

Understanding mechanisms of inhaled toxicants: implications for replacing default factors with chemical-specific data

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Abstract

Assessing risk of inhaled materials is a challenging endeavor because of the profound interspecies differences in respiratory tract anatomy, physiology, and biochemistry. Recent advances in the availability of mechanistic data and mathematical models for describing dosimetry behavior of particles and gases has led to improvements in default approaches to risk assessment of inhaled materials. An overview of some of the more well-understood differences between species in factors controlling dosimetry and response, and the default approach of the U.S. Environmental Protection Agency that accounts for many of these factors, are presented. The default methodology also creates a framework which inhalation toxicologists can use to direct research at reducing uncertainty in risk assessments that might otherwise be handled through default uncertainty factors. The optimal approach to risk assessment is to develop chemical-specific mode of action and dosimetry data that can be used quantitatively to replace the entire default approach. The toxicology of vinyl acetate and recent efforts to develop data to supplant assumptions made in the default approach are presented. The conclusion is drawn that the future of inhalation toxicity risk assessment lies in reducing uncertainties associated with interspecies extrapolation and that to do this effectively requires approaches to toxicology that are outside of routine testing paradigms, and are aimed at elucidating mechanisms of action through hypothesis-driven research.

Keywords: Nose; Lung; Inhalation toxicity; Risk assessment; Dosimetry modeling

1. Introduction

Chemical dose-response assessments have for years attempted to adequately account for a variety of factors that contribute to interspecies

differences in chemical-induced toxicity. The toxicology of the respiratory tract is a particularly challenging subject for risk assessors owing to the many anatomical, physiological, and biochemical differences between test species and humans. The nasal cavity, despite being positioned as the first potential site of deposition and reaction for inhaled substances, has been studied extensively only since the early 1980s [1]. Such studies have led to an appreciation of the significance of portal-of-entry effects in this re-

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gion. Generally, the state-of-the-science of contemporary bioassays is now such that much more mechanistic data are available for toxicological assessment. Concurrently, mathematical models have become useful to characterize interspecies dosimetry¹ differences as well as dose-response functions. These models are significantly reducing the uncertainty associated with human health estimations derived from rodent toxicity data. This review article summarizes briefly, and superficially in many cases, how mechanistic data can be brought to bear on the difficult subject of comparative risk assessment of respiratory tract toxicants. Emphasis is placed on how flexible approaches, such as the U.S. Environmental Protection Agency's methods for derivation of inhalation reference concentrations, provide a framework that allows for iterative application of mechanistic data to dose-response assessment and can direct data gathering to address areas of uncertainty and default assumptions.

2. Factors controlling comparative inhaled disposition

The various species used in inhalation toxicology studies that serve as the basis for dose-response assessment do not receive identical doses in a comparable respiratory tract region (extrathoracic, ET; tracheobronchial, TB; or pulmonary, PU) when exposed to the same aerosol or gas [2]. Such interspecies differences are important because the adverse toxic effect is likely more related to the quantitative pattern of deposition and subsequent clearance and redistribution than to the exposure concentration. This section describes the major factors that control comparative inhaled dose. Detailed description of such factors is beyond the scope of this short review and the reader is referred to several extensive discussions of particle deposition [3–6] and gas absorption [3,6–10] in the respiratory tract. Although the various factors are discussed as distinct entities, it is important to appreciate that the factors influencing chemi-

cal disposition are dynamic and interactive, thus an accurate description of the processes requires integration. These factors are in turn influenced by exposure concentration, duration, and frequency.

2.1. Particles

Particles are deposited in the respiratory tract by mechanisms of impaction, sedimentation, interception, diffusion, and electrostatic precipitation. Subsequent clearance of a deposited dose is dependent on the initial site of deposition, the physicochemical properties of the particles (e.g., solubility), and on the time since deposition. Clearance routes include dissolution into respiratory tract tissues, absorption into the blood, removal to the gastrointestinal tract via the nasopharynx or mucociliary escalator, endocytosis by macrophages or epithelial cells, and absorption into the lymphatic channels.

2.2. Gases

The major processes affecting gas transport involve convection, diffusion, absorption, dissolution, and chemical reactions. The bulk movement of an inhaled gas in the respiratory tract is induced by a pressure gradient and is termed convection. Convection can be broken down into components of advection (horizontal movement of a mass of air relative to the airway wall) and eddy dispersion (air mixing by turbulence so that individual fluid elements transport the gas and generate flux). Molecular diffusion is superimposed at all times on convection due to local concentration gradients. Absorption removes gases from the lumen and affects concentration gradients. Chemical reactions in the respiratory tract can increase absorption by acting as a sink to drive the concentration gradient. Systemic metabolism can also drive the concentration gradient for insoluble gases that are removed from the respiratory tract tissue by perfusion. Thus, the rate of transfer from the environment to the tissue, the capacity of the body to retain the material, and elimination of the parent and metabolites by chemical reaction, metabolism, exhalation, and excretion influence the disposition of gases.

¹ Dosimetry modeling is used as a more comprehensive term than physiologically based pharmacokinetic (PBPK) modeling to capture not only model structures used to address volatile organic chemicals but also irritant gases and particles.

