

Differences in the Pathways for Metabolism of Benzene in Rats and Mice Simulated by a Physiological Model

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Studies conducted by the National Toxicology Program on the chronic toxicity of benzene indicated that B6C3F₁ mice were more sensitive to the carcinogenic effects of benzene than were F344 rats. A physiological model was developed to describe the uptake and metabolism of benzene in rats and mice. Our objective was to determine if differences in toxic effects could be explained by differences in pathways for benzene metabolism or by differences in total uptake of benzene. Compartments incorporated into the model included liver, fat, a poorly perfused tissue group, a richly perfused tissue group, an alveolar or lung compartment and blood. Metabolism of benzene was assumed to take place only in the liver and to proceed by four major competing pathways. These included formation of hydroquinone conjugates (HQC), formation of phenyl conjugates (PHC), ring-breakage and formation of muconic acid (MUC), and conjugation with glutathione with subsequent mercapturic acid (PMA) formation. Values for parameters such as alveolar ventilation, cardiac output, organ volumes, blood flow, partition coefficients, and metabolic rate constants were taken from the literature.

Model simulations confirmed that during and after 6-hr inhalation exposures mice metabolized more benzene on a μ mole per kilogram body weight basis than did rats. After oral exposure, rats metabolized more benzene than mice at doses above 50 mg/kg because of the more rapid absorption and exhalation of benzene by mice. Model simulations for PHC and PMA, generally considered to be detoxification metabolites, were similar in shape and dose-response to those for total metabolism. However, simulations for the metabolites representative of the putative toxication pathways, HQC and MUC, indicated that after both oral and inhalation exposures mice would produce more of these metabolites than rats at all concentrations. This was due to the greater rates of metabolism to these metabolites for mice compared to rats. Increased metabolism of benzene through the HQC and MUC pathways in mice is consistent with the observed susceptibility of this species to benzene toxicity.

Introduction

Benzene is an important and widely used industrial solvent (1). Epidemiology studies have shown an increase of leukemias and aplastic anemia in workers exposed to benzene (2). To estimate the potential carcinogenic effects of benzene, the National Toxicology Program (NTP) orally exposed F344/N rats and B6C3F₁ mice to benzene in corn oil for 2 years (3). Mice were identified in these studies as the more sensitive species. An increased incidence of

lymphomas, lung tumors, and glandular tumors was noted.

It is generally recognized that metabolism of benzene is necessary for the expression of toxicity. What is not known, however, is the ultimate toxic metabolite or combination of metabolites. Figure 1 is a simplified scheme for the metabolism of benzene. The metabolites outlined in the scheme were based on the work of Sabourin et al. (4-6) and Bechtold et al. (7) who studied the metabolism of benzene in F344 rats and B6C3F₁ mice after oral or inhalation exposure. Included in this scheme are two putative toxic metabolites, benzoquinone and muconaldehyde. The pathways for the formation of these reactive metabolites suggest that levels of the stable metabolites, hydroquinone conjugates and muconic acid, could be used as markers for the formation of benzoquinone and muconaldehyde. For example, a species with a high capacity for formation of hydroquinone conjugates might be

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assumed to produce more benzoquinone compared to a species with a low capacity for formation of hydroquinone conjugates. The two other pathways in Figure 1 represent pathways leading to formation of two important groups of detoxification metabolites, the phenyl and mercapturic acid conjugates.

Toxicokinetic models can be used to offer insights into the mechanisms that produce toxic effects and to explain the differences noted in the toxic effects between species. Models can also be used for extrapolation. Typical extrapolations include those from animal studies to human exposures, from a single to continuous exposure, and from high to low concentrations.

The objectives of our study were to develop a physiological model to simulate the metabolism of benzene after oral or inhalation exposure and to compare results of model simulations conducted for rats and mice. We wanted to determine if differences in toxic effects seen in rats and mice could be explained by differences in pathways for metabolism or by differences in total metabolism.

