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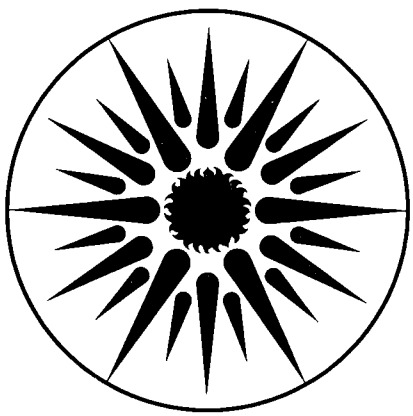

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An Analysis of Exposure Rate Effects for Benzene Using a Physiologically Based Pharmacokinetic Model

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ABSTRACT

A new physiological pharmacokinetic model was used to explore the effect of exposure rate on the rate of formation of several crucial metabolites of benzene. Metabolite formation was compared following exposure to benzene over the course of an 8 hour work-day and following a single exposure for 15 minutes. These exposures were based on the permissible exposure limit (PEL) and short-term exposure limit (STEL) of the benzene standard set by the Occupational Safety and Health Administration (OSHA). The model was parametrized using *in vitro* and *in vivo* experimental data on benzene toxicokinetics and metabolism. Ranges, rather than fixed values, were assigned to the parameters. Model predictions show that the amount of hydroquinone, catechol and muconaldehyde formed in the body following a peak exposure to 32 ppm of benzene over 15 minutes are on average 20% higher than those formed following an equivalent dose of 1 ppm over an 8 hr period. The health consequences of these findings and the implications for policy concerning short-term exposure limits are discussed.

INTRODUCTION

The final standard set by the Occupational Safety and Health Administration (OSHA) for workplace exposure to benzene includes a permissible exposure limit (PEL) of 1 ppm and a short-term-exposure limit (STEL) of 5 ppm (OSHA, 1987). The PEL regulates airborne exposures, averaged over an entire 8-hr workshift, to protect from the cumulative effect of long-term exposures. The STEL, on the other hand, is directed at transient or "peak" exposures averaged over 15-minute periods. The STEL is used to augment the PEL in situations where control of the shift-long exposures does not provide sufficient protection from the toxic effects elicited by the compound (Paxman and Rappaport, 1990; Zielhuis et al., 1988).

A previous review of the data cited by OSHA in its final standard for exposure to benzene found that no clear scientific basis was provided to support the promulgation of the STEL (Paxman and Rappaport, 1990). While leukemia and bone marrow toxicity were related to cumulative exposures of benzene received by workers, no evidence was presented that the rate of exposure at a given cumulative exposure contributed to the effects. Likewise, the cited animal studies did not indicate that exposures over shorter time scales played any role in causing toxicity. Based on a preliminary study of benzene toxicokinetics, Paxman and Rappaport (1990) concluded that the impact of peak exposures would be minimal. Insufficient evidence existed, at that time, on the adverse health effects resulting from the rate of exposure, i.e. exposure to benzene from an acute exposure of 32 ppm over 15 minutes was of greater toxicity than an equivalent exposure of 1 ppm averaged over 8 hours.

In this paper, we reexamine the toxicokinetic effects of the rate of exposure to benzene in rats using a newly proposed physiologically based pharmacokinetic (PBPK) model (Bois et al., 1991). The PBPK approach allows for a quantitative analysis of the distribution of benzene and its metabolites to various tissues, including the bone marrow. After describing briefly the model calibration we compare the metabolic profiles arising from either 8 hour time-weighted-average (TWA) or 15 minute peak exposures. The effects of exposure rate on metabolite formation are discussed in terms of the public health and policy implications.

METHODS

Model

The model has three components which together describe the pharmacokinetics of benzene and the formation of major metabolites for the F-344 male rat. The first component models the distribution and elimination of benzene (Figure 1). This physiological model includes five compartments: well perfused, poorly perfused, fat, bone marrow and liver tissues. Two possible sites of metabolism for benzene are present: the liver and the bone marrow. The bone marrow was included for its relevance to human leukemia risk. Unchanged benzene can leave the body through the lungs. Initial ranges for the parameter values of this component were either derived from the literature (volumes, blood flows, partition coefficients) (Fiserova-Bergerova, 1983; Fiserova-Bergerova et al., 1984; Gerlowski and Jain, 1983) or adjusted by us (Bois and Spear, 1990; Bois et al., in press) on the basis of experimental data published by Rickert et al. (Rickert et al., 1979) and Sabourin et al. (Sabourin et al., 1987).

The second component describes the metabolic transformations of benzene and its byproducts in the liver and the bone marrow (Figure 2). This description is currently the most widely accepted (Cooper and Snyder, 1988; Kalf, 1987). All the reactions implied, but one, are modeled by the Michaelis-Menten equation, which adequately describes simple saturable enzyme kinetics. The transformation of benzene oxide into phenol, which occurs by spontaneous hydrolysis, is modeled by a first order reaction. Muconaldehyde, muconic acid and catechol are formed from benzene glycol (Cooper and Snyder, 1988; Kalf, 1987; Sabourin et al., 1989). In rats, catechol can also be formed by oxidation of phenol (Sawahata and Neal, 1983). The GSH pathway leads to the formation of pre-phenylmercapturic and phenylmercapturic acids. Initial ranges for the parameter values were obtained from reports of *in vitro* experiments (Gilmour et al., 1986; Guengerich, 1982; Jerina and Daly, 1974; Koop et al., 1989; Sawahata and Neal, 1983; Sekura and Jakoby, 1979), or fixed by us.

