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Dose Concepts for Inhaled Vapors and Gases

ALAN R. DAHL

Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, P.O. Box 5890, Albuquerque, New Mexico 87185

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Dose Concepts for Inhaled Vapors and Gases. DAHL, A. R. (1990). *Toxicol. Appl. Pharmacol.* **103,** 185–197. "Dose" for any toxicant is a shifting concept; thus, there is a frequent need for modifiers such as "administered" or "effective," when referring to dose. When applied to inhaled gases and vapors, "dose" is even more vague. The wide range of physicochemical properties associated with gases and vapors, the different target tissues that may be affected, and the variety of mechanisms involved in producing toxic effects combine to make a useful dose descriptor for one gas inadequate for another. Although models have been developed to describe dose for inhaled gases and vapors having a broad range of properties, it is often not clear which models are appropriate for which inhalant. In an attempt to resolve this dilemma, a classification scheme for inhaled gases and vapors, based on physicochemical properties, is offered in this report. In addition, various concepts of dose for inhaled gases and vapors are discussed with reference to the classification scheme. Finally, avenues for further research in the area of gas and vapor dosimetry are suggested.

The purpose of this article is to explore aspects of dose for inhaled gases[1] and to suggest ways of thinking about inhaled gases that might serve as unifying concepts on the one hand, and as the basis for continued debate and development of the concept of dose for inhaled gases on the other.

Models for inhaled gas dosimetry include mathematical models for dosimetry of "reactive" gases (Overton and Miller, 1988), physiological or mathematical models for uptake of "metabolizable" vapors (Andersen, 1981, 1982; Filser and Bolt, 1981; Fiserova-Bergerova, 1983; Fiserova-Bergerova *et al.,* 1984; Paterson and Mackay, 1986), and models describing nasal or lung uptake as functions of tissue metabolism (Morris and Cavanaugh, 1987), physical properties of the vapor molecules (Ainsworth and Shephard, 1960; Oberst, 1961), or respiratory parameters (Aharonson *et al.,* 1974; Frank *et al.,* 1969; Hatch and Swann, 1961; Morgan and Frank, 1977).

The existence of so many different models indicates that predicting "dose" for inhaled gases is not a simple task. In part, the difficulty stems from the limited applicability of each of the various models. For instance, mathematical models such as that described by Overton and Miller (1988) were developed

[1] In this article, the term "gas" is used in the broad sense to denote substances that are in the gaseous state, but are not necessarily "true gases." A true gas is a gaseous substance at a temperature above its critical temperature (that temperature above which the substance cannot be liquified simply by increasing its pressure), whereas "vapor" usually refers to the gaseous state of a material in contact with the solid or liquid state. Inhalation toxicologists often refer to vapors of substances with boiling points below room temperature as gases, thus applying a broader usage of the term gas.

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to estimate dose to lung tissue of reactive oxidant gases, such as ozone and NO_2. It would be pointless to attempt to apply such a model to a "nonreactive" gas such as styrene vapor. At the other extreme, physiological models, such as the one described by Andersen (1982), completely neglect interaction of the inhalant with the respiratory tract mucosa. Such models are only useful for metabolizable gases such as the vapors of styrene and chloroform.

Unfortunately for those interested in modeling gas uptake, the physicochemical properties of gases run the gamut, with no clear breaks, from virtually inert to extremely reactive. Thus, it is not entirely obvious which, if any, currently used uptake models might be applied to which gases.

Categorization of Gases

Any categorization of gases for the purpose of grouping by mode of uptake must be somewhat arbitrary. In some cases, enzyme-catalyzed metabolism plays a major role in gas uptake; in others, uptake results predominantly from uncatalyzed chemical reactivity; in still others, both enzyme metabolism and uncatalyzed reactivity are important. The scheme presented here is an attempt to establish physicochemical boundaries—with appropriate transition areas between boundaries—to provide the toxicologist with appropriate considerations regarding the uptake of a gas.

Gases can be roughly divided into three categories, on the basis of their chemical properties (Fig. 1). The considerations necessary to define dose for a gas are largely dependent upon the category to which it belongs.

The first category is that of "stable" molecules. These are defined here as chemical compounds or elements for which the free energy of a chemical reaction that occurs in a physiological system is greater than 3 kcal/mol. At thermodynamic equilibrium with its reaction products, less than 1% of stable gases will be present as products,[2] and these usually will not contribute substantially to toxic effects.

The second category is that of reactive gases. These are gases which have *both* free energies of reaction of less than −3 kcal/mol *and* halftimes for uncatalyzed chemical reactions in physiological systems of 10 min or less. Ten minutes is the approximate time needed for five circulations of blood in the human body (Biology Data Book, 1974). Compounds with less than 10-min half-lives will probably not attain substantial systemic concentrations relative to their concentrations at the point of entry, namely, the respiratory tract. Instead, they will produce a concentration gradient that decreases from the point of entry to the more distal points of the systemic distribution. Less than 1% of a reactive gas will be present as the parent molecule at thermodynamic equilibrium.

