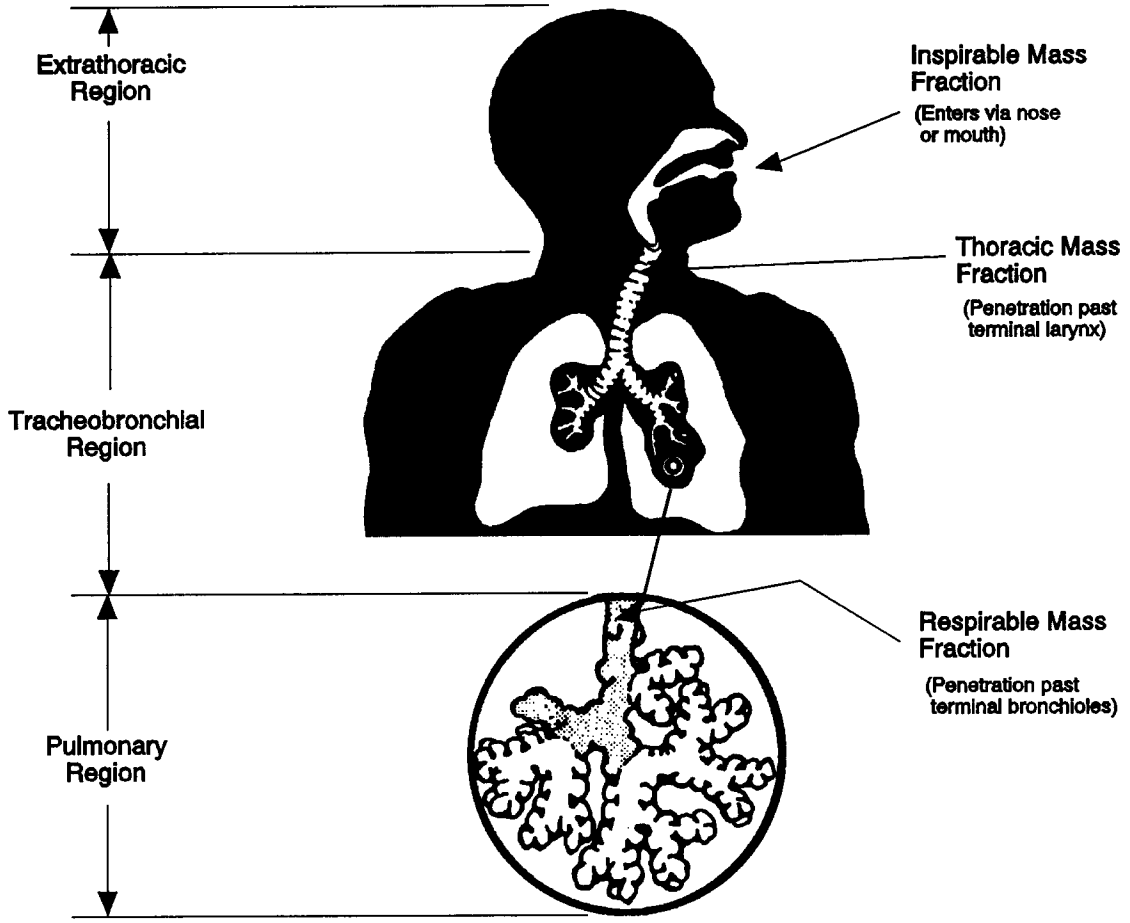

Figure 3-1. Diagrammatic representation of three respiratory tract regions.

- The airway diameter affects the aerodynamics of the air flow and the distance from the agent molecule or particle to the airway surface.
- The cross-sectional area of the airway determines the airflow velocity for a given volumetric flow.
- Airway length, airway diameter, and branching pattern variations affect the mixing between tidal and reserve air.

Differences in airway sizes and branching among species therefore may result in significantly different patterns of transport and deposition for both particles and gases. Alveolar size also differs between species, which may affect deposition efficiency due to variations on the distance between the airborne particle or molecule and alveolar walls (Dahl et al., 1991a).

Effect on Particle Deposition Mechanisms

Air flow in the extrathoracic region is characterized by high velocity and abrupt directional changes. Therefore, the predominant deposition mechanism in the ET region is inertial impaction. In this process, changes in the inhaled airstream direction or magnitude of air velocity streamlines or eddy components are not followed by airborne particles because of their inertia. Large particles (>5 μm in humans) are more efficiently removed from the airstream in this region.

Impaction remains a significant deposition mechanism for particles larger than 2.5 μm aerodynamic equivalent diameter (d_{ae}) in the larger airways of the TB region in humans and competes with sedimentation, with each mechanism being influenced by mean flow rate and residence time, respectively. As the airways successively bifurcate, the total cross-sectional area increases. This increases airway volume in the region, and the air velocity is decreased. With decreases in velocity and more gradual changes in air flow direction as the branching continues, there is more time for gravitational forces (sedimentation) to deposit the particle. Sedimentation occurs because of the influence of the earth's gravity on airborne particles. Deposition by this mechanism can occur in all airways except those very few that are vertical. For particles $\approx$4 μm d_{ae}, a transition zone between the two mechanisms, from impaction to predominantly sedimentation, has been observed (U.S. Environmental Protection Agency, 1982b). This transition zone shifts toward smaller particles for nose breathing.

Differences in airway size and branching pattern are a major source of interspecies variability in inhaled dose for the TB region. Larger airway diameter results in greater turbulence for the same relative flow velocity (e.g., between a particle and air). Therefore, flow may be turbulent in the large airways of humans, whereas for an identical flow velocity, it would be laminar in the smaller experimental animal. Relative to humans, experimental animals also tend to have tracheas that are much longer in relation to their diameter. This could result in increased relative deposition in humans because of the increased likelihood of laryngeal jet flow extending into the bronchi. Human airways are characterized by a more symmetrical dichotomous branching than that found in most laboratory mammals, which have highly asymmetrical airway branching (monopodial). The more symmetrical dichotomous pattern in humans is susceptible to deposition at the carina because of its exposure to high air

flow velocities toward the center of the air flow profile. These comparative airway anatomy differences are summarized in Table 3-2.

Sedimentation becomes insignificant relative to diffusion as the particles become smaller. Deposition by diffusion results from the random (Brownian) motion of very small particles caused by the collision of gas molecules in air. The terminal settling velocity of a particle approaches 0.001 cm/s for a unit density sphere with a physical diameter of 0.5 μm, so that gravitational forces become negligible at smaller diameters. The main deposition mechanism is diffusion for a particle whose physical (geometric) size is <0.5 μm. Impaction and sedimentation are the main deposition mechanisms for a particle whose size is greater than 0.5 μm. Hence, $d_{ae} = 0.5$ μm is convenient for use as the boundary between the diffusion and aerodynamic regimes. Although this convention may lead to confusion in the case of very dense particles, most environmental aerosols have densities below 3 g/cm^3 (U.S. Environmental Protection Agency, 1982b). Diffusional deposition is important in the small airways and in the PU region where distances between the particles and airway epithelium are small. Diffusion has also been shown to be an important deposition mechanism in the ET region for small particles (Cheng et al., 1988, 1990).

These mechanisms for particle deposition in the respiratory tract are schematically represented in Figure 3-2. Experimental deposition data and extrapolated estimates on humans that illustrate these same concepts are shown by the curves for PU (alveolar) and TB deposition in Figure 3-3. Deposition fraction is shown plotted against particle diameter. It is important to note that over half of the total mass of a typical ambient mass distribution would be deposited in the ET region during normal nasal breathing, with most of this being coarse particles (U.S. Environmental Protection Agency, 1986c). With mouth-only breathing, the regional deposition pattern changes dramatically compared to nasal breathing, with ET deposition being reduced and both TB and PU deposition enhanced. Oronasal breathing (partly via the mouth and partly nasally), however, typically occurs in healthy adults while undergoing moderate to heavy exercise. Therefore, the appropriate activity pattern of subjects for risk assessment estimation remains an important issue. Miller et al. (1988) examined ET and thoracic deposition as a function of particle size for ventilation rates ranging from normal respiration to heavy exercise. A family of estimated deposition curves were generated as a function of breathing pattern. Anatomical and functional differences

TABLE 3-2. COMPARATIVE LOWER AIRWAY ANATOMY AS REVEALED ON CASTS

Mammal/ Body Mass	Gross Structure: Left Lung Lobes	Gross Structure: Right Lung Lobes	Gross Structure: Airway Branching	Gross Structure: Trachea length/diameter (cm)	Gross Structure: Major Airway Bifurcations	Typical Structure (Generation 6): Average Airway L/D (ratio)	Typical Structure (Generation 6): Branch Angles (Major Daughter/ Minor Daughter) (degrees)	Typical Number of Branches to Terminal Bronchiole	Respiratory Bronchioles
Human/70 kg	Upper and lower	Upper, middle, and lower	Relatively symmetric	12/2	Sharp for about the first 10 generations, relatively blunt thereafter	2.2	11/33	14-17	About 3-5 orders
Rhesus monkey/2 kg	Superior, middle, and inferior	Superior, middle, and inferior, azygous	Monopodial	3/0.3	Mixed blunt and sharp	2.6	20/62	10-18	About 4 orders
Beagle dog/ 10 kg	Apical, intermediate, and basal	Apical, intermediate, and basal	Strongly monopodial	17/1.6	Blunt tracheal bifurcation, others sharp	1.3	8/62	15-22	About 3-5 orders
Ferret/ 0.61 kg	NR[a]	NR	strongly monopodial	10/0.5	Sharp	2.0	16/57	12-20	About 3-4 orders
Guinea pig/ 1 kg	Superior and inferior	Superior, middle, and inferior	Monopodial	5.7/0.4	Very sharp and high	1.7	7/76	12-20	About 1 order
Rabbit/ 4.5 kg	Superior and inferior	Cranial, middle, caudal, and postcaval	Strongly monopodial	6/0.5	Sharp	1.9	15/75	12-20	About 1-2 orders
Rat/0.3 kg	One lobe	Cranial, middle, caudal, and postcaval	Strongly monopodial	2.3/0.26	Very sharp and very high throughout lung	1.5	13/60	12-20	Rudimentary
Golden hamster/ 0.14 kg	Superior and inferior	Cranial, middle, caudal, and postcaval	Strongly monopodial	2.4/0.26	Very sharp	1.2	15/63	10-18	About 1 order

[a]NR = Not reported.

Source: Phalen and Oldham (1983); Patra (1986); Crapo (1987).

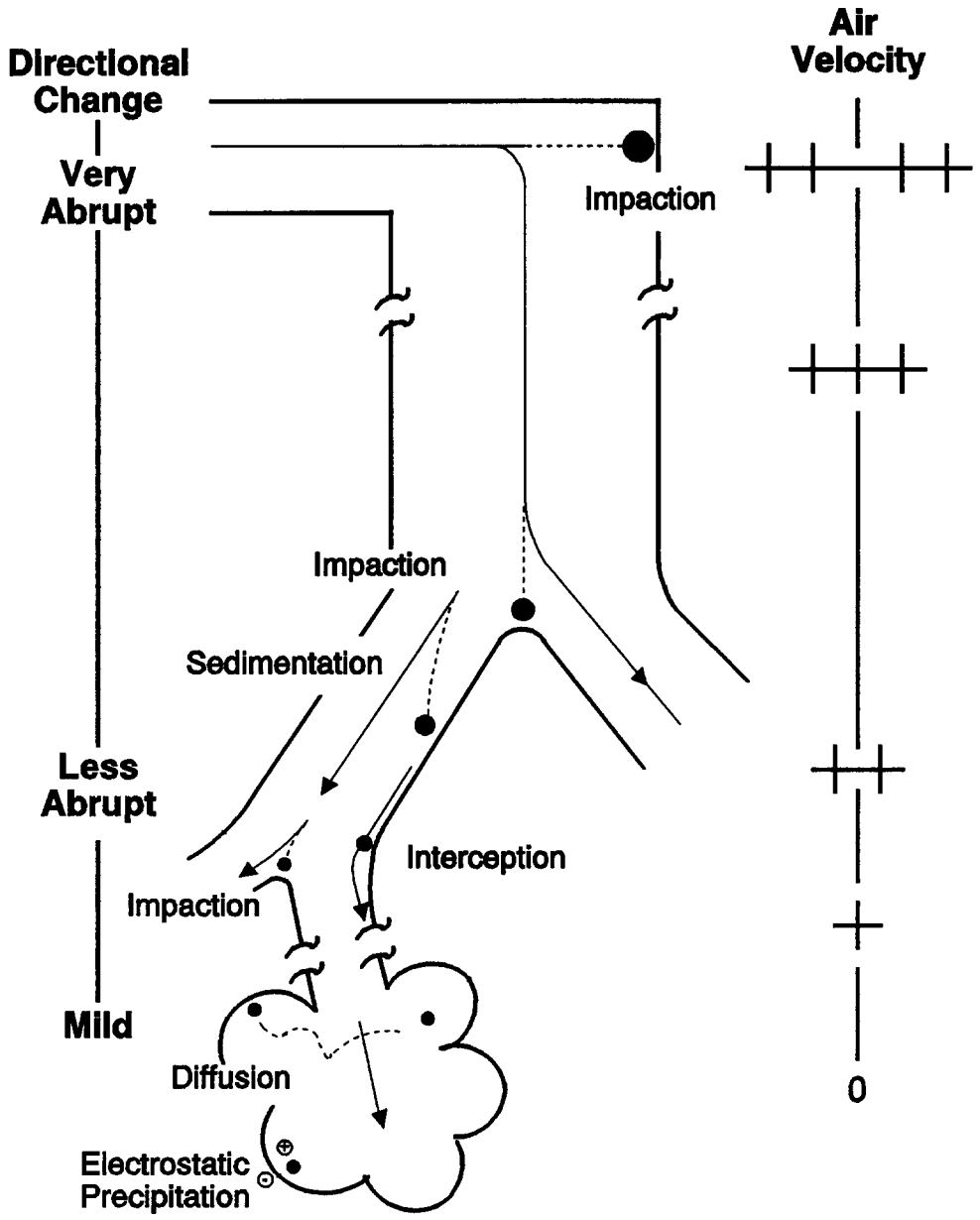

Figure 3-2. Schematic representation of selected parameters influencing regional deposition of particles in the respiratory tract.

Source: Adapted from Casarett (1975); Raabe (1979); Lippmann and Schlesinger (1984).

between adults and children are likely to yield complex interactions with the major mechanisms affecting respiratory tract deposition, again with implications for risk assessment. Age-dependent dosimetric adjustments may be possible, pending data availability for children.

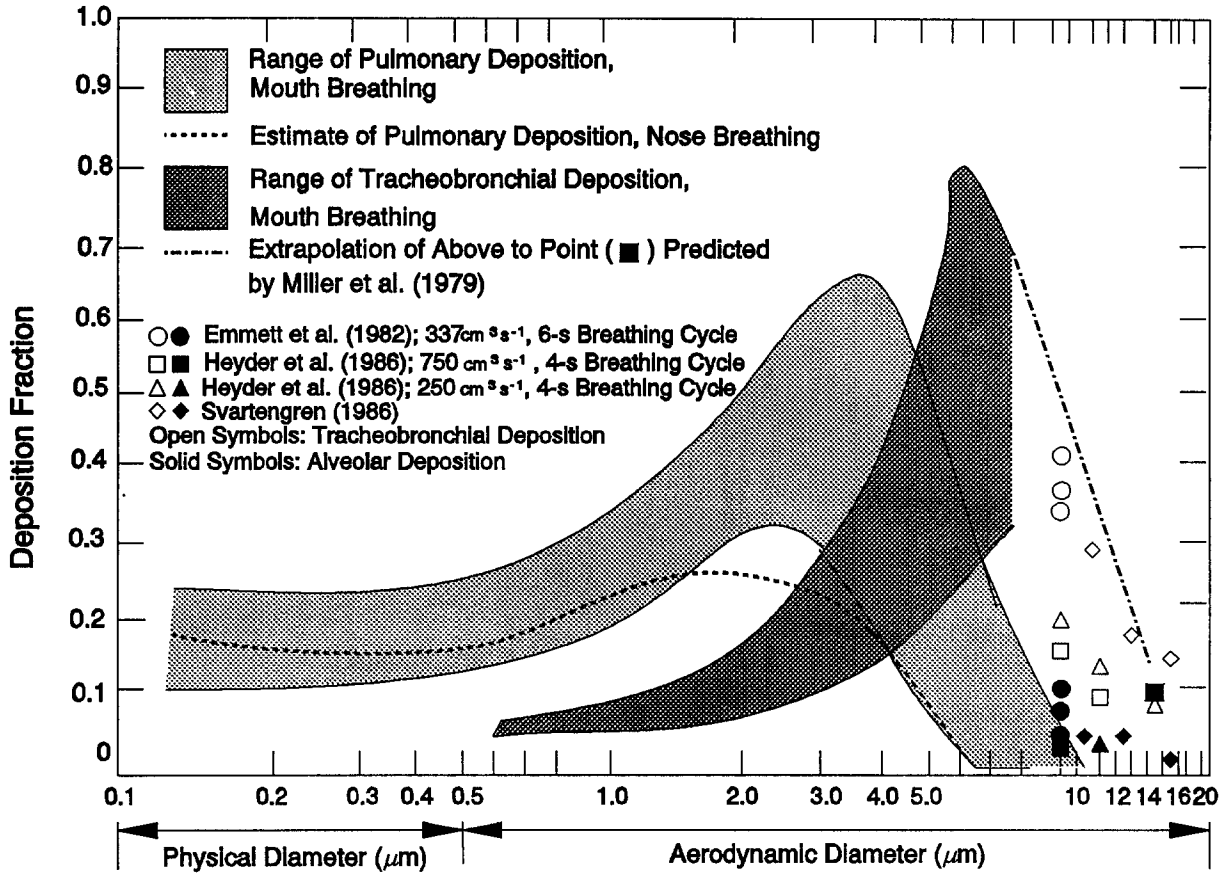

Figure 3-3. Regional deposition in humans of monodisperse particles by indicated particle diameter for mouth breathing (pulmonary and tracheobronchial) and nose breathing (pulmonary). Deposition is expressed as fraction of particles entering the mouth or nose. The PU band indicates the range of results found by different investigators using different subjects and flow parameters for PU deposition following mouth breathing. The TB band indicates intersubject variability in deposition over the size range measured by Chan and Lippmann (1980). The extrapolation of the upper bound of the TB curve in the larger particle size range also is shown and appears to be substantiated by data listed in the legend.

Source: U.S. Environmental Protection Agency (1986c).

Effect on Gas Deposition and Uptake

The major processes affecting gas transport involve convection, diffusion, absorption, dissolution, and chemical reactions. These mechanisms are schematically represented in Figure 3-4. Predictions of lower respiratory tract distribution of ozone from a detailed dosimetry model that accounts for many of these processes is shown in Figure 3-5.

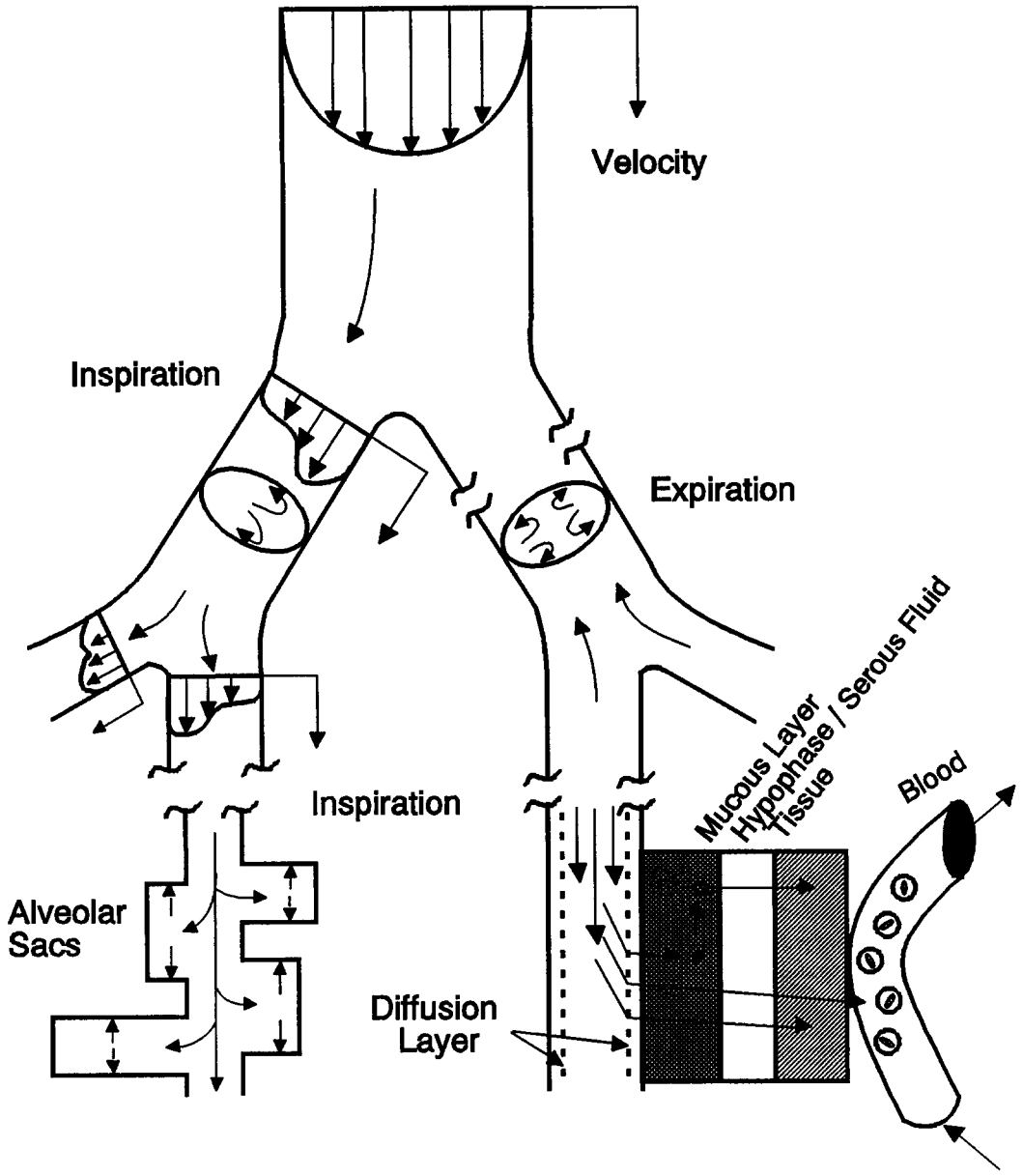

Figure 3-4. Schematic representation of selected parameters influencing regional deposition of gases in the respiratory tract.

Source: Overton (1984).

Beginning at the trachea, the model predicts the net ozone dose (flux to air-liquid interface) slowly decreases distally in the tracheobronchial region and rapidly decreases in the pulmonary region (U.S. Environmental Protection Agency, 1993b).

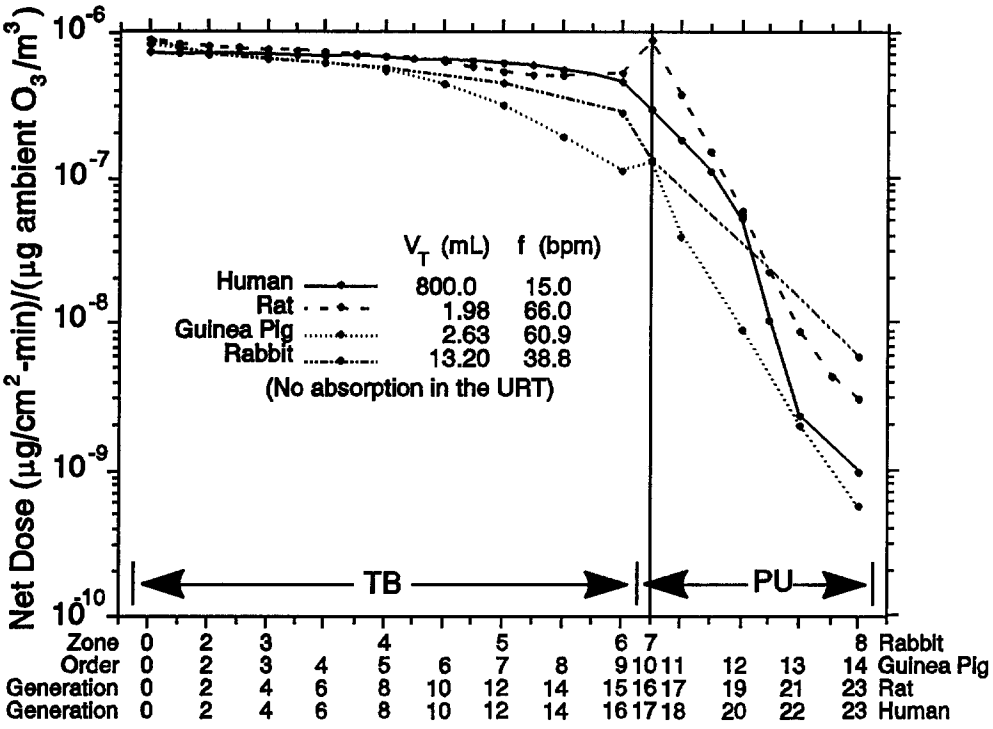

Figure 3-5. Net dose of ozone versus sequential segments along anatomical model lower respiratory tract paths for human, rat, guinea pig, and rabbit. In general, each segment represents a group of airways or ducts, with common features as defined by the designers of the anatomical model (human and rat: generation; guinea pig: order; rabbit: zone). For a given species the plotted dots represent a predicted dose that corresponds to a given segment. The dots have been joined by lines for ease of interpreting the plots; these lines do not represent predicted values except where they intercept the dots. TB = tracheobronchial region. PU = pulmonary region.

Source: Overton and Miller (1988).

The bulk movement of inspired gas in the respiratory tract is induced by a pressure gradient and is termed convection (U.S. Environmental Protection Agency, 1982b). 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 (bulk flow) due to local concentration gradients. Absorption removes gases from the lumen and affects concentration gradients.

The average concentration of a gas in a tube (i.e., an "idealized" airway) can be described by one-dimensional convection and dispersion. A pulse of substance moves down a tube with an average air velocity equal to the medium's (air's) average velocity, and its spread in the axial direction is governed by an effective dispersion coefficient that can be described by Fick's law of diffusion (Overton, 1984). This effective dispersion coefficient is larger than the molecular diffusion coefficient except in the PU region. As illustrated in Figure 3-4, perpendicular transport in this region can carry a gas molecule into the alveoli, but because of the alveolar walls, there is minimal net axial transport with respect to that in the central channel. The average axial transport is slowed because only a fraction of the molecules in the cross-sectional average can move axially, generally resulting in a dispersion process with a dispersion coefficient less than the molecular diffusion coefficient, although it is possible for longitudinal mixing to be enhanced by the presence of alveolar septa leading to dispersion coefficients that are actually greater than the molecular diffusivity (Federspiel and Fredberg, 1989). The dispersion coefficient is a function of the molecular diffusion coefficient, the total air volume, and the generation's alveolar airspace volume (Overton, 1984). The dispersion coefficient is also influenced by the absorption process (Dayan and Levenspiel, 1969).

Molecules are transferred from the flowing gas into the liquid layer lining the airway wall by molecular diffusion. A simple description for this process postulates a thin, stagnant air layer based on the assumption that the air velocity becomes very small as the air-liquid interface is approached. Transfer through this layer depends on the gas-phase diffusion coefficient, layer thickness, and the gas concentrations at the boundaries of the layer. If the molecules are absorbed, then the concentration of the gas in the diffusion layer is decreased at the liquid boundary. As the ability of the liquid to remove the gas increases, the relative concentration at the gas-liquid boundary decreases, and the mass transfer from the gas phase to the liquid phase increases. For poorly soluble, hydrophobic, and nonreactive gases, little gas is removed by the airways. The transport into and chemistry of the adjacent surface liquid and tissue layers will be described in Section 3.1.2.2, which describes the physicochemical characteristics of gases and vapors. These next layers can serve as a "sink" to help "drive" the delivery of gas across this layer. Capillary blood flow (i.e., perfusion) is important to the gas uptake in that it removes the gas or its chemical reaction products on the

other side of these liquid and tissue layers. Therefore, addressing species differences in alveolar ventilation, regional perfusion rates, and cardiac output is critical to estimating initial absorbed dose. The importance of regional differences (e.g., the distance from the air to the capillaries in the tracheobronchial region is 7 to 20 times that in the pulmonary region [Overton and Miller, 1988]) and interspecies differences in the anatomic relationship of the airspace to capillary blood should be considered. Transfer also is enhanced by a reduction in diffusion layer thickness that is dependent on the nearby rate of airflow; the higher the flow velocity, the thinner the layer, again emphasizing the significance of airway morphology.

