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Identification of 2-Bromohydroquinone as a Metabolite of Bromobenzene and *o*-Bromophenol: Implications for Bromobenzene-Induced Nephrotoxicity[1]

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ABSTRACT

2-Bromohydroquinone was identified as a metabolite of both bromobenzene and *o*-bromophenol in the rat *in vivo* and *in vitro*. Identification was based on high-pressure liquid chromatography and gas chromatography-mass spectrometry. Formation of 2-bromohydroquinone by rat liver microsomes from both bromobenzene and *o*-bromophenol was increased by treatment of rats with either phenobarbital or 3-methylcholanthrene. Covalent binding of *o*-bromophenol to rat liver microsomes was inhibited by glutathione and ascorbate but not by superoxide dismutase or catalase. Liver microsomes converted *o*-bromophenol to 2-bromohydroquinone and covalently bound material, whereas kidney and lung microsomes metabolized *o*-bromophenol less rapidly. Administration of 2-bromohydroquinone to rats caused a dose- and time-dependent decrease in hepatic and renal glutathione levels, an increase in blood urea nitrogen levels and histopathological changes in kidney without causing any alterations to the liver. The histological changes in the kidney were indistinguishable from those observed after either bromobenzene or *o*-bromophenol administration. However, the dose of 2-bromohydroquinone required to elicit a similar nephrotoxicity was less than 10% of that of bromobenzene. Thus, 2-bromohydroquinone may play a role in the nephrotoxicity observed after bromobenzene administration. Although the nature of the nephrotoxic metabolite of 2-bromohydroquinone is not known, our present results suggest that 2-bromohydroquinone or a conjugate thereof may be formed in the liver and transported to the kidney where it elicits toxicity.

Administration of bromobenzene (9.3 mmol/kg i.p.) to rats produces necrosis of the proximal convoluted renal tubules (Reid, 1973). The production of this renal necrosis is associated with the covalent binding of radiolabeled material to kidney protein (Reid, 1973). Studies on the metabolism and covalent binding of [^{14}C]bromobenzene *in vitro* and *in vivo* suggested that the renal necrosis was caused by a metabolite formed in the liver and transported by the blood to the renal tubules. The nature of this proposed metabolite is unclear.

We have recently demonstrated that whereas a major bromobenzene metabolite, *p*-bromophenol, gives rise to covalently bound material in liver, lung and kidney *in vivo*, it is neither hepatotoxic nor nephrotoxic in rats (Monks *et al.*, 1982). Moreover, i.p. administration of 4-bromocatechol, a major metabolite of both bromobenzene and *p*-bromobenol (Lau *et al.*, 1984a), does not cause any apparent histological changes in the kidney of Balb/c mice or rats (unpublished results). Thus, the kidney toxicity caused by bromobenzene does not appear to be mediated by *p*-bromophenol or its major metabolite, 4-bromocatechol.

In contrast, there is evidence that *o*-bromophenol may be converted to toxic metabolites. For example, isolated hepatocytes convert *o*-bromophenol, a metabolite of bromobenzene, to toxic metabolites which deplete cellular glutathione and cause cell death (Thor *et al.*, 1982). Moreover, *o*-bromophenol (1.92 mmol/kg i.p.) in rats also causes a decrease in kidney glutathione levels and a subsequent increase in BUN (Lau *et al.*, 1984b). Pretreatment of rats with piperonyl butoxide decreases the incidence of elevated BUN levels after *o*-bromophenol administration. Metabolites of *o*-bromophenol become covalently bound to kidney protein of untreated rats to a greater extent than to liver protein, and phenobarbital pretreatment increases the covalent binding of *o*-bromophenol metabolites to kidney protein but not to liver protein. The increases in covalent binding to kidney protein correlate with increases in BUN levels (Lau *et al.*, 1984b). In contrast, liver microsomes convert *o*-bromophenol to covalently bound material whereas kidney microsomes do not. These results thus support the view of Reid (1973) that an intermediate may be generated in the liver and transported to the kidney.

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ABBREVIATIONS: BUN, blood urea nitrogen; HPLC, high-performance liquid chromatography; GCMS, gas chromatography-mass spectrometry.

In the present study we have demonstrated that the major metabolite formed from *o*-bromophenol is 2-bromohydroquinone, and that this metabolite or a conjugate thereof may be responsible for the nephrotoxicity observed after bromobenzene and *o*-bromophenol administration.

Methods

Materials. Bromobenzene and *o*-bromophenol (redistilled before use), 4-bromoresorcinol, *p*-bromophenol and 3-methylcholanthrene were obtained from the Aldrich Chemical Company, Inc. (Milwaukee, WI). [^{14}C]Bromobenzene (23 mCi/mmol) and *o*-[^{14}C]bromophenol (23 mCi/mmol) were purchased from the Amersham Corp. (Arlington Heights, IL), and purified by HPLC before use (>99% pure). Glutathione, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and NADP were obtained from Boehringer-Mannheim Biochemicals (Indianapolis, IN). 2-Bromohydroquinone was a product of ICN Pharmaceuticals, Inc. (Plainview, NY). Sodium phenobarbital was a product of J. T. Baker Chemical Co. (Phillipsburg, NJ). Magnesium chloride, catalase and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Superoxide dismutase was a product of Miles Laboratories, Inc. (Elkhart, IN). β-Glucuronidase-arylsulfatase was obtained from Calbiochem-Behring Corp (La Jolla, CA). Piperonyl butoxide (90–95%) was purchased from Fluka AG (Buchs SG, Switzerland). Bromobenzene-2,3-dihydrodiol and bromobenzene-3,4-dihydrodiol were isolated and identified as previously described (Monks *et al.*, 1984). 4-Bromocatechol and 3-bromocatechol were synthesized according to the method of Rosenmund and Kuhnhenn (1923) as described by Lau *et al.* (1984a).

