CIIT ACTIVITIES

Institute of Toxicology

Vol. 15, No. 6 June, 1995

Determinants of Benzene Metabolism and Disposition

Paul M. Schlosser, Elaina M. Kenyon, Mark J. Seaton, and Michele A. Medinsky

The metabolism of benzene and the resulting dosimetry of benzene and its metabolites almost certainly are primary factors In determining the health risks to humans from benzene exposures. The mechanisms and pathways *that* determine the concentrations of benzene and its metabolites In various tissues have been the subject of considerable debate. This article describes insights Into benzene dosimetry and metabolism obtained at CIIT over tha past three years and subsequent implications for benzene-induced myelotoxicity and hematotoxicity. The metabolic pathways and kinetics of benzene metabolism have been explored *In vitro*, providing sufficient data to develop a mathematical model of the reactions that occur. Differences in *In vitro* benzene metabolism among mice, rats, and Individual humans have been investigated. Mice metabolize benzene faster than rats, while the range of rates exhibited by human tissue samples spans *that* of mice and rats. Some qualltatiw differences have also been observed between *In vitro* and *In vivo* benzene metabolism. Thew differences can be explained, however, by incorporating the regional distribution of liver enzymes Into a physiologically based pharmacokinetic (PBPK) model. By developing mathematical models capable of describing large data sets and observing the range of metabolic activities in samples of human liver, we expect to provide dramatically bettor tools for the assessment of human health risks from exposures to benzene.

Benzene was used extensively in the past for production of paints, resins, rubber. inks, and dyes. It is currently 1% of gasoline and a feedstock for the manufacture of synthetic organic chemicals (Ayres and Taylor, 1989). Benzene is a constituent of gasoline fumes, automobile exhaust, and both mainstream and sidestream tobacco smoke (Wallace, 1990). It is listed as an air toxic in the Clean Air Act Amendment of 1990. Exposure to high levels of benzene for extended periods of time is associated with aplastic anemia and acute myelogenous leukemia (AML) in humans (Infante et *al.*, 1977). Workplace exposures to benzene are now limited through safety regulations (Runion and *Scott*, 1985), but occupational and environmental exposures to benzene still occur (Hricko, 1994; Tompa *et al.*, 1994).

The effects of chronic exposure to low levels of benzene are not known. Uncertainties in exposure assessment in epidemiological studies have made it difficult to obtain a reliable low-dose extrapolation (Health Effects Institute, 1993). Also, a confirmed animal model does not exist for benzene-induced AML, the effect of most concern in humans. These factors lead to considerable uncertainty *about* the risk to humans of low-level benzene exposure.

Benzene is myelotoxic and hematotoxic in mice and rats (MacEachern et al., 1992; Snyder *et al.*, 1978). Because myelotoxicity and hematotoxicity have been observed prior to the onset of AML in humans, the incidence and degree of these acute effects in humans are presumed to correspond to risk of human AML. Further, because an animal model for benzene-induced AML has not been identified, the incidence of benzene-induced myelotoxicity and hematotoxicity in mice and rats is taken to be a surrogate for AML when considering the relationship between benzene exposure levels, tissue metabolite concentrations, and disease response. Based on the observation by Sammett et *al.* (1979) that partial hepatectomy in rats is protective against the hematotoxicity of benzene, *the* conversion of benzene in the liver to one *or* more toxic metabolites is believed to play a significant role in the induction of hematotoxicity. If this is true, then the risk to humans depends on the concentration, or dose, of these toxic metabolites at the site of toxicity, the bone marrow. Working under this hypothesis, research at CIIT has focused on understanding the liver metabolism of benzene and *on* determining how this metabolism influences the dosimetry of benzene and its metabolites in *vivo*. In particular, our *goal* is to develop a quantitative tool—a mathematical simulation model—that relates metabolic rates in the liver to the concentrations of benzene metabolites reaching the bone marrow. This tool is to be developed using data from rats and mice, for which extensive data sets exist or *can* be obtained. In addition to the collection and analysis of rodent data, we have been measuring the rate of benzene metabolism by liver samples from human donors. The rodent data will be used to develop and validate the simulation model. Then, by changing the model parameters to reflect human physiology and metabolic capacities, we will be able to predict the exposure-dose relationship for benzene metabolites in human bone *marrow*.

The primary pathways for benzene

(Continued *on* page 2)

ISSN 8755-4259

CIIT ACTIVITIES
Chemical Industry Institute of Toxicology

Published monthly by the Chemical Industry Institute of Toxicology. Correspondence should be directed to Willanna Griffin, CIIT information Services, P.O. Box 12137, Research Triangle Park, NC 27709. Telephone, (919)558-1200; FAX, (919) 558-1300

Roger O. McClellan, D.V.M.
President

Benzene Metabolism (from page 1)

metabolism in the liver are depicted in Figure 1. Oxidative reactions lead to the formation of benzene oxide (a reactive intermediate), phenol, hydroquinone, and small amounts of catechol and trihydroxy benzene. These oxidized metabolites may be further transformed by conjugation to compounds that are readily excreted in the urine. Benzene oxide can also be converted to an opened-ring metabolite, mucon-aldehyde, a precursor of muconic acid, which is excreted in the urine.

The conjugated metabolites of benzene are generally thought not to be responsible for its toxic effects, and a significant amount of research has focused on identifying which of the intermediate metabolites is the bad actor. Administration of either phenol or hydroquinone alone to rodents fails to reproduce the hematotoxic and myelotoxic effects of benzene exposure (Cox, 1991; Kari et *al.*, 1992). These observations led investigators to the hypothesis that neither of these metabolites is responsible for benzene toxicity and to the conjecture that muconaldehyde, the only semistable toxic metabolite that is not produced from phenol, might be responsible. However, as with phenol and hydroquinone, muconaldehyde alone does not reproduce the toxic effects of benzene (Guy et *al.*, 1991). Instead, benzene toxicity apparently results from the combined *effects* of several metabolites: muconaldehyde and hydroquinone *act* additively (Guy *et al.*, 1991), and phenol and hydroquinone act synergistically (Barale et *al.*, 1990; Eastmond et el., 1987). The risk from benzene exposure may depend *on* the dosimetry of all three metabolites, with catechol and trihydroxy benzene playing lesser roles.

To develop a simulation model for predicting *in* vivo dosimetry and metabolism in humans based on *in vitro* data, we first need to understand *in vitro* metabolism and *in* vivo metabolism and dosimetry separately in a laboratory species such as the mouse. Only then can we merge the two levels of understanding, *in vivo* and *in vitro*, and resolve any apparent differences between the two. In this article, we first describe the ongoing research *on* liver metabolism, along with some tentative physiological predictions based on *in* vitro results. Secondly, the development of what is currently an independent mathematical model of *in* vivo dosimetry and metabolism is discussed. Finally, some implications of the current results and a synopsis of future directions are presented.