Benzene Physiological Model Structure

The physiological model constructed to describe the metabolism of benzene, the differential equations driving the model, and experimental data used to derive model parameters have been described elsewhere (8,9). This model is an adaption of that proposed by Ramsey and Anderson (10). A diagram of the model is presented in Figure 2 and the parameters incorporated into the model to describe disposition of benzene by rats and mice are presented in Table 1. As indicated in Table 1, the physiological parameters such as cardiac output, alveolar ventilation, blood flow to organs, organ volumes, body weight, and the chemical parameters such as blood/air and tis-

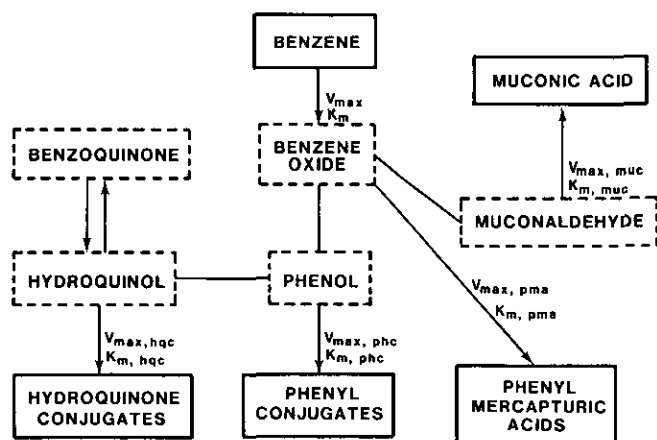


FIGURE 1. Scheme for metabolism of benzene. Biochemical rate constants are outlined in Table 1. This scheme for metabolism of benzene takes place in the liver compartment described in Fig. 2. (□) Represent metabolites of benzene that were measured and used in model simulations; (▭) represent intermediate metabolites that were not incorporated into the model.

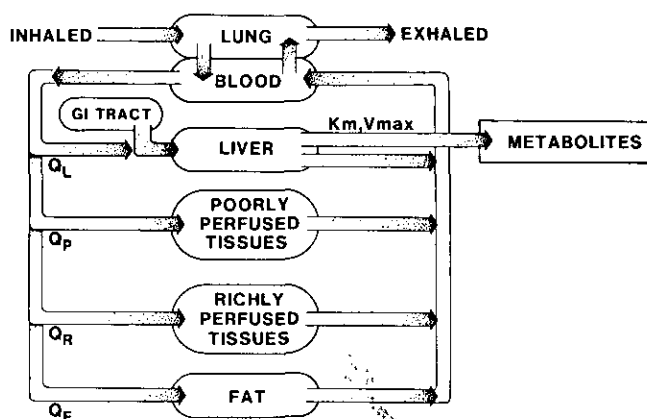


FIGURE 2. Physiological model of benzene metabolism. Metabolism of benzene was presumed to take place in the liver compartment. Pathways for benzene metabolism incorporated into the model are shown in Fig. 1.

sue/blood partition coefficients for benzene were taken from the literature. Values for gastrointestinal-liver transport and the metabolic rate constants were adjusted until results from model simulations compared favorably to data determined by Sabourin et al. (5,9). Methods for these determinations have been described previously (8).

In this model benzene can be inhaled or ingested. Inhaled benzene will be absorbed into the blood in proportion to the benzene blood/air partition coefficient presented in Table 1. Once in the blood, benzene will be transported to other organs in proportion to blood flow to that organ (or group of tissues) and the benzene tissue/blood partition coefficient. Ingested benzene is assumed to be translocated from the gastrointestinal tract to the liver by a first order process.

Tissue compartments included in the model were a) liver, the only organ where metabolism of benzene takes place; b) group of poorly perfused tissues including muscle and skin, c) a group of richly perfused tissues including bone marrow, kidney, and intestines; and d) a fat compartment. The poorly perfused tissues are important because of the large body mass they represent, the rapidly perfused tissues because of the large blood flow to these tissues, and the fat compartment because of its large partition coefficient. Large tissue mass, high blood flow, and increased solubility will each result in significant amounts of benzene being distributed to that compartment. However, the kinetics of distribution will be different for each compartment.

