Methods in dose-response assessment at RIVM

In this part of the report an overview is given of the methods and models in dose-response assessment that are currently used or investigated at RIVM. The participating labs are ACT (advisory centre toxicology), LCM (lab. carcinogenesis and mutagenesis), PAT (lab. for pathology), TOX (lab. of toxicology), BFT (unit biotransformation, pharmacokinetics and toxicokinetics) and CWM (centre for mathematical methods) and LGM (lab. for drugs and medical instruments)

Table 2 presents the techniques that are used or are investigated by the different labs

ACT and LCM use TOXRISK to calculate the acceptable dose of genotoxic carcinogens corresponding an acceptable risk. In most cases the linearised multistage and the multistage model are used. The other models that are available such as tolerance distribution models and other hit-models are only applied to compare the acceptable dose calculated by these methods with the multistage or linearised multistage model.

CWM and LCM are investigate time-to-tumour models. Especially the MVK model because it is based on biological mechanisms that are expected to be relevant for the formation of tumours. However, the data LCM uses, do sparsely include time to response data. That is why the MVK model is rarely applied to estimate human risk. In collaboration with CWM the MVK model is improved and applied to a chronic toxicity study on benzo(a)pyrene.

LGM, uses for drug assessment allometric scaling and the AUC method. They also use some empirical effects models. Collaboration of this lab with the other mentioned labs might improve dose-response assessment of toxic compounds, because at LGM a large quantity of human data are available.

ACT, TOX, and CWM have evaluated methods that have been developed to estimate the threshold dose (NOAEL, BM, BED, Gaylor). A theoretical evaluation of these methods has been made. In the near future the methods will be evaluated experimentally with available data sets. These evaluations will help to choose the best method for threshold dose estimation.

PAT is mainly a user of the accepted standard methods to estimate a acceptable dose or an ADI. PAT collaborates with CWM in the development of an animal to human extrapolation model for immunotoxicity data.

BFT, TOX and CWM are involved in PBPK modelling. These labs develop PBPK models and perform research on validating this kind of modelling for human risk assessment. The dynamic part of the development of the effect is sparsely implemented in PBPK modelling yet, but it is a item that will be treated in the future. These labs are aware that dynamic modelling is the next step in improving quantitative risk estimation.

These labs are also involved in risk assessment. The complete chain is being modeled starting from the source to the toxic effect, thus exposure modelling and dose-response modelling integrated, i.e. chain modelling. An example is the chain model of cadmium (Slob and Kranjc, 1994).

Recommendations

In this report an overview of methods and models for dose-response assessment was presented. Our intention was to make clear which models are available and which are used for dose-response assessment.

The methods and models in this report can be divided into three main parts:
1) Threshold estimation
2) Extrapolation models
3) Dose-response models
For each issue recommendations will be given below

Threshold estimation

The determination of the NOAEL is the standard method to establish a safe dose for non-genotoxic agents. This method has restrictions, as is explained in chapter 2. Alternative methods have been developed to overcome these restrictions. It is said that the latter methods have wider applicability. Whether this is true, should be investigated.

To check the various methods, it is proposed to use one data set for all methods. The estimated thresholds can then be compared. Such a comparison gives an indication of the relation between the various threshold estimates. It might also be interesting to investigate the profit of the confidence intervals that are calculated in relation to risk assessment.

Extrapolation models

For extrapolation several methods and models exist. The most simple one is the safety factor method. However, the extrapolation factor to translate the animal data to human data is arbitrary. To improve this extrapolation step, the internal dose concept in animals and humans should be estimated. This may indicate a difference in susceptibility. PBPK modelling can be used to estimate the internal doses.

Experience exists concerning PBPK modelling, however, PBPK modelling is not perfect yet. Calibration and validation as well as development of general PBPK models need more attention.

It is suggested that PBPK modelling can be used to indicate intra-individual variation. If variation of several parameters is implemented in the model then a distribution of various internal doses can be simulated.

PBPK modelling does marginally take into account the toxico-dynamics of the compound. If PBPK modelling is used for risk estimation, it is assumed that in the same effect is induced in humans as in animals. However, humans and animals often respond differently to toxic agents. An improvement of risk estimation would be toxico-dynamic modelling together with PBPK modelling, because differences in kinetics between humans and animals can be quantified as well as differences in the mechanism between animals and humans. This kind of modelling requires both mechanistic and modelling research and will be time consuming in the beginning.

Dose-response modelling
Models that need more attention are biologically based models such as the MVK model. Although the biologically based models, e.g. the MVK model, represent still an over-simplification of the process of carcinogenesis, they are considered very promising and deserve further validation in order to obtain a realistic, i.e. biologically based, tool for future quantitative cancer risk assessment.