Differences in the In *Vitro* Metabolism of Benzene among Species and Individual Humans

The metabolism of benzene is fairly complicated in that it involves a number of reactions (Figure 1). To predict the dosimetry of the various metabolites, we need to know the rate of each reaction as a function of *the* metabolite concentrations. While these rates can be determined *in vivo* for laboratory animals, our objective is to predict human metabolism, where *in vivo* experiments are precluded and the availability of tissue samples is limited. Differences exist among species as well as among individual humans in the rates of m e tabolism. Since these differences affect individual risk, we seek to understand and quantify the range of rates for benzene metabolism in humans based on those measurements that can be obtained with human liver samples.

While the rates of metabolism of a

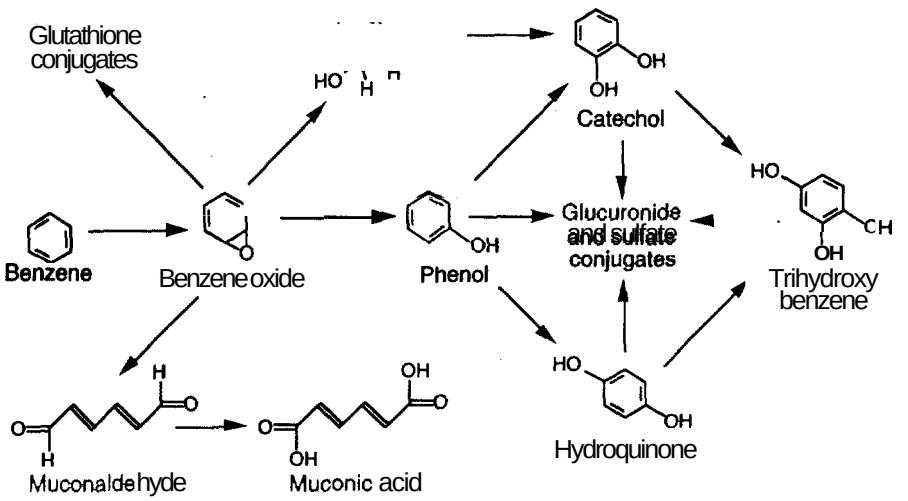


Fig. 1
Primary pathways for benzene metabolism in the liver. The oxidized metabolites phenol, hydroquinone, catechol, and trihydroxy benzene, along with the ring-open metabolite muconaldehyde, are all toxic to the bone marrow (myelotoxic) and affect the circulating blood (hemstotoxic). The conjugated metabolites and muconic acid are readily excreted in the urine.

xenobotic typically differ among species, the biochemical mechanisms of benzene biotransformation are expected to be nearly the same in all species. Therefore, our goal is to develop a mechanistically based mathematical model of metabolism in which all species and individual differences are explained by differences in parameter values such as enzyme activities. Since *in* vivo dosimetry data in mice are also being obtained and already exist for rats, these species can be used to test the extrapolation of *in vitro* rates to *in* vivo rates. Extrapolation among species or individuals can be tested by using a large *in vitro* data set for rat liver metabolism in comparison with the mouse data. In particular, we can determine if a model developed from mouse data, taken together with measurements of rat and mouse enzyme activities, *can* be used to predict rat metabolism. Finally, a smaller data set obtained with human tissues can be used to determine model parameter values and their ranges for humans.

Oxidative Metabolism In *Vitro*

By using different fractions from liver cells and supplying only the substrates and cofactors necessary for specific metabolic reactions, we *can* observe subsets of the reactions depicted in Figure 1 and reduce the complexity of developing a model for all these reactions to more manageable pieces. One specific goal is to develop quantitative descriptions or reaction rate equations for the oxidative reactions. By performing incubations with the microsomal fraction of liver and supplying NADH and NADPH as cofactors, we can observe the set of oxidative reactions in isolation. As an example, the disappearance of benzene due to oxidation by liver samples from two humans, F-344 rats, and B6C3F$_1$ mice is depicted in Figure 2. In these experiments, subcellular fractions of liver samples containing oxidation enzymes were incubated *in vitro* with benzene, and the amount of benzene oxidation was observed. As can be seen, mouse microsomes metabolize benzene faster than rat microsomes, which correlates with the fact that mice are more sensitive than rats to the hematotoxic effects of benzene. More importantly, the data from the two human samples illustrate the wide range in metabolic activity for benzene oxidation among humans, which spans the activity from mice and rats. Given that the same enzyme, cytochrome P450 (CYP) 2E1, is primarily responsible for benzene oxidation in mice and rats (Nakajima *et al.*, 1993) and has a similar role in humans (Seaton *et al.*, 1994), understanding the difference in metabolic rates between mice and rats should help to explain the range of rates observed for humans. In this case, the rate of a reaction may differ between two individuals, while the enzymatic mechanism is the same.

TABLE 1

K_m VALUES OF SEVERAL REACTIONS FOR MICE, RATS, AND HUMANS

Species	Reaction			
	BD expoxidation (μM)[a]	BMO hydrolysis (mM)[a]	BMO glutathlone (mM)[a]	Acrylonitrile expoxidation (μM)[b]
Mouse	2.00 ± 0.17	1.59 ± 0.03	35.3 ± 6.2	13 ± 1
Rat	3.75 ± 0.20	0.26 ± 0.10	13.8 ± 0.3	11 f 1
Human	5.14 ± 2.59	0.24 ± 0.10 to 1.65f0.12	10.4 ± 1.0	1 2 f 1 to 1 8 f 1

[a]From Csanády *et al.* (1992); [b]From Kedderis *et al.* (1993).

An initial mathematical simulation model of benzene oxidation by mouse and rat liver microsomes was described by Schlosser et *al.* (1993). This model incorporates a mechanism whereby benzene, phenol, hydroquinone, and catechol all interact through competition for a common reaction site *on* the oxidizing enzyme, CYP. At least two CYP isozymes are responsible for benzene oxidation, with CYP 2E1 being predominant (Koop *efal.*, 1989; Nakajima *efal.*, 1993). The model of Schlosser *et al.* (1993) considers only a single enzyme, with effective parameters for the total metabolism of benzene to phenol, hydroquinone, catechol, and trihydroxy benzene. The same model structure describes metabolism by both mouse and rat microsomes, but the parameter values for the two species are quite different.

The activity or maximal rate (V_{max}) of reactions catalyzed by an enzyme *such* as CYP 2E1 may vary significantly among animal species, but other kinetic parameters such as saturation or affinity *constants* (*e.g.*, K_m) may be very similar. For example, Csanády *et al.* (1992) examined the kinetics of 1,3-butadiene (BD) epoxidation, butadiene monoepoxide (BMO) hydrolysis, and BMO conjugation with glutathione *in* vitro with liver samples from mice, rats, and humans; apparent K_m values for these reactions are listed in Table 1. Values for acrylonitrile epoxidation obtained by Kedderis *et* a/. (1993) are also listed in Table 1. While differences in K_m values among species and in *some* cases differences among individual humans are observed, those differences are often quite small and may not be statistically significant. Further, the differences in K_m values among species for a given reaction are, in all cases, quite small compared with the differences in K_m values between reactions. In the *case* of BMO hydrolysis and conjugation with glutathione, species differences of over an order of magnitude in the respective V_{max} values were observed (values not shown). Therefore we might expect saturation parameters such as K_m generall to have similar values in different species.

