Available online at www.sciencedirect.com
SCIENCE DIRECT
Toxicology 214 (2005) 256–267
TOXICOLOGY
www.elsevier.com/locate/toxicol

# Acute inhalative exposure assessment: Derivation of guideline levels with special regard to sensitive subpopulations and time scaling

Hans Mielke [a], Anna Gundert [b], Klaus Abraham [a,*], Ursula Gundert-Remy [a]

[a] *Federal Institute for Risk Assessment, Thielallee 88-92, 14195 Berlin, Germany*
[b] *Department of Mathematics and Computer Science, Free University Berlin Arnimallee 2-6, 14195 Berlin, Germany*

Available online 1 August 2005

**Abstract**

Risk assessment for acute airborne exposure to volatile organic compounds (VOCs), including exposure to chemical warfare agents, requires consideration of local and systemic effects at high concentrations. The operating procedure developed by the US Acute Exposure Guideline Level (AEGL) committee has gained special attention, in part because of the international collaboration in the project. The procedure defines three levels (AEGL-1: discomfort; AEGL-2: irreversible or other serious, long-lasting adverse effects; AEGL-3: life-threatening effects or death) for different exposure times (10 and 30 min, and 1, 4 and 8 h). In this article, the methodology for deriving AEGL values is reported. Extending the areas covered by the existing AEGL methodology, sensitive subpopulations are dealt with in more detail. Sensitive persons are expected to suffer from stronger effects when exposed to a given external concentration. Using a kinetic model with the sample substance dichloromethane (DCM), the higher internal exposure of children is quantified and compared to a healthy, young adult. The difference is shown to depend on age, on dose, and on duration of exposure. Furthermore, several ways are presented to derive AEGL values for exposure times which differ from the exposure duration in animal studies ('time scaling'). In comparison to the conventional procedure, the alternative approaches are based on mechanistic models of the toxicodynamic effect. Use of these models results in AEGL values which are biologically justified.


*Keywords:* Acute exposure; Inhalation; Risk assessment; Sensitive subpopulation; Kinetic model; Dichloromethane

## 1. Introduction

Hazardous substances can be released by industrial explosions, fires, transport accidents, or by chemical spills. In addition, hazardous substances may also be released following an act of chemical terrorism, or may be used as warfare agents. The substances may contaminate soil, water, and air depending on their physical state. People living in the area of release are at risk from airborne exposure to the chemical. In order to give guidance to those responsible for management decisions in the event of an accident, and for those in charge of developing emergency-response plans, several committees world-wide have been working to define concentrations in the air, from safe up to life threatening levels.

In the European Union, the Seveso II Directive (EC, 1996) which is the regulatory consequence following the tragic Seveso accident, requires not only emergency response planning but also land use

* Corresponding author. Tel.: +49 30 8412 3369; fax: +49 30 8412 3763.
*E-mail addresses:* h.mielke@bfr.bund.de (H. Mielke), gundert@math.fu-berlin.de (A. Gundert), k.abraham@bfr.bund.de (K. Abraham), u.gundert-remy@bfr.bund.de (U. Gundert-Remy).

0300-483X/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2005.06.018

planning to prevent major impact on the residents living near industrial plants. In the United States of America, in 1995, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL committee) was established to develop Acute Exposure Guideline Levels (AEGLs) based on a scientific review and interpretation of relevant toxicological data. AEGLs are levels of hazardous substances in the air following their release (http://www.epa.gov/oppt/aegl/). Meanwhile, a European project (ACUTEX, http://www.acutex.info) has been initiated which is aimed at developing innovative approaches to define a set of acute toxic levels to be used in both land use planning and emergency planning.

In the following, we give a summary of the standing operating procedure (SOP) of the AEGL committee (NRC, 2001). AEGL values are derived for airborne concentrations due to accidental release of chemicals in the air. The route of exposure is inhalation and the exposure duration is short-term. In addition to the SOP procedures, the focus of this paper is on sensitive subpopulations, which are not considered in much detail in the SOP. We present physiologically based toxicokinetic modelling as a tool to use biological knowledge in the derivation of AEGL values (Section 3). Furthermore, we demonstrate how mechanistically based toxicodynamic models may be used to extrapolate findings from the exposure time used in a study to other exposure times ('time scaling', Section 4).

## 2. The acute exposure guideline level (AEGL) approach

### 2.1. Definition of the levels

AEGLs are airborne concentrations of chemicals which define threshold exposure limits for the general public. Three different levels, defined by the degree of the severity of toxic effect, are derived for five exposure periods (10 and 30 min, and 1, 4, and 8 h).

*AEGL-1* is the airborne concentration (expressed as ppm or as mg/m$^3$) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. However, the effects are not disabling and are transient and reversible after exposure.

*AEGL-2* is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

*AEGL-3* is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

### 2.2. Selection and documentation of the most relevant studies

The studies are assessed using internationally accepted standards. However, many toxicological studies which contain important information on the acute toxicological properties of chemicals were not performed according to current standards, the studies have to be assessed on their own, requiring evaluation of the data in a case-by-case approach. If a study is qualified as being performed using scientifically valid methods and the findings are described in a clear and comprehensible way, it may be used as the relevant or 'key' study for the derivation of AEGLs, even if it was performed several decades ago. A key study has to be a primary reference source. In addition, all supporting data, and any further information relevant for the derivation of AEGLs (e.g. mechanism of action) has to be taken from primary sources.

Of the studies on a specific chemical, those are preferred in which the inhalation route of exposure has been used. Human studies are always judged to be more relevant than animal studies because of the uncertainty of interspecies extrapolation. The exposure concentration in experimental studies in animals or in humans has to be determined as the chamber concentration by valid analytical methods. If anecdotal case reports or epidemiological studies following accidental chemical release are used to derive AEGLs, the assessment of chemical exposure must be based on scientifically acceptable methods and procedures.

In the absence of sound inhalation toxicity data, high confidence in route-to-route extrapolation is the prerequisite for using data derived from non-inhalation studies. In particular, oral exposure data may be used and scaled to the inhalation route only if the AEGL endpoint is systemic toxicity, and if degradation in stomach and intestine as well as first pass effects do not play an important role.

### 2.3. Defining the points of departure

The AEGL values are defined as the highest exposure concentrations at which the effects qualifying AEGL-1, AEGL-2 and AEGL-3 are not observed. In the traditional risk-assessment practice, the no-observed-adverse-effect level (NOAEL) is used as the departure

for deriving acceptable exposure levels for humans or for assessing the margin of safety (MOS), taking into account the exposure from different sources.