The third category contains metabolizable gases. These are gases for which the free energy of reaction is less than −3 kcal/mol, but that have halftimes for uncatalyzed chemical reactions in physiological systems of greater than 1000 min. For these gases, uncatalyzed reactions are so slow that they would behave like stable gases were it not for enzyme-catalyzed reactions.

The relationships among the types of gases are illustrated in Fig. 1. Note that between stable and reactive gases, there is a transition area containing gases for which the free en-

[2] The free energy of a reaction, ΔF, is related to the equilibrium constant K as follows,

$$\Delta F = -RT \ln K,$$

where R = the gas constant = 1.987×10^{-3} kcal °K^{-1}; T = temperature (°K) at which equilibrium is achieved; and, K = the equilibrium constant for a reaction such as

$$A + B \rightleftarrows C + D \qquad K = \frac{[C][D]}{[A][B]}.$$

If ΔF = 3 kcal/mol, then K = 0.0077, so that ~99% of the parent molecules are present at equilibrium. If ΔF = −3 kcal/mol, then K = 130, so that ~1% of the parent molecules are present at equilibrium.

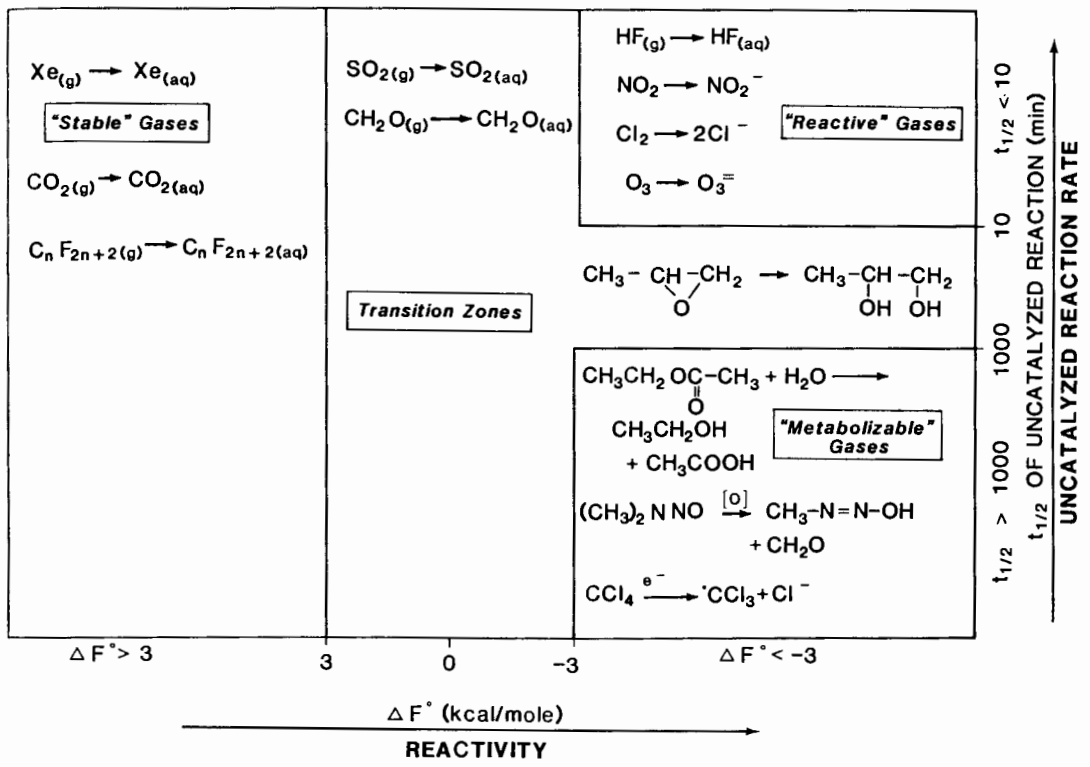


FIG. 1. Thermodynamic and kinetic classification of gases. The superscripts (g) and (aq) stand for gaseous and dissolved aqueous states, respectively. For the formation of aqueous formaldehyde and sulfur dioxide, only the first reaction that occurs is written. The solvated molecules then can undergo further reaction, depleting their concentration in mucus and preventing reentrainment into the gas phase. Those secondary reactions account for the observation that these gases behave as "reactive" gases upon inhalation. See footnote 2 for explanation of horizontal axis.

ergy of reaction is between 3 and −3 kcal/mol. Also, between the categories of reactive and metabolizable gases is a transition area wherein the uncatalyzed reaction halftimes are between 10 and 1000 min. Such transition areas are necessary in any categorization of gas molecules, because changes in physicochemical properties among gases are not distinguished by sharp cutoffs, but, instead, display a continuous gradient in which a particular physicochemical characteristic becomes increasingly predominant. Therefore, there will be gases that display mixed modes of uptake and disposition, each of which must be considered in describing dose.

