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Metabolite-Based Internal Doses Used in a Risk Assessment of Benzene

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Risk assessments of benzene have been based upon both human and animal studies. In this paper, metabolite information is used to construct an internal dose (a surrogate of the biologically effective dose) for a given administered dose. The relationship between the administered dose and this internal dose is nonlinear and is well described by a Michaelis-Menten function. The administered doses from the National Toxicology Program's rodent carcinogenicity study of benzene are transformed into internal doses, and these internal doses are used in conjunction with a multistage model to compare previous estimated virtually safe doses (VSD) associated with small added health risks. The ratio of VSD for the administered dose risk assessment to the VSD from the internal dose risk assessment was approximately 1.0 for the F344/N rats and ranged from 2.5 to 5.0 for B6C3F$_1$ mice in the National Toxicology Program study. For an occupational exposure of 1 ppm, a risk estimate of 0.7 excess cancers/1000 exposed with an upper bound of 3.5/1000 was obtained for a total metabolite internal dose risk assessment. Risk estimates based upon internal doses constructed from levels of the toxic metabolites of benzene are also presented. The implication of a dose-rate study of benzene metabolism for risk assessment is discussed, and finally, suggestions for better characterization of the dose-response function for benzene are provided.

Introduction

Benzene is an important chemical in the manufacturing of a variety of products including petroleum and rubber products. Because of its prevalent use and occupational exposures, benzene has been extensively studied for adverse human health effects. Benzene is a well-established leukemogen and causes other health effects, including decreased bone marrow cellularity and increased chromosomal aberrations in lymphocytes and marrow. The health effects of benzene are summarized in a variety of publications (*1,2*).

As with any identified health hazard, the regulation of benzene exposure becomes an important issue. An important component of regulatory decisions is the projection of health risks at current occupational exposure levels. Two general classes of studies are frequently used in developing these risk projections for benzene.

Studies in the first class are epidemiological investigations in which observations are made on human populations exposed to benzene. The populations frequently studied are workers who are potentially exposed to high levels of benzene. Such studies include benzene exposures in rubber hydrochloride workers (*3,4*), chemical plant workers (*5*), and shoe workers (*6*). A variety of risk estimation models have been applied to these data (*7–11*), and risks in the range of 20 to 80 excess leukemia deaths per 1000 workers exposed to 10 ppm for 30 years have been predicted.

The second class of studies are animal experiments often involving the chronic administration of benzene. These studies assess toxicity with respect to dose response, dose rate, and presumed mechanism of action. Dose-response studies are frequently used to do risk projection. Dose-rate studies, on the other hand, examine a question of considerable relevance in epidemiological studies: Is short-term, high-level exposure more toxic than a chronic low-level exposure?

In dose-response studies, high doses of benzene are administered by gavage or inhalation to laboratory animals with the typical end point of neoplasia being examined. The most common cancers observed in the benzene studies are Zymbal gland carcinomas, squamous cell carcinomas, lymphomas, and alveolar/bronchiolar carcinomas (*12,13*). Risk projections based upon these data have been reported (*9*). These calculations attempt to predict the doses associated with very small levels of added cancer risk (e.g., 1 in 10^6). The dose associated with the small added risk is the so-called virtually safe dose (VSD). Use of these studies to predict human health risks are criticized for a variety of reasons, including the extrapolation of animal response to human response (e.g., humans do not have Zymbal glands so what risk does a compound causing rodent Zymbal gland carcinomas pose for

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humans?) and the extrapolation of effects at high exposures to those in low-dose regions.

The extrapolation from high doses to low doses is part of a larger problem. The external exposure to a compound is not necessarily linearly related to dose of the compound at the target sites. If a chemical (or its metabolites) interact with DNA, then it may be more meaningful to relate tumorigenic response to DNA adducts (*14,15*). Alternatively, the toxic effect may not be directly related to levels of the parent compound but may be associated with levels of particular metabolite(s) of the parent compound (*16*). The nonlinearity of the response to the administered dose (AD) may simply represent the nonlinearity relating the AD to the relevant internal dose (ID) caused possibly by the saturation of pathways in the metabolism of the administered compound.

