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# BENZENE METABOLISM BY THE ISOLATED PERFUSED LUNG

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*Benzene is an occupational hazard and environmental toxicant whose toxic effects are dependent on its metabolism by cytochrome P-450. Most physiologically based pharmacokinetic models assume that benzene is metabolized only in the liver. They may not be completely accurate in predicting metabolism, especially following inhalation exposure, if metabolism by the lung is important. In the current study, the metabolizing capability of the lung was examined in an in vivo simulation using the isolated perfused lung. Lungs from the rabbit, rat, and mouse were used to mimic benzene metabolism following exposure via the pulmonary vasculature. With the isolated perfused mouse lung, three concentrations (55 μM, 120 μM, and 200 μM) were used to evaluate concentration dependence. To evaluate the ability of the lung to metabolize inhaled benzene, the isolated perfused mouse lung was exposed to benzene (~175 ppm) via the trachea. Benzene was metabolized in all species, with phenol being the major metabolite. Phenylsulfate was also detected in perfusate from rabbits and mice but at much lower levels. Benzene metabolism was concentration dependent in mice. The ability of the lung to metabolize benzene during inhalation exposure was demonstrated in the isolated perfused mouse lung. These results demonstrate that the lung can metabolize benzene in an in vivo simulation when exposed via the pulmonary vasculature or via inhalation.*

Benzene is a toxic occupational hazard and environmental pollutant. It is used in manufacturing products such as rubber, lubricants, detergents, drugs, and pesticides. It is also present in gasoline. The main concern for humans chronically exposed to low levels of benzene is acute myelogenous leukemia (Aksoy, 1980; Infante et al., 1977; Yin et al., 1996). Studies have shown an increase in various types of tumors, including lung tumors, in mice exposed to benzene (Farris et al., 1993; Maltoni et al., 1989; National Toxicology Program, 1986; Snyder et al., 1988). Increases in tumors at various sites have also been demonstrated in rats (Maltoni et al., 1989; National Toxicology Program, 1986). Although benzene is not recognized by any regulatory agency as producing lung tumors, there is limited epidemiological evidence that suggests lung cancer may result from occupational benzene exposure (Aksoy, 1980; Yin et al., 1996).

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The toxicity of benzene is dependent on its metabolism via the cytochrome P-450 enzyme system. Benzene is first metabolized to benzene oxide (Jerina et al., 1968). Phenol is formed from the nonenzymatic rearrangement of benzene oxide (Jerina et al., 1968) and is metabolized to hydroquinone and catechol (Sawahata & Neal, 1983). Hydroquinone and catechol are further metabolized by myeloperoxidase in bone marrow to form *p*-benzoquinone and *o*-benzoquinone (Eastmond et al., 1987; Sadler et al., 1988).

Conjugation reactions are also important in benzene metabolism. These include glutathione conjugation of benzene oxide, and sulfate and glucuronide conjugation of phenol, hydroquinone, catechol, and trihydroxybenzene (Henderson et al., 1989). The most abundant conjugated metabolite in primates exposed to benzene is phenylsulfate, while hydroquinone glucuronide, muconic acid, phenylglucuronide, hydroquinone sulfate, and catechol sulfate are detected in smaller amounts (Sabourin et al., 1992).

Most physiologically based pharmacokinetic (PBPK) models assume that benzene metabolism mainly occurs in the liver (Bois et al., 1991; Cole et al., 2001; Medinsky et al., 1989a, 1989b; Sinclair et al., 1999; Travis et al., 1990). These models are fairly accurate when predicting metabolism following oral exposure, but their accuracy has been questioned following occupational (Sherwood & Sinclair, 1999) and inhalation exposure (Cole et al., 2001). These authors suggest that metabolism by other organs may be important (Cole et al., 2001; Sherwood & Sinclair, 1999). One possibility may be the lung.

There are several reasons why the lung may be important in benzene metabolism. Previous studies conducted in this lab have demonstrated that the lung is capable of metabolizing benzene in vitro (Chaney & Carlson, 1995; Powley & Carlson, 1999, 2000). The lung is a target organ of benzene toxicity in animals (Farris et al., 1993; Maltoni et al., 1989; National Toxicology Program, 1986; Snyder et al., 1988). If the toxic metabolites of benzene are formed during inhalation, they could have a direct effect on this tissue. Evidence to support this hypothesis is provided by experiments that showed an increased micronuclei frequency in the lungs of mice exposed to benzene via inhalation (Ranaldi et al., 1998). Furthermore, an increase in deletion mutations was found in the lungs of mice following inhalation exposure (Mullin et al., 1998).

The lung contains the cytochrome P-450 isozymes thought to be involved in benzene metabolism, namely, CYP2E1, CYP2B, and CYP2F (Buckpitt et al., 1995; Forkert, 1995; Lag et al., 1996; Martin et al., 1993; Raunio et al., 1998; Wheeler et al., 1992).

Finally, the anatomical location and physiological function of the lungs provide the opportunity for significant exposure since it is the first site of contact following inhalation and receives 100% of the cardiac output. Therefore, the lung can contribute to systemic levels of metabolites, and it has the opportunity to metabolize benzene as a first-pass organ—that is, there may be local biotransformation and toxicity.

The hypothesis tested in the current research was that the lung has the capability of metabolizing benzene in an in vivo simulation following ex-

posure via the pulmonary vasculature and via inhalation. To test this hypothesis, the isolated perfused lung preparation was used. This is an advantage compared to whole-animal studies that can only look at total metabolism and not metabolism by any one organ. This preparation allows the flexibility to expose the lungs via the pulmonary vasculature and via inhalation. In the latter case, metabolism of benzene while it is being inhaled (first-pass effect) can be determined.