Animals. Male Sprague-Dawley rats (Taconic Farms, Inc., Germantown, NY; 150–160 g) were used for all experiments and were allowed food and water *ad libitum* before the experiments. Some rats were treated with phenobarbital once a day for 4 days (i.p.; 80 mg/kg in 0.5 ml of 0.85% saline) and some rats were treated with 3-methylcholanthrene once a day for 4 days (i.p.; 20 mg/kg in 0.5 ml of sesame oil).

Preparation of microsomes. Animals were killed by cervical dislocation 24 hr after the last injection of either phenobarbital or 3-methylcholanthrene. The livers, lungs and kidneys were perfused *in situ*, removed immediately and placed on ice. The tissues were then homogenized with Tris-KCl buffer, pH 7.4 (0.15 M KCl; 20 mM Tris) and microsomes were prepared as described by Hinson *et al.* (1977).

Microsomal incubations. Mixtures of 6 mg of rat liver, lung or kidney microsomal protein, 8 mM glucose-6-phosphate, 0.5 mM NADP, 15 mg of glucose-6-phosphate dehydrogenase, 1.0 mM magnesium chloride, 0.5 mM *o*-[^{14}C]bromophenol (2200 dpm/nmol) or 1.0 mM [^{14}C]bromobenzene (2200 dpm/nmol) and 0.05 M phosphate buffer, pH 7.4, in a final volume of 3.0 ml were incubated at 37°C for 15 min unless otherwise noted. Incubations were terminated by placing the reaction vessels in a Dry Ice-acetone bath. Covalent binding of radiolabeled reactive metabolites to microsomal protein was determined as previously described (Monks *et al.*, 1982).

Isolation and identification of 2-bromohydroquinone. Ten milliliters of a 20-ml liver microsomal incubation mixture (3-methylcholanthrene-pretreated rats; 1.0 mM bromobenzene) was extracted with 50 ml of ethyl acetate. The mixture was centrifuged and the aqueous phase was frozen in a Dry Ice-acetone bath. The ethyl acetate phase was decanted and anhydrous sodium sulfate added. The mixture was shaken with a vortex mixer and centrifuged. The dried ethyl acetate phase was transferred to another tube and evaporated to dryness under nitrogen. The residue was dissolved in 160 μl of ethyl acetate and purified by HPLC. All HPLC assays were performed with a model 6000A chromatograph (Waters Associates, Milford, MA) equipped with a dual wavelength UV absorbance detector (254 and 280 nm) and a Partisil 5 ODS-3 reversed phase column (Whatman). The mobile phase was a linear gradient of methanol-water-acetic acid ranging from 20:79:1 to 79:20:1 (v:v:v) over 40 min at a flow rate of 1 ml/min. Under these conditions, an unknown peak which had a retention time identical with that of authentic 2-bromohydroquinone (fig. 1) was collected and