2.3. Physicochemical characteristics

For a given aerosol, the 2 most important parameters determining deposition are its mass mean aerodynamic diameter (MMAD) and the distribution of the particles about the mean. MMAD can be affected by the hygroscopic nature of some particles. As water is absorbed, the density and dimensions of the particle change. Solubility of particles influences their dissolution and clearance. For gases, the properties of water solubility and reactivity influence their interaction with the respiratory tract and uptake. Reactivity includes both the propensity for dissociation and the ability to react either spontaneously or via enzymatic reaction in the respiratory tract. Systemic metabolism, perfusion rates, and elimination rates modulate the uptake of insoluble gases.

2.4. Anatomic factors

Anatomic differences among species, especially rodents and humans, are important factors controlling interspecies differences in regional toxicity of inhaled materials. Species differences in gross anatomy, nasal airway epithelia (including type and location), and the distribution and composition of mucous secretory products have been noted [11,12]. Differences in structure of the upper respiratory tract (URT) and resultant differences in airflow dynamics and lesion distribution have been described [13–15]. In general, quadruped species such as rodents and dogs have a very complicated nasal turbinate structure, which increases airflow turbulence, and highly developed olfactory systems. On the other hand, primates, including humans, have relatively poorly developed turbinate structures and greatly reduced olfactory systems. To illustrate the latter difference, approximately 50% of the total surface area of the rat nasal cavity is lined with olfactory epithelium while this figure is approximately 10% for humans [13,16]. The effect of turbinate structure on URT deposition of vapors can be illustrated by comparative studies with acetone which is considered a relatively nonreactive, and poorly metabolized vapor. In a data set summarized by Morgan and Monticello [17],

acetone deposition efficiency in the rat, guinea pig, dog and human was 12–45%, 7–20%, 52–60%, and 18%, respectively. The highest rates of deposition were found in the dog which, among the species compared, has the most complex set of anterior turbinates.

There are also clear differences between species with regard to the anatomy and geometry of the lower airway (for recent reviews, see [18,19]) that influence deposition and uptake of inhaled substances. The most obvious difference between rodents and humans is the branching pattern of the bronchi and bronchioles of the lung. In rodents, the branching pattern is asymmetric which presents a relatively unimpeded flow of inspired air to the lower respiratory tract. The branching pattern in humans, on the other hand, is symmetric presenting an airflow pattern more susceptible to deposition at branch points. Airway dimensions such as length and diameter are also different across species.

As in the URT, other anatomical differences between rodents and humans include epithelial cell types and numbers. While in the rat the predominant cell types lining the trachea are serous and ciliated cells, the predominant cell types in bonnet monkeys, are ciliated and basal cells. The distal airways of humans contain several generations of nonrespiratory bronchioles, followed by several generations of respiratory bronchioles and alveolar ducts. In contrast, the terminal bronchioles of rodents are generally contiguous with corresponding alveolar ducts [18]. The differences in cell types populating the alveolar regions are less dramatic. There appears to be a significant homogeneity in populations of epithelial, endothelial, interstitial, and macrophage cells of the alveolar regions and in the percentage of alveolar surface area covered by Type I and Type II cells [18,20]. Thus, toxicants that target the cell types or anatomical features that are specific to an individual species are likely to present difficulties for interspecies extrapolation of dosimetry and response.

2.5. Physiological factors

One of the most obvious and significant physiological differences between rodents and

humans is the fact that humans are oronasal breathers while rodents are obligate nose breathers. This has important ramifications for particle and gas deposition in the upper airways of humans as oral breathing increases with exertion [21]. Both particles and gases can escape the scrubbing effect of the URT under these conditions resulting in a greater delivery of material to the peripheral airways.

Differences in ventilation rates affect the tidal volume and ventilation-to-perfusion ratios across species. Cardiac outputs and tissue volumes also vary. These differences are important factors that interact with the anatomic differences described above to result in dramatic differences in deposition and uptake across species.

Mechanisms for clearing particles from the TB region are largely dependent on dissolution and physical clearance of poorly soluble materials by transport to the digestive tract along the mucociliary escalator or removal by macrophages and epithelial cells following phagocytosis. Clearance in the PU is accomplished by dissolution, absorption into the blood or lymphatic channels, and macrophage-mediated removal. In fact, chemotactic attraction of macrophages shows interspecies differences in response. For example, rat macrophages respond best to complement-derived chemotactic factors whereas hamster-derived macrophages respond best to *N*-formyl peptides [22]. As a result, rats have a greater capacity to clear materials such as carbonyl iron. These data raise the question of what differences arise with human macrophage clearance characteristics. Unfortunately, these are areas not yet addressed by quantitative research and can only be considered qualitatively when evaluating toxicity data.