Isolated perfused organ preparations are useful tools in analyzing xenobiotic metabolism. Isolated perfused rabbit lung experiments have been performed in this laboratory to examine the metabolism of several compounds (Page & Carlson, 1993; Trela et al., 1988, 1989; Yang & Carlson, 1991). Both isolated perfused mouse (Kanekal et al., 1990, 1991; Skelly & Shertzer, 1983, 1985) and rat lung (Bond & Mauderly, 1984; Moller et al., 1987; Molliere et al., 1987; Tornquist et al., 1985) have demonstrated the ability to metabolize xenobiotics.

Benzene metabolism in multiple species was examined with the isolated perfused lung. Adult male NSA mice, NZW rabbits, and Sprague-Dawley rats were used. Isolated perfused rabbit and rat lungs were exposed via the pulmonary vasculature to 50 μ*M* and 205 μ*M* benzene, respectively. Isolated perfused mouse lungs were exposed via the pulmonary vasculature to three concentrations of benzene (55 μ*M*, 120 μ*M*, and 200 μ*M*) to determine the concentration dependence of benzene metabolism. Isolated perfused mouse lungs were also exposed by inhalation to ~175 ppm benzene. This concentration is higher than typical occupational and environmental exposures (ASTDR, 1997) but is lower than concentrations used in carcinogenicity studies of inhaled benzene in mice (Farris et al., 1993; Snyder et al., 1988).

## MATERIALS AND METHODS

### Animals

Adult male Non-Swiss Albino (NSA) mice (20–25 g, Harlan Sprague-Dawley, Indianapolis, IN), New Zealand White (NZW) rabbits (2–3 kg, Covance, Kalamazoo, MI), and Sprague-Dawley rats (150–174 g, Harlan Sprague-Dawley, Indianapolis, IN) were used. Mice were housed in groups in solid plastic-bottom cages. Rabbits were housed individually in plastic cages. Rats were housed in groups in stainless steel cages with wire mesh floors. Animals were kept in environmentally controlled rooms with a 12-h light:dark cycle. Mice and rats were given rodent laboratory chow (number 5001, Purina Mills, Inc., St. Louis, MO) and water ad libitum. Rabbits were provided with rabbit laboratory chow (HF number 5326, Purina Mills, Inc., St. Louis, MO) and water ad libitum.

### Chemicals

Chemicals used included bovine serum albumin, D-glucose, papaverine, and sulfatase from Sigma Chemical Co. (St. Louis, MO); calcium chloride from MCB Reagents (Cincinnati, OH); glacial acetic acid, magnesium sulfate, potassium hydroxide, and sodium chloride from Mallinckrodt

Specialty Chemicals (Paris, KY); sodium bicarbonate from J. T. Baker (Phillipsburg, NJ); sodium pentobarbital (Nembutal) (50 mg/ml) from Abbott Laboratories (North Chicago, IL); and heparin from Elkins-Sinn Inc. (Cherry Hill, NJ).

The unlabeled metabolite standards solution contained butylated hydroxytoluene, catechol, and hydroquinone, from Sigma Chemical Co. (St. Louis, MO); ethyl acetate and 1,2,4-trihydroxybenzene from Aldrich Chemical Co. (Milwaukee, WI); and phenol from Mallinckrodt Specialty Chemicals (Paris, KY).

[$^{14}$C]Benzene (specific activity = 52–60 mCi/mmol) was obtained from American Radiolabeled Chemical (St. Louis, MO) or Moravek Biochemicals (Brea, CA). The benzene was not diluted with nonlabeled benzene in any of the experiments, so the specific activity remained the same in all studies.

### Isolated Perfused Rabbit Lung

The surgery and perfusion technique for the isolated perfused rabbit lung preparation have been described previously by Trela et al. (1988). The only modification is the addition of papaverine (0.1 m*M* final concentration) to the perfusate. One-milliliter samples of perfusate were taken at 0, 1, 2, 3, 4, 5, 7, 9, 15, 20, 25, and 30 min. Following removal of perfusate, ascorbic acid (3.5 m*M* final concentration) was added to the sample. After 30 min the experiment was stopped.

### Isolated Perfused Rodent Lung

**Surgery** The surgery for the isolated perfused rodent lung preparation is a modification based on the procedure described by Skelly and Shertzer (1983). Briefly, mice and rats were anesthetized with an ip injection of sodium pentobarbital (250 mg/kg body weight for mice; 65 mg/kg body weight for rats) and heparin (4000 U/kg body weight). An incision was made below the rib cage. The aorta and posterior vena cava were severed. Small holes were cut in the diaphragm to collapse the lungs. A cut was made up the right side of the animal's rib cage to the chin, and the trachea was exposed. The trachea was cannulated using a feeding tube (number 24 for mice; number 18 for rats). After severing the trachea, the cannula was pulled while supporting tissue was cut away until the trachea, heart, and lungs were removed intact.

A small clamp was placed on the apex of the heart. The left atrium was excised. A small incision was made in the right ventricle. A cannula was inserted into the incision and moved into the pulmonary artery and secured with a loop of string around the heart. For the mouse, a number 24 feeding needle with the ball removed was used as a pulmonary cannula. A small loop of thread was tightly secured ~½ cm from the end of the cannula. In the rat, the pulmonary artery was cannulated using a number 18 feeding needle. This cannula was secured by tightening a loop of thread around the pulmonary artery and aorta. The total time required to perform the surgery was ~10–15 min.