Between stable and reactive or metabolizable gases are those gases for which 1–99%[2] of the parent molecule is present at thermodynamic equilibrium. For such gases, substantial effects from both parent molecules and reaction products must be considered.

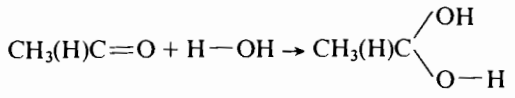
The transition area between reactive and metabolizable gases and vapors includes compounds with half-lives of between 10 and 1000 min for uncatalyzed reactions. Molecules with half-lives in this range may undergo both uncatalyzed reactions and enzyme-catalyzed reactions to significant extents. Therefore, both fates need to be considered for molecules of such gases.

Evaluation of Gases: Stable, Reactive, or Metabolizable

Figure 2 shows a scheme by which an inhaled gas can be categorized as stable, reactive, or metabolizable, by answering a series of questions.

Question 1: Is the gas stable, according to the criteria discussed above (Fig. 1)? For many gases, stability, or the lack thereof, is "intuitively obvious"; for example, ozone is reactive and xenon is stable. But, how stable are nickel carbonyl, methylene fluoride, or methyl silicate? When presented with an unfamiliar gas, the first task of the inhalation toxicologist is to assess the stability of the gas upon inhalation. First, the spontaneous reactions likely to occur in a physiological system are considered. Then, using published bond strengths (March, 1985; CRC Handbook, 1978), the free energy changes associated with many such reactions can be estimated.[3]

[3] Such calculations give only approximate equilibrium values, but are nevertheless useful for getting an estimate of the relative abundance of parent molecules and products in solution when direct data are unavailable. Using bond energy values (March, 1985), the free energy of reaction for the hydration of acetaldehyde is calculated as (bonds shown are made or broken in the reaction)



Bonds broken	Energy	Bonds made	Energy
C=O	+177 kcal	C−O	−88 kcal
H−O	+110.5 kcal	O−H	−110.5 kcal
		C−O	−88 kcal
	Net +287.5 kcal		Net −286.5 kcal

Net change in energy: $\Delta F = +1$ kcal.
From $\Delta F = -RT \ln K$,

$$K_{(20°C)} = 0.18 = \frac{[CH_3(H)C(OH)_2]}{[CH_3(H)CO][H_2O]}.$$

Because the concentration of water, $[H_2O]$, = 55.6 mol/liter, the ratio of the concentration of hydrated acetaldehyde to acetaldehyde is

$$\frac{[CH_3(H)C(OH)_2]}{[CH_3(H)CO]} = 0.18 \times 55.6 = 10.0,$$

which holds when 91% of the acetaldehyde is hydrated. The experimental value is reported as 56% hydrated acetaldehyde (March 1985). This level of agreement between calculations and experimental data is about as good as can be expected. Discrepancies of a factor of 10 or more can easily result, because the equilibrium constant is very sensitive to slight changes in the calculated value of ΔF.

For reactions not involving bond making and breaking—hydration reactions, for example—the required data may be more difficult to obtain, and one may need to resort to direct measurement.

If a gas is stable, only physical or reversible effects, such as asphyxiation or anesthesia, would be expected. If it is not stable, it must be further categorized as to whether it is reactive or metabolizable.

Question 2: What is the uncatalyzed reaction rate? Gases that are quickly converted to nonvolatile products in mucus will have effects that are largely confined to the nasal tissues. Ruthenium tetroxide (Snipes and Kanapilly, 1983), methylphosphonic difluoride (Dahl and Bechtold, 1985), and formaldehyde (Chang *et al.*, 1983) fall into the category of "fast" reactors. Unfortunately, we do not know the meaning of fast in this context. While most reaction rates could potentially be determined, the average residence time in the mucus for an inhaled vapor molecule is not known.[4] The absence of detailed uncatalyzed reaction kinetics for inhaled pollutants has been noted by others (Ultman, 1988).

For gases with uncatalyzed reaction rates designated as "moderate," effects are largely confined to the respiratory tract. Oxidizing gases such as ozone (Overton and Miller, 1988) and chlorine (Klonne *et al.*, 1987) fall into this category, as do vesicant war gases such as phosgene (Cameron *et al.*, 1946). As was the case for fast reactors, we do not know

[4] Yamada *et al.* (1988) have shown that 5-nm. ultrafine particles deposit with a 50% efficiency in casts of human noses. Gas molecules are on the order of 0.1 nm in diameter, and their diffusion rates are orders of magnitude faster than are those of the ultrafine particles. Thus, nasal deposition of inhaled gas molecules must approach 100%. Deposited gas molecules, unlike particles, may then reentrain in the air before they react or are carried away in the blood. The average residence time in the mucus for inhaled gas molecules, relative to their rates of reaction, is a key parameter for predicting nasal uptake. Unfortunately, this parameter has not yet been determined for any gas. This is an important area of research that needs to be explored.