2.6. Biochemical factors

Interspecies differences in biochemical mechanisms of airway activation, detoxication, and responses to inhaled toxicants are largely uncharacterized and present many opportunities for reducing uncertainties in risk assessments of chemicals that undergo metabolic activation or detoxication. Interspecies comparison of metabolic capabilities of the nasal cavity was recently

completed [23,24] which shows that cytochrome P450 activities for a variety of substrates are metabolized less efficiently in microsomes from human nasal mucosa than that of rodents. On the other hand phase II enzymes, such as nasal tissue epoxide hydrolase and glutathione *S*-transferases, appear to be less active in rodents than in humans. A variety of other non-cytochrome P450 xenobiotic-metabolizing enzymes have also been identified and localized within specific cell types of the nasal epithelium [25], but interspecies differences in the activity of these enzymes is largely unknown. Carboxylesterase activity is particularly prominent in the nasal cavity of rodents [25–27]. Research on the carboxylesterase-mediated metabolism of esters suggest that humans have a reduced capacity compared to rodents [28,29].

With regard to the lower airways, comparative data on metabolic capabilities are also sparse. Tissue content and activities of cytochrome P450 and glutathione *S*-transferases in human lung are generally lower than most species and considerably lower than that of mouse [30,31]. These results suggest significant differences exist in both phase I and phase II xenobiotic-metabolizing enzymes. Chemical- and mechanism-specific knowledge of these differences could contribute to reductions in uncertainties in risk assessments.

3. Default dose-response approach

The methods to derive inhalation reference concentrations (RfCs) are based on the same conceptual approach as that of the oral reference dose (RfD). There is one major exception. The RfC methods incorporate a dosimetric adjustment factor (DAF_r) for either respiratory tract region effects or remote toxicity [3]. The DAF_r is used to adjust for species differences in dosimetry. It is a multiplicative factor that represents the laboratory animal to human ratio of a given dose and is applied to laboratory animal exposure effect levels to calculate the human equivalent concentration (HEC). The HEC is expected to be associated with the same delivered dose to the observed target tissue as in the experimental species. The DAF_r calculated depends on (1) the physicochemical characteris-

tics of the inhaled toxicant (particle or 1 of 3 gas categories), (2) the location of the observed toxicity (i.e., either 1 of 3 respiratory tract regions or at remote sites), and (3) the type of dosimetry model (default or optimal) available for a particular chemical [3,32].

The derivation of an RfC from laboratory animal data is generally as follows. First, because many inhalation toxicity studies using laboratory animals are intermittent exposure regimens, a concentration (C) times time (t) product ($C \times t$) prorated adjustment is used to normalize these exposures to a continuous exposure as

$$\text{NOAEL}_{[\text{ADJ}]}^* (\text{mg}/\text{m}^3) = \frac{E (\text{mg}/\text{m}^3) \times D (\text{h}/\text{day}/24 \text{ h}) \times W (\text{days}/7 \text{ days})}{1} \quad (1)$$

where the $\text{NOAEL}_{[\text{ADJ}]}^*$ is the no-observed-adverse-effect level (NOAEL) or analogous effect level obtained with an alternate approach such as the benchmark dose approach, which is adjusted for duration of experimental regimen; E is the experimental exposure level; D is the number of (h exposed/day)/24 h; and W is the number of days (days of exposure/week)/7 days. The above duration adjustment is also applied to lowest-observed-adverse-effect levels (LOAELs).

The benchmark dose approach has been proposed as an improvement on the NOAEL/LOAEL approach. In general terms it is the use of a specific mathematical model (e.g., Weibull, logistic, polynomial) to determine a concentration associated with a predefined response (e.g., 10% response of a dichotomous outcome) and an estimate of its lower bound. This approach has a number of advantages in that estimates derived take into account the slope of the exposure-response curve and the statistical rigor of experimental design. Guidance on the application of this approach to derivation of RfD and RfC estimates is presented elsewhere [33,34]. The use of the benchmark dose approach does not obviate the requirement for uncertainty factors (UFs) with the exception of the UF for LOAEL to NOAEL extrapolation.

The rationale for the default duration adjustment is that the resultant continuous human exposure concentration should be the ($C \times t$)

equivalent of the laboratory animal exposure level. An advantage of dosimetry models is that because they incorporate and integrate various physicochemical and physiological determinants of chemical disposition, and thus dynamically simulate intermittent or continuous exposure, the use of this duration adjustment is obviated. Consideration of the basis of this adjustment is beyond the scope of this presentation and has been reviewed elsewhere [35].

The RfC methods then calculate the HEC by applying a DAF_r to the laboratory animal exposure effect level in order to account for species differences in dosimetry as

$$\text{NOAEL}_{[\text{HEC}]}^* (\text{mg}/\text{m}^3) = \text{NOAEL}_{[\text{ADJ}]}^* (\text{mg}/\text{m}^3) \times \text{DAF}_r \quad (2)$$

where the $\text{NOAEL}_{[\text{HEC}]}^*$ is the NOAEL human equivalent concentration; $\text{NOAEL}_{[\text{ADJ}]}^*$ is defined in Eq. (1); and DAF_r is a dosimetric adjustment factor for either an effect in a specific respiratory tract region, r (ET, TB, PU, or TOTAL) or remote effects. The DAF_r is either the regional deposited dose ratio (RDDR $_r$) for particles or the regional gas dose ratio (RGDR $_r$) for a given gas category and type of effect [3]. The DAF_r is constructed using default normalizing factors for the physiological parameters of interest. For example, because insoluble particles deposit and clear along the surface of the respiratory tract, the deposited dose in a specific region (e.g., TB) is commonly normalized to the surface area of that region. Extrarespiratory or remote effects are usually normalized to body weight.