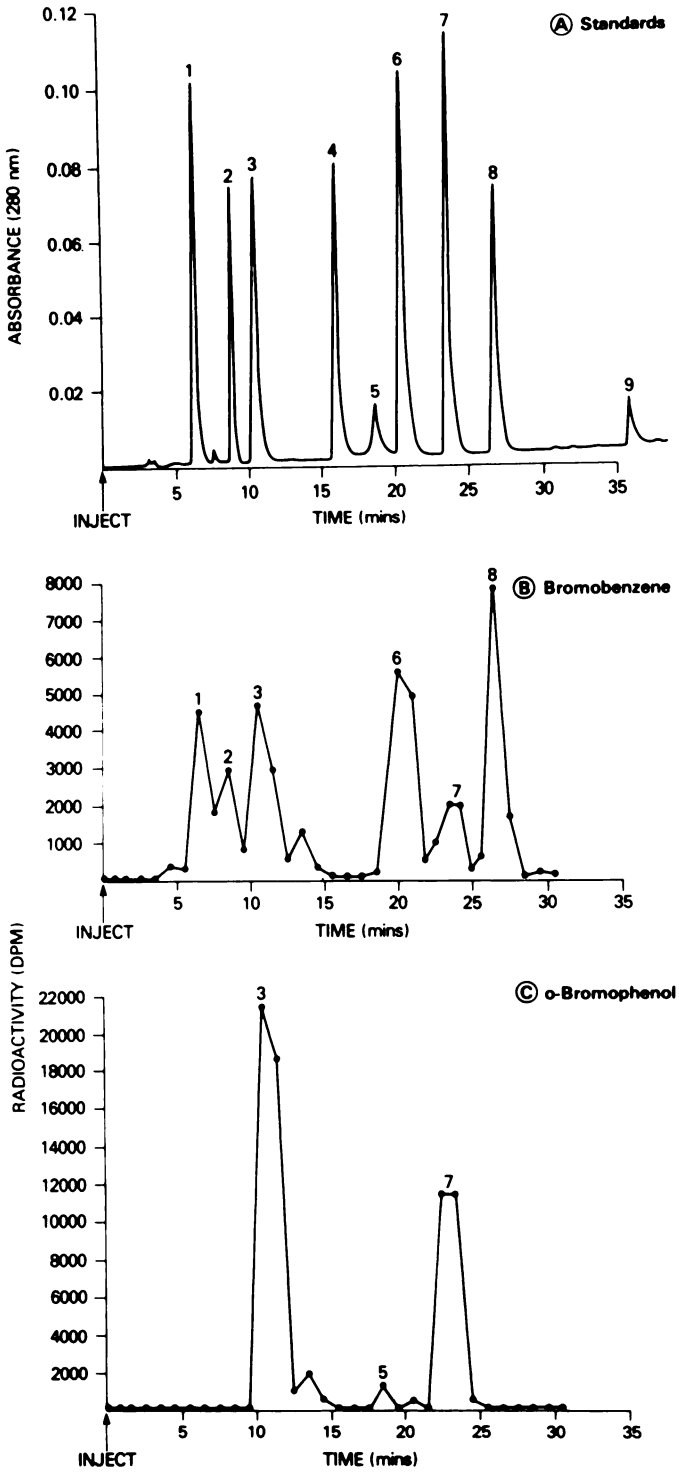

Fig. 1. HPLC elution profile of authentic bromobenzene metabolites (A), extracts from microsomal incubations of bromobenzene (B) and *o*-bromophenol (C). The mobile phase consisted of a linear gradient of methanol-water-acetic acid (20:79:1) to methanol-water-acetic acid (79:20:1) over 40 min at a flow rate of 1 ml/min. The peaks correspond to the following compounds with retention time in minutes in parentheses: 1, 2,3-dihydrodiol (6.3); 2, 3,4-dihydrodiol (8.3); 3, 2-bromohydroquinone (10.5); 4, 4-bromoresorcinol (16.0); 5, 3-bromocatechol (19.0); 6, 4-bromocatechol (20.5); 7, *o*-bromophenol (23.5); 8, *p*-bromophenol (26.8); 9, bromobenzene (35.9).

the methanol evaporated under nitrogen. The residual aqueous phase was extracted into ethyl acetate (1:1; v:v), and the extract evaporated to dryness under nitrogen. The residue was dissolved in 50 μl ethyl acetate. A 2-μl aliquot of this extract was analyzed by GCMS. A V. G. Micromass model 16F mass spectrometer was set in the electron-ionization mode. The accelerating voltage was 4 kV, the electron energy was 20 eV and the source temperature was 240°C. The spectrometer was interfaced with a Hewlett Packard model 5710A gas chromatograph that was equipped with a methyl silicone (SP 2100) Diamond fused silica capillary column (30 m × 0.3 mm). The oven temperature was programmed from 50°C to 250°C at 8°C per min and the injector temperature was 250°C. Helium was used as the carrier gas at a flow rate of 2 ml/min. Under these conditions both the isolated metabolite and authentic 2-bromohydroquinone had a retention time of 10.5 min.

Assay for 2-bromohydroquinone. One-milliliter samples of microsomal incubation mixtures were extracted into 3 ml of ethyl acetate and vortex mixed. The mixture was centrifuged and the organic phase collected after freezing the aqueous layer in a Dry Ice-acetone bath. The organic phase was then dried over anhydrous sodium sulfate, centrifuged and evaporated under nitrogen to a final volume of 200 μl. A 50-μl aliquot was then analyzed by HPLC as described above. Fractions were collected at 1-min intervals and radioactivity determined by liquid scintillation spectroscopy. Under these conditions recovery of authentic 2-bromohydroquinone was 62 ± 1%.

Detection of 2-bromohydroquinone in rat urine. [^{14}C]Bromobenzene (1.28 mmol/kg i.p.; 400 dpm/nmol) was given to groups of three rats pretreated with either phenobarbital or 3-methylcholanthrene. The 0- to 24-hr urine from rats in each group was pooled and collected in a light-protected flask containing 50 mg of ascorbic acid. The amount of 2-bromohydroquinone in urine of rats receiving either bromobenzene or *o*-bromophenol was measured before and after enzyme hydrolysis by β-glucuronidase-arylsulfatase. Urine (0.5 ml) was diluted 1:1 with acetate buffer, pH 4.5, and hydrolyzed with 50 μl of glusulase (β-glucuronidase 8.1 IU/ml; arylsulfatase 2.3 IU/ml) at 37°C for 16 hr. After incubation, 0.5 ml of the mixture was extracted twice into 4 ml of ethyl acetate. The organic extracts were combined and evaporated under nitrogen to a final volume of 200 μl, and a 50-μl aliquot was analyzed by HPLC.

