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## SYMPOSIUM OVERVIEW

# Comparative Dosimetry of Inhaled Materials: Differences among Animal Species and Extrapolation to Man[1,2]

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Dose is a fundamental concept in the science of toxicology; yet, for all of its importance, the determination of dose for inhaled materials presents issues with which inhalation toxicologists are still grappling. Amid a myriad of lesser factors, the two major ones influencing dose for inhalants are the physicochemical properties of the inhaled materials and the species of the animal doing the inhaling. The speakers in this symposium presented the most recent advances in research related to descriptions of dose for inhaled particles, particle-associated organic compounds, reactive vapors, and metabolizable vapors. They also discussed methods for predicting inhaled doses in humans based on measured doses in test animals and advances in the search for biomarkers of inhaled carcinogens.

The factors that affect the fate of inhaled particles were reviewed and the particle deposition and clearance patterns of experimental animals and people were compared. Advances in determining dose for reactive vapors that are largely absorbed in the nose (exemplified by formaldehyde) or in the lung (exemplified by ozone) were discussed in terms of relating experimental data to predictive models. The uptake of metabolizable vapors, as affected by both the physicochemical properties of the vapors and the metabolic capacities of test animals and people, was explored by using specific examples of vapors commonly encountered in the environment. Problems in making interspecies comparisons were also addressed. Finally, methods for indexing the dose of inhaled carcinogens by using toxic metabolites, DNA or hemoglobin adducts, oncogene activation, gene mutations, and chromosomal changes as biomarkers were reviewed, with an emphasis on the use of such markers in risk assessments.

### *Comparative Deposition, Clearance, and Retention of Particle-Borne Toxicants (R. B. Schlesinger)*

Experimental animals are employed in toxicological studies of inhaled particles, with the ultimate goal of extrapolating the results to humans. To adequately apply the results of such studies to human risk assessment, however, it is essential to consider interspecies differences in particle disposition. Different species exposed to the same particulate atmo-

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sphere may not receive identical doses in comparable respiratory tract regions. Extrapolation of the results of a study with any one species, therefore, may provide unrealistic estimates of the human respiratory tract or systemic dose and, thus, of the relationship between exposure and potential human health effects.

The fate of inhaled particles is dependent both upon their pattern of deposition—the sites within which such particles initially come into contact with airway surfaces and the amount removed from the inhaled air at such sites—and their clearance—the rates and routes by which deposited particles are physically translocated from the respiratory tract. For toxicants that exert their primary action on the surface contacted, the initial deposition pattern is the major predictor of response. However, in many cases, it is the net result of deposition and clearance processes, namely retention—the number of particles remaining in the respiratory tract at specific times after exposure—that determines the degree of any hazard from exposure.

The extent and loci of particle deposition depend upon a number of host factors. The major ones are respiratory tract anatomy and ventilatory characteristics. The human respiratory tract anatomy differs from that of most other mammals used in inhalation toxicology studies, although the implications of this difference for particle deposition have not been adequately appreciated. The respiratory tracts of most mammals have comparable anatomical components, but there are considerable differences in the structure of some of these components. In the upper respiratory tract, for example, there are species differences in the relative shapes and sizes of nasal airways (Patra, 1986; Schreider, 1986). In general, laboratory animals have much more convoluted nasal turbinate systems than do humans, and the length of the nasopharynx in relation to the entire length of the nasal passages also differs between species.

Another major difference between humans and most other mammals commonly used in inhalation toxicology studies is the pattern of tracheobronchial tree branching (Patra, 1986; Schlesinger and McFadden, 1981); the branching is much more symmetrical in humans than in most other laboratory animals. The branching pattern affects the depth of penetration of inhaled particles, because the number of branch divisions from the trachea to each distal bronchiole may differ, and influences localized patterns of deposition within individual airway generations. There are also large interspecies differences in the structure of the pulmonary (alveolar) region (Gehr *et al.*, 1981; Tyler, 1983), including differences in the number of generations of bronchioles and the extent of bronchiolar alveolarization. Alveolar size also differs between species; this may affect deposition efficiency, because of variations in the distance between airborne particles and airway walls.

Breathing pattern during exposure to particle-containing gases or vapors influences the sites and relative amounts of regional deposition. Differences in tracheobronchial tree structures between species also affect airflow patterns. Furthermore, minute ventilation (even when adjusted for differences in body weight) differs between species and, in turn, affects both the number of particles available for deposition and their depth of penetration into the lungs (Schum and Yeh, 1980). Finally, most experimental animals are obligate nasal breathers. Coupled with the aforementioned greater complexity of the nasal passages, the result is greater particle deposition in the upper respiratory tract of experimental animals than occurs in humans breathing orally or even nasally. The extent of upper respiratory tract removal affects the number of particles available for penetration into the lungs and, therefore, influences dose to the rest of the respiratory system. Thus, the relationship between respiratory tract deposition in obligate-nasal-breathing experimental animals and orally breathing humans should be defined.