Once the HEC is calculated, the UFs as shown in Table 1 are applied (as required) to calculate the RfC as

$$\text{RfC} = \text{NOAEL}_{[\text{HEC}]}^* / (\text{UF} \times \text{MF}) \quad (3)$$

The UFs are generally an order of magnitude, although incorporation of dosimetry adjustments or other mechanistic data has routinely resulted in the use of reduced UFs for RfCs. The composite UF applied to an RfC will vary in magnitude depending on the number of extrapolations required. An RfC will not be derived when use of the available data involve greater than 4 areas of extrapolation. The composite UF when 4 factors are used is reduced from 10 000 to 3000

Table 1
Guidelines for the use of UFs in deriving inhalation RfC

| Standard UFs | Processes considered in UF purview |
|---|---|
| <p>H, human to sensitive human Use \leq 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population.</p> | <p>Pharmacokinetics/pharmacodynamics Sensitivity Differences in mass Activity pattern Does not account for idiosyncrasies</p> |
| <p>A, laboratory animal to human Use \leq 3-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of average healthy humans. Use of a 3 is recommended with default dosimetric adjustments. More rigorous adjustments may allow additional reduction. Conversely, judgment that the default may not be appropriate could result in an application of a 10-fold factor.</p> | <p>Pharmacokinetics/pharmacodynamics Relevance of laboratory animal model Species sensitivity</p> |
| <p>S, subchronic to chronic Use \leq 10-fold factor when extrapolating from less than chronic results on experimental animals or humans when there are no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs.</p> | <p>Accumulation of chemical or cumulative damage Pharmacokinetics/pharmacodynamics Severity of effect Recover Duration of study Dependence of effect on duration.</p> |
| <p>L, LOEL_(HEC) to NOAEL_(HEC) Use \leq 10-fold factor when deriving an RfC from a LOAEL_(HEC) instead of a NOAEL_(HEC). This factor is intended to account for the uncertainty in extrapolating from the LOAEL_(HEC)s to NOAEL_(HEC)s.</p> | <p>Severity Pharmacokinetics/pharmacodynamics Slope of dose-response curve Relationship of endpoints Functional vs. histopathological evidence</p> |
| <p>D, incomplete to complete database Use \leq 10-fold factor when extrapolating from valid results in experimental animals when the data are 'incomplete'. This factor is intended to account for the inability of any single animal study to adequately address all possible adverse outcomes in humans.</p> | <p>Lack of second species Data gaps (lack of potential end points) Comprehensiveness of critical and supporting studies Exposure uncertainties</p> |
| <p>Modifying factor (MF) Use professional judgment to determine whether another uncertainty factor (MF) that is \leq 10 is needed. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and database not explicitly treated above (e.g., the number of animals tested or quality of exposure characterization). The default value for the MF is 1.</p> | |

Source: Refs. 3 and 45.

in recognition of the lack of independence of these factors. The lack of independence is evident in Table 1 which shows the various pharmacokinetic and pharmacodynamic processes typi-

cally believed to be encompassed by each UF.

By definition, a database for derivation of a dose-response estimate for noncancer toxicity should ensure that both appropriate and

adequate numbers of endpoints have been evaluated. The minimum requirement for derivation of an RfC with low confidence is a well-conducted subchronic inhalation bioassay that evaluated a comprehensive array of endpoints, including an adequate evaluation of respiratory tract effects, and established an unequivocal NOAEL and LOAEL. Chronic inhalation bioassay data in 2 different mammalian species, developmental studies in 2 different mammalian species, and a 2-generation reproductive study may be required to establish high confidence. The rationale supporting these data requirements is that, since the objective of the RfC is to serve as a lifetime estimate, all potential endpoints at various critical life stages must be evaluated. Well-defined and conducted subchronic toxicity studies are considered to be reliable predictors of many forms of chronic toxicity, with the notable exceptions of carcinogenic, teratogenic, and reproductive effects. The specific requirement for adequate respiratory tract evaluation arises from the increased potential for the portal-of-entry tissue to interact intimately with chemicals. Dosimetry data that indicate distribution to extrapulmonary tract sites is insignificant (e.g., a highly reactive and irritant gas which causes respiratory tract damage) may obviate the requirement for reproductive and developmental data. If these minimum database requirements are not met, an RfC is not derived.

4. RfC framework for interspecies dosimetry adjustments

As illustrated in Fig. 1, it is ultimately desirable to have a comprehensive biologically based dose-response model that incorporates the mechanistic determinants of chemical disposition, toxicant-target interactions, and tissue responses integrated into an overall model of pathogenesis in order to characterize the exposure-dose-response continuum. Unfortunately, the data to construct such comprehensive model structures do not exist for the majority of chemicals. Without dosimetry, default methods for dose-response estimation are limited to the rudimentary ('black-box') level and necessarily incorporate