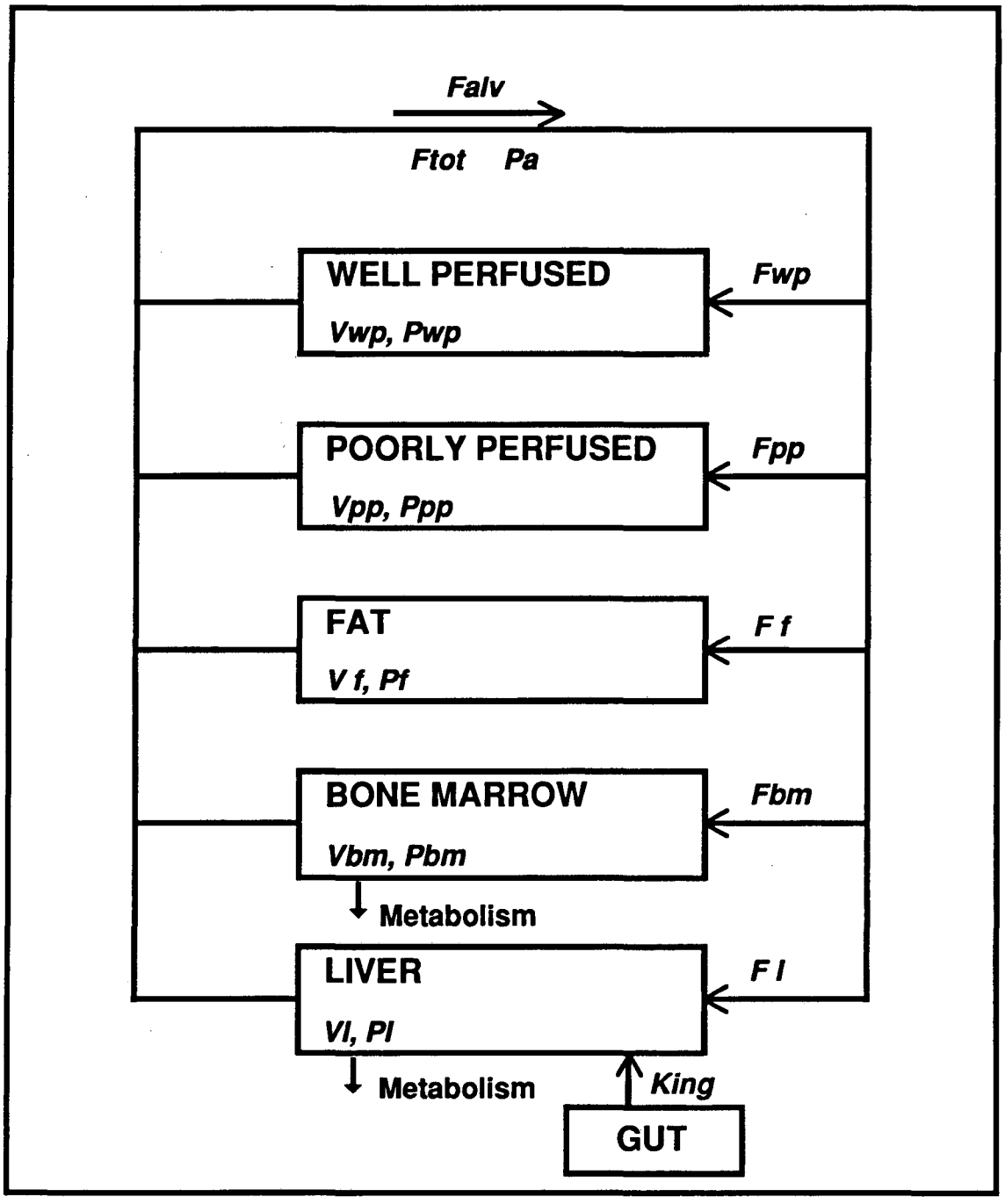

Figure 1: Schematic representation of the physiological model used to simulate the distribution and metabolism of benzene. The symbols are given in Table 1.

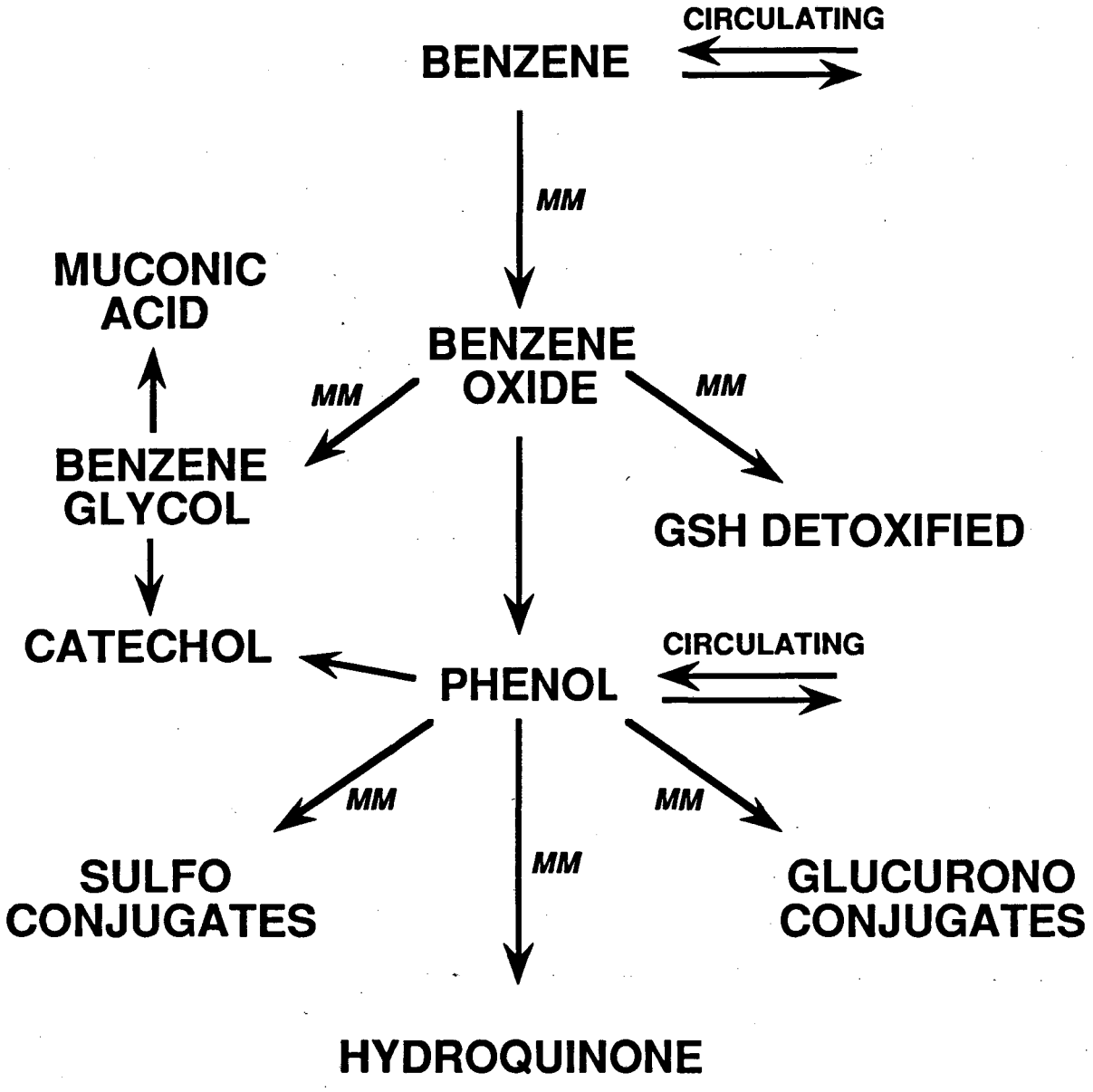

Figure 2: Benzene metabolic pathways simulated by the model. MM stands for Michaelis-Menten type reaction. The distribution of benzene and phenol is described by two specific pharmacokinetic models – see Figure 1 and text.