The deposition of inhaled particles occurs by similar physical mechanisms in both humans and experimental animals. However,

there are interspecies differences in the relationship between particle size and deposition efficiency (the amount of deposition expressed as a percentage of the total number of particles inhaled for specific particle sizes) in different regions of the respiratory tract, and for the respiratory tract as a whole (Schlesinger, 1985). But even in cases for which deposition expressed as a percentage of the total number of particles inhaled is similar, the mass deposition may differ for different species exposed to the same atmosphere. When examined in terms of body or lung mass (weight), smaller animals will receive greater initial particle burdens per unit weight and per unit exposure time than will larger ones (Phalen *et al.*, 1977). For example, the deposition of 1-$\mu$m particles in the rat will be 5–10 times that in humans, if defined on a per unit-lung-weight basis.

Particles that do deposit on airway surfaces either 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. Insoluble particles depositing in the upper respiratory tract and tracheobronchial tree may be cleared by mucociliary transport, whereby a fluid (mucous) lining over the epithelium is moved by the action of cilia. Soluble material may dissolve in the mucus. Clearance from the respiratory (alveolar) region occurs by a number of mechanisms and pathways; the relative importance of each appears to depend upon the physicochemical properties and amounts of material deposited. A major pathway for clearance of insoluble particles involves phagocytosis by resident alveolar macrophages. These cells may then be cleared from the pulmonary region after reaching the distal terminus of the mucociliary transport system or by migrating through the interstitium to the lymphatic system. Soluble particles will dissolve in alveolar lining fluid and enter the blood or lymph directly.

Because dosimetry depends upon clearance rates and routes, adequate toxicological assessment necessitates relating clearance kinetics in laboratory animals to those in humans. Although the basic mechanisms of clearance from the respiratory tract are similar in most species, regional clearance rates may vary substantially. For example, clearance from the alveolar region of mice and rats is much faster than that in dogs and humans, which have similar clearance rates (Snipes, 1989). It is likely that dissolution rates, and rates by which dissolved substances are transferred into blood, are related solely to the properties of the material being cleared and are essentially independent of species (Griffith *et al.*, 1983; Bailey *et al.*, 1985). On the other hand, different rates of mucociliary transport in the conducting airways (Felicetti *et al.*, 1981) or of macrophage-mediated clearance from the alveolar region (Bailey *et al.*, 1985) may result in species-dependent rate constants for these pathways. In terms of mucociliary transport, mucous velocities, in the larger airways at least, seem to be proportional to body weight, while interspecies differences in functional properties of macrophages include differences in phagocytic efficiency and differences in mobility, both random and in response to chemotactic factors that may be released upon deposition of certain particles.

The end result of deposition and clearance processes is retention, which may differ for the same particles inhaled by different species. Thus, especially during chronic exposures, identical lung burdens may not be obtained in all animals, and if the toxic response is related to the lung burden, the biological effects of the inhaled material may also differ. The latter must be considered whenever experimental animals are used in inhalation toxicology studies, because such species differences may make it difficult to extrapolate from the results of studies with laboratory animals to the results expected in humans. There is no one species that is an ideal surrogate for humans in terms of being similar in all aspects of those factors that affect particle disposition. Some species may deposit the particles similarly; others may deposit them differently but may have similar mucociliary or long-term

clearance. There is, thus, a definite need to obtain reliable, baseline comparative data that will allow more precise interspecies extrapolations of deposition and clearance, so that better estimates of risk to humans may be obtained from particle inhalation studies with experimental animals.

### *Comparative Dosimetry of Inhaled Reactive Vapors (H. d'A. Heck)*

Reactive vapors are defined as volatile compounds that can undergo *nonenzymatically catalyzed* chemical reactions in biological systems. Such compounds include aldehydes, epoxides, isocyanates, halohydrins, halomethyl ethers and ketones, $\beta$-lactones, and inorganic oxidizing agents. Reactive vapors are frequently toxic to the respiratory tract, but systemic toxicity may be induced depending on the solubility and reactivity of the vapor.

Reactive vapors are inherently unstable; hence, to measure delivered dose, a fragment of the reactive vapor covalently bound to a target molecule, rather than the vapor itself, is often identified in the tissue of interest. The amount of bound fragment is then related to the inhaled concentration of the reactive vapor by using a pharmacokinetic model, thereby linking the delivered and administered doses. In principle, a variety of target molecules, including DNA, RNA, proteins, peptides, or lipids, could be used to measure delivered dose. The criteria for selecting an appropriate target include relevance to the toxic effect, biological half-life of the target molecule, concentration of target sites relative to the concentration of the toxicant, and the sensitivity and facility of the analytical method.