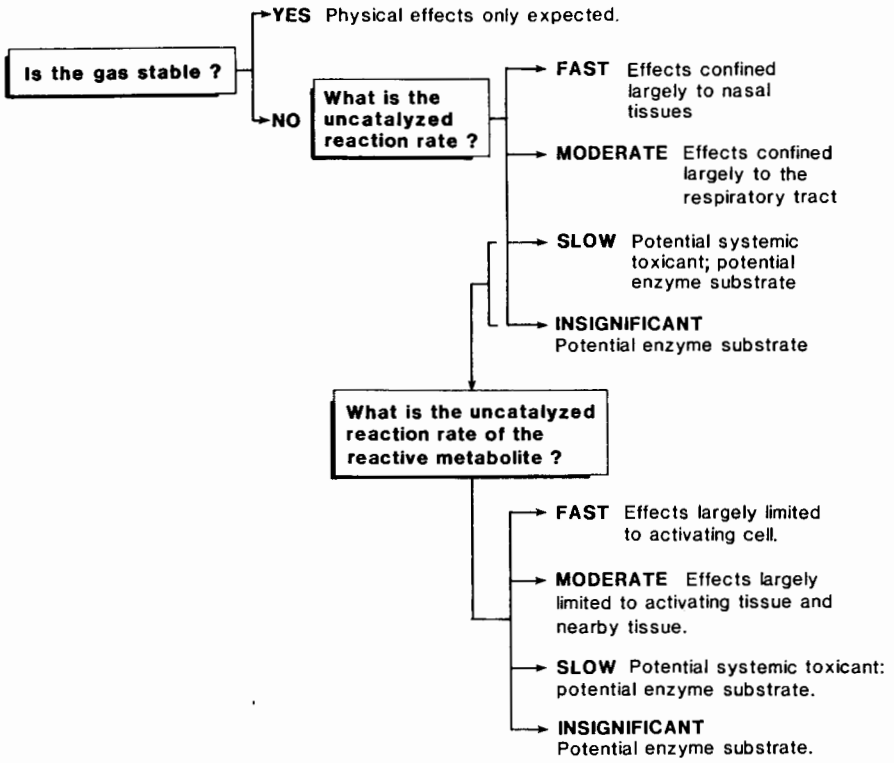


FIG. 2. Decision flow chart for classifying gases.

exactly how to define moderate reaction rates.

"Slowly" reactive gases are those that pass beyond the respiratory tract to become systemic toxicants, but, due to their reactivity, may produce a concentration gradient between the respiratory tract and distal anatomic tissues. These are potential enzyme substrates and fall in the transition area between reactive and metabolizable gases. Many epoxides (Gervasi *et al.*, 1985) fall into this category.

Finally, there are those numerous gases for which uncatalyzed reactions occur at insignificant rates. These are potential enzyme substrates and fall into the category of potentially metabolizable gases.

Question 3: For metabolizable gases, what is the spontaneous reaction rate of the metabolite(s)? Gases with slow or insignificant, uncatalyzed reaction rates either will be excreted largely unchanged—in which case they behave toxicologically like stable gases —or will be metabolized. In the latter case, the metabolites are potential toxicants, and it is the "dose" of these metabolites, rather than of the parent compound, that is the toxicologically relevant measurement.

The cells and tissues at risk from reactive metabolites depend not only on the source of the metabolites, but also on their kinetic properties (see also Guengerich and Liebler, 1985). The toxic effects of metabolites that react at fast rates are confined to the activating enzyme or cell. The carbenes formed from metabolites of benzodioxoles fall into this category (Dahl and Brezinski, 1985). Once again, the term fast cannot be defined precisely in this context.

If metabolite reaction rates are moderate, effects will largely be limited to the activating tissue and to nearby tissues. Dimethylnitrosamine (Umbenhauer and Pegg, 1981) and vinyl chloride (Guengerich *et al.*, 1981) form metabolites that fall into this category.

Slow-reacting metabolites are themselves potential substrates for further metabolism. For example, epoxide metabolites of alkenes

often have spontaneous reaction half-lives of several hours (Gervasi *et al.*, 1985) and are substrates for epoxide hydrolase enzymes.

If the spontaneous rate of reaction is insignificant, the metabolites may be eliminated unchanged. Alternatively, the metabolites may be substrates for still further metabolism. For a complete toxicokinetic assessment, the fate of inhaled gas molecules must be determined up to the point at which the metabolites (or their uncatalyzed reaction products) are quantitatively eliminated, fall into the category of stable compounds (e.g., CO_2), or are metabolized to products that are incorporated into biomolecules (e.g., propionic acid and acetic acid).

Dose Measurements for Inhaled Gases

Defining dose. The severity of an effect of different toxicants or of the same toxicant among different individuals does not necessarily reflect the quantity of the dose. For example, in a sensitized individual, exposure to a very small quantity of hapten molecules might precipitate a cascade of immunological events that leads to a toxic effect far more severe than that brought about in an unsensitized individual by direct toxicity from the inhaled gas molecules, their reaction products, or their metabolites.