However, it is well known that the NOAEL depends on the number of animals, the spacing of doses and the array of effects under observation. To overcome the drawbacks of deriving a NOAEL from experimental data (Leisenring and Ryan, 1992), several authors have proposed the statistical approach of modelling the dose/concentration-effect relationship and deriving the 95% lower confidence limit of the 'benchmark dose/concentration' which results in a prespecified level of effect ('benchmark response', typically in the range between 1% and 10%: Barnes et al., 1995; Gaylor et al., 1998, 1999; Fowles et al., 1999).

The benchmark approach requires data appropriate for this type of analysis, e.g. data sets from inhalation studies to estimate $LC_{50}$ values. However, it is difficult to set rules on the effect level for which the benchmark concentration (BMC) is to be calculated. Several authors explored databases comparing the 95% lower confidence limits of the BMC and also maximum likelihood estimates for 1%, 5% and 10% response levels with NOAELs. In these analyses, two statistical models for the dose-effect relationship were explored, the probit model and the Weibull model. Based on the analysis of several authors (Allen et al., 1994; Fowles et al., 1999; Gaylor, 1996), the NAC/AEGL Committee decided generally to use the lower confidence limit of the BMC for an effect level of 5%. For endpoints other than lethality, the type of data analysis is less sophisticated.

### 2.4. Deriving AEGL endpoints

The effect which defines an AEGL-1 level has to be selected on a case-by-case basis. It is difficult to define the degree of 'discomfort' which is an AEGL-1 level. This level is not a level of awareness to the exposure by smelling or tasting or some other sensation. Although awareness may cause discomfort in some individuals, this is not associated with adverse health effects.

The same line of argument is applicable for the selection of an AEGL-2 value. As the AEGL-2 level is below the concentration causing an irreversible or long-lasting serious effect and below a level causing impairment of the ability to escape, it is difficult to derive because of the continuum of the increasing seriousness of an effect. Hence, if a reversible effect is observed in a data set at a distinct exposure level and irreversible effects are occurring at a higher level, the reversible effect is selected as the basis for developing an AEGL-2 level.

For the derivation of AEGL-3 levels, in most cases the highest exposure level has been selected in the past which does not cause life-threatening effects or death. In some cases based on experience, levels causing severe toxicity have been used as surrogates for life-threatening effects or death.

### 2.5. Potential dosimetry correction

As pointed out, human studies are of prime interest in the process of deriving AEGL values, and are used whenever data quality and validity allow. However, in many human studies, exposure level measurements are insufficient and poorly documented, precluding the use of the information on the health effect for deriving an AEGL value. In the extrapolation from dose/concentration-effect relationships in animal studies to the human situation, several aspects have to be considered. For the inhalation route, the observed effect is either a local one, which may be present in the (upper or lower) airways or in lung tissue, or a systemic effect on other tissues/organs after the chemical has entered the systemic circulation. The US Environmental Protection Agency with their reference concentration (RfC) method for chronic exposure (EPA, 1994), and also the US National Research Council (NRC, 1993) have proposed scaling methods for both types of possible effects of inhaled chemicals. However, validation of the proposed methodologies is lacking and concerns have been raised that the derived values may underestimate or overestimate the exposure of humans, because for inhalation toxicology the absorbed dose depends on various factors. These include the physical state of the chemical (gas, vapour, aerosol or particles), water solubility, and reactivity.

Category 1 gases are highly water soluble and/or rapidly irreversibly reactive resulting in fast deposition on mucous membranes and missing penetration to blood. Category 2 gases are moderately water soluble and intermediate in their reactivity, resulting in distribution throughout the respiratory tract and absorption into the systemic circulation. Category 3 gases have low water solubility and reactivity, resulting in the absence of local effects and in high alveolar absorption (EPA, 1994). Furthermore, the deposition depends on the anatomical situation, which obviously is different in rodents as compared to man for the extrathoracic, the tracheobronchial, and the pulmonary compartment. In addition, the deposition is expected to depend on the relative ventilation rate, which also differs with species.

Because of the lack of validation for the proposed methodologies and the complexity of the issue, the

AEGL committee decided not to use a dosage adjustment procedure for inhalation data.

### 2.6. Uncertainty and adjustment factors for interspecies variation and intraspecies variability

A number of authors have reviewed the history and the current status of uncertainty and adjustment factors (Renwick, 1993; Meek et al., 1994; Dourson, 1996; Gundert-Remy and Sonich-Mullin, 2002). In brief, originally an arbitrary factor of 100 was selected by the Joint Expert Committee of the Food and Agriculture Organisation of the World Health Organisation (WHO) within the concept of 'acceptable daily intake' (ADI) in the late 1950s. In the following decades, data analysis revealed that this factor may account for the difference in kinetics and in dynamics between animals (mostly rodents) and man, as well as for the variability among the human population due to interindividual differences in kinetics and dynamics. Following discussion in the International Programme on Chemical Safety (IPCS of WHO, ILO and UN), the concept of uncertainty factors has been changed to a concept of adjustment factors which takes into consideration scientific knowledge about the individual chemicals (Gundert-Remy and Sonich-Mullin, 2002).

Within the development of AEGL values, the interspecies factor is set to 3 in all cases where the interspecies variability is low (if data of more than one species is available, interspecies variation may be calculated to be less than 3, allowing for a lower interspecies factor to be used), and the most susceptible species is used in the selected key study. If a species other than the most sensitive one is used, the factor is 10. This is also the case for inadequate data, large variation between species, or where humans are known to be more susceptible. Another aspect is the mechanism of action: if it is known to be the same in animal and man, the factor is reduced from 10 to 3, the latter accounting for possible toxicokinetic differences between species.

Less clear guidance is given by the SOP for developing AEGLs concerning the selection of intraspecies uncertainty factors. In general, the aim is to protect the general population including susceptible individuals, but 'hypersusceptible' subjects may not be protected. Regarding susceptibility, the SOP refers to children and to subjects with pre-existing diseases (e.g. impaired renal, hepatic, pulmonary or cardiac function). The factor of 10 (frequently used as a default) seems to be protective for susceptible individuals in most cases. For chemicals for which the mode of action is known, the sensitive groups may be identified, and professional judgement may then be used for the selection of the uncertainty factor in a weight-of-evidence decision. The problem of sensitive subpopulations is discussed in more detail below (Section 3).

### 2.7. Modifying factors

Modifying factors are applied in addition to the other assessment factors. They may range from 1 (the default value) to 10. There is limited experience with these factors, which may be used to account for (1) a limited data set, (2) cases in which the target effect is more severe than the effect defined by the AEGL description, and (3) to account for the differential toxicity of chemical isomers.