In this article, we discuss two approaches to modeling the dosimetry of reactive vapors. In the first approach, a physiologically based pharmacokinetic model is used to estimate the extent of the reaction of formaldehyde with DNA in the nasal mucosa of F344 rats, rhesus monkeys, and adult humans. In the second approach, a convection–diffusion–chemical reaction model (Overton *et al.*, 1987) is used to estimate the dose of ozone delivered to the lower respiratory tract tissues of rats and humans. These models differ significantly in their definitions of dose and in their methods for calculating the quantity of toxicant delivered to target macromolecules or tissues.

Formaldehyde (HCHO) is an upper respiratory tract (URT) toxicant that is absorbed primarily in the nasal cavity. Dosimetry measurements of HCHO have been performed by analyzing the concentration of DNA–protein cross-links produced in the nasal mucosa of F344 rats and rhesus monkeys exposed to [$^{14}$C]formaldehyde (Casanova *et al.*, 1989; Heck *et al.*, 1989). Cross-link formation in rats was described in terms of a one-compartment pharmacokinetic model that combines the anterior nose and the nasal turbinates in a single unit (Fig. 1). Cross-linking in monkeys was interpreted by using a three-compartment model involving the anterior nose, turbinates, and nasopharynx as separate units (Fig. 1). HCHO was assumed to be absorbed from the nasal airstream into each compartment and, within each compartment, to be eliminated by saturable reactions (metabolism) or by parallel, nonsaturable processes, one of which is DNA–protein cross-link formation (Fig. 2). The kinetic constants relating delivered-to-administered concentrations were estimated by fitting the models to the nonlinear concentration–response curves observed for cross-link formation. Lower concentrations of cross-links were measured in the nasal tissues of monkeys than in those of rats, at all airborne concentrations.

Because the pharmacokinetic models for HCHO are based on identifiable (and measurable) physiologic parameters, the doses observed should be predictable across species by applying the appropriate scaling factors. Cross-link concentrations in monkeys were predicted from those measured in rats by using conventional allometric scaling methods, but the predictions agreed only semiquantitatively with the experimental results. This was probably due, in part, to the marked differences in URT

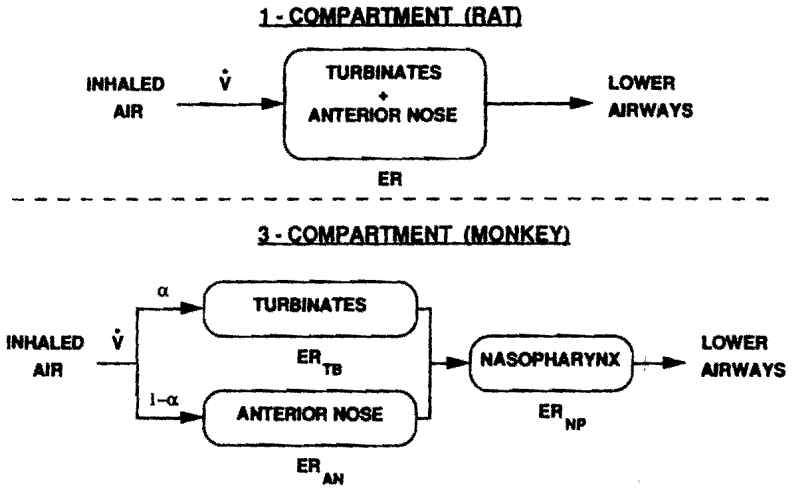


FIG. 1. Compartmental models of the nasal conducting airways illustrating parameters pertinent to HCHO absorption: $\dot{V}$, minute volume; $\alpha$, fraction of airstream contacting the turbinates of the monkey nose; ER or $ER_i$, extraction ratio [fraction of HCHO removed by a particular compartment from the airstream, where $i$ = turbinates (TB), anterior nose (AN), or nasopharynx (NP)].

anatomy and physiology between rats and monkeys. The human nose is structurally similar to the monkey nose; hence, monkey-to-human scaling may be more accurate than rat-to-monkey scaling. Concentrations of cross-links in the human nose were predicted from those measured in monkeys; the results suggested that HCHO is likely to induce lower concentrations of cross-links in humans than in monkeys. The calculations support the use of allometric scaling in the *absence* of suitable pharmacokinetic data, but there is still doubt about the quantitative validity of the results, owing to uncertainties in the magnitudes of the interspecies conversion factors, especially for highly divergent species such as rodents and primates.

Ozone is a lower respiratory tract (LRT) toxicant that reacts rapidly with certain functional groups, especially carbon–carbon double bonds, in biological constituents present in nasal mucus and cells. Measuring $O_3$ dosimetry is experimentally challenging and continues to be an important goal (Santrock *et al.*, 1989). $O_3$ doses delivered to specific regions and tissue components of the LRT have been calculated by using a mathematical model that involves convection, diffusion, and chemical reactions in the lower airways (Fig. 3) (Overton *et al.*, 1987). Key elements of the model are the dimensions of the tracheobronchial airways and pulmonary regions and the thickness and composition of the liquid lining (mucus or surfactant) covering the cells of the LRT.

