



An Interaction of Benzene Metabolites Reproduces the Myelotoxicity Observed with Benzene Exposure

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An Interaction of Benzene Metabolites Reproduces the Myelotoxicity Observed with Benzene Exposure. EASTMOND, D. A., SMITH, M. T., AND IRONS, R. D. (1987). *Toxicol. Appl. Pharmacol.* **91**, 85-95. Benzene-induced myelotoxicity can be reproduced by the coadministration of two principal metabolites, phenol and hydroquinone. Coadministration of phenol (75 mg/kg) and hydroquinone (25-75 mg/kg) twice daily to B6C3F1 mice for 12 days resulted in a significant loss in bone marrow cellularity in a manner exhibiting a dose-response. One explanation for this potentiation is that phenol stimulates the peroxidase-dependent metabolism of hydroquinone. Addition of phenol to incubations containing horseradish peroxidase, H_2O_2 , and hydroquinone resulted in a stimulation of both hydroquinone removal and benzoquinone formation. Stimulation occurred with phenol as low as 100 μM and with very low concentrations of horseradish peroxidase. When boiled rat liver protein was added to identical incubations containing [^{14}C]hydroquinone, the level of radioactivity recovered as protein bound increased by 37% when phenol was added. Similar results were observed when [^{14}C]hydroquinone was incubated in the presence of activated human leukocytes. Hydroquinone binding was increased by approximately 70% in the presence of phenol. Phenol-induced stimulation of hydroquinone metabolism and benzoquinone formation represents a likely explanation for the bone marrow suppression associated with benzene toxicity. © 1987 Academic Press, Inc.

Chronic exposure to benzene is associated with aplastic anemia, pancytopenia, leukemia, and other blood dyscrasias in man (Goldstein, 1977; IARC, 1982). Nevertheless the significance of exposure to low concentrations of benzene (<10 ppm) remains controversial. This is a serious health concern because benzene is an extremely important industrial chemical, to which 2 million U.S. workers are potentially exposed (NIOSH, 1974), and a ubiquitous pollutant (USEPA, 1980). Due to the limitations inherent in epidemiological and animal carcinogenicity studies, understanding the mechanism by which benzene exerts its hematopoietic toxicity may be the only way to determine whether

low-level chronic exposure to benzene poses a significant health threat (Goldstein, 1953). Although the mechanism of toxicity is as yet unknown, studies have shown that benzene itself is probably not the actual toxicant but is converted by the liver to a metabolite(s) which travels to the bone marrow and exerts its toxic effects (Sawahata *et al.*, 1985; Sammett *et al.*, 1979; Andrews *et al.*, 1977). *In vivo* metabolic studies following benzene exposure have identified phenol as the primary metabolite of benzene with hydroquinone, catechol, *trans,trans*-muconic acid, and 1,2,4-trihydroxybenzene as significant secondary metabolites (Rusch *et al.*, 1977; Snyder *et al.*, 1981; Gad-El-Karim *et al.*,

1985a). After benzene exposure, phenol concentrations within the bone marrow rise and fall relatively quickly, whereas hydroquinone and catechol concentrations, although moderately low initially, rise with time and persist at relatively high concentrations (Rickert *et al.*, 1979; Irons *et al.*, 1982; Greenlee *et al.*, 1981a,b). A perplexing problem is that the administration of the principal metabolites of benzene has failed to mimic the myelotoxicity characteristic of benzene exposure (Gad-El-Karim *et al.*, 1985b; Tunek *et al.*, 1981; Mitchell, 1971; Snyder and Engelsberg, 1982). This failure of the known benzene metabolites to reproduce benzene-induced myelotoxicity led us to hypothesize that toxicity may be the result of a combination of metabolites rather than the result of any single metabolite.

The isolation of phenol, hydroquinone, and catechol in the bone marrow following benzene exposure suggested the possibility that the critical metabolic events resulting in toxicity may involve an interaction of these phenolic compounds during localized metabolism within the bone marrow. Human bone marrow contains appreciable levels of myeloperoxidase for which phenol is a known substrate and hydroquinone and catechol are probable substrates (Yamazaki, 1958; Himelhoch *et al.*, 1969; Eastmond *et al.*, 1986). Evidence for the involvement of peroxidases in the metabolic activation of various xenobiotics such as acetaminophen, diethylstilbestrol, and certain aromatic amines has accumulated in recent years (Moldeus *et al.*, 1982; Metzler, 1984; Wise *et al.*, 1984). Furthermore, the target organ specificity exhibited by benzene for peroxidase-containing organs during recent animal carcinogenesis bioassays such as the zymbal gland, the harderian gland, and mammary glands also suggests a role for peroxidases in the metabolic activation of benzene (Haseman *et al.*, 1984; Osborne *et al.*, 1980; Morrison and Allen, 1966; Cavalieri and Rogan, 1985). Polymorphonuclear leukocytes (PMNs), when stimulated by