### 2.8. Time scaling

AEGL values are developed for distinct exposure durations (10 and 30 min, and 1, 4, and 8 h). Unfortunately, toxicological studies in which the toxicology of the compound is investigated after different exposure times are available only for a few chemicals. So in most cases it is necessary to extrapolate from the exposure duration used in the key study to the AEGL exposure times. This procedure is called 'time scaling', in the AEGL methodology the rule of ten Berge is used. A detailed discussion of this rule and possible alternatives is provided in Section 4.

### 2.9. Output of the AEGL process

Using the methodology described above, technical support documents (TSDs) are produced for selected chemicals (taken from a priority list) which are reviewed by the National Advisory Committee (NAC). The final release is in a book series of the NRC entitled 'Acute Exposure Guideline Levels for Selected Airborne Chemicals', but is also available online (www.epa.gov/oppt/aegl). For some chemical warfare agents (GA, GB, GD, GF, VF, sulfur mustard), the derivation of AEGL values is presented in volume 3 (AEGL-Committee, 2003).

## 3. Sensitive subpopulations

The AEGL values represent threshold levels aimed at protecting the human population. But from the view of risk assessment, this is a very heterogeneous group: from the unborn to elderly, male and female including pregnant women, individuals with pre-existing diseases, poor nutritional status, obesity, or prior exposures, and

those using medicines or social drugs like alcohol and cigarettes. Under the conditions of an airborne exposure to industrial chemicals lasting a few hours, particular subpopulations may be more susceptible than healthy young adults (the so-called 'normal' population). At identical levels of external exposure, a stronger effect may be caused by one or more of the following factors:

(1) Higher inhalation uptake due to higher ventilation rates on a body weight basis leading to higher internal concentrations. This factor is independent of the particular chemical or mechanism of action.
(2) Slower hepatic elimination of the parent compound, or faster metabolism of the parent compound and slower renal elimination of toxic metabolites, leading to higher internal concentrations of the relevant compound regarding the toxic effect (differences in toxicokinetics). This leads to a higher susceptibility if the particular chemical underlies the type of metabolism/elimination described. These mechanisms are expected to be especially relevant for long exposure times (impaired excretion resulting in accumulation of the toxicants).
(3) Higher sensitivity of the tissue due to a shift of the concentration-effect relationship, leading to a stronger response at the same level of external exposure for local effects, or at the same level of internal exposure for systemic effects (differences in toxicodynamics, depending on the mechanism of action of a particular chemical).

Sensitive subpopulations have to be identified for the chemical of interest (no subpopulation is particularly sensitive towards exposure to every chemical). In a second step, the higher risks of these subpopulations have to be quantified. This can be done by deriving an intraspecies factor for the particular subpopulation which has to be applied additionally to the intraspecies factor considering the 'normal' variability in healthy young adults. In order to derive a reasonable factor, the quantitative distribution of the response in terms of mean and variability has to be known for the specific subgroup and for the 'normal' population. A serious problem is often the lack of data for exact quantification of the variability of responses in humans. Therefore, in many cases only an informed guess is possible.

The sensitive subpopulation may respond in two ways: The effect may be stronger than in the total population but qualitatively the same. Examples of this are asthmatics in case of local effects on the respiratory tract, or patients with coronary heart disease in case of the systemic effect of an asphyxiant. In both of these groups of patients, the higher sensitivity can range from mild to extreme, depending on the level of severity. Another possibility is that the effect does not occur in the normal population but only in the sensitive subpopulation (e.g. allergic responses, developmental effects in unborn children). An additional problem may be the identification (e.g. for evacuation) of individuals known to be sensitive to a certain chemical, e.g. undiscovered coronary heart disease in case of exposure to asphyxiants.

Furthermore, a general decision has to be made, namely what proportion of the total population (or of the subgroup) should be protected? This decision has to be based on political and ethical, rather than scientific considerations. It is a very complex question involving cost-benefit analysis and issues of acceptance of risks in the society. In the case of a rare mutation, for example occurring in 1 out of 40,000 people, some would argue not to protect these individuals. In case of newborns which constitute a part of less than 1 out of 1000 in the population of industrialised countries, emotional aspects play a greater role, especially if possible irreversible effects lead to a long period of suffering or decreased quality of life. Preterm neonates constitute an even smaller part of the population.

### *3.1. Example: kinetics of dichloromethane in children*

Due to age-dependent differences in anatomy and physiology, children may experience a different internal exposure during inhalation of volatile organic compounds (VOCs) which is relevant for systemic effects. Regarding an acute inhalation exposure scenario, the most important differences are:

- A higher ventilation rate on a body-weight basis, reflecting the higher energy expenditure (ongoing growth, relatively higher caloric losses due to higher surface/body mass ratio, and relatively higher physical activity in older children), leading to relatively higher alveolar uptake.
- Correspondingly higher circulation rates (cardiac output and tissue flow rates), leading to faster distribution and faster metabolism in the case of flow limited metabolism.
- Different relative organ volumes, especially regarding the brain and the liver (relative respective volumes decreasing during childhood). Whether this leads to higher or lower internal concentrations depends on the properties of the chemical.

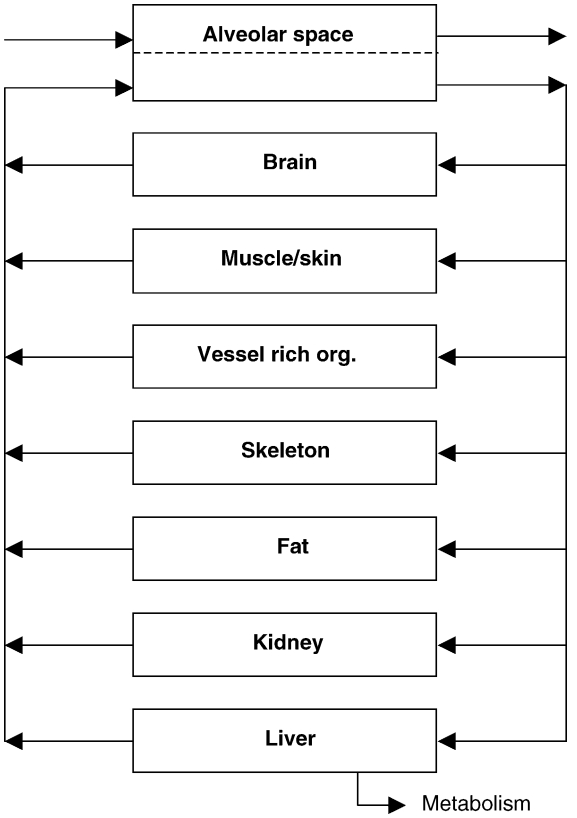


Fig. 1. Structure of the kinetic simulation model.