The model predicts that a sharp maximum in the *tissue* dose of $O_3$ will occur at the ter-

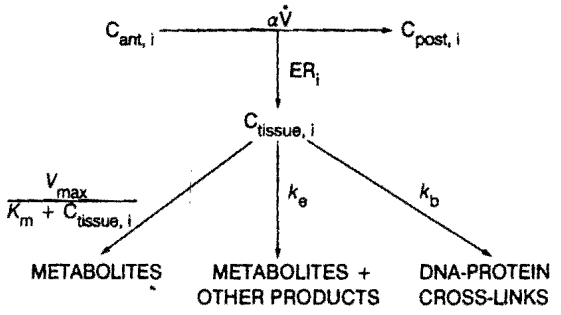


FIG. 2. Reaction scheme for HCHO in compartment $i$: $C_{ant,i}$ and $C_{post,i}$, airstream concentrations of HCHO at anterior and posterior ends, respectively, of compartment $i$; $C_{tissue,i}$, concentration of HCHO in tissue of compartment $i$; $V_{max}$ and $K_m$, apparent maximal velocity and Michaelis constant, respectively, for elimination of HCHO by a saturable metabolism; $k_e$, pseudo-first-order rate constant for removal of HCHO by nonsaturable processes other than DNA–protein cross-link formation; $k_b$, pseudo-first-order rate constant for DNA–protein cross-link formation.

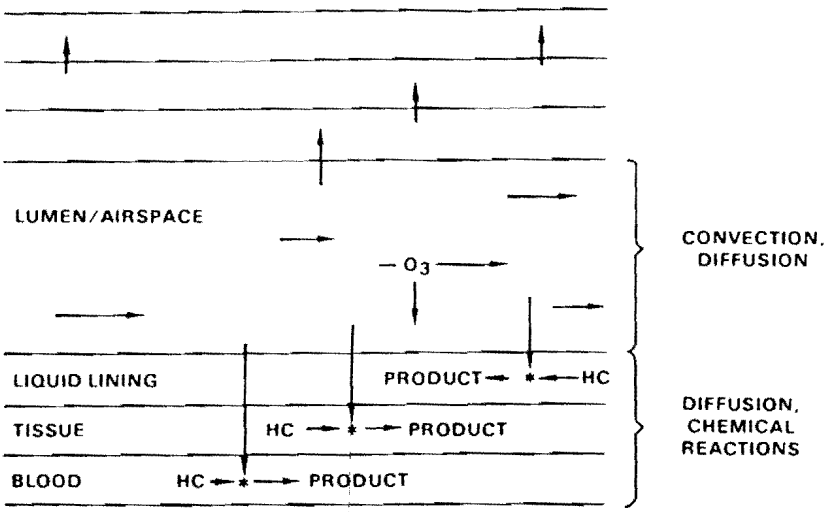


FIG. 3. Diagram illustrating some of the important physical and chemical processes taken into account by the dosimetry model of $O_3$; HC, a biological constituent that reacts with $O_3$. Reprinted with permission from Overton *et al.* (1987).

minal bronchiole–alveolar junction, which is the major site of tissue damage in experimental animals exposed to $O_3$. The model also predicts that rats will receive a higher tissue dose than humans at a given airborne concentration. This mathematical model provides a novel and potentially very useful approach to the problem of interspecies comparisons and facilitates examinations of different exposure scenarios. Unfortunately, the absolute accuracy of dose predictions is presently unknown, and the doses calculated by using the model are not readily validated.

Dosimetry measurements are very important for understanding how species differ in their responses to reactive vapors and for predicting human risks. The database is currently quite limited, however, and much more information is needed with respect to other compounds and species (especially nonrodents). Further advances may be anticipated in several developing areas, including airflow characterization of the upper respiratory tract, integration of dosimetric pharmacokinetic models with models of toxic responses (such as tumor growth models), and experimental validation of model predictions.

*Comparative Uptake and Fate of Inhaled Organic Vapors (M. A. Medinsky)*

Volatile organic chemicals are of interest to toxicologists, because they may have carcinogenic or other toxic effects. They are present in air, household and industrial products, cigarette smoke, and gasoline vapors, and everybody receives some degree of exposure. The dosimetry of these volatile organics is frequently complicated by the fact that, for many organics, the metabolites are more toxic than the parent chemical. Because of the great diversity in the biological activities and physical properties of these volatile organics, it is useful to examine, in a general way, the determinants most important for influencing their uptake, distribution, and elimination. These determinants include those of a physiological nature, chemical factors, metabolic determinants, and those governing the binding of the parent chemical or metabolite to tissue macromolecules.