The role of CYP 2E1 in benzen metabolism was further explored by Seato *et al.* (1994), who compared biotrans formation by liver samples from 10 human as well as from mice and rats. Plots of th disappearance of benzene and th formation of phenol and hydroquinone ar shown in Figure 3. Benzene metabolisı correlated strongly with CYP 2E1 activi across species and individuals, suggestin that a mathematical model could b developed in which this activity is the on difference among individuals or specie Such a model was developed and describe by Seaton et *al.* (1994) based on the mod of Schlosser *et* a/. (1993). This model al assumed that a single enzyme wa responsible for oxidation, with benzen phenol, and hydroquinone competing for t enzymatic reaction site. From Figure 3, v see that the model does a fairly good job predicting data to which it has not been

The idea of a universal metabolism mod in which CYP 2E1 activity alone explains dividual or species differences in rates of be zene metabolism, is quite appealing. The da in Figure 3 not only suggest this possibil but also that a first-generation model capat of describing oxidative metabolism by hum liver might be developed almost complet from mouse and rat data. Yet Schlosser *et* (1993) obtained dissimilar values of satu tion and competition parameters for rats a mice. How do we explain these conflicti results of Schlosser *et al.* (1993), where sa ration parameter values appear to be qu different between mice and rats, and of Seat *et al.* (1994), where the kinetics of benze oxidation appear very similar in mice, rats, a humans? The model of Seaton *et* a/. (19 describes the results of benzene incubatic starting at a single concentration (4 μM) a

(Continued on page

zene Metabolism (from page 3)

tion of CYP 2E1 activity. When additional se and rat data are considered, including lts from benzene and phenol incubations vo concentrations for each substrate, a le-enzyme model is no longer capable of cribing the entire data set, even for a single cies. In fact, more careful examination of re 3 reveals that there are different indi- als with nearty identical CYP 2E1 activi- but significantly different extents of me- lism.

f a single-enzyme model is incapable of cribing a larger data set (one that includes lts from incubations with phenol as a sub- e), how can the correlation evident in Fig- 3 be explained and utilized? The rate of zene oxidation to phenol is determined sly by CYP 2E1 activity, which limits the lability of phenol during benzene incuba- , and this limited availability may control rate at which benzene-derived phenol is sequently converted to hydroquinone. On other hand, when phenol is the substrate, vailability is not limited by CYP 2E1 activ- nd a second CYP may be involved. There- the rate of conversion to hydroquinone be much more dependent on the activity CYP other than 2E1. Koop et al. (1989) ved that several CYP isozymes can con- phenol to hydroquinone. The hypothesis a CYP other than 2E1 contributes to phe- oxidation is currently being tested through development of a two-enzyme model, re the activity of one enzyme is taken to hat of CYP 2E1. Candidates for the sec- enzyme include CYP 1A1, CYP 1A2, CYP 6, and CYP 2C11 (Nakajima *et al.*, 1993). e parameter values have been set by ching the model to a data set that includes benzene and phenol incubations at mul- concentrations, the dependence of el-predicted benzene metabolism on CYP activity will be tested and compared to the data in Figure 3. If a two-enzyme model is able to describe the existing multiple-substrate data sets, then inhibition experiments can be performed to directly test and compare the dependence of benzene and phenol metabolism on CYP 2E1 activity.

Conjugation *In Vitro*

As in the case of oxidation, the conjugation reactions can also be studied separately. Phenol sulfation and hydroquinone glucuronidation are the primary routes for conjugation of benzene metabolites in rodents. Once conjugated, the metabolites are readily excreted in the urine, so conjugation reactions are considered to be detoxication pathways. The circulating levels of the oxidized metabolites are determined by the balance between oxidative rates and conjugation rates. Therefore determining the rates of conjugation reactions relative to the oxidative reactions and determining how those rates vary among individual humans are equally important as determining the rates of the oxidative reactions. The rates of conjugation reactions can be measured using the cytosolic and microsomal subcellular fractions, respectively, obtained after centrifugation of liver homogenates. We examined differences in the *in vitro* rates of conjugation among species or individuals using pooled samples of rat and mouse liver and individual samples of human liver. We observed a threefold variation among human samples in the rate of phenyl sulfate formation (range, 0.3 to 0.9 nmole/mg/min; Table 2). For laboratory animals, phenol sulfation was much faster in rat cytosol (1.2 nmole/mg/min) then in mouse cytosol (0.5 nmole/mg/min). Hydroquinone glucuronidation varied by almost threefold among human samples (range, 0.1 to 0.3 nmole/mg/min; Table 2) and was more rapid in mouse liver microsomes (0.22 nmole/mg/min) compared with rat liver microsomes (0.08 nmole/mg/min). No correlation was observed among the rates of oxidation, sulfation, and glucuronidation for rats, mice, or individual humans. Therefore we can assume that these rates vary independently in risk assessment calculations, where the range of metabolism rates among humans is important.

In addition to interindividual variability among human samples in the activities of phenol sulfation, hydroquinone glucuronidation, and CYP 2E1 oxidation (*Seaton et al.*, 1994), species differences observed among humans, mice, and rats are interesting and deserve comment. The mouse is more sensitive than the rat to the toxic effects of ben-

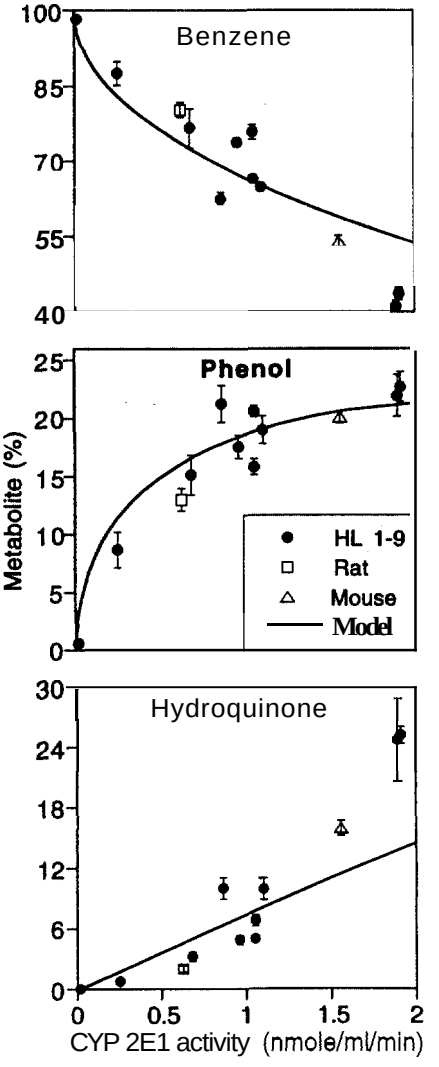
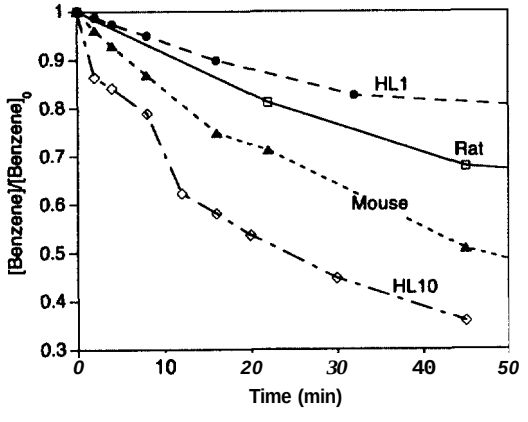