**Perfusion Procedure for Experiments With Exposure Via the Pulmonary Vasculature** Perfusate was injected into the pulmonary artery cannula to clear blood from the lungs. The perfusate, a modified Krebbs–Henseleit buffer (pH 7.4), contained bovine serum albumin (4.5%), glucose (5 m*M*), sodium chloride (0.118 *M*), potassium chloride (4.8 m*M*), calcium chloride (3.3 m*M*), potassium phosphate (monobasic, 1.5 m*M*), sodium bicarbonate (25 m*M*), and papaverine (0.1 m*M*). If the lungs did not appear to be ~90% cleared of blood they were discarded. The square ends of the cannulas were placed on the ends of 1-ml syringes inserted through a rubber stopper (number 5 rubber stopped for mice; number 10 rubber stopper for rats). The stopper was then placed in an artificial thorax (Figure 1). Compressed breathing air was supplied to the lungs via the syringe leading to the tracheal cannula. Before entering the lungs the air was humidified by a bubbler containing water. The breathing air, respirator, and vacuum pump were turned on. System parameters for perfusion rate, ventilation rate, respirator rate and voume, and pressure within the artificial thorax are presented in Table 1. Perfusion of the pulmonary vasculature was initiated by turning on pump 2. The lungs were allowed to perfuse for 5 min with blank perfusate to remove any residual blood and to allow equipment adjustments to be made. Lungs were sighed periodically by closing off the check valve connected to the exit end of the tubing from the tracheal cannula to assure that the lungs were fully inflating. After the lungs were cleared of blood, the perfusate was replaced with perfusate containing the desired concentration of [$^{14}$C]benzene. Pump 2 was then activated to start the flow of perfusate containing [$^{14}$C]ben-

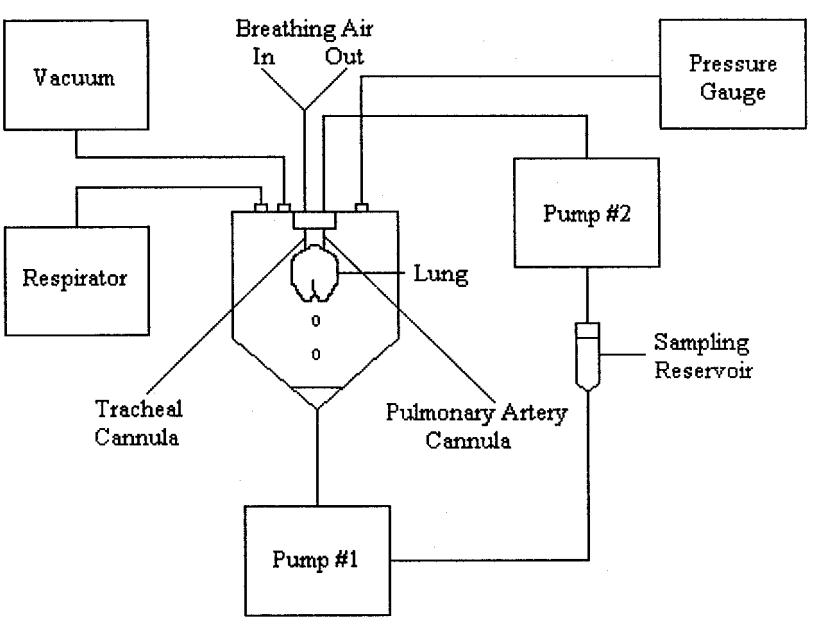


**FIGURE 1.** Isolated perfused lung.

**TABLE 1.** System parameters for isolated perfused lung system

| Parameter | Rabbit | Rat | Mouse |
|---|---|---|---|
| Perfusion rate (ml/min) | 150 | 3.53 | 1.21 |
| Perfusate volume (ml) | 100 | 20 | 5 |
| Airflow (ml/min) | 67 | 180 | 32 |
| Respirator (cycles/min) | 50 | 60 | 90 |
| Pressure range (cm $H_2O$) | –5 to –15[a] | –5 to –15[a] | –5 to –15[a] |

[a]Pressure range was adjusted for each experiment to insure proper inflation/deflation of the lungs. However, final pressures were within this range.

zene. Pump 1 was used to recirculate perfusate from the bottom of the artificial thorax to the sampling reservoir. One-milliliter samples of perfusate were taken from the sampling reservoir at 0, 5, 10, 15, and 20 min. As with the isolated perfused rabbit lung experiments, ascorbic acid (3.5 m*M* final concentration) was added to the perfusate samples. The experiment was terminated after 20 min.

**Perfusion Procedure for Inhalation Exposure Experiments** When the lungs were exposed to benzene via the trachea, the perfusate was not recirculated. Following the 5-min equilibration period, generation of benzene vapor (discussed later) began. Perfusate samples were taken over 1-min intervals at 0, 1, 3, 6, 10, 15, and 20 min, and ascorbate (3.5 m*M*) was added. The experiments were terminated at 20 min.

**Generation of Benzene Vapor for Inhalation Exposure Experiments** The generation system consisted of a microinfusion pump (Harvard Apparatus, South Natick, MA) and a 50-ml round-bottom flask containing glass beads held in place over a hotplate at 50°C. [$^{14}$C]Benzene was infused into the round bottom-flask. Breathing air (32 ml/min) mixed with the volatilized benzene in the round-bottom flask before flowing to the tracheal cannula. Because the benzene was in solution with methanol, this water-soluble carrier was removed from the airstream while allowing the benzene to pass. This system was made of two 10-ml syringes in series filled with gauze joined together by a 5-ml syringe with the ends removed and made airtight with Teflon tape.