- Immature metabolism in the very young. The impact depends on whether the parent compound or the metabolites are the toxicologically active compound. The impaired renal function of the very young may also be important.

A physiologically based pharmacological model (PBPK model) allows considering several factors varying with age, together with various external concentrations and exposure duration. A model with seven compartments (brain, liver, kidney, adipose tissue, muscle/skin, vessel rich organs and skeleton, Fig. 1) was used as described in detail elsewhere (Abraham et al., 2005a). Anatomical (organ weight) and physiological data sets (inhalation rate, blood flow rate through the organs) were derived from the literature for the ages 0 (newborn), 1, 5, 10 and 15 years as well as for the male adult ('reference man'). Model simulations presented here were made for dichloromethane (DCM) with an alveolar concentration equal to that in ambient air ('category 3' gas; 'wash in–wash out' phenomenon unimportant: EPA, 1994; Johanson, 1991) and acute toxicity (unconsciousness) due to the parent compound. Properties regarding partition coefficients (air/blood and blood/tissue) and hepatic metabolism via CYP2E1 (Michaelis–Menten kinetics: $V_{max}$, $k_m$) were taken from the literature (Gargas et al., 1986; Andersen et al., 1987). To include the immature metabolism at the age of 0 and 1 year, 14% and 50% CYP2E1 activity per liver volume, respectively, was assumed in comparison to older children and adults having 100% activity (estimated from data of Vieira et al., 1996). The second pathway involving the Glutathione *S*-transferase (GST) was not considered, as it is quantitatively irrelevant for DCM degradation.

A concentration range between 1 and 10,000 ppm in ambient air and an exposure period of up to 8 h were chosen (according to the AEGL scenario). The calculations were made for the arterial concentrations as well as for the ratios of these concentrations (child/adult), using the Matlab software (Matlab 7.0, The MathWorks, Natick, MA, USA).

Simulated concentrations in arterial blood are shown in Figs. 2a and 3a for the different age groups at concen-

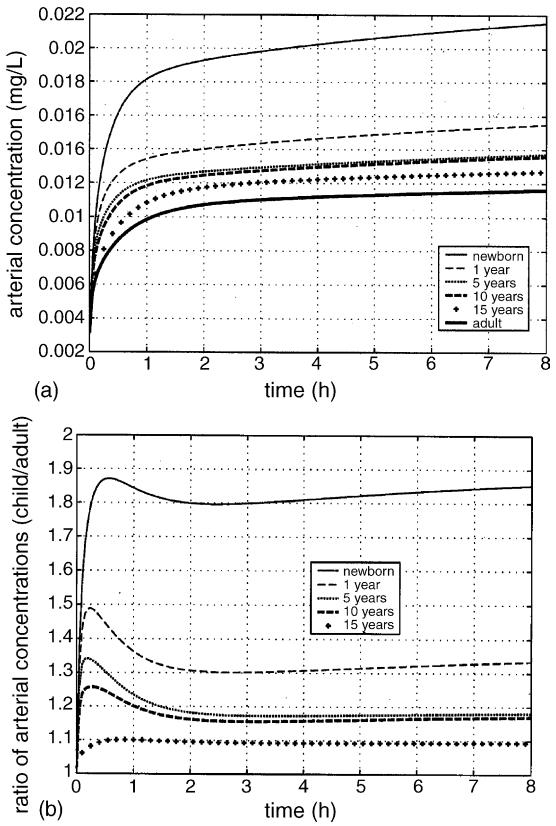


Fig. 2. Model calculations for the inhalation exposure to dichloromethane, with concentrations in ambient air of 1 ppm. Concentration–time profiles in arterial blood during exposure for 8 h in children (newborn, 1, 5, 10, and 15 years old) and adults (a), and corresponding ratios of arterial concentrations (child/adult) (b).

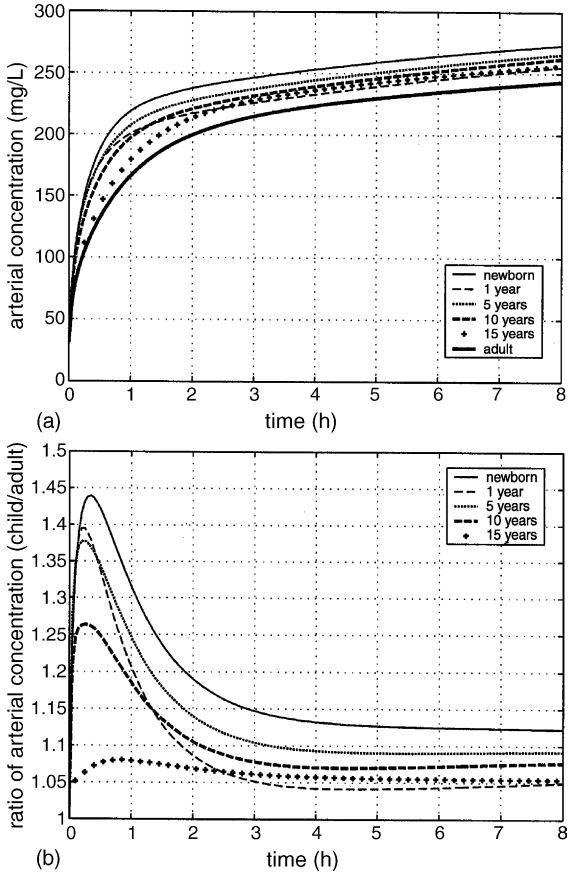


Fig. 3. Model calculations for the inhalation exposure to dichloromethane, with concentrations in ambient air of 10,000 ppm. Concentration–time profiles in arterial blood during exposure for 8 h in children (newborn, 1, 5, 10, and 15 years old) and adults (a), and corresponding ratios of arterial concentrations (child/adult) (b).