Although the preceding figures have only illustrated these concepts for the lower respiratory tract, the influence of anatomy on comparative deposited dose is also important in the ET region. Species differences in gross anatomy, nasal airway epithelia (e.g., cell types and location) and the distribution and composition of mucous secretory products have been noted (Harkema, 1991; Guilmette, 1989). The geometry of the upper respiratory tract exhibits major interspecies differences (Gross and Morgan, 1992). Figures 3-6 and 3-7 show diagrams of the ET region that illustrate the differences between Rhesus monkeys and F344 rats. Cross-sections for the four levels shown on the transverse section are at comparable locations in the monkey and rat. Figure 3-8 shows the influence these differences have on airflow patterns in the region. In both species shown in Figure 3-8, studies have demonstrated complex inspiratory flow streams, exhibiting regions of simple laminar, complex secondary (vortices, eddies, swirling), and turbulent flows (Morgan et al., 1991). Differences in nasal air flow patterns between these two species and humans (Hahn et al., 1993) is an important consideration for extrapolation of dose associated with nasal toxicity. Good correlation has been shown between routes of flow, regional secondary flows, turbulence, and impaction of airstreams on the airway wall, with the reported distribution of formaldehyde-induced nasal lesions in these species, illustrating the influence of the nasal anatomy on gas deposition for this reactive and soluble gas (Morgan et al., 1991; Kimbell et al., 1993).

In order to model the effects that the intricate morphological structure of the respiratory tract have on the nature of gas mixing and flows, representations of the mechanical mixing imparted by tube bifurcations, turbulence, and secondary flows due to molecular diffusion must be formulated. Location, diameter, and length of airways are considered to be the

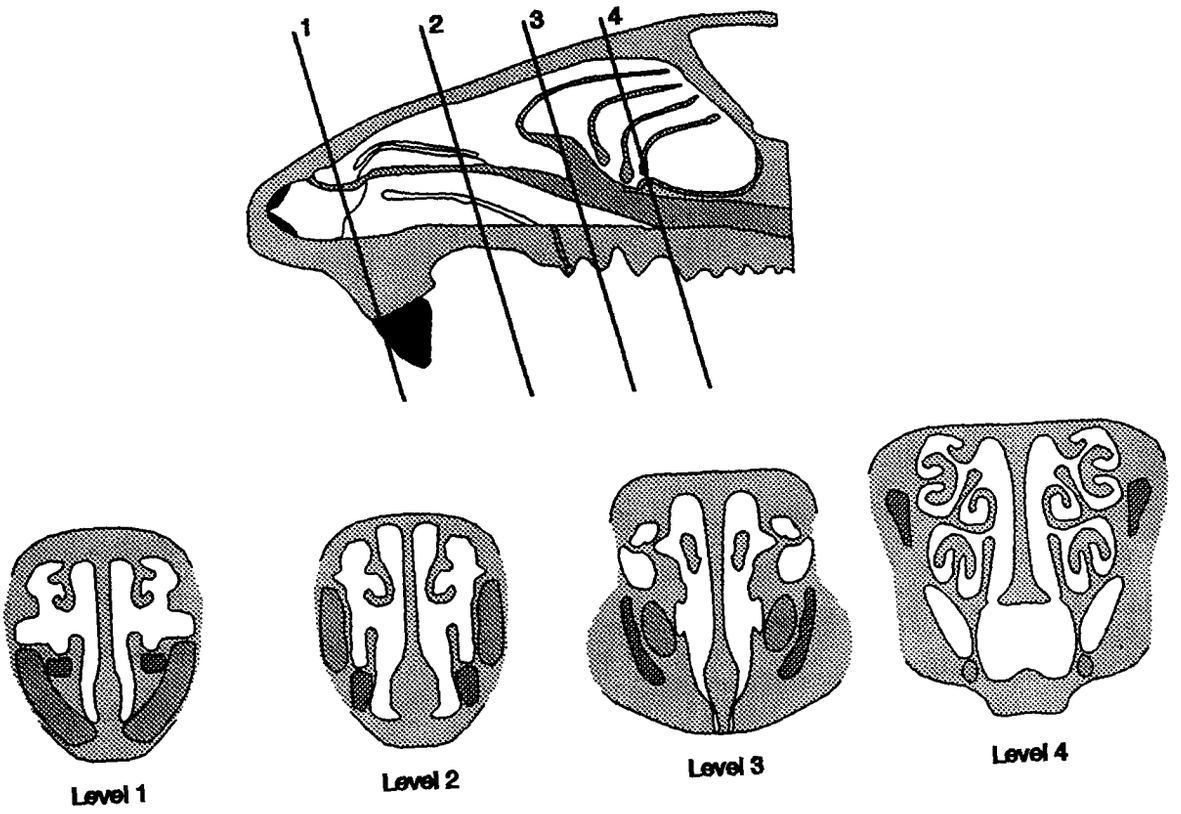

Figure 3-6. Diagram of the nasal passages for the F344 rat modified from Morgan et al. (1984). Cross-sections are shown at the four levels indicated and correspond to comparable locations for the rhesus monkey illustrated in Figure 3-7. Note the greater complexity of the posterior (ethmoid) region of the rat nose compared to that of the monkey. Much of this region is covered by olfactory epithelium, reflecting the macrosmatic nature of rodents.

relevant measurements for gas transport (Overton, 1984). Because of the morphology of the respiratory tract and air flow patterns, the relative contribution of these gas transport processes is a function of location in the respiratory tract and point in the breathing cycle (i.e., depth and rate) (U.S. Environmental Protection Agency, 1982b; Overton, 1984). The interspecies differences in the nature and structure of the respiratory tract, as summarized in Table 3-2, critically influence the differences in transport and deposition of gases across

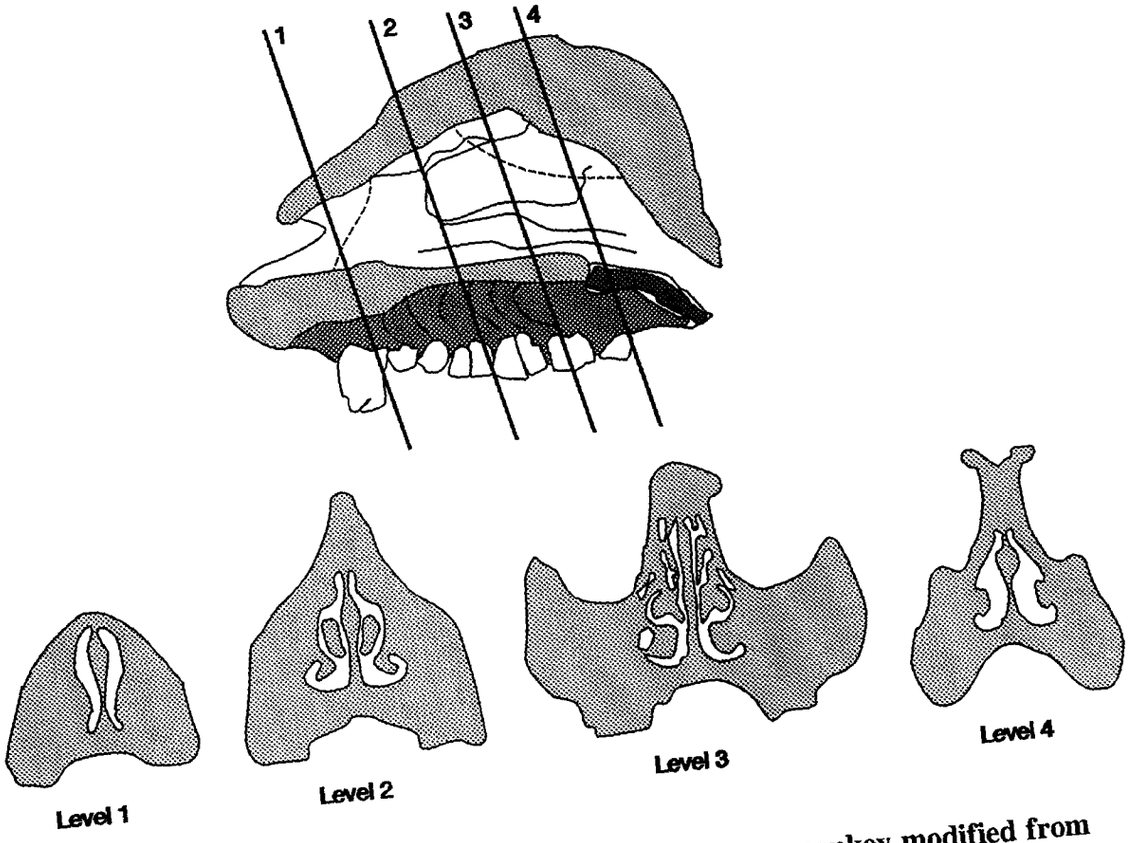

Figure 3-7. Diagram of the nasal passages for the Rhesus monkey modified from Monticello et al. (1989). Broken lines on the transverse section indicate the junction of squamous with the transitional/respiratory (anterior line) epithelia and the respiratory with the olfactory epithelium (dorsal line). Cross sections are shown at the four levels indicated and correspond to comparable locations for rat illustrated in Figure 3-6.

species. The airways also show a considerable degree of within species size variability and this is most likely the primary factor responsible for the deposition variability seen within single species (Schlesinger, 1985). Sex also influences airway anatomy. Additionally, age has dramatic influences on respiratory dynamics.

The differences in respiratory tract anatomy summarized in this Section 3.1.1 are the structural basis for the species differences in gas and particle deposition. In addition to the structure of the respiratory tract, the regional thickness and composition of the airway epithelium (a function of cell types and distributions) is an important factor in gas absorption

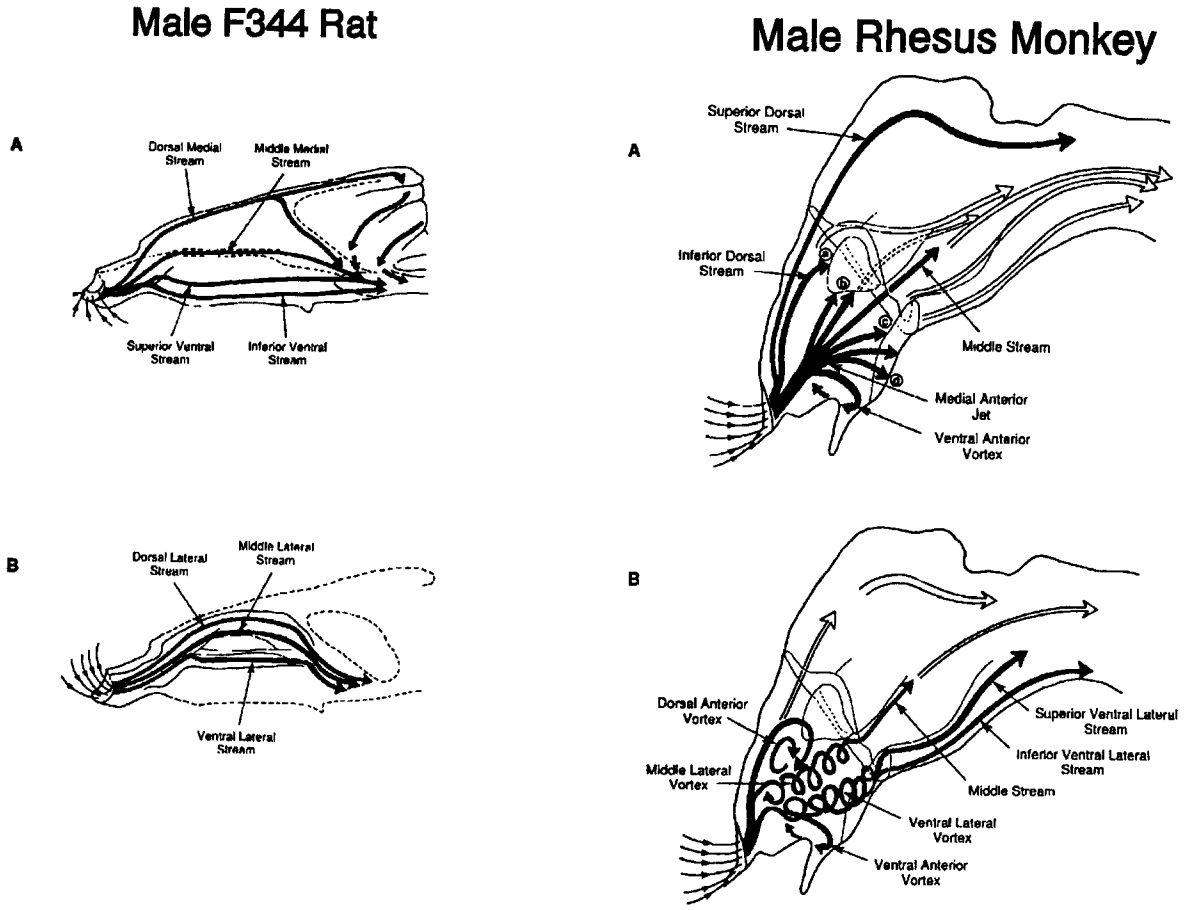

Figure 3-8. Inspiratory airflow patterns in upper respiratory tract of F344 rat and Rhesus monkey. A = major medial streams; B = major lateral streams. Black and white arrows depict high and low velocity airstreams, respectively.

Source: Morgan et al. (1991).

and contributes to the solubility and extent of reaction of the gas. Other anatomic and physiologic factors that influence gas uptake include (1) ventilation, which affects the tidal volume and ventilation to perfusion ratios; (2) body build, which affects the volume of distribution (including cardiac output and tissue volume); and (3) metabolic capacities. These are all factors to evaluate when estimating inhaled dose, interpreting injury response, and extrapolating effects between species.

3.1.1.2 Clearance Mechanisms

Deposited material is removed from the respiratory tract by clearance mechanisms, which vary depending on the site of deposition and the properties of the inhaled toxicant. The speed and efficiency by which the inhaled toxicants are cleared can be critical determinants of their toxic potential. Rapid removal lessens the time available to cause critical damage to the respiratory tract tissue and to permit systemic absorption of agents that have target organs other than the respiratory tract (Menzel and Amdur, 1986). The clearance mechanisms involved include (1) exhalation of volatiles; (2) mucociliary transport; (3) macrophage phagocytosis; (4) chemical reactions; (5) metabolism by various cell types; and (6) dissolution and absorption into the blood, lymphatic, or lung fluids.

Inhalation represents a route of exposure in which a variety of interrelated factors influence not only the nature of the effects (respiratory versus systemic) but also the manner by which they occur. The influence of target cell populations in the respiratory tract on the nature of the response is a factor unique to the inhalation route of exposure. Unlike the liver, a first-pass organ in oral exposures that has a more homogenous population of limited types of cells, the respiratory tract has more than 40 cell types (Sorokin, 1970). Xenobiotics, which exert their action by direct effects of the parent compound or by metabolites, can manifest profound differences in the nature and degree of response, depending on the route of exposure and subsequent availability to interact with various cell populations.

The likelihood of adverse effects in the respiratory tract can be affected by (1) production, distribution, and reactivity of metabolites by and among specific cell types; (2) the degree to which detoxication systems are overwhelmed (e.g., glutathione depletion); (3) efficiency and sensitivity of repair processes (e.g., type II cell proliferation); (4) efficiency of clearance processes; (5) airway mechanics; and (6) mechanism of action (e.g., pharmacologic or immunologic) (Bond, 1989; Boyd, 1980; Calabrese, 1983; Gram et al., 1986; Trush et al., 1982; Nadel et al., 1986; Marin, 1986).

Exhalation of volatile agents (including from administration routes other than inhalation) is an important excretory pathway that is dependent on tissue levels and exposure regimen. For inhalation exposures, the exposure duration influences the amount of chemical entering the systemic circulation, the amount metabolized, and the concentration of the chemical in tissues. Using a simulation model, Fiserova-Bergerova et al. (1984) demonstrated that for

chemicals that are not metabolized, tissue concentrations of "poorly soluble" ($H_{oil/gas} < 10$) chemicals change very minimally after 2 h of exposure. The pulmonary uptake rate approaches zero at the end of a 2-h exposure and apparent equilibrium is established. "Easily soluble" chemicals ($10 \leq H_{oil/gas} \leq 10,000$) require more than 1 day of exposure to reach apparent equilibrium and "highly soluble" chemicals ($H_{oil/gas} > 10,000$) require more than 1 year of exposure. If the chemical is metabolized, pulmonary uptake and the amount metabolized increase with exposure duration, but the effect of metabolism may be more complex if exposure concentrations are so high that metabolic pathways approach saturation kinetics and cause metabolism to deviate from first order kinetics.

Conversely, pulmonary clearance decreases with increasing biosolubility (refers to solubility of gases and vapors in biologic materials) and thereby affects the accumulation of chemicals during intermittent exposure regimens. Simulation of an 8 h/day, 5 days/week schedule for a 3-week exposure duration to a 70 kg man showed that poorly soluble chemicals (as defined previously) have no tendency to accumulate in the body, although easily and highly soluble chemicals do have a tendency to accumulate because the intermissions between exposures are not long enough to allow the chemical to be removed from adipose tissue (Fiserova-Bergerova et al., 1984). Excursions in exposure concentrations had a great effect on tissue concentrations of poorly soluble chemicals, but had little effect on tissue concentrations of highly soluble chemicals. Concentrations in well-perfused tissues were more affected by excursions in exposure concentrations than concentrations in muscle or adipose tissues.

The results of these simulation efforts emphasize the uncertainty that the dual function (i.e., uptake and exhalation) of the respiratory system adds to any attempt to estimate either respiratory tract or extrarespiratory (remote) "dose" of volatile agents. These simulations also emphasize the need for careful consideration of the uptake, metabolism, and excretion parameters for these agents when attempting the exposure duration and concentration conversions discussed in Chapter 4, and when ruling out the possibility of a respiratory tract endpoint when using oral data as part of the data base.

There are numerous defense systems that protect the respiratory tract. While some defense systems are truly protective, it must be kept in mind that many "activate" inhaled agents and may be responsible for adverse effects. Defense systems can be physical in nature

(e.g., filtration of particles by nasal hair), mechanical (e.g., expiration), enzymatic, or cellular (e.g., phagocytosis).

Nasal hair can be envisioned as a first line of defense since it can help prevent contact of toxicants (e.g., particles) with underlying epithelia. However, trapping of agents in the diffusion layer underlying cilia in the nose can serve as a source of irritation and more serious adverse effects. Some agents (e.g., formaldehyde, acrolein) have been shown to cause severe lesions in nasal epithelial cells (Morgan et al., 1986). The mouth also can be envisioned as another first-line defense system. Mouth-breathing in humans can result in solubilization of vapors in saliva and deposition of particles. Swallowing can reduce pulmonary exposure but increase presentation of the agent systemically via gastrointestinal tract absorption. Once an agent penetrates to the tracheobronchial region, agent deposition and/or solubilization occurs in the mucous blanket covering the surface epithelium.

Deposited particles can be cleared from the respiratory tract completely or they may be translocated to other sites within this system. Clearance mechanisms are regionally distinct, in terms of both routes and kinetics (Dahl et al., 1991a). Particles deposited on the anterior nares are cleared by mechanical processes such as nose wiping, blowing (humans), or sneezing (animals/humans). Particles in this area can have long biological half-lives. Those deposited in the nasopharynx or oropharynx, however, are swallowed within minutes and passed through the esophagus down to the gastrointestinal tract.

Particles deposited in the TB region are transported out of the respiratory tract by the mucociliary system, an interaction between the mucous secretions and the cilia that provide the mechanisms of movement. Such transport occurs along the area from the larynx to the terminal bronchioles. Insoluble particles are transported up to the esophagus where they are swallowed. Soluble particles may dissolve in the mucus. Generally, the biological half-lives of insoluble particles deposited in the TB region are on the order of hours.

Clearance of particles from the PU region of the lung generally takes the longest, usually a rapid phase of hours, and slower phases with biological half-lives of days, months, or years, depending on particle size and solubility. A major clearance process for "insoluble" particles is phagocytosis by alveolar macrophages. These cells then may be removed from the PU region after reaching the distal terminus of the mucociliary transport system or by migrating through the interstitium to the lymphatic system. Highly soluble particles will

dissolve in alveolar lining fluid and enter the blood or lymph directly (Johanson and Gould, 1977; Dahl et al., 1991a).

It is likely that dissolution rates and rates by which dissolved substances are transferred into blood are related mostly to the physicochemical properties of the material being cleared and are essentially independent of species. On the other hand, different rates of mucociliary transport in the conducting airways or of macrophage-mediated clearance from the PU region may result in species-dependent rate constants for these pathways (Dahl et al., 1991a). For example, clearance of insoluble particles from the PU region of mice and rats is much faster than that in dogs and humans, which have similar clearance rates of inhaled particles (Snipes, 1989a,b).

As discussed in Chapter 2, an overload phenomenon can occur with excessive particle exposures that can alter the clearance kinetics of lung dust burdens and confound the interpretation of toxicological effects (Morrow, 1992).

Conceptually, uptake of a gas requires that it move from the airway lumen through the surface-liquid lining layer, the tissue layer, and the capillary endothelium, to reach the blood. This passage is influenced by the physiochemical properties of the gas as well as the biochemistry and thickness of the layers between the lumen and blood. For reactive gases, the sequence in which anatomic sites are affected appears to be more dependent on concentration than on exposure duration. However, at a given local anatomic site and at a specific concentration, the stages in the pathogenesis of the lesion relate to the duration of exposure (U.S. Environmental Protection Agency, 1986d, 1993b). The rate of mucous transport also affects the gas transport mechanisms in the diffusion layer at the gas/liquid interface along the airways. The rate varies with the depth of the airways (greater velocities in the proximal airways) and across species. For example, a very highly reactive gas may not reach the blood if it reacts biochemically with mucus and the mucus layer has sufficient volume (thickness) to serve as a sink. This same gas may not react with the saturated lipid of surfactant; and if deposited significantly in the PU region, could reach alveolar tissue. The thickness and efficiency of the epithelial barrier also influences absorption. Both of these main factors (liquid lining and epithelial barrier) are present in all species but have species-specific differences, only a few of which have been quantified. Mucus is a complex secretion with contributions from various epithelial cells. The numbers and distribution of

these cells may affect the composition and properties of the mucus, which in turn interacts with the physicochemical properties of the agent. The species differences in the thickness of the alveolar epithelial cells could account for variations observed in the diffusion of gases into the bloodstream (Crapo et al., 1983). The lung also is a very efficient excretory organ for volatile organic chemicals after the exposure ceases or is lowered. The efficacy of PU excretion correlates directly with the saturated vapor pressure of the chemical and indirectly to water solubility.

Cell Types

A variety of other cellular defense mechanisms can be marshaled, which can diminish or sometimes exacerbate toxic insult. The numerous cell types found in different species contribute to the varying clearance patterns from the respiratory regions and differences in the nature of the response. Table 3-3 presents the distributions of various cell types across species commonly used in inhalation toxicologic investigations. Different mammalian species have different amounts and isozyme distribution of cytochrome P-450 in their Clara cells, which could account for differences in metabolism of some agents. Recent investigations have also shown species differences in cellular organization at the terminal respiratory bronchioles/alveolar duct junctions and in the ultrastructure of the same cell type across species (St. George et al., 1988). The possible functions of these cell types are provided in Table 3-4, and the differences seen in the cell types across species are summarized in Table 3-5. Such species differences are important to consider when determining if the laboratory animal is an appropriate model for the chemical's mechanism of action. For example, the rat may be an inappropriate species for the evaluation of hypersensitivity because of its lack of mast cells.

Alveolar macrophages are the predominant cell type responsible for clearance of particles from the PU region. Particles are phagocytized and transported within macrophages to the mucociliary escalator. This alveolar macrophage clearance of the PU region is considerably slower (weeks to years) than clearance in the TB region. Gases and soluble particles that escape phagocytosis by alveolar macrophages can be dissolved in the lining fluid. This dissolution would be governed by physicochemical characteristics such as

TABLE 3-3. NORMAL SURFACE AIRWAY EPITHELIUM: CELL TYPES

	Humans	Monkey	Dog	Ferret	Guinea Pig	Rabbit	Rat	Hamster	Mouse
Epithelial									
Ciliated	+	+	+	+	+	+	+	+	+
Mucous	+	+	+	+	+	+	+	+	+
Serous	a	-	-	-	-	-	b	c	-
Clara	+	+	+	+	+	+	+	+	+
Endocrine	+	+	-	-	+	+	+	+	+
Type I	+	+	+	+	+	+	+	+	+
Type II	+	+	+	+	+	+	+	+	+
Transitional	d	-	-	-	-	-	e	g	f
Special type	h	-	+	-	-	-	-	-	-
Brush	-	-	-	+	+	+	+	-	+
Intermediate	+	-	+	+	-	-	+	+	+
Basal	+	+	+	+	+	+	+	+	+
Migratory									
Lymphocyte	+	i	-	-	-	+	+	+	+
Globule leukocyte	-	i	i	-	-	-	+	-	-
Mast cell	h	+	i	-	+	-	-	-	-
Macrophage	+	(+)	+	(+)	(+)	(+)	+	(+)	(+)
Neural									
Neuroepithelial body	+	+	-	-	-	+	+	-	+
Nerve terminals	h	+	-	+	+	+	+	+	j

\+ = Reported present;
(+) = Not specifically reported in sources cited;
\- = Unidentified;
a = Fetal tissue;
b = In specific pathogen-free rats;
c = Only young animals;
d = Ciliomucous, mucoserous, endocrine-mucous;
e = Seromucous;
f = Ciliomucous, seromucous;
g = Ciliomucous;
h = Not in "normal" biopsy material;
i = "Migratory cell";
j = Bronchiolus only.

Source: Jeffery (1983), Crapo et al. (1983).

TABLE 3-4. SOME SPECIFIC LUNG CELL TYPES AND THEIR FUNCTIONS

Cell Types	Location and Function
Epithelium	
Clara cells	Nonciliated cells of the tracheobronchial region; high xenobiotic metabolic activity; secretory; function not well-defined; may serve as precursor of goblet and ciliated cells
Ciliated cells	Most common epithelial cells in airways; may secrete mucous-like substances; controls perciliary fluid
Type II alveolar	Generally covers <5% of alveolar surface; secrete surfactant; replace injured Type I cells; high xenobiotic metabolic activity
Type I alveolar	Large and covers considerable surface area per cell; covers ≥95% of alveolar surface; forms the alveolar epithelium and facilitates gas exchange; low metabolic activity; incapable of self-reproduction
Mucous	Mucus-secreting
Serous	Mucus-secreting; perciliary fluid; stem cell
Brush cells	Chemoreceptor cells; preciliated
Globule leukocyte	Immunoglobulin transportation; releases inflammatory mediators
Endocrine	Secreto- and vaso-regulatory
Submucosal	
Goblet (mucus) cells	Epithelial linings; common in trachea and bronchioles; contribute to mucus production
Serous cells	Mucus-secreting; perciliary fluid; stem cell/proliferative
Endocrine cells	Secretes amines and neuropeptides
Lymphocytes	Immunoresponsive
Myoepithelial	Expulsion of mucus
Bronchoalveolar mast cells	Migratory cells located throughout respiratory tract; release mediators of bronchoconstriction when antigens bind to IgE antibodies on surface
Macrophage	Phagocytic; secrete mediators of inflammatory reactions; modulate lymphocytes and otherwise participate in immune response
Endothelial cells	Approximately 40% of lung parenchyma cells; metabolize blood-borne substances; proliferative
Fibroblasts (interstitial)	Predominant in alveolar wall and constitutes the basement membrane; become activated during disease states and produce elastin and collagen; proliferation leads to fibrosis, modulation of growth, bronchial tone, and mucosal secretion

Source: Jeffery (1983), Bowden (1983), Marin (1986), Nadel et al. (1986), Plopper et al. (1983), Burri (1985), Brain (1986).

TABLE 3-5. MAIN SPECIES DIFFERENCES IN EPITHELIAL CELLS AND GLANDS

Epithelial Morphology

- Thickness and pseudostratification
- Thickness and structure of "basement membrane"

- Mucus-secreting cells
  - Number
  - Histochemistry
  - Predominant ultrastructure type

- Clara cells
  - Morphology (smooth endoplasmic reticulum)
  - Distribution

- Endocrine cell frequency

- Ciliated cells
  - Extent of coverage
  - Structure of rootlet
  - Lamellar bodies
  - Glycogen stores

- Presence of brush cell

- Basal cells
  - Number
  - Shape
  - Tonofilaments

- Presence of Globule Leukocytes
- Innervation
  - Extent
  - Distribution
  - Type

Gland Morphology

- Amount
- Distribution
- Main histochemical cell type
- Presence of collecting duct
- Innervation

Source: Jeffery (1983).

reactivity, water solubility, lipophilicity, and ability to serve as substrate for activation and/or detoxification enzymes.