As another example, an inhaled gas may serve as a catalyst for a toxic event. Ferrocene vapor, for instance, is metabolized, in part, to an unstable intermediate that releases ferrous ion inside the metabolizing cell. The released ferrous ions may then catalyze lipid peroxidation (J. D. Sun, personal communication).

Perhaps the greatest potential disparity between dose and effect occurs with carcinogens. Tumors can metastasize to many locations within the body and may even be transplanted to congenic animals never exposed to the causative agent.

For the purposes of this article, measurements of dose must involve determinations of the physical presence of the inhaled molecules themselves, or of any portion of the parent molecule. Thus, quantitation of DNA adducts is a determination of dose, because at least a part of the inhaled molecule is present in the analyte. Quantitation of fragments of DNA, in cases where residues from the inhaled gas are no longer present, is not a measurement of dose, but a determination of an effect.

In inhalation toxicology, dose measurements for gases (Fig. 3) range in refinement from measurements of the gas concentration of an exposure atmosphere to calculations of the "effective" dose—the inhaled molecules, or their reaction products, that participate in the particular effect under consideration.

Dose as the inhaled gas concentration. The simplest of the dose concepts, inhaled gas concentration, is directly related to effects that do not involve strong chemical binding of the gas, its reaction products, or its metabolites, to biomolecules. When tissues are at equilibrium with high concentrations (>1000 ppm) of stable gases (e.g., xenon), metabolizable gases (e.g., chloroform vapors), or those in the transition area between metabolizable and reactive (e.g., propylene oxide; Hine *et al.*, 1981) effects such as narcosis, anesthesia, and asphyxiation may result. Such effects are thought to be brought about by reversible, "physical" interactions of gas molecules with biomolecules (Tichy, 1983).

Dose, in terms of inhaled gas or vapor "concentration," is usually expressed as mass of the gas per unit volume (e.g., μg/liter or μmol/m^3), or as a ratio of molecules of the study gas to total gas molecules (e.g., percentage or ppm). Expressing the dose of a gas simply as gas concentration, or as both concentration and exposure duration, does not account for variables such as changes in respiratory parameters that sometimes result from exposure, for saturation of enzyme-catalyzed pathways (Andersen *et al.*, 1987), or for depletion of coreactant biomolecules (Overton and Miller, 1988). Thus, except for stable gases, linearity between dose (i.e., inhaled concentration) and toxic effects, over a

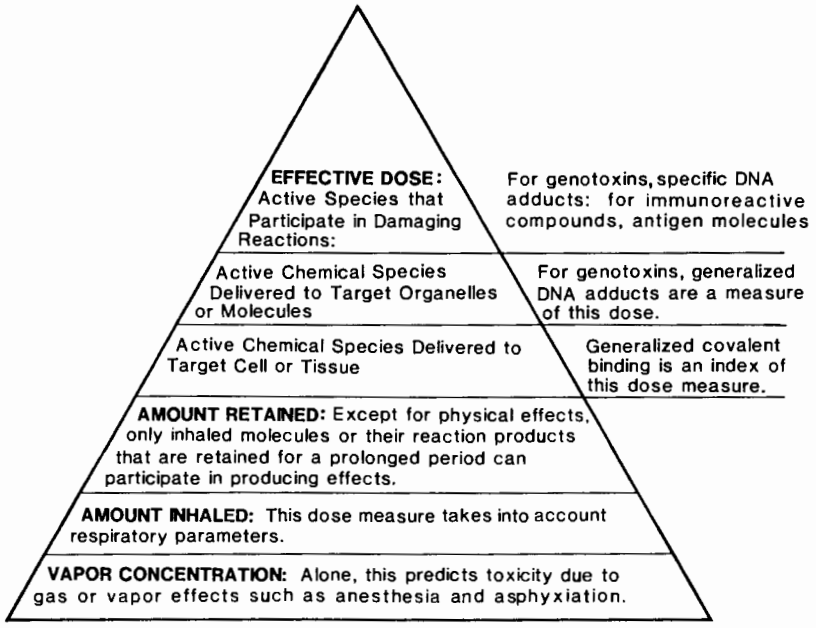


FIG. 3. Pyramid of refinements in "dose" measurements for inhaled gases.

wide range of inhaled concentrations and exposure durations, would not be expected.

Dose as the amount of gas inhaled. The amount of gas inhaled can be estimated by multiplying the appropriate minute volume ($\dot{V}_I$) by the vapor concentration (C) and the exposure duration (t):

$$\dot{V}_I \times C \times t = \text{amount of gas inhaled.}$$

The minute volume can be estimated from published sources (e.g., Likens and Mauderly, 1979), or can be determined during exposure (Mauderly, 1986; Medinsky *et al.*, 1985). Determination during exposure has obvious advantages, both because respiratory parameters sometimes change dramatically in response to an inhalant and because minute volumes vary from one subject or condition to another. These changes form the basis for the RD50 (the concentration causing 50% respiratory rate depression) measure of irritancy for inhaled vapors (Kane *et al.*, 1979) and can be very important. The difference in relative changes in respiratory parameters between mice and rats in response to inhaled formaldehyde is probably the basis for the difference in the tumorigenicity of formaldehyde in the two species (Chang *et al.*, 1983).