Any benzene that does not partition into any of the above compartments will return to the lungs through the venous circulation. Depending on the alveolar concentration of benzene, benzene in the venous blood can partition into the alveolar space and be exhaled. During a prolonged inhalation exposure, an equilibrium can be established between the alveolar concentration and venous concentrations of benzene. However, there are situations in which there will be a net efflux of benzene from the blood into the alveolar space, such as after an oral exposure or after the end of an inhalation exposure. This is because the concentration of benzene in the alveolar

Table 1. Parameters used in the physiological simulation model for benzene metabolism in F344 rats and B6C3F₁ mice.

Parameter	Value	
	Rats	Mice
Physiological		
Aveolar ventilation, L/hr · kg ^a	32.4	55.3
Cardiac output, L/hr · kg ^b	19.4	34.8
Gastrointestinal transfer, fraction/hr	0.25	3.0
Body weight, kg	0.288	0.030
Blood flow (fraction of cardiac output) ^b		
To liver	0.25	0.25
To fat	0.09	0.09
To poorly perfused tissues ^c	0.15	0.15
To richly perfused tissues ^d	0.51	0.51
Organ volumes (fraction of body weight) ^e		
Blood	0.056	0.056
Liver	0.03	0.03
Fat	0.11	0.11
Poorly perfused tissues	0.74	0.74
Richly perfused tissues	0.06	0.06
Chemical		
Molecular weight, g/mole	78.2	78.2
Partition coefficients ^f		
Liver/blood	1.0	1.0
Fat/blood	28.0	28.0
Poorly perfused tissues/blood	0.6	0.6
Richly perfused/blood	1.0	1.0
Blood/air	18.0	18.0
Biochemical parameters ^g		
V _{max} , μmole/hr · kg	122	200
K _m , μmole/L	40	1.0
V _{max} , phc, μmole/hr · kg	174	333
K _m , phc, μmole/L	5	3.0
V _{max} , pmc, μmole/hr · kg	104	90
K _m , pmc, μmole/L	15	4.0
V _{max} , hqc, μmole/hr · kg	1.7	27
K _m , hqc, μmole/L	0.5	0.1
V _{max} , muc, μmole/hr · kg	3.5	12
K _m , muc, μmole/L	0.5	0.1

^aExperimentally determined (9).

^bData from Ramsey and Andersen (10).

^cIncludes muscle, skin and bone.

^dIncludes kidneys, brain, heart, spleen, intestines, bone marrow, etc.

^eData from Dutcher et al. (11) and Birnbaum et al. (12).

^fData from Thomas (13).

^gRefer to Fig. 1

space will be lower than the concentration of benzene in the blood for both oral and inhalation exposure.

In the model, metabolism of benzene occurs only in the liver. Michaelis-Menten kinetics are used to model the formation of the benzene metabolites outlined in Figure 1. Both the total amount of benzene metabolized and the total amounts of the individual metabolites formed are tracked in this model. Initially benzene is metabolized to benzene oxide, which is further metabolized by one of four pathways. The first metabolite is formed by rearrangement of the benzene oxide to phenol followed by conjugation of phenol to phenyl sulfate or phenyl glucuronide. Thus, for the purposes of model simulations, the total amounts of phenyl sulfate and phenyl glucuronide were combined and expressed as total phenyl conjugates. A second pathway for benzene oxide metabolism consisted of reaction with glutathione and subsequent modification of the mercapturic acids. This pathway was represented by combining the total amounts of prephenyl mercapturic acid and phenyl mercapturic acid.

The third pathway for benzene metabolism involved rearrangement of benzene oxide to phenol, oxidation to hydroquinone, and conjugation to hydroquinone glucuronide or sulfate. For this pathway total amounts of hydroquinone glucuronide and hydroquinone sulfate formed were combined. The fourth pathway was metabolism of benzene oxide to muconic acid presumably through a muconaldehyde intermediate. Although benzene could be metabolized to other metabolites such as catechol or trihydroxybenzene, only very low levels of these metabolites were found in animal studies (4-6) and thus pathways for formation of these metabolites were not included in our model.

