NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT
BILTHOVEN, THE NETHERLANDS

Report no. 620110.002

Relevance of effect modelling for the risk assessment of substances

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January 1996

This study was conducted by order of the Directorate General for Environmental Protection, Directorate for Chemicals, Safety and Radiation Protection within the framework of project number 620110.

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SUMMARY

Dose-related effects play an important role in the risk assessment of substances. The preventive risk assessment (standard-setting) generally utilises only one data point of the dose-effect curve: the No-observed-adverse-effect-level. The actual risk assessment, however, requires insight into the dose-effect *curve* for the evaluation of the risk of current exposure. The shape of a dose-effect curve is determined by toxicokinetics and toxicodynamics of a substance. Toxicokinetics deals with the absorption, distribution, metabolism and excretion of substances. Toxicodynamics deals with the mode of action by which a substance exerts its toxic effects. Toxicokinetic and toxicodynamic processes can be dynamically described by mathematical modelling. This report describes how toxicokinetic modelling (PBPK modelling) and toxicodynamic modelling (effect modelling) can contribute to improvement of preventive and actual risk assessment. Combined toxicokinetic and toxicodynamic modelling facilitates the risk assessment at any human exposure level (no high-to-low-dose extrapolations), for any exposure pattern (no concentration-time extrapolations) and for different exposure routes (no route-to-route extrapolation).

The report concludes with a proposal for a study on toxicokinetic/toxicodynamic modelling. Based on the relatively high exposure levels and the varying exposure of the human population, benzene is selected as a relevant substance. A major advantage of benzene as a subject for study is that toxicokinetic and toxicodynamic models can be taken from the literature. As an example of a more generic issue, a toxicodynamic model for the process of carcinogenesis will be used to illustrate the effects of different exposure patterns on tumour formation.

SAMENVATTING

Dosis-gerelateerde effecten spelen een belangrijke rol bij de risico-evaluatie van stoffen. De preventieve risicoschatting (normstelling) gebruikt in het algemeen slechts één punt van de dosis-effect curve: het Geen-waargenomen-schadelijk-effect-niveau oftewel de "No-observed-adverse-effect-level". Voor de actuele risicoschatting is echter inzicht in de dosis-effect *curve* van belang om het risico bij een bepaalde actuele blootstelling te kunnen schatten.
De vorm van de dosis-effect curve wordt bepaald door de toxicokinetiek en toxicodynamie van een stof. De toxicokinetiek behelst de absorptie, distributie, metabolisme en excretie van een stof. De toxicodynamie omvat de manier waarop een stof tot toxische effecten leidt. Door mathematische modellering kunnen toxicokinetische en toxicodynamische processen dynamisch worden beschreven. Dit rapport gaat in op hoe toxicokinetische modellering (PBPK-modellering) en toxicodynamische modellering (effect-modellering) kunnen bijdragen aan een verbetering van de preventieve en actuele risicoschatting. Het gecombineerd toxicokinetisch/toxicodynamisch modelleren maakt de risico-evaluatie van stoffen mogelijk voor alle humane blootstellingsniveaus (geen hoge-dosis-lage-dosis extrapolatie), voor elk blootstellingsprofiel (geen concentratie-tijd extrapolaties) en voor verschillende blootstellingsroutes (geen route-to-route extrapolatie).

Het rapport besluit met een onderzoeksvoorstel voor toxicokinetisch/toxicodynamisch modelleren. Gebaseerd op de relatief hoge humane blootstellingsniveaus en de variaties in blootstelling, is benzeen geselecteerd als een relevante stof. Een belangrijk voordeel van benzeen is dat toxicokinetische en toxicodynamische modellen uit de literatuur kunnen worden verkregen. Als een voorbeeld van een meer generiek probleem, zal een toxicodynamisch model voor het proces van de carcinogenese worden gebruikt, om de effecten van verschillende blootstellingsprofielen op tumorinductie te illustreren.