Toxicity studies. 2-Bromohydroquinone in 0.5 ml of ethanol-0.9% saline (1:1) was given to rats by i.p. injection at doses of 0.27, 0.53 and 0.80 mmol/kg. Ethanol-0.9% saline (1:1) (0.5 ml i.p.) was injected into control rats. After 24 hr, a sample of blood (400 μl) was taken from the retro-orbital sinus. Plasma was separated by centrifugation and the degree of renal damage was assessed by measuring BUN levels using Sigma Kit 535A according to Sigma technical bulletin 535. Livers and kidneys were removed and histology slides made and stained with hematoxylin and eosin by American Histolabs (Rockville, MD).

Glutathione depletion. *Time course.* 2-bromohydroquinone (0.8 mmol/kg i.p.) was given to rats which were subsequently killed by decapitation at time zero and at 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 hr thereafter. A second group of control rats were killed at 5 hr to take into account any possible temporal variation in glutathione levels (Jaeger *et al.*, 1973; Hassing *et al.*, 1979; Monks *et al.*, 1982). Livers and kidneys were removed immediately from animals and total nonprotein thiol levels measured by the method of Ellman (1959) as described previously (Mitchell *et al.*, 1973).

Dose response. 2-bromohydroquinone in 0.5 ml of ethanol-0.9% saline (1:1) was injected i.p. into rats at doses of 0.27, 0.53 and 0.80 mmol/kg. Animals were killed 2 hr after administration of the compound and liver and kidney glutathione levels measured (Mitchell *et al.*, 1973).

Results

Identification of 2-bromohydroquinone. Figure 1A shows the HPLC elution profile of a mixture of bromobenzene and some of its known and possible metabolites. Figure 1B shows the radiochromatographic profile of an extract from an incubation mixture containing liver microsomes and [^{14}C]bromobenzene. In addition to radioactive peaks corresponding to previously identified metabolites of bromobenzene (Lau *et al.*, 1984a; Monks *et al.*, 1984), a radiolabeled peak was eluted at the same retention time as authentic 2-bromohydroquinone. Figure 1C shows the radiochromatographic profile of an extract from an incubation mixture containing liver microsomes and *o*-[^{14}C]bromophenol in which the major radiolabeled peak eluted at the same retention time as authentic 2-bromohydroquinone; a minor peak was observed which corresponds to the retention time of authentic 3-bromocatechol.

Figure 2A shows the GCMS of authentic 2-bromohydroquinone and figure 2B the GCMS of a metabolite of bromobenzene isolated from a microsomal incubation. The major ions in both spectra were *m/z* 188, 190 (M^+); 108 (M − HBr) and 80 (M − HBr − CO). The similarities in the HPLC retention time and the fragmentation pattern of the GCMS spectrum between the metabolite and an authentic compound indicated that the unknown metabolite was 2-bromohydroquinone.

Microsomal formation of 2-bromohydroquinone. Although covalent binding of radiolabel from [^{14}C]bromophenol to microsomal protein was greater in liver microsomes from phenobarbital-treated animals than in liver microsomes from 3-methylcholanthrene-treated animals (table 1), the formation of *o*-bromophenol was greater in liver microsomes from 3-methylcholanthrene-treated animals. Formation of 2-bromohydroquinone from bromobenzene was not detected in incubation mixtures containing liver microsomes from untreated rats. However, liver microsomes obtained from phenobarbital- or 3-methylcholanthrene-treated rats catalyzed the formation of 2-bromohydroquinone from [^{14}C]bromobenzene to similar extents.

As shown in table 2, liver microsomes from both phenobarbital- and 3-methylcholanthrene-treated rats increased the formation of 2-bromohydroquinone from *o*-[^{14}C]bromobenzene. Treatment with phenobarbital also increased the covalent binding of metabolites derived from *o*-bromophenol but treatment with 3-methylcholanthrene decreased metabolite binding.

Covalent binding and 2-bromohydroquinone formation from *o*-[^{14}C]bromophenol in microsomes of liver, lung and kidney from phenobarbital-treated rats is shown in table 3. Liver microsomes formed substantial amounts of 2-bromohydroquinone and covalently bound material. In contrast, kidney and lung microsomes formed relatively small amounts of 2-bromohydroquinone or covalently bound material. Covalent binding from *o*-[^{14}C]bromophenol was inhibited 90% by the addition of either 2 mM ascorbate or 1 mM glutathione (table 4). Ascorbic acid also caused a concominant increase in 2-bromohydroquinone levels whereas glutathione caused a 40% decrease in 2-bromohydroquinone levels. In contrast, superoxide dismutase and catalase had little effect on either covalent binding or 2-bromohydroquinone formation (table 4).