Uptake: The amount of gas retained (for reactive gases) or metabolized (for metabolizable gases). "Uptake" is the amount of gas inhaled that is not exhaled. At steady state, uptake results exclusively from metabolism or the uncatalyzed reaction of the inhaled gas within the physiological system. Before steady state is attained, uptake may also result from absorption of the inhaled molecules into various tissues. The factors that affect absorption of xenobiotics into tissues are the subject of continuing research (Bickel, 1988).

Except for reversible physical effects, only gas molecules whose components are retained over a prolonged period participate in producing effects. This is equivalent to saying that if an inhaled gas molecule is exhaled unchanged, it cannot have produced a chemical effect involving covalent binding to biomolecules. The chemical species producing an effect may be a reactive gas itself, a reactive primary metabolite, or, in some cases, molecules several metabolic steps removed from the inhalant.

Dose expressed as the amount of gas retained, which is equivalent to uptake, may be normalized to exposure duration (in which case it becomes a dose *rate*), to body weight,

or to the concentration of inhaled vapor. Normalization to exposure duration or to gas concentration, however, is appropriate only if the rate of uptake is linear with concentration and time over the ranges under consideration. Such normalization gives uptake in units of, for example, nmol/min kg^{-1} ppm^{-1} (Dahl *et al.,* 1988). Uptake for a given exposure scenario can be calculated from normalized rates by multiplying these rates by the exposure gas concentration and the exposure duration. Gas uptake rates are also reported as "clearance" rates (Andersen, 1982) or as "maximum, first-order rate constants" (Andersen, 1981). These expressions arise from the decay-type kinetics determined from static kinetic methodology.[5]

For gases other than stable gases, for which only reversible effects are expected, measuring the amount retained or metabolized usually provides the dose measurement of most importance when comparing the relative toxicities of different gases, or of the same gas in different species. For reactive gases with fast-reaction kinetics, retention of molecular components will approach 100%, unless the reaction products are themselves volatile. For reactive gases with longer half-lives, initial retention will be governed by the same factors affecting uptake of metabolizable gases (Dahl *et al.,* 1988; Fiserova-Bergerova *et al.,* 1984).

For gases that are metabolized to active compounds, neither the inhaled gas concentration nor the total amount inhaled is nearly as relevant as measurements of dose, as is the amount metabolized. In some cases, the amount of gas converted to a particular reactive metabolite may be an even more apposite dose measurement. Thus, there is reason to look beyond total metabolism or total retention and to examine the detailed fate of the retained molecule and the nature of its metabolites or uncatalyzed reaction products.

Reactive gas or active metabolite molecules delivered to target cells or tissues. It is difficult to absolutely quantify an active chemical species delivered to a target cell to tissue, because, by definition, the relevant molecules are reactive and their concentrations are strongly time dependent, making direct measurement extremely difficult. Covalent binding, although not necessarily an absolute dose measurement, is a useful index of dose when comparing exposure of different tissues or animal species to reactive metabolites or reactive gases.

The use of the covalently bound portion of an inhaled gas molecule as an index of total exposure for a particular tissue or cell must be carefully considered, however. The degree of binding to a particular biological entity will depend both on the chemical nature of the reacting xenobiotics and on the nature of the biomolecules. For example, if binding involves electrophilic attack on nucleophilic nitrogen or sulfur, tissues poor in these nucleophiles, such as adipose tissue, may bind little of the inhalant, no matter how high the exposure dose. Even comparisons of covalent binding in the same tissue, but in different species, may not give accurate indices of relative dose. Hemoglobin structures, for example, vary substantially among species (Satoh *et al.,* 1987), and these differences may significantly affect binding, for example, of certain epoxides (Henderson *et al.,* 1989).

Reactive gases or reactive metabolite molecules delivered to target biomolecules or organelles. The amount of a reactive toxicant that penetrates to the target tissue is very close

[5] Maximum, first-order rate constants, k, can be converted to concentration-normalized uptake rates, as follows:

$$k \cdot \frac{1}{V_m} \cdot V = \text{the uptake rate } (\mu\text{mol kg}^{-1}\text{ hr}^{-1}\text{ per ppm}),$$

where units of k are kg^{-1} hr^{-1} (Andersen, 1981); V_m = volume of 1 mol of air (1/mol); and, V = volume of container in which k was determined (liters). Clearance rates, Cl, can be converted to gas concentration-normalized uptake rates, as follows:

$$Cl \cdot \frac{1}{V_m} = \text{the uptake rate } (\mu\text{mol kg}^{-1}\text{ hr}^{-1}\text{ per ppm}),$$

where units of Cl are 1 kg^{-1} min^{-1} (Andersen, 1982).