Recently, studies of the production of benzene metabolites as a function of administered doses of benzene have been reported (*17,18*). In this paper, the benzene metabolite study (*17*) is used to derive an internal dose, which is then modeled as a function of the administered dose. This relationship is applied to the NTP dose-response carcinogenicity study of benzene, and previously calculated VSDs are examined. In addition to reporting this risk projection, the implications of a dose-rate study of benzene (*18*) and metabolite levels are discussed.

Constructing and Modeling an Internal Dose

In the Sabourin et al. study (*17*), F344/N rats, Sprague-Dawley (S-D) rats and B6C3F$_1$ mice were exposed to benzene by one of three routes (gavage, inhalation and IP). We shall be concerned with the gavage exposures as the NTP gavage study will be our source of carcinogenicity data. The gavage dose administered to the rodents (Table1) covered a range of 0.5 to 300 mg/kg for the F344/N rats, 0.5 to 150 mg/kg for the B6C3F$_1$ mice, and 0.5 and 150 mg/kg for the S-D rats. The elimination of benzene was measured collecting all exhalant using a CO_2 trap (KOH) and an ethanol trap (EOH), urine, feces, carcass, and pelt. KOH, urine, feces, and pelt are all indicators of benzene metabolism, and EOH is an indicator of unmetabolized benzene. Basic results from this study include: a) at doses $\geq$ 15 mg/kg, an increasing percentage of benzene was exhaled unmetabolized in both rats and mice, which implies saturation of uptake or metabolism; and b) at doses $\geq$ 50 mg/kg, total metabolite production in the mice plateaus with respect to the administered dose. These results indicate that a nonlinear function will be important for relating administered dose to an internal dose. An internal dose might be the total amount of benzene metabolized. The quantity can be represented as follows:

$$ID = AD \times \frac{\left[KOH + Urine + Feces + Carcass + Pelt\right]}{\left[KOH + Urine + Feces + Carcass + Pelt + ETOH\right]} \quad (1)$$

Thus, the ID is constructed as the fraction of the AD that corresponds to metabolized benzene.

The plots of ID versus AD for the B6C3F$_1$ mice and the F344 rats are presented in Figures 1 and 2, respectively. From these plots, the relationship between the ID and the AD appears nonlinear (although the rat data may be adequately described by a line). A reasonable model for such a relationship would be a Michaelis-Menten function, i.e.,

$$ID = \frac{\alpha AD}{\beta + AD} \quad (2)$$

Such a function was to fit to these data, and estimates of the parameters α and β are given in Table 2. The fitted Michaelis-Menten function is superimposed over the data points in Figures 1 and 2.

Table 1. Details of gavage doses administered in the Sabourin et al. study of benzene (*17*).

	Gavage dose, mg/kg					
Species	0.5	5	15	50	150	300
B6C3F$_1$	x	x	x	x	x	
F344	x	x	x	x	x	x
S-D	x				x	

Risk Calculations Using AD and ID

Risk projections will be made using the NTP gavage study of benzene (*13*). This study indicated a tumorigenic response for a variety of sites over a range of doses for which metabolite information is now available. The doses used in the NTP study and the ID corresponding to these ADs are given in Table 3. For the rat data, the relative doses in the AD scale is maintained in the ID dose scale. In other words, a doubling on the AD scale (e.g., from 50 mg/kg to 100 mg/kg) is matched by an approximate doubling on the ID scale (e.g., from 29.75 mg/kg to 55.75 mg/kg). This relative dosing is not preserved for the mice data.