GLOSSARY OF TERMS

ADI	Acceptable daily intake
AUC	Area-under-the-curve
C_{max}	Maximum plasma concentration
$C \times T = k$	"Concentration times time gives constant toxic effect", known as Haber's Law. To extrapolate external dose and exposure time, Haber's Law gives equal weight to dose ('concentration') and exposure duration ('time'). See also report 659101 002
effect modelling	toxicodynamic modelling
HBAEL	Health-based acceptable exposure limit
MAC	Maximum acceptable concentration, used in occupational toxicology
NOAEL	No-observed-adverse-effect-level. The highest dose at which no statistically significant adverse effects are found.
PBPK	Physiologically based pharmacokinetic modelling
toxicokinetics	discipline dealing with the absorption, distribution, metabolism and excretion of substances
toxicodynamics	discipline dealing with the mode of action by which a substance exerts a toxic effect
TWA	Time-weighted-average

1. INTRODUCTION

In risk evaluations of substances, toxic effects are related to the external dose. For the preventive risk assessment (standard setting) the relation between observed health effects and administered dose is studied according to international guidelines. To get insight in which dose-levels result in adverse health effects, the use of three or four dose levels is common. The main objective of standard toxicity studies is the establishment of the No-observed-adverse-effect-level (NOAEL). The NOAEL is the highest dose which does not result in a statistically significant adverse effect. By means of uncertainty factors the chronic NOAEL as observed in experimental animals is extrapolated to a human "safe" level, such as the Acceptable Daily Intake (ADI). The NOAEL, which can be considered as a semi-quantitative estimate of a dose at which toxicity is absent, is thus the only point of the dose-effect curve that is used for the preventive risk assessment.

For the actual risk assessment i.e. the evaluation of human risks at a current exposure, the qualitative as well as the quantitative aspects of dose-related effects are important. Unfortunately, the experimental design of toxicity studies often impairs the construction of dose-effect *curves*. As a consequence, the toxicity at doses other than tested in the experiment is difficult to predict. Also, the standard toxicity tests do not provide information on the toxicity of a substance in the case of other exposure routes or exposure patterns. In general, human exposure does not resemble the exposure of experimental laboratory animals. While experimental animals are exposed during defined time intervals to fixed concentrations of substances, human exposure duration and exposure concentration varies during life-time. Moreover, in general, the dose levels of experimental animals exceeds the "real-life" exposure of humans. Since exposure patterns may have a marked influence on the eventual toxicity of substances, insight is needed in how variations of dose levels and dose intervals influence the toxic effects.

2. TOXICOKINETICS, TOXICODYNAMICS AND TOXIC EFFECTS

By relating administered external doses to the toxic effects, a dose-effect curve can be constructed. Preferably, enough data should be available to obtain a dose-effect curve with a good fit. By interpolation or extrapolation, the expected effect at doses, other than administered in the experiment, can be predicted. However, as has been mentioned before, the dose-effect curve is only valid for the specific experimental conditions used to construct the dose-effect curve.

A dose-effect curve is the result of actions of the body on the substance and actions of the substance on the body. This is schematically represented in Fig.1. The input of this scheme is the external dose, which is defined as the amount of substance which enters the cavities of the body. The output is the ultimate toxic effect, i.e. the adverse health effect caused by the substance. Between external dose and effect, two boxes are situated: toxicokinetics and toxicodynamics.

Toxicokinetics deals with the absorption, distribution, metabolism and excretion of the substance (and its metabolites) and can be regarded as the action of the body on the substance.
Toxicodynamics deals with the mode of action by which the compound (or a metabolite) exerts a toxic effect. Appropriate toxicodynamic factors include the identification of the important toxic entities (e.g. metabolites), the nature of the molecular target (e.g. a receptor, DNA) and the presence and activity of protective (e.g. glutathione) or repair mechanisms.

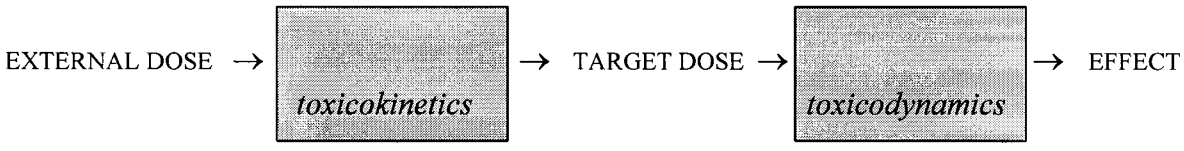

Fig. 1 The influence of toxicokinetics and toxicodynamics on the effect

The "target dose" which is the output of toxicokinetics and the input of toxicodynamics, is the amount of substance which reaches the biological "target" in the body at each point in time. As an example, consider the exposure to benzene. The amount of inhaled benzene in the lungs per unit of time can be considered as the external dose. Toxicokinetics deals with the uptake of benzene in the blood (part of benzene is exhaled), the transport through the body, metabolism in the liver and excretion of parent compound and metabolites. The target dose may be the total of genotoxic and/or carcinogenic benzene metabolites reaching the blood-forming cells. Toxicodynamics describes the critical interactions between these metabolites and the "target" cells. These interactions eventually lead to the final toxic effect (in humans acute myelogenous leukemia).