Before each experiment the gauze in the methanol removal system was wetted, and airflow was initiated for ~10 min. Airflow was measured at all connecting points using a rotameter to insure that the system was airtight. Following the start of the experiment and after the 5-min equilibration period the microinfusion pump was started. One-half-milliliter samples of air were taken from a sample point immediately before the tracheal cannula through a septum using a gas-tight syringe to determine the benzene concentrations in the inspired air. These samples were taken at 1, 3, 6, 10, 15, and 20 min.

**Quantification of Benzene Concentration in the Air** Air samples were analyzed using a Varian 3700 gas chromatograph with a flame-ionization

detector. The 2-m glass column was packed with 20% SP-2100 and 0.1% Carbowax 1500 on 100/120 Supelcoport. The injector port and detector were at 50°C and 200°C, respectively. The column temperature was increased from 35°C to 39°C at a rate of 2°C/min in order to separate benzene and methanol. The carrier gas, nitrogen, flow rate was 30 ml/min. Methanol had a retention time of ~1 min while benzene had a retention time of ~2.2 min.

**Perfusate Metabolite Assay** Prior to analysis, perfusate samples were filtered (Ultrafree-MC, 0.22-μ*M*-pore Durapore membrane; Millipore Corporation, Bedford, MA) by centrifuging for 15 min at 12,000 rpm (Eppendorf centrifuge 5415). A mixture of unlabeled standards (0.1 ml) was then filtered in the same tube at 12,000 rpm for 4 min. The resulting solution was added to the filtered perfusate and stored at -4°C until analysis.

**Quantification of Metabolites** The components of the sample were separated using a Shimadzu high-performance liquid chromatography (HPLC) system with a Beckman octadecylsilane (ODS) column (5 μm; 4.6 mm × 25 cm) and a Beckman Ultrasphere ODS guard column (5 μm; 4.6 mm × 4.5 cm). The mobile phases were deionized water purified by a Milli-Q Reagent Water System (A) and HPLC-grade methanol (B). Both phases were filtered through a 0.2-μ*M* Nylaflo filter and degassed prior to use. Flow composition was as follows; 0–5 min, 0% B; 5–10 min, increase to 20% B; 10–15 min, 20% B; 15–20 min, increase to 50% B; 20–30 min, 50% B; 30–36 min, increase to 100% B; 36–45 min, 100% B; 45–50 min, decrease to 0% B; 50–55 min, 0% B. Throughout the analysis the flow rate was 1 ml/min. The sample loops had volumes of 200 μl or 250 μl. The retention time of phenol was ~25 min, and for phenylsulfate it was ~5.5 min.

HPLC samples were analyzed by an ultraviolet (UV) detector to identify fractions containing metabolites. The fractions were subsequently analyzed by a Packard 505TR flow scintillation analyzer (Packard Instrument Co., Inc., Meriden, CT). Each milliliter of HPLC eluent mixed with 3 ml of Ultima Flo AP scintillation cocktail (Packard Instrument Co., Inc., Meriden, CT). The radioactive metabolites were identified by the time of their elution compared to the UV peaks of the unlabeled standards. Metabolites were quantified as pmol metabolite. Conjugated benzene metabolites were identified by incubating 0.5 ml of perfusate with sulfatase (14.3 U/ml sample) for 4 h at 37°C.

**Data Analysis** Although numerous animals were used to develop the bioassays, three mice produced data of sufficient quality for complete data analysis for both systemic and inhalation exposure. Six rabbits and two rats were used in systemic exposure experiments.

## RESULTS

### Benzene Metabolism in Isolated Perfused Rabbit Lung During Exposure Via the Pulmonary Vasculature

Benzene metabolism was monitored in the isolated perfused rabbit lung during exposure via the pulmonary vasculature. Perfusate was recirculated for

30 min. Benzene disappeared rapidly from the system (Figure 2). This disappearance was determined to be first order based on semilog plots of the data. These graphs are not shown but are available (Powley, 2001). Perfusate samples contained phenol (Figure 3) and phenylsulfate. The phenol data are presented as amount of this metabolite because of changing volumes in the perfusate with sampling. The amounts have been adjusted for this change. Because the phenylsulfate peak could not be clearly separated by HPLC from a contaminant in the initial [$^{14}$C]-benzene, accurate quantification of phenylsulfate was difficult and is, therefore, omitted from the figure. This contaminant was not identified. Completely eliminating the contaminant using HPLC to clean up the benzene proved to be impossible. The amount of phenol found in the perfusate increased rapidly over the first 9 min and remained relatively constant throughout the remainder of the experiment.

### Benzene Metabolism in Isolated Perfused Rat Lung During Exposure Via the Pulmonary Vasculature

Benzene metabolism was examined during exposure via the pulmonary vasculature using the isolated perfused rat lung in recirculating mode. As was seen in experiments using the isolated perfused rabbit lung, benzene disappeared quickly from the system (Figure 4). The benzene disappeared in a first order manner similar to the isolated perfused rabbit lung. Again, this observation is based on semilog plot of the data (data not shown).

Analysis of perfusate samples by HPLC/liquid scintillation counting indicated that benzene was metabolized during exposure via the pulmonary vasculature (Figure 5). Phenol was quickly produced during the first 5 min of perfusion, but formation slowed during the remaining 15 min as the benzene concentration in the perfusate rapidly decreased. If other metabolites were formed during the experiment, they were below the limits of detection.