The third component describes the distribution of phenol. This component is similar to the benzene PBPK model with the addition of two compartments: the lung and the g.i. tract, where first pass effects for phenol have been shown (Cassidy and Houston, 1980; Cassidy and Houston, 1984). Phenol can be conjugated by sulfo- or glucurono-transferases in the latter two organs besides the bone marrow and liver. As described in the previous paragraph, phenol can be metabolized into hydroquinone or catechol in the liver and bone marrow. The compound can also leave the body by first order rate excretion.

A list of the model parameters is presented in Table 1. To account for known dependencies between physiological parameters (for example between alveolar ventilation rate and cardiac output), scaling factors were used. The scaling factors are the actual parameters sampled and the ranges apply to them.

The differential equations for the parts of the model are tightly dependent and are solved simultaneously using the Gear routine for stiff differential systems. All computations were performed on a Sun 4 workstation. A listing of the equations is available upon request.

Monte Carlo simulations

Ranges, rather than fixed values, were assigned to the parameter scaling factors, to reflect their intrinsic variability across the population and the uncertainty in current knowledge. As explained above, the parameter ranges were either taken from the literature or fixed by us. Monte Carlo simulations (Bois et al., 1991; Bois et al., in press; Bois et al., 1990; Farrar et al., 1989; Hammersley and Handscomb, 1964; Portier and Kaplan, 1989; Press et al., 1986; Spear et al., in press) were used to calibrate the model and predict surrogate exposure levels (in this case, cumulative formation of the metabolites). The goal of these simulations is to verify that the parameter ranges produce results that bracket the experimental data. This ensures that, although not known exactly, the "true" parameter values are likely to be included in our ranges (assuming that the model is physiologically realistic).

Table 1: Ranges of the parameter scaling coefficients[a] used for the model of benzene pharmacokinetics and metabolism in male rats.

Scaled Parameter	Multiplier	Scaling Coefficient Range
Total blood flow (F_{tot})	$Bw^{0.75}$	0.22 - 0.28
Alveolar ventilation rate (F_{alv})	F_{tot}	0.5 - 1.2
Blood flows		
gastro-intestinal tract and liver (F_l)	F_{tot}	0.23 - 0.33
bone marrow (F_{bm})	F_{tot}	0.01 - 0.05
fat (F_f)	F_{tot}	0.04 - 0.13
poorly perfused tissue (F_{pp})	F_{tot}	0.10 - 0.18
well perfused tissue (F_{wp})	F_{tot}	- [b]
Volumes		
lung (V_{lu})	Bw	0.003 - 0.009
arterial blood (V_{ab})	Bw	0.01 - 0.03
venous blood (V_{vb})	Bw	0.05 - 0.07
liver (V_l)	Bw	0.03 - 0.05
bone marrow (V_{bm})	Bw	0.02 - 0.04
gastro-intestinal tract (V_{gi})	Bw	0.01 - 0.04
fat (V_f)	Bw	0.07 - 0.13
poorly perfused tissue (V_{pp})	Bw	- [c]
well perfused tissue (V_{wp})	Bw	0.03 - 0.07
Blood/air partition coefficient for benzene (Pb_a)	1	8.0 - 24.5
Tissue/blood partition coefficients for benzene		
liver (Pb_l)	1	0.63 - 2.9
bone marrow (Pb_{bm})	1	3.1 - 11.6
fat (Pb_f)	1	24.0 - 33.0
poorly perfused tissue (Pb_{pp})	1	0.6 - 2.0
well perfused tissue (Pb_{wp})	1	0.6 - 3.0
Lung/air partition coefficient for phenol (Pp_a)	1	1.0 - 10.0
Tissue/blood partition coefficients for phenol		
lung (Pp_{lu})	1	0.5 - 2.0
liver (Pp_l)	1	0.5 - 2.0
bone marrow (Pp_{bm})	1	0.3 - 5.0
gastro-intestinal tract (Pp_{gi})	1	0.5 - 2.0
fat (Pp_f)	1	0.3 - 5.0
poorly perfused tissue (Pp_{pp})	1	0.5 - 2.0
well perfused tissue (Pp_{wp})	1	0.5 - 2.0
Intestinal absortion rate for benzene (Kb_{ing})	1	0.0047 - 0.03
Intestinal absortion rate for phenol (Kp_{ing})	1	0.005 - 0.05
Phenol excretion rate (K_{exc}) [d]	1	0.001 - 0.10
Vmax for benzene to benzene oxide		
liver ($Vmax1_l$)	$Bw^{0.75}$	0.0005 - 0.002
bone marrow ($Vmax1_{bm}$)	$Vmax1_l$	0.05 - 0.18
Vmax/Km ratios for benzene to benzene oxide		
liver ($Km1_l$)	1	1.0 - 41.0
bone marrow ($Km1_{bm}$)	1	0.017 - 2.7
Rate for benzene oxide to phenol (Kbp)	1	0.35 - 5.0

Table 1 continued.

Scaled Parameter	Multiplier	Scaling Coefficient Range
Vmax for benzene oxide to benzene glycol		
liver ($Vmax2_l$) [d]	$Bw^{0.75}$	0.001 - 0.1
bone marrow ($Vmax2_{bm}$)	$Vmax2_l$	0.01 - 0.2
Vmax for benzene oxide to GSH compounds		
liver ($Vmax3_l$)	$Bw^{0.75}$	0.0001 - 0.001
bone marrow ($Vmax3_{bm}$)	$Vmax3_l$	0.01 - 0.20
Vmax for phenol to sulfo-conjugate		
lung ($Vmax4_{lu}$) [d]	$Vmax4_l$	0.2 - 10.0
liver ($Vmax4_l$) [d]	$Bw^{0.75}$	0.0001 - 0.02
bone marrow ($Vmax4_{bm}$)	$Vmax4_l$	0.01 - 0.20
gastro-intestinal tract ($Vmax4_{gi}$) [d]	$Vmax4_l$	0.2 - 10.0
Vmax for phenol to glucurono-conjugate		
lung ($Vmax5_{lu}$) [d]	$Vmax5_l$	0.2 - 10.0
liver ($Vmax5_l$) [d]	$Bw^{0.75}$	0.0001 - 0.01
bone marrow ($Vmax5_{bm}$)	$Vmax5_l$	0.01 - 0.20
gastro-intestinal tract ($Vmax5_{gi}$) [d]	$Vmax5_l$	0.2 - 10.0
Vmax for phenol to hydroquinone		
liver ($Vmax6_l$) [d]	$Bw^{0.75}$	0.0001 - 0.01
bone marrow ($Vmax6_{bm}$)	$Vmax6_l$	0.01 - 0.20
Rate for phenol to catechol [d]		
liver (Kpc_l)	1	0.0006 - 0.01
bone marrow (Kpc_{bm})	Kpc_l	0.01 - 0.15
Rate for benzene glycol to muconaldehyde (Kbgm) [d]	1	0.004 - 0.05
Rate for benzene glycol to catechol (Kbgc) [d]	1	0.02 - 0.5
Vmax/Km ratios for benzene oxide to benzene glycol		
liver ($Km2_l$)	1	0.5 - 10.0
bone marrow ($Km2_{bm}$)	1	0.05 - 1.0
Vmax/Km ratios for benzene oxide to GSH compounds		
liver ($Km3_l$)	1	0.2 - 2.0
bone marrow ($Km3_{bm}$)	1	0.02 - 0.2
Vmax/Km ratios for phenol to sulfo-conjugate [d]		
lung ($Km4_{lu}$)	1	0.01 - 10.0
liver ($Km4_l$)	1	0.1 - 30.0
bone marrow ($Km4_{bm}$)	1	0.001 - 1.0
gastro-intestinal tract ($Km4_{gi}$)	1	0.05 - 50.0
Vmax/Km ratios for Phenol to glucurono-conjugate [d]		
lung ($Km5_{lu}$)	1	0.001 - 0.2
liver ($Km5_l$)	1	0.001 - 0.2
bone marrow ($Km5_{bm}$)	1	0.0001 - 0.02
gastro-intestinal tract ($Km5_{gi}$)	1	0.001 - 0.2
Vmax/Km ratios for phenol to hydroquinone [d]		
liver ($Km6_l$)	1	0.01 - 2.0
bone marrow ($Km6_{bm}$)	1	0.001 - 0.2