Though Fig. 1 may suggest that toxicokinetics and toxicodynamics are a sequence of events, it should be noted, that neither in definition nor in time-frame toxicokinetics and toxicodynamics are clearly separated. Toxicokinetic and toxicodynamic processes are closely coupled and will occur simultaneously rather than sequentially. Bioactivation of compounds to toxic metabolites may be considered as a part of the metabolism and thus part of the toxicokinetics. However, since bioactivation of a parent compound may be essential for the eventual toxic effect, it can also be considered as part of the toxicodynamics. When a certain metabolite appears to be the key toxic entity, the toxicokinetics of this metabolite as well as the toxicodynamic action are coupled to the toxicokinetics and toxicodynamics of the parent compound.

The qualitative and quantitative outcome of toxicokinetics and toxicodynamics highly depends on the dose rate. Variations in dose (rate) influence the toxicokinetics: uptake or elimination mechanisms which are often dose-dependent may become (de)saturated. Variation in dose patterns may result in different plasma peak concentrations (C_{max}) and different target doses while external doses may be the same. Exposure conditions thus determine not only the external dose but also through the toxicokinetics the target dose and

Time-frame of exposure	
continuous exposure	an un-interrupted period of exposure
duration	how long exposure occurs
peak exposure	a relatively high single exposure of less than 24 h
intermittent exposure	periods of exposure which are temporally separated e.g. in work-shifts. In particular, intermittent exposure may be presented as a block-pulse exposure. Intermittent exposure is composed of:
pulse	exposure with a limited duration
period	the time-interval between pulses

Note: A schematical description of exposure as a block-pulse is often a simplification of reality. Block-pulse diagrams assume distinct time-intervals in which a pulse with a constant dose/exposure-level occurs. A gradual variation of exposure-duration and exposure heights are more likely.

chronic exposure	an exposure during at least 90% of the total life-time (for rats: 18-month study)
semi-chronic exposure	an exposure during at least 10% of the total life-time (for rats: 90-days study)
sub-acute exposure	an exposure during approximately 4% of the total life-time (for rats: 28-days study)
acute exposure	single exposure; can be compared to "peak exposure"
timing of exposure	period(s) of a lifetime at which an individual is exposed

Dose-frame of exposure	
exposure	refers to potential dose (this is the concentration in air, drinking water, food or consumer's product)
constant exposure	the height of exposure has a constant level in time
varying exposure	the height of exposure varies with time
dose rate	the amount of substance per unit body mass administered per time interval (e.g. mg/kg/day)
potential dose	the amount of substance in the medium in contact with the body
external dose	the amount of substance which enters the cavities of the body
internal dose	the amount of substance which is taken up by the body
systemic dose	the amount of substance which is available in the body after first-pass metabolism
target-dose	the amount of substance which reaches the target in the body

Fig. 2 Exposure patterns: associated definitions

thus, the toxicodynamics. As a consequence, toxic effects which are related to the target-dose will also change qualitatively and quantitatively.
For each substance and each individual different exposure patterns exist. The exposure may be incidental, regular or continuous during a defined period or during life-time. Also heights of exposure may vary. To illustrate the large variation in exposure patterns and in dose rate, some terms associated with different types of exposure patterns are presented in Fig. 2.

The effect of dose rate on toxicokinetics and toxicodynamics can be illustrated by the effect of pulse exposure. Intermittent exposure (i.e. temporally separated doses) is quite common in work shifts. Fig. 3 illustrates the influence of intermittent exposure versus continuous exposure on the accumulation of chemicals in the body. It is clear that the target dose-level varies with exposure duration. In this example, continuous exposure leads to a higher target dose than intermittent exposure. Since the target dose determines the toxicological effects, it is therefore important to gain insight in the concentration-time relations of both toxicokinetics and toxicodynamics.