***In vivo* formation of 2-bromohydroquinone.** 2-Bromohydroquinone was identified in the urine of rats receiving either bromobenzene (1.28 mmol/kg i.p.) or *o*-bromophenol (1.74 mmol/kg i.p.) (table 5). With both substrates, more 2-bromohydroquinone was excreted into the urine of rats pretreated with phenobarbital than with 3-methylcholanthrene. As only a small amount of 2-bromohydroquinone (<1% of the dose) was present in organic extracts before hydrolysis of the urine with β-glucuronidase-arylsulfatase, nearly all the 2-bro-

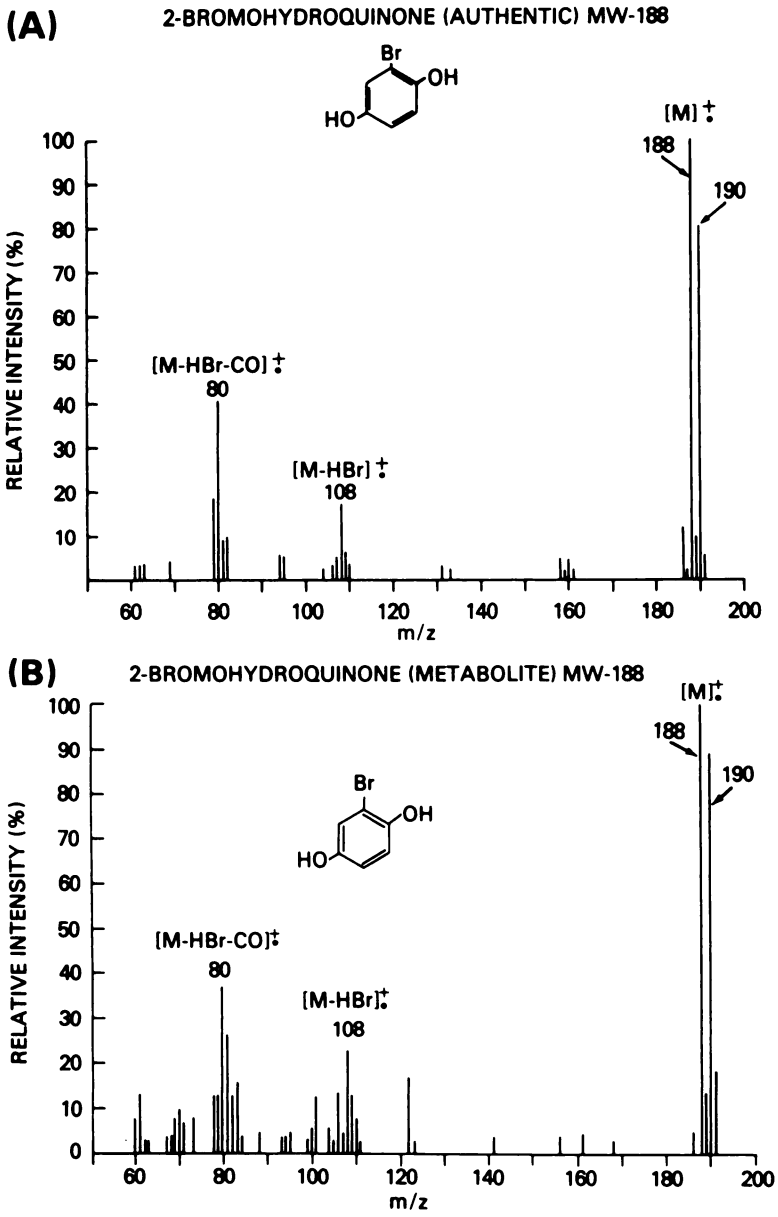

Fig. 2. The GCMS of (A) authentic 2-bromohydroquinone and (B) of a metabolite of bromobenzene, formed in microsomes from 3-methylcholanthrene-treated rats.

TABLE 1

Effects of various treatments on the *in vitro* covalent binding and formation of *o*-bromophenol and 2-bromohydroquinone from 1 mM [^{14}C]bromobenzene in liver microsomes

Treatment	Covalent Binding	*o*-Bromophenol	2-Bromohydroquinone
	nmol/mg protein/15 min		
None	0.87 ± 0.08[a]	0.14 ± 0.01	N.D.[b]
Phenobarbital	13.2 ± 0.7	3.9 ± 0.5	10.2 ± 0.7
3-Methylcholanthrene	4.0 ± 0.3	12.5 ± 1.3	12.7 ± 1.1

[a] Mean value ± S.D. of triplicate incubations.
[b] N.D., nondetectable.

TABLE 2

Effects of various treatments on the *in vitro* covalent binding and formation of 2-bromohydroquinone from 0.5 mM *o*-[^{14}C]bromophenol in liver microsomes

Treatment	Covalent Binding	2-Bromohydroquinone
	nmol/mg protein/15 min	
None	4.74 ± 0.24[a]	14.9 ± 1.0
Phenobarbital	8.45 ± 0.71	55.7 ± 4.0
3-Methylcholanthrene	3.40 ± 0.24	42.4 ± 1.1

[a] Mean value ± S.D. of triplicate incubations.

mohydroquinone detected in urine was present as its corresponding glucuronide and/or sulfate conjugate.

2-Bromohydroquinone toxicity. Administration of 2-bromohydroquinone i.p. to rats resulted in a dose-dependent decrease in hepatic and renal glutathione levels (fig. 3). Maximum depletion of glutathione occurred at a dose of 0.80 mmol/kg in both tissues. At this dose, kidney and liver glutathione levels were depleted by 35 and 28%, respectively. The time course of glutathione depletion is shown in figure 4. The nadir of depletion for both tissues was at about 2 hr postdosing. Kidney glutathione levels tended to recover more rapidly than did liver glutathione levels, the latter remaining at 60% of control values for as long as 24 hr after administration of 2-bromohydroquinone (fig. 4).