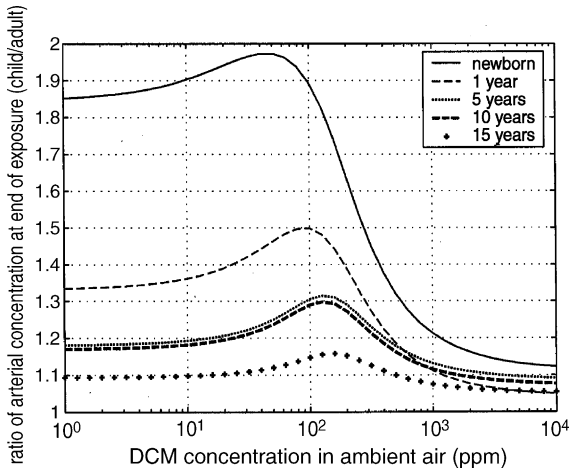


Fig. 4. Ratios of arterial concentrations (child/adult) after a dichloromethane exposure duration of 8 h, depending on the concentration in ambient air, in children of different ages (newborn, 1, 5, 10 and 15 years old).

trations in ambient air of 1 and 10,000 ppm, respectively. During the observation period of 8 h, arterial concentrations continuously increase. Comparing the six age groups, the levels are highest in the newborn. At concentrations in ambient air of 1 ppm, with increasing age the concentrations more and more resemble those in the adult. At external concentrations of 10,000 ppm, the curve of the arterial concentration in the 1-year-old child 'crosses' those of the older children. Interestingly, for exposure durations longer than 2.5 hours the concentrations in this age group are closer to those of the adult than in any other age group. In addition, the ratios of the arterial concentrations (child/adult) in Figs. 2b and 3b reveal a dose- and duration-dependent phenomenon. At an external concentration of 1 ppm, the newborn to adult ratio is highest with values between 1.8 and 2.0 reached shortly after begin of exposure. Age-dependently, the ratios are lower for the older children. In contrast, the ratios are generally lower at 10,000 ppm with a peak within 30 min and a decrease of the ratios thereafter. More than 2.5 h after begin of exposure, all ratios are lower than 1.2, and the lowest values are calculated for the 1-year-old child.

In Fig. 4, the ratios are shown to depend on the DCM concentration in ambient air (values for 8 h exposure). The maximum ratio of nearly 2.0 is calculated for the newborn exposed to 60 ppm. With increasing age, the maximum ratios become lower and occur at higher concentrations in ambient air. In the 15-year-old child, the maximum ratio is about 1.15 with an exposure to 150 ppm. As the highest values were calculated for the newborn to adult ratio, further calculations are performed for this age group only. The complex relationship between this ratio, the external DCM concentration (1 to 10,000 ppm) and the exposure duration (up to 8 h) are presented as three-dimensional graph (Fig. 5). This figure shows that the most pronounced difference to the adult in terms of the AEGL scenario occurs about 10 min after exposure to external concentrations up to 100 ppm DCM. With higher concentrations, the ratios continuously decrease.

This three-dimensional graph can be understood as kind of a 'fingerprint' of a chemical giving the concentration- and duration-dependent ratios for the most sensitive age group in case of a 'category 3' gas and toxicity due to the parent compound. Compared to calculations for styrene using the same model (Abraham et al., 2005a), the dose-dependency was less pronounced for

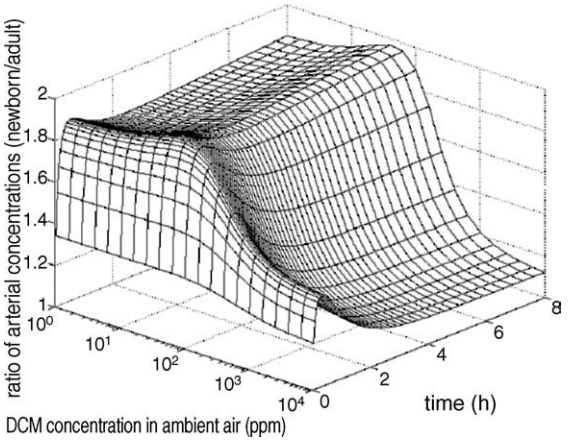


Fig. 5. Ratio of arterial concentrations (newborn/adult) as three-dimensional graph, against the duration of exposure (0 to 8 h) and the dichloromethane concentration in ambient air (note the logarithmic scale).

DCM, mainly due to the much lower blood:air partition coefficient (8.9 instead of 55.6). General considerations of chemical properties influencing the child/adult ratios can be found in Abraham et al. (2005b).

The dose-dependent ratios result from non-linear Michaelis–Menten metabolism which in children becomes saturated at lower external concentration due to higher internal exposure following the relatively higher ventilation rates. This phenomenon is pronounced in the newborn (and the 1-year-old child) due to the 'immature' metabolic capacity. With DCM concentrations above 500 ppm, the metabolism becomes saturated in all age groups, reducing the disadvantage of low enzyme activity in the newborn. With very high external concentrations (where metabolism is relatively unimportant), the internal concentrations of children and adults are more or less the same after a longer period of exposure, but not during the first hours of exposure (effect of relatively high ventilation rate and high concentration gradient in the lung).

For risk assessment of systemic effects due to the parent compound resulting from inhalation of VOCs, these ratios can be used to establish data-derived kinetic safety factors in order to include the newborn as a relatively sensitive subpopulation. The simulations presented are of particular importance for risk assessment in the context of the AEGL procedure: whereas other approaches are aimed at setting 'safe' levels (which by definition are levels below the no adverse effect level), acute exposure guideline levels (AEGLs) are concentrations which are related to varying degrees of health impairment. Therefore, a broad range of concentrations has to be covered, including those leading to saturated metabolism.

### 3.2. Physical activity

As inhalation is the main route of exposure, local effects on mucous membranes of the airways as well as the internal exposure relevant for systemic effects both greatly depend on the ventilation rate. Under normal conditions, the ventilation rate reflects the metabolic needs of the body. In the case of physical activity (e.g. heavy work, sport activities), the ventilation may be increased many-fold, in general leading to a much higher internal exposure compared to resting conditions. This is well known from model calculations and experimental measurements during inhalation of VOCs (Astrand, 1983). If physical activity was considered as a factor in the derivation of AEGL values, it would be more important than nearly any other factor for short-term exposure, and heavy physical activity would be much more relevant regarding higher susceptibility than most other conditions. The AEGL concept does not consider the possibility of higher internal exposure of rescue workers or of running subjects, e.g. those trying to escape from an accident.

## 4. Time scaling using dynamic models

For time extrapolation it would be most convenient to have scientifically equally valid studies for all required exposure times. Usually, such studies are not available. Instead, simple equations, such as the rule of ten Berge et al. (1986) are applied, implicitly assuming that time, concentration, and effect are in an easily computable algebraic relation. For extrapolation to time periods that do not differ much, this procedure may not be too inaccurate. Nevertheless, it seems desirable to search for more plausible strategies.