The multistage model is frequently used to model tumorigenic response data and has been used in a previous risk assessment of benzene (*9*). The basic form of this model for a four dose group experiment is

$$P(d_i) = 1 - exp\left\{-\left(q_0 + q_1 d_i + q_2 d_i^2 + q_3 d_i^3\right)\right\} \quad (3)$$

where $P(d_i)$ corresponds to the lifetime probability of tumor development in an animal given dose d_i, and q_0, q_1,

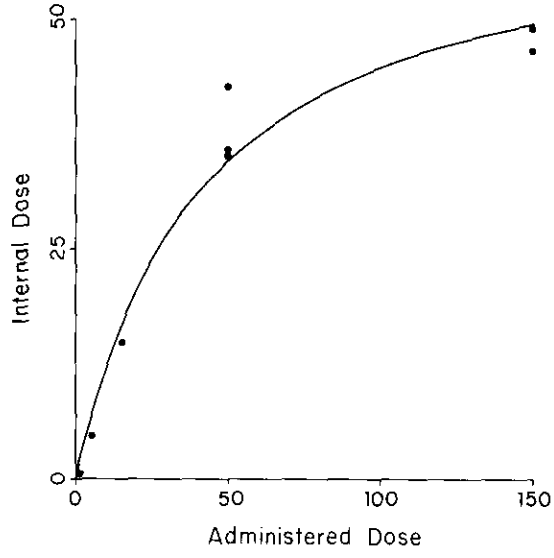

FIGURE 1. Plot of internal dose versus administered dose for B6C3F$_1$ mice with superimposed Michaelis-Menten fitted curve. Some of the plotted points represent multiple observations.

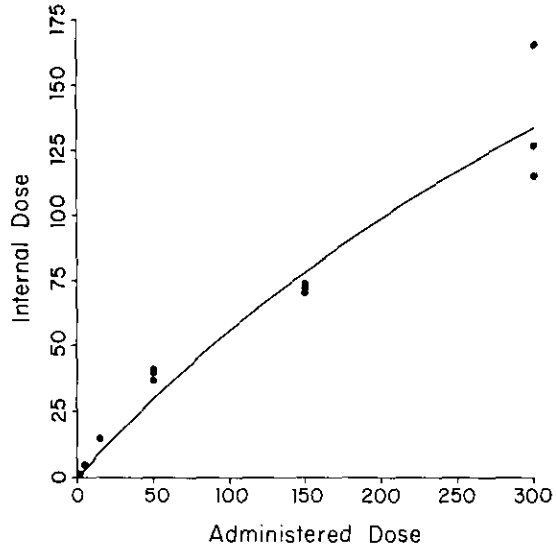

FIGURE 2. Plot of internal dose versus administered dose for F344/N rats with superimposed Michaelis-Menten fitted curve. Some of the plotted points represent multiple observations.

Table 2. Michaelis-Menten parameter estimates and SEs for fitting the ID-AD relationship.

Species	α	β
B6C3F$_1$	63.3	41.3
	(3.5)[a]	(6.3)
F344/N	443.6	695.6
	(179.7)	(376.7)

[a]Numbers in parentheses are SEs.

Table 3. NTP benzene gavage administered doses (AD) and corresponding internal doses (ID) based upon a Michaelis-Menten function.

Species	Sex	AD, mg/kg	ID, mg/kg
B6C3F$_1$	Male	0	0
		25	23.87
		50	34.67
		100	44.79
B6C3F$_1$	Female	0	0
		25	23.87
		50	34.67
		100	44.79
F344/N	Male	0	0
		50	29.75
		100	55.75
		200	99.06
F344/N	Female	0	0
		25	15.39
		50	29.75
		100	55.75

q_2, and q_3 are nonnegative parameters that are estimated. Maximum likelihood parameter estimates for q_0, q_1, q_2, and q_3 were calculated, and the lower bound on a dose associated with an added risk of 1 in 10^6 was obtained (*19*). We are not advocating the calculation of VSDs for risk levels of one in a million. The dose associated with such a small risk is clearly below the lowest tested dose group, and is very dependent on the choice of the dose-response function. Our interest is solely to compare the effects of a change from an AD to an ID on the estimation of VSDs for a model and risk level used in previous risk assessments of benzene (*9*). Once this safe dose is determined for the risk assessment based upon the ID scale, the conversion back to the AD dose scale can be done directly by inverting the Michaelis-Menten function, i.e.,

$$AD = \frac{\hat{\beta} \times ID}{\hat{\alpha} - ID} \tag{4}$$

where the estimates of α and β are used in this formula.