### Benzene Metabolism in Isolated Perfused Mouse Lung During Exposure Via the Pulmonary Vasculature

This study was designed to examine the ability of the isolated perfused mouse lung to metabolize benzene when exposed via the pulmonary vasculature. The effect of substrate concentration was examined using 200 μ*M*, 120 μ*M*, and 55 μ*M* benzene. This low and narrow range was selected based on our previous experiments using microsomes (Powley & Carlson, 1999, 2000) and to go as close to environmentally relevant concentrations as our analytical techniques would allow. Similar to the results from the experiments using rabbit and rat lung, benzene disappeared rapidly in the isolated perfused mouse lung (Figure 6). At all concentrations examined the disappearance of benzene was first order for the first 10 min. However, after 10 min the rate of disappearance of benzene decreased and no longer

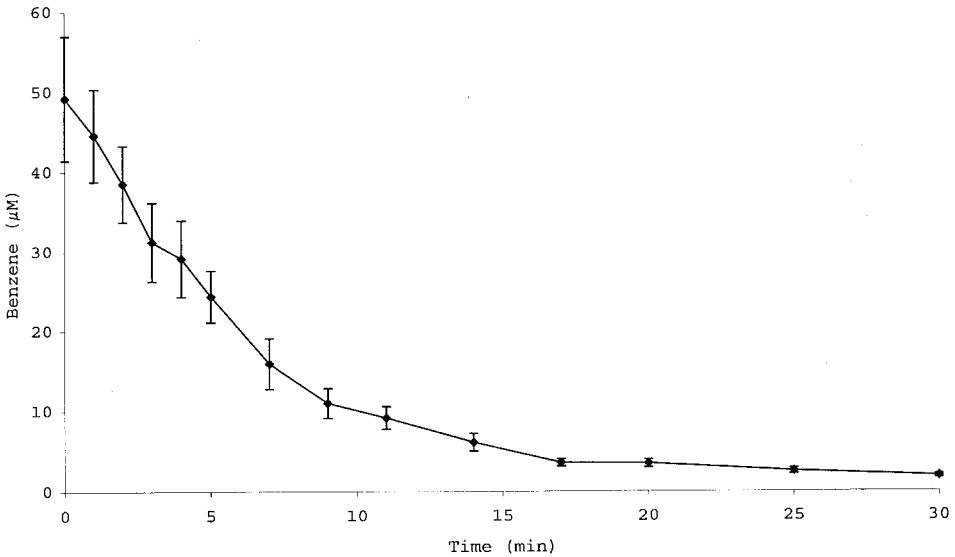


**FIGURE 2.** Time course of perfusate benzene concentration in the isolated perfused rabbit lung during exposure via the pulmonary vasculature. Data are presented as mean ± SE in units of μ*M* benzene ($n = 6$).

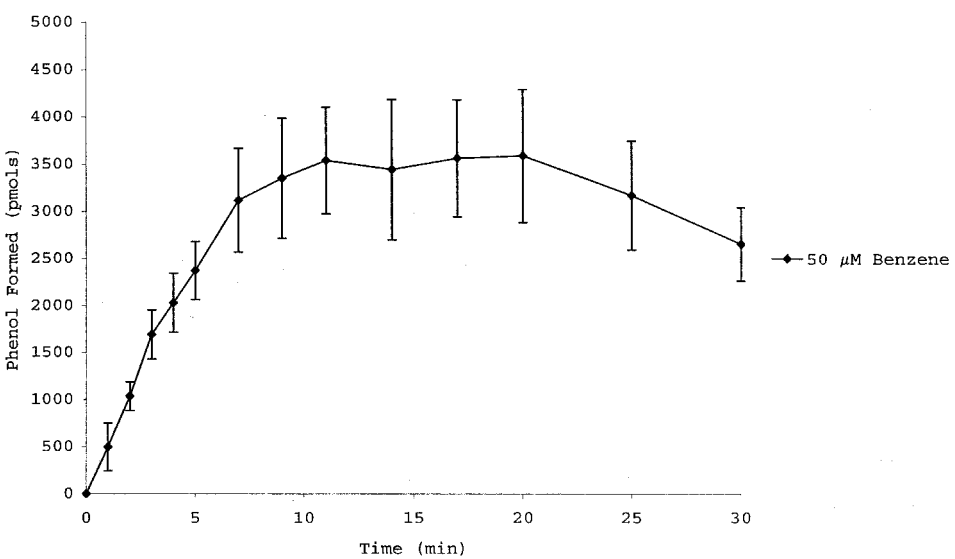


**FIGURE 3.** Time course for phenol formation in perfusate samples in the isolated perfused rabbit lung during exposure via the pulmonary vasculature. Data are presented as mean ± SE with units of pmol phenol formed ($n = 6$).