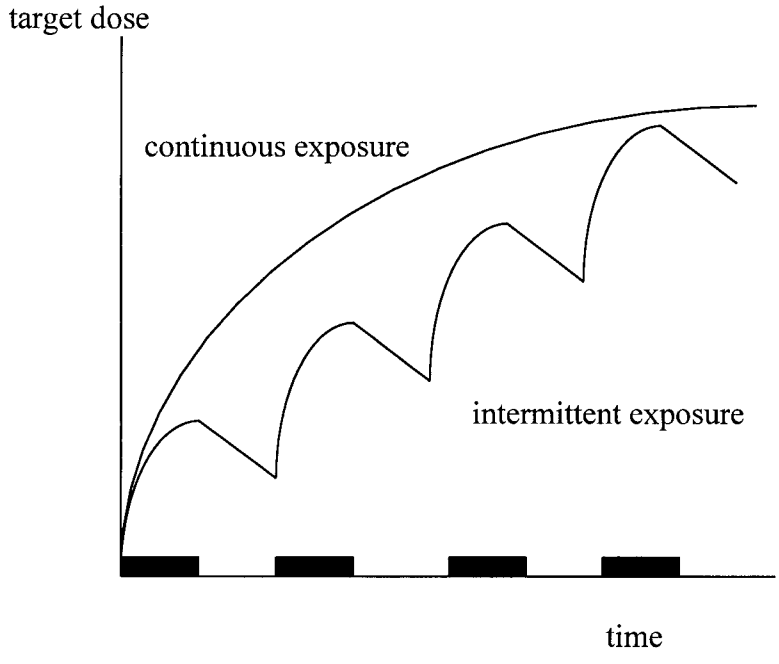

Fig. 3 The influence of exposure duration on the accumulation of chemicals in the body: intermittent versus continuous exposure.
Solid bars represent the exposure duration.

3. CONCENTRATION -TIME EXTRAPOLATIONS: CURRENT PRACTICE

3.1. Preventive risk assessment

3.1.1. Inhalatory administration

For practical reasons, the exposure of experimental animals to inhalable substances is often intermittent. Generally, the animals are exposed during working-hours, so for example 6 hours a day and 5 days a week. For the preventive risk estimation the $NOAEC_{intermittent}$ is extrapolated to a $NOAEC_{continuous}$ according to:

$$NOAEC_{continuous} = NOAEC_{int\ ermittent} * (\frac{6}{24}) * (\frac{5}{7})$$

This linear extrapolation of external dose rate and exposure time, gives equal weight to dose and exposure duration. This extrapolation method was suggested by Haber (1868-1934), who used the empirical rule *Concentration x Time = constant toxic effect* for the extrapolation of exposure-concentration and exposure duration.

Though a linear extrapolation may be valid in specific situations, in general, $C \times T = k$ has limited validity (RIVM report 659101 002). Haber's rule implies linear toxicokinetics; in particular, the area under the exposure concentration-exposure time curve (AUC of the exernal dose) is proportional to the AUC of the internal dose. Furthermore, $C \times T = k$ implies toxicodynamics in which the effect-size is proportional to the AUC of the internal dose. At high peak exposures enzymes may become saturated, in which situation toxicokinetics cannot be linear. Though for some long-term assays, the AUC may reasonably predict the toxic effects, other effects may depend more on the C_{max} rather than on the AUC (e.g. the teratogenicity of valproic acid, Nau (1986)). Therefore, Haber's rule will not hold, in general.

In occupational toxicology, maximum acceptable concentrations (MAC) are derived for the workplace. For the derivation of MAC-values, the toxicity of a substance as well as the feasibility to reduce exposure is taken into account. The MAC-value is applied as an 8-hour time-weighted-average (TWA). In practice, however, the actual concentrations of chemical substances in the workplace air fluctuate frequently and markedly within that 8 hours. Therefore, also peak limitations (5-60 min exposure) exist. The peak limitations vary from 2-10 times the MAC-value, depending on the half-life, the kinds of effects and the onset of effects.

3.1.2. Oral administration

Preferably, health-based acceptable exposure limits (HBAELs) such as the ADI, are derived from chronic toxicity data. When no chronic toxicity data are available, the NOAEL of a semichronic study may be used. To extrapolate a semichronic NOAEL to a chronic NOAEL, an uncertainty factor of 10 is usually applied. In the case of rats it is assumed that the

NOAEL for 24 months of exposure (chronic) will be equal or less than one tenth of the NOAEL of 3 months of exposure (semichronic).
Maybe unintentionally, this assumption resembles the outcome of a $C \times T = k$ extrapolation. Assuming $C \times T = k$, the chronic NOAEL would result from dividing the semichronic NOAEL by a factor of 8:

$$NOAEL_{chronic} = NOAEL_{semichronic} * (\frac{3}{24})$$

Often, when doses are administered by oral intubation, experimental animals receive 5 doses a week, instead of 7 doses a week. In general, this regular interruption of dosing is not taken into account. Recently, the IPCS (1994) recommended to use a $C \times T = k$ -like conversion to extrapolate the 5 days a week oral doses to 7 days a week doses (multiply the NOAEL with 5/7).

