An Overview of Benzene Metabolism

Robert Snyder and Christine C. Hedli

Environmental and Occupational Health Sciences Institute, Rutgers, The State University of New Jersey and the University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School, Piscataway, New Jersey

Benzene toxicity involves both bone marrow depression and leukemogenesis caused by damage to multiple classes of hematopoietic cells and a variety of hematopoietic cell functions. Study of the relationship between the metabolism and toxicity of benzene indicates that several metabolites of benzene play significant roles in generating benzene toxicity. Benzene is metabolized, primarily in the liver, to a variety of hydroxylated and ring-opened products that are transported to the bone marrow where subsequent secondary metabolism occurs. Two potential mechanisms by which benzene metabolites may damage cellular macromolecules to induce toxicity include the covalent binding of reactive metabolites of benzene and the capacity of benzene metabolites to induce oxidative damage. Although the relative contributions of each of these mechanisms to toxicity remains unestablished, it is clear that different mechanisms contribute to the toxicities associated with different metabolites. As a corollary, it is unlikely that benzene toxicity can be described as the result of the interaction of a single metabolite with a single biological target. Continued investigation of the metabolism of benzene and its metabolites will allow us to determine the specific combination of metabolites as well as the biological target(s) involved in toxicity and will ultimately lead to our understanding of the relationship between the production of benzene metabolites and bone marrow toxicity. — Environ Health Perspect 104(Suppl 6):1165–1171 (1996)

Key words: benzene, metabolism, toxicity, hydroquinone, 1,2,4-benzenetriol, benzoquinone, covalent binding, oxidative stress, bone marrow, liver

Introduction

The identification of benzene metabolites and their quantification began in the 19th century. However, it was not until carbon-14 was available for the synthesis of [14C]benzene that it was possible to perform accurate measurements and to be sure that all the stable metabolites could be detected. Thus, in 1953 Parke and Williams (1) reported that upon administering [14C]benzene to rabbits they could recover 32.6% of the dose in urine as phenol, catechol, hydroquinone, 1,2,4-benzenetriol, trans-trans-muconic acid, and l-phenylmercapturic acid, 44.9% in the expired air as unchanged benzene and small amounts of carbon dioxide, and 5 to 10% in the feces and tissues. Their total recovery was approximately 84 to 89%. They went on to suggest that benzene toxicity, i.e., benzene-induced bone marrow depression, might be caused by some of these metabolites.

Review of Benzene Toxicity

A discussion of the role of benzene metabolites in benzene toxicity requires a brief review of benzene toxicity (2,3). Benzene toxicity to humans exposed in the workplace has been characterized as either early-reversible hematotoxicity or, with prolonged exposure to high doses, irreversible bone marrow damage. Studies of worker populations in factories in which benzene was employed as a solvent (2) showed a range of hematotoxic effects including anemia, leukopenia, and thrombocytopenia. In some cases, more than one cell type was decreased. A decrease in the levels of all the classes of blood cell types in the circulation is termed pancytopenia and is usually associated with irreversible bone marrow aplasia. Aplastic anemia is most cases fatal. In those who survive aplasia, the marrow appears to be dysplastic. Myelodysplastic syndrome, which has been called “preleukemia,” is probably an early stage of acute myeloid leukemia. Thus, those interested in benzene metabolism need to determine how benzene metabolites contribute to the production of this series of events associated with toxicity.

Benzene Metabolism

The evidence is quite strong that benzene metabolism plays a critical role in benzene toxicity (2,3). Thus, inhibition of benzene metabolism by toluene, a competitive inhibitor, results in a decrease in benzene metabolism and a reduction in benzene toxicity (4). Decreasing the hepatic metabolism of benzene by partial hepatectomy also reduced benzene toxicity, suggesting that hepatic metabolism plays an important role in toxicity (5). In addition to hepatic metabolism, it appears that secondary metabolism of benzene metabolites in bone marrow contributes to toxicity (6–11). Thus, elucidation of the metabolic pathway for benzene biotransformation is essential for a full understanding of the mechanism of toxicity.