Benzene Physiological Model Simulations

Using the parameters in Table 1 and the model structure described in Figure 2 (3), we conducted simulations of uptake of benzene by rats and mice over a range of oral

or inhalation exposures. The total amount of benzene metabolized as well as the formation of individual metabolites was simulated over a 48-hr period. Results of model simulations for total benzene metabolized after exposure of rats and mice to a range of concentrations are presented in Figure 3. The oral simulations represent metabolism of benzene following a single bolus gavage with various doses of benzene. The inhalation exposures represent metabolism of benzene during and after a single 6-hr inhalation exposure. To facilitate comparisons between rats and mice, model simulations were expressed as μmole benzene metabolized per kilogram body weight. Thus, all comparisons described are in terms of relative amounts.

Simulations demonstrated that for mice, formation of more benzene metabolites could be achieved by inhalation exposure over a 6-hr period as compared to oral exposures. For rats, similar amounts of benzene were metabolized after inhalation exposure or oral administration. It is apparent that for any given inhaled benzene concentration, mice will metabolize more benzene as compared to rats (Fig. 3). Total metabolism of benzene in rats and mice is similar for oral exposures up to 50 mg benzene/kg. Above that, rats metabolize more benzene than do mice.

Similar pictures are presented for the phenyl conjugates as indicated in Figure 4 and the phenyl mercapturic acids in Figure 5. Model simulations indicated that mice produce more of these metabolites after inhalation exposure compared to rats on a per kilogram body weight basis. Except for very low oral doses, rats produce more of these metabolites as compared to mice when expressed on a per kilogram body weight basis.

In looking at the hydroquinone conjugates (Fig. 6) and muconic acid (Fig. 7), markers for toxic metabolites, the simulations are very different than those seen for the detoxification metabolites or for total metabolism. In the case of the hydroquinone metabolites, mice produced far greater amounts of these metabolites after all inhalation or oral exposures compared to rats. For example,

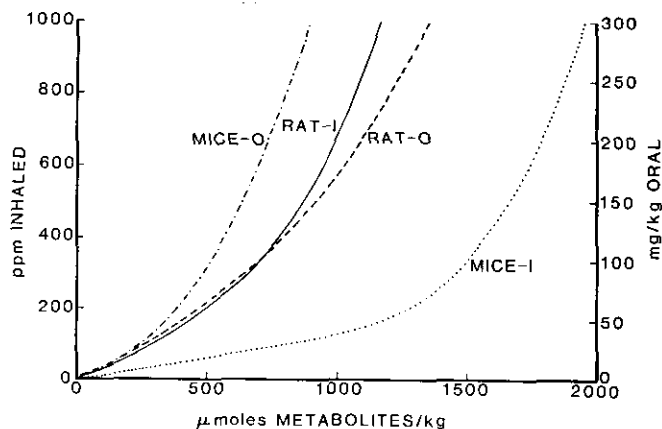


FIGURE 3. Total metabolism of benzene in rats and mice compared after oral administration (O) or a single 6-hr inhalation exposure (I). Lines represent results of model simulations for rats and mice.

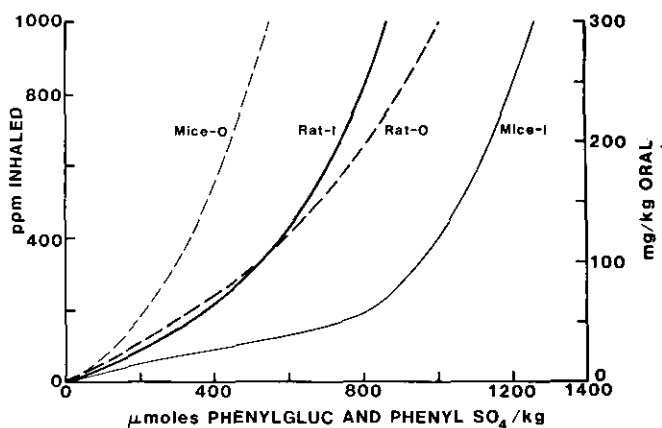


FIGURE 4. Phenyl conjugates excreted by rats and mice compared after oral administration (O) or a single 6-hr inhalation exposure (I). Lines represent results of model simulations for rats and mice.