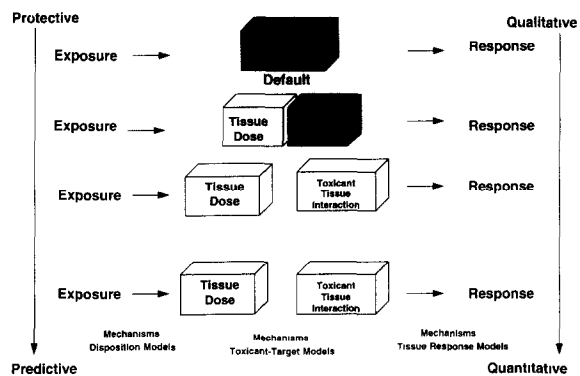


Fig. 1. Schematic characterization of comprehensive exposure-dose-response continuum and the evolution of protective to predictive dose-response estimates [3].

large UFs to ensure that the estimates are protective in the presence of substantial data gaps. With each progressive level, incorporation and integration of mechanistic determinants allow elucidation of the exposure-dose-response continuum and, depending on the knowledge of model parameters and fidelity to the biological system, a more accurate characterization of the pathogenesis process. Due to the increase in accuracy of the characterization with each progressive level, dose-response estimates also progress from more conservative (protective) to factually based (predictive) [32].

Mathematical dosimetry models that incorporate mechanistic determinants of disposition of chemicals have been useful in describing relationships between exposure concentration and target tissue dose, particularly as applied to describing these relationships for the dose-response component of risk assessment. The default dosimetric adjustments used in the RfC methods were based on comprehensive model structures and reduced to forms requiring a minimal number of parameters by utilizing the dominant determinants of disposition for various categories of compounds and simplifying assumptions [3,32].

For example, because a theoretical model of particle deposition requires detailed information on all of the influential parameters (e.g., respiratory rates, exact airflow patterns, complete measurement of the branching pattern of the respira-

tory tract) across the various species used in risk assessment, an empirical model (i.e., a system of equations fit to experimental data) was developed as the default model [3].

The default DAF calculated for gases, as for particles, is different for each respiratory tract region or for remote effects. In addition, the DAF for gases is dependent on which of 3 categories classifies the gas. The scheme used to categorize gases (Fig. 2) was constructed based on the physicochemical characteristics of water solubility and reactivity as major determinants of gas uptake. Reactivity is defined to include both the propensity for dissociation and the ability to react either spontaneously or via enzymatic reaction in the respiratory tract. The scheme does not apply to stable gases that exert their effects by reversible 'physical' interactions of gas molecules with biomolecules (e.g., 'displacement' of oxygen by carbon dioxide).

As an example, Fig. 3 shows the schematic for the default model used to characterize respiratory

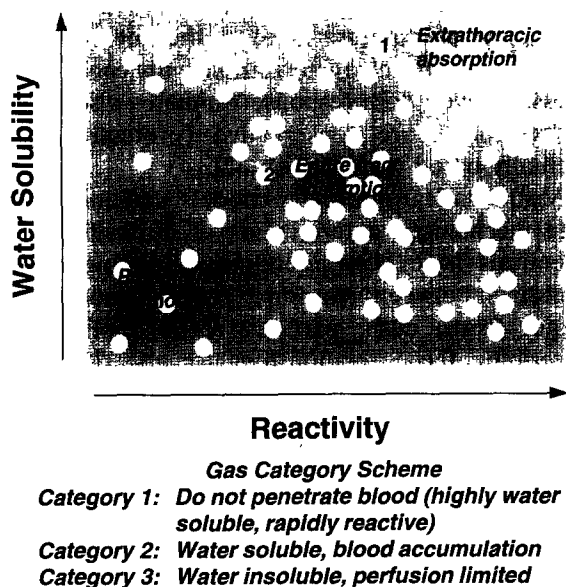


Fig. 2. Gas categorization scheme based on water solubility and reactivity as major determinants of gas uptake [3,32]. Reactivity is defined to include both the propensity for dissociation as well as the ability to serve as a substrate for metabolism in the respiratory tract. Definitive characteristics of each category and the anticipated location (region) for respiratory tract uptake are shown.

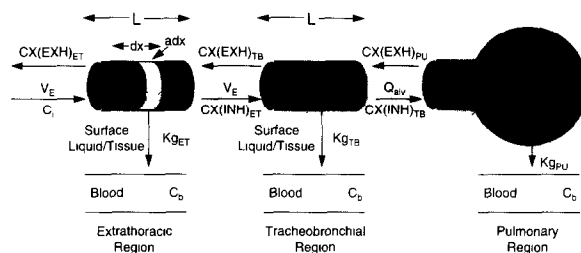


Fig. 3. Schematic of model to estimate default DAF_r for gases in category 1 [3,32]. a, airway perimeter; C_{alv} , pulmonary region gas concentration; C_b , blood concentration; C_i , inhaled concentration; $CX(EXH)_{ET}$, concentration existing from ET region upon exhalation; $CX(EXH)_{PU}$, concentration existing from PU region upon exhalation; $CX(EXH)_{TB}$, concentration exiting from TB region upon exhalation; $CX(INH)_{ET}$, concentration exiting from ET region upon inhalation; $CX(INH)_{TB}$, concentration exiting from TB region upon inhalation; dx , differential of axial distance into airway; ET, extrathoracic respiratory region; K_{gET} , overall mass transport coefficient of the ET region; K_{gPU} , overall mass transport coefficient of the PU region; K_{gTB} , overall mass transport coefficient of the TB region; alv, alveolar ventilation rate; V_E , minute ventilation.