Certain cell types can be stimulated to release mediators, such as mast cell release of histamine. Histamine can cause bronchoconstriction, which can be protective, by limiting the amount of pollutant inhaled, or can be toxic, by limiting oxygen uptake. Synthesis or metabolism of prostaglandins (leukotrienes) also can affect airway and vascular caliber. The chemotactic factors released can recruit phagocytic cells involved in clearance. It should be recognized that the respiratory tract contains a variety of different cell types that possess different metabolizing potential and are distributed in a manner that varies among species. Lists of common cell types and their functions are provided in Tables 3-3 and 3-4. Macrophages, for example, constitute a cellular protection system and not only protect inner surfaces of the respiratory tract from damage caused by particles and microorganisms, but also have the potential to cause damage themselves because the proteases and mediators that are useful in destroying microbes or physical agents can also destroy healthy tissue (Rossi, 1986) (Brain, 1986). Although recruitment of macrophages to the lung is related to the toxicant dose, the adaptive increase in macrophages can be exceeded (Bowden, 1986). This threshold may vary among species. The alteration of macrophage functioning has the potential to shift the balance between protective and adverse effects.

Epithelial secretions in response to injury may recruit scavenger cells such as polymorphonuclear leukocytes, which can biotransform inhaled agents. More recent data on cellular morphometrics and interspecies differences in cell populations (Mercer and Crapo, 1987; St. George et al., 1988) will aid in dosimetry adjustments for clearance, metabolism, and uptake. As an example, modeling for the metabolic capacity of the human lung instead of considering it only as a physical barrier can result in disparate estimates of extrapulmonary dose (see Section 3.2). Estimates from models that account for respiratory tract metabolism may better fit experimental data on systemic dose surrogates for some chemicals.

Concurrent with the action of inhaled agents upon critical cell types in the respiratory tract, a portion of the dose in the PU region is likely to be transported across the alveolar epithelium and enter systemic circulation. Changes in permeability can result from the action of some of the mediators and proteases mentioned. The greater the amount reaching the systemic circulation, the greater the likelihood for adverse effects in other systems (e.g.,

liver, kidney, central nervous system). The rapidity and extent to which systemic absorption occurs and the time-to-steady-state blood levels are influenced by (1) ventilation rates and airway mechanics, (2) blood transit time in capillary beds (i.e., perfusion limited), (3) metabolic conversion in the respiratory tract and other organs, (4) alveolar surface area, (5) thickness of the air-blood barrier, and (6) the blood:air and blood:tissue partition coefficients. Many of these factors vary among species and, therefore, should be considered in key study identification.

After the inhaled agent enters systemic circulation, the liver may produce additional metabolites that, if the half-life is sufficiently long, may re-enter the lungs and exacerbate the portal-of-entry effects or produce additional adverse effects (Boyd and Statham, 1983; Yost et al., 1989). Some other agents, that do not require bioactivation, have been shown to damage the lung when applied systemically (Kehrer and Kacew, 1985).

Metabolism

The effect of respiratory tract metabolism on the toxicity of inhaled materials is thought to be important for many chemicals because (1) high concentrations of xenobiotic metabolizing enzymes occur in the nose and substantial concentrations occur in the lower respiratory tract; (2) the respiratory tract tissues are the first exposed to inhaled chemicals and are exposed to the highest concentrations (barring tissue-specific uptake); (3) the products of respiratory metabolism may have different toxicities from those of hepatic metabolism; and (4) tissues at risk to toxic metabolites formed in the respiratory tract are different from those formed in the liver (Dahl et al., 1988). The metabolic capacity of the lower respiratory tract has been recognized for many years and nasal metabolism has recently been shown to be significant for some compounds (Dahl et al., 1988). Accordingly, it is useful to consider that inhaled chemicals may be extensively metabolized in the nose or in the lower respiratory tract and both the metabolites and the parent compound may be cleared via the blood or by mucociliary clearance.

Metabolism of potentially toxic inhaled compounds is achieved by a variety of enzyme reactions involving oxidation, reduction, hydrolysis, and conjugation. The enzymes may work individually, concurrently, or consecutively to detoxicate or, in some cases, activate inhaled foreign compounds (Ohmiya and Mehendale, 1984; Minchin and Boyd, 1983; Dahl

et al., 1987). These enzymes may vary in activity across species and organs (Ohmiya and Mehendale, 1984; Ziegler, 1980; Tynes and Hodgson, 1985; Plopper et al., 1983; Litterst et al., 1975). Depending on the chemical being metabolized, each of these enzymes may play a role in either an activation or detoxication pathway. The balance between activation and detoxification governs the rate of delivery of bioactive metabolite to the macromolecular target site (Dahl et al., 1991a).

The oxidation and reduction reactions are catalyzed primarily by the cytochrome P-450 and flavin-containing monooxygenases (FAD-MO). The cytochrome P-450 isoenzymes are ubiquitous hemoproteins located in the endoplasmic reticulum of a variety of cells and are responsible for the oxidation of foreign compounds. Isoenzyme specificity, inducibility, catalytic activity, and localization in the rabbit and rat lung (Domin and Philpot, 1986; Vanderslice et al., 1987) have been elucidated. Until recently, it was thought that the cytochrome P-450 isoenzymes were the only primary monooxygenases in the lung. However, recent studies have shown that the FAD-MO play an important role in detoxication of foreign compounds. FAD-MO have also been demonstrated to exist in various isoenzymic forms, with substrate specificity and mechanisms different from those of cytochrome P-450 (Ziegler, 1988).

The Clara cells lining the respiratory and terminal bronchioles are thought to be the primary site of cytochrome P-450 because of the presence of endoplasmic reticulum. However, the ultrastructure of the Clara cell varies across species (Plopper et al., 1980). In the ox, cat, and dog, more than 60% of the cytoplasmic volume is glycogen with a relatively small proportion of the cell volume containing endoplasmic reticulum or mitochondria. Therefore, species differences in Clara cell ultrastructure can be reflected in significant differences in xenobiotic metabolism potential (Plopper et al., 1983; St. George et al., 1988). Differences in localization of cytochrome P-450 activity have been suggested as a likely basis for some differences in respiratory tract toxicity (O'Brien et al., 1985).

Epoxide hydrolases and carboxy esterases are hydrolytic enzymes found in both the nasal cavity and lower respiratory tract tissues. The epoxide hydrolases further metabolize potentially toxic oxidation products after initial cytochrome P-450-dependent metabolism of aromatic compounds or alkenes. The carboxy esterases hydrolyze carboxylic esters to the respective alcohols and carboxylic acids. At least two types of aldehyde dehydrogenases have

been detected in the nasal cavity and may be important in modifying the toxicity of volatile aldehydes such as formaldehyde and acetaldehyde (Casanova-Schmitz et al., 1984). Aldehyde dehydrogenase also occurs in the lower respiratory tract, particularly in the Clara cells of the distal bronchioles.

Individually or in concert with the cytochrome P-450 isoenzymes, conjugation reactions are catalyzed by the glutathione-*S*-transferases that transform potentially toxic parent compounds or activated metabolites into nontoxic water soluble compounds. The glutathione-*S*-transferases may catalyze conjugation reactions with toxic metabolites formed by the cytochrome P-450, rendering them harmless and easier to excrete from the body. However, GSH conjugation with certain substrates (e.g., 1,2-dibromoethane and several other related haloalkenes) has been shown to provide reactive species capable of producing nephrotoxicity (Monks and Lau, 1989). The cofactor required for these reactions is glutathione (GSH). The GSH is synthesized in the lung, as well as in other major organs, and also is reduced from the oxidized state (GSSG) to the reduced state (GSH) by GSH reductase. Under extreme conditions of GSH depletion in the lung, it has been hypothesized that extrapulmonary GSH is mobilized and transported to the lung from the liver (Berggren et al., 1984). The GSH has been identified in isolated Type II epithelial cells, Clara cells, and ciliated cells of the lung, but it is not known if it is present in all pulmonary cells. The GSH also is the cofactor utilized by the enzyme GSH peroxidase. The GSH peroxidase catalyzes the metabolism of hydrogen peroxide and organic peroxides formed by the ozonization of unsaturated fatty acids. Other key antioxidant components in the lung include ascorbic acid, α-tocopherol, superoxide dismutase, and catalase (Massaro et al., 1988).

3.1.2 Physicochemical Characteristics of the Inhaled Toxicant

The physicochemical characteristics of the inhaled agent will influence the deposition and retention within the respiratory tract, translocation within the respiratory system, distribution to other tissues, and ultimately, the toxic effect. Therefore, it is important to consider characteristics of the inhaled agent as well when attempting to evaluate and extrapolate the effects of a particular exposure.

3.1.2.1 Particles

For a given particle exposure, the two most important parameters determining deposition are the mean diameter and the distribution of the particle diameters. The size, density, and shape of the particles influence their aerodynamic behavior and, therefore, their deposition (Raabe, 1979; U.S. Environmental Protection Agency, 1982b, 1986c). The definition of diameter for a spherical particle is unambiguous, but for irregularly shaped particles, a variety of definitions exist. Nonspherical particle size often is described by its aerodynamic properties. Fibrous material may be described by actual length, actual diameter, coil length, coil diameter, aspect ratio, or coil-to-aspect ratio.

Information about particle size distribution aids in the evaluation of the effective inhaled dose (Hofmann, 1982). Recommendations defining the particle size ranges for inspirability to the various regions have been published by an ad hoc working group of the International Standards Organization (1981). Particle diameter and size distribution should be provided to the risk assessor to completely characterize the aerosol in order to estimate respiratory tract deposition with any confidence and to evaluate relevance to toxicologic potential. Appendix H provides definitions of particle size diameters and distributions. Appendix G presents a dosimetry model that accounts for interspecies differences in regional respiratory tract deposition and illustrates the influence of particle size and distribution on deposition.

3.1.2.2 Gases and Vapors

The deposition site and rate of uptake of a volatile chemical are determined by its reactivity and solubility characteristics. Therefore, the pharmacokinetics of gases and vapors are governed by

- Rate of transfer from the environment to the tissue,
- Capacity of the body to retain the material, and
- Elimination of the parent compound and metabolites by chemical reaction, metabolism, exhalation, or excretion.

As mentioned in Section 3.1.1.1, the transport processes in the liquid and tissue layers adjacent to the airway lumen influence the relationship of the gas with the air-liquid

boundary. Physicochemical characteristics of the gas that contribute to the relative importance of these processes include its chemical reactivity and solubility.

The chemical reactions of the gas with both the liquid and tissue layers may be important. For example, reactions with the liquid layer could result in an increased flux from the airway but reduce (relative to no reactions) the delivery of the gas to the tissue. If the gas is the only toxic molecule, then this reaction would protect the tissue. Conversely, if the reaction products are toxic, then reactions with the tissue layer would increase the delivery of toxic molecules to the tissue (Overton, 1984). Chemical reactivity with the biological constituents of the tissue is similarly important to the gas's toxic potential to the respiratory tract tissue and to the amount of gas and reaction products that enter the blood for potential extrarespiratory toxicity. Theoretically, knowledge of all the chemical species involved and the reaction rates of the reactants and products is necessary to characterize a system for dosimetry. Sometimes the complexities may be reduced into relative classifications (e.g., slow, fast, instantaneous) using approximation techniques for time and spatial dependence (Overton and Miller, 1988).

Gases that are not soluble or reactive are relatively inert to the airways and penetrate to the alveoli. Examples are nitrogen and volatile hydrophobic chemicals. The major factor driving the uptake of these gases is the removal of the gas from alveolar air by capillary blood. The concentration in alveolar air and capillary blood is generally considered to reach equilibrium. Therefore, uptake of alveolar gases depends on blood:air partitioning, ventilation/perfusion ratio, and air and blood concentrations.

For gases that are soluble, uptake is linearly related to solubility (Overton and Miller, 1988). There are many different expressions for the solubility of gases, differing in terms of units as well as in terms of what chemical form of the gaseous species in the liquid phase is related to the gas-phase quantities. As long as the concentration of dissolved gas is small, and the pressure and temperature are not close to the critical temperature and pressure, then Henry's Law is obeyed (Overton and Miller, 1988). It should be noted that the Henry's Law constant is independent of chemical reactions so that it refers to the parent molecular form of the gas in water and air, and not the total quantity absorbed in water to air quantities. Considering the importance of chemical reactions as described above, solubilities as indicated by Henry's Law constants may not be appropriate to fully describe uptake. Further,

extrapolation of Henry's Law constants from water data to biological fluids and tissues is not always appropriate, particularly for organic compounds.

Because uptake and disposition of inhaled vapors and gases are driven by the equilibration of their partial pressures in tissues with their partial pressures in ambient air, solubility may be aptly described by Ostwald solubility coefficients at body temperature. Ostwald solubility coefficients and partition coefficients (concentration ratios of the volatile chemical in two phases with equilibrated partial pressures) have the same values (Fiserova-Bergerova et al., 1984). Partition coefficients are essentially a measure of the affinity of a chemical for one medium compared to another at equilibrium. The blood:air (or blood:gas) partition coefficient is a critical determinant in the uptake and achieved blood concentration of volatile organic chemicals (Dahl et al., 1991a). Absorption generalizations based on molecular weight are not recommended. As an example, the difference in solubility between methanol and ethane, which have similar molecular weights, is a result of the presence of the hydroxyl group on methanol. Interspecies comparisons necessitate consideration of the effects of the differences in anatomy and physiology described previously, but it can generally be stated that the less water soluble and less reactive the gas, the more similar the deposition will be between humans and laboratory animals. The tissue:gas partition coefficient of a chemical has been shown to correlate with its fat:gas and blood:gas partition coefficients so that linear correlation equations may provide a useful means of estimating tissue:gas and blood:gas partition coefficients (Fiserova-Bergerova and Diaz, 1986).

Similarly, the fat:air partition coefficient can serve as an index of whether high concentrations of the chemical will occur in the fat. The fat compartment plays an important role in accumulating and storing lipophilic chemicals both during and after exposure. The chemical stored in fat becomes available for redistribution by the systemic circulation after the end of exposure when the arterial blood concentration decreases relative to the fat. This "postexposure" phenomenon due to fat solubility can be an important factor influencing the amount of chemical metabolized, because that chemical that leaches from the fat compartment after exposure is available for metabolism, which can continue for a signifiant period of time after removal from the exposure atmosphere. Therefore, interspecies differences in body fat can induce interspecies differences in uptake, distribution, accumulation, and toxicity of lipophilic chemicals.

Metabolism of the parent compound can modulate uptake of inhaled gases from the respiratory tract and is also probably the most important determinant of tissue dosimetry when metabolites are the toxic moiety. The cells and tissues at risk from toxic metabolites depend not only on the source of the metabolites but also on their kinetic properties. The toxic effects of metabolites that react at fast rates are confined to the activating enzyme or cell. If metabolite reaction rates are moderate, effects will largely be restricted to the activating tissue and to nearby tissues. Slow-reacting metabolites may themselves be potential substrates for further metabolism.

The effect of concentration and exposure time on the above parameters of reactivity and metabolism should be addressed. Uncatalyzed reactions follow pseudo-first-order kinetics if the gas is inhaled at "low" concentrations (Overton and Miller, 1988). "High" vapor concentrations can qualitatively change the chemical fate and toxicity. Depletion of biological coreactants, or just an increase in the concentration of the chemical to the point at which reactions can no longer be treated as pseudo-first-order, may qualitatively change the fate and potentially the toxicity of an inhaled gas. For chemicals metabolized according to Michealis-Menten kinetics, metabolism may be saturated at high concentrations and become described by zero-order kinetics. Further, saturation of metabolic pathways can alter the metabolites formed and the resultant toxicity of the metabolized compound.

Such effects of inhaled vapor concentration on metabolism are not limited to systemic enzymes, but also occur in localized areas within the respiratory tract. In general, the concentrations of inhalants in the respiratory tract mucus will be higher than anywhere else in the body, barring selective tissue uptake. Therefore, the xenobiotic metabolizing enzymes of the respiratory tract will reach maximum reaction velocities at inhaled concentrations far lower than those needed to bring extrarespiratory (systemic) enzymes to maximum velocities. Therefore, it is likely (except at extremely low inhaled gas concentrations) that local metabolizing areas within the respiratory tract, particularly the nasal tissues, will not follow linear enzyme kinetics (Dahl, 1990).

The physicochemical gas characteristics of reactivity and solubility will interact with physiologic parameters such as pulmonary ventilation, cardiac output (perfusion), metabolic pathways, tissue volumes, and excretory capacities. The relative contribution or interaction of these is, in turn, affected by the exposure conditions (concentration and duration), so that as

emphasized previously, integration of these various factors is necessary to estimate the deposited (on airway surfaces) and absorbed doses in order to assess toxicity.

3.2 MODELING COMPARATIVE DOSIMETRY OF INHALED TOXICANTS

The preceding discussion provides an overview of the various factors that affect the disposition (deposition, uptake, distribution, metabolism, and elimination) of inhaled toxicants. Major determinants include (1) the respiratory tract anatomy and physiology and (2) the physicochemical characteristics of the inhaled toxicant. The relative contribution of each of these factors is a dynamic relationship. Further, the relative contribution of these determinants is also influenced by exposure conditions such as concentration and duration.

As discussed in Chapter 1, a comprehensive description of the exposure-dose-response continuum is desired for accurate extrapolation from experimental conditions and dose-response assessment. Therefore, an extrapolation model should incorporate all of the various deterministic factors described in the previous section into a computational structure. Clearly, many advances in the understanding and quantification of the mechanistic determinants of chemical disposition, toxicant-target interactions, and tissue responses are required before an overall model of pathogenesis can be developed for a specific chemical. Such data do exist to varying degrees, however, and may be incorporated into less comprehensive models that nevertheless are useful in describing delivered doses or in some cases, target tissue interactions.

Because much information on the mechanistic determinants of target tissue dose, toxicant-target interactions, and tissue responses is likely lacking for any given chemical to which this RfC methodology will be applied, the default dosimetry adjustments are derived from models that incorporate only the major determinants of chemical disposition. The defaults are determined categorically for particles versus gases, and within gases, for those more reactive (defined as including local metabolism) and soluble than nonreactive and insoluble. It is recognized, however, that these are default dosimetry models, so that use of models that incorporate a more comprehensive description of the exposure-dose-response continuum may take precedence when such a model is judged to provide a more accurate

description. The next sections describe the rationale for the default models and dosimetry adjustments provided in detail in Chapter 4 and the Appendices G, I, and J. Examples of more robust models are provided to illustrate considerations of the appropriateness of the default versus alternative model structures. The summary for this section then provides considerations for judgement of the relative value of different modeling structures. This judgment may be based on whether the structure of the alternative model is superior to that of the default, (e.g., incorporates additional known mechanistic determinants) or if it empirically results in a better correlation between "dose" and "effect".

3.2.1 Particle Deposition Model Based on Available Data

The preceding discussion in this chapter described the various mechanisms and anatomical dependencies of deposition in the respiratory tract. A theoretical model to describe deposition would require detailed information on all of these parameters (e.g., exact airflow patterns, complete measurements of the branching structure of the respiratory tract, pulmonary region mechanics) across the various species used in toxicity studies.
As described in Appendix G, an empirical model was instead developed due to the limited availability of these types of data. An empirical model is a system of equations fit to experimental data. Measurement techniques for deposition are such that deposition can be defined only for the major respiratory tract regions (i.e., ET, TB and PU) and not for localized areas such as the respiratory versus olfactory epithelium. The choice of the experimental data and description of the model are provided in Appendix G.

The default model used in the RfC methodology estimates regional deposition. "Dose" may be accurately described by deposition alone if the particles exert their primary action on the surface contacted (Dahl et al., 1991a), but since the RfC is defined as a dose-response estimate for chronic exposures, a more appropriate dose metric for particle exposures may be to take into account clearance of the deposited dose and thereby calculate the retained dose and the dose rate to extrarespiratory tissues. Incorporation of clearance kinetics into the dosimetric adjustments awaits development of data enabling comparable modeling of clearance across species. Often the physicochemical properties or mechanisms of action of the inhaled toxicant (particle or gas) can be used to gauge the relative importance of the various factors controlling inhaled dose. For example, the model of Yu and Yoon (1990) for

diesel exhaust incorporates clearance components such as transport of deposited particles to the lymphatic system. A model that described the retained dose for diesel particles was necessary because the toxicity is related to particle overload.

3.2.2 Gas Categorization Scheme Directs Default Gas Modeling

Numerous model structures have been used to describe toxicant uptake in the respiratory tract. The type of model often reflects the physicochemical characteristics of the gases to which they are applied. For example, the model of Miller et al. (1985) for the respiratory tract uptake of ozone (highly reactive and moderately water soluble) is a detailed, distributed parameter model. Key elements incorporated into this convective-diffusion-chemical reaction model include (1) anatomic dimensions of the airspace and tissue thickness (2) dispersion in the airspace, (3) reactivity in the liquid lining (mucus or surfactant) covering the cells of the lower respiratory tract, and (4) lateral mass transport resistance from the airspace to the blood (Overton et al., 1987). Models for highly reactive and highly soluble gases (e.g., formaldehyde, hydrogen fluoride) have emphasized the requirement to account for scrubbing of the gas from the airstream by the upper respiratory tract (Aharonson et al., 1974; Morgan and Frank, 1977; Morris and Smith, 1982; Hanna et al., 1989; Cassanova et al., 1991). Such models are not applicable to a nonreactive gas such as styrene, however.

The chemical-specific or class-specific nature of these models has been dictated by the physicochemical characteristics of the subject gases, and therefore, any single model is not applicable to the broad range of gases that the RfC methodology must address. Dahl (1990) categorized gases as stable, reactive, or metabolizable based on their thermodynamic and kinetic properties. Various concepts of "dose" can be related to these properties and the mechanism of action (e.g., macromolecular bound fraction as dose for reactive gases versus inhaled dose for stable asphyxiants). A gas categorization scheme was constructed based on physicochemical characteristics as determinants of gas uptake as shown in Figure 3-9. A similar scheme has been developed by the International Commission on Radiological Protection (1993). The definition of reactivity includes both the propensity for dissociation as well as the ability to serve as a substrate for metabolism in the respiratory tract. The scheme does not apply to inert gases that exert their effect by reversible "physical" interactions of

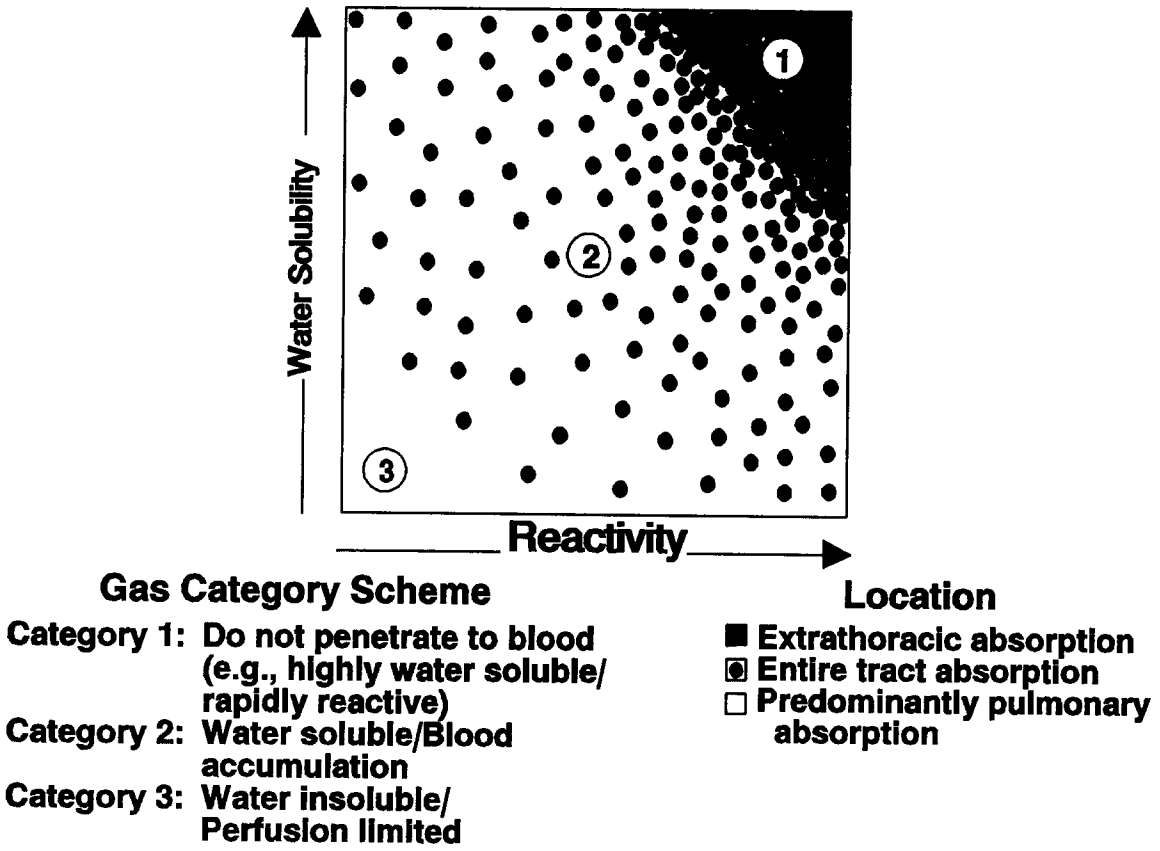

Figure 3-9. Gas categorization scheme based on water solubility and reactivity as major determinants of gas uptake. Reactivity is defined to include both the propensity for dissociation as well as the ability to serve as a substrate for metabolism in the respiratory tract. Definitive characteristic of each category and anticipated location (region) for respiratory tract uptake are shown.

gas molecules with biomolecules (e.g., "displacement" of oxygen by carbon dioxide). Consideration of this mechanism was discussed in Section 2.1.2.3.

The dominant determinants are used to construct a conceptual framework that directs development of the default dosimetry model structures discussed in Appendices I and J. These model structures are reduced further by simplifying assumptions to forms requiring a minimal number of parameters in order to derive the default adjustments used in Chapter 4 for each category that are commensurate with the amount of data typically available for RfC chemicals.

The two categories of gases with the greatest potential for respiratory effects are (1) gases that are highly water soluble and/or rapidly irreversibly reactive and (2) water soluble gases which may also be rapidly reversibly reactive or moderately to slowly irreversibly metabolized in respiratory tract tissue. The objective of the default modeling approach is to describe the effective dose to the three major regions of the respiratory tract (ET, TB, PU) by addressing the absorption or "scrubbing" of a relatively water soluble and/or reactive gas from the inspired airstream as it travels from the ET to PU region. That is, the dose to the peripheral regions (TB and PU) is affected by the dose to the region immediately proximal. The appropriateness of assessing proximal to distal dose representative of the scrubbing (uptake) pattern is supported by the proximal to distal progression pattern of respiratory tract toxicity with increasing concentration that is observed with many chemicals (Jarabek, 1994). At low concentrations of highly water soluble and/or irreversibly reactive gases, observed effects are largely isolated to the ET region. At higher concentrations, more severe effects occur in the ET region and toxicity is also observed to progress to the peripheral regions. The severity of toxicity also progresses distally with increased exposure concentrations. As for the default particle deposition model described in Appendix G, the default gas models do not describe respiratory tract uptake in detail to the level of local airflow distribution (e.g., respiratory versus olfactory epithelium), but they do adequately describe the scrubbing of the chemical from the inhaled airstream on a regional scale.

The defining characteristic for Category 1 gases is that they are so highly water soluble and/or rapidly irreversibly reactive in the surface-liquid/tissue of the respiratory tract that they do not develop a significant backpressure (i.e., reversal in the concentration gradient at the gas-liquid interface) from the surface-liquid tissue phase during exhalation. Category 1 gases are also distinguished by the property that the gas does not significantly accumulate in the blood which would reduce the concentration driving force and, hence, reduce the absorption rate. The default model structure is based on these characteristics. Examples of gases classified as Category 1 are hydrogen fluoride, chlorine, formaldehyde, and the volatile organic acids and esters.

Gases in Category 2 are moderately water soluble and rapidly reversibly reactive or moderately to slowly irreversibly metabolized in respiratory tract tissue. Ozone, sulfur

dioxide, xylene, propanol, and isoamyl alcohol are examples of Category 2 gases. The boundaries between categories are not definitive. Some compounds may appear to be defined by either Category 1 or Category 2 because water solubility and reactivity are a continum. Thus, although sulfur dioxide is reversibly reactive, which would categorize it as a Category 2 gas, it is also highly soluble such as to be a Category 1 gas. Similarly, ozone is highly reactive yet only moderately water soluble. More explicit delineation of categories will be made upon review of the empirical data and the predictability of the model gases that may appear to fit more than one category.