It should be noted that in common toxicity tests more underlying assumptions towards toxicokinetics and toxicodynamics are made, besides the concentration-time extrapolations mentioned above. For example, the external dose is defined as mg/kg body weight/day which assumes that the substance is homogeneously and instantaneously distributed over the body. Furthermore, it is assumed that the particular mode of dose administration does not influence the toxic effect. However, the administration of the test substance through the diet is completely different from the "bolus" oral intubation method. Though the total external dose may be the same, the (AUC of the) internal dose may be totally different between these two modes of administration. In that case, $C \times T = k$ will not hold.

3.2. Actual risk assessment

For an accurate risk evaluation of chemical substances, data on actual exposure are needed. If no direct measurements on the actual exposure are available, the exposure can be estimated by an exposure model. With the RIVM model CONS-EXPO (Van Veen, 1995) the exposure and uptake through the oral, inhalatory and dermal routes can be estimated. The RIVM model STEM (Slob, 1993) estimates oral exposure from concentrations in food products.

If the actual exposure exceeds accepted exposure limits, adverse health effects cannot be excluded. For an accurate risk assessment, qualitative and quantitative insight in dose-related effects is a requisite. Unfortunately, the experimental design of routine toxicity tests impairs the construction of good dose-effect curves. But even when a relatively good dose-effect curve can be constructed, the described relation of the external dose with the observed effect is only valid for that typical experiment. The actual events in the organism (toxicokinetics and toxicodynamics) are hidden in a "black box" (see Fig. 3A).
The limited use of experimental dose-effect curves is especially apparent with the extrapolation to low-dose regions. Toxicity experiments with laboratory animals are always conducted at relatively high doses. Quantitative models are required to extrapolate adverse effects at high doses in rodents to predict potential risks at low doses in humans.

Also when exposure conditions are different than tested (e.g. short-term instead of long-term exposure, a different exposure route etc.), the dose-effect curve will have limited use only.