[a] Scaled parameter = multiplier × scaling coefficient. Units: body weights in kg, flows (F) in L/min, volumes (V) in L, Vmax in mmol/min, Vmax/Km and first order rates in min^{-1}. The body weight was 0.3kg for benzene experiments , 0.23kg for phenol experiments and 0.38kg for the NTP bioassays (1980; 1986).

[b] Values for F_{wp} were computed at each run so that the sum of the blood flows was equal to the total flow.

[c] Values for V_{pp} were computed at each run so that the sum of the volumes was equal to 90% of the body volume.

[d] The scaling coefficients for these parameters were sampled log-uniformly.

Each Monte Carlo simulation is performed by first picking at random, from their ranges, values for the 68 scaling factors of the model. The sampling was either uniform or log-uniform as indicated in Table 1, depending on the uncertainty in the parameter considered (the latter is used when the upper limit of a range was 50 times, or more, larger that the lower limit). After sampling the scaling coefficients and deriving the corresponding parameter values, the model was run to simulate experimental data.

A final set of 200 best fitting simulations out of 1000 was obtained, each with a particular random set of parameters. The goodness of fit was measured by the log-likelihood of the data (Edwards, 1972; Kalbfleisch, 1985). For each simulation the log-likelihood (*LL*) is given by:

$$LL = \sum_{i=1}^{N} -\frac{n_i}{2} \cdot \ln\left(1 + \frac{(y_i - \hat{y}_i)^2}{s_i^2}\right) \qquad (1)$$

where N is the number of mean experimental data points used ($N = 96$ in the full data set and 83 in the reduced data set); n_i and s_i^2 are respectively the number of experimental repetitions and the variance (with n_i degrees of freedom) for each data point; y_i is the experimental data point value, and $\hat{y}_i$ the corresponding model-predicted value (Edwards, 1972, p.115-119). The log-likelihood is similar to a measure of the sum of squared deviates, weighted by the variance of each experimental data point. The higher the value of the log-likelihood, the better the fit.

The results of these 200 calibrating simulations are presented in the next section. Finally, predictions of metabolite levels for various exposure scenarios (TWA vs. peak exposure) were made with the model using the same 200 sets of parameters.

Box plots are used to display the distribution of the computed results. They give the 10th percentile (lower bar), the 25th percentile (bottom of the box), the median (middle bar), the 75th percentile (top of the box) and the 90th percentile (upper bar). Simulation results falling in the lowest or the highest 10% of the distribution are displayed individually with circles.

RESULTS

Model Calibration

In addition to the data of Cassidy and Houston (1984) and Sabourin et al. (1988; 1989; 1987) used to parametrize our previous version of the model (Bois et al., 1991), we took advantage of additional data from Sawahata and Neal (1983) and of the constant C×T (concentration by time) exposure data of Sabourin et al. (1989, Table 4). We report here only the calibration results for these additional data, as the results for the other data are similar to those previously reported (Bois et al., 1991).

Sawahata and Neal (1983) observed the formation of catechol during the incubation of [^{14}C]phenol with liver microsomes from male Sprague-Dawley rats pretreated with phenobarbital. The amount of catechol formed was about 5% of the amount of hydroquinone generated. We computed the ratio of catechol over hydroquinone formation during the simulations of Cassidy and Houston (1984) intraduodenal administration of phenol to Sprague-Dawley rats. The parameters Kpc_l and Kpc_{bm} were adjusted until the ratios observed in the simulations bracketed the experimental value of 5%.

The second data set modeled was published by Sabourin et al. (Sabourin et al., 1989). Male F-344/N rats were exposed nose-only to three different vapor concentrations of [^{3}H]benzene and the time of exposure was varied such that the product (C×T) of the vapor concentration and the time of exposure was constant. Actual exposure regimens were 51 ppm for 6 hr, 153 ppm for 2 hr, and 588 ppm for 0.5 hr. Four rats were sacrificed at selected time intervals during and for 8 hr following the exposures. The last sacrifices were performed at 14 hr, 10 hr and 8.5 hr from the beginning of exposure for the 6 hr, 2 hr and 0.5 hr exposures respectively. Urine samples were collected from the bladder and the urinary metabolites analyzed.

