Species Differences in the Metabolism of Benzene

Rogene F. Henderson

Inhalation Toxicology Research Institute, Albuquerque, New Mexico

The pathways of metabolism of benzene appear to be qualitatively similar in all species studied thus far. However, there are quantitative differences in the fraction of benzene metabolized by the different pathways. These species differences become important for risk assessments based on animal data. Mice have a greater overall capacity to metabolize benzene than rats or primates, based on mass balance studies conducted *in vivo* using radiolabled benzene. Mice and monkeys metabolize more of the benzene to hydroquinone metabolites than do rats or chimpanzees, especially at low doses. Nonhuman primates metabolize less of the benzene to muconic acid than do rodents or humans. In all species studied, a greater proportion of benzene is converted to hydroquinone and ring-breakage metabolites at low doses than at high doses. This finding should be considered in attempting to extrapolate the toxicity of benzene observed at high doses to predicted toxicity at low doses. Because ring-breakage metabolites and hydroquinone have both been implicated in the toxicity of benzene, the higher formation of those metabolites in the mouse may partially explain why mice are more sensitive to benzene than are rats. Metabolism of benzene in humans, the species of interest, does not exactly mimic that of any animal species studied. More information on the urinary and blood metabolites of occupationally exposed people is required to determine the fractional conversion of benzene to putative toxic metabolites and the degree of variability present in human subjects. — Environ Health Perspect 104(Suppl 6):1173–1175 (1996)

Key words: benzene, species differences, metabolism

Introduction

Two-year bioassay studies of the toxicity of benzene indicated that $B6C3F_1$ mice develop more and more varied tumors in response to similar benzene exposures than do Sprague-Dawley rats (*1,2*). To determine if species differences in metabolism of benzene contribute to species differences in response to benzene, studies have been conducted comparing metabolism of benzene in rats and mice and comparing the rodent metabolism to that in primates, including humans. This paper reviews many of those studies.

This paper was presented at Benzene '95: An International Conference on the Toxicity, Carcinogenesis, and Epidemiology of Benzene held 17–20 June 1995 in Piscataway, New Jersey. Manuscript received 16 January 1996; manuscript accepted 14 June 1996.

The research on benzene metabolism conducted at the Inhalation Toxicology Research Institute was supported by the National Institute of Environmental Health Sciences through an Interagency Agreement, 222-ES-20092, and by the American Petroleum Institute through a Funds-in-Agreement, DE-FI04-94AL97353, with the U.S. DOE/OHER under contract DE-AC04-76EV01013.

Address correspondence to Dr. R.F. Henderson, Inhalation Toxicology Research Institute, PO Box 5890, Albuquerque, NM 87185. Telephone: (505) 845-1164. Fax: (505) 845-1198. E-mail: rhenderson@lucy.tli.org

Abbreviation used: PMA, phenylmercapturic acid.

Studies using radiolabeled benzene determined the mass balance of benzene in the body of different species following exposures by various routes and to a range of doses (*3–8*). The questions addressed by the studies were *a*) How much of benzene administered by inhalation, intraperitoneally or orally is absorbed and metabolized by different species? *b*) What is the ultimate fate of the absorbed benzene—in which tissues or excreta and in what form? *c*) How does total dose affect the answers to the first two questions?

Species Differences in Absorption and Total Metabolism of Benzene

Mice have a higher capacity to metabolize benzene than either rats or cynomolgus monkeys (*6,7*). After 6-hr exposures to low concentrations (7–10 ppm) of benzene, mice retained 20% of the benzene they inhaled compared to 3 to 4% for rats or monkeys. This fact, plus the higher respiratory rate per body weight in the mice, results in mice receiving approximately twice the internal dose as rats after short-term exposures to concentrations of benzene up to about 150 to 200 ppm (Table 1) (*9,10*). At higher exposure concentrations, metabolism becomes saturated in both species, but particularly in the mouse. A 7-fold increase in the exposure concentration (from 130 to 925 ppm) results in only about a 3-fold increase in internal dose in the mouse, and 15% of the absorbed dose is exhaled as unmetabolized benzene (Table 1). Nonetheless, the mouse metabolizes a greater fraction (85%) of its internal dose of 152 mg/kg than the rat, which was only able to metabolize about half its internal dose of 116 mg/kg (*7,9*) following exposure to 925 ppm. If the dose is not delivered slowly over a 6-hr period as in the inhalation studies but is given as a bolus dose, either orally or by intraperitoneal injection, only about half of a dose of 150 mg/kg is metabolized by either species, and the other half is exhaled as unmetabolized benzene. Even a bolus dose of 50 mg/kg results in an increase in the fractional amount of exhaled benzene over what is observed at lower doses in both rats and mice (3,4).