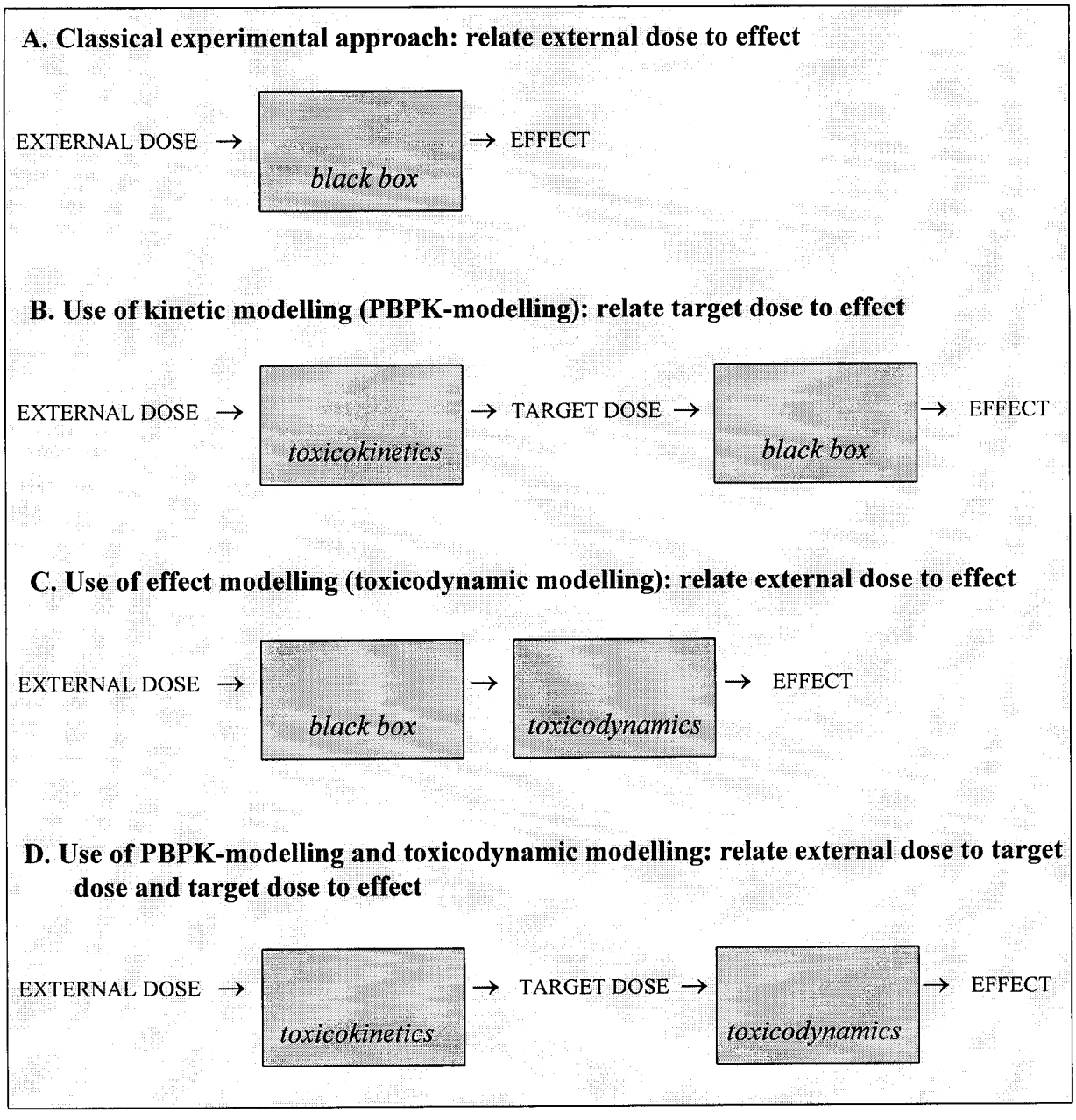

Fig. 3 Overview of approaches in current risk assessment

Another bottle-neck in the actual risk assessment of substances is the extrapolation of animal data to the human population. For the preventive risk assessment, assumptions towards the relative sensitivity are made (usually a factor of 10). However, for the actual risk assessment, more detailed information may be needed to perform a proper extrapolation of measured effects in experimental animals to actual risks for (certain groups of) the human population.

When dose-effect curves are based on *human* data instead of animal data, they may be valuable for the risk assessment of substances. Since a good epidemiological descriptive "model" describes the effects in the human population, such a model can be used to estimate the actual risk. However, epidemiological data often have limited value, because exposures are usually not well-defined.

For some substances, physiologically based pharmacokinetic (PBPK) models are currently used in the preventive and actual risk assessment of substances. With a PBPK-model the toxicokinetic fate (uptake, distribution, metabolism and excretion) of a substance can be dynamically described. The model is constructed within a physiological framework of the organism (blood flows through organs). Physiological and compound-specific kinetic parameters determine the *output* which is the (biologically active) concentration of the substance or its metabolite in target tissue (target dose). For example, a PBPK-model for benzene predicts the concentration in blood-forming cells of the parent compound (benzene) and its mutagenic metabolites. A PBPK-model can easily illustrate how variations of exposure patterns or exposure routes influence the target dose. For the risk assessment, the target dose is related to the toxic effect. No insight is provided, however, on the biological working mechanism by which a parent compound or its metabolite exerts its toxicity (see Fig. 3B). Consequently, though PBPK-modelling "translates" changing exposure conditions towards changing target doses, the eventual toxic effect cannot be predicted.
Under the assumption that the target dose is directly proportional to the toxic effects, PBPK-models can be used for actual risk assessment. However, the validity of such a linear relationship between target-dose and effect is questionable. Therefore, to obtain real dose-*effect* curves, one needs to have insight in the toxicodynamic processes as well.

For the process of carcinogenesis, some toxicodynamic models have been developed. An example is the Moolgavkar-Venzon-Knudson model (MVK-model) in which the critical steps of tumorigenesis are described. Fig. 3C illustrates how effect-modelling can aid the risk assessment. In most applications of this model, the external dose is directly related to the toxic effect (tumorigenesis). Biologically relevant effects (genotoxicity, cell proliferation) which are believed to be causally related to tumour formation are directly scaled to the external dose. In the ideal situation however, one should also know how the external dose relates to the target dose. The ideal combination of toxicokinetic and toxicodynamic modelling is presented in Fig. 3D. By combining toxicokinetic and toxicodynamic modelling, an accurate risk assessment can be carried out for all possible exposure patterns. Combined toxicokinetic and toxicodynamic knowledge has been used for example in the risk assessment of nitrate (Zeilmaker et. al, 1995). In this nitrate/nitrite model, the kinetics of nitrate and nitrite in the body was connected to the critical biological event, i.e. the formation of MetHb in the blood.