Fig. 3
Metabolism of ~ 4 µM benzene to phenol and hydroquinone from 16-min incubations with 1 mg/ml liver microsomal protein from a number of individual humans (HL1-9), rats, and mice, shown as a function of cytochrome P450 (CYP) 2E1 activity. The amount of metabolite is displayed as a percentage of the total substrate and metabolite concentration (other metaboiltes not shown). Data (symbols) and model (solid line) are from Seaton *et al.* (1994). (Adapted by permission of Oxford University Press.)



l. 2
mparison of benzene metabolism with liver microsomes from B6C3F1 mice, F344 s, and humans (HL1 and HL10). [Benzene]/[Benzene]$_0$ is the proportion of ben- e remaining as a function of time relative to the initial benzene concentration. ubations were performed with ~ 4 µM benzene and 1 mg/ml microsomal protein, described in Schlosser et al. (1993). Mouse and rat data were originally reported Schlosser et al. (1993); human data were reported in Seaton et al. (1994).

zene (Snyder and Kalf, 1994). Our results reveal a greater capacity for activation and less for detoxication in mice compared with rats, whereas human values include slow activation and detoxication (Table 2, HL 2), moderate activation and rapid detoxication (Table 2, HL 6), and rapid activation and slow detoxication (Table 2, HL 10). Observations in mice and rats are in agreement with the suggestion that susceptibility to benzene toxicity results from a balance between oxidative and conjugative reactions. Moreover, these observations agree with *in vivo* data from Sabourin et a/. (1987) and physiological model simulations by Medinsky *et al.* (1989). Those studies demonstrated greater urinary excretion of phenyl sulfate and less excretion of hydroquinone conjugates in rats compared with mice following benzene exposure, consistent with Mgher CYP 2E1 activity and lower phenol sulfotransferase activity measured *in vitro* in mouse liver.

A Physiological Compartmental Model

As discussed in the introduction, benzene-induced hematotoxicity and myelotoxicity and the risk of AML in humans are probably a function of the circulating levels of oxidized metabolites that occur during benzene exposure. Gwen the range of activities for oxidation, sulfation, and glucuronidation indicated in Table 2, what phenol and hydroquinone blood levels would occur? We have developed a physiological compartmental model for estimating steady-state blood concentrations of phenol and hydroquinone that might have been achieved during continuous exposure of those 10 individuals to 0.01 µM benzene in blood (Seaton et *al.*, 1995). This Mood concentration is likely to result from exposure to benzene at the current permissible exposure limit of 1 ppm under the Occupational Safety and Health Act (Wallace, 1990).

The steady-state phenol and hydroquinone concentrations for the individual humans, rats, and mice are depicted in Figure 4. According to model calculations, predicted steady-state phenol concentrations varied six-fold among humans and correlated inversely with measured microsomal activity of CYP 2E1. This inverse relationship is most likely due to the fact that CYP 2E1 is also responsible for phenol oxidation to hydroquinone in this model. In particular, while the rate of phenol formation increases with CYP 2E1 activity, the rate of removal also increases with CYP 2E1 activity, resulting in a net decrease in concentration. Steady-state hydroquinone concentrations varied fivefold among humans. With laboratory animals, model simulations predicted that steady-state concentrations of phenol and hydroquinone would be higher in mice than in rats during exposure to this very low benzene dose. These predictions are in agreement with *in vivo* observations from studies with higher benzene concentrations (Sabourin *et al.*, 1987). Predicted steady-state concentrations in mice were higher than in humans, whereas rat values fell among the range of predictions for humans. These simulations suggest that the rat may be a *good* model for humans with respect to tissue dosimetry of benzene metabolites.

Higher levels of oxidized metabolites in mice are due in part to the faster blood flow per unit body weight in this small species compared with humans. More benzene is brought to the liver per unit time for metabolism. The model predictions for higher concentrations of oxidized metabolites in mouse blood, if correct, also suggest that development of leukemia in humans is a function of tissue response in addition to dosimetry. In particular, mice exhibit levels of the putative leukemogenic benzene metabolites that are at least as high as is predicted in humans at exposure levels associated with AML in humans, but these levels do not give rise to AML in mice. Therefore mouse bone marrow apparently responds to these agents in a manner that is qualitatively different from humans.

Variability in rates of conjugation plays a definite role in determining steady-state blood concentrations of phenol and hydroquinone. Although the *in* vivo rate *constants* for benzene oxidation vary 13-fold among human samples, the steady-state concentration of phenol in blood varies only 6-fold. Moreover, steady-state concentrations of hydroquinone do not correlate with rates of oxidation reactions and appear to be influenced more by competition between phenol sulfation and phenol oxidation and detoxication due to hydroquinone glucuroni-dation. While the effect of the hydroquinone:phenol concentration ratio in blood on susceptibility to benzene toxicity is not dear, a synergistic relationship between these metabolites has been demonstrated *in vitro* (Smith *et al.*, 1989; Irons *et a* 1992; Kolachana etal., 1993; Chapman eta 1994).

The results described above suggest significant interaction between Phase I (oxidation) and Phase II (conjugation) pathways in determining blood and tissue levels of benzene metabolites. The quality of these predictions must be tested experimentally in laboratory animals in *vivo* before utilizing them for human risk assessment. Once this has been done, a model like that described here, taken together with the observed ranges of enzyme activities among humans, *can* be used to predict risk to individual humans with given enzyme activity profiles and subsequently the range of risk to human populations.

TABLE 2
INITIAL RATES FOR BENZENE OXIDATION, PHENOL SULFATION, AND HYDROQUINONE GLUCURONIDATION IN INDIVIDUAL HUMAN LIVER SAMPLES AND IN POOLED LIVER SAMPLES FROM MICE AND RATS[a]

	Initial rate		
Liver sample	Benzene oxidation (nmole/mg/min)	Phenol sulfatlon (nmole/mg/min)	Hydroquinone glucuronidatlon (nmole/mg/min)
Human			
HL 1	0.344	0.309	nd[b]
HL 2	0.926	0.581	0.117
HL 6	1.433	0.867	0.281
HL 7	1.499	0.919	0.167
HL 10	4.442	0.485	0.106
Mouse	1.558	0.485	0.218
Rat	0.625	1.195	*0.077*

[a]Results from Seaton et a/. (1995). [b]Not determined due to insufficient sample.