Metabolic Pathway for Biotransformation

Figures 1 and 2 show alternative routes by which the first step of benzene metabolism, namely, phenol formation, can occur. Cytochrome P4502E1, and perhaps other cytochromes P450, can generate H2O2 when acting as oxidases of nicotineamide adenine dinucleotide phosphate (NADPH). The hydroxyl radical formed from H2O2 can hydroxylate benzene to yield phenol. An alternative mechanism for phenol formation is seen in Figure 2, which is designed to reflect on the fate of the benzene oxide–oxygen system. When benzene oxide is the first product, it can rearrange nonenzymatically to form phenol. Alternatively, benzene oxide can be hydrated via epoxide hydrolase to yield 1,2-benzene dihydrodiol, which can in turn

CYP450 + NADPH + H+ + O2 → CYP450 + NADP + H2O

H2O2 → 2 OH

Benzene + OH → Phenol

Figure 1. An alternative route of phenol formation from benzene: hydroxylation of benzene by hydroxyl radicals generated from H2O2.
be oxidized via dihydrodiol dehydrogenase to form catechol. The reaction of benzene oxide with glutathione catalyzed by glutathione S-transferase leads to the formation of the premercapturic acid. It is likely that benzene oxide or its oxepin are precursors to ring opening (12). Phenol can be further hydroxylated to form hydroquinone or catechol. In theory, 1,2,4-benzene triol may be formed by the hydroxylation of either hydroquinone or catechol; but Inoue et al. (13) suggested that catechol is not a precursor of 1,2,4-benzene triol in humans.

**Urinary Metabolites**

The metabolites of benzene found in urine are shown in Figure 3. Any of the phenolic metabolites may be conjugated with either sulfate or glucuronide. In addition to 1-phenolmercapturic acid reported by Parke and Williams (1), other mercapturates include 6-N-acetyl cysteiny1-S-2,3-cyclohexadienol (14,15) and 2,5-diOH-phenol-mercapturic acid (16). The urine also contains two ring-opening products, i.e., trans-trans-muconic acid (1,17) and 6-OH-t,t-2,4-hexadienoic acid (18), and the residue of a covalently bound DNA adduct, i.e., 2-OH-p-phenylenediamine (19,20).

**Microsomal Metabolism**

The metabolism of benzene by purified, reconstituted rat liver CYP450E1 is characterized by the requirement of the presence of cytochrome P450 to obtain maximum metabolism (21). At low benzene concentrations (Table 1), a much larger percentage of hydroquinone is formed than at higher benzene concentrations. The addition of epoxide hydrolase also stimulates hydroquinone formation at the expense of phenol. The role of epoxide hydrolase in stimulating the second hydroxylation of benzene is not clear. It may assist in stabilizing CYP450E1 to continue to hydroxylate phenol, the concentration of which rises as benzene metabolism proceeds. Alternatively, it may reflect a hitherto unrecognized metabolic activity leading to hydroquinone formation. Further study of this problem is required.

**Potential Mechanisms of Toxicity**

The production of benzene metabolites, largely in the liver, is followed by their transport to the bone marrow and other organs. There are many possibilities for causing bone marrow toxicity. Iron and coworkers (22,23) suggested that covalent binding of hydroquinone to spindle fiber protein could explain inhibition of cell replication by benzene. Damage to DNA could result in bone marrow depression leading to aplastic anemia, which in survivors leads to marrow dysplasia and ultimately to acute myeloid leukemia (24). Figure 4 diagrams two mechanisms by which benzene metabolites could cause damage to DNA. One pathway focuses on
the metabolic activation of benzene to species that covalently bind to DNA to produce mutagenic events that are expressed as leukemia. The second mechanism involves the production of metabolites that cause oxidative stress, subsequent oxidative damage to DNA, and a mutagenic effect that has the same consequences.

**Covalent Binding and Toxicity**

We have known for almost two decades that benzene is metabolized to species capable of covalent interaction with cellular macromolecules. Snyder et al. (25) first demonstrated that proteins in the bone marrow and other tissues of mice treated with radiolabeled benzene in vivo contained covalently bound radiolabel (Table 2). Lutz and Schlatter subsequently demonstrated covalent binding of benzene metabolites to DNA in rat liver (26). Our studies of the covalent interaction of benzene metabolites with cellular macromolecules suggested that this phenomenon might play an important role in the expression of toxicity. Sammet et al. (5) showed that in rats partial hepatectomy correlated with both protection against benzene toxicity and reduced levels of covalent binding of benzene metabolites in bone marrow; Longacre et al. (27) showed that the levels of covalently bound metabolites measured in the hematopoietic tissues were higher in mouse strains that were more sensitive to benzene toxicity than in those that were less sensitive. Rushmore et al. (28) extensively investigated covalent binding in an isolated mitochondrial system; they showed that the benzene metabolites are capable of covalent binding to DNA and inhibiting protein and RNA synthesis. To chemically characterize the DNA adducts formed, adducts were prepared in vitro by reacting deoxynucleosides or deoxynucleoside monophosphates with either P-benzozquinone or hydroquinone in the presence of an oxidizing agent. The combination of UV, fluorescence, mass, and nuclear magnetic resonance spectrometry was first used by Jowa et al. (29) to identify 3'-OH-1,2-benzathene-2'-deoxyguanosine as a major deoxyguanosine adduct (Figure 5). Pongracz et al. (30,31) and Levay et al. (32) subsequently combined these spectroscopic methods of structural analysis with the sensitive DNA-[32P]post-labeling method of adduct detection to identify (3'-OH)-3,N4-benzathenedeoxyctydindine-3'-phosphate, (3'-OH)-1,N6-benzathene-2'-deoxyadenosine-3'-phosphate and (3'-OH)-1,N6-benzathene-2'-deoxyguanosine-3'-phosphate following the in vitro reaction of p-benzozquinone and calf thymus DNA. The structures of the deoxyribonucleoside forms of these adducts are shown in Figure 5.