Because they are not as reactive in the respiratory tract tissue as Category 1 gases, gases in Category 2 have the potential for significant accumulation in the blood and thus have a higher potential for both respiratory and remote toxicity. Thus, the model structure used to describe uptake of these gases is a hybrid of that for Category 1 and Category 3. The PBPK model component of the structure is necessary to evaluate the steady-state blood concentration which allows calculation of both absorption flux on inhalation and the desorption flux during exhalation. The derivation of the model structures and their reduction to forms with a minimal number of parameters are described in detail in Appendix I.

Gases in Category 3 are relatively water insoluble and unreactive in the ET and TB surface liquid and tissue and thus result in relatively small dose to these regions. The uptake of Category 3 gases is predominantly in the pulmonary region and is perfusion limited. Styrene is an example of a Category 3 gas. The site of toxicity of these gases is generally at sites remote to the respiratory tract and a compartmental approach can be used to describe distribution to various systemic tissues. Thus, the default model for Category 3 gases is similar in structure to the PBPK model used by Ramsey and Andersen (1984) to describe styrene distribution. The model structure and the derivation of the default dosimetric adjustment based on this model are described in detail in Appendix J.

3.2.3 Summary Considerations for Judging Model Structures

Although a comprehensive description of the exposure-dose-response continuum is desired for accurate extrapolation from experimental conditions and dose-response assessment, often the data base is inadequate. The preceding chapter discussion illustrates that data on the mechanistic determinants of chemical disposition, toxicant-target interactions, and tissue

responses vary in degree of availability for chemicals and species. Depending on the relative importance of these various determinants, models with less detail may be used as a default to adequately describe differences in dosimetry for the purposes of interspecies extrapolation often required for the chemicals at which the RfC methodology is directed. The default dosimetry models incorporated in the methodology represent structures that are commensurate with the available data for both chemical-specific (e.g., reactivity and solubility) and species-specific (e.g., respiratory tract airway dimensions, surface areas, ventilation rates, deposition data, distribution of cell types, metabolic capacities) parameters.

An understanding of the basis for model structures also allow development of a framework for the evaluation of whether an alternative model structure is considered optimal relative to the default. For example, an alternate model structure might be considered more optimal than the default for extrapolation when default assumptions or parameters are replaced by more detailed, biologically-motivated descriptions or actual data, respectively. For example, a model could be considered more optimal if it incorporates more chemical or species-specific information or if it accounts for more mechanistic determinants. These considerations are summarized in Table 3-6.

TABLE 3-6. HIERARCHY OF MODEL STRUCTURES FOR DOSIMETRY AND INTERSPECIES EXTRAPOLATION

Optimal 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

The sensitivity of the model to these differences in structure may be gauged by their relative importance in describing the response function for a given chemical. A model which incorporates many parameters may not be any better at describing ("fitting") limited response data than a simpler model. In these instances, the principle of parsimony might dictate the use of the simpler model.

Woodruff et al. (1992) used Monte Carlo analyses to assess the impact that structure and parameterization of PBPK models has on output predictions and variability. Nonphysiologically based (NPB) models of three or two compartments were compared with PBPK models that either used five compartments (PBPK5) to describe the body (well-perfused, poorly-perfused, fat, bone marrow, and liver tissue compartments) or that "lumped" the body into three (fat, bone marrow, and central) compartments (PBPK3). Comparisons were run for different data sets from inhalation to benzene. The two main influences on variability of model output predictions were (1) the quantity and type of data used to calibrate the model and (2) the number of parameters in the model. While some differences existed between the models' average predictions when calibrated to the same experimental data, these differences were smaller than the differences between the predictions made by the same model fitted to different data sets. An excessive number of parameters was shown to lead to overparameterization and cause large variability in the output. The similarities in the average predictions of the NPB and PBPK models supported the use of NPB models in some cases. The NPB models have fewer parameters and are potentially easier to fit. The PBPK models did show greater reliability for extrapolation but NPB models provided reliable results with less effort needed in fitting data when the objective was to interpolate from the current data.

Issues addressed in the review by Woodruff et al. (1992) and others (Hattis et al., 1989; Farrar et al., 1989; Portier and Kaplan, 1989; Bois et al., 1990) regarding evaluation of the uncertainty in input parameters and the variability of predictions due to alternate structures and data sets, should be considered when evaluating different available model structures for replacing the default adjustments.

4. QUANTITATIVE PROCEDURES

This chapter presents the quantitative procedures for dose-response[1] assessment for noncancer toxicity. Once key studies have been identified in the available data base for a chemical and evaluated for adequacy and limitations in terms of experimental design and analysis, dose-response assessment for noncancer toxicity involves the designation of the critical effects for each individual study, dosimetric adjustment of the associated exposure concentrations to human equivalent concentrations (HECs), and an analysis of the overall data array of these effects for that which is most representative of the threshold region. It is this no-observed-adverse-effect level (NOAEL) for the critical effect, together with uncertainty factors (UFs), that is used for the derivation of the inhalation reference concentration (RfC). An RfC has a numerical value, and hence, a quantitative nature. As discussed throughout this document, numerous theories, assumptions, and empirical data provide the quantitative framework for these RfC calculations. To account for inherent uncertainties in the chemical-specific data base and essential qualitative judgments, levels of confidence are assigned, enhancing the interpretation of a numerical RfC.

This chapter begins in Section 4.1 with a discussion of the minimum data base criteria to develop an RfC and of how to evaluate the available data to determine that a sufficient number of appropriate endpoints were addressed to ascertain the potential for noncancer toxicity. Guidance on how to designate effect levels (i.e., assign exposure levels as NOAELs or lowest-observed-adverse-effect levels [LOAELs]) is provided in Section 4.2. The remainder of the chapter is dedicated to the dosimetric adjustments used to extrapolate the experimental data to an HEC, according to whether the observed toxicity is in the respiratory tract or is extrarespiratory (sites remote to the portal of entry) and according to the type of inhaled toxicant. Conversion of experimental units to the units of the RfC (mg/m^3) and

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[1]Although the strict definitions of "dose", "response", and "effect" are recognized and discussed explicitly in Section 1.2., the conventions of the NAS paradigm will be used in this document, with the RfC being synonymous with a "dose-response" assessment. Thus, in the broader sense, the term "dose" may encompass administered dose (i.e., exposure concentration), delivered dose, or target tissue dose. Likewise, "response" in the qualitative sense, is an indication of an adverse influence regardless of whether the data were measured as quantal, count, continuous, or ordered categorical. The reader is referred to Section 1.2 for a detailed discussion of the general principles of dose-response and assessment for noncancer toxicity.

adjustment for discontinuous experimental exposure duration are described in Sections 4.3.2 and 4.3.3, respectively. The dosimetric adjustments for particles are discussed in Section 4.3.5 and for gases in Section 4.3.6. Duration and dosimetric adjustment of human data is discussed in Section 4.3.7. Once the effect levels are converted to HECs, the choice of the critical effect and principal study is made according to the guidance in Section 4.3.8. The operational derivation of an RfC is provided and the choice of UFs and assignment of confidence levels discussed in Sections 4.3.8.1 and 4.3.8.2, respectively.

4.1 MINIMUM DATA BASE CRITERIA

Noncancer toxicity is defined as health effects other than cancer and gene mutations that are due to the effects of environmental agents on the structure or function of various organ systems. Therefore, by definition, a data base for derivation of a dose-response estimate for noncancer toxicity should ensure that both appropriate and adequate numbers of endpoints have been evaluated.

As shown in Table 4-1, the minimum laboratory animal toxicologic data base 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 portal-of-entry (respiratory tract) effects, and established an unequivocal NOAEL and LOAEL. For a higher confidence RfC, chronic inhalation bioassay data, two-generation reproductive studies, and developmental studies in two different mammalian species are usually required. Considerations related to evaluating the comprehensiveness of the available data according to these criteria follow in Section 4.1.1. Oral data may be used, according to the criteria and guidance provided in Section 4.1.2, when inhalation data are not available. Typically, the level of confidence in a given RfC will be higher if it is derived from human data and supported by laboratory animal data. A more detailed discussion of how to assign confidence levels is provided in Section 4.3.9.2.

4.1.1 Evaluation of Comprehensiveness

Data bases vary considerably in their completeness. The rationale supporting the minimum data base requirements for derivation of an RfC, as outlined above, is that

TABLE 4-1. MINIMUM DATA BASE FOR BOTH HIGH AND LOW CONFIDENCE IN THE INHALATION REFERENCE CONCENTRATION (RfC)

	Mammalian Data Base[a]	Confidence	Comments
1.	A. Two inhalation bioassays[b] in different species	High	Minimum data base for high confidence
	B. One two-generation reproductive study		
	C. Two developmental toxicity studies in different species		
2.	1A and 1B, as above	Medium to high	
3.	Two of three studies, as above in 1A and 1B; one or two developmental toxicity studies	Medium to high	
4.	Two of three studies, as above in 1A and 1B	Medium	
5.	One of three studies, as above in 1A and 1B; one or two developmental toxicity studies	Medium to low	
6.	One inhalation bioassay[c]	Low	Minimum data base for estimation of an RfC

[a]Composed of studies published in refereed journals, reports that adhered to good laboratory practice and have undergone final QA/QC, or studies rated by the Office of Pesticide Programs as "core-minimum". It is understood that adequate toxicity data in humans can form the basis of an RfC and yield high confidence in the RfC without this data base. Pharmacokinetic data that indicate insignificant distribution occurs remote to the respiratory tract may decrease requirements for reproductive and developmental data.
[b]Chronic data.
[c]Chronic data preferred but subchronic acceptable.

well-defined and well-conducted subchronic toxicity studies are generally considered to be reliable predictors of many forms of chronic toxicity, with the notable exceptions of carcinogenic, teratogenic, and reproductive effects. Testing is required in two different species, in the absence of a relevant laboratory animal model, in order to address potential species sensitivity. The additional specific requirement for adequate respiratory tract evaluation arises from the increased potential for the portal-of-entry tissue to interact intimately with chemicals. The observation that approximately 70% of the RfCs derived to date (October, 1994) have been based on respiratory tract endpoints is consistent with this increased potential.

It should be recognized, however, that for some substances, results of other studies may suggest the possibility of effects not detected in the subchronic studies. Current toxicity testing strategies are hierarchical sequences of tests designed to develop a profile of a chemical's toxicity (Environ Corporation, 1985). Initial testing tiers consist of relatively rapid, inexpensive tests designed to identify acute toxicity. This information is not directly useful in predicting chronic adverse effects in humans, but can be used to guide decisions as to type and extent of other testing required, such as subchronic, chronic, or reproductive bioassays. The toxicity "profiles" or information required as a minimum data base also are somewhat structured according to this hierarchy. The magnitude of data insufficiency varies on a case-by-case basis and should be defined by the nature of the plausible or possible pathogenesis processes (i.e., defined according to the possible mechanism[s] of action for the observed effect[s]). For example, the U.S. Food and Drug Administration (1982) suggests that if a chemical tested in a subchronic study is found to cause focal hyperplasia, metaplasia, proliferative lesions or necrosis, then a carcinogenicity study in two rodent species is warranted. Likewise, if reproductive effects are found, then teratology testing also should be conducted. If acute or subchronic data demonstrate reproductive organ toxicity or neurotoxic effects, standard 2-generation reproductive assays, developmental testing, and a neurotoxicity battery may be required for appropriate characterization. Pharmacokinetic data that indicate insignificant distribution to sites remote from the respiratory tract at exposure concentrations under consideration for derivation of an RfC can mitigate the requirements for reproductive and developmental data, except when these endpoints are suggested as potential targets by other inhalation data. Route-to-route extrapolation of oral data, according to the criteria provided in the next section, may provide a qualitative gauge by which to judge the relative sensitivity of these endpoints to those under consideration for the respiratory tract or other target tissues.

The quantitative relationships between these various endpoints and how to evaluate the entire data array for determination of the principal studies on which to base the derivation of the RfC are discussed in Section 4.3.8.

4.1.2 Route-to-Route Extrapolation

When the data base for a given chemical is not adequate via inhalation, route-to-route extrapolation is often practiced by some risk assessors using empirically derived factors that are not necessarily applicable to the case at hand. For most route-to-route extrapolations, the lack of data, lack of ability to interpret data, and underutilization of existing data due to insufficient models and statistics reduce or eliminate the validity of these extrapolations (Gerrity and Henry, 1990).

Data from other routes of exposure may be useful to derivation of an RfC only when respiratory tract effects and/or "first-pass" effects (a pharmacologic phenomenon) can be ruled out. First-pass effects refer to the metabolism that can take place in the portal-of-entry tissue, prior to entry into the systemic circulation. For example, after oral administration, many chemicals are delivered to the liver via the portal vein from the gastrointestinal (GI) tract before they enter into the systemic circulation.

The respiratory tract can also exhibit a first-pass effect after inhalation due to its various cell types and metabolic enzyme systems. This first-pass action can alter the disposition of the parent and metabolites, thereby modulating the dose to remote or systemic target tissues in a route-dependent fashion. Therefore, unless this first-pass effect and dosimetry are adequately understood, there can be substantial error introduced in route-to-route extrapolation that does not account for these parameters. In the absence of data to determine dosimetry via inhalation, when a chemical is thought to be susceptible to first-pass effects (e.g., metabolized), or where a potential for portal-of-entry effects is indicated but not well characterized (e.g., respiratory toxicity after acute exposures or skin irritation after dermal administration), then route-to-route extrapolation for derivation of an RfC is not appropriate. For a more detailed discussion of important parameters to consider, refer to Gerrity and Henry (1990), the National Research Council (1986, 1987), and Pepelko and Withey (1985).

Oral toxicity data are the most common data available as alternatives to inhalation data. Oral data should not be used for route-to-route extrapolation in the following instances:

(1) when groups of chemicals are expected to have different toxicity by the two routes; for example, metals, irritants, and sensitizers;

(2) when a first-pass effect by the respiratory tract is expected;

(3) when a first-pass effect by the liver is expected;

(4) when a respiratory tract effect is established, but dosimetry comparison cannot be clearly established between the two routes;

(5) when the respiratory tract was not adequately studied in the oral studies; and

(6) when short-term inhalation studies, dermal irritation, in vitro studies, or characteristics of the chemical indicate potential for portal-of-entry effects at the respiratory tract, but studies themselves are not adequate for an RfC development.

Dose-response data from other routes of exposure, such as intravenous, intraperitoneal, subcutaneous, dermal, and intramuscular routes also may be available. Intravenous data may provide reliable information for certain chemicals (e.g., metabolizable or stable but not rapidly reactive) on blood levels but such information would have to be supplemented by knowledge of the quantitative relationship between inhalation exposure concentration and blood levels in order to be useful. The other routes generally have a much more limited usefulness in route-to-route extrapolation because the pharmacokinetics are, in general, poorly characterized.

The ability to perform quantitative route-to-route extrapolation is critically dependent upon the amount and type of data available. Regardless of the toxic endpoint being considered, a minimum of information is required to construct plausible dosimetry for the routes of interest. This information includes both the nature of the toxic effect and a description of the relationship between exposure and the toxic effect. Illustration for this rationale is provided by Figures 4-1 and 4-2.

Figure 4-1 shows physiologically based pharmacokinetic (PBPK) model simulations of the concentrations required to result in a comparable "administered dose" (mg/kg/BW) after gavage with different vehicles (oil or water), oral exposure via drinking water, or inhalation for different durations (6 or 24 h). For gavage and drinking water studies, dose was defined as the total amount of chloroform entering the gastrointestinal tract. Administered dose for inhalation studies was defined as the product of inhaled air concentration (mg/L) and the alveolar ventilation rate (L/h). Absorption efficiency was assumed to be 100%. For a

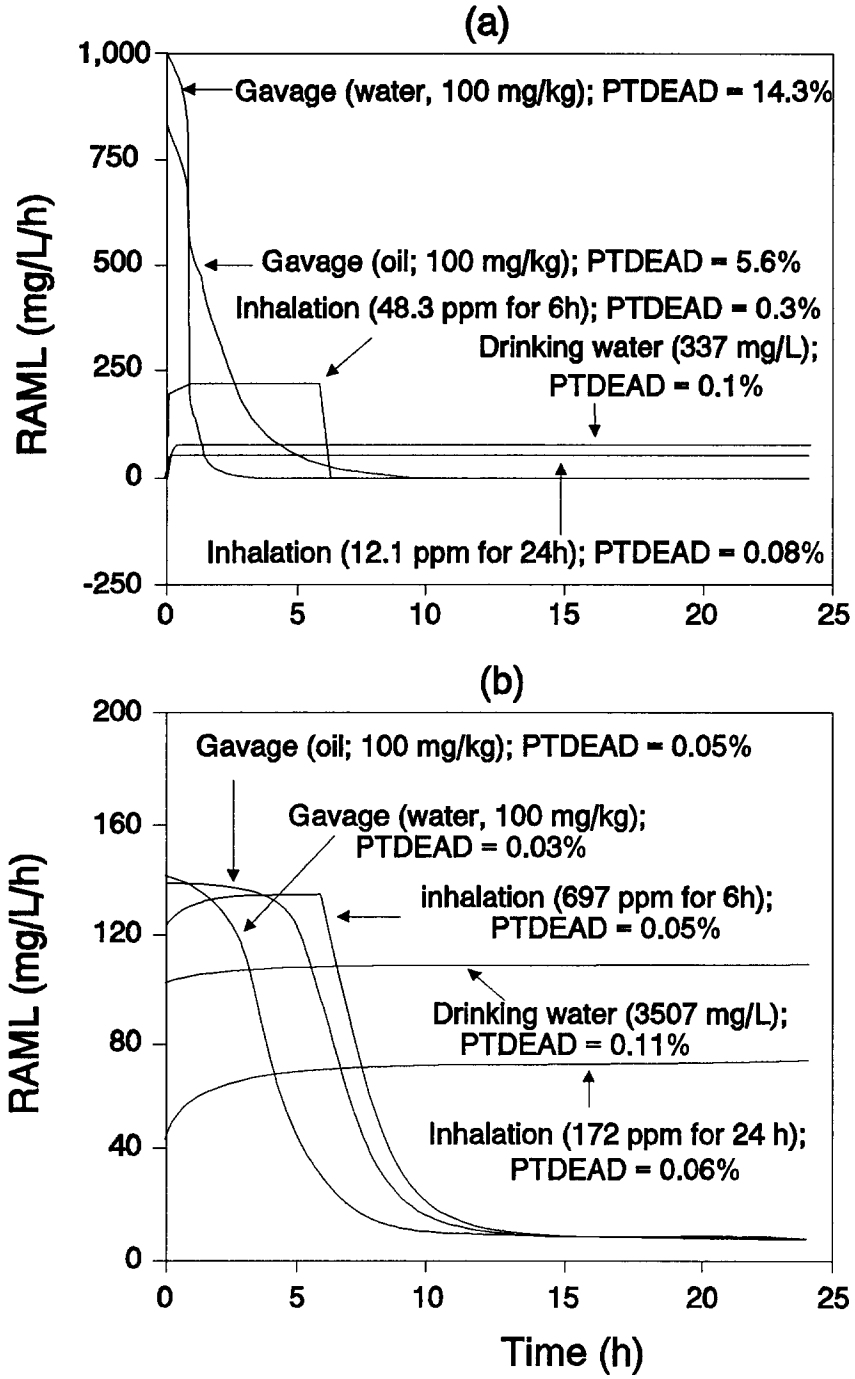

Figure 4-1. Multiple route comparisons for (a) mice and (b) humans administered chloroform at a dose of 100 mg/kg body weight. Actual concentrations of chloroform in air or in drinking water used to deliver a total body burden comparable to a gavage dose of 100 kg/mg and the percentage of liver cells killed (PTDEAD) as a result of the exposures are labeled for each simulation. Model simulations are of the rates of metabolism in the liver (RAML, mg/L of liver/h) for 24 h.

Source: Corley and Reitz (1990).

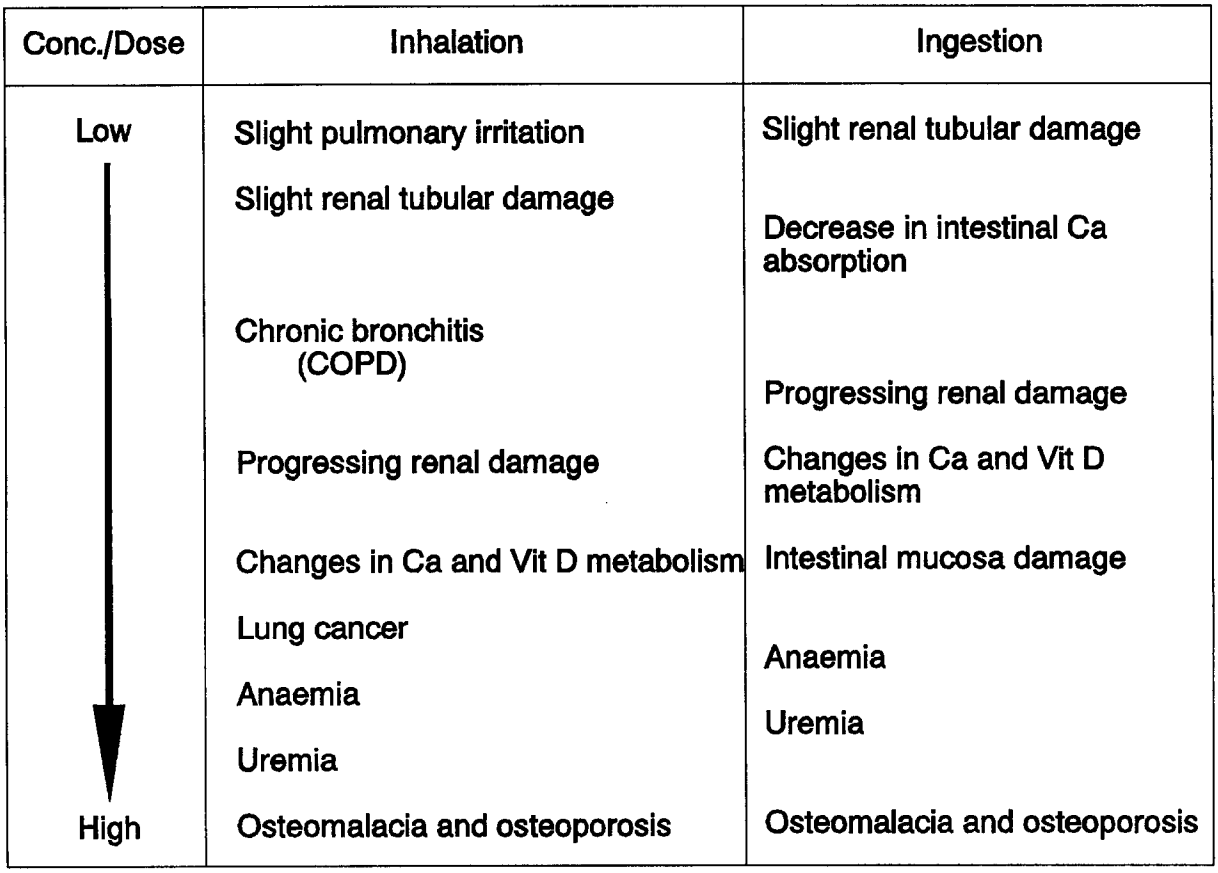

Figure 4-2. Differential effects of inhaled and ingested cadmium with increasing inhaled and ingested doses.

Source: Oberdörster (1990).

detailed discussion of the PBPK model structure and parameter values, refer to Corley and Reitz (1990). The figure is used here to highlight the differences in administered dose by various routes required to achieve the same internal dose in a target tissue, in this case, the liver. The model predicts the percentage of hepatocytes killed in the liver due to the metabolism of parent compound. Note the different profiles of metabolism via the different routes in Figure 4-1. The degree of cytotoxicity predicted by the model simulations was in the order of gavage (water) > gavage (corn oil) ≫ inhalation (6 h) > drinking water > inhalation (24 h). This figure also illustrates the interspecies differences in the processes involved. For example, to produce a total body burden of chemical comparable to that

achieved by an exposure dose of 100 mg/kg/day of chloroform, the concentration of the 24-h inhalation exposure was increased from 12 ppm in the mouse to 172 ppm for the humans.

Figure 4-2 shows the qualitative relationships of differential effects after either inhalation or ingestion of cadmium (Cd) (Oberdörster, 1990). It is apparent that the exposure route influences the target organ effects. Respiratory effects have only been observed after inhalation exposures and GI tract effects only after oral exposure. Portal-of-entry effects are obviously of importance. In contrast, remote effects such as those on the kidney, bone, and the hematopoietic system are observed after exposure by either route. However, the portal of entry also modulates the dose rate to the remote tissues. Using a simplified steady-state PBPK model of a few basic transfer kinetics (e.g., 90% of inhaled soluble Cd is absorbed; 5% of ingested) for soluble Cd compounds (e.g., cadmium oxide, cadmium chloride), Oberdörster (1990) estimated that 1 $\mu g/m^3$ of inhaled (24-h) Cd was equivalent to 21.5 μg (daily ingested) and 1,000 μg (daily ingested) for renal and respiratory effects, respectively. The great disparity in potency by different routes for Cd emphasizes the point that dosimetry should be established for each relevant route when either contact site or remote toxicity is a concern.

Therefore, the actual impact of exposure by different routes can only be estimated by taking account of factors that influence absorption at the portal of entry, such as (1) physicochemical characteristics of the chemical (e.g., dissociation state, molecular weight, partition coefficient, reactivity, solubility), (2) exposure factors (e.g., concentration, duration, regimen), and (3) physiologic parameters (e.g., barrier capacity as related to variability in species, blood flow, cell types and morphology, metabolism, pH, specialized absorption sites, storage in cells), and those parameters that influence dose remote to the portal of entry, including metabolism, clearance, tissue binding, tissue blood flows, tissue:blood partition coefficients, and tissue volumes.

Evaluation of the adequacy of the available data to address the factors outlined above is the basis for the decision tree shown in Figure 4-3 (Gerrity and Henry, 1990). As discussed above, route-to-route extrapolation for quantitative dose-response assessment should only be considered when concern for contact-site (portal-of-entry) toxicity has been ruled out (Option 2 of Figure 4-3 is sufficient only for hazard identification). Although the fact that

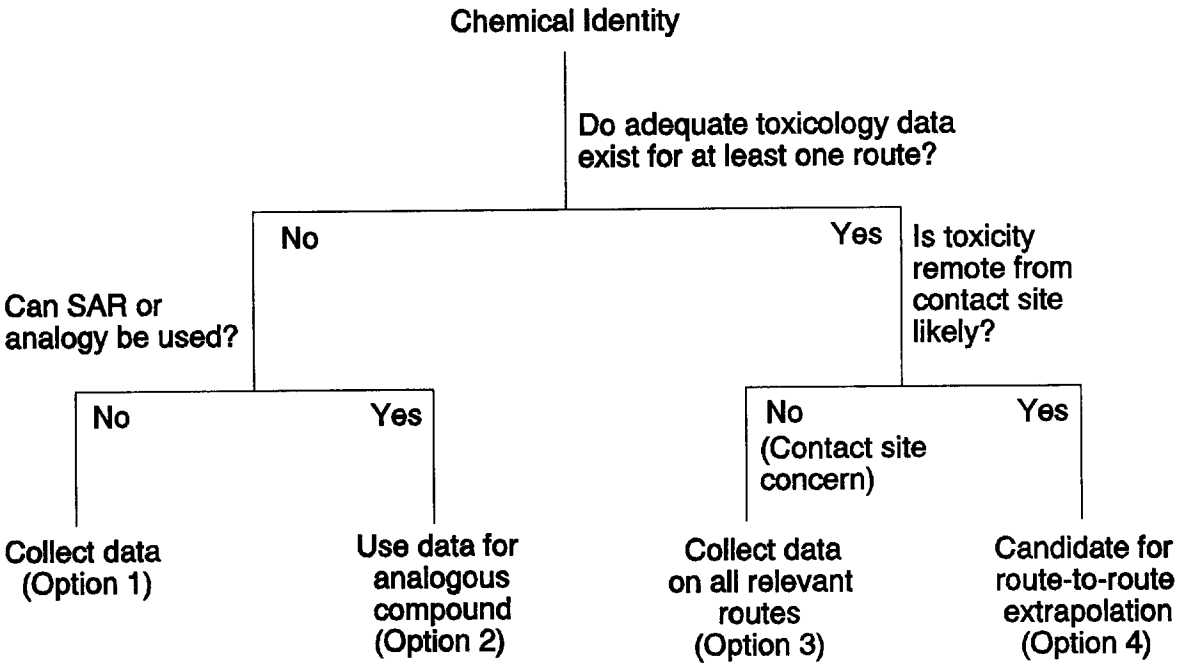

Figure 4-3. Decision tree for route-to-route extrapolation (see text below for a discussion of the options listed). SAR = structure activity relationship.

Source: Gerrity and Henry (1990).

the effect of a chemical is observed in the portal of entry does not necessarily preclude route-to-route extrapolation, the requirements for quantitative data via each route (Option 3 of Figure 4-3) in order to perform such an extrapolation usually obviate the reason (i.e., lack of data) for which it was being considered in the first place.