Biologically based models describe a toxic process mechanistically. Therefore, extrapolation outside the tested dose range with these mechanistic models will be more reliable. It is as yet unclear whether the use of biologically based models will be confined within the range of observations, partly outside it (and combined with a linear 'model free' extrapolation to low doses), or whether these models will also be used for the direct estimation of risks associated with low exposures.

Biologically based models are based on animal data, and merely on one or a few data sets. For appropriate use these models need to be validated on several animal data sets and if possible on human data. The validation step, however, is often given little attention. It is recommended that validation of the model is required, before application.

In risk assessment biologically based modelling receives much attention. To improve this kind of modelling, there should be a concerted effort of all kinds of disciplines to develop mechanistic models. It involves the development of new bioassay designs and the collection of various kinds of data to derive a mechanism and then estimate parameters in models that are a result of these studies.

Risk management
Most recommendations point at more PBPK modelling and biologically based modelling to improve risk assessment. These techniques, have the potency to improve risk assessment, however, the development of such models is time consuming.
Therefore it is proposed to develop a decision tree to decide when a PBPK model is required for risk assessment and when for example the safety factor method will do. This recommendation is interesting for risk management.

Exposure assessment
In this report, only methods in dose-response assessment are described. However, for risk estimation exposure assessment is also required (see chapter 1). Thus to improve quantitative risk estimation on both levels efforts are needed. Integration or combination of knowledge, available in the labs at RIVM involved in both exposure assessment and dose-response assessment, may be helpful to improve quantitative risk estimation.

ACT, BFT, TOX and CWM focus also on the exposure. BFT and ACT developed a model for consumer's exposure (Van Veen, 1994). CWM developed also other models in this field (Wortelboer, 1994). TOX has developed a model for air pollutants (Van Scheindelen in preparation). TOX and CWM also developed a model for the distribution of nitrate uptake by food in the human population, based on the Food Consumption Program (VCP).

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Table 1a. Methods and models presently available in human dose-response assessment. The methods and models are presented in order of increasing complexity.

Methods and Models in Human Dose-response assessment

	SF	RSF	Haber	Route	NOAEL	DSD	HK	BM	Gaylor	Dis	Hit	Time	MVK	PBPK	AUC	Allo	SE	FE	DM
Methods available for assessment of:																			
Threshold					X	X	X	X	X				X						
Acceptable risk	X	X								X	X	X	X				X	X	X
Toxicity standard	X									X	X	X	X				X	X	X
Risk/fixed exposure								X	X	X	X	X	X				X	X	X
Risk/variable exposure													X						
Methods especially for extrapolation																			
Route-to-Route				X										X	X				
Interspecies	X	X												X	X	X			
Intraspecies	X	X												X	X				
High-to-Low dose														X	X				
Exposure scenario			X											X	X				

Explanation of abbreviations and the paragraphs that discuss the various methods :

SF   Safety factor method (2.3.1)
Route   Route-to-Route extrapolation (2.3.4)
DSD   Dose-severity diagrams (2.2.3)
Dis   Tolerance distribution models (2.4.1)
MVK   Moolgavkar-Venzon-Knudson (2.4.2)
Allo   Allometry (2.3.3)
DM   Dutch linear method (2.4.2)

RSF   Renwick's safety factor method (2.3.2)
NOAEL   No-observed-adverse-effect-level (2.2.1)
BM   Benchmark (2.2.2)
Hit   Hit model (2.4.1)
PBPK   Physiologically based pharmacokinetic modelling (2.3.5)
SE   Sigmoid E_{max} (2.4.1)

Haber   Haber's law (2.3.4)
HK   Hoekstra's bounded effect dose (2.2.2)
Gaylor   Gaylor's linear extrapolation method (2.2.2)
Time   Time-to-response models (2.4.1)
AUC   Area Under Curve (2.3.5)
FE   Fixed effect (2.4.1)

Threshold: method to estimate the threshold
Tox standard   method to set a human toxicity standard; only after estimation of threshold
Risk/variable exp method to determine risk under variable exposure concentration and duration
Interspecies   extrapolation across species: animal to human
High to low dose extrapolation from high to low dose

Acceptable risk: method to determine a dose that induces an acceptable risk (10^{-6} or 10^{-4})
Risk/fixed exp   method to determine risk after fixed exposure duration and concentration
Route-to-route   extrapolation across various exposure routes
Intraspecies   extrapolation across humans: human vs. human
Exposure scenario extrapolation across exposure concentration and duration

Table 1b Requirements for the application of dose-response assessment methods.