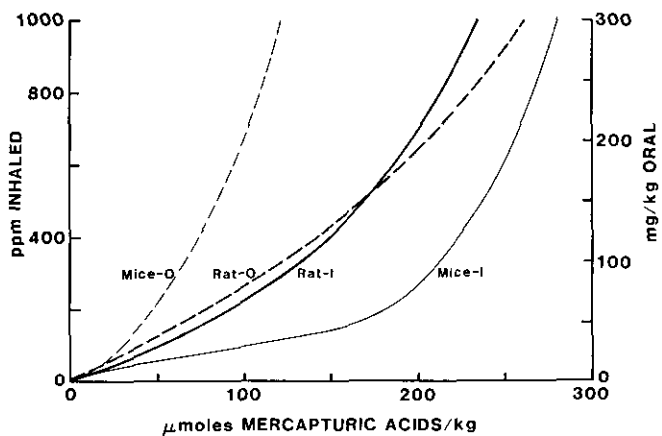


FIGURE 5. Phenyl mercapturic acids excreted by rats and mice compared after oral administration (O) or a single 6-hr inhalation exposure (I). Lines represent results of model simulations for rats and mice.

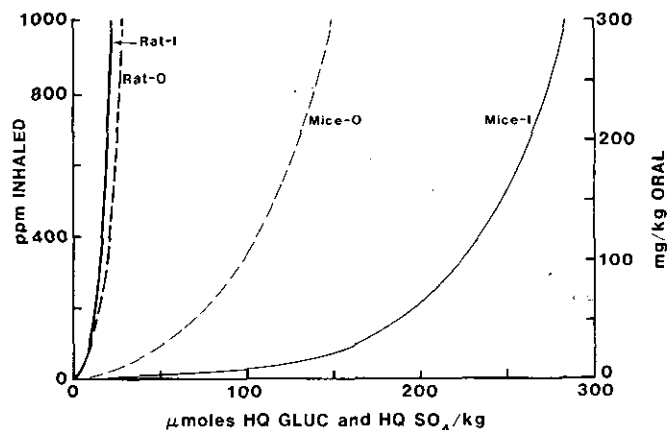


FIGURE 6. Hydroquinone conjugates excreted by rats and mice compared after oral administration (O) or a single 6-hr inhalation exposure (I). Lines represent results of model simulations for rats and mice.

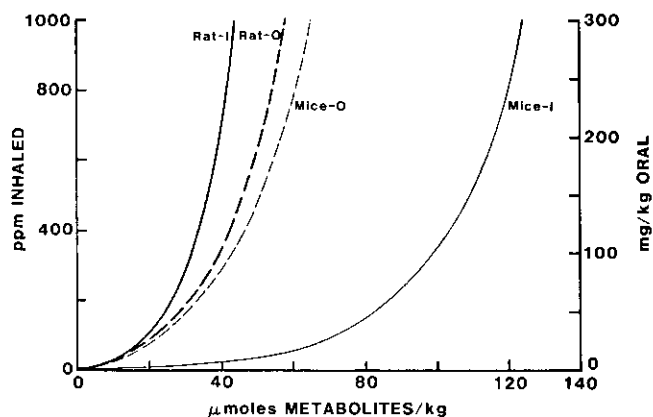


FIGURE 7. Muconic acid excreted by rats and mice compared after oral administration (O) or a single 6-hr inhalation exposure (I). Lines represent results of model simulations for rats and mice.

amounts of hydroquinone conjugates produced by rats at 300 mg/kg oral exposures were similar to levels produced by mice after only 12 mg/kg exposure. Since hydroquinone is considered to be a marker for benzoquinone, one of the putative toxic metabolites, these model simulations are consistent with the observed differences in toxicity between rats and mice orally exposed to benzene.