If respiratory tract toxicity can be ruled out and remote site toxicity is of interest, then route-to-route extrapolation becomes a possibility (Option 4 of Figure 4-3). Methods for route-to-route extrapolation range in accuracy and therefore, inherent uncertainty. The simplest approach is to use default absorption values for each exposure route appropriate to the chemical class in question. Such values have only been developed for a limited class of volatile organic chemicals. Because this approach entails an increased uncertainty compared to those that use pharmacokinetic data and PBPK modeling, use of default absorption values is considered inadequate for quantitative dose-response assessment.

Direct measurement of absorption efficiency for the routes of interest is an improvement on the use of default values, but the approach still ignores many of the potentially important factors mentioned above, invoking additional uncertainty that would have to be accounted for when calculating a dose-response estimate. Measurement of bioavailability by the use of a validated internal marker provides greater certainty. Comparative excretion data when the associated metabolic pathways are equivalent by each route and regimen of interest or comparative systemic toxicity data when such data indicate equivalent effects by each route and regimen of interest may also provide useful information. However, the associated uncertainty would have to be accounted for in the estimate derived using an extrapolation based on such data.

The preferred method for performing route-to-route extrapolation involves the development of a PBPK model that describes the disposition (deposition, absorption, distribution, metabolism, and elimination) of the chemical for the routes of interest (Gerrity and Henry, 1990). Such models account for fundamental physiologic and biochemical parameters and processes such as blood flows, ventilatory parameters, metabolic capacities, and renal clearance, tailored by the physicochemical (e.g., blood:air and tissue:blood partitions) and biochemical properties (e.g., binding, depletion of co-factors) of the chemical in question.

The use of a PBPK model is predicated on the assumption that an effective (target-tissue) dose achieved by one route in a particular species is expected to be equally effective when achieved by another exposure route or in some other species. A key determination is the choice of the dose surrogate for the toxic effect. The more accurately the exposure-dose-response continuum is characterized, and therefore the correlation of the chosen dose surrogate with toxic effect, the more accurate this approach will be with respect to use in quantitative extrapolation. For example, a measure of target-tissue dose for a chemical with pharmacologic activity could be the tissue concentration divided by some measure of the receptor-binding constant for that chemical. The behavior of a substance administered by a different exposure route can be determined by adding equations that describe the nature of the new input function. Similarly, because known physiologic parameters are used, different species (e.g., humans versus laboratory animal species) can be modeled by replacing the appropriate constants. It should be emphasized that PBPK models must be used in

conjunction with toxicity and mechanistic studies in order to relate the effective dose associated with an effect for the test species and conditions to other scenarios. The use of an existing model structure, essentially a template, can greatly reduce the effort required for model development of analogous chemicals.

4.1.3 Not-Verifiable Status

When the available data do not meet the minimum data base requirements as discussed above or when the existing data can not be synthesized into a compelling toxicity profile without great uncertainty (see Section 4.3.8), the data base on a given chemical is designated as "not-verifiable" and no RfC estimate is calculated. This status would require reanalysis when new data become available.

4.2 DESIGNATION OF EFFECT LEVELS

The designation of effect levels, or the association of adversity[2] with exposure concentrations, is one of the most difficult procedures of any dose-response analysis for noncancer toxicity. The critical effect for an individual study is often described as either the adverse effect that first appears in the dose scale as dose is increased, or as a known precursor to the first adverse effect. The premise of this designation is the underlying threshold phenomenon and it assumes that if this critical effect is prevented then all observed adverse effects at subsequent concentrations are prevented. In the simplest terms, a NOAEL and a LOAEL are determined for the specified adverse effect from the exposure levels of a given individual study for each of the various species tested. The NOAEL is the highest level tested at which the specified adverse effect (i.e., a biologically and statistically defined adverse effect) is not produced and is, thus, by definition, a subthreshold level (Klaassen, 1986). The NOAEL/LOAEL is a function of the exposure levels used in the experimental

[2]Here adverse effects are considered to be functional impairments or pathological lesions that may affect the performance of the whole organism or that reduce an organism's ability to cope with an additional challenge (Federal Register, 1980). One of the major problems encountered with this concept is the reporting of "observed effect levels" as contrasted to "observed adverse effect levels." The terms "adverse" and "not adverse" are at times satisfactorily defined, but because more subtle responses continue to be identified due to increasingly sophisticated testing protocols, scientific judgment is needed regarding the exact definition of adversity.

design, or is a function of designating a specified health effect measure (e.g., 10% incidence of a lesion) as the outcome of interest in the case of some alternative approaches presented in Appendix A[3], and therefore, does not necessarily reflect the "true" biological threshold.

Table 4-2 presents the four types of effect levels that may be applicable when evaluating an individual study. Historically, the distinction between adverse effects and nonadverse effects has been and remains problematic. For example, although disease is a dynamic process (injury, adaptation, or healing), a pathologist records a morphologic change at a single point in time and these "freeze-frame" data are used to determine the probable cause and pathogenesis (past) and probable progression or outcome (future). Designation of an effect level (i.e., the designation of adversity) requires interpretation of the data based on an ability to deduce the preceding events that have led to the observed change and to predict the outcome or progression. The relationship between structural alterations to altered function is not always simple, however.

Determining whether altered morphology is an adaptive response or truly an expression of toxicity (functional impairment) can be extremely difficult and even controversial (Burger et al., 1989; Ruben and Rousseaux, 1991). In some cases, structural alteration can occur, but normal function can continue in target tissues with functional reserve such as the lung, liver, and kidney. Not all tissues demonstrate this high reserve. The central nervous system can compensate to only a limited degree and where the damage occurs is vitally important for the function of the system. Therefore, "focal" damage may be adverse in some but not all target tissues. Also, the lack of observed functional change may be due to failure to detect subtle or unknown functional changes rather than to their absence.

A similar morphologic alteration may have both functional and physiologic significance, but often it is difficult to differentiate toxicity from physiologic response by morphologic means alone. Not all functional abnormalities manifest themselves morphologically. Temporal-spatial patterns are particularly challenging when evaluating toxicologic pathology. Problems concerning time include reversibility, adaptation versus toxicity, progression versus

[3]There are alternative approaches under development (presented and discussed in Appendix A) aimed at deriving estimates of exposures that are analogous in intent to the establishment of a NOAEL. The NOAEL/LOAEL approach outlined is not intended to discourage alternative or more sophisticated dose-response procedures when sufficient data are available, but rather to present key issues necessarily involved (e.g., dosimetric adjustment and data array analysis) in any approach for the assessment of noncancer toxicity.

TABLE 4-2. FOUR TYPES OF EFFECT LEVELS[a] (RANKED IN ORDER OF INCREASING SEVERITY OF TOXIC EFFECT) CONSIDERED IN DERIVING INHALATION REFERENCE CONCENTRATIONS FOR NONCANCER TOXICITY

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

[a]Note that these levels represent points on a continuum and are not discrete.
[b]Adverse effects are defined as any effects resulting in functional impairment and/or pathological lesions that may affect the performance of the whole organism, or that reduce an organism's ability to cope with an additional challenge.
[c]Frank effects are defined as overt or gross adverse effects (e.g., severe convulsions, lethality, etc.).

regression, and peracute lethal toxicity. Problems concerning space are limited to missing the lesion completely or missing a relevant area because of sampling method. For example, histologic examination of the nasal cavity should select four tissue sections, not one, to achieve a thorough examination (Young, 1981). Further, due to the proximal to distal inspiratory airstream, some examination of the upper respiratory tract is indicated when respiratory toxicity from an inhaled irritant is evident in the lower respiratory tract.

Due to the structural-functional and temporal-spatial problems discussed above, an approach that integrates pathological studies (ultrastructural, histochemical, cellular, and molecular) with functional methods is recommended (Ruben and Rousseaux, 1991). Morgan (1991) has provided guidance on the identification and interpretation of URT lesions in toxicologic studies. A systematic but flexible approach to evaluation of lesions in the URT is

recommended, one that considers selection of section level in context with the physicochemical characteristics of the inhaled gas (e.g., water solubility and reactivity), the role of factors that may account for lesion distribution (e.g., dosimetry and tissue susceptibility), and development of a pathogenesis profile or a chronological order of events (e.g., degenerative, adaptive, and adaptive/regenerative changes versus time). The nasal diagrams proposed by Mery et al. (in press) offer an approach to recording data and mapping lesions that aids this type of interpretation strategy. This approach is also likely the best to compile the data and precludes the restraint to interpretation and mathematical modeling presented by data scored categorically for severity (e.g., + = mild, ++ = moderate; and +++ = severe) and/or without sufficient section detail with respect to lesion location (Jarabek, 1994).

In the early stages of respiratory disease, there is considerable uncertainty concerning how to differentiate between acute reversible effects, which are the immediate consequence of an exposure episode, and potential progression to chronic, nonreversible respiratory pathology. The boundary between adaptive and toxic responses also remains controversial for some respiratory tract lesions (Burger et al., 1989). These are important issues both in terms of evaluation of respiratory tract effects per se, as well as for decisions concerning the critical effect in inhalation studies. Inhalation-specific issues such as evaluation of pulmonary function, sensory irritation, and allergic sensitization data are discussed in Section 2.2.

Designation of effect levels usually contains an element of scientific judgment in addition to objective criteria. Considerable experience and precedent for such decisions have accrued over the last several years in the process of developing oral reference doses, RfCs, and other health-related benchmark estimates. Table 4-3 presents guidance as to how general effects would usually be designated as different (adverse) effect levels. In general, effects that may be considered marginal are designated as adverse only to the extent that they are consistent with other structural and functional data suggesting the same toxicity. For example, altered liver enzymes (statistically out of normal range) would only be considered adverse in context with altered structure (pathology) and liver weight changes.

TABLE 4-3. EFFECT LEVELS CONSIDERED IN DERIVING INHALATION REFERENCE CONCENTRATIONS IN RELATIONSHIP TO EMPIRICAL SEVERITY RATING VALUES (Ranks are from lowest to highest severity.)[a]

Effect or No-Effect Level	Rank	General Effect
NOEL	0	No observed effects.
NOAEL	1	Enzyme induction or other biochemical change, consistent with possible mechanism of action, with no pathologic changes and no change in organ weights.
NOAEL	2	Enzyme induction and subcellular proliferation or other changes in organelles, consistent with possible mechanism of action, but no other apparent effects.
NOAEL	3	Hyperplasia, hypertrophy, or atrophy, but no change in organ weights.
NOAEL/LOAEL	4	Hyperplasia, hypertrophy, or atrophy, with changes in organ weights.
LOAEL	5	Reversible cellular changes including cloudy swelling, hydropic change, or fatty changes.
(LO)AEL[b]	6	Degenerative or necrotic tissue changes with no apparent decrement in organ function.
(LO)AEL/FEL	7	Reversible slight changes in organ function.
FEL	8	Pathological changes with definite organ dysfunction that are unlikely to be fully reversible.
FEL	9	Pronounced pathologic changes with severe organ dysfunction with long-term sequelae.
FEL	10	Death or pronounced life shortening.

[a]Adapted from DeRosa et al. (1985) and Hartung (1986).
[b]The parentheses around the "LO" in the acronym "LOAEL" refer to the fact that any study may have a series of doses that evoke toxic effects of rank 5 through 7. All such doses are referred to as adverse effect levels (AELS). The lowest AEL is the (LO)AEL.

4.3 CALCULATION OF HUMAN EQUIVALENT CONCENTRATIONS

A key element of extrapolation of laboratory animal inhalation data to humans is estimation of the "dose" (i.e., agent mass deposited per unit surface area or tissue volume) delivered to specific target sites in the respiratory tract or made available to uptake and metabolic processes for systemic distribution considered with mechanistic determinants of toxicant-target interactions and tissue responses (Martonen and Miller, 1986; Andersen et al., 1991). To this end, PBPK and other mathematical dosimetry models have evolved into particularly useful tools for predicting disposition differences for risk assessment (Miller et al., 1987b). Their use is predicated on the assumption that an effective (target-tissue) dose in a particular species is expected to be equally toxic when achieved in some other species. However, it is likely that species differences in sensitivity occur due to such species-specific factors as host defense, repair processes, and genetics, so that the use of a 10-fold UF to account for intraspecies variability, despite application of dosimetric adjustments, requires additional research.

This section outlines the methods for calculating HEC estimates by using adjustment factors that have resulted from similar modeling efforts of species-specific dosimetry differences. The factors are used to adjust the observed exposure effect levels (i.e., NOAELs, LOAELs, etc.) in laboratory animals to estimate a concentration that would be an equivalent exposure to humans (i.e., $NOAEL_{[HEC]}$s, $LOAEL_{[HEC]}$s, etc). These HECs then are the basis for comparison and choice of the critical effect and study.

As discussed in Section 3.2, the equations presented in this chapter are default adjustments based on dosimetry models that incorporate only the major determinants of particle or gas disposition. The use of models that may incorporate a more comprehensive description of the exposure-dose-response continuum is considered the optimal approach in each case. It should also be noted that because PBPK models allow for explicit handling of intermittent exposure regimens (e.g., model can simulate 6 h/day, 5 days/7 days exposure and predict resultant internal dose), the duration adjustment discussed in Section 4.3.2 is obviated by the use of these models.

Figure 4-4 is a flowchart for the default calculation of HECs and provides an outline for the contents of this section. Conversion of units from ppm to mg/m^3 is required before dosimetric adjustments can be applied. This calculation is discussed in Section 4.3.1. The

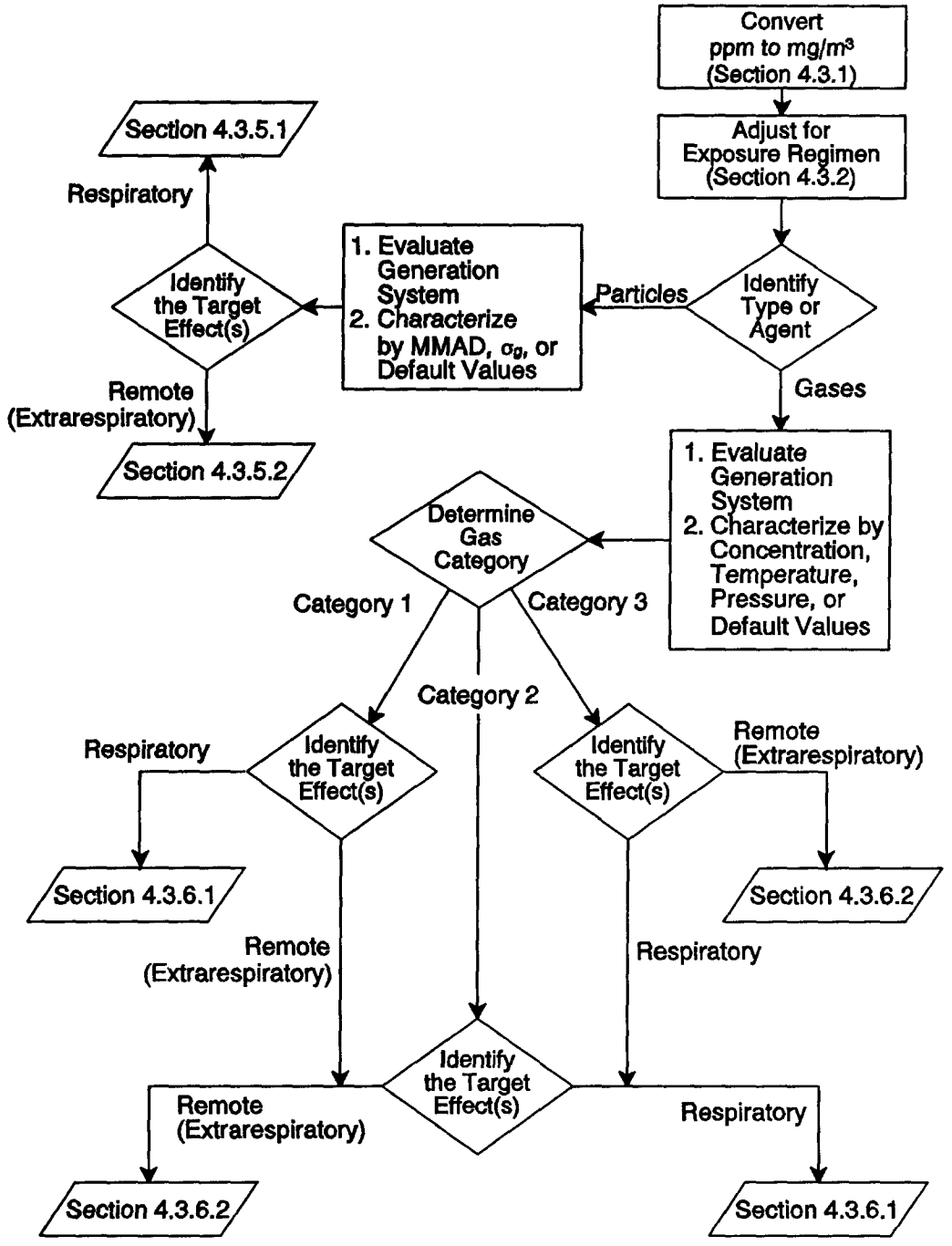

Figure 4-4. Flowchart for calculation of human equivalent concentrations.

next step in calculating a HEC is to convert the exposure regimen of the experiment in question to that of the human exposure scenario; that is, a continuous (24-h/day) lifetime (70-year) exposure. The third step of the approach is to apply the dosimetric adjustments appropriate for the type of toxicant to be assessed (particle or gas, and if a gas, what category) and the effect to be assessed (respiratory tract or extrarespiratory toxicity) resulting from an inhalation exposure. The default dosimetric adjustments to derive HECs for respiratory tract effects and extrarespiratory effects of particles are provided in Section 4.3.5. For gases, the determination of the appropriate gas category according to the scheme provided in Section 3.2.2 is required to determine which dosimetric adjustment to apply to calculate an HEC. Because the boundaries between the categories are not definitive (see discussion in Section 3.2.2 and Appendix I), but instead were made to allow derivation of default model structures, identification of the target effect(s) is used to further define the gas category. Thus, remote (extrarespiratory) effects of Category 1 gases and respiratory effects of Category 3 gases are treated according to the default dosimetric adjustments for each of these respective effects of Category 2 gases (Section 4.3.5 and 4.3.6). The default dosimetric adjustments to derive HEC values for respiratory effects of Category 1 gases are provided in Section 4.3.5. The default dosimetric adjustment to derive HEC values for extrarespiratory effects of Category 3 gases is provided in Section 4.3.6.

Although the presentation in this section divides the dosimetry calculations into those applied to extrapolate respiratory tract effects versus extrarespiratory effects, it should be recognized that there is no strict compartmentalization of effects for a chemical. A given inhaled chemical could cause both respiratory tract and extrarespiratory effects. Therefore, the decision on which of the equations to use in this chapter is governed by the endpoint of interest in concert with the properties of the chemical to be assessed.

4.3.1 Conversion to Standard Units

In the rare event that investigations using particulate exposures would report the concentration in ppm, a mass-density relationship should be used to convert the exposure concentration to mg/m^3. Inhalation toxicity studies on gases typically employ exposure levels expressed as mg/m^3 or ppm. Exposure levels for gases, including the NOAEL selected for RfC derivation, should be expressed in standard units of mg/m^3. For exposure levels

expressed as ppm, the Ideal Gas Law should be used to derive the corresponding mg/m^3 level:

$$\frac{mg}{m^3} = ppm \times \frac{g\text{-mole}}{22.4L} \times \frac{MW}{g\text{-mole}} \times \frac{273\,^\circ}{T} \times \frac{P}{760\ mm\ Hg} \times \frac{10^3 L}{m^3} \times \frac{10^3 mg}{g}, \qquad (4\text{-}1a)$$

where:

ppm = concentration expressed on a volumetric basis $\frac{L}{10^6 L}$,

MW = molecular weight in grams,

22.4 L = the volume occupied by 1 g-mol of any compound in the gaseous state at 0 °C and 760 mm Hg,

T = actual temperature in degrees Kelvin, and

P = actual pressure in mm Hg.

At 25 °C and 760 mm Hg, 1 g-mole of a perfect gas or vapor occupies 24.45 L. Therefore, under these conditions, the conversion becomes

$$mg/m^3 = \frac{ppm \times MW}{24.45}. \qquad (4\text{-}1b)$$

4.3.2 Temporal Relationships of Toxicity and Duration Adjustment

Many inhalation toxicity studies using laboratory animals use discontinuous exposure regimens. Often exposures are for 6 to 8 h/day and 5 days/week. Inhalation reference concentrations are constructed to reflect a benchmark level for continuous exposure. By extension, the RfC also is assumed to be protective for discontinuous exposures at the same air concentration. A normalization to some given exposure (e.g., 24 h/day for a lifetime of 70 years) is needed to adjust for the wide variety of experimental exposures to permit comparisons between studies. As discussed earlier, the RfC proposed herein is to reflect lifetime continuous exposure, making this scenario the objective of normalization. Attention should be paid to the degree this scenario deviates from the experimental, and to

the physicochemical (solubility and reactivity) parameters of the inhaled agent and species-dependent factors (e.g., distribution volumes and metabolic pathways) that might temper this conversion.

To calculate duration-adjusted exposure levels in mg/m^3 for experimental animals, the equation is

$$NOAEL^{\star}_{[ADJ]}\ (mg/m^3) = E\ (mg/m^3) \times D\ (h/24\ h) \times W\ (days/7\ days), \qquad (4\text{-}2)$$

where:

$NOAEL^{\star}_{[ADJ]}$ = the NOAEL or analogous effect level obtained with an alternate approach as described in Appendix A, adjusted for duration of experimental regimen;

E = experimental exposure level;

D = number of hours exposed/24 h; and

W = number of days of exposure/7 days.

NOTE: 1. This same duration adjustment is applied to LOAELs.
2. This duration adjustment is not applied when PBPK models are used (see Section 4.3.3).
3. Duration adjustment for human data is discussed in Section 4.3.6.

The rationale for this linear prorate adjusment is that the resultant human exposure concentration should be the concentration (C) × time (T) equivalent of the experimental animal exposure level. This adjustment is weakly founded because steady-state conditions may not be reached in laboratory animals for some chemicals and intermittent regimens and because the influence of dose-rate is different for different toxicity mechanisms (e.g., an effect mediated by peak blood concentration versus integrated tissue dose). Thus, depending on the mechanism of action, such duration adjustment may be inappropriate. Toxic effects of gases such as irritation, narcosis or asphyxia may be much more dependent on concentration than duration. An attempt should always be made to take into account the mechanisms of toxic action as related to the temporal parameters of duration and frequency, although C × T is rarely investigated after subchronic or chronic durations. Unless more information is available on a case-by-case basis, this default is used.

As the effect in question increases in its severity, the validity of this equation becomes more tenuous. The toxicity of an exposure is dependent upon the character of the "concentration-time" (C × T) curve, which may be described by a hyperbola whose arms converge asymptotically toward the axes of the coordinates (Bliss, 1940). Bliss and James (1966) have shown that such curves can be extrapolated with minimal error when the time points in the experiment are located on the segment of the curve asymptotically approaching the axes of the coordinates (i.e., high concentration acute exposures or low concentration chronic exposures). The exposure duration should ideally embrace the time span in which the rate of onset of specific toxic effects sharply change, reflecting the degree of arc in the curve of the (C × T) relationship.

Fiserova-Bergerova et al. (1980), using a compartmentalized model based on first-order kinetics, demonstrated that duration of exposure to a gas can have profound effects on the fractions of uptake exhaled or metabolized. Concentrations in tissues reflected the concentration fluctuations in exposure, but the fluctuation in tissues was greater during exposure to low solubility gases than to lipid soluble vapors (blood:air partition coefficients of 0.5 and 10.0, respectively), due to the faster equilibration of partial pressures of the low solubility gases. Fluctuations between tissue and exposure concentrations were diminished if the substances were metabolized. Because a toxic effect is usually related to tissue concentration, consideration should be given to these duration and solubility effects. Extrapolation on the basis of C × T should be attempted only if a steady-state was attained. Likewise, linear extrapolation from one concentration exposure to another is scientifically supportable only if all processes involved in the uptake and elimination of the inhaled agent are first order. Differences are caused primarily by concentration-dependent metabolic clearance.

4.3.3 Use of Pharmacokinetic and Pharmacodynamic Data

Pharmacokinetic and pharmacodynamic data (described in Section 1.2) can be used in a range of applications, from providing adjustments to external exposures based on correlations of exposure to effect, through gathering insight on various important mechanistic insights and calculation of kinetic parameters, to developing a comprehensive exposure-dose-response description that incorporates major determinants of toxicant disposition, toxicant-target

interaction and tissue response. These data can also be used to ascertain what laboratory animal species is most appropriate, based on similarity of major mechanistic determinants, for extrapolation to humans.

Empirical equations such as correlation equations (e.g., that relate the extent of external exposure with the amount of internal biologic markers) can be used to describe kinetic processes by a simple mathematical expression. These data, as described in Section 4.1.2, may be useful as a qualitative index of uptake for a given route, but they provide no insight into the other parameters controlling disposition of a toxicant (distribution, metabolism, excretion) over time and therefore their use is rather restricted.

Experimental data that track the concentrations of various kinetic parameters during and following exposures can be used to determine various measures of the intensity of tissue exposure. The parameters that are proportional to the relevant measure of tissue exposure are referred to as tissue dose metrics (Andersen, 1987). These metrics include estimates of time integrals of tissue exposure to a parent toxicant or its metabolite(s) (e.g., area under the blood [AUBC] or tissue curve [AUTC]), concentrations of these materials in tissues, or receptor occupancy caused by the presence in tissues. This information provides little insight into the mechanistic determinants or the biological effect of the parent or its metabolite(s). The choice of which metric to use as an appropriate measure should be based on some knowledge of the mechanism by which the toxic effects are induced.

This mechanistic knowledge does not necessarily have to be exhaustive, but can rather be related to certain general aspects of the nature and causes of a particular toxic interaction. For example, is the effect related to chemical reactivity or to occupancy of cellular receptor molecules? Is the effect associated with the parent or with a metabolite? If it is a metabolite, does the metabolite have a sufficiently long half-time in the body to circulate freely throughout the body or is it so reactive that it likely produces its damage locally? Are the effects themselves reversible cytotoxic phenomena or irreversible changes? Is there sufficient time for the target tissue to recover from the damage within the exposure frequency interval?

If the critical damaging toxicant-target interaction is caused by direct chemical reaction in which the toxicant reacts with and consumes cellular constituents, the degree of damage should be related to the time integral of tissue exposure to the reactive chemical (e.g., AUTC). This definition would likely need to incorporate quantitative information on the

synthesis and normal catabolism of the macromolecules involved to describe chronic exposures accurately. If the toxicant interacts with tissue by noncovalent binding to cellular receptor molecules, the response of the cell is dependent on the occupancy of the receptor and occupancy is determined by the binding constant for the chemical and the free concentration of the toxicant in the cell.

The use of categorization schemes based on the physicochemical properties or mechanisms of action of the inhaled toxicant have been proposed and different concepts of "dose" related to these (National Research Council, 1986; Andersen, 1987; O'Flaherty, 1989; Dahl, 1990). Considerations such as these are described in Section 3.2. and went into the development of the default dosimetry adjustments provided in the following Sections 4.3.5 through 4.3.7. Details on the development of the dosimetry models are provided in Appendices G, I and J. The default adjustments are determined categorically for particles versus gases, and within gases, for those more reactive and soluble than nonreactive and insoluble. 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. Because these are default dosimetry adjustments, the use of models that may incorporate a more comprehensive description of the exposure-dose-response continuum is considered the optimal approach for extrapolation to HECs when such a model is judged to provide a more accurate description. This judgment may be based on whether the structure of the alternative model is superior to that of the default, (e.g., incorporates known mechanistic determinants) or if it empirically results in a better correlation between "dose" and "effect". The reader is referred to Section 3.2 for a discussion of modeling comparative dosimetry and to Section 3.2.3 for summary considerations regarding judging model structures.

Use of more comprehensive models obviate the need for the duration adjustment described above in Section 4.3.2 because such models employ parameters that describe time-dependent determinants of toxicant disposition such as metabolic clearance, distribution volumes and elimination constants. These models can therefore be used to simulate both the experimental exposure regimen as well as the exposure scenario for the human. PBPK and linear pharmacokinetic models have both been used to evaluate and to adjust for different work place exposure durations (Droz, 1985; Andersen et al., 1987b; Saltzman, 1988). For example, in order to extrapolate laboratory animal data using a PBPK model, the laboratory

animal regimen (e.g., 6 h/day, 5 days/week) is simulated and the resultant appropriate dose metric (e.g., AUTC) calculated. This is done assuming steady-state conditions for chronic studies if it is likely that these conditions were met for 90% of the time (see Section 4.3.5), or the entire exposure can be simulated with the model. The model is then used with the human parameters to ascertain the exposure concentration that results in an equivalent dose metric under the human exposure scenario (e.g., 24 h/day). This exposure concentration back-extrapolated from the equivalent dose metric is the HEC.