In Vivo Metabolism of Benzene and Phenol In Mice

The underlying *goal* of our *in* vivo metabolism studies in mice has been the development of a quantitative, mechanistically based description of the relationship between a phase benzene exposure concentration (ppm) and the amount of active metabolite being delivered to the target tissue. Oxidative metabolism by CYP 2E1 is required for manifestation of the hemato-toxic and genotoxic effects of benzene, but the dosimetry of active benzene metabolites in the target tissue, bone marrow, is a net result of competing toxification (oxidation) and detoxification (conjugation) reactions. Therefore we need to understand how these processes interact *in* vivo to determine tissue concentrations of benzene metabolites.

(Continued on page

nzene Metabolism (from page 5)

Phenol metabolism is of interest in this ıtext since phenol is a major oxidized me- olite of benzene, and there are distinct erences in both carcinogenic and ıotoxic responses between benzene and ınol in mice. For example, while benzene :arcinogenic in a number of tissues in :e following both oral and inhalation ex- ;ure (Huff et *al.*, 1989), no increased in- ence of tumors was found in a two-year tional Cancer Institute (NCI) bioassay in ch male and female B6C3F1 mice were ıosed to phenol in drinking water at lev- of up to 5000 ppm (NCI, 1980). Ben- ıe has been consistently found to induce ronuclei in mouse bone marrow cells lf, 1987). In contrast, both weakly posi- and negative results have been reported micronuclei induction in mouse bone rrow cells by phenol following intraperi- eal and oral administration (ATSDR, 39).

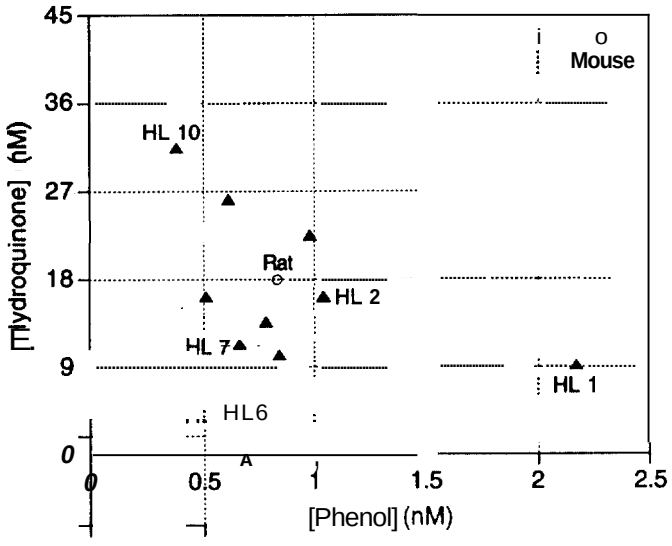
These dramatic differences in carcino- ıicity and genotoxicity between benzene ı its major oxidized metabolite, phenol jure 1), have puzzled benzene research- and led to different hypotheses about metabolites responsible for benzene tox- y. Insight into a possible metabolic basis the high potency of benzene as both an mal carcinogen and genotoxicant and the k of genotoxicity and carcinogenicity of mol can be gained by comparing the uri- y metabolite profiles after comparable l doses of benzene and phenol. This nparison is presented in Table 3, using data from CIIT for urinary excretion of phenol metabolites following oral gavage of phenol in male B6C3F1 mice (Kenyon *et al.*, 1995) and similar data reported by Sabourin *et al.* (1989) following oral gavage with benzene. Differences in the excretion of hydroquinone glucuronide are quite marked, particularly at the lower dose (~1 mg/kg). Hydroquinone glucuronide excretion in urine was sixfold and twofold greater after benzene compared with phenol at doses of approximately 1 and 10 mg/kg, respectively. The *in vitro* studies described above indicate that hydroquinone is produced by sequential oxidation of benzene via phenol. How then could more hydroquinone be produced after administration of benzene than after phenol?

The differences in hydroquinone formation after benzene and phenol administration are of interest for three reasons. First, hydroquinone itself is a inducer of micronuclei and chromosomal aberrations in various test systems (IPCS, 1992). Thus greater production of hydroquinone after benzene administration compared with phenol administration, together with production of other putative toxic metabolites such as muconaldehyde that are unique to benzene, provide a possible metabolic basis for the carcinogenic and genotoxic effects observed with benzene but not with phenol. Secondly, these data are in contrast to *in vitro* data (liver microsomes and isolated hepatocytes) that suggest greater production of hydroquinone after phenol compared with benzene (Schlosser et *al.*, 1993; Schrenk and Bock, 1990). The implication here is that a mechanistic basis for this difference should be incorporated into our quantitative *in vivo* description of the relationship between benzene exposure and target tissue dosimetry. Finally, this difference points out the need for caution when interpreting *in vitro* metabolism studies.

We hypothesize that differences in the urinary metabolite profile of phenol compared with benzene following oral administration result from quantitative zonal differences in the distribution of metabolizing enzymes within the liver acinus, as illustrated in Figure 5. Phenol absorbed from the gut lumen has relatively greater opportunity for conjugation than for oxidation, initially in gastrointestinal mucosal cells (sulfation and glucuronidation) and subsequently in the periportal hepatocytes of zone 1 (sulfation) when absorbed into the portal circulation. The greater opportunity for phenol conjugation over oxidation is due to the relatively greater abundance of conjugating enzymes compared with oxidative enzymes in the areas where phenol would be distributed when initially absorbed (Gebhardt, 1992; Morris and Pang, 1987). The net effect of this hypothetical scenario is that less phenol reaches hepatic zone 3, where oxidative enzyme activity is greatest, resulting in less hydroquinone production.

In contrast to phenol, benzene is only a substrate for CYP 2E1, and CYP 2E1 activity is much greater in the pericentral hepatocytes of zone 3 (Tsutsumi et *al.*, 1989). Thus metabolism of benzene would be minimal as benzene passes through periportal and midzonal hepatocytes prior to reaching hepatocytes in zone 3, where CYP 2E1 oxidation capacities are relatively greater (Gebhardt, 1992; Moms and Pang, 1987). Consequently, administration of benzene may result in the delivery of more free phenol to zone 3 oxidative enzymes than administration of phenol itself. The metabolic implications of these hypothetical scenarios are reflected in the observed urinary metabolite profiles presented in Table 3. Phenyl sulfate excretion is approximately twofold higher and phenyl glucuronide excretion approximately four- to ninefold higher after phenol compared with benzene exposure, and oxidation products such as hydroquinone are higher after benzene exposure.