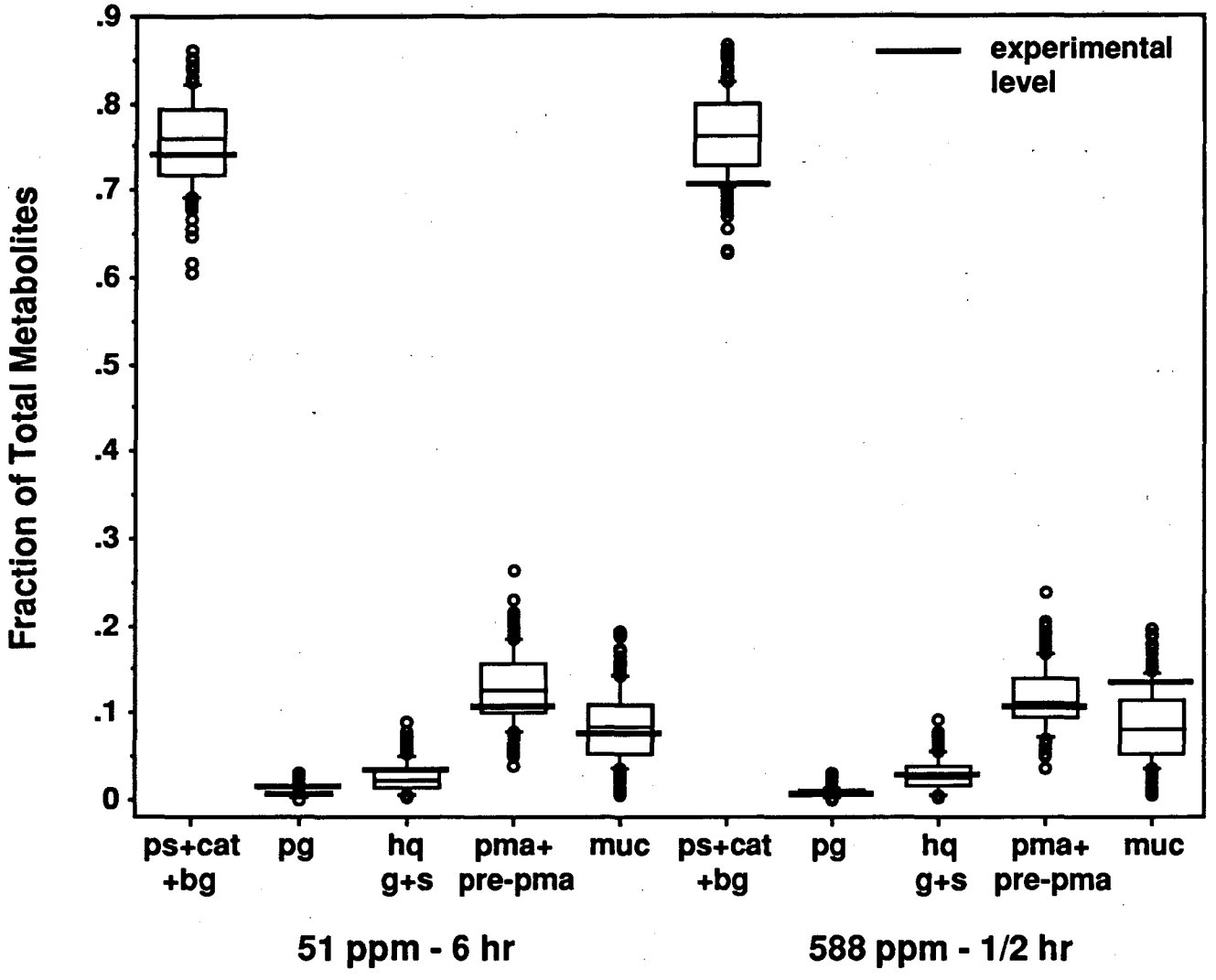

Figure 3: Distribution box plots of simulated values for the fractions of metabolites formed after two schedules of constant C×T benzene inhalation in F-344 rats (Sabourin et al., 1989). Abbreviations: bg: benzene glycol; cat: catechol; hq g+s: hydroquinone sulfate and glucuronide; muc: muconic acid; pma: phenylmercapturic acid; pre-pma: pre-phenylmercapturic acid; ps: phenyl sulfate. The experimental observations are represented by thick horizontal lines.

Figure 3 displays the results of our simulations for the 51 ppm and 588 ppm exposure data. The amounts of phenyl sulfate, phenyl glucuronide, hydroquinone sulfate and glucuronide, pre-phenylmercapturic acid, phenylmercapturic acid, and muconic acid formed were observed. The phenyl sulfate measured experimentally was contaminated by catechol sulfate, due to the proximity of the two peaks in the HPLC

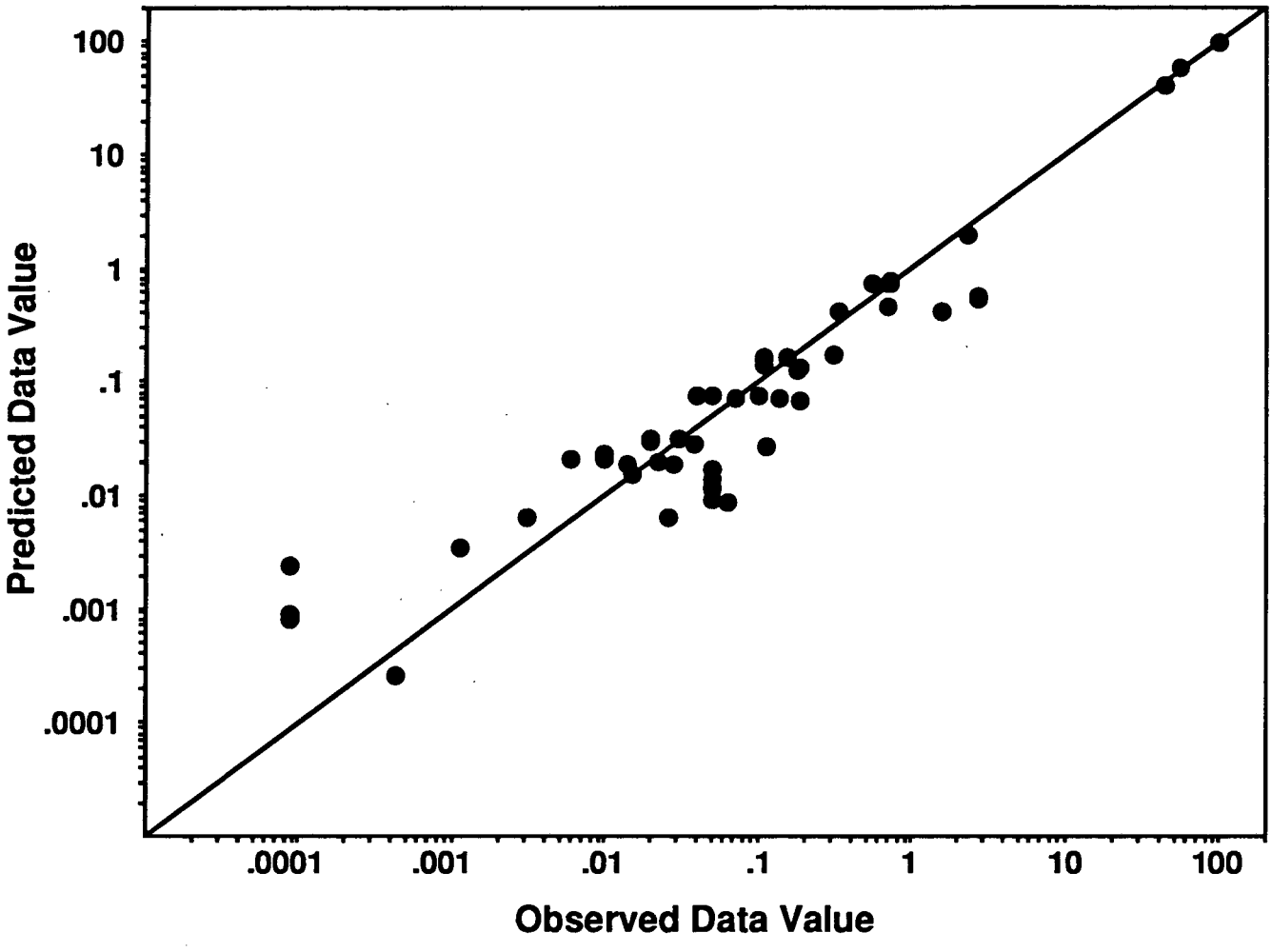

Figure 4: Predictions made by the best-fitting simulation run versus observed experimental values (Cassidy and Houston, 1980; Sabourin et al., 1988; Sabourin et al., 1989; Sabourin et al., 1987; Sawahata and Neal, 1983). For a perfect fit, the points would fall on the identity line.