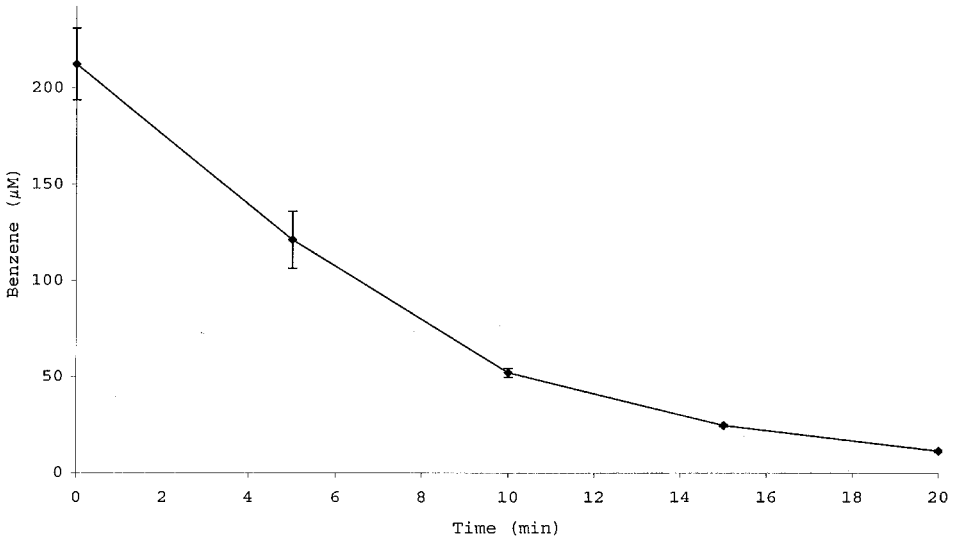


**FIGURE 4.** Time course of perfusate benzene concentration in the isolated perfused rat lung during exposure via the pulmonary vasculature. Data are presented as mean ± SE in units of μ*M* benzene ($n = 2$).

appeared to be first order, especially at the two lower substrate concentrations. These observations were based on semilog plots of the data (data not shown). Benzene was metabolized to phenol (Figure 7) and phenylsulfate. However, due to the inability to fully separate phenylsulfate from a contaminant using HPLC, phenylsulfate could not be reliably quantified. Phenol

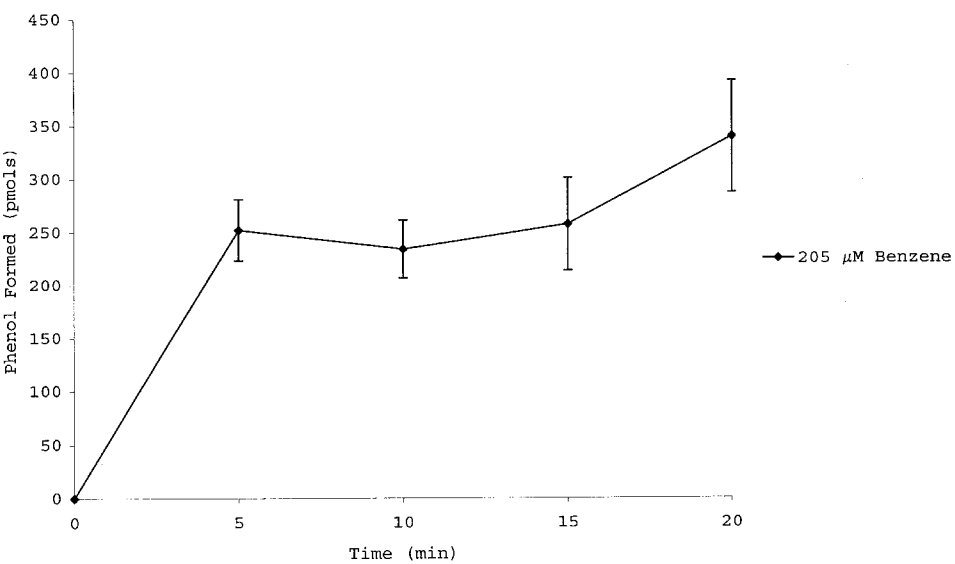


**FIGURE 5.** Time course for phenol formation in perfusate samples in the isolated perfused rat lung during exposure via the pulmonary vasculature. Data are presented as mean ± SE with units of pmol phenol formed ($n = 2$).

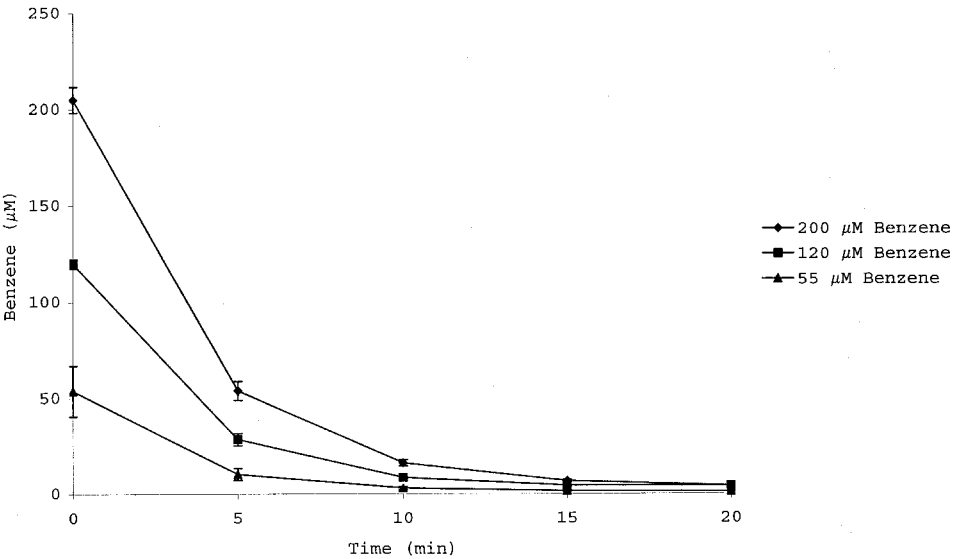


**FIGURE 6.** Time course of perfusate benzene concentration in the isolated perfused mouse lung during exposure via the pulmonary vasculature. Data are presented as mean ± SE in units of μ*M* benzene ($n = 3$ for each benzene concentration).

was formed in similar amounts at the two lower benzene concentrations. With 200 μ*M* benzene, phenol formation was greater. The greatest amount of phenol was formed from 10 to 15 min, and then it slightly decreased for the remainder of the perfusion.