certain agents, undergo a unique type of oxidative metabolism known as the "oxidative burst" which is characterized by a cyanide-insensitive increase in oxygen consumption, increased hexose monophosphate shunt activity, and a release of oxygen radicals, myeloperoxidase, and other lysosomal enzymes (Karnovsky and Robinson, 1981). Recent studies in our laboratory have shown that isolated human myeloperoxidase and PMNs, when stimulated to undergo the oxidative burst, are capable of converting the phenolic compounds, phenol, 1-naphthol, and diethylstilbestrol to reactive protein-binding species (Eastmond *et al.*, 1986, 1987a,b). These observations suggest that a secondary peroxidase-dependent metabolism of phenol, hydroquinone, and catechol may be occurring within the bone marrow and that this mechanism may be responsible for benzene-induced myelotoxicity. The object of this study was to test whether benzene-induced myelotoxicity could be reproduced by the coadministration of two of its metabolites and to identify a potential mechanism whereby myeloperoxidase may be involved in this interaction.

METHODS

Chemicals and enzymes. Phenol and hydroquinone were purchased from Aldrich Chemical Co. (Milwaukee, WI). Hydrogen peroxide (30% solution), horseradish peroxidase (HRP; type VI, 275 U/mg), dextran 70, cytochrome c (type III), and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma Chemical Co. (St. Louis, MO). [2,3,5,6-¹⁴C]Hydroquinone (22 mCi/mmol) was purchased from Amersham, (Arlington Heights, IL). [U-¹⁴C]Phenol (30.5 mCi/mmol) was obtained from Pathfinder Laboratories (St. Louis, MO). All other chemicals or solvents were generally of the highest grade available and obtained through local suppliers. Distilled water was purified using the Millipore Q system from Millipore (Bedford, MA).

Animals. Male B6C3F₁ mice, 4 to 6 weeks old on arrival, were obtained from Charles River Breeding Laboratories (Wilmington, MA) and held in quarantine for 2 weeks. Animals were housed five to six per cage with hardwood bedding and were given food (NIH-07, Ziegler

Bros., Gardners, PA) and water *ad libitum*. Serologic examination confirmed the absence of antibody titers to pathologic murine viruses. Groups of six mice treated intraperitoneally twice daily at 6-hr intervals with solutions of metabolites freshly prepared in phosphate-buffered saline as previously described (Wierda and Irons, 1982).

Bone marrow cellularity. It is the previous experience of this laboratory that bone marrow cellularity is more sensitive and reliable as a single indicator of hematopoietic status than peripheral leukocyte counts, which are susceptible to a variety of influences in addition to bone marrow toxicity (Irons *et al.*, 1979). Bone marrow cellularity was determined by flushing and thoroughly suspending bone marrow from left femurs with 4 ml Isoton (Coulter Electronics). A 400- μ l aliquot was then diluted 1:50 with Isoton containing 0.3 ml Zapoglobin (Coulter) to lyse erythrocytes and counted on a Model ZF Coulter counter.

Metabolism and binding studies with horseradish peroxidase. The standard incubation contained HRP (0.06 μ g/ml), H_2O_2 (1 mM), and hydroquinone (75 μ M) in phosphate buffer (0.1 M, pH 7.4). Incubations were initiated by the addition of H_2O_2 (immediately following hydroquinone addition) and were performed at 37°C in a shaking water bath. The reaction was terminated at 2 min by the addition of 5% trichloroacetic acid and maintained on ice until HPLC or protein-binding analyses were performed. Determination of the binding of [14 C]hydroquinone or phenol equivalents to protein was performed as previously described (Eastmond *et al.*, 1986). HPLC analyses by electrochemical detection were performed as described by Eastmond *et al.* (1986) with the following modifications. Working potentials of +0.7 and -0.4 V were used for hydroquinone and 1,4-benzoquinone detection, respectively. The mobile phase consisted of 99% 0.1 M ammonium acetate buffer (pH 4.0) and 1% acetonitrile at a flow rate of 1 ml/min. The column was maintained at 50°C.