In case a biologically based toxicodynamic model is available for the chemical in question, the method of deriving AEGL values could be as follows. In a first step, take from an experimental study the dose and exposure time leading to the effect intensity to be described. Second, compute the value on the effect scale related to this dose and exposure time. Third, for a different exposure duration, determine the external concentration for which the same effect intensity is predicted.

In the following, we demonstrate three different ways to compute 'AEGL values'. We do not propose the results for use as actual AEGL values. The aim is rather to explore possible procedures. In fact, we exemplify the procedures by considering the CNS effects of DCM which are assumed to be caused by the substance itself, not by a metabolite. In particular, we disregard the formation of carbon monoxide although it might be more

Table 1
'AEGL-2' values calculated using the three effect models discussed

| Model | Exposure time | | | | |
|---|---|---|---|---|---|
| | 10 min | 30 min | 1 h | 4 h | 8 h |
| ten Berge rule | 2135 | 1480 | 1175 | 719 | 359 |
| Direct link | 1788 | 1223 | 980 | 747 | 694 |
| Indirect response 1 | 2952 | 1333 | 1010 | 747 | 693 |
| Indirect response 2 | 18901 | 5541 | 2632 | 729 | 502 |

All values are in ppm. The values are external concentrations at which the same CNS effect is predicted by the respective model as for an exposure for 230 min at 751 ppm. The indirect response model was used with parameters $k_{injury} = 1h^{-1}$, $S_{max} = 10$, $SC_{50} = 100$ mg/L; $k_{repair}$ was chosen to be $10h^{-1}$ in the first run (indirect response 1) and $0.3h^{-1}$ in the second run (indirect response 2).

relevant for the effect severity than DCM itself. Considering the AEGL-2 level only, we used human data (Reitz et al., 1997) as key study for the CNS effect, namely 230 min at 751 ppm. The values computed by the three different procedures are collected in Table 1.

### 4.1. Algebraic method as used within the AEGL methodology

Guided by observations, Haber (1924) proposed the rule that the product of exposure level C and time period t is approximately constant ($C \times t = \text{const}$). Mechanistically, this relationship means that the exposure concentration is not the only determinant for toxicity and that the exposure duration plays an equally important role. Though Haber's rule has been used for decades (Rinehart and Hatch, 1964), a paper by ten Berge et al. (1986) suggested a generalisation to fine-tune the importance of time in relation to concentration. The new rule differs from Haber's rule in that the concentration $C$ is raised to some power $n$, which is specific for the chemical and the endpoint:

$$C^n \times t = \text{const}$$

In their analysis, ten Berge et al. (1986) derived values for $n$ ranging from 0.8 to 3.5 for 20 chemicals.

Although there are not many publications on time scaling, and limited evidence, the NAC/AEGL decided to make use of the ten Berge rule. The exponent $n$ is derived from experimental data, usually animal experiments. If no data is available, default values are used. A default of $n = 1$ is used for the extrapolation from shorter to longer exposure duration and a default of $n = 3$ for the extrapolation from longer to shorter exposure duration. The DCM 30-min AEGL-2 value, for instance, is computed as $\sqrt[3]{((751\,\text{ppm})^3 \times 230\,\text{min})/30\,\text{min}}$, the 8-h value as $(751\,\text{ppm} \times 230\,\text{min})/480\,\text{min}$.

The AEGL committee has decided to flat-line the values across short exposure periods when extrapolating from longer to shorter exposure periods in order to prevent excessively high concentrations being calculated. For sensory irritants (often AEGL-1), the committee takes the view that the AEGL value may be constant over the whole range of periods.

### 4.2. Biologically motivated method—direct link model

In a direct link model it is assumed that the effect is directly related to the concentration in the target organ. A frequently used direct link model is the $E_{max}$ model (Holford and Sheiner, 1981). Here, the effect is supposed to be a consequence of the chemical docking at a receptor. The extent of effect $E$ is given by the number of receptors occupied by the chemical and may be computed from the concentration $C$ by the formula

$$E = \frac{E_{max} \times C}{EC_{50} + C}$$

where $E_{max}$ is the maximal possible extent of effect and $EC_{50}$ is that concentration in the brain at which half of the receptors is occupied. For small concentrations (compared to $EC_{50}$), this essentially is the linear model $E = \text{const} \times C$: when there are many free receptors, then doubling the amount of chemical will double the number of receptors occupied. For high concentrations, increase of effect will slow down and converge to $E_{max}$: when all receptors are occupied the maximum effect is reached.

We only consider the CNS effects of DCM, so the brain is the target organ. Using the pharmacokinetic model described above, we simulate the DCM concentration in the adult brain, which after 230 min at 751 ppm is calculated to be 13.6 mg/L, corresponding to some effect intensity E which depends on $E_{max}$ and $EC_{50}$. For each exposure duration, we determine the exposure concentration at which our model predicts the same effect intensity $E$. Since the effect intensity is in one-to-one correspondence with the brain concentration this is equivalent to the exposure concentration at which the same brain concentration of 13.6 mg/L is predicted (see Fig. 6b). Hence, these calculations do not depend on the knowledge of the actual $E_{max}$ and $EC_{50}$ values and, more generally, not even on the concrete direct link model.

### 4.3. Biologically motivated method—indirect response model

In an indirect response model it is assumed that the effect is not directly related to the concentration of the