*Physiological determinants.* Physiological determinants, such as organ weights and physiological processes, have been shown to be related to body weight through the allometric expression, property $\propto$ (body weight)$^a$, where the property of interest is proportional

to a power function of body weight (Adolph, 1949). For many tissue weights, such as those of liver, kidney, blood, and heart, the allometric component, $a$, is approximately equal to 1. Thus, the weight of these organs increases in direct proportion to increasing body weight. For flow-related physiological processes, such as blood flows, clearance, or ventilation, the allometric exponent lies between 0.65 and 0.8. These flow rates, when expressed per unit of body weight, are slower in larger animals than in smaller ones. Differences in the allometric relationships for weights and flows have an impact on kinetics and tissue dosimetry that is important to take into account when extrapolating across species. For example, the terminal half-time ($t_{\frac{1}{2}}$) of a chemical (Eq. (1)) tends to be shorter in smaller animals than in larger ones:

$$t_{\frac{1}{2}} = 0.693 \times \frac{\text{volume of distribution}}{\text{clearance}}. \quad (1)$$

The terminal $t_{\frac{1}{2}}$ decreases with body weight because, as size decreases, the volume of distribution, a weight-related parameter, decreases faster than clearance, a measure of flow. Thus, tissue exposure to volatile organics may be prolonged in larger animals such as man, in comparison to the exposure of tissues in smaller animals such as rodents. For example, after an inhalation exposure to a volatile organic in which equivalent, steady-state blood concentrations are achieved, the clearance of the organic from blood will be slower in larger animals than in smaller ones. This interspecies difference in systemic clearance is illustrated by comparing the time course for blood concentrations of styrene in rats and humans exposed to 80 ppm styrene for 6 hr (Ramsey and Andersen, 1984) (Fig. 4). Note the similar, achieved concentrations in the two species at the end of exposure. However, the disappearance of styrene from rat blood is much more rapid than the comparable decline of that in humans. The slower decline in humans is due to the slower rate of processes such as blood flow and metabolism, in addition to the larger fat volume of the human

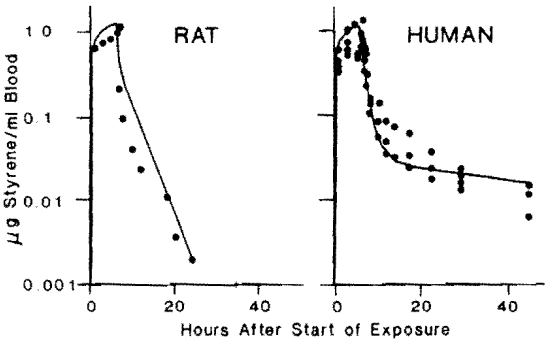


FIG. 4. Blood concentrations of styrene in rats and humans exposed to 80 ppm styrene for 6 hr. Circles are experimentally determined values. Lines are the results of physiological model simulations. Data taken from Ramsey and Andersen (1984).

compared to that of the rat. Differences in flow-related processes across species also influence the uptake of inhaled organics into blood. It will take longer for steady-state blood concentrations to be achieved in larger animals than in smaller ones (National Academy of Sciences, 1986).

The prolonged tissue exposure to volatile organics in humans compared to that in smaller animals such as rodents may have implications for volatile organics for which a metabolite is the toxic species. The total time interval over which metabolism can take place might be longer in larger animals compared to that in smaller ones.

*Partition coefficients.* Partition coefficients are a measure of the affinity of a chemical for one medium compared to another at equilibrium. Partition coefficients of organic vapors are often determined *in vitro* by using methods such as vial equilibration (Gargas *et al.*, 1989; Sato and Nakajima, 1979). Partition coefficients used in physiologically based models are often described in terms of blood:air (Eq. (2)), tissue:air (Eq. (3)) or tissue:blood (Eq. (4)) relationships,

$$P_b = [\text{Blood}]/[\text{Air}], \quad (2)$$

$$P_t = [\text{Tissue}]/[\text{Air}], \quad (3)$$

and

$$P_{tb} = P_t/P_b, \quad (4)$$

TABLE 1

PARTITION COEFFICIENTS FOR SOME VOLATILE ORGANIC CHEMICALS[a]

| Chemical | Blood/air | Fat/air | Muscle/air |
|---|---|---|---|
| Isoprene | 3 | 72 | 2 |
| Benzene | 18 | 500 | 11 |
| Styrene | 40 | 2000 | 40 |

[a] Data taken from Gargas *et al.* (1989).

where $[X_i]$ = the concentration of the volatile organic in that medium at equilibrium.