Leukocyte experiments. Leukocytes were isolated from the blood of healthy human volunteers by dextran sedimentation as described by Markert *et al.* (1985). Cell counts were performed using a hemocytometer and viability was determined to be >95% by trypan blue exclusion. Prior to further experimentation, the ability of PMNs to produce superoxide anion radical when stimulated by PMA was determined as previously described (Markert *et al.*, 1985). Incubations to determine the protein binding of [14 C]hydroquinone during the oxidative burst by PMNs were performed as follows: Cell suspensions containing PMNs (5×10^6), [14 C]hydroquinone (30 μ M), and PMA (1 μ g/ml) in modified Dulbecco's phosphate buffer (pH 7.1) were incubated at 37°C in a shaking water bath. The reactions were terminated at 20 min by the addition of 5% trichloroacetic acid after which the samples were maintained on ice and centrifuged. The

binding of hydroquinone equivalents to leukocyte protein was performed as previously described (Eastmond *et al.*, 1986).

Statistical analyses. Statistical analyses, unless otherwise stated, were performed using Dunnett's *t* test for multiple comparisons following one- or two-way analyses of variance. In experiments where the variances were not homogeneous, log transformation of the data was performed prior to analysis. Critical values were determined using a 0.05 probability of type I error. Analysis of bone marrow cellularity data consisted of an F test for equality of variance followed by a pooled *t* test.

RESULTS

Toxicity studies. The administration of phenol (0–150 mg/kg) twice daily for 12 days did not result in a suppression of bone marrow cellularity. Hydroquinone administration (100 mg/kg, 2 $\times$ /day) produced a transient mild suppression in cellularity that was evident after 3 days but was essentially the same as that of controls after 12 or even 36 days of continued administration (Fig. 1A). Neither compound produced any obvious dose-response (data not shown). However, the concomitant administration of phenol (75 mg/kg) and hydroquinone (25–75 mg/kg) twice daily resulted in a significant decrease in bone marrow cellularity. The decrease in cellularity was evident after 12 days of administration and exhibited a clear dose-response relationship (Fig. 1B). The administration of catechol, either alone or in combination with phenol or hydroquinone, did not contribute to the suppression of bone marrow cellularity (data not shown).

Metabolism studies. HRP, a model peroxidase enzyme, was shown to convert hydroquinone to 1,4-benzoquinone in a reaction that was dependent upon H_2O_2 . The addition of phenol to these incubations resulted in a 30% stimulation of hydroquinone removal from the incubation (Table I). Hydroquinone removal was accompanied by an increase in 1,4-benzoquinone recovered in the medium in a stoichiometric fashion (Table 1). This stimulation was dose related and ob-

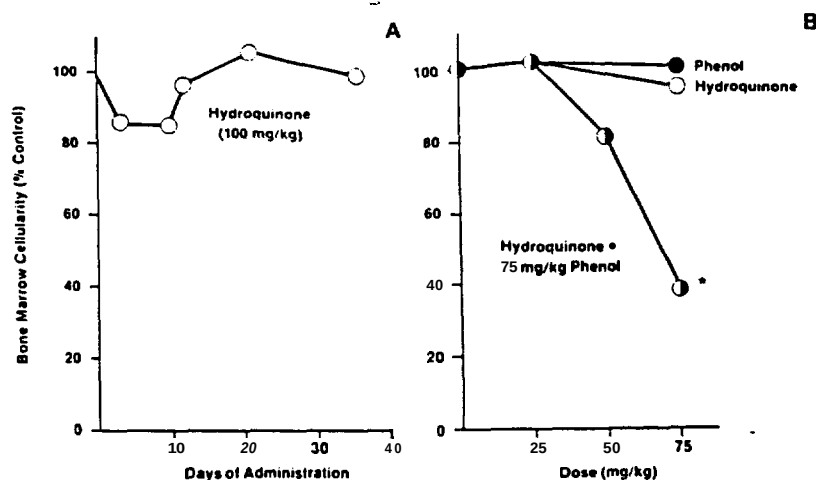


FIG. 1. Influence of phenol on suppression of bone marrow cellularity by hydroquinone. B6C3F1 male mice, 8–10 weeks of age, were administered phenol, hydroquinone, or both intraperitoneally at the doses indicated, twice daily, 6 hr apart. (A) Hydroquinone alone (○) over 36 days of administration. (B) Hydroquinone (25–75 mg/kg) in the presence of phenol (75 mg/kg) (●); phenol alone (●) after 12 days of administration. Each point represents the mean femoral cellularity of five or six animals expressed as a percentage of control (untreated) mice which averaged 1.63×10^7 (1.02×10^6 SE) nucleated cells per femur. Asterisk indicates significant difference from controls ($p < 0.001$).