Larger amounts of muconic acid, a marker for the putative toxic metabolite muconaldehyde, were also seen in mice after oral or inhalation exposure compared to rats. After oral administration these results are not as dramatic as those seen for hydroquinone. Considering that after oral exposure rats metabolize more total benzene compared to mice when expressed on a μ mole per kilogram body weight basis, the larger amounts of muconic acid for mice indicated that a greater portion of benzene metabolism is through the muconic acid pathway in mice.

Discussion and Extrapolation

The major results of model simulations were that metabolism to hydroquinone conjugates and muconic acid represent significant metabolic pathways in mice. In contrast, rats metabolize benzene primarily to the phenyl conjugates and the phenyl mercapturic acids. This differential metabolism of benzene by rats and mice is consistent with the increased susceptibility to the toxic effects of benzene exhibited by mice compared to rats as noted in chronic oral toxicity studies conducted by the National Toxicology Program (3). Increased formation of hydroquinone conjugates and muconic acid may be an indication that increased concentrations of either benzoquinone, unconjugated hydroquinone, muconaldehyde, or a combination of these metabolites are present in the target tissues of mice compared to rats.

Currently, the model structure presented here does not enable us to predict the concentration of these unconjugated and reactive metabolites in tissues such as the target cells of the bone marrow. We are simulating only the formation of the end products of benzene metabolism, the water soluble urinary metabolites. As such we have assumed for purposes of simplification that metabolism

of benzene occurs only in the liver. This may or may not be true. It is likely that the target organs such as bone marrow are capable of metabolism of benzene, phenol, or hydroquinone. Thus, distribution of these compounds to tissues such as bone marrow is an important process to consider in the formation of physiological models in the future.

One of the most useful features of a physiological model is insight into the factors that are critical in the uptake and metabolism of a chemical. For example, the rate constants for metabolism of benzene by rats and mice are very different. As indicated in Table 1, the maximum rates for metabolism of benzene oxide to the hydroquinone conjugates and muconic acid are higher for mice compared to rats. These higher maximum rates result in formation of more of these metabolites.

The rate constants for benzene metabolism in Table 1 can be divided into two groups. The first group includes metabolites with a small apparent K_m , hydroquinone, and muconic acid. The second group includes metabolites with a large apparent K_m , the phenyl conjugates, and mercapturic acids. K_m is defined in Michaelis-Menten kinetics as the concentration of the substrate at which the reaction velocity is half-maximal. In addition, the detoxification metabolites have larger V_{max} than do the toxication metabolites. This suggests that metabolism to hydroquinone and muconic acid are low-capacity, high-affinity pathways while metabolism to phenyl conjugates and mercapturic acids follows a higher capacity, lower affinity enzyme system.

The implication of the relationship between these parameters can be seen in Figures 8A and 8B. These figures compare total metabolism and the formation of individual metabolites by mice as a function of 6-hr inhalation exposure to various concentrations of benzene. Above approximately 75 ppm the phenyl conjugates are the predominant metabolites produced. Equal amounts of the hydroquinone conjugates and the mercapturic acids are formed. Muconic acid is produced in the smallest quantity. However, for concentrations below 200 ppm, the effect of the differences in the K_m values of the pathways is apparent (Figure 8B). At concentrations below approximately 35 ppm, the hydroquinone conjugates are produced in the highest quantity. Above that concentration the production of the phenyl conjugates exceeds that of the hydroquinone conjugates, which increases in a non-linear manner. Similarly, at concentrations below approximately 75 ppm, muconic acid is produced in greater amounts than the mercapturic acids. Above 75 ppm, formation of mercapturic acids exceeds that of muconic acid.