Table 1 contains partition coefficients for several volatile organic chemicals. The blood:air partition coefficient is a critical determinant in the uptake and achieved blood concentrations of volatile organic chemicals (Fig. 5). As the blood:air partition coefficient increases from 3 for isoprene to 40 for styrene (Table 1), the concentration of the volatile organic in the systemic circulation increases for exposures at equivalent airborne concentrations. Similarly, the fat:air partition coefficients for isoprene, benzene, and styrene (Table 1) indicate that the highest fat concentrations of volatile organics will be achieved by very lipophilic chemicals such as styrene. The fat compartment plays an important role in accumulating and storing the volatile organic, both during and after exposure. This stored chemical becomes available for distribution by the systemic circulation to the metabolizing organs after the end of exposure. The importance of postexposure metabolism is demonstrated in Fig. 6, which shows that for isoprene, the chemical with the smallest fat:air partition coefficient, most of the metabolism occurs during the 6-hr exposure. For benzene, which has a larger partition coefficient than isoprene, the majority of metabolism still occurs during exposure; however, approximately one-third of the benzene metabolism occurs after the end of the exposure. For the highly lipid-soluble organic, styrene, over 50% of metabolism takes place after the end of exposure. Figure 6 illustrates the importance of fat as a storage

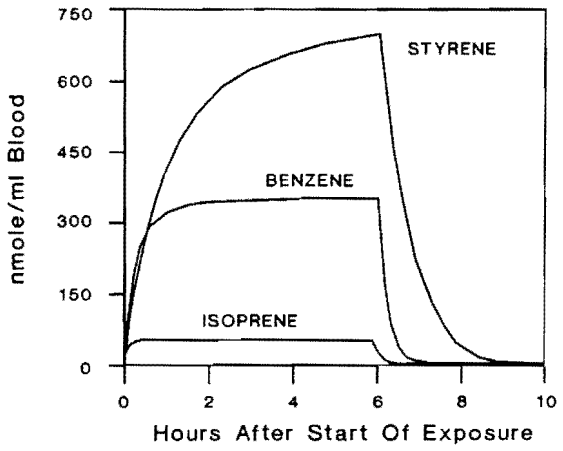


FIG. 5. Effect of blood/air partition coefficient on steady-state blood concentrations of three volatile organics, both during and after a 6-hr inhalation exposure to 600 ppm.

compartment for volatile organics and emphasizes that metabolism of the volatile organic can continue for a significant period of time after the individual is removed from the exposure atmosphere.

*Metabolic determinants.* Metabolic determinants such as $V_{max}$ (maximum metabolic capacity) and $K_m$ (affinity of the enzyme for a substrate) can be key determinants in the exposure of tissues to the toxic chemical species of volatile organics. For many volatile organics, the metabolite of the chemical, and not

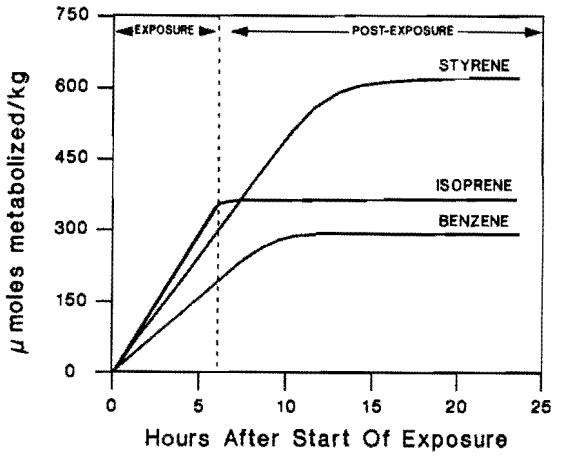


FIG. 6. Effect of fat/air partition coefficient on postexposure metabolism of three volatile organics, both during and after a 6-hr inhalation exposure to 600 ppm.

the chemical itself, is responsible for the toxicity. The importance of metabolic determinants is illustrated here for benzene. Sabourin *et al.* (1988, 1989) and Medinsky *et al.* (1989) determined that the major, stable benzene metabolites produced by $B6C3F_1$ mice and F344 rats were sulfate and glucuronide conjugates of phenol and hydroquinone, glutathione conjugates of phenol, and muconic acid, an opened-ring metabolite. Distinct species differences in the formation of these metabolites were noted (Fig. 7). When rats were exposed to 600 ppm benzene for 6 hr, the primary metabolite produced was the sulfate conjugate of phenol. Smaller amounts of the glutathione conjugates and muconic acid were formed. A very small amount of hydroquinone sulfate and virtually no hydroquinone glucuronide were detected. In mice exposed to the same concentration of benzene, the profile of metabolites was very different. Significant quantities of other metabolites in addition to phenyl sulfate were produced, including hydroquinone glucuronide and phenyl glucuronide. Studies by Sabourin *et al.* (1989) and Medinsky *et al.* (1989) showed distinct, concentration-related differences in the profiles of metabolites, in addition to the species-related differences. These studies indicated that, based on present knowledge, allometric scaling could not be used to predict the behavior of benzene in one of these rodent species based on its metabolic capacity in the other species.