servable with phenol concentrations as low as $100 \mu\text{M}$ (Fig. 2). At low peroxidase concentrations, the phenol-induced stimulation of hydroquinone metabolism increased to nearly 200%. Furthermore, when boiled rat liver

protein was added to similar incubations containing [^{14}C]hydroquinone, the level of radioactivity recovered bound to protein (an indication of the formation of a highly reactive product from hydroquinone) was increased by 37% when phenol was added to these incubations (Table 2). However, the addition of nonradiolabeled hydroquinone to similar incubations containing [^{14}C]phenol reduced the binding of phenol equivalents by 85% indicating that hydroquinone was acting as a competitive inhibitor of phenol metabolism by HRP (Table 3). Both sets of incubations, those containing phenol and those without, were shown to be peroxidase dependent and required the presence of both H_2O_2 and HRP.

Leukocyte experiments. Similar results were observed when freshly isolated human PMNs were stimulated by PMA to undergo the oxidative burst. The addition of phenol to incubations containing [^{14}C]hydroquinone, PMA, and PMNs resulted in an approximately 70% increase in [^{14}C]hydroquinone equivalents binding to leukocyte protein over

TABLE I

PHENOL-INDUCED STIMULATION OF HORSE RADISH PEROXIDASE-CATALYZED METABOLISM OF HYDROQUINONE

Treatment	Hydroquinone remaining (μM)	Benzoquinone formed (μM)
Complete ^a	22 $\pm$ 3 ^b	47 $\pm$ 5 ^b
+10 mM phenol	6 $\pm$ 1 ^c	71 $\pm$ 3 ^c
Negative controls ^d	75 $\pm$ 1 ^c	3 $\pm$ 1 ^c

^aThe incubation and HPLC conditions are as described under Materials and Methods.

^bMean and standard deviation of five incubations.

^cDiffers significantly from complete incubation using a two-tailed Dunnett multiple range test at $p < 0.05$.

^dComposite of the complete incubation and incubation + phenol in which H_2O_2 and HRP were omitted.

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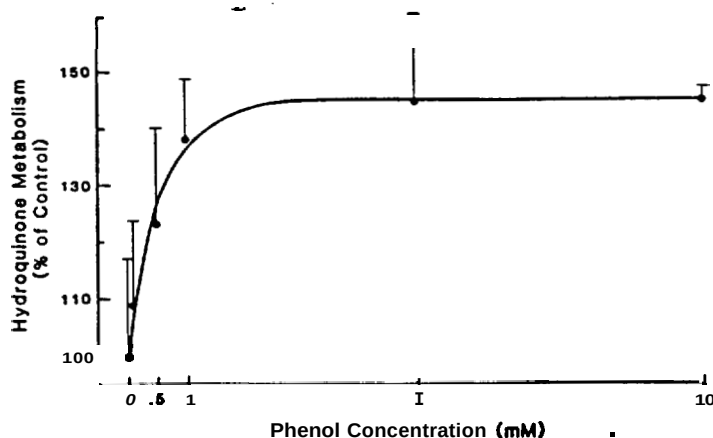


FIG. 2. Effects of various phenol concentrations on hydroquinone metabolism. Incubation conditions are as described under Materials and Methods. The data represent the means and standard deviations of three experiments. The 0 and 0.1 phenol treatment groups differ significantly ($p < 0.05$; paired t test).

that seen when [^{14}C]hydroquinone was incubated in the absence of phenol (Table 4). The addition of PMA to the PMN-containing incubations resulted in a ninefold increase in hydroquinone binding (from 0.9 to 6.6 nmol/mg protein) over that observed in the absence of PMA. However when phenol was also included in the incubations, hydroquinone binding increased to 10.8 nmol/mg protein, a 12-fold increase over the binding observed in the absence of PMX.