4. ADVANTAGES OF TOXICOKINETIC AND TOXICODYNAMIC MODELLING FOR RISK ASSESSMENT

As discussed above, both toxicokinetic and toxicodynamic modelling can contribute in improving risk assessment. Combined toxicokinetic/toxicodynamic modelling will show the time-profile of the uptake of the parent compound in the organism, the subsequent distribution and metabolism, the time-profile of the target-dose (parent compound or metabolites) and the eventual toxic cellular effect leading to adverse health effects. This is especially advantageous for the low dose regions for which, in general, no adequate animal data are available. Since a model *dynamically* describes toxicokinetics and/or toxicodynamics, variations in dose and dose rate will directly influence the outcome of the model. If necessary, the entry of the substance through different exposure routes can be implemented in the model.
When the models can be provided with human parameter values or sound estimates of these values, uncertainty factors for interspecies extrapolation will not be necessary. Even a risk assessment for well characterised groups at risk within the human population can be carried out, provided that one has insight in the variations of human sensitivity.

Summarizing, toxicokinetic and toxicodynamic modelling may facilitate an accurate actual risk assessment:
- at any human exposure level (no high-to-low-dose extrapolations)
- at any exposure pattern (no concentration-time extrapolations)
- regardless the exposure route (no route-to-route extrapolations)
- directly for humans (no interspecies extrapolation)
- for certain sub-groups of the human population with different sensitivity (no intraspecies extrapolation)

Finally, since toxicokinetic and toxicodynamic modelling describe the whole biological process, also the preventive risk assessment (standard setting) will be more accurate and realistic.

5. A STUDY PROPOSAL

5.1. Some considerations

5.1.1. Construction of a toxicokinetic model

For the construction of a toxicokinetic model, the toxicokinetics of a substance must be reasonably well understood. Insight is required in the toxicokinetic fate of a substance in the body and body compartments. What is the route of exposure, which body compartments are most relevant, does biotransformation (metabolism) occur etc.? In the model, uptake, distribution, metabolism and excretion of the substance are mathematically described. This requires several input parameters. Some of these parameters are physiological (blood flows, organ volumes), whereas other parameters are substance-specific (partition coefficients, metabolic parameters).

Since the objective of toxicokinetic modelling is the improvement of risk assessment in humans, a human model is preferred above an animal model. Some of the parameters needed, however, may be obtained or estimated from studies with experimental animals. In the case that kinetic processes in humans clearly deviate from those in experimental animals (e.g. different metabolic pathways), the model should contain human toxicokinetic parameters. Human parameters may be obtained from human studies with volunteers or from experiments with human tissues. Though some of the metabolic constants may be relatively easy to obtain, it may often be difficult to obtain good estimates of all human parameters.

5.1.2. Construction of a toxicodynamic model

For the construction of a toxicodynamic model, insight is needed in the critical biological events leading to the ultimate toxic effects. The toxic entity of the substance (parent compound or one or several metabolites) must be identified. Furthermore, the molecular interaction of this toxic entity with the cellular "receptor" should be clear. The input parameters of a toxicodynamic model consist of physiological parameters (amount of "receptor"-molecules, maximum regeneration capacity) and substance-specific input parameters (metabolic parameters, affinity to receptors, inducible repair-mechanisms).

The construction of a toxicodynamic model thus requires insight in the critical biological events in the cell, leading to adverse health effects for the organism. It is often not immediately clear, however, which biological events are most critical and how these events lead to the eventual health effect. Defining the *conceptual* framework of the toxicodynamic model is therefore the most important step in toxicodynamic modelling. When the concept of the biological events leading to the adverse health effects is defined, toxicodynamic parameters are required to complete the model. As has been indicated before, the objective is risk assessment for *humans*. Therefore, the also the toxicodynamic model should contain not only physiological human parameters but also human toxicodynamic parameters. Building a toxicodynamic model, parallel with determining all toxicodynamic parameters, will be a very time-consuming exercise. One should realize that some molecular processes may be rather difficult to measure in vivo and/or in vitro. Fortunately, many of the necessary parameters and constants can be derived or estimated from the literature. Human parameters may be obtained from experiments with human tissues or with human volunteers. In the case that human data are not available, animal parameters for which it can reasonably be assumed that they resemble the human counterpart may be used.

5.1.3. Choosing a relevant subject for modelling studies

Considering the research necessary to develop a toxicokinetic and toxicodynamic model, the investment for a proper risk evaluation is rather large. Therefore, it is of great importance to develop models only for those substances which are highly relevant for the human population. These may be substances for which for example human exposure is relatively high. Other substances may cause adverse effects in specific sensitive sub-groups of the human population at or slightly above accepted health limits.