to being the ultimate "effective" dose. For genotoxins, DNA adducts are an example of this level of dose measurement. Like total covalent binding, total DNA adducts are an index of dose, rather than an absolute measure, because only a fraction of the active chemical species delivered to the chromosomes is likely to be measured as adducts. Another example of this refinement of dose is delivery of cyanide to mitochondria by cyanogenic vapors (e.g., acetone cyanohydrin). Hydrogen cyanide exerts its toxic effects on the oxidative phosphorylation enzymes in the mitochondria. A cyanogenic compound may deliver a relatively large amount of hydrogen cyanide to a particular cell, but much of the cyanide may be detoxicated to thiocyanate by the enzyme rhodanese, or may bind to noncritical biomolecules, or both. Thus, it is only the portion of the cyanogenic compound that penetrates to the mitochondria that can participate in the primary toxic effect.

Gas or reactive metabolite molecules participating in ultimate toxic reactions: Effective dose. Measurement of DNA adducts that lead to a particular mutation is an example of measurement of the ultimate effective dose for genotoxins. For immunoreactive compounds, the antigenic molecules that bind to the components of the gas as haptens would constitute the effective dose prompting antibody formation. Differences in potencies among various inhaled gases and vapors almost certainly result, in part, from differences affecting the fraction of inhaled molecules participating in the effective dose. Understanding the mechanisms leading to differences in effective dose is a major research need in inhalation toxicology.

For effective dose to be clearly defined, the effect under consideration must also be defined. In a series of papers reporting refinements in the measurements of effective dose for inhaled formaldehyde (Casanova and Heck, 1987; Casanova-Schmitz *et al.*, 1984; Chang *et al.*, 1983; Starr and Buck, 1984; Swenberg *et al.*, 1983), the dose of interest was the formaldehyde that participated in a particular effect: production of squamous cell carcinomas of the nasal cavity. Because the effective dose was not a constant fraction of the inhaled formaldehyde for different inhaled concentrations (Casanova-Schmitz *et al.*, 1984), effective dose measures were deemed to be more reliable indicators of risk for nasal squamous cell carcinoma than were less refined dose measures such as the vapor concentrations or the amount inhaled (Fig. 3) (Starr and Buck, 1984).

The Effects of Exposure Parameters, Vapor Pressure, and Solubility on Dose

Effects of gas concentration and exposure time. Up to this point in the article, it has been assumed that metabolizable gases are inhaled in the "linear" range for metabolism (Andersen *et al.*, 1982) and that reactive gases are inhaled under exposure conditions such that substantial decreases in the concentrations of biological coreactants do not occur during exposure (Overton and Miller, 1988). These assumptions may not hold at high inhaled concentrations or for prolonged exposures.

Uncatalyzed reactions follow pseudo-first-order kinetics if the gas is inhaled at "low" concentrations (Overton and Miller, 1988; Ultman, 1988).[6] "High" vapor concentrations can qualitatively change the chemical

[6] If kinetics are first order in the reactant gas molecule, then the reaction rate is given by

$$\text{Rate} = k\,[\text{gas}],$$

and the rate is proportional only to the concentration of gas [gas]. Usually, however, an endogenous molecule is also involved in the reaction, and—as is the case for water, for example—the mucus concentration of the coreactant is high, relative to the gas concentration, or it remains constant throughout the reaction time, because it is continually replaced. Technically, the reaction rate is dependent on both the concentration of the vapor and of the coreactant but, since the coreactant concentration is essentially constant, its concentration can be incorporated directly into the rate constant, k. This defines a "pseudo-first-order" reaction.

fate and toxicity of inhaled vapors. Depletion of biological coreactants, or just an increase in the concentration of the xenobiotic 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 example, formaldehyde carcinogenicity is probably far less at low concentrations than would be predicted from exposures to high concentrations (Starr and Buck, 1984; Starr *et al.*, 1985). This break in the dose response may be explained by sharp decreases in the nasal concentrations of available glutathione at high inhaled concentrations of formaldehyde (Casanova and Heck, 1987).

For metabolizable compounds, it has been assumed in the foregoing discussion that the inhaled concentrations fall into the linear range, wherein Michaelis–Menten kinetics predict that at low inhaled concentrations the reaction rate is given by rate = (V_{max}/km) [gas]. At high concentrations, metabolism is saturated and is governed by zero-order kinetics: rate = V_{max}. Saturation of metabolic pathways can qualitatively alter the metabolites formed and the toxicity of a metabolizable compound. Thus, at low concentrations, methylene chloride is predominantly metabolized by a cytochrome P450 pathway to noncarcinogenic carbon monoxide. At higher concentrations, this pathway becomes saturated and a pathway involving glutathione conjugation becomes important. The glutathione conjugate is a putative carcinogen (Andersen *et al.*, 1987).