### Benzene Metabolism in Isolated Perfused Mouse Lung During Inhalation Exposure

The ability of the lung to metabolize inhaled benzene was examined using the isolated perfused mouse lung in nonrecirculating mode and exposure to benzene vapor via the tracheal cannula. The concentrations of benzene in air and in the perfusate are shown in Figure 8. The air concentration increased rapidly for the first 10 min, and remained fairly constant for the remainder of the 20-min exposure. The benzene concentration for the final 10 min was approximately 175 ppm. The concentration of benzene in the perfusate increased in a similar fashion to the air concentration. There was an early, sharp increase in the concentration from 3 min until 10 min. The concentration continued to increase over the final 10 min of the 20-min exposure, but it did so at a slower rate. The concentration of benzene in the perfusate at the 20-min time point was 2.1 μ*M*.

Since experiments were conducted without recirculating the perfusate, data are presented as rate of phenol formation (pmol/min). In this preparation the lung was able to metabolize inhaled benzene (Figure 9). The rate of phenol formation increased gradually over the first 10 min. Following this increase, the rate of phenol did not change significantly over the final 10 min of the experiment.

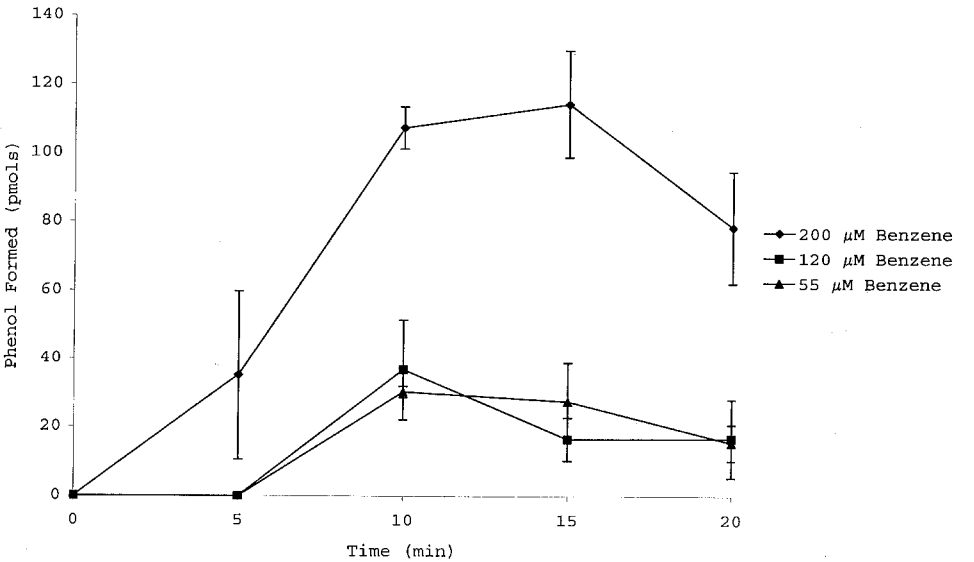


**FIGURE 7.** Time course for phenol formation in perfusate samples in the isolated perfused mouse lung during exposure via the pulmonary vasculature. Data are presented as mean ± SE with units of pmol phenol formed ($n = 3$ for each benzene concentration).

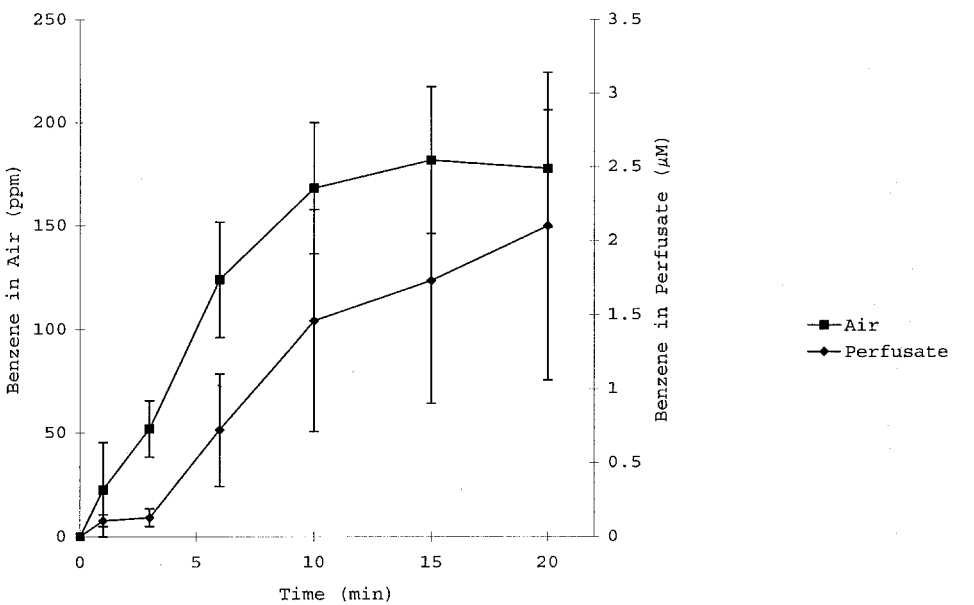


**FIGURE 8.** Time course of air and perfusate benzene concentrations in perfusate samples in the isolated perfused mouse lung during inhalation exposure. Data are presented as mean ± SE with units of μ*M* benzene ($n = 3$).