Figure 8B suggests that the proportion of total metabolism due to individual metabolites at high concentrations (e.g., above 200 ppm) is not predictive of metabolism at lower concentrations. Since the putative toxic metabolites are preferentially produced at low concentrations, this means that linear extrapolation of metabolite profiles from high concentrations to low concentrations will underestimate the contribution of the putative toxic metabolites to total metabolism. If toxicity is proportional to the flux of benzene through the putative toxication

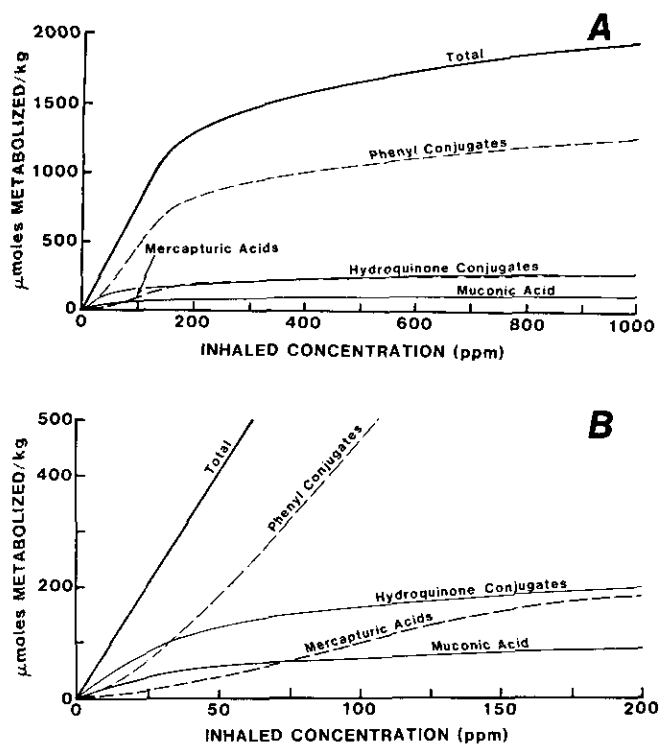


FIGURE 8. Comparison of benzene metabolites produced by mice after inhalation of benzene for 6 hr. Lines indicate model simulations for total metabolism or individual groups for concentrations up to 1000 ppm benzene (A) or 200 ppm benzene (B).

pathways of muconaldehyde and benzoquinone formation, and if there is no threshold in the concentration of metabolites required to elicit a toxic response, then linear extrapolation of toxic effects obtained at high doses may underestimate the risk at low exposures.

The physiological model can also be used to gain insights into differences in the uptake of a chemical as related to different exposure routes. For example, after oral exposure rats metabolize more benzene than do mice, yet after inhalation exposure mice metabolize more benzene at all exposure concentrations (Fig. 1). In both cases the identical V_{\max} and K_m for overall metabolism of benzene are used for inhalation or oral exposure, although different metabolic parameters are used for rats and mice. In the model simulations, the differences in uptake between rats and mice were due to the differences in the gastrointestinal translocation rate constants for these species (Table 1). This is demonstrated in Figure 9A in which the total metabolism of benzene by mice is simulated following oral exposure to 50 mg benzene/kg. In these simulations, the gastrointestinal-liver translocation rate constant was varied from 4 hr^{-1} to 0.5 hr^{-1} . The simulations demonstrate that decreasing the rate constant for gastrointestinal transport of benzene increased the total time over which benzene was transported to the liver. This slower delivery of the same total amount of benzene ultimately results in more benzene being metabolized. Any benzene that is not metabolized will be exhaled (9).