In summary, uptake of volatile organic chemicals is a complex process that can be described in the context of physiological, chemical, and biochemical determinants. Physiological factors that are important in the tissue dosimetry of volatile organics can generally be extrapolated across species. Because flow-related determinants tend to extrapolate across species as a fractional power of body weight, the time course for systemic uptake and elimination and for tissue exposure to absorbed chemicals will be prolonged for species with larger body weights (humans) compared to those of species with smaller body weights (rodents) exposed to the identical concentration of the chemical. Chemical determinants of uptake can vary significantly among volatile organic chemicals, but generally are similar across species for particular chemicals. Chemical determinants such as blood:air and tissue:blood partition coefficients govern the systemic and tissue concentrations of volatile organics and their metabolites. Metabolic capacity is probably the most important determinant of tissue dosimetry, because metabolites of many volatile organics are more toxic than the parent chemical. Unfortunately, at the present time, metabolic rates are the most difficult to predict across species, because

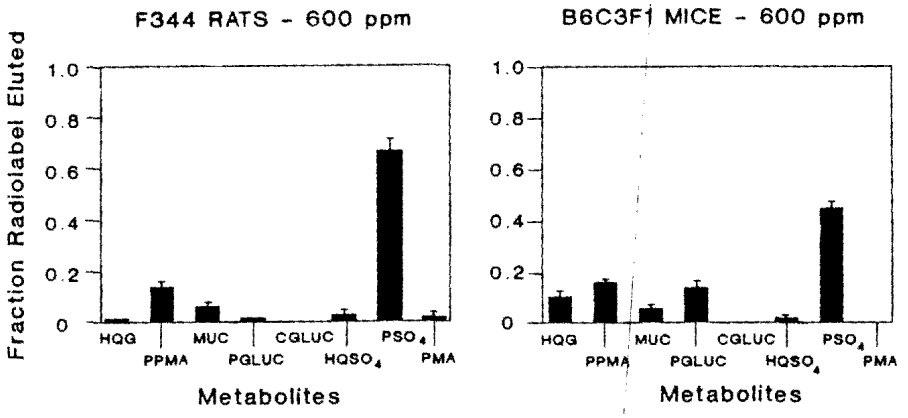


FIG. 7. Differences in the urinary excretion of benzene metabolites by rats and mice exposed to 600 ppm benzene for 6 hr. HQG, hydroquinone glucuronide; PPMA, pre-phenylmercapturic acid; MUC, muconic acid; PGLUC, phenyl glucuronide; CGLUC, catechol glucuronide; $HQSO_4$, hydroquinone sulfate; $PSO_4$, phenyl sulfate; and PMA, phenylmercepaturic acid.

of our lack of knowledge of the factors that are critical for understanding species differences in the metabolism of organic chemicals.

*Molecular Dosimetry of Inhaled Carcinogens: Implications for Epidemiology and Risk Assessment (G. W. Lucier)*

There is a great deal of uncertainty in the estimation of human risks from low-dose exposure to chemical carcinogens when high-dose animal data are used as the basis for extrapolation. It is increasingly evident that molecular approaches can contribute a great deal to reducing the uncertainties that are inherent in the risk assessment process when gross biological endpoints such as tumors are used. This knowledge has led to the development of approaches designed to incorporate biomarkers into toxicological and epidemiological studies.

The framework for incorporating molecular data or "biomarkers," as they are frequently called, into the risk assessment process is illustrated in Fig. 8. The essence of this illustration is that there are numerous biological and biochemical events that ultimately determine an adverse health effect following exposure to a toxic chemical. The chemical must first be internalized, leading to its presence in blood or tissues. There are numerous cases where toxic chemicals have been detected at extraordinarily low concentrations in blood. For example, recent developments in analytic methodology have lowered the limits of detection for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to the low, part-per-trillion range, allowing detection of this compound in the general population. Once internalized, many chemicals are metabolized by a wide variety of drug-metabolizing enzymes that are present in virtually every tissue of the body (Lucier *et al.*, 1979). These enzymes include the multiple forms of cytochrome P450, epoxide hydrolase glutathione transferase, glucuronyltransferase, and sulfotransferase, among others. Depending on the chemical being metabolized, each of these enzymes may play a role in either an activation or a detoxication pathway. For example, glutathione transferase detoxifies electrophilic arene oxides of polycyclic aromatic hydrocarbons (Jerina and Bend, 1975), but catalyzes the formation of a DNA-reactive metabolite of ethylene dibromide (Guengerich *et al.*, 1987).