Species Differences in Excretion Patterns

The pattern of excretion of the internal dose of benzene is similar in the rat and mouse, with most of the dose excreted in the urine (Table 1). A small amount is found in feces, a small amount remains in the body, and, at low exposure concentrations, only a small fraction is exhaled unmetabolized. In exposed monkeys, the mass balance was not complete because the animals were not killed at the end of the exposures to determine the remaining body burdens and

Table 1. Internal dose and subsequent excretion pathways in rats and mice exposed to [^{14}C]benzene.[a]

Exposure concentration, ppm	Internal dose, mg/kg		Excretion pathways, % initial dose							
			Urine		Feces		Exhaled air		Carcass	
	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice
11	3.3	7.5	91	88	3	8	4	3	2	1
130	24	60	88	89	2	3	5	1	5	7
925	116	152	48	79	3	5	48	15	1	1

[a]Exposures were for 6 hr at the indicated concentrations; excreta were collected for 48 hr after the exposure.

exhaled breath was not collected; therefore, there is not a complete accounting of all of the radioactivity of the benzene administered to monkeys (*6*). However, the data we have suggest that the monkey does not have a high capacity to metabolize benzene. Monkeys dosed intraperitoneally with 5, 50 or 500 mg [^{14}C]benzene/kg excreted only 56, 37, and 13% of the radioactivity in the urine, respectively, suggesting that the monkey's capacity to metabolize benzene is exceeded at relatively low doses. On the other hand, chimpanzees administered 1 mg/kg of [^{14}C]benzene intravenously excreted essentially all of the radioactivity in the urine.

Because most of the internal dose in animals exposed to low doses of benzene appears to be excreted as metabolites in the urine, one can get a picture of the overall metabolism of benzene in these animals by observing the metabolic profile in the urine. The urinary profiles in benzene-exposed mice, rats, and primates are shown in Table 2. For the low-dose treatments, a major species difference between rats and mice is the higher fraction of benzene converted to hydroquinone and its conjugates in the mice. The urine of the monkeys, on the other hand, contains approximately the same fraction of hydroquinone as found in the urine of mice. One must keep in mind, however, that a smaller fraction of inhaled benzene is metabolized in the monkey compared to the mouse. The urinary profile of the chimpanzee is similar to that of the rat in regard to the hydroquinone fraction.

A second species difference is that rodents (both rats and mice) metabolize more of the benzene to ring-breakage metabolites (as indicated by muconic acid) than primates (Table 2). Finally, one observation is consistent for all species studied. A smaller fraction of the benzene is converted to hydroquinone and ring-breakage metabolites at higher doses than at low doses (*3,7,8*). A possible reason for the lower fractions of hydroquinone produced at higher benzene exposures has been suggested by Schlosser et al. (*11*), who found that high levels of benzene inhibit the metabolism of phenol. However, the mechanism by which the fraction of ring-breakage metabolites produced at high benzene doses is decreased is not known.

Table 2. Urinary metabolites (% of total) following exposure to [^{14}C]benzene.

	Exposure concentration, 6 hr, ppm		
	5	50	600
Mice			
Phenyl conjugates	37	37	67[a]
Hydroquinone conjugates	33	40	11[a]
Pre-PMA	6	1	15[a]
Muconic acid	23	21	5[a]
Rats			
Phenyl conjugates	58	72	74[a]
Hydroquinone conjugates	12	3	2[a]
Pre-PMA	10	11	17[a]
Muconic acid	19	14	4[a]
	IP dose, mg/kg		
	5	50	500
Monkey			
Phenyl conjugates	61	73	78[b]
Hydroquinone conjugates	27	15	9[b]
Catechol conjugates	8.0	6.0	9.9
Pre-PMA	–	–	–
Muconic acid	4.4	3.1	1.3[b]
	IV dose		
	1 mg/kg		
Chimpanzee			
Phenyl conjugates	75		
Hydroquinone conjugates	8		
Catechol conjugates	–		
Pre-PMA	0.5		
Muconic acid	5.5		

[a]Differs from 5 ppm, $p \leq 0.05$. [b]Differs from 5 mg/kg, $p \leq 0.05$. From Sabourin et al. (*3,7,8*).