tract uptake of category 1 gases. Category 1 gases are defined as highly water soluble and rapidly reactive. Because of these properties, category 1 gases (e.g., hydrogen fluoride, chlorine, formaldehyde, and the organic esters) are likely to interact with the respiratory tract. The objective of the default modeling approach is to describe the effective dose to the 3 regions by addressing the absorption or 'scrubbing' of the gas from the inspired airstream as it travels from the ET to PU region. The approach used to model the uptake is based on the concept of an overall mass transfer coefficient, K_g [3,32]. The concept of the K_g is based on a concentration gradient analysis similar to Fick's Law of diffusion and is utilized to describe transport through several different phases, such as air and the liquid/tissue phase of the respiratory tract. A fractional penetration model is used to determine the fraction of the inhaled concentration in each region. For example, the uptake in the ET region and the output to the TB region (fractional penetration, fp_{ET}) is dependent on the K_{gET} , so that uptake in the ET region is defined as $1 - fp_{ET}$. A ventilation-perfusion model is used to estimate the uptake in the PU region by sub-

stituting the concentration of the air exiting the TB region for the inhaled concentration. The rate of mass absorbed at the gas-surface interface of the airway in a region (r) is simply the product of the absorbed fraction, $(1 - fp_r)$, and the total mass inhaled during a single breath, $\dot{V}_E C_i$, where C_i is the inhaled concentration. The \dot{V}_E is used as the default volumetric flow rate because it approximates the flow rate at which the animal was breathing during the experimental exposure. The alveolar ventilation rate is used to calculate the absorption rate for the PU region.

The DAF_r for each region is then calculated based on equations describing the relationship between K_g and $1 - fp_r$ for each region, the ventilation rate, and regional surface area. The assumption that absorption is distributed equally within a region allows the description on a regional basis. Although this is a drastically reduced number of parameters in comparison to distributed parameter model descriptions, the default model does require K_g values for different animal species and gases. It is important to note that K_g is both species and chemical specific. Values of K_g obtained in a single animal species may be scaled within a species for a different gas in the same category by decomposing K_g to the individual gas-phase and surface-liquid/tissue phase transport resistances [3]. The default equations can be further reduced by applying additional simplifying assumptions regarding the likely values of K_g . The derivation of the equations and DAF_r for each region, including the models for the 2 other gas categories, are provided in detail elsewhere [3].

An understanding of the basis for the default adjustments allows development of a framework for the evaluation of whether an alternative model structure may be considered optimal relative to the default. Depending on the relative importance of these various determinants, models with less detail (i.e., less of the determinants depicted in Fig. 1) may be used to adequately describe difference in dosimetry for the purposes of interspecies extrapolation. An alternative model might be considered more appropriate than the default for extrapolation when default assumptions or parameters are replaced by more detailed, biologically motivated description or actual data, respectively. For example, a model could be preferable if it incorporates more chemical- or species-specific information or if it accounts for more mechanistic determinants. These considerations are summarized in Table 2. The sensitivity of the model to these differences in structure may be gauged by its relative importance in describing the response function for a given chemical.

5. Opportunities to replace default approaches with mechanistic data: vinyl acetate as an example

Improvements in the design of standard inhalation toxicity studies and development of adjunct mechanistic data should increase the accuracy of extrapolation and thereby reduce uncertainty. Given the default paradigm presented above, studies can be conducted to address

Table 2
Hierarchy of model structures for dosimetry and interspecies extrapolation

Optimal^a model structure

Structure describes all significant mechanistic determinants of chemical disposition, toxicant-target interaction, and tissue response
Uses chemical-specific and species-specific parameters
Dose metric described at level of detail commensurate to toxicity data

Default model structure

Limited or default description of mechanistic determinants of chemical disposition, toxicant-target interaction, and tissue response
Uses categorical or default values for chemical and species parameters
Dose metric at generic level of detail

Source: Refs. 3 and 32.

^a Optimal is defined as preferable or more appropriate relative to the default.

specific areas of uncertainty highlighted by the default approach and the range of UFs used in the default approach. Aside from using the RfC methodology as a guide in this process, all studies should be designed to answer specific questions; i.e., they should be hypothesis driven. Too often, toxicologists are resorting to standard study protocols with little thought given to the health question being addressed. Presented below is an example of the nasal toxicant vinyl acetate and how data on mode of action can be used to address many of the default factors used in the conventional RfC methodology.