						Methods and Models in Human Dose-Response Assessment													
	SF	RSF	Haber	Route	NOAEL	DSD	HK	BM	Gaylor	Dis	Hit	Time	MVK	PBPK	AUC	Allo	SE	FE	DM
Requirements																			
Dose range			X	X	X	X	X	XXX	XXX	XXX	XXX	XXX	XXX	XXX	X	X	X	X	X
Effects																			
* qualitative effects	X	X				X													
* quantitative effects	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Models & Methods																			
* mathematical model								X	X	X	X	X	X	X	X	X	X	X	
* mechanistic concept													X	X		X			
* kinetic data		X												X	X				
* dynamic data		X											X						
* physiological data														X		X			
* confidence intervals							X	X	X										
* time-to-tumour data													X						

XXX = extended dose range

SF	Safety factor method (2.3.1)	RSF	Renwick's safety factor method (2.3.2)	Haber	Haber's law (2.3.4)

SF — Safety factor method (2.3.1)
Route — Route-to-Route extrapolation (2.3.4)
DSD — Dose-severity diagrams (2.2.3)
Dis — Tolerance distribution models (2.4.1)
MVK — Moolgavkar-Venzon-Knudson (2.4.2)
Allo — Allometry (2.3.3)
DM — Dutch linear method (2.4.2)

RSF — Renwick's safety factor method (2.3.2)
NOAEL — No-observed-adverse-effect-level (2.2.1)
BM — Benchmark (2.2.2)
Hit — Hit model (2.5.1)
PBPK — Physiologically based pharmacokinetic modelling (2.3.5)
SE — Sigmoid E$_{max}$ (2.4.1)

Haber — Haber's law (2.3.4)
HK — Hoekstra's bounded effect dose (2.2.2)
Gaylor — Gaylor's linear extrapolation method (2.2.2)
Time — Time-to-response models (2.4.1)
AUC — Area Under Curve (2.3.5)
FE — Fixed effect (2.4.1)

Table 2 Overview of dose-response models and methods that are used or under investigation at labs in RIVM

Lab	Toxrisk	TTT	MVK	SF	NOAEL	BM	BED	Gaylor	PBPK	AUC	Allo	SE	FE	DM
ACT	Y			Y	Y/X	X	X	X						
PAT				Y	Y									Y
TOX				X/Y	X/Y	X	X	X	Y/X					
BFT										Y/X	Y/X			
LAM	Y	X	X											Y
CWM		X	X		X	X	X	X	Y/X					
LGM					X				X	Y/X	Y/X	Y	Y	

X   = under investigation
Y   = applied for risk assessment

TOXRISK = computer program consisting of hit models, tolerance distribution models, mvk model
TTT   = time-to-tumour models
MVK   = Moolgavkar-Venzon-Knudson model
SF   = safety factor method
NOAEL   = no-observed-adverse-effect-level
BM   = benchmark method (Crump)
BED   = bounded-effect dose (Hoekstra)
GAYLOR = Gaylor's linear extrapolation method (Gaylor)
PBPK   = physiologically based pharmacokinetic modelling
SE   = sigmoid E max
FE   = fixed effect
DM   = dutch linear method

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5 APPENDICES

Appendix A

Effect categories and their definition

Effect category	Definition
FEL	Frank Effect Level. That exposure level which produces unmistakable adverse effects, such as irreversible functional impairment or mortality, at a statistically or biologically significant increase in frequency or severity between an exposed population and its appropriate control
LOAEL	Lowest-Observed-Adverse-Effect-Level. The lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group
NOAEL	No-Observed-Adverse-Effect-Level. That exposure level at which there are no statistically or biologically significant increases in frequency compared to its appropriate control. Effects produced at this level, are not considered to be adverse
NOEL	No-Observed-Effect-Level. The exposure level at which there are no statistically or biologically significant increases in frequency or severity of effects between the exposed population and its appropriate control

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Appendix B.

EPA classification of adversity of toxicological effects. The rating corresponds to the severity of the effects.

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rating	effect
1	Enzyme induction or other biochemical change with no pathological changes and no change in organ weight.
2.	Enzyme induction and subcellular proliferation or other changes in organelles, but no other apparent effects.
3.	Hyperplasia, hypertrophy, or atrophy, but no changes in organ weights.
4.	Hyperplasia, hypertrophy, or atrophy, but changes in organ weights.
5.	Reversible cellular changes: cloudy swelling hydropic change, or fatty changes.
6.	Necrosis or metaplasia with no apparent behavioural sensory, or physiologic changes.
7.	Necrosis, atrophy, hypertrophy, or metaplasia with a detectable decrement of organ functions. Any neuropathy with a measurable change in behaviour, sensory, or physiologic activity.
8.	Necrosis, atrophy, hypertrophy, or metaplasia with definite organ dysfunction. Any neuropathy with gross changes in behaviour, sensory, or motor performance. Any decrease in reproductive capacity. Any evidence of fetotoxicity.
9.	Pronounced pathologic changes with severe organ dysfunction. Any neuropathy with loss of behavioural or motor control or loss of sensory ability. Reproductive dysfunction. Any teratogenic effect with maternal toxicity.
10.	Death or pronounced life shortening. Any teratogenic effect without signs of maternal toxicity.

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