procedure used (Sabourin et al., 1988; Sabourin et al., 1988), and it is likely that benzene glycol eluted at the same time. So, the total catechol, benzene glycol and phenyl sulfate amounts computed in the model were summed for comparison with the phenyl sulfate levels reported by Sabourin et al. (1989). Of the 200 best simulations performed, many still lead to large over- or under-estimates of the experimental data. Nevertheless, the simulations bracket each experimental data point observed. In addition, the data themselves have inherent variability (the average coefficient of variation for all the data points is 43%). The plot of predicted versus observed data points (Figure 4) shows the overall fit to the data. For a perfect fit to the experimental data the points would fall on the "identity line" of the plot, each computed value being identical

with its experimental counterpart. The largest deviations occur for 3 points in the low range. As discussed by Bois et al. (1991), there is a conflict between these data, obtained by Sabourin et al. (1988) and those of Cassidy and Houston (1984). Still, the overall model fit is acceptable given the diversity of the data, their wide range of values and the complexity of the model. The next step is to use the 200 parameter sets to make predictions of unobserved quantities.

Comparison of TWA to Peak Exposures

OSHA's permissible exposure limit (PEL) for benzene is 1 ppm (0.0033 mg/l or 4.17×10^{-5} mMol/l). The model was used compare the kinetic profiles of benzene oxide, catechol, hydroquinone, muconic acid or phenylmercapturic acid and phenol following an acute exposure of 32 times the PEL (i.e. 32 ppm) for 15 min. versus a continuous exposure at the PEL for 8 hr. The product of vapor concentration and exposure duration (C×T) for these two exposures is the same, i.e. 8 ppm-hr. The profiles were simulated for an entire 24 hr period, including exposure. The areas under the curve (AUC) for each metabolite were computed by numerical integration at the same time as the corresponding instantaneous quantities.

Figure 5 presents the relative differences between the AUC for the quantities of various metabolites formed in the body during the 15 min and 8 hr exposures. The AUCs are equivalent to C×T measures of exposures for the metabolites. For phenol, the relative difference in the AUCs of blood concentrations is presented. These results are model predictions based on the 200 best parameter sets found previously. For all metabolites but benzene glycol, the AUC is significantly higher (paired t-test on the absolute differences, $p < 0.0001$) after the 15 min exposure than after the 8 hr exposure. The relative difference, although significant, is low on average for benzene oxide and phenol (1 and 4 percent respectively). For the other surrogates of effective exposure (phenylmercapturic acid, hydroquinone, catechol and muconaldehyde), the relative difference is, on average, 20 percent. Since we used 200 plausible parameter sets, these differences have a range of values. For phenol and hydroquinone the range of the differences includes values as high as 60 or 130 percent higher in AUC during the short-term exposure. Additional simulations show that a 5 min exposure to 96 ppm gives similar median effects (20 percent increases in AUCs the four previous metabolites) but with a wider range (up to 250 percent increase for hydroquinone).

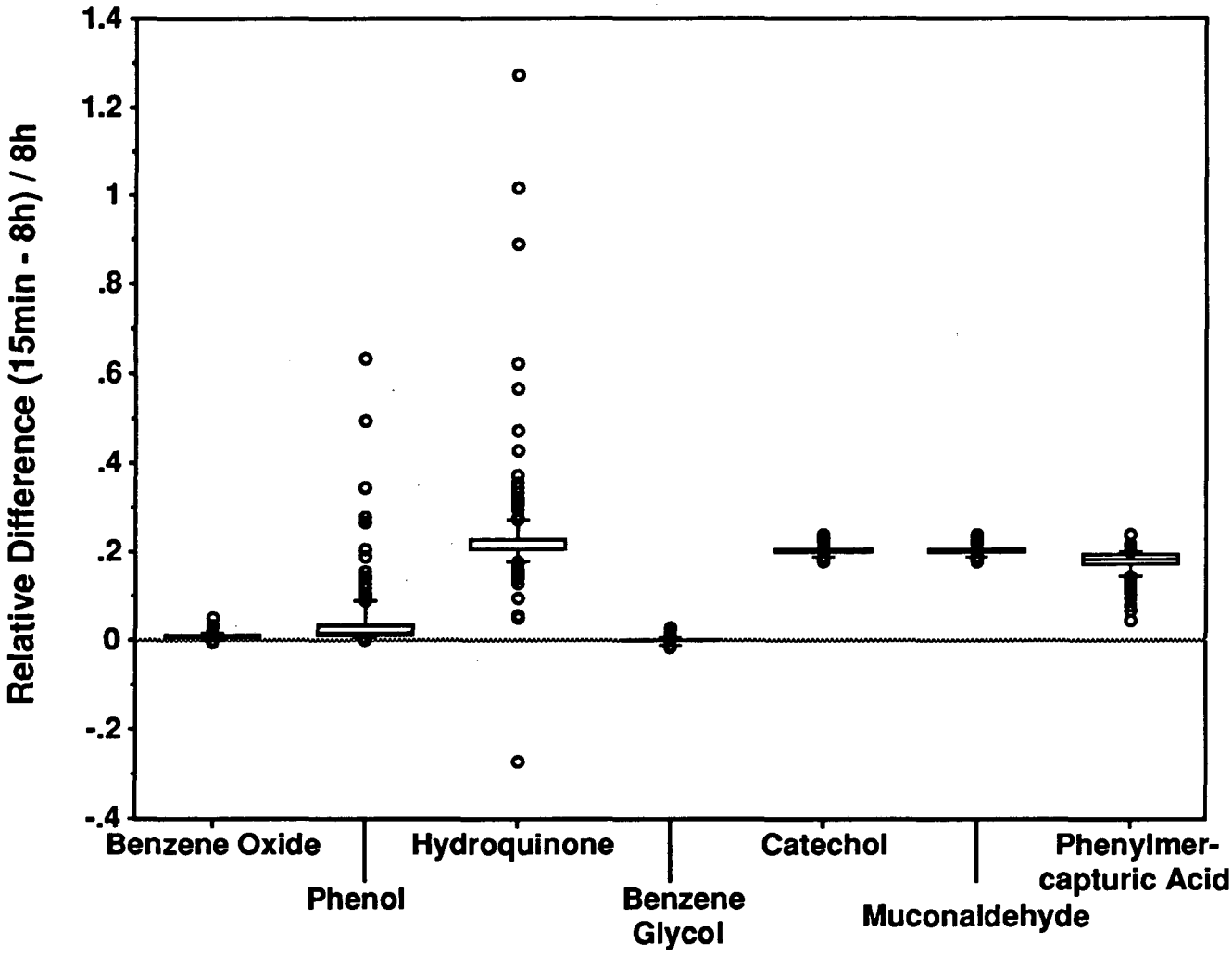

Figure 5: Relative differences in the areas under the curve for the kinetic profiles of various metabolites of benzene in the body of F-344 rats after constant C×T exposures to 1 ppm for 8 hr or 32 ppm for 15 min. These differences were obtained by simulation, using the 200 best model parameter sets.