DISCUSSION

The failure of the individual benzene metabolites to reproduce the myelotoxicity observed following benzene exposure has been an enigma. This failure of the known benzene metabolites to reproduce benzene-induced myelotoxicity has led some investigators to examine reactive intermediates formed during ring opening such as *trans,trans*-muconaldehyde. The administration of near lethal doses of *trans,trans*-muconaldehyde has been reported to result in moderate bone marrow damage (Witz *et al.*, 1985) but not the extensive damage that accompanies benzene exposure (Green *et al.*, 1981). We therefore postu-

lated that toxicity was due to a combination of metabolites rather than any one metabolite. Here we report that the coadministration of phenol and hydroquinone results in the extensive bone marrow damage characteristic of benzene toxicity, whereas the coadministration of combinations of the other phenolic metabolites had no significant effect on bone marrow cellularity.

Metabolism studies employing the model peroxidase enzyme HRP and isolated human PMNs support the hypothesis that the synergistic effects of phenol and hydroquinone *in vivo* are due to a localized peroxidase-mediated metabolism of these compounds within the bone marrow. The presence of phenol in incubations containing HRP induced a significant stimulation in hydroquinone oxidation to 1,4-benzoquinone and an increase in binding of hydroquinone to protein. The HRP-catalyzed metabolism of hydroquinone and the binding of [^{14}C]hydroquinone equivalents to protein was increased by 30–40% by the addition of phenol into the incubations. Although a 30–40% stimulation of hydroquinone metabolism may seem insufficient to account for the major difference in bone marrow damage observed when hydroquinone is

TABLE 2

PHENOL-DEPENDENT STIMULATION OF [¹⁴C]HYDROQUINONE BINDING TO RAT LIVER PROTEIN

Treatment	nmol hydroquinone equiv. bound/mg protein	Sample size
Complete ^a	10.4 ± 3.2	10
+Phenol	14.4 ± 3.7 ^a	10
Complete	9.4 ± 2.9 ^c	6
-H ₂ O ₂	2.4 ± 1.4 ^d	6
-HRP	3.2 ± 0.5 ^d	6
+Phenol ^e	13.4 ± 3.5	6
+Phenol - H ₂ O ₂	2.5 ± 1.6 ^f	6
+Phenol - HRP	3.3 ± 0.9 ^f	6

^aThe incubation conditions are as described under Materials and Methods.

^b Differs significantly from the corresponding complete incubation using Student's *t* test at *p* < 0.05.

^c Mean and standard deviation.

^d Differs significantly from the complete incubation using a one-tailed Dunnett multiple range test at *p* < 0.05.

^e Phenol (10 mM) was added to the complete incubation.

^f Differs significantly from the +phenol treatment using a one-tailed Dunnett multiple range test at *p* < 0.05.

administered separately and with phenol. This stimulation can be increased by simple modifications of the incubation conditions. By reducing the concentration of HRP and thereby reducing the amount of metabolism, the phenol-induced stimulation of hydroquinone metabolism increased from 30 to nearly 200%. Similar incubations employing isolated human myeloperoxidase showed a phenol-induced stimulation of hydroquinone metabolism of greater than 400% (data not shown). This is particularly relevant to benzene toxicity in light of results reported here and elsewhere that the administration of hydroquinone by itself can cause mild bone marrow damage (Gad-El-Karim *et al.*, 1985b). The stimulation of hydroquinone metabolism by low concentrations of phenol and at low peroxidase concentrations, conditions analogous to those occurring in the bone marrow, suggests that this mechanism

TABLE 3

INHIBITION OF PEROXIDASE-MEDIATED PHENOL BINDING BY HYDROQUINONE

	nmol [¹⁴ C]phenol equiv. bound/mg protein ^a	Percentage
Complete ^b	115	100
Complete + 75 μM hydroquinone		
Complete - H ₂ O ₂	40	35 ^c
Complete - HRP	27	23

^a Means of duplicate experiments.

^b The complete incubation contained HRP (0.06 μg/ml), H₂O₂ (1 mM), [¹⁴C]phenol (10 mM), and 0.9 mg/ml boiled rat liver postmitochondrial supernatant in phosphate buffer (0.1 M, pH 7.4). The incubations were performed for 2 min at 37°C in a shaking incubator and terminated by the addition of 5% trichloroacetic acid. Protein binding was determined as described under Materials and Methods.

^c Represents an 85% inhibition from the complete incubation after correcting for background binding.

of metabolic activation of hydroquinone could be occurring within the bone marrow following exposure to benzene.