5.1. UF: duration adjustment and choice of dosimetrics

As discussed above, the underlying assumption of Eq. 2, that the resultant continuous human equivalent exposure concentration should be the $C \times t$ product equivalent of the experimental animal exposure, is essentially 'Haber's Law'. Accordingly, a constant, in this case a fixed effect level (i.e., a constant severity and/or incidence level) is assumed to be related to the $C \times t$ product. Toxicity, however, can depend on the magnitude, duration, and frequency of exposure. If detoxication or elimination occurs between successive doses, then the $C \times t$ product may not be an appropriate dose metric and the duration adjustment becomes too conservative. The choice of an appropriate measure of dose must be defined by the nature of the pathogenesis process for the effect under consideration. Concentration and time study designs may provide insight on whether concentration, duration, or the product most influence the toxicity. Because dosimetry models can integrate time- and con-

centration-dependent processes (e.g., distribution and metabolism rates) that are important mechanistic determinants of toxicity, they can eliminate the need for the default $C \times t$ product duration adjustment and provide insight on the proper dose metric to use [35]. These models can be linked to pharmacodynamic models that address factors influencing tissue response and further refine the choice of dose metric. In the case of vinyl acetate, it is the acetic acid produced within sustentacular cells of olfactory epithelium that initiates cytotoxicity. Cell death is likely a consequence of heavy tissue proton burdens that overwhelm the natural cellular buffering and proton transport mechanisms [36,37]. Such a mechanism suggests that response is dependent on the intracellular proton concentrations as a dose metric. PBPK models have the capability to account for many of these factors in a chemical- and species-specific manner (Table 3). A recent review of these methods for nasal toxicants illustrated a variety of approaches to using dosimetry models to address these issues [38]. A model of vinyl acetate deposition and metabolism has recently been developed [37] (Fig. 4). The model construct is similar to that developed by Morris et al. [39]. Dosimetrics for acetic acid and acetaldehyde formation are generated for specific sites within the nose such as respiratory vs. olfactory epithelium. A human-equivalent model can be developed in which human-specific data on airflow characteristics [40] and metabolism [41] are incorporated into the model. These attributes of the model enable a more close mechanistic link between the specific tissue affected and the tissue dosimetry of the toxic metabolite causing olfactory degeneration (acetic acid). This is an improvement over

Table 3
Advantages of PBPK/dosimetry modeling approach

-
- Allows integration and extrapolation using diverse data
 - Predicts complex kinetic behavior
 - Capability to 'lump' or 'split' model structure to explore dose response
 - Enables interspecies dosimetric comparisons
 - Allows parameter scaling across species
 - Facilitates hypothesis generation
 - Identifies areas of needed research
-

Vinyl Acetate PB-PK Model Structure

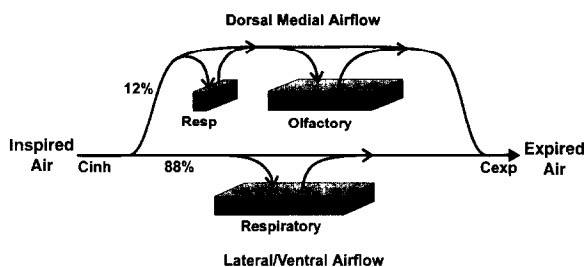


Fig. 4. Schematic diagram of the vinyl acetate PBPK model. The model structure, developed by Morris [39] is based on airflow patterns and anatomic, physiologic and biochemical data specific to the nasal cavity.

the default RfC dosimetry approach in a number of ways including providing site-specific information on metabolism (as opposed to a regional K_g). The use of this model should therefore result in a reduction in the residual UF of 3.

5.2. UF: completeness of database

An incomplete data set will frequently result in the application of a 10-fold UF. Vinyl acetate has been tested in 90-day and 2-year inhalation studies in both rats and mice in which the pathology of URT lesions has been characterized by extensive histopathology examination. A study design using adequate numbers of animals and interim sacrifices, ensured reasonable statistical power. These studies showed the critical effect is degeneration of the olfactory epithelium with NOAEL of 50 ppm. A significant incidence of nasal tumors was observed at the highest concentration of 600 ppm. Occupational exposure data show no adverse effects of exposure at mean exposure levels of approximately 8.6 ppm. Inhalation developmental toxicity studies showed there was no embryolethality or developmental toxicity at levels below maternally toxic levels. Although an inhalation reproductive toxicity study has not been conducted, a multigeneration drinking water study was negative. Thus, with the possible exception of an inhalation reproductive toxicity study, this database was considered complete. However, the lack of

evidence for systemic toxicity, coupled with estimates of nasal metabolism that suggest complete metabolism within the nose and dosimetry data indicating insignificant remote distribution of vinyl acetate (discussed below), obviate the need for studies of reproductive toxicity. Thus, there is no need for an UF to account for these data gaps.

5.3. UF: subchronic to chronic

In the case of vinyl acetate, there is no need for the application of an UF to account for the lack of a chronic inhalation bioassay. However for many chemicals such data will not be available and will require the uncertainty of unknown outcomes from chronic exposure to be addressed. Adjunct data on metabolism that bear on the question of accumulation of toxic metabolites and lesion repair rates could be important factors for reducing this uncertainty. In cases where toxic metabolites or lesions do not accumulate over time one might expect that toxic levels of exposure would be similar despite the length of exposure. This is the case for many irritants. Lesion repair often begins during exposure and may not be observable at time of sacrifice. As an example, recovery of olfactory epithelial damage was observed to commence prior to cessation of methyl bromide exposures [42]. Incorporation of interim sacrifices into standard inhalation bioassay study designs could provide information on the chronological dynamics of lesion formation and repair. In an empirical approach to the issue of differences in toxic exposure levels between subchronic and chronic exposures and the appropriateness of UFs, Nessel et al. [43] summarized the $LOAEL_{subchronic} / LOAEL_{chronic}$ for 9 studies, the majority of which showed portal-of-entry effects. The mean ratio was 4.5 suggesting that a factor 10 might be excessive for these types of chemical.