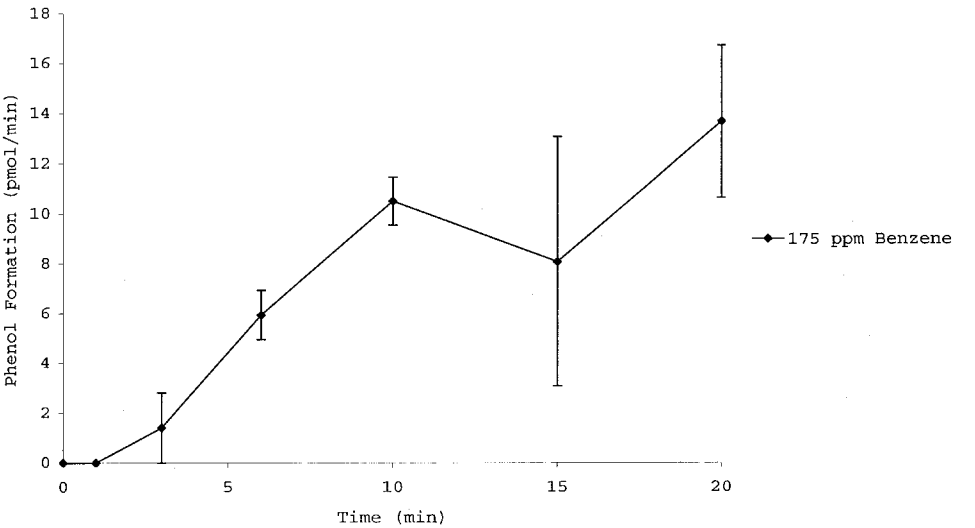


**FIGURE 9.** Time course for phenol formation in perfusate samples in the isolated perfused mouse lung during inhalation exposure. Data are presented as mean ± SE with units of pmol phenol formed ($n = 3$).

## DISCUSSION

To test the hypothesis that the lung can metabolize benzene in an in vivo simulation, experiments were performed using the isolated perfused lung preparation. The lungs were exposed to benzene either via the pulmonary vasculature to imitate systemic exposure, or via the trachea to simulate inhalation exposure. Furthermore, the concentration dependence of benzene metabolism was examined by varying the substrate concentration in the mouse study. Benzene metabolism during exposure via the pulmonary vasculature was assessed in the rabbit, rat, and mouse lung. Although substrate disappeared from the system quickly, metabolism did occur when the benzene was administered via the pulmonary artery. In this recirculating system a very small percentage of the benzene added to the perfusate was metabolized to phenol. There were qualitative differences between these species in the metabolites detected in perfusate. In the isolated perfused rabbit exposed to 50 μ*M* benzene and mouse lung exposed to 55, 120, and 200 μ*M* benzene, both phenol and phenylsulfate were detected in perfusate. In contrast, during exposure to 205 μ*M* benzene only phenol was detected in the isolated perfused rat lung. This may indicate that the capacity to conjugate via sulfation is lower in the rat than in the rabbit or mouse.

The effect of substrate concentration on benzene metabolism was examined in the isolated perfused mouse lung using three benzene concentrations. Both phenol and phenylsulfate were detected in perfusate samples, demonstrating the ability to metabolize benzene by both toxification and de-

toxification reactions. This is also true of benzene metabolism in the isolated perfused mouse liver (Hedli et al., 1997). Of particular interest in our study is the effect of benzene concentration on the amount of phenol formed. The amounts of phenol formed after exposure to 55 μ*M* and 120 μ*M* benzene were approximately equal. With 200 μ*M* benzene, the amount of phenol formed was greater than at the two lower concentrations. The lack of a difference between the two lower concentrations but greater metabolism at the higher level may indicate that more than one cytochrome P-450 is involved, although experimental variability cannot be ruled out. An additional consideration is the possibility that the balance of P-450-mediated and conjugative pathways acting to remove the phenol may be concentration dependent.

The ability of the lung to metabolize inhaled benzene was also evaluated using the isolated perfused mouse lung. During exposure to ~175 ppm, phenol formation was detected. Since experiments were conducted without recirculating the perfusate, data are presented as rate of phenol formation (pmol/min). The increase in the rate of phenol formation closely followed the increase in uptake of benzene in the perfusate. These experiments showed that the lung is capable of metabolizing benzene when exposed via inhalation, which may be significant if local toxicity occurs following biotransformation. There are several studies showing that the lung is a target organ of benzene toxicity following inhalation exposure (Snyder et al., 1988; Farris et al., 1993; Ranaldi et al., 1998; Mullin et al., 1998). It has been suggested that physiologically based pharmacokinetic (PBPK) models do not accurately predict benzene metabolism following inhalation exposure but can more reasonably predict metabolism following oral exposure. The results of these studies indicated that lung may be important in metabolism of inhaled benzene during its uptake. Future studies would be aided by measurement of the amount of benzene exhaled to assist in distinguishing between the benzene taken up by the lungs and that lost from the isolated perfused lung system.

In summary, the results from isolated perfused lung experiments demonstrate that the lung can metabolize benzene in an in vivo simulation. This metabolism is dependent on substrate concentration. Metabolism of benzene occurred during exposure via the pulmonary vasculature and inhalation. This may be significant if local toxicity occurs following biotransformation. These conclusions suggest that the lung plays a role in benzene metabolism and should be included as a metabolizing organ to improve current physiologically based pharmacokinetic models and allow a more accurate assessment of the risk to humans from benzene exposure.

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