The observation that phenol stimulates hydroquinone metabolism rather than acting as

TABLE 4

PHENOL-INDUCED STIMULATION OF HYDROQUINONE BINDING DURING THE PMA-STIMULATED INDUCATIVE BURST OF HUMAN LEUKOCYTES

Treatment	nmol hydroquinone equiv. bound/mg protein ^a
Complete ^b	6.6 ± 0.7 ^b
+10 mM phenol	10.8 ± 2.7
-PMA ^d	0.9 ± 0.3 ^c

^a The incubation conditions are as described under Materials and Methods.

^b Mean and standard deviation of four incubations.

^c Differs significantly from the complete incubation using a two-tailed Dunnett multiple range test at *p* < 0.05.

^d Incubations containing phenol without PMA showed no difference from the -PMA incubations.

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a competitive inhibitor of the peroxidase enzyme is somewhat anomalous. However, phenol and other phenolic compounds have been previously reported to stimulate the peroxidase-mediated oxidation of a variety of compounds (Yamazaki, 1958; Danner *et al.*, 1973; Kalyanaraman *et al.*, 1985). The mechanism has been proposed to involve the ability of the phenoxy radical (derived enzymatically from phenol) to directly oxidize hydroquinone to the semiquinone radical, thereby increasing the speed of a rate-limiting step in the overall oxidation of hydroquinone to 1,4-benzoquinone (Yamazaki, 1958; Kalyanaraman *et al.*, 1985). Consistent with this mechanism are the results demonstrating an inhibition of peroxidase-mediated [¹⁴C]phenol binding by hydroquinone (Table 3) which indicate that hydroquinone acts as a competitive inhibitor of phenol oxidation by HRP.

The ability of stimulated PMNs to convert hydroquinone into a reactive protein binding species and the 70% increase in this binding induced by phenol suggest that leukocyte-mediated metabolism by myeloperoxidase may be an important metabolic activation pathway in benzene toxicity. This has been proposed for other xenobiotics as well, including estrogens, arylamines, and phenolic compounds (Klebanoff, 1977; Tsuruta *et al.*, 1985; Eastmond *et al.*, 1987a,b). Recent studies by Trush and co-workers (Trush *et al.*, 1985; Cathers and Trush, 1986) have also demonstrated that benzo[a]pyrene-7,8-dihydrodiol is converted in a peroxidase-dependent reaction to a binding and mutagenic species during the oxidative burst of human leukocytes and have suggested that this mechanism may be involved in the hematopoietic toxicity observed with benzo[a]pyrene administration. The bone marrow contains 90% of the body's granulocytic leukocytes (Andrews *et al.*, 1979), many of which are capable of undergoing the oxidative burst and contain considerable levels of myeloperoxidase (Zakhireh and Root, 1979; Bainton *et al.*, 1971). Recent experiments by Khan *et*

al. (1984) have presented evidence that myelotoxicity of benzene is accompanied by an increased production of thiobarbituric acid-reactive products and increased levels of superoxide dismutase in the bone marrow. Each of these results suggest a potential role for O₂⁻ and free radical generation during benzene toxicity such as that which occurs during the oxidative burst of PMNs.

The increased conversion of hydroquinone to 1,4-benzoquinone by myeloperoxidase in the presence of phenol and the ability of these two compounds to produce myelotoxicity *in vivo* provides evidence supporting previous reports that 1,4-benzoquinone is the proximate species responsible for benzene toxicity (Kracke and Parker, 1934; Greenlee *et al.*, 1981; Irons *et al.*, 1981; Irons, 1985). 1,4-Benzoquinone has been shown to bind to protein and DNA and interfere with critical cellular processes such as microtubule assembly and the synthesis of DNA and RNA (Rushmore *et al.*, 1984; Tunek *et al.*, 1980; Irons *et al.*, 1981; Irons, 1985; Schwartz *et al.*, 1985; Post *et al.*, 1984). In addition, the *in vivo* administration of other quinones such as lapachol and Adriamycin have been reported to cause bone marrow damage (Morrison *et al.*, 1970; Carter, 1975). Snyder and Engelsberg (1982) were not able to reproduce the results of an earlier report demonstrating myelotoxicity following 1,4-benzoquinone administration *in vivo* (Kracke and Parker, 1934). The most likely explanation for the failure of *in vivo* administered 1,4-benzoquinone to mimic the hematopoietic toxicity of benzene is due to its reactive properties. Administered 1,4-benzoquinone reacts rapidly with blood and plasma proteins and does not reach the bone marrow in sufficient concentration to produce myelotoxicity (Irons *et al.*, 1981).