The safe doses calculated from the AD and ID risk assessments are presented in Table 4. The ID safe doses have been converted to the AD dose scale. The ratio of AD-based safe dose to the ID-based safe dose is also presented in this table. This ratio is essentially 1.0 for the rat data. This result is as expected since the AD relative dose spacing was preserved on the ID scale. The ratio for mice ranged from 2.5 to 5.0 for the mice. Thus, the nonlinearity between the ID and AD was converted into a VSD at least 2.5 times smaller than would have been predicted based solely upon the AD risk assessment.

Historically, linear projections of risk into low-dose regions have been performed (*11*). A natural question about internal dose construction is: Does the tumorigenic response exhibit a linear relationship with this dose measure? We examined the shape of the tumor-response dose relationships in the NTP study for both administered and internal doses. The results of this examination are pre-

Table 4. The 95% lower bound estimates for the dose associated with an added risk of 10^{-6}.[a]

	Safe dose		
	AD	ID	Dose (AD)/dose (ID)
B6C3F$_1$ male mice			
All squamous cell carcinomas	0.129×10^{-3}	0.256×10^{-4}	5.3
Alveolar/bronchiolar carcinomas	0.238×10^{-3}	0.780×10^{-4}	3.1
Lymphoma, NOS	0.357×10^{-3}	0.139×10^{-3}	2.6
B6C3F$_1$ female mice			
All squamous cell carcinomas	0.667×10^{-3}	0.281×10^{-3}	2.4
Mammary gland carcinoma, NOS	0.270×10^{-3}	0.120×10^{-3}	2.3
Alveolar/bronchiolar carcinomas	0.366×10^{-3}	0.141×10^{-3}	2.6
Malignant lymphoma	0.271×10^{-3}	0.611×10^{-4}	4.4
F344/N male rats			
Zymbal gland carcinomas	0.383×10^{-3}	0.319×10^{-3}	1.2
All squamous cell carcinomas	0.339×10^{-3}	0.282×10^{-3}	1.2
F344/N female rats			
Zymbal gland carcinomas	0.238×10^{-3}	0.217×10^{-3}	1.1
All squamous cell carcinomas	0.479×10^{-3}	0.435×10^{-3}	1.1
Squamous cell carcinoma of the preputial gland	0.123×10^{-3}	0.125×10^{-3}	1.0

[a]Estimates from AD-based and ID-based risk assessments.

sented in Table 5. These shape assessments are simply rough descriptions based upon examining the tumorigenic response-benzene dose patterns.

It is worth noting the potential influence of shape on risk projection when using internal doses versus using administered doses. As an example, we consider all squamous cell carcinomas in male B6C3F$_1$ mice. This response is particularly relevant because it has been used in other benzene risk assessments when extrapolating animal to human risk (*9*). In Figure 3, the observed incidence of all squamous cell carcinomas is plotted against the administered dose for male mice. The curve representing the best fitting multistage model is overlayed on this figure. This dose-response relationship appears almost linear (slightly sublinear or even sigmoidal?). A similar figure for this tumor site using the internal dose scale is presented in Figure 4. In this figure, the dose-response curve is very nonlinear (hockey stick shape). (The shape of the dose-response relationship for all squamous cell carcinomas differs for the other three sex-species conditions. This relationship is nonlinear on both the administered dose and internal dose scalings for female mice and is roughly linear for both dose scalings in both sexes of the rats.) Given the linear dose-response using the administered dose and hockey stick dose-response relationship using the internal dose, one might expect that the risk assessment based upon the ID would lead to a larger safe dose associated with a given specified small risk. This is in fact observed for the point estimates of the dose associated with a 10^{-6} risk (dose estimates: 6.9×10^{-4} for