Although in vitro studies have established that reactive metabolites of benzene covalently bind to DNA, in vivo evidence of covalent binding has been more difficult to demonstrate. Initial studies involving administration of radiolabeled benzene to rats by Lutz and Schlatter (26) as well as later studies by Artellini et al. (33) and Mazullo et al. (34) using rats and mice, demonstrated covalent binding to DNA of several animal tissues including liver, bone marrow, spleen, kidney, stomach, and lung. Attempts to identify adduct formation in vivo using the [32P]post-labeling method have not, however, been highly successful.
These problems may be related to the low covalent binding index of benzene, the complex nature of the bone marrow, and difficulties in establishing both an optimal treatment regimen and an animal model system that accurately reflects all the toxic responses to benzene observed in humans.

The most consistent demonstrations of benzene metabolite-induced DNA adduct formation in a cellular model have been made using human promyelocytic (HL-60) cells in culture, a line of myeloid cells that has the capacity to differentiate in response to specific chemical stimulants into any of the four classes of hematopoietic cells of the myelomonocytic lineage, i.e., granulocytes, monocytes, eosinophils, or macrophages (35). Studies using this model and the \[^{32}P\] postlabeling method of adduct detection by Levay et al. have led to the detection of DNA adducts in benzene metabolite-treated cells that are not chromatographically identical with those formed following the \textit{in vitro} reaction of p-benzoquinone and DNA (32). Although these investigations showed that benzene metabolites interact synergistically to produce DNA adducts (36) and that peroxidase activation of hydroquinone is required for adduct formation (37), no attempts were made to link adduct formation with any end point of toxicity other than cytotoxicity. We recently evaluated the significance of DNA adduct formation in toxicity by studying the effects of benzene metabolites on DNA adduct formation and retinoic acid-induced granulocytic differentiation in this model (38).

Table 3 shows that while treatment of HL-60 cells with 50 μM hydroquinone for 1 to 4 hr induced the formation of a single DNA adduct that increased with increasing time of exposure, no adducts were detected in cells treated with 50 to 500 μM 1,2,4-benzene triol for up to 4 hr. Using the same incubation conditions, treatment of the cells with either hydroquinone or 1,2,4-benzene triol prior to inducing differentiation with retinoic acid significantly inhibited their capacity to differentiate, as assessed by evaluating cell morphology, using light and electron microscopy, and two indicators of cell functional capacity—phagocytosis and nitroblue tetrazolium reduction. These data indicate that DNA adduct formation may play a role in inhibiting cell differentiation in hydroquinone but not in 1,2,4-benzene triol-treated cells, and support the contention that various metabolites contribute to different components of the mechanism of toxicity.

**Oxidative Stress and Toxicity**

The potential for oxidative stress to contribute to benzene toxicity is closely tied to specific benzene metabolites. Hydroquinone may be oxidized to p-benzoquinone, which is highly reactive and can covalently bind to cellular macromolecules (above) or to glutathione. Alternatively, benzene metabolites may engage in redox cycling, which involves autooxidation of a reduced form of the metabolite to yield an oxidized species plus reactive oxygen. The bone marrow, which is a richly oxygenated organ, has the capability to generate reactive oxygen species. The four-electron reduction of oxygen (39) may generate superoxide anion, hydrogen peroxide, and hydroxyl radical. The oxidized metabolite may undergo flavoprotein reduction to yield the starting material that may reenter the redox cycle.

It might be postulated that hydroquinone–p-benzoquinone would be likely to undergo redox cycling. Recent studies by Boersma et al. (40) argue to the contrary. Figure 6 shows that the reduction of p-benzoquinone may proceed via a reductase such as CYPI450 reductase in two steps. The first product would be the semiquinone anion.