We also used our model to simulate the formation of benzene oxide, phenol, phenylmercapturic acid, and hydroquinone in the bone marrow, the target organ of benzene carcinogenicity in humans (Figure 6). Again, a statistically significant increase in the relative difference between AUCs is observed (paired t-test on the absolute differences, $p < 0.0001$). This increase is similar to the one found in the whole body, or slightly higher (25 percent for hydroquinone). The spread of values (uncertainty) is also higher, with possible differences for hydroquinone AUC ranging from -50 percent (indicating a higher AUC during the 8 hr exposure) to 300 percent.

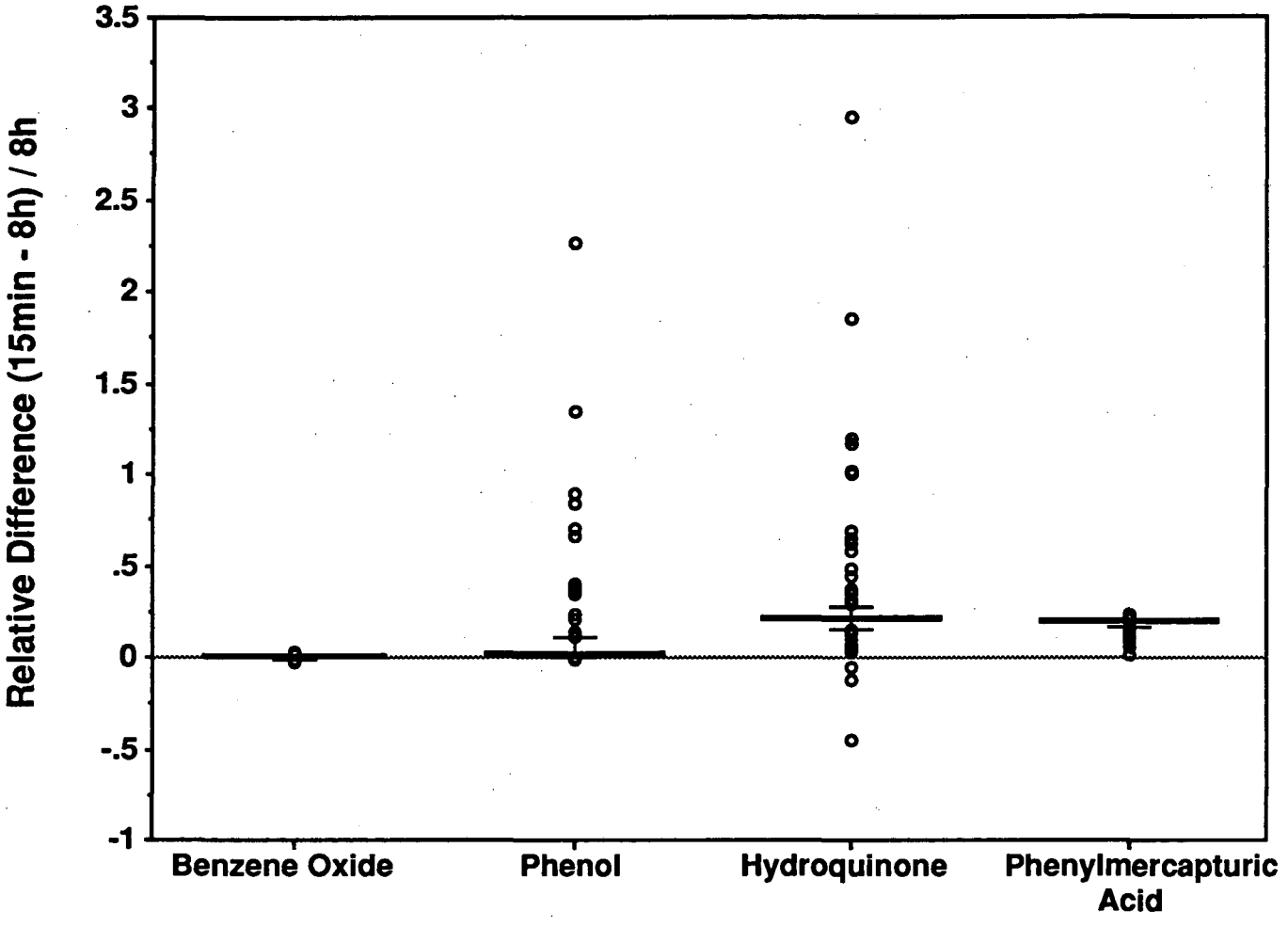

Figure 6: Relative differences in the areas under the curve for the kinetic profiles of four metabolites of benzene in the bone marrow of F-344 rats after constant C×T exposures to 1 ppm for 8 hr or 32 ppm for 15 min. These differences were obtained by simulation, using the 200 best model parameter sets.

DISCUSSION

The final standard for workplace exposure to benzene (OSHA, 1987) includes an 8 hr TWA PEL of 1 ppm and a STEL of 5 ppm over 15 minutes. In justifying the STEL, OSHA contended that the long term adverse effects of benzene were related to peak air concentrations as well as TWA levels. Thus, OSHA argued that the peak concentration of benzene was intrinsically important to the production of damage in the bone marrow, which can ultimately lead to leukemia. However, OSHA did not provide clear evidence of dose-rate effects from benzene exposure in humans or experimental animals (Paxman and Rappaport, 1990).

In this paper we have used a new PBPK model to explore the biological significance of different rates of exposure for benzene chronic toxicity. Our model is unique in that it enables us to quantitatively predict the formation of individual metabolites, and provides information not previously accessible to the toxicologist. The approach taken to parametrize the model is robust, because wide parameter ranges rather than point estimates are used to derive distributions for the model predictions. Extensive discussions of the Monte Carlo techniques used to parametrize the model have been presented elsewhere (Bois et al., 1991; Bois et al., in press; Spear et al., in press). To our knowledge, this is the first application of PBPK in assessing exposure rate effects for the purpose of setting exposure limits.