The effect of 2-bromohydroquinone on BUN is shown in figure 5. At a dose of 0.80 mmol/kg, BUN levels became elevated between 5 and 6 hr after administration of 2-bromohydroquinone. Thereafter BUN levels continue to rise even at 24 hr

TABLE 3

Covalent binding and 2-bromohydroquinone formation from o-[^{14}C] bromophenol in liver, lung and kidney microsomes

Microsomes were prepared from rats pretreated with phenobarbital as described under "Methods"

	Covalent Binding	2-Bromohydroquinone
	nmol/mg protein/15 min	
Liver	8.45 ± 0.71[a]	55.96 ± 0.80
Kidney	0.07 ± 0.006	1.09 ± 0.41
Lung	0.43 ± 0.006	1.35 ± 0.93

[a] Mean value ± S.D. of triplicate incubations.

after dosing. Table 6 shows the dose-dependent increase in BUN levels after administration of 2-bromohydroquinone. Elevated BUN levels corresponded with histological alterations in the kidney as evaluated by the method of Reid (1973) as previously described by Lau *et al.* (1984b). The renal lesions consisted of extensive coagulative necrosis of the renal tubular cells in the corticomedullary region. The collecting ducts and tubules exhibited occasional hyaline casts while some of the tubules in the mid and outer cortex were markedly dilated and contained proteinaceous fluid.

Discussion

In the present study we have identified 2-bromohydroquinone as an *in vivo* and *in vitro* metabolite of both bromobenzene and *o*-bromophenol in the rat. Moreover, administration of 2-bromohydroquinone to rats caused a dose-related increase in BUN levels and histopathological alterations in the kidney which were indistinguishable from those observed after either bromobenzene (9.3 mmol/kg) (Reid, 1973) or *o*-bromophenol administration (1.9 mmol/kg) (Lau *et al.*, 1984b). Thus, the dose of 2-bromohydroquinone required to elicit toxicity was less than 10% of that of bromobenzene.

The present results lend credence to the hypothesis that *o*-bromophenol was metabolized in the liver to an intermediate which was subsequently transported to the kidney. Thus, while liver microsomes formed a substantial amount of 2-bromohydroquinone from *o*-bromophenol, kidney microsomes formed very little (table 3). In contrast, administration of 2-bromohydroquinone to rats caused specific nephrotoxicity, without producing any histopathological alterations in the liver. The data, therefore, suggest that 2-bromohydroquinone, or a conjugate thereof, was formed in the liver and transported to the kidney where it was converted to the ultimate nephrotoxic metabolite(s). In this respect, glutathione was shown to decrease the levels of 2-bromohydroquinone in microsomal incubations with *o*-bromophenol (table 4). Concomitant with this decrease was the appearance of water-soluble metabolites, presumably glutathione conjugates. Moreover, 2-bromohydroquinone *in vivo* decreased both hepatic and renal glutathione levels (figs. 3 and 4), suggesting that an oxidized form of 2-bromohydroquinone reacted with reduced glutathione to form a glutathione conjugate(s). Indeed, preliminary experiments indicated that 2-bromohydroquinone may be converted to various glutathione adducts *in vitro* and that these conjugates are nephrotoxic (Monks *et al.*, 1983).

Similar amounts of 2-bromohydroquinone were formed from both bromobenzene and *o*-bromophenol in microsomes from either phenobarbital- or 3-methylcholanthrene-treated rats (tables 1 and 2). The covalent binding derived from both substrates, however, was higher in phenobarbital microsomes. The covalently bound radiolabel derived from [^{14}C]bromobenzene, however, probably represents the formation of several reactive metabolites (Lau *et al.*, 1984a). With *o*-bromophenol as the substrate, the mechanism for the covalent binding to liver microsomes *in vitro* is also unclear. Ascorbic acid decreased the amount of covalently bound reactive material derived from *o*-bromophenol by more than 90% and increased the accumulation of 2-bromohydroquinone (table 4). As ascorbic acid reduces semiquinones and quinones to catechols (Eistner and Kramer, 1973; Nishikim, 1975), the covalently bound material may have arisen from 2-bromo-1,4-quinone formed by oxidation of 2-bromohydroquinone. It is also possible that the covalently bound material represents the formation of an intermediate epoxide(s) of *o*-bromophenol (fig. 6) before its rearrangement to 2-bromohydroquinone. In this respect both a 2,3- or 3,4-epoxidation of *o*-bromophenol could give rise, upon subsequent rearrangement, to 2-bromohydroquinone (fig. 6). Moreover, while 3-bromocatechol appears to be a minor metabolite of *o*-bromophenol (fig. 1C), there is no evidence that *o*-bromophenol is metabolized to either 2- or 4-bromoresorcinol. However, the mechanism of oxidation of 2-bromohydroquinone in microsomal incubations remains unclear. Neither superoxide dismutase nor catalase affected 2-bromohydroquinone levels, which suggests that superoxide anion and hydrogen peroxide do not mediate the oxidation of 2-bromohydroquinone to a corresponding quinone. Similar results on the effects of superoxide dismutase and catalase on covalent binding and hydroquinone formation from phenol have recently been reported (Sawahata and Neal, 1983). The possibility that 2-bromohydroquinone is oxidized by either superoxide anion or hydrogen peroxide generated in microsomes before they become accessible to the exogenously added enzymes, however, cannot be ruled out.