4.3.4 Default Dosimetric Adjustment and Physiological Parameters

As described in Sections 3.2 and 4.3.3., the dosimetric adjustment factors described in in the following sections are default approaches to be used when more sophisticated or chemical-specific models are not available. The HEC is calculated with the default dosimetric adjustment factor as:

$$NOAEL^{\star}_{[HEC]}\ (mg/m^3) = NOAEL^{\star}_{[ADJ]}\ (mg/m^3) \times DAF_r \qquad (4\text{-}3)$$

where:

$NOAEL^{\star}_{[HEC]}$ is the NOAEL or analogous effect level obtained with an alternate approach as described in Appendix A, dosimetrically adjusted to an HEC,

$NOAEL^{\star}_{[ADJ]}$ is defined in Equation 4-2, and

DAF_r is a dosimetric adjustment factor for respiratory tract region, r (ET, TB, PU, TH, or TOTAL), either the regional deposited dose ratio ($RDDR_r$) for particles or the regional gas dose ratio ($RGDR_r$) for gases.

The DAF represents a multiplicative factor used to adjust an observed exposure concentration in a particular laboratory species to an exposure concentration for humans that would be associated with the same delivered dose. The calculation of the $RDDR_r$ for particles and the $RGDR_r$ for gases is described in section 4.3.5 and 4.3.6, respectively.

Depending on whether the observed toxicity is in the respiratory tract or at remote (extrarespiratory) sites, the DAF_r is used in conjunction with default normalizing factors for the physiological parameter of interest. Because insoluble particles deposit and clear along the surface of the respiratory tract, dose per unit surface area is a commonly used

normalizing factor for respiratory effects due to particulate deposition; body weight is often used to normalize dose to remote target tissues. In some cases, it may be appropriate to normalize by regional volumes or target organ weights. For gases, use of mass flux (mass per surface area-time) is considered a reasonably accurate predictor of the peak localized concentration driving the absorption gradient for respiratory tract effects. For example, if the observed toxicity is in the TB region, the dose deposited in that region for each species is normalized to the TB surface area for each species.

Default values of surface area (SA) for the various respiratory tract regions of five commonly tested animal species are provided in Table 4-4. Selection of the values was based on a meeting of experts in laboratory animal and human morphometric measurements convened in August 1991 (Jarabek, 1991). At that time, a thorough review of the literature had been conducted and the group was presented with summary tables of surface area measurements; animal information (as available) including strain, body weight, sex and age; tissue preparation, and morphometric measurement technique. Based on discussion among the expert group members, values were identified as most representative of a species and designated as the default. These values do not always correspond exactly to the published value that is cited in Table 4-4, most generally due to rounding.

TABLE 4-4. DEFAULT SURFACE AREA VALUES FOR RESPIRATORY EFFECTS[a]

	ET (cm^2)	Source	TB (cm^2)	Source	PU (m^2)	Source
Human	200.0	Guilmette et al. (1989)	3,200.0	Mercer et al. (1994a)	54.0	Mercer et al. (1994b)
Mouse	3.0	Gross et al. (1982)	3.5	Mercer et al. (1994a)	0.05	Geelhaar and Weibel (1971), Mercer et al. (1994b)
Hamster	14.0[b]		20.0	Yu and Xu (1987)	0.3	Lechner (1978)
Rat	15.0[c]	Gross et al. (1982)	22.5	Mercer et al. (1994a)	0.34	Mercer et al. (1994b)
Guinea Pig	30.0	Schreider and Hutchens (1980)	200.0	Schreider and Hutchens (1980)	0.9	Tenney and Remmers (1963)
Rabbit	30.0	Kliment (1973)	300.0	Kliment (1973)	5.9	Gehr et al. (1981)

[a]ET = Extrathoracic.
TB = Tracheobronchial.
PU = Pulmonary.
[b]No measurements of hamster ET surface area were found in the literature. This value is estimated based on similarity of the other regional surface areas to the rat.
[c]Additional unpublished measurements of the surface area beyond the ethmoid turbinates are included.

Body weight is the recommended normalizing factor for remote (extrarespiratory) effects. The default body weight values for the same five animal species are provided in Table 4-5. The body weight for the human is the weight used by the International Commission on Radiological Protection (Snyder et al., 1975) for the Reference Man. The values in Table 4-5 are taken from U.S. Environmental Protection Agency (1988a) and provide recommended values for body weights when evaluating subchronic or chronic studies in a variety of strains for each species. Often information on the strain used in a particular study can be obtained from the principal investigator in the rare event that it is not provided in the journal articles. If different strains are used than those in Table 4-5 and the body weight is reported, choose the strain with the most comparable body weight. Documents on recommended values for use in risk assessment (U.S. Environmental Protection Agency, 1988a) and for use in physiologically based models (U.S. Environmental Protection Agency, 1988b) are useful sources of default values for parameters such as ventilation rates and body weights for use in these equations when these values are not supplied in individual investigations. Available allometric equations (Adolph, 1949; Weibel, 1972; U.S. Environmental Protection Agency, 1988a), relating body size to the parameters of interest such as ventilatory rates and lung surface areas also may be appropriate. It must be emphasized that the use of default or derived values must be consistent with the dosimetric modeling parameters and approaches used in adjusting concentrations to human equivalent values, such as the parameters used to calculate the $RDDR_r$ and $RGDR_r$.

The default ventilation values for minute volume [$\dot{V}_E$ = tidal volume (V_T) $\times$ breathing frequency (f)] are calculated using the allometric scaling equations provided in U.S. Environmental Protection Agency (1988a). The general form for the equation is:

$$\log (\dot{V}_E) = b_o + b_1 \log(BW) \qquad (4\text{-}4)$$

where log refers to the natural logarithm, $\dot{V}_E$ is in L/min and body weight (BW) is in kg. The species specific parameters (b_0 and b_1) are listed in Table 4-6. At the present time, the default body weight for the human is defined to be 70 kg, and the $\dot{V}_E$ is defined to be (13.8 L/min) 20 m^3/day.

TABLE 4-5. BODY WEIGHT (kg) DEFAULT VALUES—RATS

Strain	Sex	Subchronic	Chronic
Fisher 344	F	0.124	0.229
Fisher 344	M	0.180	0.380
Sprague-Dawley	F	0.204	0.338
Sprague-Dawley	M	0.267	0.523
Long-Evans	F	0.179	0.344
Long-Evans	M	0.248	0.472
Osborne-Mendel	F	0.201	0.389
Osborne-Mendel	M	0.263	0.514
Wistar	F	0.156	0.297
Wistar	M	0.217	0.462

BODY WEIGHT (kg) DEFAULT VALUES—MICE

Strain	Sex	Subchronic	Chronic
B6C3F1	F	0.0246	0.0353
B6C3F1	M	0.0316	0.0373
BAF1	F	0.0204	0.0222
BAF1	M	0.0223	0.0261

BODY WEIGHT (kg) DEFAULT VALUES—HAMSTER

Strain	Sex	Subchronic	Chronic
Syrian	F	0.095	0.145
Syrian	M	0.097	0.134
Chinese and Djungarian	F	0.025	0.038
Chinese and Djungarian	M	0.03	0.041

BODY WEIGHT (kg) DEFAULT VALUES—GUINEA PIGS

Strain	Sex	Subchronic	Chronic
Not specified	F	0.39	0.86
Not specified	M	0.48	0.89

BODY WEIGHT (kg) DEFAULT VALUES—RABBITS

Strain	Sex	Subchronic	Chronic
New Zealand	F	3.10	3.93
New Zealand	M	2.86	3.76

Source: U.S. Environmental Protection Agency (1988a).

TABLE 4-6. INTERCEPT (b_0) AND COEFFICIENT (b_1) VALUES USED IN ALGORITHM (Equation 4-4) TO CALCULATE DEFAULT MINUTE VOLUMES BASED ON BODY WEIGHT

	b_o	b_1
Rat	-0.578	0.821
Mouse	0.326	1.050
Hamster	-1.054	0.902
Guinea pig	-1.191	0.516
Rabbit	-0.783	0.831

Source: U.S. Environmental Protection Agency (1988a).

4.3.5 Dosimetric Adjustments for Particle Exposures

Inhalation toxicologists have advanced their ability to measure the deposition of particles in the various regions of the respiratory tract across species. Initially the data were primarily total deposition values for polydisperse and sometimes unstable aerosols, but data now exist for insoluble monodisperse aerosols of different sizes under different breathing conditions (U.S. Environmental Protection Agency, 1982b). Data are available for many experimental species of interest on the regional deposition of aerodynamic particle size ranges and on the necessary physiologic parameters (e.g., ventilation parameters and regional surface areas) incorporated in dose adjustments (Overton et al., 1987; Miller et al., 1987b; Miller et al., 1988; Raabe et al., 1988; Patra et al., 1986; Patra, 1986). Deposition data are usually reported as the deposition fraction for each respiratory tract region of the species of interest. Deposition fraction is the ratio of the number or mass of particles deposited in the respiratory tract to the number or mass of particles inhaled. Deposition data also may be expressed as efficiencies, that is the amount deposited in a particular region normalized for the amount entering that region.

Knowledge also has been gained in the technology and methods for generating and characterizing aerosols. State-of-the-art inhalation toxicology studies characterize the particulate exposure by the particle diameter (e.g., aerodynamic equivalent diameter [d_{ae}], aerodynamic resistance diameter [d_{ar}], mass median aerodynamic diameter [MMAD]), and the geometric standard deviation (σ_g). Appendix H provides information on converting

reported particle units to those used in the calculation of the dosimetric adjustment factor and guidance on default values.

These advances in quantitation of species-specific regional respiratory tract deposition and characterization of physiologic parameters have been used in the development of an empirical model that accounts for dosimetry differences using deposition data typical for aerodynamic particles. This application is an adaptation (Miller et al., 1983b; Graham et al., 1985) and an extension (Miller et al., 1988; Jarabek et al., 1989, 1990) of previous work. A series of empirical equations were fit to experimental measurements of regional particle deposition in various laboratory species and humans as described in Appendix G. These equations are used to estimate fractional deposition and, in conjunction with normalizing factors such as body weight or surface area, are used to adjust for dosimetric differences between species in the calculation of an HEC. The approach is limited at this time to relatively insoluble and nonhygroscopic particles.

The derivation of the $NOAEL_{[HEC]}$ for insoluble, approximately spherical particles is described as

$$NOAEL^{\star}_{[HEC]}\ (mg/m^3) = NOAEL^{\star}_{[ADJ]}\ (mg/m^3) \times RDDR_r, \qquad (4\text{-}5)$$

where:

$NOAEL^{\star}_{[HEC]}$ = the NOAEL or analogous effect level obtained with an alternate approach as described in Appendix A, dosimetrically adjusted to an HEC;

$NOAEL^{\star}_{[ADJ]}$ = is defined in Equation 4-2; and

$RDDR_r$ = a multiplicative factor used to adjust an observed inhalation particulate exposure concentration of an animal (A) to the predicted inhalation particulate exposure concentration for a human (H) that would be associated with the same dose delivered to the r^{th} region or target tissue:

$$RDDR_r = \frac{(RDD_r/\text{Normalizing Factor})_A}{(RDD_r/\text{Normalizing Factor})_H}.$$

The *r regions* and potential target tissues identified by this calculation are the three respiratory tract regions (extrathoracic [ET], tracheobronchial [TB], or pulmonary [PU]).

Definitions of the three regions are provided in Chapter 3 of this document. The $RDDR_r$ can also be calculated for the thoracic (TH) region (TB plus PU regions) or the total (TOT) respiratory tract (all three respiratory tract regions). Total deposition (deposition summed for all three regions) is assumed to be available for transport to other organs and is used to calculate the RDDR for extrarespiratory (ER) effects.

It is frequently desirable to use a *normalizing factor* when comparing doses across species. Because insoluble particles deposit and clear along the surface of the respiratory tract, dose per unit surface area is a commonly used normalizing factor for particulate deposition in the respiratory tract. In some cases, it might be desirable to normalize by regional volumes, organ weight, or body weight. It might also be appropriate to examine the dose ratio with no normalizing factor. The appropriate normalizing factor to use may also be judged according to the guidance provided in Section 4.3.3 on the use of pharmacokinetic and pharmacodynamic data, with heed to the cautionary notes provided in the following sections.

Regional deposited dose (RDD_r) is estimated as

$$RDD_r = 10^{-6} \times C_i \times \dot{V}_E \times F_r, \tag{4-6}$$

where:

RDD_r = dose deposited in region r, mg/min,
C_i = concentration, mg/m^3,
$\dot{V}_E$ = minute volume, mL/min,
F_r = fractional deposition in region r.

The $RDDR_r$ may be expressed as a series of four ratios:

$$RDDR_r = \frac{(10^{-6} \times C_i)_A}{(10^{-6} \times C_i)_H} \times \frac{(\text{Normalizing Factor})_H}{(\text{Normalizing Factor})_A} \times \frac{(\dot{V}_E)_A}{(\dot{V}_E)_H} \times \frac{(F_r)_A}{(F_r)_H}. \tag{4-7}$$

For the purposes of calculating the $RDDR_r$, the exposure concentration for the laboratory animal (A) and human (H) are assumed to be the same because it is assumed that the observed effect in the laboratory animal is relevant to human health risk. Therefore, the $RDDR_r$ provides a factor to adjust for the difference in dose delivered to the target tissue

under the same exposure scenario. The first term in Equation 4-7, therefore, equals one and will not be discussed further.

The second term in Equation 4-7 is the ratio of the normalizing factors for the human and laboratory animal of interest. For effects in any or all of the three regions of the respiratory tract, surface area (see Table 4-4) is the recommended normalizing factor. To evaluate extrarespiratory effects, body weight (see Table 4-5) is the recommended normalizing factor. The third term of Equation 4-7 is the ratio of minute volumes (see Equation 4-4).

The final term in the $RDDR_r$ equation is the ratio of regional fractional deposition in laboratory animals and humans. By means of nonlinear regression, empirical equations have been fit using experimentally measured regional deposition in both laboratory animals and humans. Details on the estimation procedures are provided in Appendix G. These equations provide predictions for approximately spherical, nonhygroscopic, insoluble particles in the aerodynamic size range (particle diameter > 0.5 μm). Deposition fractions should not be calculated using these equations if the particles deviate enough from spherical that they are not reasonably described by an aerodynamic diameter (e.g., fibers) or if the particles are smaller than 0.5 μm (see Appendix H for a discussion of particle-related issues). Predicted deposition of hygroscopic particles may be approximated by these equations using the equilibrium particle size, if known. Other techniques to estimate fractional deposition are required for particles falling outside the assumptions of this empirical model.

The $RDDR_r$ is most easily calculated using the software available as a supplement to this document. For near monodisperse particles ($\sigma_g \leq 1.3$), deposition fractions may be calculated as described in Appendix G and the $RDDR_r$ calculated by hand. For polydisperse particles ($\sigma_g > 1.3$), however, deposition fractions are calculated by integrating the product of the monodisperse deposition probabilities and the log-normal distribution. This calculation must be done by computer. The software to perform the $RDDR_r$ calculation is written in C and will run on any DOS-based personal computer. A math coprocessor chip is not required. Figures 4-5 to 4-8 illustrate the four screens of the program. The first three figures show how the program display screens will look during data entry, while Figure 4-8 reproduces the RDDRs that would be calculated using these input data.

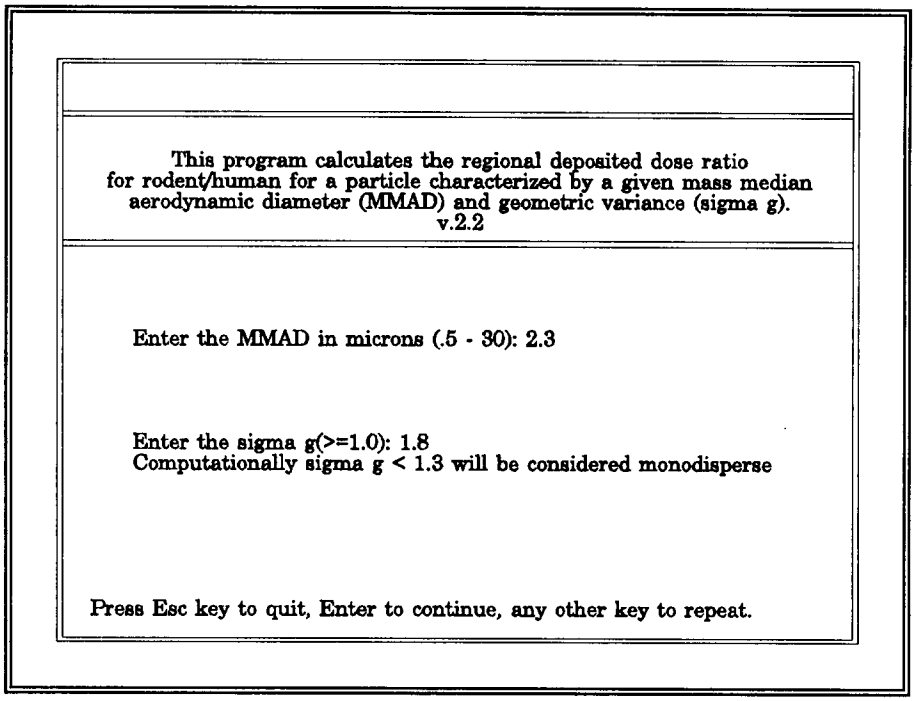

Figure 4-5. Display Screen 1 of the computer program that calculates regional deposited dose ratios.

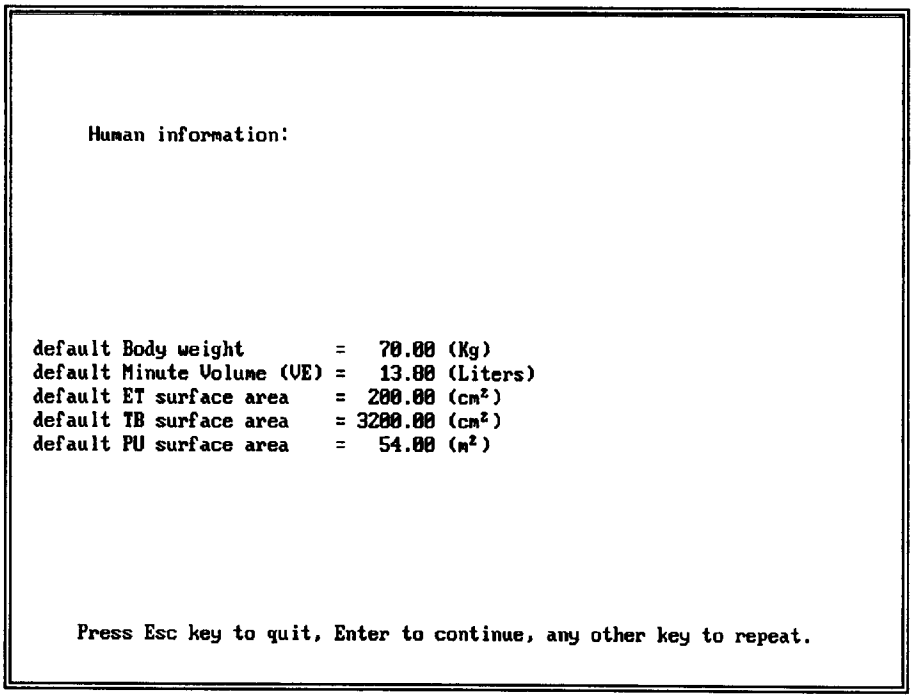

Figure 4-6. Display Screen 2 of the program that calculates regional deposited dose ratios.

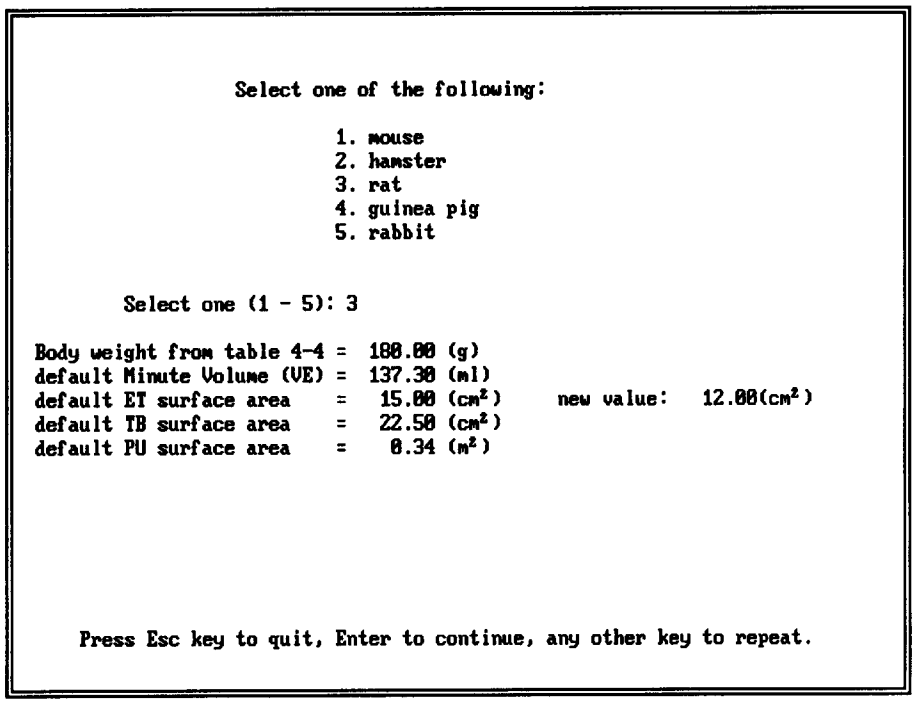

Figure 4-7. Display Screen 3 of the computer program that calculates regional deposited dose ratios.

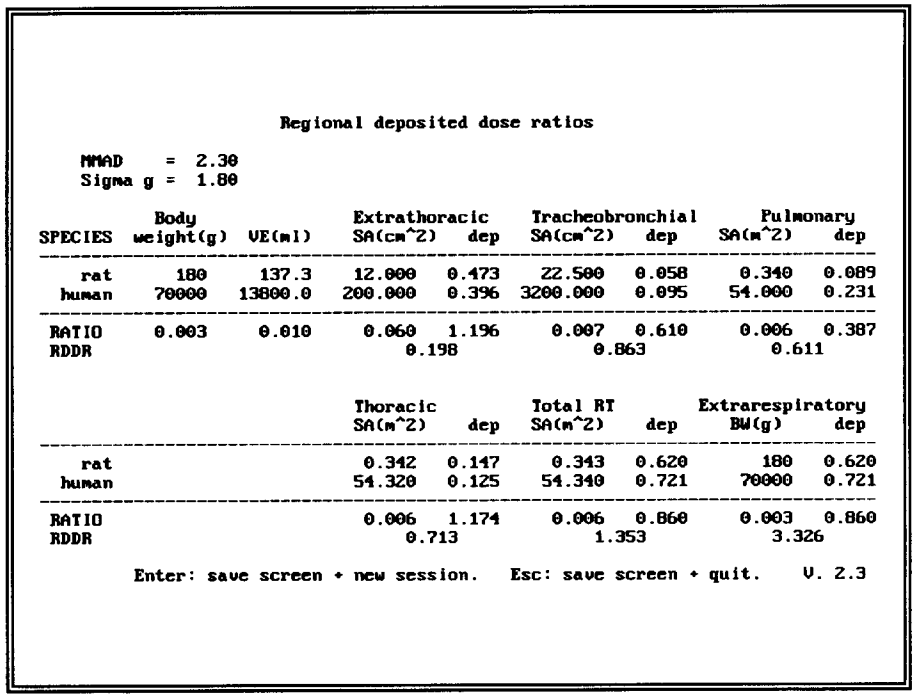

Figure 4-8. Display Screen 4 of the computer program that calculates regional deposited dose ratios.

To begin the program, type RDDR in upper or lower case letters. The program will then prompt you to enter the MMAD and σ_g for which you want to calculate an $RDDR_r$ (Figure 4-5). Although most studies do report particle sizes as aerodynamic diameter, some studies do not. **Using incorrect units in the program will result in incorrect estimates of the deposition fraction.** Particle size definitions, a discussion on conversion among units, and guidance on default values when there is inadequate information in a study to determine the MMAD and σ_g are provided in Appendix H.

The second screen (Figure 4-6) will print the default values for the minute volume, the three respiratory tract surface areas, and the body weight for the human. As each one is listed, the user has the option of changing the default value for the calculations. Although the software is written so that default values may be changed, it should be noted that body weight, surface areas, and minute volumes are all inter-related and should be changed so that all values are consistent with each other. It is also not recommended to make changes without being able to provide detailed documentation to support alternative values.

The third screen (Figure 4-7) provides a list of 5 animal species from which one must be selected. The body weight (selected from Table 4-5) must be entered. The program then calculates and lists the default minute volume and the default surface areas. Similar to the humans, any of these values may be changed if desired. The same cautions and caveats for changing human default values apply to the laboratory animals.

In the fourth screen (Figure 4-8), the input parameters are listed; the ratios described in Equation 4-7 are printed; and the calculated RDDRs are listed for the three respiratory tract regions, the thoracic region, the total respiratory tract and for extrarespiratory effects. This screen may be output to an ASCII file and printed using DOS commands. The "PRINT SCREEN" key will work for a stand-alone PC with its own local printer. Use of the "PRINT SCREEN" key with networks depends on how files are treated in the buffer.

The program may be run sequentially and calculations made by hand to determine an $RDDR_r$ based on a human activity pattern. First, the minute volumes to be included in the activity pattern and the fractional time spent at each minute volume must be determined. Then, the program must be run for each minute volume (keeping all other data the same—

MMAD, σ_g, and surface area for the human, and species, minute volume, and surface area for the animal). Then,

$$RDDR_{r_{[ACT]}} = \frac{a}{t_{[1]} \times \dot{V}_{E_{H[1]}} \times F_{r_{H[1]}} + t_{[2]} \times \dot{V}_{E_{H[2]}} \times F_{r_{H[2]}} + \ldots + t_{[n]} \times \dot{V}_{E_{H[n]}} \times F_{r_{H[n]}}} \quad (4\text{-}8)$$

where $t_{[i]}$ is the fractional time spent breathing minute volume [i],

$$t_{[1]} + t_{[2]} + \ldots + t_{[n]} = 1, \quad \text{and} \quad (4\text{-}9)$$

$$a = \frac{(SA_r)_H}{(SA_r)_A} \times \dot{V}_{E_A} \times F_{r_A}. \quad (4\text{-}10)$$

All of the needed values can be read from Screen 4. The calculated value, a, should have the same input values (i.e., surface areas, all animal input information) on each Screen 4 generated for the activity pattern, but the human values for deposition ($F_{r_{H[i]}}$) and minute volume ($\dot{V}_{E_{H[i]}}$) will be different.

Although the default normalizing factor used in the program for the respiratory tract RDDRs is surface area and the default normalizing factor for the extrarespiratory RDDR is body weight, there are situations in which an alternative normalizing factor might be appropriate. In this case, the deposition ratio and the $\dot{V}_E$ ratio from the 4th screen of the computer program (Figure 4-8) may be multiplied by hand calculations of the normalizing factor in humans divided by the normalizing factor in animals to determine the $RDDR_r$. Alternatively, when the default surface area values are listed in Screens 2 and 3 (Figures 4-6 and 4-7), they may be changed to the values of the new normalizing factor. A caveat when "tricking" the program this way is to pay attention to the units of the normalizing factor. In the program, ET and TB surface areas are entered in cm^2 while the PU surface area is entered in m^2. The program converts units internally when calculating the TH and total RDDRs. Unless the units of the proposed normalizing factor bear the same relationship to one another as the surface areas, the program calculated TH and total RDDRs with the alternative normalizing factor will be incorrect. At the present time, because $\dot{V}_E$ calculations depend on entering correct body weight data, if the program is "tricked" by

entering information other than body weight to estimate an extrarespiratory RDDR, then the correct $\dot{V}_E$ must be used to replace the program calculated "default" $\dot{V}_E$.

The next two sections provide a summary of the default values used for respiratory tract effects and extrarespiratory tract effects. As discussed above, details on estimation of the deposition fractions are described in Appendix G.

4.3.5.1 Respiratory Effects

The general dosimetric approach for insoluble particles outlined above provides the basis for estimating HECs. When the toxic effect of interest is in the respiratory tract, the equivalent dose across species is assumed to be the particle mass (mg) per minute per unit surface area (cm^2 or m^2) of the respiratory tract region of concern.