Dose-rate Effects

The kinetics of benzene metabolism were investigated to determine whether a particular exposure regimen, at a constant exposure dose of 8 ppm-hr, is likely to lead to large differences in tissue levels of the crucial metabolites. Significant differences would implicate exposure intensity as a causal factor in the adverse health effects associated with exposure to benzene. Such an outcome would suggest the need for a short-term exposure limit for benzene in the workplace.

Our results point to an increased formation, as measured by the area under the curve, of certain benzene metabolites in the peak exposure regimen compared to the 1 ppm TWA exposure. Whereas no differences were detected in the primary metabolites benzene oxide and phenol, statistically significant differences were

observed in the formation of secondary metabolites including hydroquinone, muconaldehyde, and catechol. These metabolites are precisely the crucial components of benzene toxicity to the bone marrow, as discussed by Bois et al. (1991). Taken together, the data suggest differential selectivity of metabolic pathways under the different exposure scenarios. We are currently investigating which quantitative features of the metabolic pathways are critical in producing the observed behavior.

In the present formulation of the model, the quantity of catechol and muconaldehyde formed in the bone marrow are not computed. The amount of existing data on the formation of these metabolites in that organ is inadequate. However, we were able to obtain site-specific estimates of the formation of phenol, hydroquinone and phenylmercapturic acid. For a peak exposure, an increased formation of the latter two was observed in the bone marrow, as seen in the whole body. The uncertainty in these results, reflected in the distribution box-plots, arises because we took a set of parameter values (the best 200 after model calibration) rather than fixed estimates. Additional data, particularly on the levels of individual metabolites in the bone marrow, would help reduce the variance of the estimated numbers and increase the precision of the results.

Sabourin et al. (1989) concluded from their constant C×T exposure experiments that, within the range of C×T factors studied, the rate of the inhalation exposure to benzene did not affect the AUC of metabolites in the tissue of rats. The experimental C×T factor was approximately 300 ppm–hr, while we investigated a factor of 8 ppm–hr, more relevant to human exposure conditions. Note that we have calibrated our model with Sabourin et al. data and that therefore no conflict exists between the findings of Sabourin and ours. It is likely that at the high levels studied by Sabourin et al. the saturation of some enzymatic reactions occurs, which prevents the onset of the effect observed at low levels. Additional studies are under way to fully understand the mechanisms leading to the increased damaging potential of peak exposures. While Hattis (1990) has discussed the possibility of such an effect for abstract systems of coupled non-linear reactions, our results demonstrate its occurrence in the specific case of benzene metabolism.

Previously Paxman and Rappaport (1990) had shown that while the concentration of benzene in the blood reflected the 32 fold greater ambient air concentration of a peak exposure, only a 3 fold increase, at its highest concentration, reached the bone marrow; the area under the tissue concentration-time curve is the

same for benzene under both exposure conditions. It was determined that the impact of peak exposures would be small because the peak concentration of benzene would be within the capacity of the cytochrome P450 liver enzyme system to maintain first order metabolism and that the predicted maximum blood concentrations of metabolites would be below that which have been shown to induce toxicity. Rappaport and Spear (1988) demonstrated that the transmittance of exposure variability (such as between peak and TWA) is attenuated as it approaches the target organ depending upon physicochemical and biological properties, in a process termed "physiological damping". Therefore, with a limited analysis, the evidence was insufficient to implicate the rate of exposure as a causal factor in the chronic health effects of benzene. While mostly substantiating the previous work on the STEL, our quantitative description of the nonlinear kinetics of benzene metabolism using the PBPK approach leads to somewhat different conclusions.

Implications for Health Effects and Exposure Control Policy

A short-term exposure limit (STEL) is directed at controlling transient or "peak exposures" averaged over 15 minute periods. The STEL is used to augment the PEL when the latter is not sufficiently protective against the toxic effects caused by benzene. Although OSHA has not always provided a clear rationale for setting them, STELs generally are used to prevent acute effects associated with peak exposures, even when the 8 hr TWA concentration is below the PEL (Paxman and Rappaport, 1990 ; Zielhuis et al., 1988). The relationship of the intensity of exposure at a given TWA to chronic health effects, such as leukemia, has not been adequately investigated.

To justify a STEL on the basis of chronic effects, either of two conditions are seemingly required. First, the peak exposure must be transmitted to the bone marrow as a corresponding large tissue concentration of benzene or metabolites. Second, the rate at which damage is produced in the bone marrow must be disproportionately large during periods of highest tissue concentration. These conditions would result in greater toxicity from transient peak exposure than from continuous low exposure at an equivalent dose (Rappaport, 1985; Rappaport, 1988; Rappaport and Spear, 1988). The augmented concentration of benzene metabolites in the body and bone marrow observed in the present study suggests that the peak concentration is transmitted directly or indirectly to the target tissues. The occurrence of the second

condition, rate of tissue damage, is unclear; however, the possibility of nonlinear synergistic effects between metabolites (Barale et al., 1990; Robertson et al., 1991) could provide the required mechanism.

Whether the amount of metabolites formed actually represents an enhanced risk of leukemia remains to be seen. In a previous paper it was shown that the PEL was substantially below the level at which acute effects, especially narcosis, would occur (Paxman and Rappaport, 1990). Nonetheless, given the demonstrated role of the metabolites in benzene's carcinogenicity (Eastmond et al., 1987), an increased metabolite formation suggests an increased risk of leukemia. A peak exposure may thus result in a disproportionate amount of damage relative to the TWA exposure.

Identifying differential health sequelae from various types of exposures will require additional toxicological and epidemiological data. Policy, however, must often be made with incomplete information. Setting a STEL remains a policy decision for OSHA. Offsetting the health information, OSHA must also consider the economic and technological feasibility of its standards (Robinson and Paxman, 1991). A STEL places greater strains on the limited resources available for air sampling by industrial hygienists by increasing compliance monitoring, and consideration must be given to the scheduling of workshifts to prevent worker exposure to peak blasts of benzene. These augmented management responsibilities represent substantial health and safety costs that would not be incurred under a PEL. Thus, a STEL should be underpined with adequate scientific evidence. Since a peak benzene exposure may present a greater risk of leukemia for the worker, then the increased monitoring requirements for the STEL seem justified. Given the magnitude of the observed effect at the peak exposure condition, the benzene STEL of 5ppm averaged over 15 minutes appears sufficiently health-protective.

The methodology presented here provides a toxicokinetic analysis of benzene and the formation and distribution of its metabolites. For benzene and other toxicants, this type of analysis should be useful to policy makers in two ways: 1) by providing a qualitative and quantitative estimate for the allowable ambient concentration that will insure health protection; and 2) by identifying gaps in the data that require further research. With the increasing legal and economic complexity of environmental and occupational health standards, regulatory agencies are required to provide ever more scientific justifications for their regulations. The use of mechanistic PBPK models should be useful for that purpose.

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