Table 5. Shapes of the tumorigenic response—AD/ID curves.[a]

	AD scale	ID scale
B6C3F$_1$ male mice		
All squamous cell carcinomas	L/−	−
Alveolar/bronchiolar carcinomas	+	L
Lymphoma, NOS	L	L
B6C3F$_1$ female mice		
All squamous cell carcinomas	−	−
Mammary gland carcinoma, NOS	L	−
Alveolar/bronchiolar carcinomas	+	L
Malignant lymphoma	+	+
F344/N male rats		
Zymbal gland carcinomas	L	L
All squamous cell carcinomas	L	L
F344/N female rats		
Zymbal gland carcinomas	L	L
All squamous cell carcinomas	L	L
Squamous cell carcinoma of the preputial gland	+	+

[a]Abbreviations: (L) linear; (+) superlinear; (−) sublinear.

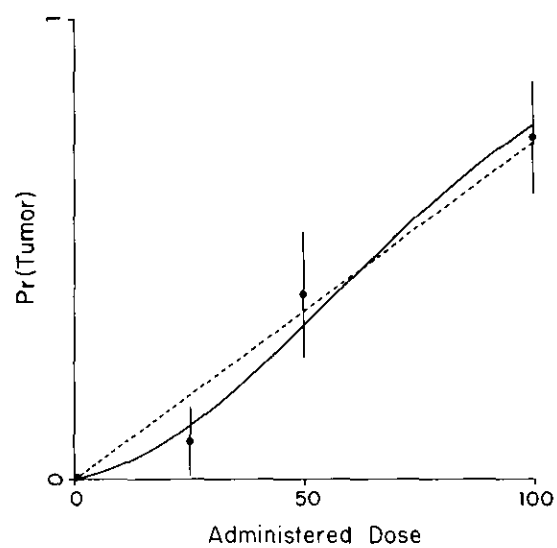

FIGURE 3. *Plot of probability of all squamous cell carcinomas versus administered dose for* B6C3F$_1$ *male mice with superimposed multistage model fitted curve (solid line), and linear regression through the origin (dashed line). Quantal response data points along with 95% confidence limits are presented as solid circles with vertical lines.*

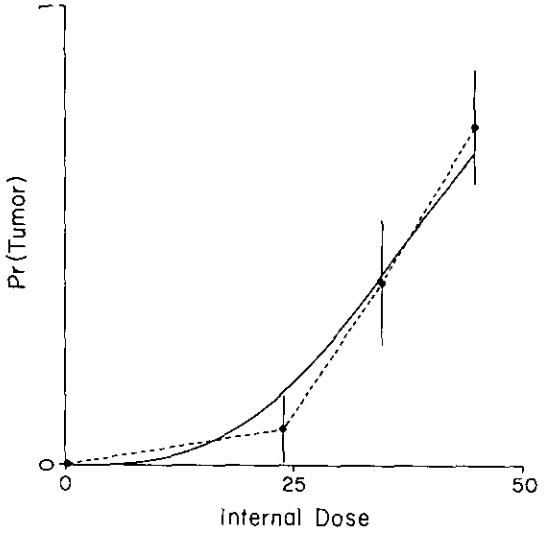

FIGURE 4. Plot of probability of all squamous cell carcinomas versus internal dose for B6C3F$_1$ male mice with superimposed multistage model fitted curve (solid line). Dashed lines connect the observed quantal responses at each dose value. The quantal response data points along with 95% confidence limits are presented as solid circles with vertical lines.

AD; 4.3×10^{-1} for ID-based risk estimates transformed back to the AD scale). When the linearized form of the multistage model is used to derive the lower bound on the dose associated with 10^{-6} risk (*19*), the ID-based estimate is five times smaller than the AD-based estimate. A forced linearized multistage model fit is inappropriate for describing the internal dose-tumorigenic response relationship if the data are truly nonlinear and may lead to misleading results. In fact, the routine use of any complicated computer model for low dose extrapolation is worth questioning.