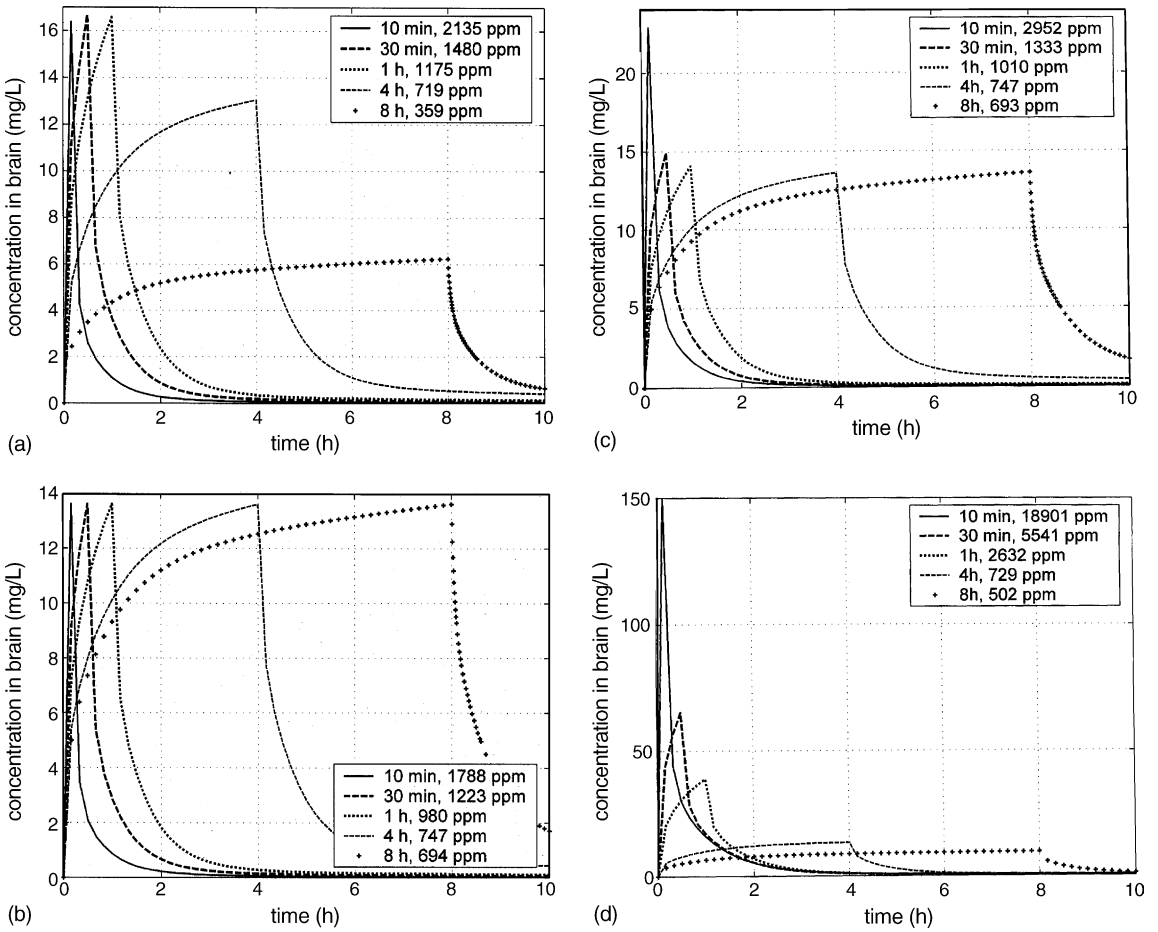


Fig. 6. Comparison of internal (brain) concentration-time course for the three effect models: (a) ten Berge rule, (b) direct link model, (c) indirect response model 1 and (d) indirect response model 2. For all models, the external concentrations for the five exposure times are chosen to result in the same extent of effect.

chemical but can be described by some other quantity driven by the chemical's concentration in the brain. Jusko and Ko (1995) present a family of physiologic indirect effect models that are capable of accounting for the pharmacodynamics of drugs that act by way of inhibition or stimulation of the production or loss of endogenous substances or mediators. These models were applied successfully in pharmacology in a diversity of cases.

For instance, the biological response ($R$) may be the result of concentration dependent injury and its limited repair, described by the differential equation

$$\frac{dR}{dt} = k_{\text{injury}} \times \left(1 + \frac{S_{\max} \times C}{SC_{50} + C}\right) - k_{\text{repair}} \times R$$

This model has a couple of free parameters which (contrary to the direct link model) do influence the outcome. To demonstrate the influence of the choice of parameters, we chose two sets of parameter values. As it turned out that the choice of $S_{\max}$ and $k_{\text{injury}}$ has little influence on the outcome, we fixed $k_{\text{injury}} = 1\text{h}^{-1}$ and $S_{\max} = 10$ as well as $SC_{50} = 100\,\text{mg/L}$. For $k_{\text{repair}}$, we chose first $k_{\text{repair}} = 10\text{h}^{-1}$ (indirect response model 1) and then $k_{\text{repair}} = 0.3\text{h}^{-1}$ (indirect response model 2). These are arbitrary choices for demonstration purposes only.

### 4.4. AEGL values resulting from the different time scaling procedures

The results of the computations are compiled in Table 1. To visualise and compare the values derived by the different rules, the time course of DCM concentrations in brain is depicted in Fig. 6(a) ten Berge rule, (b) direct link model, (c) indirect response model 1 and (d) indirect response model 2. Note that in Fig. 6b the maximum brain concentration is the same for all exposure times.

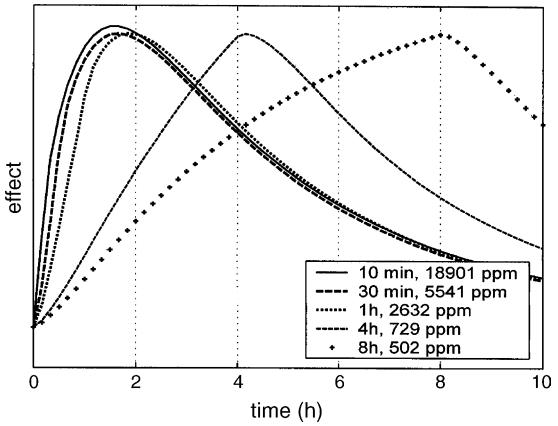


Fig. 7. Time course of effect in the brain compartment for the indirect effect model with parameters $k_{injury} = 1h^{-1}$, $k_{repair} = 0.3h^{-1}$, $S_{max} = 10$, $SC_{50} = 100$ mg/L (indirect response model 1). Note that the maximum effect may occur distinctly after the end of external exposure.

The three strategies for time extrapolation resulted in quite different 'AEGL-2' values. For short exposure times, the direct link model gives the most conservative estimate. The values computed using the indirect response model depend very much on the choice of parameters, especially for short exposure times. So the use of this model is not recommended unless there is strong evidence for the parameter values used.

With the direct link model, it is clear that the maximum effect is reached at the end of exposure, since after the end of exposure the brain concentration decreases. However, with the indirect response model, this is not necessarily the case: Since the effect is 'produced' by some mechanism which is driven by the brain concentration, the effect may increase as long as the brain concentration is sufficiently high. This phenomenon can be observed in Fig. 7 (effect–time course for the indirect response model 2, compare with Fig. 6d): Although the 18,901 ppm exposure ends after 10 min, the maximum effect is only reached after around 90 min. This is in contrast to the situation with longer exposure to lower concentrations, e.g. 8 h, 502 ppm. For time extrapolation it seems to be appropriate to consider the maximum produced effect intensity rather than the effect at end of exposure.