Returning to the question of benzene toxicity, Smith et *al.* (1989) hypothesized that benzoquinone, formed from the myeloperoxidase-mediated oxidation of hydroquinone in the bone marrow, is responsible for benzene-induced hematotoxicity. The net effects of heterogeneous distribution of enzymes in the liver and the resulting preferential oxidation of benzene and conjugation of phenol are a higher blood level of hydroquinone after benzene administration compared with phenol and more hy-



g. 4
eady-state blood concentrations of phenol and hydroquinone for 10 individual mans, B6C3F1 mice, and F344 rats when the blood concentration of benzene is nstant at 0.01 µM. This blood concentration is approximately what would be ex- cted for a continuous exposure to 1 ppm benzene. The steady-state phenol and droquinone concentrations are calculated from *in vitro* oxidation and conjugation tivities, such as those listed in Table 1, using a physiological compartment model, , reported by Seaton et *al.* (1995). (Adapted by permission of Oxford University ess.)

TABLE 3
COMPARISON OF URINARY METABOLITES EXCRETED AS PHENOL SULFATE, PHENOL GLUCURONIDE, AND HYDROQUINONE GLUCURONIDE FOLLOWING GAVAGE ADMINISTRATION OF PHENOL OR BENZENE IN MALE B6C3F$_1$ MICE

Chemical administered	Dose (mg/kg)	Total metabolites in urine (%)[a]		
		Phenol sulfate	Phenol glucuronide	Hydroquinone glucuronide
Benzene[b]	1	26 ± 0.3	4.2 ± 0.3	43 ± 0.8
Phenol[c]	1.4	56 ± 0.9	39 ± 1.1	7.2 ± 0.4
Benzene[b]	10	32 ± 0.8	5.9 ± 0.3	36 ± 0.4
Phenol[c]	9.4	57 ± 7.7	26 ± 6.4	15 ± 1.6

[a]Values are means ± SE.
[b]Urinary metabolite data following benzene exposure are from Sabourin et *al.* (1989).
[c]Data are from Kenyon *et al.* (1995).

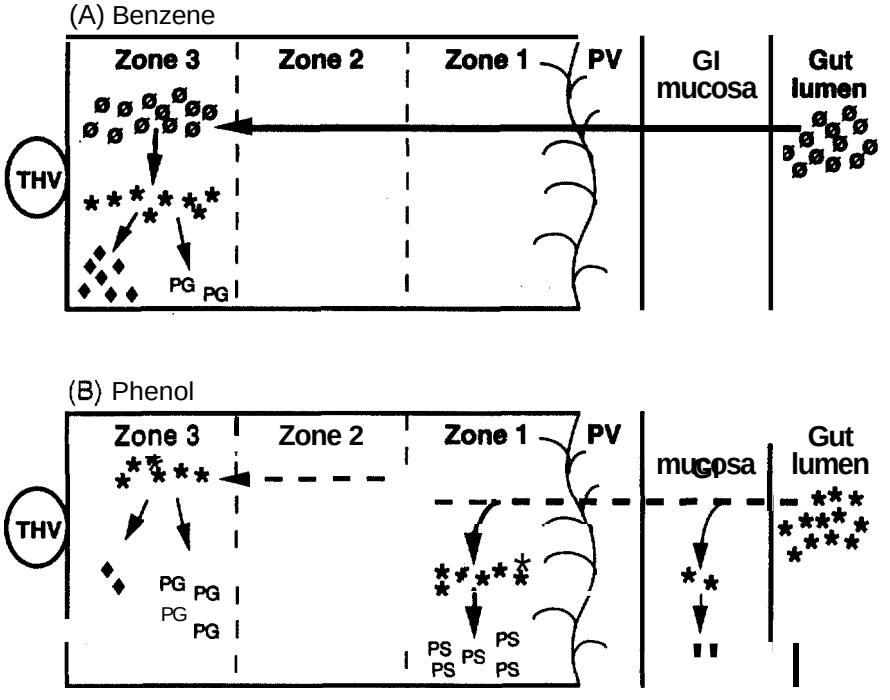


Fig. 5
Hypothesized major relative hepatic zonal differences in metabolism of benzene (A) compared with phenol (B) following gavage administration during an initial pass through the liver. Symbols and abbreviations denote benzene (ø), phenol (*), hydroquinone (•), portal vein (PV), and the terminal hepatic venuk (THV). Benzene and phenol absorbed from the gastrointestinal (GI) tract are sequentially available for metabolism in the GI mucosa and periportal (zone 1) and pericentral (zone 3) regions of the liver. The overall capacity of GI mucosal metabolism is low relative to the liver. Periportal locaiizatlon of the enzymes (sulfotransferase) responsible for phenol sulfation suggests that less free phenol would reach the pericentral *hepatocytes* where higher levels of CYP 2E1 activity are localized. The necessity for oxidation of benzene prior to conjugation suggests that more benzene will reach the pericentral hepatocytes for oxidation to phenol and hydroquinone. Phenol sulfate, PS; phenol glucuronide, PG.

droquinone in bone marrow available foro dation to benzoquinone. Localization of e zymes in the liver provides explanations differences both in observed toxicity and in *vitro* metabolism of benzene and phen Thus both the location and the quantities benzene-metabolizing enzymes must be corporated into in *vivo* models to pred risks for humans exposed to benzene.

The concept that the metabolic fate o metabolite derived from a precursor diffe from that of a preformed metabolite is r new. It was, in fact, proposed by Pang a Gillette in 1978 for drugs. The kinetics o drug and its metabolite can differ due to d ferences in diffusional barriers between dr and hepatocyte, the rate of communicati of the drugs between hepatocytes, and t distribution of drug-metabolizing enzyr systems within the liver parenchyma (Pa and Terrell, 1881). Using the drug-metat lite pair phenacetin and acetaminophe Pang and colleagues noted differences clearance of the chemicals from an isolat perfused liver depending on whether per sion was conducted in a normal (portal centrilobular) or retrograde (reversed) dir tion. They attributed these differences preferential localization of P450 demethylation enzymes and sulfation e zymes in different zones of the liver and su gested that existing models of well-stirr livers were oversimplifications and cou only be used in limiting cases when the d tribution of enzymes was viewed as ope tionally uniform.

Using a combination of experimen and mathematical modeling approache Pang and Terrell (1981) determined tt substrate concentration at any point is fluenced by preceding events during t metabolic processing of a substrate alo the length of the liver sinusoid. Enzyme sy tems such as sulfation, when present u stream along the sinusoidal flow pa modify residual substrate available for me bolic activities such as oxidation witt downstream hepatocytes. In the case benzene and phenol, we propose that t residual phenol available for oxidation in t centrilobutar region is very low when pt nol is administered directly because mo of it is conjugated upstream of this regic In contrast, when benzene is administere the phenol is produced in the centrilobul region and thus is more available for su sequent oxidation to hydroquinone.