Individual Benzene Metabolites

Overall metabolite production as an ID is confounded somewhat since at higher doses of benzene the metabolism may shift to the detoxification pathways. This may represent a shift in the balance of activation/deactivation pathways. Using the levels of the toxic metabolites helps to avoid this problem and brings us one step closer to the biologically effective dose. A preliminary risk assessment of benzene has been conducted using levels of toxic metabolites to construct an internal dose. A dose-response study of individual metabolites has been conducted (*18*). In this study, B6C3F$_1$ mice and F344/N rats were exposed by gavage (at doses 1, 10, and 200 mg/kg) and by inhalation (at doses 5, 50, and 600 ppm); animals were sacrificed at fixed time points, and levels of individual benzene metabolites were determined for the liver, lung, blood, and urine. For each dose, site, and metabolite, a concentration-time profile was obtained. We focus our attention on two indicators of the toxic pathways of benzene metabolism (*18*), namely the metabolites muconic acid (MUC) and hydroquinone glucuronide (HQG). Internal doses were constructed by summing the area under the metabolite concentration-time curves (AUCs) for MUC and HQG over all tissue and fluid samples. As was previously done, a Michaelis-Menten function was used to model the ID-AD relationship (for HQG: $\alpha = 1.84$, $\beta = 261.45$; for MUC: $\alpha = 8.14$, $\beta = 365.21$). Similar calculations were done for the rats (for HQG: $\alpha = 0.03$, $\beta = 25.63$; for MUC: $\alpha = 0.29$, $\beta = 45.91$). The internal doses based upon HQG and MUC will be denoted ID(HQG) and ID(MUC), respectively. Values for these internal doses are presented for the NTP administered doses in Table 6. From this table, the ID-AD relationship appears roughly linear for the mice and very nonlinear for the rats. The nonlinearity in the ID(HQG)-AD relationship for rats may be artificial because the observed levels of HQG are extremely small with little to no HQG detected even at high administered doses. There are only nine data points fit by the Michaelis-Menten curve, and these points exhibit tremendous variability especially at the highest AD. The linearity in the ID-AD fitted curves for mice may also have similar problems. In the spirit of exploration and recognizing the possible problems in the ID-AD fitted curves, we used these ID(HQG) and ID(MUC) doses to see the effects this information has on benzene risk estimation. The details of this risk estimation are given in the section on the "Extrapolation to Human Exposure."

Benzene Dose-Rate Studies

From the risk estimation discussed previously, we see the potential importance of the ID-AD relationship in obtaining safe dose estimates. Short-term, high-level exposure may lead to greater toxic response than chronic, low-level exposure. The data to address such an issue has been described (*18*), and we would like to summarize some of the early analyses of these data and discuss the

Table 6. NTP benzene gavage administered doses and corresponding internal doses based upon AUC for hydroquinone glucuronide and muconic acid.[a]

Species	Sex	AD, mg/kg	ID(HQG), mg/kg	ID(MUC), mg/kg
B6C3F$_1$	Male	0	0	0
		25	0.16	0.52
		50	0.30	0.98
		100	0.51	1.75
B6C3F$_1$	Female	0	0	0
		25	0.16	0.52
		50	0.30	0.98
		100	0.51	1.75
F344/N	Male	0	0	0
		50	0.019	0.15
		100	0.023	0.20
		200	0.025	0.23
F344/N	Female	0	0	0
		25	0.014	0.10
		50	0.019	0.15
		100	0.023	0.20

[a]Abbreviations: AD, administered dose; ID, internal dose; HQG, hydroquinone glucuronide; MUC, muconic acid.

potential implication of such data on the risk estimation process.

In these dose-rate experiments, $B6C3F_1$ mice and F344/N rats were exposed by inhalation at one of three dose rates: a) 600 ppm for 30 min; b) 150 ppm for 2 hr; or c) 50 ppm for 6 hr. Animals were sacrificed at fixed time points, and levels of individual benzene metabolites were determined for the liver, lung, blood, and urine. For each dose rate, tissue or body fluid, and metabolite, a concentration-time profile was obtained. The area under the concentration-time curve again was used as a summary of the total metabolite burden.