Species Differences in Blood, Bone Marrow, and Other Tissue Concentrations of Benzene Metabolites

It is important to know if the metabolic profile in the urine matches that in the blood and in the major target tissue for benzene, the bone marrow. The profile of benzene metabolites found in the bone marrow of rats versus mice following a 6-hr exposure to 50 ppm benzene is shown in Table 3 (*8*). The major metabolites found in the bone marrow of rats are phenyl conjugates or muconic acid. (Catechol conjugates were not separated from phenyl conjugates in this study.) Hydroquinone conjugates were detected only in the mouse bone marrow and not in the rat. Muconic acid concentrations were also higher in the mouse than in the rat.

Table 3. Benzene metabolites in bone marrow of rodents[a] (% of total).

	B6C3F$_1$ mice	F344 rats
Hydroquinone glucuronide	23	ND
Muconic acid	12	4
Phenyl glucuronide	1.5	ND
Phenyl or catechol sulfate	63	96

ND, not detected. [a]Exposures from 6 hr to 50 ppm benzene. From Sabourin et al. (*8*) *and* Henderson et al. (*9*).

Blood, liver, and lung levels of benzene metabolites in rats and mice were calculated as the "area under the curve," i.e., the integrated dose of the metabolite during and following a 6-hr exposure to 50 ppm benzene (Table 4) (*8,9*). Hydroquinone conjugates could not be detected in the blood or tissues of rats but were equal to or about half the level of phenyl conjugates in mice. Muconic acid levels were especially high in mouse livers. Thus, the urinary profiles of benzene metabolites were similar to those found in the tissues of the rodents.

Recent research by McDonald et al. (*12*) indicates that the formation of 1,2-benzoquinone protein adducts in the blood and bone marrow of benzene-exposed rodents is favored in rats, while the formation of 1,4-benzoquinone adducts is favored in mice. The authors suggest that this is due to the higher amounts of epoxide hydrolase activity compared to cytochrome P450 activity in the livers of rats compared to those of mice. The higher fractions of benzene converted to hydroquinone in mice versus rats would predict a higher amount of the 1,4-benzoquinone being formed in the mouse. In rats, however, we do not have evidence for a large amount of catechol formation, and catechol would be the expected precursor for 1,2-benzoquinone formation.

In studies using liver microsomes (*11*), the percent of benzene converted to phenol was about 20% in both rat and mouse microsomes, but the fraction of benzene converted to hydroquinone was 31% in the mouse compared to 8% in the rat. Conversion to catechol was 0.5% in the rat and 2% in the mouse liver microsomes, while very little muconic acid was formed in either species. These data are consistent with the higher formation of hydroquinone in mice versus rats in *in vivo* studies but

Table 4. Benzene metabolites in F344 rats and B6C3F$_1$ mice exposed to 50 ppm benzene for 6 hr ($\bar{X} \pm$ SE) (area under the curve, nmol/g/hr).

	Hydroquinone conjugates	Muconic acid	Phenyl conjugates
Blood			
Rats	ND[a]	4.3 ± 2.5	144 ± 32
Mice	105 ± 14	8.0 ± 5.5	225 ± 49
Liver			
Rats	ND	65 ± 10	93 ± 29
Mice	190 ± 31	1200 ± 95	150 ± 34
Lung			
Rats	ND	14 ± 3	112 ± 15
Mice	110 ± 17	110 ± 13	230 ± 37

[a]ND, not detected. From Henderson et al. (*9*).

contrasts with the high level of muconic acid found in livers of mice exposed *in vivo*. The study by Schlosser et al. (*11*) also predicted that most of the catechol formed in either the rat or the mouse comes from oxidation of phenol rather than hydrolysis of benzene oxide. This was based on the fact that the same amount of catechol was formed in rodent liver microsome incubations whether the substrate was benzene or phenol. The conclusion that most of the catechol comes from phenol contrasts with the conclusion of McDonald et al. (*12*), who suggested that the preponderance of 1,2- over 1,4-benzoquinone adducts in benzene-exposed rats compared to mice is due to the higher rate of formation of catechol via hydrolysis of benzene oxide in the rat. The quantitative contribution of the two pathways for catechol formation in the two species remains to be elucidated.