A combination of kinetic and dynamic modelling may assist in improving time–concentration exposure extrapolation in data rich cases, which should have some indication on the preferred toxicodynamic model. If the $E_{max}$ model is applicable, the calculation can be performed without any assumption of the model parameters $E_{max}$ and $EC_{50}$. The indirect response model is a biologically motivated model which seems to be justified conceptually for toxicodynamic endpoints as it assumes that a concentration dependent injury can be repaired to a certain extent, facts which are known for many toxicological endpoints. However, the model outcome depends on the model parameters and seems to be especially sensitive on $SC_{50}$ and $k_{rep}$ neither of which is known in most cases.

## Acknowledgements

Supported by European Union (contract No. EVG1-CT-2002-00071) within 5th Framework programme. The help of Brigitte Bartel in preparing the manuscript is gratefully acknowledged.

## References

Abraham, K., Mielke, H., Huisinga, W., Gundert-Remy, U., 2005a. Elevated internal exposure of children in simulated acute inhalation of volatile organic compounds: effects of concentration and duration. Arch. Toxicol. 79, 63–73.

Abraham, K., Mielke, H., Huisinga, W., Gundert-Remy, U., 2005b. Internal exposure of children by acute inhalation of volatile organic compounds: the influence of chemical properties on the child/adult concentration ratio. Basic Clin. Pharmacol. Toxicol. 96, 242–243.

AEGL-Committee, 2003. Acute Exposure Guideline Levels for Selected Airborne Chemicals. The National Academies Press, Washington, DC.

Allen, B.C., Kavlock, R.J., Kimmel, C.A., Faustman, E.M., 1994. Dose-response assessment for developmental toxicity. II. Comparison of generic benchmark dose estimates with no observed adverse effect levels. Fundam. Appl. Toxicol. 23, 487–495.

Andersen, M.E., Clewell III, H.J., Gargas, M.L., Smith, F.A., Reitz, R.H., 1987. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol. Appl. Pharmacol. 87, 185–205.

Astrand, I., 1983. Effect of physical exercise on uptake, distribution and elimination of vapors in man. In: Fiserova-Bergerova, V. (Ed.), Modeling of Inhalation Exposure to Vapor: Uptake, Distribution, and Elimination. CRC Press, Boca Raton, Florida, pp. 107–130.

Barnes, G.B., Daston, G.P., Evans, J.S., et al., 1995. Benchmark dose workshop: Criteria for use of a benchmark dose to estimate a reference dose. Regul. Toxicol. Pharmacol. 21, 296–306.

Dourson, M., 1996. Uncertainty factors in noncancer risk assessment. Regul. Toxicol. Pharmacol. 24, 107.

EC, 1996. Council Directive 96/82/EC of 9 December 1996 on the control of major-accident hazards involving dangerous substances (Official Journal L 010 of 14 January 1997 pp. 0013–0033).

EPA, 1994. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. EPA/600/8-90/066F, Environmental Criteria and Assessment Office, Office of Research and Development, Research Triangle Park, NC.

Fowles, J.R., Alexeeff, G.V., Dodge, D., 1999. The use of benchmark dose methodology with acute inhalation lethality data. Regul. Toxicol. Pharmacol. 29, 262–278.

Gargas, M.L., Clewell, H.J., Andersen, M.E., 1986. Metabolism of inhaled dihalomethanes in vivo: differentiation of kinetic constants for two independent pathways. Toxicol. Appl. Pharmacol. 82, 211–223.

Gaylor, D.W., 1996. Quantalization of continuous data for benchmark dose estimation. Regul. Toxicol. Pharmacol. 24, 246–250.

Gaylor, D.W., Kodell, R.L., Chen, J.J., Krewski, D., 1999. A unified approach to risk assessment for cancer and noncancer endpoints based on benchmark doses and uncertainty/safety factors. Regul. Toxicol. Pharmacol. 29, 151–157.

Gaylor, D.W., Ryan, L., Krewski, D., Zhu, Y., 1998. Procedures for calculating benchmark doses for health risk assessment. Regul. Toxicol. Pharmacol. 28, 150–164.

Gundert-Remy, U., Sonich-Mullin, C., 2002. The use of toxicokinetic and toxicodynamic data in risk assessment: an international perspective. Sci. Total Environ. 288, 3–11.

Haber, F., 1924. Zur Geschichte des Gaskrieges. In: Haber, F. (Ed.), Fünf Vorträge aus den Jahren 1920–1923. Springer-Verlag, Berlin, pp. 76–92.

Holford, N.H., Sheiner, L.B., 1981. Pharmacokinetic and pharmacodynamic modeling in vivo. CRC Crit. Rev. Bioeng. 5, 273–322.

Johanson, G., 1991. Modeling of respiratory exchange of polar-solvents. Ann. Occup. Hyg. 35, 323–339.

Jusko, W.J., Ko, H.C., 1995. Physiologic indirect effect models characterize diverse types of pharmacodynamic effect. Clin. Pharm. Ther. 56, 406–419.

Leisenring, W., Ryan, L., 1992. Statistical properties of the NOAEL. Regul. Toxicol. Pharmacol. 15, 161–171.

Meek, M.E., Newhook, R., Liteplo, R.G., Armstrong, V.C., 1994. Approach to assessment of risk to human health for priority substances under the Canadian Environmental Protection. Act. Environ. Carcinogen. Ecotoxicol. Rev. 12, 105–134.

NRC, 2001. Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals. National Academy Press, Washington, DC.

NRC, 1993. Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances. National Academy Press, Washington, DC.

Reitz, R.H., Hays, S.M., Gargas, M.L., 1997. Addressing Priority Data Needs for Methylene Chloride with Physiologically Based Pharmacokinetic Modeling. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Renwick, A.G., 1993. Data-derived safety factors for the evaluation of food additives and environmental contaminants. Food Addit. Contam. 10, 275–305.

Rinehart, W.E., Hatch, T., 1964. Concentration-time product (CT) as an expression of dose in sublethal exposures of phosgene. Am. Ind. Hyg. Assoc. J. 25, 545–553.

ten Berge, W.F., Zwart, A., Appelman, L.M., 1986. Concentration-time mortality response relationship of irritant and systemically acting vapors and gases. J. Hazard. Mater. 13, 301–309.

Vieira, I., Sonnier, M., Cresteil, T., 1996. Developmental expression of CYP2E1 in the human liver – hypermethylation control of gene expression during the neonatal period. Eur. J. Biochem. 238, 476–483.