When the toxic effect of interest is in the respiratory tract, the normalizing factor described in Equation 4-7 should be replaced specifically by the surface area (SA) of the respiratory tract region of interest.

$$RDDR_r = \frac{(10^{-6} \times C_i)_A}{(10^{-6} \times C_i)_H} \times \frac{(SA_r)_H}{(SA_r)_A} \times \frac{(\dot{V}_E)_A}{(\dot{V}_E)_H} \times \frac{(F_r)_A}{(F_r)_H} \qquad (4\text{-}11)$$

The default surface area values are provided in Table 4-4.

It is preferable, when possible, to estimate the $RDDR_r$ for one of the three defined respiratory tract regions (ET, TB, or PU). Sometimes the nature of the effect or the detail of reporting precludes distinguishing between a TB and a PU effect so that an $RDDR_r$ for the TH region would be preferred, or it might be possible only to identify the region of interest as the entire respiratory tract. Either some aggregation must be used in calculating the $RDDR_r$, or the $RDDR_r$ for the region that results in the most conservative HEC could be selected. There are several techniques to aggregate the deposition information for calculation of TH or total respiratory tract RDDRs. The resulting RDDR can vary substantially, and in some cases the determination of which species is more sensitive (human or laboratory animal) may change. This is due to differences in fractional deposition (reflecting the complexities of the mechanisms governing deposition) in the different regions (see Chapter 3) and to the differences in regional surface areas, which may span several orders of magnitude. The

formula used to calculate the total respiratory tract RDDR ($RDDR_{TOT}$) is given below. Calculation of the thoracic RDDR ($RDDR_{TH}$) differs only in the exclusion of terms related to the ET region.

First, for each species, regional fractional deposition (F_r) per unit surface area (SA_r) is calculated and weighted by the percent of the respiratory tract (TH region) accounted for by that region.

$$\frac{F_{ET}}{SA_{ET}} \times \frac{SA_{ET}}{SA_{ET} + SA_{TB} + SA_{PU}} \tag{4-12}$$

Then, simplifying this expression and summing over the three (or two in the case of the calculation for the TH region) regions gives

$$\frac{F_{TOT}}{SA_{TOT}} = \frac{F_{ET} + F_{TB} + F_{PU}}{SA_{ET} + SA_{TB} + SA_{PU}}, \tag{4-13}$$

yielding

$$RDDR_{TOT} = \frac{(10^{-6} \times C_i)_A}{(10^{-6} \times C_i)_H} \times \frac{(SA_{TOT})_H}{(SA_{TOT})_A} \times \frac{(\dot{V}_E)_A}{(\dot{V}_E)_H} \times \frac{(F_{TOT})_A}{(F_{TOT})_H}. \tag{4-14}$$

4.3.5.2 Remote (Extrarespiratory) Effects

The respiratory tract might not be the target organ for an inhaled compound. The dose actually delivered to other regions of the body will be affected by metabolism, clearance, and distribution patterns. Particles depositing in the respiratory tract will clear rapidly (ET can be within seconds of inhalation) or slowly (PU clearance may take weeks or months) to the GI tract or be absorbed into the interstitium, lymphatics, or into the blood from the respiratory tract. Once deposited, however, very few particles will clear by exhalation (sneezing or coughing). Therefore, it is not unreasonable to estimate extrarespiratory deposition by total

deposition in the respiratory tract when information on dose delivered to nonrespiratory tract organs is unavailable.

The current default normalizing factor for extrarespiratory effects is body weight. In the case of extrarespiratory effects of particles, the equivalent dose across species is assumed to be the mass of particles (mg) deposited per unit body weight (kg). Until clearance and distribution parameters can be incorporated, it is assumed that 100% of the deposited dose to the entire respiratory system is available for uptake to the systemic circulation. Although this assumption will most likely result in an overestimate of the dose delivered to the extrarespiratory target tissue, it is not possible to predict a priori the impact on the dose ratio and resulting HEC (e.g., if the overestimate is of similar magnitude in both the laboratory species and human, the HEC will be relatively unaffected). Use of deposited dose is more accurate than using exposure concentration, however. Therefore, Equation 4-7 may be rewritten as:

$$RDDR_{ER} = \frac{(10^{-6} \times C_i)_A}{(10^{-6} \times C_i)_H} \times \frac{BW_H}{BW_A} \times \frac{(\dot{V}_E)_A}{(\dot{V}_E)_H} \times \frac{(F_{TOT})_A}{(F_{TOT})_H} \qquad (4\text{-}15)$$

The default values for body weight are shown in Table 4-5. The body weight for the human is the weight used by the International Commission on Radiological Protection (Snyder et al., 1975) for the Reference Man.

4.3.5.3 Additional Issues for Particle Dosimetry

The $RDDR_r$ for particle exposures consists of components to account for differences between species due to ventilation ($\dot{V}_E$), the dose metric (surface area, body weight, or other appropriate factor) and predicted regional deposition fractions. Although the use of this dosimetric adjustment provides a step towards more quantitative risk assessment, there are some limitations in the available data which, at present, preclude extension of the model to certain scenarios of interest for risk assessment. As additional information becomes available, the particle dosimetry equations will be refined and updated. This section discusses current recommendations for addressing particle dosimetry when the exposure information is outside the defined conditions for the model.

Hygroscopicity, Solubility, and Nonspherical Particles

The empirical equations used to estimate the predicted regional deposition fractions are derived from exposures using monodisperse, approximately spherical, nonsoluble, and nonhygroscopic particles. The cases outside the defined conditions for the equations include polydisperse particle size distributions, nonspherical particles, and soluble and/or hygroscopic particles. Also, Gerde et al. (1991) have shown that highly lipophilic chemicals and chemicals either absorbed or precipitated onto particles behave fundamentally differently and may require other modeling approaches.

As described in Appendix G, deposition fractions may be estimated for polydisperse spherical particles by integrating the monodisperse deposition fraction over the size distribution of polydisperse particle. The calculations made for this document assume a lognormal particle size distribution (Raabe, 1971). When particle size distribution for an exposure is reported as MMAD and σ_g, it may be assumed that the particle size distribution is described by the lognormal distribution (because MMAD and σ_g are the first two moments for a lognormal distribution). If exact size distribution information is given or the particles are described as coming from a different, well-parameterized distribution, then an exact calculation must be performed.

Nonspherical particles may be described in terms of their equivalent aerodynamic diameter and, if this information is provided, deposition fractions may be calculated as described in this chapter and Appendix G. Deposition fractions may not be calculated using these equations if an aerodynamic diameter is not provided.

Many particles are hygroscopic and/or soluble. Hygroscopic particles may change size, shape, and density as they traverse the warm, humid airways of the respiratory tract. Soluble particles might or might not undergo hygroscopic changes as they travel along the airways. Solubility will change the physicochemical interactions of the particle with the surface upon which it deposits. Hygroscopicity is a phenomenon related to deposition whereas solubility is related to clearance. This discussion, therefore, will focus on hygroscopicity and its potential effects on predicted fractional deposition.

The RDD of a hygroscopic aerosol will often be different from that of nonhygroscopic particles, although both had similar size distributions upon inhalation (Martonen et al., 1985). The factors influencing changes in inhaled hygroscopic particle characteristics are being

studied experimentally and through development and analysis with complex theoretical models (Martonen and Patel, 1981; Martonen, 1982; Ferron and Hornik, 1984; Martonen et al., 1985; Eisner et al., 1990), but application in risk assessment awaits definition of the primary factors influencing hygroscopic growth on species- and agent-specific bases. The factors include initial particle geometry and density, material hygroscopic growth characteristics, respiratory parameters, and temperature and relative humidity profiles. Observations on the data from modeling efforts to date indicate that hygroscopic particles in the diffusion-dominated regime have reduced TH deposition relative to nonhygroscopic particles of identical preinspired size, whereas those hygroscopic particles affected by inertial and gravitational forces have an increase in TH deposition relative to nonhygroscopic particles (Martonen et al., 1985). These observations may be explained by changes in the particle size after inspiration. Accordingly, the calculated deposition efficiency for nonhygroscopic particles would underestimate the TH deposited dose for the larger (affected by inertial and gravitational forces) hygroscopic particles, and overestimate the deposited dose for the smaller diffusion-dependent hygroscopic particles. The TH deposited dose of inhaled nonhygroscopic particles, however, is always less than the initial total dose (exposure dose). Also, the relative changes in deposition will be in a similar direction in experimental animal species and humans. Dosimetric adjustment by the default insoluble (nonhygroscopic) empirical deposition equations is recommended as a conservative default for the hydroscopic particles, pending modification by the elucidation of the hygroscopic models.

Ventilation

It is recognized that this approach is based on deposition efficiency data obtained or derived under a particular set of ventilatory parameters (i.e., the experimental parameters for the laboratory animals and human subjects), coupled with default ventilation parameters ($\dot{V}_E$). The assumption in this application is that it is valid to linearly extrapolate from these experimental values to the default sets of ventilation parameters. The validity of this assumption is being investigated. The effect of activity pattern on ventilation and the allometric relationships between lung weight, lung surface area, and body weight have been investigated (Adolph, 1949; Weibel, 1972; U.S. Environmental Protection Agency, 1988a;

1993b; Federal Register, 1992b). A discussion of the impact that breathing pattern has on the human deposition estimates can be found elsewhere (Miller et al., 1988).

Differences Between Experimental and Ambient Exposures

The human ambient exposure scenario, when known, may be characterized by a different MMAD and σ_g than that used to derive the health risk assessment. Comparisons between ratios calculated with a MMAD and σ_g the same as the animal exposure and calculated with the human estimate using the anticipated ambient MMAD and σ_g may provide some insight on the uncertainty of this extrapolation.

Clearance and Retention

In addition to inspired air concentration, $\dot{V}_E$, surface area, and deposition efficiency, the effective dose of inhaled particulate matter will vary with bioavailability. The fraction of particulate matter dissolved and assumed to be bioavailable can be expected to increase with greater particle solubility, as well as with longer residence time in the lungs. Until clearance and distribution parameters can be systematically incorporated, 100% of the deposited dose to the entire respiratory tract is assumed to be available for uptake to the systemic circulation. As discussed, this assumption will most likely result in an overestimate of the dose delivered to the extrarespiratory target tissue, although it is not possible to predict a priori the impact on the dose ratio and resultant HEC. Use of deposited dose is more accurate than using exposure concentration, however. Models have recently been used to simulate clearance and estimate retention in various species (Snipes, 1989a,b; Yu and Yoon, 1990). The EPA has recognized the importance of incorporating clearance components to its dosimetric adjustments in order to calculate regional retained dose ratios (RRDRs) for estimates of long-term lung burdens, but such models for classes of particles and different species used in testing are not fully developed. In those cases where clearance and distribution have been experimentally determined and a validated model exists, the more comprehensive model should be used. For example, the model of Yu and Yoon (1990) was used to calculate the HEC for diesel engine emissions (IRIS, 1992).

Population Variability

The calculation of an $RDDR_r$ currently uses point estimates for all the terms in Equation 4-7 and its variants; that is, a default $\dot{V}_E$ for each species, a default regional surface area, and an estimate of fractional deposition. These single values are assumed to be representative of the average value of that term for a member of the laboratory animal species or human population. In fact, as discussed in Chapter 3, there are many sources of intraspecies variability that contribute to the range of responses observed to a given external exposure to an inhaled toxicant. Host factors affect both the delivered dose of the toxicant to the target tissue and the sensitivity of that tissue to interaction with the toxicant. The procedures described in the preceding sections of this chapter on particle dosimetry provide some limited capabilities to examine the effects of population variability on the $RDDR_r$ by simply changing the default $\dot{V}_E$ and surface areas in an iterative fashion. As indicated in these sections and in Appendix G, however, because of the correlations between $\dot{V}_E$, surface area, and body weight, such changes should be made with extreme caution. Although the point estimates of the parameters used to predict deposition efficiencies (details in Appendix G) are used to calculate fractional regional deposition, the empirical model also provides estimates of variability that can be used to generate confidence intervals reflective of population variability. Using iterative computational procedures, it is possible to generate envelopes of regional fractional deposition that can be used with distributions of $\dot{V}_E$, surface areas, and body weights to provide ranges of $RDDR_r$s. Actual implementation of this procedure is not straightforward due to the complex nature of the correlation structures. In future versions of the dosimetric model used to calculate $RDDR_r$, it should be possible to estimate a distribution for the $RDDR_r$ reflective of population variability in both laboratory animals and humans.

Susceptible Subpopulations

The data used to estimate regional fractional deposition are based on experimental measurements made in healthy laboratory animals and humans breathing under normal or approximately normal conditions. It is recognized that deposition patterns might vary in potentially susceptible subpopulations such as children, the elderly, or people with respiratory diseases (see Chapter 2). Limited data are available at present for fitting deposition

efficiency equations for any of these subpopulations. If it is assumed that the same efficiency relationships may be used, then the model may be used to examine predicted RDDRs (in healthy children, for example) by scaling surface area and ventilation for size. This approach is consistent with deterministic models of deposition in which airway geometry and ventilation are scaled to children's dimensions, but the mechanisms of deposition are unchanged. Although the approaches are consistent, the predicted deposition patterns might vary with measured data.

4.3.6 Dosimetric Adjustments for Gas Exposures

The approach described in Section 4.3.5 for the insoluble particles illustrates the feasibility of interspecies dosimetry calculations to extrapolate the toxicological results of inhaled toxicants to human exposure conditions for dose-response evaluation. Dosimetry data facilitate evaluation of concentration-response data with respect to dose-response relationships. As described in Section 3.2, predictive physiologically-based modeling for relatively insoluble and reactive gases has been demonstrated (Overton and Miller, 1988). Predictive physiologically based modeling has also been demonstrated for gases and vapors of organic solvents that may be metabolically activated (Fiserova-Bergerova, 1983; Andersen et al., 1987a; Overton, 1989), and for reactive and soluble gases (Aharonson et al., 1974; Morgan and Frank, 1977; Hanna et al., 1989; Casanova et al., 1991; Morris and Blanchard, 1992). As discussed in Section 3.2.2, the chemical-specific or class-specific nature of these models has been dictated by the physicochemical characteristics of the subject gases and no single model structure is applicable to the broad range of gases that the RfC methodology must address. A gas categorization scheme was thus developed as a way to create separation between types of gases so that model structures for each type could be developed. The scheme developed in Section 3.2.2 should be used to categorize the type of gas for dosimetric adjustment. The derivation of the model structure and its reduction to a form with a minimal number of parameters as the basis of the default dosimetry adjustments for gases in Category 1 and 2 are presented in Appendix I. The model structure and basis for the default adjustment for gases in Category 3 are presented in Appendix J. The reader is referred to these sections for proper understanding of the framework of default dosimetric equations presented herein.

Consideration also should be given to the discussion by the National Research Council (1986) and Dahl (1990) on interspecies extrapolation based on mechanism of action. Three classes of mechanism were distinguished based on whether the parent compound, stable metabolite, or reactive metabolite produces the toxic effect; measures of dose for each of these classes were suggested. These factors are often species-specific and dose-dependent, as well as being chemical-specific and, therefore, require a substantial data base (beyond that which exists in most circumstances) in order to model comparative species dosimetry of gases based on mechanism of action. O'Flaherty (1989) presented a framework within which to consider measures of delivered dose and discusses procedures for interspecies conversion of kinetically equivalent doses. Identification of the limiting anatomic and physiologic parameters, physicochemical characteristics, and exposure concentration and duration conditions will facilitate the application of these factors to improve the interspecies default dose adjustments. This understanding can also be used to gauge the appropriation of the default adjustments on a case-by-case basis.

Basically, the $RGDR_r$ is used as the DAF_r in Equation 4-3 to dosimetrically adjust the experimental NOAEL to an HEC as

$$NOAEL^{\star}_{[HEC]}\ (mg/m^3) = NOAEL^{\star}_{[ADJ]}\ (mg/m^3) \times RGDR_r, \qquad (4\text{-}16)$$

where:

$NOAEL^{\star}_{[HEC]}$ = the NOAEL or analogous effect level obtained with an alternative approach as described in Appendix A, dosimetrically adjusted to an HEC;

$NOAEL^{\star}_{[ADJ]}$ = is defined in Equation 4-2; and

$RGDR_r$ = $(RGD)_A/(RGD)_H$, the ratio of regional gas dose in laboratory animal species to that of humans for region (r) of interest for the toxic effect.

The default equations to derive the $RGDR_r$ for the different gas categories according to toxicity in the respiratory tract versus remote sites follow in Section 4.3.6.1 and 4.3.6.2, respectively. Because the boundaries between the categories are not definitive (see discussion in Section 3.2.2 and Appendix I), but instead were made to allow derivation of default model

structures, identification of the target effect(s) is used to further define the gas category. Thus, remote (extrarespiratory) effects of Category 1 gases and respiratory effects of Category 3 gases are treated according to the default dosimetric adjustments for each of these respective effects of Category 2 gases (Section 4.3.5 and 4.3.6). The default dosimetric adjustments to derive HEC values for respiratory effects of Category 1 gases are provided in Section 4.3.5. The default dosimetric adjustment to derive HEC values for extrarespiratory effects of Category 3 gases is provided in Section 4.3.6. Note that the gas categorization scheme dose not apply to inert gases that exert their effects by reversible "physical" interactions of gas molecules with biomolecules (e.g., "displacement" of oxygen by carbon dioxide or narcosis by some parent compounds). Consideration of the inert gases is discussed in Section 2.1.2.3.

4.3.6.1 Respiratory Effects

The two categories of gases with the greatest potential for respiratory tract effects are gases in Category 1 and 2. Category 1 gases are defined as gases that are highly water-soluble and/or rapidly irreversibly reactive in the respiratory tract. Reactivity is defined to include both the propensity for dissociation as well as the ability to serve as substrate for metabolism in the respiratory tract. Gases in Category 2 are defined as gases that are moderately water-soluble that may be rapidly reversibly reactive or moderately to slowly irreversibly reactive in respiratory tract tissue. Examples of gases in Category 1 are hydrogen fluoride, chlorine, formaldehyde, and the organic acids and esters. Examples of gases in Category 2 are ozone, sulfur dioxide, xylene, propanol, and isoamyl alcohol.

Respiratory Effects—Category 1 Gases

Category 1 gases are distinguished by the property that the gas does not significantly accumulate in the blood which would reduce the concentration driving force into the respiratory tract tissue and hence reduce the absorption rate. This characteristic allowed the default approach to be developed based on the integration of attributes of two empirical models as discussed in Appendix I. The approach takes into account the loss of chemical in the airstream to the upper respiratory tract as it progresses to the lower respiratory tract and

separate equations are provided to calculate dose in each region. The rationale and full derivation of the equations is provided in Appendix I.

Extrathoracic Effects. For Category 1 gases that have an effect in the upper respiratory tract, the following equation is used to calculate the ET regional gas dose ratio ($RGDR_{ET}$).

$$RGDR_{ET} = \frac{(Dose_{ET})_A}{(Dose_{ET})_H} = \frac{(\frac{\dot{V}_E}{SA_{ET}})_A(1 - e^{\frac{-K_{g_{ET}}SA_{ET}}{\dot{V}_E}})_A}{(\frac{\dot{V}_E}{SA_{ET}})_H(1 - e^{\frac{-K_{g_{ET}}SA_{ET}}{\dot{V}_E}})_H}, \quad (4\text{-}17)$$

where:

$\dot{V}_E$ = minute volume (mL/min = cm^3/min),
S_{ET} = surface area of the extrathoracic region (cm^2), and
$K_{g_{ET}}$ = overall mass transport coefficient in the extrathoracic region (cm/min).
A, H = subscripts denoting laboratory animal and human, respectively.

When the overall mass transport coefficient in the ET region ($K_{g_{ET}}$) is not known or can not be reasonably approximated with experimental data for either species, the following equation is used to calculate the default $RGDR_{ET}$ (see Section I.2.4.1):

$$RGDR_{ET} = \frac{(Dose_{ET})_A}{(Dose_{ET})_H} \cong \frac{(\frac{\dot{V}_E}{SA_{ET}})_A}{(\frac{\dot{V}_E}{SA_{ET}})_H}. \quad (4\text{-}18)$$

Tracheobronchial Effects. For Category 1 gases that affect the lower respiratory tract, the scrubbing in the upper airways of the chemical is taken into account, and the concentration of the air exiting the ET region is used in the derivation of dose to the TB region.

The following equation is used to calculate the TB regional gas dose ratio ($RGDR_{TB}$):

$$RGDR_{TB} = \frac{(Dose_{TB})_A}{(Dose_{TB})_H} = \frac{\left(\frac{\dot{V}_E}{SA_{TB}}\right)_A}{\left(\frac{\dot{V}_E}{SA_{TB}}\right)_H} \quad \frac{(fp_{ET})\ A}{(fp_{ET})\ H} \quad \frac{(1 - e^{\frac{-K_{g_{TB}} SA_{TB}}{\dot{V}_E}})_A}{(1 - e^{\frac{-K_{g_{TB}} SA_{TB}}{\dot{V}_E}})_H}, \qquad (4\text{-}19)$$

where:

SA_{TB} = surface area of the tracheobronchial region (cm^2),

$K_{g_{TB}}$ = overall mass transport coefficient in the tracheobronchial region (cm/min), and

fp_{ET} = the fraction of inhaled chemical concentration penetrating the ET region and thereby available for uptake in the TB region, calculated as

$$fp_{ET} = e^{-\frac{K_{g_{ET}} SA_{ET}}{\dot{V}_E}}. \qquad (4\text{-}20)$$

If the penetration fraction is unknown due to the lack of data on $K_{g_{TB}}$, it is reasonable to assume that K_g is large, which is consistent with the definition of Category 1 gases, such that the exponential term of Equation 4-19 reduces to zero. The same result may be achieved by determining the conditions in which the third ratio of the right hand side of Equation 4-19 reduces to 1. These conditions will be a function of the default values for respiratory tract surface area and minute volume as well as the absolute value of the overall mass transport coefficient. Using the definition of fp_{ET} results in the following dose ratio:

$$RGDR_{TB} = \frac{(RGD_{TB})_A}{(RGD_{TB})_H} = \frac{\left[\frac{\dot{V}_E}{SA_{TB}}\right]_A}{\left[\frac{\dot{V}_E}{SA_{TB}}\right]_H} \quad \frac{(e^{-K_{g_{ET}}\frac{SA_{ET}}{\dot{V}_E}})_A}{(e^{-K_{g_{ET}}\frac{SA_{ET}}{\dot{V}_E}})_H}. \tag{4-21}$$

When the overall mass transport coefficient in the extrathoracic region ($K_{g_{ET}}$) is not known or can not be reasonably approximated with experimental data for either species, $K_{g_{ET}}$ is further assumed to be one, and Equation 4-21 reduces further such that only minute volume and surface areas are needed to evaluate the dose ratio:

$$RGDR_{TB} = \frac{(RGD_{TB})_A}{(RGD_{TB})_H} = \frac{\left[\frac{\dot{V}_E}{SA_{TB}}\right]_A}{\left[\frac{\dot{V}_E}{SA_{TB}}\right]_H} \quad \frac{\left[e^{-\frac{SA_{ET}}{\dot{V}_E}}\right]_A}{\left[e^{-\frac{SA_{ET}}{\dot{V}_E}}\right]_H}. \tag{4-22}$$

If $K_{g_{ET}}$ is available for each species, Equation 4-21 would be the preferred default equation.

Pulmonary Effects. The gas concentration that reaches the PU region was affected by the amount of uptake in both the ET and TB regions so that the derivation for the PU gas dose ratio ($RGDR_{PU}$) incorporates the penetration fraction both for the ET and TB regions, respectively. The following equation is used to calculate $RGDR_{PU}$:

$$RGDR_{PU} = \frac{(Dose_{PU})_A}{(Dose_{PU})_H} = \frac{(\frac{K_{g_{PU}} SA_{PU}}{K_{g_{PU}} SA_{PU} + \dot{Q}_{alv}})_A}{(\frac{K_{g_{PU}} SA_{PU}}{K_{g_{PU}} SA + \dot{Q}_{alv}})_H} \frac{(\frac{\dot{Q}_{alv}}{SA_{PU}})_A}{(\frac{\dot{Q}_{alv}}{SA_{PU}})_H} \frac{(fp_{TB})_A}{(fp_{TB})_H} \frac{(fp_{ET})_A}{(fp_{ET})_H}, \tag{4-23}$$

where:

$\dot{Q}_{alv}$ = alveolar ventilation rate (mL/min = cm^3/min),

SA_{PU} = surface area of the pulmonary region (cm^2),

K_{gPU} = overall mass transport coefficient in the pulmonary region (cm/min),

fp_{ET} = the fraction of inhaled chemical concentration penetrating the extrathoracic region and thereby available for uptake in the tracheobronchial region, calculated as in Equation 4-20, and

fp_{TB} = the fraction of inhaled chemical concentration penetrating the tracheobronchial region and thereby available for uptake in the pulmonary region, calculated as

$$fp_{TB} = \frac{CX_{TB}}{CX_{ET}} = e^{\frac{-K_{gTB}\, SA_{TB}}{\dot{V}_E}} \tag{4-24}$$

where:

CX_{ET} = the concentration exiting the extrathoracic region, and

CX_{TB} = the concentration exiting the tracheobronchial region.

At large $K_{g_{PU}}$ values, Equation 4-23 reduces to

$$RGDR_{PU} = \frac{(RGD_{PU})_A}{(RGD_{PU})_H} = \frac{\left[\frac{\dot{Q}_{alv}}{SA_{PU}}\right]_A}{\left[\frac{\dot{Q}_{alv}}{SA_{PU}}\right]_H} \frac{(fp_{TB})_A}{(fp_{TB})_H} \frac{(fp_{ET})_A}{(fp_{ET})_H}. \tag{4-25}$$

If the penetration fractions to each of the preceding regions are unknown due to lack of data on $K_{g_{ET}}$ and $K_{g_{TB}}$, the approach to deriving a default equation for the PU region is described below.

Using the definition of fp_{ET} and fp_{TB} results in the following PU region gas dose ratio:

$$RGDR_{PU} = \frac{(RGD_{PU})_A}{(RGD_{PU})_H} = \frac{(\frac{\dot{Q}_{alv}}{SA_{PU}})_A}{(\frac{\dot{Q}_{alv}}{SA_{PU}})_H} \frac{(e^{-K_{gTB}\frac{SA_{TB}}{\dot{V}_E}})_A}{(e^{-K_{gTB}\frac{SA_{TB}}{\dot{V}_E}})_H} \frac{(e^{-K_{gET}\frac{SA_{ET}}{\dot{V}_E}})_A}{(e^{-K_{gET}\frac{SA_{ET}}{\dot{V}_E}})_H}, \quad (4\text{-}26)$$

which can be rearranged to

$$RGDR_{PU} = \frac{RGD_{PU})_A}{(RGD_{PU})_H} = \frac{\left[\frac{\dot{Q}_{alv}}{SA_{PU}}\right]_A}{\left[\frac{\dot{Q}_{alv}}{SA_{PU}}\right]_H} \frac{\left[e^{\frac{-SA_{TB}}{\dot{V}_E}}\right]_A^{(K_{gTB})_A}}{\left[e^{\frac{SA_{TB}}{\dot{V}_E}}\right]_H^{(K_{gTB})_H}} \frac{\left[e^{\frac{-SA_{ET}}{\dot{V}_E}}\right]_A^{(K_{gET})_A}}{\left[e^{\frac{-SA_{ET}}{\dot{V}_E}}\right]_H^{(K_{gET})_H}}. \quad (4\text{-}27)$$

If $(K_{g_{ET}})_A$ and $(K_{gTB})_A$ are assumed to be equal to $(K_{g_{ET}})_H$ and $(K_{gTB})_H$, respectively, then Equation 4-27 can be further simplified to

$$RGDR_{PU} = \frac{(RGD_{PU})_A}{(RGD_{PU})_H} = \frac{\left[\frac{\dot{Q}_{alv}}{SA_{PU}}\right]_A}{\left[\frac{\dot{Q}_{alv}}{SA_{PU}}\right]_H} \frac{\left[e^{-\frac{SA_{TB}}{\dot{V}_E}}\right]_A^{K_{gTB}}}{\left[e^{-\frac{SA_{TB}}{\dot{V}_E}}\right]_H^{K_{gTB}}} \frac{\left[e^{-\frac{SA_{ET}}{\dot{V}_E}}\right]_A^{K_{gET}}}{\left[e^{-\frac{SA_{ET}}{\dot{V}_E}}\right]_H^{K_{gET}}} \quad (4\text{-}28)$$

If it is further assumed that the value of K_g is equal to 1 for each region, the resulting default equation (Equation 4-28) reduces to an equation requiring only surface area and $\dot{V}_E$ parameters. It should be noted that as comparative transport studies become available, Equation 4-27 would be preferable because it includes the differences in mass transport in each region for each species.