Such effects of inhaled vapor concentration on metabolism are not limited to systemic enzymes, but also occur in localized areas within the respiratory tract. Inhaled dimethylnitrosamine, for example, shows saturation kinetics with enzymes of the olfactory tissue of the hamster when the vapor concentration changes from 0.1 to 1 to 10 ppm (Dahl *et al.*, 1989). 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 systemic enzymes to maximum velocities. It is likely that, except at extremely low inhaled gas concentrations, local metabolizing areas within the respiratory tract, particularly the nasal tissues, will not follow linear enzyme kinetics. Because the nasal tissue is often a target for inhaled toxicants (Morgan and Monticello, 1990), possible saturation of nasal enzymes must be taken into account when extrapolating doses of metabolizable gases from high to low concentrations, or vice versa.

At very high inhaled concentrations, removal of gases, either by metabolism or by uncatalyzed chemical reaction, may be insignificant relative to the inhalation rate. The gas may then show effects more typical of the reversible effects associated with stable compounds. For example, inhaled propylene oxide has significant uncatalyzed reactivity and is also an enzyme substrate. It causes nasal lesions at 300 ppm (Kuper *et al.*, 1988), but at concentrations over 4000 ppm it has a weak anesthetic effect (Hine *et al.*, 1981).

Increasing exposure duration may lead to changes similar to those observed following increases in gas concentration. Exposure to a relatively low concentration of a gas for a short period of time may not result in depletion of endogenous substrates or in blood and tissue levels at which systemic metabolism becomes saturated. The same concentration for a longer period, however, might exceed the capacity of the body to replenish endogenous coreactants or may result in a steady-state tissue concentration that saturates systemic metabolism.

Effects of vapor pressure on dose. The vapor pressure of a liquid or solid[7] at ambient temperatures determines the maximum ex-

[7] Solids can have *significant* vapor pressures: examples are dry ice, ferrocene, cyanogen bromide, chloral hydrate, osmium tetroxide, and phenol.

posure concentration for its vapor. The maximum exposure concentration in ppm may be calculated from the vapor pressure at 25°C; thus, maximum exposure concentration (ppm) = $[VP_{25°C}(\text{mm Hg})/760] \times 10^6$. Knowing the vapor pressure of a liquid or solid is important for estimating its capacity to produce reversible effects. A compound with a vapor pressure of less than 0.76 mm Hg at room temperature will attain an air concentration of less than 1000 ppm at the saturated vapor concentration. This concentration is below the limits for which narcotic or anesthetic effects are generally observed (Eger, 1974; Tichy, 1983). Thus, if a material has a vapor pressure of less than 0.76 mm, its potential to produce such effects can reasonably be ruled out.

Effects of water solubility and liquid/air partition coefficients. The importance of aqueous and lipid solubility, and of liquid/air partition coefficients, on the uptake of vapors has been reported (Dahl *et al.*, 1988; Fiserova-Bergerova *et al.*, 1984; Morgan and Frank, 1977; Morgan and Monticello, 1990). Highly water-soluble gases, such as SO_2, will be efficiently removed by the nose (Brain, 1970; Dalhamn and Strandberg, 1961). This is not solely an effect of solubility, however, since other highly soluble gases, such as acetone, "saturate" the nasal mucosa and are actually exhaled when systemic saturation (air/tissue equilibrium) occurs (Egle, 1973; Landahl and Herrmann, 1950; Morris and Cavanaugh, 1987; Morris *et al.*, 1986). The difference can be explained by the fact that SO_2 rapidly forms a nonvolatile bisulfite ion in the mucus (Ultman, 1988), whereas acetone reaches air/mucus equilibrium.

The difference in nasal uptake between SO_2 and acetone can be considered an effect of the differences in their mucus/air *partition coefficients*. A partition coefficient is the ratio of the concentration of a gas in a liquid relative to its concentration in air at thermodynamic equilibrium. The effective partition coefficient for SO_2 is very large in buffered solutions facilitating bisulfite formation. For acetone, the water/air partition coefficient is 395 (Fiserova-Bergerova, 1983).

Partition coefficients can be derived from extensive lists of Henry's law constants (Mackay and Shiu, 1981). Like Henry's law constants, partition coefficients are temperature dependent, but are essentially independent of pressure.

SUMMARY AND CONCLUSIONS

A classification of gases according to physicochemical properties has been offered. The classifications were made to facilitate choosing the appropriate model or set of concepts to apply to the considerations of dose for a particular gas. Various concepts of dose for inhaled gases were explored, beginning with inhaled concentration and ending with the amount effectively delivered to the target biomolecules. Finally, a classification pathway, by which the inhalation toxicologist can determine the class to which a particular gas belongs, was offered.

A signal theme throughout this article was the qualitative nature of discussions of dose. The need for vague terms such as fast-, moderate-, and slow-reaction kinetics indicates an almost complete void in our knowledge of the details of reaction rates for gases in mucus, and in biological fluids, in general. The inability to present means by which the actual dose of toxicologically relevant, reactive metabolites of gases can be determined indicates another gap in our knowledge of the mechanisms of toxicity of inhaled gases. These are among the most critical research areas in inhalation toxicology today.

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