Recently, it has been reported that the administration of high doses of the putative benzene metabolite *trans,trans*-muconaldehyde to rodents could result in moderate bone marrow damage (Witz *et al.*, 1985). Al-

though this finding could partially explain the mechanism of benzene toxicity. other metabolites and mechanisms are probably involved since numerous *in vivo* and cellular metabolic studies have failed to observe *trans,trans*-muconaldehyde formation during benzene exposure (Snyder *et al.*, 1981; Rusch *et al.*, 1977; Billings, 1985). *trans,trans*-Muconic acid, the subsequent oxidation product of *trans,trans*-muconaldehyde, has, however, been isolated (Parke and Williams, 1953; Gad-El-Karim *et al.*, 1985a). The high reactivity of muconaldehyde and the numerous cellular defenses within the liver, such as glutathione and aldehyde dehydrogenase (Brabec, 1981; Goldstein *et al.*, 1982), make it unlikely that this reactive aldehyde would be able to travel from the interior of the liver to the bone marrow in significant quantities without being detoxified. The *in vivo* administration of large quantities of *trans,trans*-muconaldehyde would circumvent hepatic detoxification, thereby allowing this reactive aldehyde to reach the bone marrow at cytotoxic concentrations. The detection of both phenol and hydroquinone within the bone marrow *in vivo* following benzene exposure (Rickert *et al.*, 1979), plus our findings that the administration of phenol and hydroquinone in combination produce extensive myelotoxicity, indicate that an interaction between phenolic compounds represents a likely alternative mechanism for benzene toxicity.

One perplexing problem is the inability of administered phenol to produce bone marrow toxicity *in vivo* (Gad-El-Karim *et al.*, 1985; Snyder and Engelsberg, 1982; Mitchell, 1971) since the *in vitro* biotransformation of phenol by the cytochrome P-450 monooxygenase enzymes has been reported to result in the formation of hydroquinone (Tunek *et al.*, 1980; Sawahata and Neal, 1983; Smart and Zannoni, 1984). A potential explanation for this phenomenon is that the *in vivo* hepatic metabolism of exogenously administered phenol differs from the metabolism of phenol

added to *in vitro* incubations or even that of phenol produced endogenously from benzene *in vivo*. This explanation is supported by recent studies by Gilmour *et al.* (1986) which indicate that the metabolism of phenol formed from benzene in microsomal incubations differs from the metabolism of phenol when added directly to microsomal incubations. Other important considerations could be the regimen of metabolite administration or the subsequent differential distribution of these metabolites within the body. The regimen of administration (i.e., twice a day) appears to be important in producing bone marrow suppression. The daily single administration of the metabolites did not result in significant bone marrow toxicity (data not shown). Even using the combined treatment protocol described in this study, daily administration results in a gradual diminution of bone marrow suppression over a 2- to 4-week period. However, discontinuous administration (3-4 days/week) results in prolonged bone marrow suppression for the duration of treatment. Regimen-dependent bone marrow suppression is consistent with previous reports that benzene-induced bone marrow toxicity is cell-cycle dependent (Irons *et al.*, 1979; Muirhead *et al.*, 1980; Pfeifer and Irons, 1981).

In summary, we report that myelotoxicity such as that observed following benzene exposure can be reproduced by the coadministration of phenol and hydroquinone, two major metabolites of benzene. The hematopoietic toxicity observed during benzene exposure therefore appears to depend primarily upon (1) the initial hepatic conversion of benzene to phenol and hydroquinone; (2) a subsequent selective accumulation of hydroquinone in the bone marrow; (3) a localized interaction of phenol and hydroquinone at this site, most probably through a phenol-induced stimulation of the myeloperoxidase-dependent conversion of hydroquinone to 1,4-benzoquinone; and (4) the reaction of 1,4-benzoquinone with macromolecules re-

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sulting in an inhibition of critical cellular processes. Studies within our laboratories are being conducted to elucidate the precise nature of this interaction between phenol and hydroquinone, to investigate the genotoxicity resulting from the coadministration of these two compounds, and to assess the relative contribution of both *trans,trans*-muconaldehyde and 1,4-benzoquinone to myelotoxicity produced by exposure to benzene.

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