Conclusions

We cannot yet claim to have complete elucidated the factors that determine be zene metabolism and dosimetry, let alo toxicity. Nevertheless, the research d scribed here does represent considerat

(Continued on page

Benzene Metabolism (from page 7)

progress in understanding and quantitating the factors that determine the distribution of benzene and benzene metabolites in the body. In particular, we have seen that the quantity of a benzene metabolite produced is the result of subtle interplay among various enzymes competing for substrates at a given location, the distribution of those enzymes in the liver, and relative rates of perfusion in different species. Given the complexity of this system, it is not surprising that many, sometimes contradictory, mechanisms for benzene metabolism have been proposed. At this point in time, many experimental observations must be explained by any proposed model. One example is the fact that *in vivo* administration of benzene yields higher levels of hydroquinone than *in vivo* administration of phenol but that in vitro metabolism of phenol yields greater quantities of hydroquinone than *in vitro* metabolism of benzene. The mechanisms proposed here are capable of resolving these disparate observations and can be tested experimentally. In the case of hepatic enzyme localization, experiments with isolated hepatocytes (in a well-mixed suspension) can be compared with the results of normal grade and retrograde perfused liver experiments to determine if this is indeed a significant factor.

A second important point is that the individual pieces of research described here, taken together with further planned *in vitro* experiments, will allow us to quantify the relationship between rates of biotransformation determined *in vitro* and benzene metabolite dosimetry in vivo based on quantitative, mechanistic data. Once this has been done for laboratory animals (mice and rats), we will have a sound basis for predicting metabolism and dosimetry in humans. These predictions should then lead to significantly improved risk assessments for human exposures to benzene, since they will allow us to track the amount of critical benzene metabolites that reach the target tissue, bone marrow. Risk assessments can then be based on target tissue dosimetry rather than on ambient air concentration. Given the differences among species and among individual humans, such assessments should provide much better characterizations of risk.

References

Agency for Toxic Substances and Disease Registry (ATSDR) (1989). *Toxicological Profile* for *Benzene*. ATSDR/TP-88/03.

Ayres, P. H. and Taylor, W. D. (1989). Solvents. In Principles and Methods of *Toxicology* [2nd ed.]. (A. H. Hayes, ed.) Raven Press Ltd., New York, pp. 111-135.

Barale, R., Marrazzini, A., Betti, C., Vangelisti, V., Loprieno, N., and Barrai, I. (1990). Genotoxicity of two metabolites of benzene: Phenol and hydroquinone show strong synergistic effects in *vivo*. *Mutat. Res.* 244, 15-20.

Chapman, D. E., Namkung, M. J., and Juchau, M. R. (1994). Benzene and benzene metabolites as embryotoxic agents: Effects on cultured rat embryos. Toxicol. Appl. *Pharmacol.* 128,129-137.

Cox, L. A. (1991). Biological basis of chemical carcinogenesis: Insights from benzene. *Risk Anal.* 11, 453-464.

Csanády, G. A., Guengerich, F. P., and Bond, J. A. (1992). Comparison of the biotransformation of 1,3-butadiene and its metabolite, butadiene monoepoxide, by hepatic and pulmonary tissues from human, rats and mice. *Carcinogenesis* 13, 1143-1153.

Eastmond, D. A., Smith, M. T, and Irons, R. D. (1987). An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene. *Toxicol.* Appl. *Pharmacol.* 91, 85-95.

Gebhardt, R. (1992). Metabolic zonation of the liver: Regulation and implications for liver function. *Pharmacol. Ther.* 53, 275-354.

Guy, R. L., Hu, P., Witz, G., Goldstein, B. D., and Snyder, R. (1991). Depression of iron uptake into erythrocytes in mice by treatment with the combined benzene metabolites p-benzoquinone, muconaldehyde and hydroquinone. J. *Appl. Toxicol.* 11, 443-446.

Health Effects Institute (1993). Research prioritiesto reduce uncertainties for benzene. Research *Priorities* for Mobile Air Toxics. (Health Effects Institute, ed.) Health Effects Institute, Cambridge, MA, pp. 24-43.

Hricko, A. (1994). Rings of controversy around benzene. *Environ. Health Perspect.* 102,276-281.

Huff, J. E., Haseman, J. K., DeMarini, D. M., Eustis, S., Maronpot, R. R., Peters, A. C., Persing, R. L., Chrisp, C. E., and Jacobs, A. C. (1989). Multiple-site carcinogenicity of benzene in Fischer 344 rats and B6C3F$_1$ mice. *Environ.* Health *Perspect.* 82,125-163.

Infante, P. E., Rinsky, R. A., Wagoner, J. K., and Young, R. J. (1977). Leukemia in benzene workers. *Lancet* 2, 76-78.

International Programme on Chemical Safety (IPCS) (1992). Environmental Health *Criteria* for Hydroquinone. World Health Organization, Geneva, PCS/EHC/92.8.

Irons, R. D., Stillman, W. S., Colagiovanni, D. B., and Henry, V. A. (1992). Synergistic action of benzene metabolite hydroquinone on myelopoietic stimulating activity of granulocyte/macrophage colony-stimulating in *vitro*. *Proc. Natl.* Acad. *Sci. U* SA 89,3691-3695.

Kalf, G. F. (1987). Recent advances in the metabolism and toxicity of benzene. Crit. Rev. *Toxicol.* 18, 141-159.

Kari, F. W., Bucher, J., Eustis, S. L., Haseman, J. K., and Huff, J. E. (1992). Toxicity and carcinogenicity of hydroquinone in F344/N rats and B6C3F$_1$ mice. Food Chem. *Toxicol.* 30,737-747.

Kedderis, G. L., Batra, R., and Koop, D. R. (1993). Epoxidation of acrylonitrile by rat and human cytochrome P450. Chem, Res, *Toxicol.* 6, 866-871.

Kenyon, E. M., Seeley, M. E., Janszen, D., and Medinsky, M. A. (1995). Dose, route and sex-dependent urinary excretion of phenol metabolites in B6C3F$_1$ mice. J. *Toxicol. Environ.* Health 44,219-233.

Kolachana, P., Subrahmanyam, V. V., Meyer, K., Zhang, L., and Smith, M. (1993). Benzene and its phenolic metabolites produce oxidative DNA damage in HL-60 cells in *vitro* and in the bone marrow in vivo. Cancer Res. 53, 1023-1026.

Koop, D. R., Laethem, C. L., and Schnier, G. G. (1989). Identification of ethanol-inducible P450 isozyme 3a (P450IIE1) as a benzene and phenol hydroxylase. Toxicol. Appl. *Pharmacol.* 98, 278-288.

MacEachern, L., Snyder, R., and Laskin, D. L. (1992). Alterations in the morphology and functional activity of bone marrow phagocytes following benzene treatment of mice. *Toxicol. Appl. Pharmacol.* 117, 147-154.

Medinsky, M. A., Sabourin, P. J., Luder, G., Bimbaum, L. S., and Henderson, R. F. (1989). A physiological model for simulation of benzene metabolism by rats and mice. *Toxicol. Appl. Pharmacol.* 99, 193-206.

Morris, M. E. and Pang, K. S. (1987). Competition between two enzymes for substrate removal in liver: Modulating effects due to substrate recruitment of hepatocyte activity. J. *Pharmacokinet. Biopharm.* 15, 473-496.