Another approach may be to develop toxicodynamic models for more "generic" processes. For example, the interaction of substances with membrane components, leading to e.g. irritation may be an event occurring for various substances. Another generic event may be the depletion of scavenging molecules in the cell, such as glutathione.

A pilot compound relevant for risk assessment may be benzene. The exposure to benzene is often higher than the dose associated with the $1 * 10^{-6}$ risk after life-time exposure. Moreover, in the Netherlands the preventive risk assessment of benzene has been a subject of discussion. The advantage of developing a toxicodynamic model for benzene is that several toxicokinetic (PBPK) models already exist and toxicodynamic models for this compound have been proposed (Scheding et al., 1992). Below, some information concerning benzene is summarised.

A generic process which is worth studying is carcinogenesis. For the process of carcinogenesis, Moolgavkar, Venzon and Knudson proposed a two-steps toxicodynamic model (MVK-model). This model may be used to study the influence of changing exposure patterns on the ultimate carcinogenic effect. In this way the "concentration-time" relation of genotoxic compounds can be examined. More detailed information on the use of the MVK-model is provided below.

5.2. Benzene

The most significant adverse effects from prolonged exposure to benzene are hematotoxicity, genotoxicity and carcinogenicity. In humans, benzene is known to cause acute myelogenous leukaemia.
Sources of benzene include cigarette smoke, combustion and evaporation of benzene-containing gas-oil. Mean air concentrations in urban areas are about 5-20 $\mu g.m^{-3}$. Inhalation is the dominant pathway for benzene exposure. Smoking is a large source of personal exposure, while high short-term exposures occur during refuelling of automobiles.

In the Netherlands, the risk assessment of benzene is based on a linear extrapolation method since benzene is considered to be a genotoxic carcinogen. A dose of 0.12 $\mu g.m^{-3}$ was found to be associated with a $1 * 10^{-6}$ risk after life-time exposure (RIVM, basisdocument benzeen, 1987). This value is comparable to the value of 0.1 $\mu g.m^{-3}$ used by the US-EPA (IRIS, 1995). In 1987, the WHO recommended a value of 0.25 $\mu g.m^{-3}$.
In the Netherlands a dose of 12 $\mu g.m^{-3}$ is associated with the $1 * 10^{-4}$ risk. In 1993, the C-value was set on 30 $\mu g.m^{-3}$ (Vermeire, T.G., 1993).

The exposure to benzene of the general population and the benzene exposure of industrial workers in particular, may be higher than the life-time dose associated with a $1 * 10^{-6}$ risk after a life-time exposure. It can be expected, however, that benzene exposure will fluctuate markedly depending on daily activities (smoking, refuelling, work-shifts). Until now, linear relationships are assumed between exposure concentration and duration (i.e. $C \times T = k$), although there are biological arguments that invalidate this approach.

For an accurate risk assessment of benzene at varying (low) concentrations and varying dose intervals quantitative models are required. The first "step" in biological modelling - physiologically-based pharmacokinetic modelling- has been made by several groups. A PBPK-model for benzene was developed for example by Medinsky et al. (1989). This model takes into account the toxicokinetics of benzene and the formation of its three genotoxic metabolites. The toxicodynamic action of benzene or its metabolites, however, is not modelled. The development of a toxicodynamic model which describes the key interactions of these genotoxic metabolites with the blood forming tissues, would thus be a major contribution to the risk assessment of benzene. The coupling of toxicokinetics and toxicodynamics would enable to estimate the risk of benzene at different exposure scenarios.

5.3. MVK-model

In carcinogenesis, several dose-response models exist. Many of the dose-response models used for the risk assessment of carcinogens are purely descriptive regression functions (e.g. Weibull). More mechanistic based modelling is present in the Armitage Doll model (1961), which assumes that tumours develop after a series of transformations in a cell. By incorporating the processes of cell division and cell death (c.q. differentiation) the "MVK-model" was developed (see e.g. Moolgavkar et al., 1988, Moolgavkar and Luebeck, 1990). The MVK-model assumes that two mutations (occurring in a stochastic way) are necessary for tumour formation.

The MVK-model calculates the expected tumour incidence at any age. The MVK-model may be useful to examine more general issues, such as the impact of changing the dosing-patterns on the probability of tumours. The influence of exposure patterns can be illustrated by model simulations.

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