The mechanism by which the *o*-bromophenol metabolites

TABLE 4

Effect of various additions on the *in vitro* covalent binding and 2-bromohydroquinone formation from o-[^{14}C]bromophenol in liver microsomes from phenobarbital-pretreated rats

The results represent the mean ± S.D. of triplicate incubations. Figures in parentheses represent percentge of control values.

Addition	Covalent Binding	2-Bromohydroquinone	Total
		nmol/mg protein/15 min	
None	8.45 ± 0.71	55.7 ± 4.0	64.2 ± 3.5
Glutathione (1 mM)[a]	0.89 ± 0.07*** (10.5%)	33.4 ± 2.9** (60.0%)	34.4 ± 2.9*** (53.6%)
Ascorbate (2 mM)	0.80 ± 0.10*** (9.5%)	63.0 ± 2.5 (113.1%)	63.9 ± 2.6 (99.5%)
Superoxide dismutase (15 μg/ml)	7.04 ± 0.28* (83.3%)	49.8 ± 1.7 (89.4%)	57.0 ± 1.9* (88.8%)
Catalase (200 U/ml)	7.39 ± 0.06 (86.5%)	57.2 ± 1.2 (102.7%)	64.6 ± 1.1 (100.6%)

[a] Water-soluble metabolites recovered from incubations containing 1 mM glutathione were 28.3 ± 6.1 nmol/mg of protein per 15 min; thus the total metabolites were 62.7 ± 8.92 nmol/mg of protein per 15 min.

* $P < .05$ when compared to control; ** $P < .002$ when compared to control; *** $P < .001$ when compared to control.

TABLE 5

Quantitation of 2-bromohydroquinone excreted into urine of rats receiving either bromobenzene or o-bromophenol

The urine samples from three rats were pooled and assayed as described under "Methods." 2-Bromohydroquinone was quantitated after enzyme hydrolysis of the urine with glusulase as described under "Methods." Figures represent percentage of the dose excreted in 0 to 24 hr urine.

Substrate	2-Bromohydroquinone	
	Phenobarbital treatment	3-Methtylcholanthrene treatment
Bromobenzene (1.28 mmol/kg i.p.)	1.5%	0.7%
o-Bromophenol (1.74 mmol/kg i.p.)	21.0%	5.7%

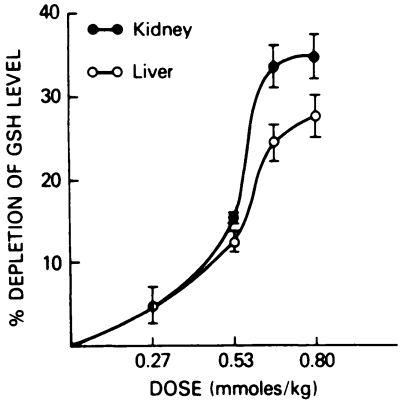

Fig. 3. The effect of increasing doses of 2-bromohydroquinone on hepatic and renal glutathione (GSH) levels in noninduced rats. Data represent mean ± S.D. (n = 5).

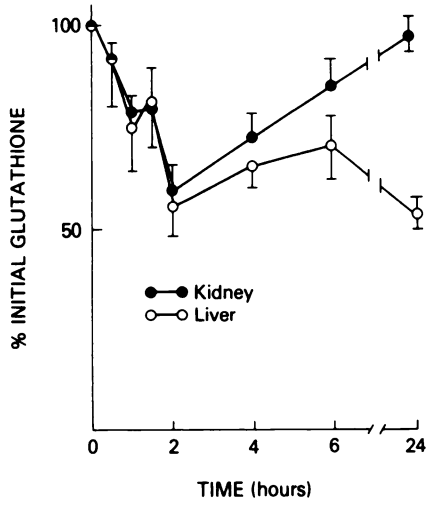

Fig. 4. The time course of glutathione depletion caused by 2-bromohydroquinone (0.8 mmol/kg i.p.) in liver and kidney of noninduced rats. Data represent mean ± S.D. (n = 5).

become covalently bound to kidneys *in vivo* (Lau *et al.*, 1984b) is also unclear. Pretreatment of rats with piperonyl butoxide (1600 mg/kg s.c.) did not protect the animals from 2-bromohydroquinone (0.80 mmol/kg i.p.)-induced nephrotoxicity (data not shown), suggesting that renal or hepatic cytochrome P-450 isozymes may not catalyze the activation of 2-bromohydroquinone to a nephrotoxic metabolite. It has recently been demonstrated that the peroxidase component of prostaglandin (H) synthase can utilize a variety of electron donors to reduce the hydroperoxide moiety of prostaglandin endoperoxide to the