Nakajima, T., Wang, R.-S., Elovaara, E., Park, S. S., Gelboin, H. V., and Vainio, H. (1993). Cytochrome P450-related differences between rats and mice in the metabolism of benzene, toluene and trichloroethylene in liver microsomes. *Biochem. Pharmacol.* 45, 1079-1085.

National Cancer Institute (NCI) (1980). Bioassay of Phenol for Possible *Carcinogenicity*. Department of Health and Human Services, Technical Report Series No. NCI-CG-TR-203.

Pang, K. S. and Gillette, J. R. (1978). Kinetics of metabolism and elimination in the perfused rat liver preparation: Differences between the elimination of preformed acetominophen and acetominophen formed from phenacetin. *J.* Phannawl. Exp. *Ther.* 207, 178-194.

Pang, K. S. and Terrell, J. A. (1981). Retrograde perfusion to probe the heterogeneous distribution of hepatic drug metabolizing enzymes in rats. J. *Pharmacol. Exp. Ther.* 216, 339-346.

Runion, H. E. and Scott, L. M. (1985). Benzene exposure in the United States 1978-1983: An overview. Am. J. Ind. Med. 7, 385-393.

Sabourin, P. J., Bechtold, W. E., Griffith, W. C., Birnbaum, L. S., Lucier, G., and Henderson, R. F. (1989). Effect of exposure concentration, exposure rate and route of administration on metabolism of benzene by

F344 rats and B6C3F$_1$ mice. ***Toxicol. Appl. Pharmacol.*** 99, 421-444.

Sabourin, P. J., Chen, B. T., Lucier, G., Birnbaum, L. S., Fisher, E., and Henderson, R. F. (1987). Effect of dose on the absorption and excretion of [^{14}C]benzene administered orally or by inhalation in rats and mice. ***Toxicol. Appl. Pharmacol.*** 87, 1-12.

Sammett, D., Lee, E. W., Kocsis, J. J., and Snyder, R. (1979). Partial hepatectomy reduces both metabolism and toxicity of benzene. J. ***Toxicol. Environ. Health*** 5,785-792.

Schlosser, P. M., Bond, J. A., and Medinsky, M. A. (1993). Benzene and phenol metabolism by mouse and rat liver microsomes. ***Carcinogenesis*** 14, 2477-2486.

Schrenk, D. and Bock, K. W. (1990). Metabolism of benzene in rat hepatocytes: Influence of inducers of phenol glucuronidation. ***Drug Metab. Dispos.*** 18,720-725.

Seaton, M. J., Schlosser, P. M., Bond, J. A., and Medinsky, M. A. (1994). Benzene metabolism by human liver microsomes in relation to cytochrome P450 2E1 activity. ***Carcinogenesis*** 15, 1799-1806.

Seaton, M. J., Schlosser, P. M., Bond, J. A., and Medinsky, M. A. (1995). ***In vitro*** conjugation of benzene metabolites by human liver: Potential influence of interindividual variability on benzene toxicity. ***Carcinogenesis*** 16(7), 1519-1527.

Smith, M. T., Yager, J. W., Steinmetz, K. L., and Eastmond, D.A. (1989). Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. ***Environ. Health Perspect.*** 82, 23-29.

Snyder, C. A., Goldstein, B. D., Sellakumar, A., Wolman, S. R., Bromberg, I., and Laskin, S. (1978). Hematotoxicity of inhaled benzene to Sprague-Dawiey rats and AKR mice at 300 ppm. ***J. Toxicol. Environ. Health*** 4, 605-618.

Snyder, R. and Kalf, G. F. (1994). A perspective on benzene leukemogenesis. ***Crit. Rev. Toxicol.*** 24, 177-209.

Tompa, A., Major, J., and Jakab, M. G. (1994). Monitoring of benzene-exposed workers for genotoxic effects of benzene: Improved-working-condition-related decrease in the frequencies of chromosomal aberrations in peripheral blood lymphocytes. ***Mutat. Res.*** 304, 159-165.

Tsutsumi, M., Lasker, J. M., Shimizu, M., Rosman, A. S., and Lieber, C. S. (1989). The intralobular distribution of ethanol-inducible P450IIE1 in rat and human liver. ***Hepatology*** 10, 437-446.

Wallace L. (1990). Major sources of exposure to benzene and other volatile organic chemicals. Risk ***Anal.*** 10, 59-64.

Left to right: Drs. Medinsky, Kenyon, Seaton, and Schlosser

The Authors

Paul Schiosser received his Ph.D. in chemical engineering from the University Rochester in 1988 and subsequently performed postdoctoral research at the Calif nia Institute of Technology. Dr. Schlosser Joined CIIT in 1991, with his initial foc primarily on the benzene project. He has an adjunct faculty position at North Ca lina State Universlty and is on the editorial review board of ***Nonlinear Times a Digest.*** He has a general interest in the modeling of complex biological-chemic physical systems. Other projects at CIIT in which he is involved are dosimetry inhaled gases, endocrine toxicology, and genetic susceptibility.

Elaina M. Kenyon, who received her Ph.D. in toxicology from the Universtty Massachusetts at Amherst, was a postdoctoral fellow at CIIT from 1992 to early 19 While at CIIT, she conducted ***in vivo*** dosimetry research on benzene and extende physiologically based pharmacokinetic (PBPK) model for benzene to predict the d simetry of benzene, phenol, and hydroquinone in bone marrow. This work was su ported in part by a National Research Service Award (NRSA) from the National Ins tute of Environmental Health Sciences. Dr. Kenyon is currently with the Health I fects Research Laboratory, U.S. Environmental Protection Agency, Research Trian Park, NC, where she is conducting research in the area of pharmacokinetics to d velop PBPK models for both volatile organics and metalloids such as arsenic.

Mark J. Seaton received his Ph.D. in pharmacology from Pennsylvania State U versity in 1993 and then came to CIIT as a postdoctoral fellow to study ***in vitro*** m tabolism of butadtene by human liver. Dr. Seaton has been more recently involv with ***In vitro*** studies of butadiene metabolism by hepatic and pulmonary cells. Tt work Is supported in part by an NRSA. He is currently performing research on t metabolic and toxic effects of coexposures to benzene and unleaded gasoline.

Michele A. Medinsky is a senior scientist at CIIT who has had a long-standi interest in assessing the health risks of airborne materials. She conducted her d sertation research at Lovelace inhalation Toxicology Research institute (ITRI) in A buquerque, NM, and received her Ph.D. In biology from the University of New Mexic Following a postdoctoral appointment at CIIT, Dr. Medinsky accepted a staff positi at ITRI and then returned to CIIT in 1989 as a staff scientist, where she serves coordinator for the benzene project and participates in several other projects cc cerned with evaluating factors influencing the disposition of inhaled chemicals. I Medinsky is a Diplomate of the American Board of Toxicology. She currently serv as a member of the NAS-NRC Committee of the Society of Toxicology.