As before, the two indicators of the toxic pathways of benzene metabolism (muconic acid and hydroquinone glucuronide) are considered. In general, the AUC for MUC and HQG was significantly greater in the lower-dose exposure groups (2 hr, 150 ppm and 6 hr, 50 ppm) than in the short-term, high-exposure group (30 min., 600 ppm) in the liver, blood and lung of the mice. The AUC for the lower exposure groups was roughly two to three times the AUC for the short-term, high-exposure group. In general, the opposite was true for the rats where the short-term, high-exposure group had a greater toxic metabolite burden than the lower exposure groups by a factor of about 1.5 to 2. This result is consistent with the observed saturation of metabolic pathways in mice and lack of saturation in rats (*17*).

The implication of this dose-rate study for human risk estimation depends upon which animal model, the mouse or the rat, provides the best analog for human metabolism of benzene. If human metabolism is clearly saturable like the mouse, then exposure measures in epidemiological studies should weigh longer, lower dose exposures more heavily than short-term, high-dose exposure. The opposite weighting scheme should be used if human metabolism is more like the rat in that high-dose exposures have not saturated the metabolic pathways.

Extrapolation to Human Exposure

The ultimate goal of any risk assessment process is to predict human risk. Epidemiological studies have many difficulties, including the assessment of exposure. This is typically not an issue in animal studies; however, there is considerable uncertainty in the extrapolation of these studies to the human condition. Not only must the question of species extrapolation be addressed, but the issue of generalizing one route of exposure (e.g., gavage) to another route of exposure (e.g., inhalation) frequently must be addressed. Both of these questions are discussed in order to use the multistage model results.

The question of extrapolating inhalation to gavage dosing is addressed by assuming that each part per million of inhaled benzene contains a certain mass of benzene per cubic meter [3.19 mg/m^3 is frequently used (9)], that the breathing rate of humans in an 8-hr period is known [10 m^3 (*9*)], and that all of the inhaled benzene is absorbed. The conversion from rodents to humans is based upon what is believed to be the dose units for equivalent risks. Following the methods of Crump and Allen (*9*), milligram/kilogram/day is considered the equivalent dose unit, and the milligram/kilogram/day dose d' for humans exposed to benzene at a level of ε ppm per work day for a lifetime is

$$d' = \varepsilon \times \left(\frac{3.19\ mg/m^3}{1\ ppm}\right) \times \left(\frac{10\ m^3}{8\text{-}hr\ day}\right) \times \frac{1}{70\ kg} \quad (5)$$

where 70 kg is assumed to be the average weight of a human. For example, a 1 ppm exposure (i.e., $\varepsilon = 1$) leads to an equivalent milligram/kilogram/day dose of 0.456. If this dose is input into the multistage model for male mice all squamous cell carcinomas, then a point estimate of 0.69 excess cancers/1000 exposed (with an upper bound of 3.52/1000) is predicted. Assuming that the ID-AD relationship is the same in humans as in mice, the human administered dose is first transformed into the internal dose scale using the Michaelis-Menten function for mice, and this internal dose is input into the ID-based multistage model for all squamous cell carcinomas. A point estimate of 4.21 excess cancers/1000 exposed (with an upper bound of 17.62/1000) is predicted using the internal dose. For all squamous cell carcinomas in the F344/N male rat, point estimates of 1.0 excess cancers/1000 (upper bound: 1.3/1000) and 2.9 excess cancers/1000 exposed (upper bound: 3.9/1000) are predicted using the administered dose and the internal dose, respectively.