5.4. UF: LOAEL to NOAEL

Since inhalation studies are difficult to conduct, the toxicologist is frequently faced with less

than adequate range finding data to support the choice of exposure concentrations for the definitive bioassay. In addition, maintaining accurate exposure levels can be technically difficult resulting in excursions above or below the target concentration. These factors, among others, can contribute to a target NOAEL becoming a LOAEL. Information which could help reduce this uncertainty includes structure activity relationships showing NOAELs for similar compounds. The benchmark dose approach offers an advantage in this situation since the model allows the estimation of NOAELs. Toxicologists designing inhalation bioassays should be knowledgeable about this approach in order to gain the complete advantages of its implementation. The approach rewards statistically robust study designs (large numbers of experimental groups, large numbers of animals per group) with better estimates of the NOAEL and lower bound estimates that are close to the maximum likelihood estimates. The cost of the improved study design must be weighed against its potential benefit. In the case of vinyl acetate, NOAELs were determined in all studies. However, because the benchmark dose approach allows interpolation between exposure levels, the benchmark estimate turned out to be higher than the lowest concentration tested (50 ppm). Therefore no UF is necessary.

5.5. UF: laboratory animal to human and intrahuman variability

As explained above, what is known about interspecies differences in respiratory tract dosimetry ('pharmacokinetic' processes) and tissue response ('pharmacodynamic' processes) suggest that these differences are significant. As noted in Table 1, pharmacokinetic and pharmacodynamic processes fall under the purview of both the interspecies and the intrahuman UFs and the factors are typically parcelled into factors of 3 each. These factors are shown incorporated into the exposure-dose-response framework in Fig. 5. The default UF for interspecies extrapolation is $10^{0.5}$ or approximately 'halved' in the RfC methods because application of the default DAF_r

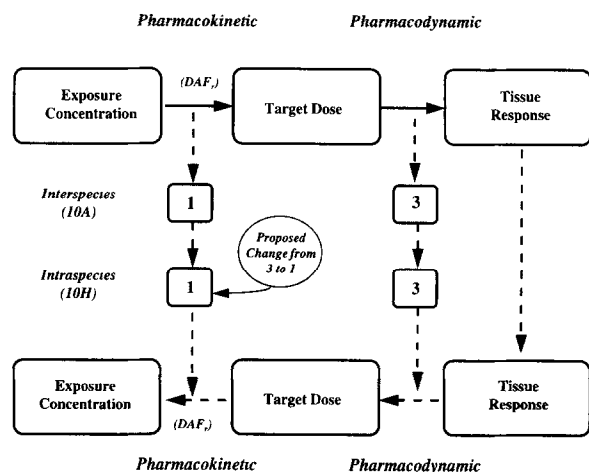


Fig. 5. Interspecies and intraspecies (intrahuman) UFs incorporated into framework of exposure-dose-response continuum (Ref. 45, as modified from [46]).

was thought to account for a portion of the 'pharmacokinetic' portion of the extrapolation. Although the default DAF_r also adjusts for factors that control target dose in humans, a portion of the uncertainty was left remaining because the adjustments are viewed as default. More robust dosimetry models can be anticipated to obviate the entire pharmacokinetic component. In the case of vinyl acetate, the proposed PBPK model [37] can be scaled to humans and used with human-specific parameter values. Use of these robust dosimetry models to simulate the experimental animal and anticipated human exposure scenario in order to calculate the HEC estimates could obviate both parcels of the pharmacokinetic component.

Models that address the determinants of response may impact the pharmacodynamic component. In the case of the URT, few diseases are known to be pre-existent and not attributable to some source of ambient exposure. Effects such as rhinitis, rhinorrhea, irritation, and swelling of the mucous membranes are shared among those caused by airborne allergens and low-level chemical exposure [44]. Thus, it is possible that atopic status or pre-existing URT disease may represent susceptibility factors for chemical exposures that cause URT damage.

With regard to the mechanism of vinyl acetate

toxicity, populations with reduced olfactory capacity and those with high nasal carboxylesterase activities would be expected to show sensitivity. Studies on human nasal carboxylesterase with vinyl acetate showed a relatively tight range of activities for both respiratory and olfactory epithelium [41]. The sample population was from 9 donors and included predominantly caucasian male donors aged 54–82 years. To the extent that there is some homogeneity within this population, an argument could be made that some reduction in the pharmacodynamic portion of the UF is warranted since the rates of metabolism used in the model reflected this variability.

6. Conclusions

This overview illustrates the many complexities involved with assessing risk of inhaled materials and highlights the many assumptions that contribute to uncertainty in these assessments. However, by conducting toxicological research in a manner that is aimed at addressing uncertainties, more meaningful and realistic assessments can be made. Physiologically based approaches to modeling dosimetry of inhaled materials is becoming more common. Basic research on mechanisms of action is needed to enable development of response models. With regard to both dosimetry and response, research on human-specific parameters appears to be the area in greatest need for research.

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