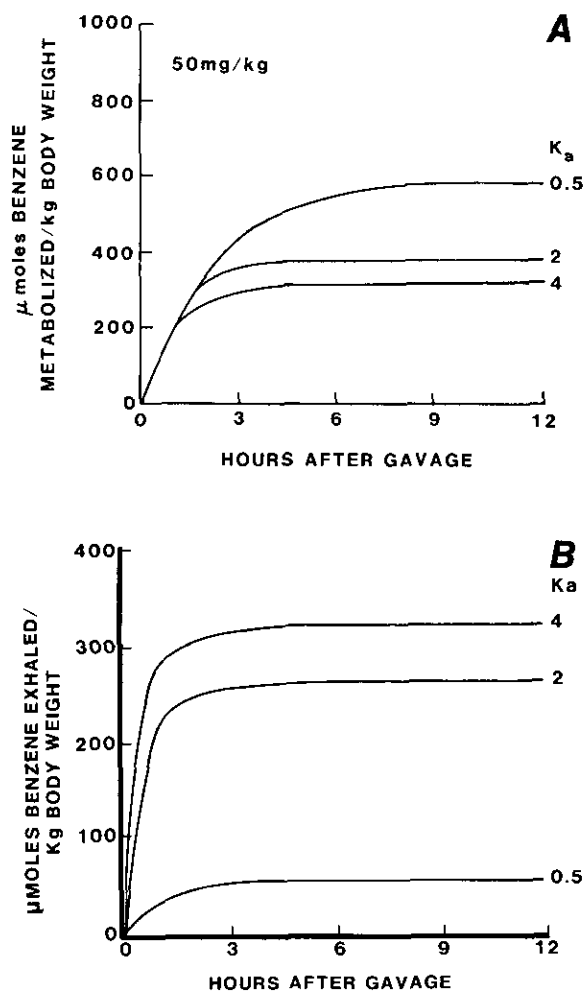


FIGURE 9. Effect of variations in the gastrointestinal transfer rate constant (K_a) on cumulative total metabolism (A) of benzene by mice after oral exposure to 50 mg benzene/kg body weight or on the exhalation of unmetabolized benzene (B).

Figure 9B demonstrates the effect of alterations in the gastrointestinal translocation rate constant on the amount of benzene exhaled. As the rate constant increased, the total amount of benzene exhaled increased. Thus, benzene that is not metabolized by the liver can partition into the blood. As this blood passes through the lungs the benzene will be exhaled proportional to the blood/air partition coefficient. Model simulations are consistent with the observations of Sabourin et al. (9) who demonstrated that with increasing oral exposure an increasing fraction of the benzene was exhaled unmetabolized.

In contrast, the uptake or metabolism of benzene after inhalation exposure is the result of the differences in V_{\max} and K_m between species (Fig. 10). Although the rate of alveolar ventilation of mice is approximately twice that of rats, suggesting that the mice will inhale more benzene than will rats; they will also exhale more benzene. Metabolism of benzene is related to the concentrations in the arterial blood going to the liver. For any given air con-

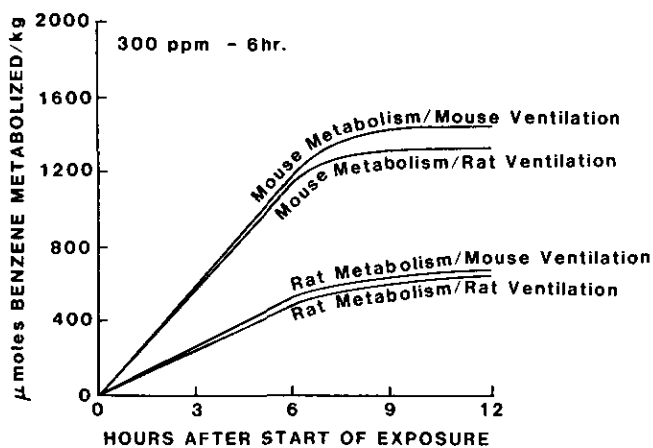


FIGURE 10. Effect of alveolar ventilation on metabolism of benzene. Lines are results of model simulations of cumulative total metabolism during and after a 6-hr exposure to 300 ppm benzene. Parameters used in the simulations are indicated in Table 1.

centration, the concentration of benzene in the blood is directly related to the blood:air partition coefficient, which is identical for rats and mice. Thus, as demonstrated in Figure 10, simulation of an inhalation exposure with rat ventilation parameters and mouse metabolic rate constants results in a total amount of benzene metabolized that is similar to results with both mouse ventilation and metabolism parameters. Simulations with mouse ventilation-rat metabolism and rat ventilation-rat metabolism produce comparable results.

In summary, a physiological model has been used to describe the uptake and metabolism of benzene. Model simulations as well as the experimental data of other investigators (4-7,9) indicate that metabolism of benzene in mice favors the pathways leading to the putative toxic metabolites, benzoquinone and muconaldehyde, whereas metabolism of benzene in rats is primarily through detoxification pathways. These predictions are consistent with the observed increased sensitivity of mice to the toxic effects of benzene compared to rats.

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