The balance between activating and detoxifying enzyme systems governs the rate of delivery of bioactive metabolite to the macromolecular target site. For carcinogens, which are initiating agents, the macromolecular interaction of interest could be a DNA adduct, and for chemicals that are tumor promoters, the critical interaction could be receptor occupancy. It should be noted that "initiation" and "promotion" are operational terms, not stages that have clearly defined mechanisms. In any event, the concentration of DNA adduct or occupied receptor has been termed the "biologically effective dose" and leads to

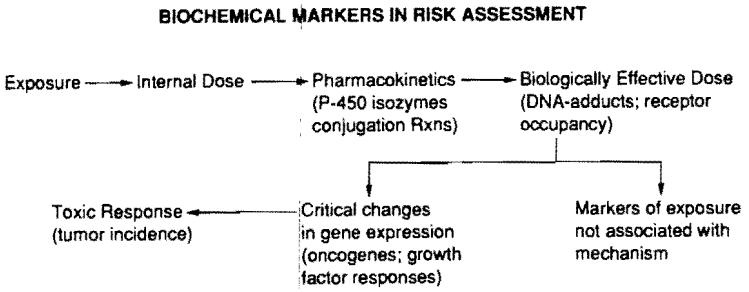


FIG. 8. Schematic representation of the sequence of events producing a toxic response after exposure to a toxicant.

changes in gene expression, which may or may not be associated with the mechanism of carcinogenesis. Changes not involved in the carcinogenesis process may provide a reliable "marker of exposure," such as occurs when arylhydrocarbon hydroxylase is induced following exposure to polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene and TCDD. Other changes in gene expression, such as activation of proto-oncogenes and alterations in cell proliferation pathways, may have direct links to mechanisms of chemical carcinogenesis (Reynolds *et al.*, 1987; Swenberg *et al.*, 1987) and may also represent possible candidates for "markers of effect" for chemical carcinogens. A detailed review and evaluation of biomarkers has recently been published by a National Research Council (NRC) Committee (1987).

One of the most compelling issues in the risk assessment process is the estimation of dose–response relationships. For reasons of economic necessity, high doses are used in animal bioassays for carcinogenicity of environmental chemicals. Extrapolation of tumor incidence arising from high-dose exposures in animals to low-dose exposure risks in humans is a highly uncertain exercise. Biomarkers, such as activated metabolites or DNA adducts, can usually be detected following exposure to much lower doses than those needed to detect increased tumor incidence. Therefore, it seems reasonable to assume that a careful evaluation of biomarkers in experiments covering a wide exposure dose range offers an opportunity to remove some of the uncertainty in the risk assessment process by providing a more reliable estimation of the shape of the dose–response curve at low doses. These kinds of studies have been termed molecular dosimetry.

There are several issues that have an impact on the use of DNA adducts as molecular dosimeters. These include adduct heterogeneity, cell specificity, and the use of surrogate markers (i.e., lymphocytes). The two most sensitive ways of detecting DNA adducts in human samples are immunochemistry (Perera, 1987; Harris *et al.*, 1985) and $^{32}$P-postlabeling (Reddy and Randerath, 1987). One of the best examples of a DNA adduct used as a molecular dosimeter is NNK (a carcinogenic metabolite of nicotine) (Belinsky *et al.*, 1987). $O^6$-Methylguanine, the promutagenic adduct of NNK, is formed more efficiently in the lung at low exposure doses than at high exposure doses, and this finding is consistent with the dose–response relationships observed for the carcinogenicity of this compound. Bond *et al.* (1989) characterized dose–response patterns for DNA adducts arising from exposure to polycyclic aromatic hydrocarbons present on particles such as diesel exhaust. These studies related relative adduct concentrations to lung tumor incidence. In another study, DNA adducts were detected in human lymphocytes by $^{32}$P-postlabeling, but the adduct profiles of smokers and nonsmokers were not different (Jahnke *et al.*, 1990). A great deal of interindividual variation exists in adduct concentrations, and this variation may reflect polymorphisms in metabolic activation/deactivation reactions in human populations. For example, one study showed that the polymorphism of glutathione transferase $\mu$ effects DNA adduct formation of some carcinogens, but not of others (Liu *et al.*, 1990).

The results of a recent National Toxicology Program study (Tennant *et al.*, 1987) suggest that as many as 40% of the chemicals that are positive for carcinogenicity in the lifetime bioassay are acting through nongenotoxic mechanisms. The implication is that effects of DNA adducts on signal transduction pathways, receptor-mediated proliferative responses, and cell cycle control are involved in the mechanism of action of many carcinogens. Therefore, one area of research needed is the evaluation of the quantitative relationships between biochemical events involved in tumor promotion and carcinogenic incidence. A central underlying need relative to molecular dosimetry studies is increased knowledge of the diverse mechanisms of chemical carcinogens.

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