Summary and Discussion

The pathways of metabolism of benzene appear to be qualitatively similar in all species studied thus far. However, there are quantitative differences in the fraction of benzene metabolized by the different pathways. Mice appear to have a greater overall capacity to metabolize benzene than rats or primates, based on mass balance studies conducted *in vivo* using radiolabeled benzene. Mice and monkeys metabolize more of the benzene to hydroquinone metabolites than do rats or chimpanzees, especially at low doses. Nonhuman primates metabolize less of the benzene to muconic acid than do rodents or humans. Because ring-breakage metabolites and hydroquinone have both been implicated in the toxicity of benzene, the higher formation of these metabolites in the mouse may partially explain why mice are more sensitive to benzene toxicity than are rats. Mice also metabolize more of inhaled benzene than do rats.

For all species studied, there is a greater fractional formation of these and ring-breakage metabolites at low doses compared to high doses. This also appears to be true for humans (Bechtold et al., unpublished data). This finding, along with many other factors, must be considered in attempting to extrapolate the toxicity of benzene observed at high doses to those expected at low doses.

Metabolism of benzene in humans, the species of interest, does not exactly mimic that of any animal species studied. More information on the urinary and blood metabolites of occupationally exposed people is required to determine the fractional conversion of benzene to putative toxic metabolites and the degree of variability present in the human subjects.

REFERENCES

1. NTP. Toxicology and Carcinogenesis Studies of Benzene in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series 289. Research Triangle Park, NC:National Toxicology Program, 1986.
2. Maltoni C, Conti B, Cotti G. Benzene: A multipotential carcinogen. Results of long-term bioassays performed at the Bologna Institute of Oncology. Am J Ind Med 4:589–630 (1983).
3. Sabourin PJ, Bechtold WE, Griffith WC, Birnbaum LS, Lucier G, Henderson RF. 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 (1989).
4. Henderson RF, Sabourin PJ, Bechtold WE, Griffith WC, Medinsky MA, Birnbaum LS, Lucier GW. The effect of dose, dose rate, route of administration, and species on tissue and blood levels of benzene metabolites. Environ Health Perspect 82:9–17 (1989).
5. Dahl AR, Sun JD, Birnbaum LS, Bond JA, Griffith WC, Mauderly JL, Muggenburg BA, Sabourin PJ, Henderson RF. Toxicokinetics of inhaled 1,3-butadiene in monkeys: comparison to toxicokinetics in rats and mice. Toxicol Appl Pharmacol 110:9–19 (1991).
6. Sabourin PJ, Muggenburg BA, Couch RC, Lefler D, Lucier G, Birnbaum LS, Henderson RF. Metabolism of [^{14}C]benzene by cynomolgus monkeys and chimpanzees. Toxicol Appl Pharmacol 114:277–284 (1992).
7. Sabourin PJ, Chen BT, Lucier G, Birnbaum LS, Fisher E, Henderson RF. Effect of dose on the absorption and excretion of [^{14}C]benzene administered orally or by inhalation in rats and mice. Toxicol Appl Pharmacol 87:325–336 (1987).
8. Sabourin PJ, Bechtold WE, Birnbaum LS, Lucier G, Henderson RF. Differences in the metabolism and disposition of inhaled [^{3}H]benzene by F344/N rats and B6C3F$_1$ mice. Toxicol Appl Pharmacol 94:128–140 (1988).
9. Henderson RF, Sabourin PJ, Medinsky MA, Birnbaum LS, Lucier G. Benzene dosimetry in experimental animals: relevance for risk assessment. In: Relevance of Animal Studies to the Evaluation of Human Cancer Risk (D'Amato R, Slaga TJ, Farland WH, Henry C, eds). New York:Wiley-Liss, 1992;93–105.
10. Medinsky MA, Sabourin PJ, Lucier G, Birnbaum LS, Henderson RF. A physiological model for simulation of benzene metabolism by rats and mice. Toxicol Appl Pharmacol 99:193–206 (1989).
11. Schlosser PM, Bond JA, Medinsky MA. Benzene and phenol metabolism by mouse and rat liver microsomes. Carcinogenesis 14:2477–2486 (1993).
12. McDonald TA, Yeowell-O'Connell K, Rappaport SM. Comparison of protein adducts of benzene oxide and benzoquinone in the blood and bone marrow of rats and mice exposed to [^{14}C/^{13}C]benzene. Cancer Res 54:4907–4914 (1994).