As in the total metabolite ID risk assessment, we focus on the projection of risk for a 1-ppm exposure now using individual metabolite (muconic acid and hydroquinone glucuronide) based internal doses. As above, all squamous cell carcinomas in $B6C3F_1$ male mice is the tumorigenic end point of interest. For simplicity, we described the tumor response-dose relationship using the best-fitting regression line through the origin. This was justifiable because the tumor response-dose relationship was approximately linear for all three dose scalings. Recall that an oral dose of 0.456 mg/kg/day was associated with a 1-ppm inhalation dose. For this dose, a risk of 3.3/1000 is projected. This is very similar to risk estimates from the multistage model. Performing calculations similar to those described for the total metabolite ID, a risk of 4.4/1000 and 4.1/1000 is projected for ID(HQG) and ID(MUC), respectively. Similar calculations for all squamous cell carcinomas in the F344/N male rats lead to risk estimates of 0.8/1000, 5.4/1000, and 3.4/1000 using AD, ID(HQG) and ID(MUC), respectively.

Discussion

The basic conclusion from using internal dose information in a multistage model-based risk assessment is that the risk estimates based upon the total metabolite internal dose leads to a safe dose that is less than one order of magnitude smaller than a safe dose from a risk assess-

ment based upon the administered doses. There are possible problems with this analysis that must be considered. First, the ID-AD relationship was derived from an experiment conducted with only one gavage dosing, whereas the NTP tumorigenic response was based upon two years of dosing. Therefore, this analysis is confounded by issues of possible repeated exposure and aging effects. Second, there is variability in the estimates of α and β in the Michaelis-Menten function that was not explicitly considered in the risk estimation. Finally, the multistage model for tumorigenesis and the Michaelis-Menten ID-AD function may not be appropriate models.

The dose-rate studies may provide insight into the importance of the type of exposure on possible benzene health risks. If human metabolism is similar to the rat, then administered dose risk estimation for chronic low-dose exposures may actually overestimate the true risk. In this scenario, concern should be focused on spike exposures. If mouse metabolism is a better model for human metabolism, then the chronic exposures should be of greater concern than the spike exposures. In human exposure-based risk assessments where a cumulative exposure measure was used, risks of 3 to 11 excess cancer deaths/1000 exposed have been suggested for a 1-ppm lifetime (30 year) exposure (*10*). This range is roughly what we observed for the internal dose risk estimates and may suggest that the mouse internal dose may be a useful model for the human internal dose.

We discussed the use of individual metabolites in the derivation of internal doses. The shape of the internal dose-administered dose curve must be reasonably well established before an individual metabolite-based internal dose is used. If data were available at some of the middle dose points (such as 15, 25, and 50 mg/kg for the gavage dosings), a much better characterization of the internal dose-administered dose relationship may be possible.

We would like to consider some ways in which the risk assessment process for benzene may be improved. Ongoing research in the construction of internal dose for a risk assessment of benzene include applying physiologically-based pharmacokinetic models for modeling the disposition of benzene and measuring hemoglobin adducts as a toxic metabolite surrogate.

The physiologically-based pharmacokinetic models provide a natural means of modeling repeated exposure so that the internal dose may reflect the exposures used in the chronic bioassays such as the NTP study. Additionally, these models can be used to compare different dose-rate exposures. It is not clear how sensitive these models are relative to the choice of physiological parameters; therefore, it is not clear that these models will provide improvement to the risk assessment process.

Hemoglobin adducts are indicators of the sum of metabolic activation/deactivation that has occurred. As such, these adducts should take into account individual differences in metabolism. These adducts may correlate with DNA adduct formation. Once such measures are developed, a natural equivalent dose for species extrapolation may be available.

From the relatively small amount of metabolite and kinetic data available, we see that risk estimates for benzene may well change as better internal dosimetry is available. The total metabolite or individual metabolite internal doses are an improvement over the administered dose scales in that these doses reflect some of the metabolic processing of an administered chemical. Clearly, this is not the total picture. Ideally, we would like to consider the relationship between the administered dose and the effect in the target cell related to the mechanism of action. A good candidate for the target cells for benzene leukemogenesis are the stem cells in the bone marrow. Additional data of this type will allow a more satisfactory risk analysis of benzene to be performed.

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