Respiratory Effects—Category 2 Gases

Category 2 or "transitional" gases have the potential for significant accumulation in the blood and thus have the potential for both respiratory and remote (extrarespiratory) toxicity. The accumulation in the blood will reduce the concentration driving force during inspiration and thereby reduce the absorption rate or dose upon inhalation. They also have the potential for significant desorption during exhalation. The model structure used as the basis for the default dosimetric adjustment for Category 1 gases was insufficient for addressing this property and a hybrid structure between that for Category 1 and Category 3 gases was constructed. The rationale and full derivation of the equations is provided in Appendix I. The default dosimetric adjustments for respiratory tract effects of Category 2 gases is presented below and those for dosimetric adjustment of remote toxicity are provided in Section 4.3.6.2.

Extrathoracic Effects. For Category 2 gases, the ET regional gas dose ratio ($RGDR_{ET}$) is given by

$$RGDR_{ET} = \frac{(RGD_{ET})_A}{(RGD_{ET})_H} = \frac{(C_i \frac{\dot{V}_E}{SA_{ET}})_A}{(C_i \frac{\dot{V}_E}{SA_{ET}})_H} \frac{(1-\frac{C_{b/g}}{C_i})_A}{(1-\frac{C_{b/g}}{C_i})_H} \frac{(1 - e^{-K_{g_{ET}} \frac{SA_{ET}}{\dot{V}_E}})_A}{(1 - e^{-K_{g_{ET}} \frac{SA_{ET}}{\dot{V}_E}})_H} \qquad (4\text{-}29)$$

where:

C_i = inhaled concentration ($mg/cm^3 = 10^{-6}$ mg/m^3), and

$C_{b/g}$ = gas concentration in equilibrium with blood concentration (mg/m^3).

However, $K_{g_{ET}}$ for Category 2 gases is by definition less than 1. Assuming $K_{g_{ET}}$ is equal to or less than 0.5, a power series expansion of the exponential term results in the following relationship:

$$RGDR_{ET} = \frac{(RGD_{ET})_A}{(RGD_{ET})_H} = \frac{(C_i \frac{\dot{V}_E}{SA_{ET}})_A}{(C_i \frac{\dot{V}_E}{SA_{ET}})_H} \frac{(1 - \frac{C_{b/g}}{C_i})_A}{(1 - \frac{C_{b/g}}{C_i})_H} \frac{(-K_{g_{ET}} \frac{SA_{ET}}{\dot{V}_E})_A}{(-K_{g_{ET}} \frac{SA_{ET}}{\dot{V}_E})_H} . \quad (4\text{-}30)$$

Assuming the same inspired concentration, simplifies the $RGDR_{ET}$ to

$$RGDR_{ET} = \frac{(RGD_{ET})_A}{(RGD_{ET})_H} = \frac{K_{g_{ETA}}}{K_{g_{ETH}}} \frac{(1 - \frac{C_{b/g}}{C_i})_A}{(1 - \frac{C_{b/g}}{C_i})_H} . \quad (4\text{-}31)$$

If the overall mass transport coefficients ($K_{g_{ET}}$) are assumed equal as in the case of Category 1 gases, the $RGDR_{ET}$ is reduced to the ratio of the blood term $(1 - C_{b/g}/C_i)$.

Two cases were developed for the derivation of the blood term (see Appendix I). The first case assumes systemic elimination is much greater than respiratory tract metabolism such that

$$RGDR_{ET} = \frac{(RGD_{ET})_A}{(RGD_{ET})_H} = \frac{K_{g_{ETA}}}{K_{g_{ETH}}} \frac{(0.25 \dot{Q}_T H_{b/g})_A}{(0.25 \dot{Q}_T H_{b/g})_H} , \quad (4\text{-}32)$$

and the second case where the respiratory tract metabolism is of equal significance to systemic elimination such that

$$RGDR_{ET} = \frac{(RGD_{ET})_A}{(RGD_{ET})_H} = \frac{K_{g_{ETA}}}{K_{g_{ETH}}} \frac{(0.5 \dot{Q}_T H_{b/g})_A}{(0.5 \dot{Q}_T H_{b/g})_H} , \quad (4\text{-}33)$$

where E_{MAX}, the maximum extraction efficiency, is equal to 0.25 $\dot{Q}_T$ and $H_{b/g}$ is the blood:gas (air) partition coefficient of the chemical. Because the constants are equal in the numerator and denominator, Equations 4-32 and 4-33 reduce to the same equation. Thus, the default regional gas dose ratio for ET effects of Category 2 gases is:

$$RGDR_{ET} = \frac{(RGD_{ET})_A}{(RGD_{ET})_H} = \frac{K_{g_{ETA}}}{K_{g_{ETH}}} \frac{(\dot{Q}_T H_{b/g})_A}{(\dot{Q}_T H_{b/g})_H} . \quad (4\text{-}34)$$

Equation 4-34 can be further reduced by the assumption that the overall mass transport coefficients ($K_{g_{ET}}$) are equal when these values are not available. The value of 1.0 is used for the ratio of $(H_{b/g})_A$ / $(H_{b/g})_H$ if $(H_{b/g})_A > (H_{b/g})_H$ or if these partition coefficient values are unknown. Gargas et al. (1989) and Jepson et al. (1994) provide discussion of techniques to derive partition coefficients and report values for volatile and nonvolatile chemicals, respectively.

Tracheobronchial Effects. The TB regional gas dose ratio ($RGDR_{TB}$) for Category 2 gases is given by

$$RGDR_{TB} = \frac{(RGD_{TB})_A}{(RGD_{TB})_H} = \frac{(C_i \frac{\dot{V}_E}{SA_{TB}})_A}{(C_i \frac{\dot{V}_E}{SA_{TB}})_H} \frac{(e^{-K_{g_{ET}} \frac{SA_{ET}}{\dot{V}_E}})_A}{(e^{-K_{g_{ET}} \frac{SA_{ET}}{\dot{V}_E}})_H} \frac{(1 - \frac{C_{b/a}}{C_i})_A}{(1 - \frac{C_{b/a}}{C_i})_H} \frac{(1 - e^{-K_{g_{TB}} \frac{SA_{TB}}{\dot{V}_E}})_A}{(1 - e^{-K_{g_{TB}} \frac{SA_{TB}}{\dot{V}_E}})_H} . \quad (4\text{-}35)$$

As in the ET region, $K_{g_{TB}}$ for Category 2 gases is by definition less than 1 and a power series expansion of the exponential term for the TB region similarly reduces the last term on the right hand side to the animal-to-human ratio of $K_{g_{TB}}$ $(SA_{TB}/\dot{V}_E)$. The exponential term for the ET term in Equation 4-35 is reduced by assuming $K_{g_{ET}}$ is the same for each species as was assumed for Category 1 gases. At values of $K_{g_{ET}}$ less than or equal 0.5, the

ET exponential term approaches one. Thus, assuming the same inspired concentrations, Equation 4-35 becomes

$$RGDR_{TB} = \frac{(RGD_{TB})_A}{(RGD_{TB})_H} = \frac{K_{g_{TB}A}}{K_{g_{TB}H}} \frac{(1 - \frac{C_{b/a}}{C_i})_A}{(1 - \frac{C_{b/a}}{C_i})_H} . \quad (4\text{-}36)$$

As above for the ET region, the case in which systemic elimination predominates is given by:

$$RGDR_{TB} = \frac{(RGD_{TB})_A}{(RGD_{TB})_H} = \frac{K_{g_{TB}A}}{K_{g_{TB}H}} \frac{(0.25\,\dot{Q}_T H_{b/a})_A}{(0.25\,\dot{Q}_T H_{b/a})_H} , \quad (4\text{-}37)$$

and the case in which respiratory tract metabolism and systemic elimination are of equal significance is given by:

$$RGDR_{TB} = \frac{(RGD_{TB})_A}{(RGD_{TB})_H} = \frac{K_{g_{TB}A}}{K_{g_{TB}H}} \frac{(0.5\,\dot{Q}_T H_{b/a})_A}{(0.5\,\dot{Q}_T H_{b/a})_H} , \quad (4\text{-}38)$$

where E_{MAX} is equal to 0.25 $\dot{Q}_T$. Because the constants are equal in the numerator and denominator, Equations 4-37 and 4-38 reduce to the same equation. Thus, the default regional gas dose ratio for TB effects of Category 2 gases is

$$RGDR_{TB} = \frac{(RGD_{TB})_A}{(RGD_{TB})_H} = \frac{K_{g_{TB}A}}{K_{g_{TB}H}} \frac{(\dot{Q}_T H_{b/a})_A}{(\dot{Q}_T H_{b/a})_H} . \quad (4\text{-}39)$$

Equation 4-39 can be further reduced by the assumption that the overall mass transport coefficients ($K_{g_{TB}}$) are equal when these values are not available. The value of 1.0 is used for the ratio of $(H_{b/g})_A$ / $(H_{b/g})_H$ if $(H_{b/g})_A > (H_{b/g})_H$ or if these partition coefficient values are unknown. Gargas et al. (1989) and Jepson et al. (1994) provide discussion of techniques

to derive partition coefficients and report values for volatile and nonvolatile chemicals, respectively.

Pulmonary Effects. The PU regional gas dose ratio ($RGDR_{PU}$) for Category 2 gases is given by

$$RGDR_{PU} = \frac{(RGD_{PU})_A}{(RGD_{PU})_H} = \frac{(C_i \frac{\dot{Q}_{alv}}{SA_{PU}})_A}{(C_i \frac{\dot{Q}_{alv}}{SA_{PU}})_H} \frac{(e^{-K_{g_{ET}} \frac{SA_{ET}}{\dot{V}_E}})_A}{(e^{-K_{g_{ET}} \frac{SA_{ET}}{\dot{V}_E}})_H} \frac{(e^{-K_{g_{TB}} \frac{SA_{TB}}{\dot{V}_E}})_A}{(e^{-K_{g_{TB}} \frac{SA_{TB}}{\dot{V}_E}})_H} \frac{(1 - \frac{C_{b/a}}{C_i})_A}{(1 - \frac{C_{b/a}}{C_i})_H} . \quad (4\text{-}40)$$

The default ratio is obtained by assuming the mass transport coefficients for the ET and the TB region are the same in each species. At values of $K_{g_{TB}} \leq 0.5$, as per the definition for Category 2 gases, the exponential term for both the ET and TB term in Equation 4-40 reduces to 1.0. Thus, assuming the same inspired concentrations, Equation 4-40 becomes

$$RGDR_{PU} = \frac{(RGD_{PU})_A}{(RGD_{PU})_H} = \frac{(\frac{\dot{Q}_{alv}}{SA_{PU}})_A}{(\frac{\dot{Q}_{alv}}{SA_{PU}})_H} \frac{(1 - \frac{C_{b/a}}{C_i})_A}{(1 - \frac{C_{b/a}}{C_i})_H} . \quad (4\text{-}41)$$

The $RGDR_{PU}$ must be evaluated for both cases described above for the ET and TB regions. In the case where systemic elimination determines the blood term, the ratio is given by

$$RGDR_{PU} = \frac{(RGD_{PU})_A}{(RGD_{PU})_H} = \frac{(\frac{\dot{Q}_{alv}}{SA_{PU}})_A}{(\frac{\dot{Q}_{alv}}{SA_{PU}})_H} \frac{(0.25 \dot{Q}_T H_{b/g})_A}{(0.25 \dot{Q}_T H_{b/g})_H} . \quad (4\text{-}42)$$

In the case where respiratory tract metabolism and systemic elimination are equally important, the ratio is given by

$$RGDR_{PU} = \frac{(RGD_{PU})_A}{(RGD_{PU})_H} = \frac{\left(\frac{\dot{Q}_{alv}}{SA_{PU}}\right)_A}{\left(\frac{\dot{Q}_{alv}}{SA_{PU}}\right)_H} \frac{(0.5\,\dot{Q}_T H_{b/g})_A}{(0.5\,\dot{Q}_T H_{b/g})_H} . \qquad (4\text{-}43)$$

Because the constants are equal in the numerator and denominator, Equations 4-42 and 4-43 the same equation. Thus, the default regional gas dose ratio for PU effects of Category 2 gases is:

$$RGDR_{PU} = \frac{(RGD_{PU})_A}{(RGD_{PU})_H} = \frac{\left(\frac{\dot{Q}_{alv}}{SA_{PU}}\right)_A}{\left(\frac{\dot{Q}_{alv}}{SA_{PU}}\right)_H} \frac{(\dot{Q}_T H_{b/g})_A}{(\dot{Q}_T H_{b/g})_H} . \qquad (4\text{-}44)$$

The value of 1.0 is used for the ratio of $(H_{b/g})_A$ / $(H_{b/g})_H$ if $(H_{b/g})_A > (H_{b/g})_H$ or if these partition coefficient values are unknown. Gargas et al. (1989) and Jepson et al. (1994) provide discussion of techniques to derive partition coefficients and report values for volatile and nonvolatile chemicals, respectively.

4.3.6.2 Remote (Extrarespiratory) Effects

As discussed above in Section 4.3.6.2, Category 2 gases have physicochemical characteristics that result in the potential for significant accumulation of the gas in the blood. Thus, these gases also have the potential to cause remote (extrarespiratory) toxicity at target tissues other than the respiratory tract. Gases or vapors in Category 3 are relatively water insoluble and unreactive in the ET and TB regions. Thus, the relatively limited dose to these respiratory tract regions does not appear to result in any significant toxicity, although some respiratory tract toxicity may be related to recirculation. The uptake of these gases is predominantly in the pulmonary region and is perfusion limited. The site of toxicity is

generally remote to the principal site of absorption in the PU region. An example of a Category 3 gases is styrene.

Remote (Extrarespiratory) Effects—Category 2 Gases

In the event that remote toxicity is associated with a gas in Category 2, the dose to the respiratory tract, and therefore to the blood, is necessary to establish the dose ratio. However, in this case, the surface area of the respiratory tract is irrelevant, only the absorption rate in mass/time (RGD_{RT}) is important such that the dose ratio becomes

$$\frac{(RGD_{RT})_A}{(RGD_{RT})_H} = \frac{(\dot{V}_E)_A}{(\dot{V}_E)_H} \frac{(1 - \frac{C_{b/a}}{C_i})_A}{(1 - \frac{C_{b/a}}{C_i})_H}, \quad (4\text{-}45)$$

As in Section 4.3.6.1, this ratio must be evaluated for each of two cases. In the case where systemic elimination determines the blood term, the regional gas dose ratio for remote (extrarespiratory) effects of Category 2 gases is given by

$$RGDR_{ER} = \frac{(RGD_{RT})_A}{(RGD_{RT})_H} = \frac{(\dot{V}_E)_A}{(\dot{V}_E)_H} \frac{(0.25\dot{Q}_T H_{b/g})_A}{(0.25\dot{Q}_T H_{b/g})_H}. \quad (4\text{-}46)$$

In the case where respiratory tract metabolism and systemic elimination are equally important, the ratio is given by

$$RGDR_{ER} = \frac{(RGD_{RT})_A}{(RGD_{RT})_H} = \frac{(\dot{V}_E)_A}{(\dot{V}_E)_H} \frac{(0.5\dot{Q}_T H_{b/g})_A}{(0.5\dot{Q}_T H_{b/g})_H}. \quad (4\text{-}47)$$

Because the constants are equal in the numerator and denominator, Equations 4-46 and 4-47 reduce to the same equation. Thus, the default regional gas dose ratio for remote (extrarespiratory) effects of Category 2 gases is:

$$RGDR_{ER} = \frac{(RGD_{RT})_A}{(RGD_{RT})_H} = \frac{(\dot{V}_E)_A}{(\dot{V}_E)_H} \frac{(\dot{Q}_T H_{b/g})_A}{(\dot{Q}_T H_{b/g})_H} . \quad (4\text{-}48)$$

The value of 1.0 is used for the ratio of $(H_{b/g})_A$ / $(H_{b/g})_H$ if $(H_{b/g})_A > (H_{b/g})_H$ or if these partition coefficient values are unknown. Gargas et al. (1989) and Jepson et al. (1994) provide discussion of techniques to derive partition coefficients and report values for volatile and nonvolatile chemicals, respectively.

Remote (Extrarespiratory) Effects-Category 3 Gases

For gases in Category 3 that exhibit their toxic effects outside of the respiratory tract, an approach for the scenario when the concentrations of the gas in the animal is periodic (or could be expected to be) with respect to time is recommended. Derivation of the procedure and Equation 4-48 for estimating $NOAEL_{[HEC]}$s for extrarespiratory effects of these gases is based on a PBPK model described in Appendix J. The procedure will give equivalent or more conservative values for the $NOAEL_{[HEC]}$s than those obtained by using the PBPK model, and can be used with compounds for which modeling would be applicable, but for which some or all values of the important parameters ($H_{b/g}$, VMAX, KM, etc.) are not available. The approach assumes that physiologic and kinetic processes can be described by a PBPK model, allometric scaling of physiologic and kinetic parameters may be used, and that all concentrations of the inhaled compound in the experimental animal are periodic with respect to time. Based on the PBPK ventilation-perfusion model concept (e.g., Ramsey and Andersen, 1984), algebraic equations that relate organ and tissue compartment concentrations to exposure concentrations under equilibrium conditions were derived for humans; for laboratory animals, equations were derived that relate time average concentrations. Because toxic effects observed in chronic bioassays are the basis for the determination of NOAELs from which RfC values for human exposures are derived, the procedure assumes that chronic laboratory animal exposure scenarios are equivalent to human lifetime exposures. The

procedure also assumes that the toxic effects observed are related to the arterial blood concentration (concentration leaving lung compartment in the model) of the inhaled compound and that $NOAEL_{[HEC]}$s should be such that the human time-integrated arterial blood concentration is less than or equal to that of the exposed laboratory animal. This latter assumption is equivalent to assuming that the laboratory animal time-averaged arterial blood concentration is equal to the human equilibrium arterial blood concentration. Note that the time average concentrations are the area under the curve over a period divided by the length (time) of a period (e.g., average concentration over 1 week). A mathematical derivation was used to obtain the proposed method of simple alebraic equations to compute $NOAEL_{[HEC]}$s. A more detailed description of the development of the procedure is given in Appendix J.

Another assumption is that the concentrations of the inhaled compound within the animal achieved periodicity with respect to time (i.e., periodic steady state—the concentration versus time profile is the same for every week). An illustration of periodicity is provided in Figure 4-9. Periodicity of the arterial concentration of the agent was not achieved until the sixth week for the plotted theoretical exposure simulation. Practically, the conditions of periodicity should be met during "most" of the exposure duration. For example, if this condition is met for 90% of the time (e.g., periodic during the last 90 weeks of a 100 week experiment), then estimates of average concentrations will be in error by less than 10%.

The following equation is used to calculate an HEC for extrarespiratory effects of gases in Category 3:

$$NOAEL^{\star}_{[HEC]}\,(mg/m^3) = NOAEL^{\star}_{[ADJ]}\,(mg/m^3) \times \frac{(H_{b/g})_A}{(H_{b/g})_H}, \qquad (4\text{-}48)$$

where:

$NOAEL^{\star}_{[HEC]}$ = the NOAEL or analogous effect level obtained with an alternative approach as described in Appendix A, dosimetrically adjusted to an HEC;

$NOAEL^{\star}_{[ADJ]}$ = is defined in Equation 4-2; and

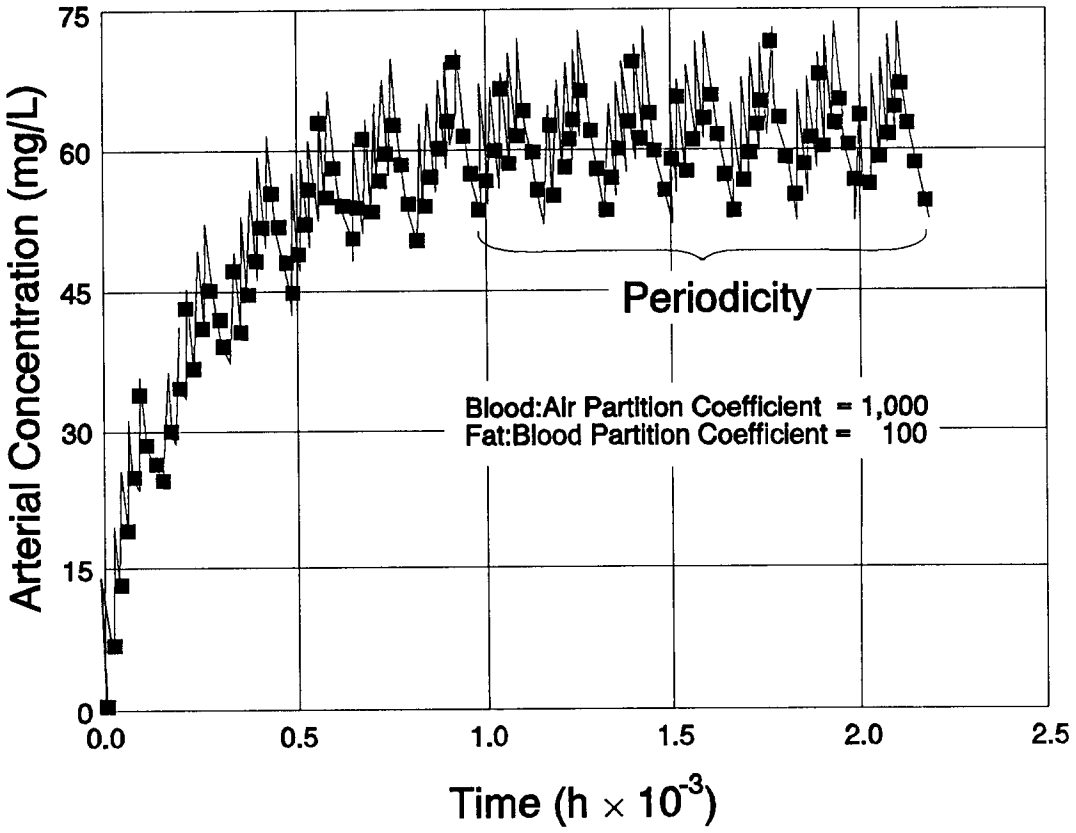

Figure 4-9. Time course of periodicity for F344 rat exposed 6 h/day, 5 days/week to theoretical gas with partition coefficients as shown (Jarabek et al., 1990).

$(H_{b/g})_A/(H_{b/g})_H$ = the ratio of the blood:gas (air) partition coefficient of the chemical for the laboratory animal species to the human value. The value of 1.0 is used for the ratio if $(H_{b/g})_A > (H_{b/g})_H$.

In the case where $H_{b/g}$ values are unknown, the default value of $(H_{b/g})_A/(H_{b/g})_H = 1$ is recommended. An analysis of the available data on rats for blood:air partition coefficients shows that the $(H_{b/g})_A$ is greater than $(H_{b/g})_H$ in most cases. Gargas et al. (1989) and Jepson et al. (1994) provide discussion of techniques to derive partition coefficients and report values for volatile and nonvolative chemicals, respectively.

Figure 4-10 provides guidance on the relationship of the blood:air and fat:blood partition coefficients with respect to achieving periodicity of an inhaled agent in the arterial blood of a 380-g F344 rat. (It should be noted that often tissue:air partition coefficients are

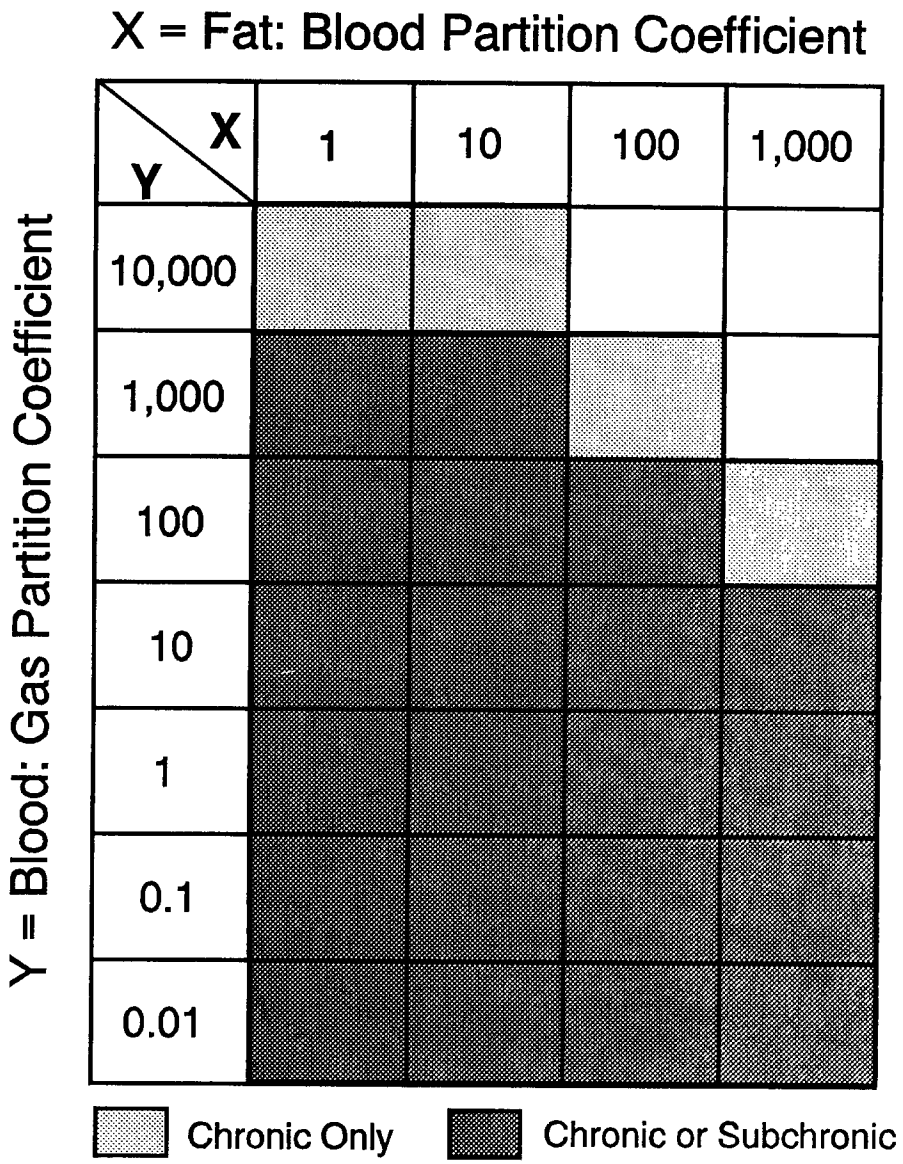

Figure 4-10. Relationship of blood:gas ($H_{b/g}$) and fat:blood partition coefficients to the attainment of periodic blood concentrations in the F344 rat. For a given combination of partition coefficients, the figure indicates (by shading) if simulated blood concentrations reached periodicity within 10% of the exposure time. The exposure regimen was 6 h/day, 5 days/week to 10 ppm. Subchronic = 90 days; chronic = 104 weeks.

reported (e.g., fat:air). The fat:blood partition coefficient can be calculated by dividing the fat:air partition coefficient by the blood:air partition coefficient.) The PBPK model as described in Appendix J was run to simulate a 6 h/day, 5 days/week exposure regimen of

10 ppm. Physiologic parameters, such as ventilation rate, were scaled as described in Appendix J. No metabolic parameters were incorporated in the model for the simulations, because the arterial blood concentration takes longer to reach periodicity without metabolism. Therefore, this figure represents the most conservative values for the partition coefficients for that exposure regimen. The blood:air and fat:blood partition coefficients were chosen based on sensitivity analyses that indicated these two parameters were important to describing the time course of the concentration of an agent in the arterial blood, and upon data availability.

The importance of the relationship between the partition coefficients and the attainment of periodicity is particularly significant when extrapolating from studies of different durations. For example, for an agent with a blood:air partition coefficient of 1,000 and a fat:blood partition coefficient of 100, it would be inappropriate to extrapolate from a subchronic exposure regimen because the criterion of attaining periodicity for 90% of the exposure duration is not met. Periodicity is attained with these same parameters when the study is carried out for a longer duration, however, so that the approach based on the ratio of animal:human partition coefficients can be used on a chronic study without violation of critical assumptions.

Similar matrices to Figure 4-10 can be developed for the relationship between partition coefficients and the attainment of periodicity of the agent in the arterial blood of each experimental species of interest. Use of physiologic parameters for other species and different exposure regimens at various concentrations will influence this relationship and should be considered when determining the extrapolation approach to use for derivation of an HEC.

Since the requirement for achieving periodicity over 90% of the exposure duration is based on the objective of limiting error in the estimate to less than 10%, a modifying factor to account for a greater amount of error should be applied (see Section 4.3.8.1) when the nature of the inhaled agent (e.g., high fat:blood partition coefficient) suggests this condition was not met.

4.3.6.3 Additional Assumptions and Default Values

As with aerosols, after evaluation of the adequacy of the generation system, the initial step in the calculation of HECs is characterization of the exposure.