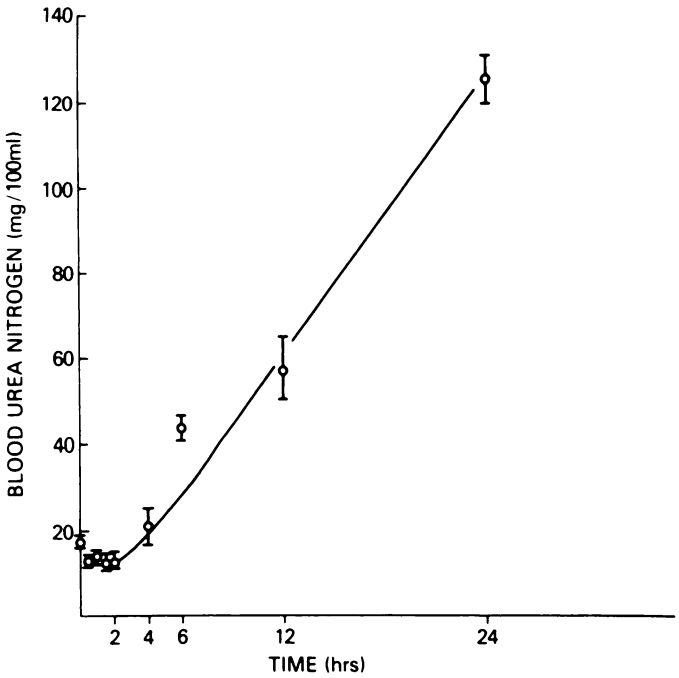

Fig. 5. The time course of BUN levels after 2-bromohydroquinone (0.8 mmol/kg i.p.) administration.

TABLE 6

Effect of increasing doses of 2-bromohydroquinone on BUN levels

2-Bromohydroquinone was given by i.p. injection in ethanol-0.9% saline (1:1, v:v; 0.5 ml) and rats were killed 24 hr later.

Dose	BUN
mmol/kg	*mg/100 ml*
0.0	13.3 ± 0.9[a]
0.27	15.2 ± 0.7
0.53	17.6 ± 4.6
0.80	101.5 ± 12.4***

[a] Mean value ± S.E.M. (n = 5–9).
*** $P < .001$ when compared with appropriate ethanol-saline control.

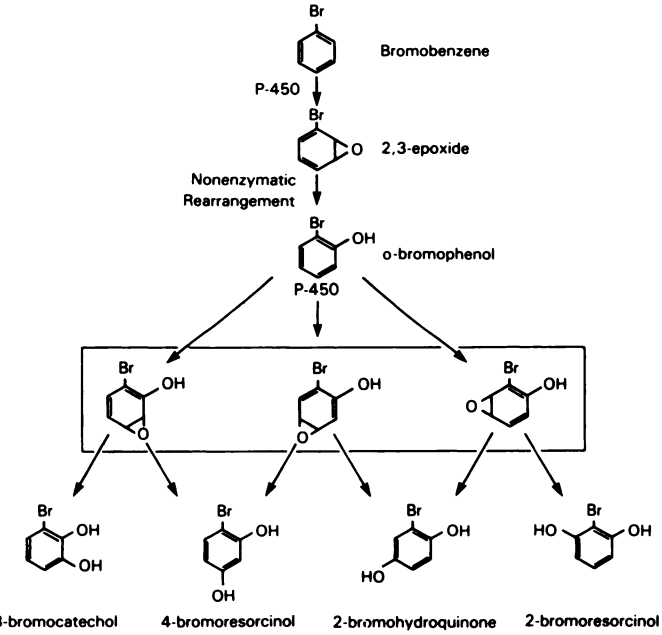

Fig. 6. Possible pathways of 2-bromohydroquinone formation from bromobenzene and o-bromophenol.

corresponding hydroxyl group (Ohki *et al.*, 1979). Such electron donors include a variety of xenobiotics (Fitzpatrick and Wynalda, 1976; Zenser *et al.*, 1979; Marnett *et al.*, 1980) which undergo simultaneous co-oxidation to potentially reactive products. Several compartments within the kidney possess the necessary enzymes for prostaglandin synthesis (Bohman, 1977; Smith and Wilkin, 1977; Smith and Bell, 1978; Hassid *et al.*, 1979; McGiff and Wong, 1979; Sraer *et al.*, 1979; Hassid and Dunn, 1980), but they are principally localized within the medullary papillae (Zusman and Keiser, 1977a,b; Zenser *et al.*, 1979; Beck *et al.*, 1980; Grenier *et al.*, 1981). Certain drugs such as acetaminophen, salicylate and phenacetin have been shown to be concentrated within the inner medulla (Bluemle and Goldberg, 1968; Duggin and Mudge, 1976), and these agents are metabolized to reactive intermediates that covalently bind to protein (Mitchell *et al.*, 1977; Kluwe *et al.*, 1978) within the inner medulla (Mudge *et al.*, 1978; Mohandas *et al.*, 1981), presumably *via* the prostaglandin (H) synthase (Mohandas *et al.*, 1981). However, in the present experiments the region where the peroxidases are most abundant does not correspond to the site of cell injury caused by 2-bromohydroquinone. Peroxidases may therefore not be directly involved in the formation of a nephrotoxic metabolite from 2-bromohydroquinone.

The possibility that oxidation and conjugation of 2-bromohydroquinone occur in one part of the kidney while conversion to the ultimate nephrotoxic metabolite occurs in the region where the nephrotoxicity occurs, is currently under investigation.

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