---

**Table 3. Effect of benzene metabolite treatment on granulocytic differentiation and DNA adduct formation in HL-60 cells.**

| Treatment                  | DNA binding by \[^{32}P\] postlabeling | Morphological evaluation by light and electron microscopy | Phagocytosis of sheep red blood cells | Functional evaluation |
|---------------------------|---------------------------------------|----------------------------------------------------------|--------------------------------------|-----------------------|
| Negative-control untreated cells | None                                   | Immature undifferentiated                                   | –                                    | –                     |
| Positive-control untreated—RA induced 50 μM HQ/0.5 to 4 hr | Not analyzed                           | Mature undifferentiated                                      | +                                    | –                     |
| 50 μM BT/2 hr              | Single adduct/time-dependent increase | Less mature cells, differentiation inhibited                | ↓                                    | ↓                     |
| 250 μM BT/2 hr, 4 hr       | Not detectable                         | Differentiation inhibited                                   | ↓                                    | ↓                     |
| 500 μM BT/4 hr             | Not detectable                         | Not analyzed                                              | Not analyzed                        | Not analyzed          |

---

**Figure 6.** Two-step reduction of p-benzoquinone by reductase (FPH2): evidence that p-benzoquinone does not support redox cycling at physiological pH. Steps 1, 2A, and 2B are sites at which reactions with oxygen to yield superoxide would occur in redox cycling. However, p-benzoquinone is stoichiometrically reduced to hydroquinone at pH 7.5, the pH of the cell (40).
radical (Step 1), which could either be reduced again (Step 2A), or may—more likely—be protonated before the second reduction (Step 2B). The pKₐ of the protonation step is 4.1, suggesting that at the pH of the cell, i.e., approximately 7.5, the anionic form would predominate. Following the second reduction, the monoanion of hydroquinone would be formed, but the pKₐ for its protonation is 9.85, indicating that it would exist mainly in the diprotonated form. Reoxidation to p-benzoquinone, the next step in redox cycling, would be inhibited because it is the monanion that is the substrate for autooxidation leading to superoxide anion formation. Thus, it is unlikely that hydroquinone-p-benzoquinone undergoes redox cycling at physiological pH.

The metabolic fate of p-benzoquinone, if it is not reduced, may be to react with glutathione (GSH) to form the premercapturic acid of hydroquinone, which may go on to form the mercapturic acid, or may undergo slow autooxidation leading to the production of reactive oxygen species (41) (Figure 7). Alternatively, p-benzoquinone may be converted to its epoxide either via CYP450 or HOOH (Figure 8, Step 1), leading to p-benzoquinone 2,3-oxide and ultimately to 1,2,4-benzenetriol (Step 2A) following either a two-electron reduction by diaphorase or two one-electron reductions by CYP450 reductase. Reaction of p-benzoquinone 2,3-oxide with GSH leads to the formation of glutathionyl-p-benzoquinone 2,3-oxide and ultimately to 1,2,4-benzenetriol (Figure 8, Step 2B).

The unique feature of glutathionyl 1,2,4-benzenetriol is its ability to spontaneously undergo autooxidation and redox cycling (Figure 9). Brunmark and Cadenas (41) showed that glutathionyl benzenetriol could be sequentially oxidized to glutathionyl-1,2,4-benzenetriol semiquinone and then to 5-OH-glutathionyl-p-benzoquinone (HGB). HGB is also the product of the reaction between p-benzoquinone 2,3-oxide and glutathionyl-1,2,4-benzenetriol semiquinone (GBS), which also yields 5-OH-benzosemiquinone. Alternatively, GBS can undergo disproportionation to form glutathionyl-1,2,4-benzenetriol, which can initiate the redox cycle again. If redox cycling is an important phenomenon in the mechanism of benzene toxicity, it is likely that 1,2,4-benzenetriol plays a significant role in the process.

In a series of studies in HL-60 cells, hydroquinone, p-benzoquinone and 1,2,4-benzenetriol were added so that researchers could study their impacts on oxidative stress and antioxidant factors (42). Table 4 shows that hydroquinone and p-benzoquinone increased superoxide, nitric oxide, and HOOH production, but that 1,2,4-benzenetriol, while increasing superoxide and HOOH, had no effect on nitric oxide production. Hydroquinone and p-benzoquinone but not 1,2,4-benzenetriol decreased catalase activity. Hydroquinone and 1,2,4-benzenetriol but not p-benzoquinone decreased superoxide dismutase and hydroquinone and benzenetriol decreased sulfhydryl levels. Thus, although all of the metabolites induce oxidative

1. Autoxidation of 2-OH-5-glutathionyl hydroquinone to yield 2-OH-5-glutathionyl-p-benzoquinone

\[ \text{GS - OH} + \text{O}_2 \rightarrow \text{GS - OH - O}^* \]

2. Alternative mechanism for 2-OH-5-glutathionyl-p-benzoquinone production via redox transition

\[ \text{GS - OH} + \text{O}_2 \rightarrow \text{GS - OH}^* \]

3. Disproportionation of the hydroxysemiquinone

\[ \text{GS - OH}^* \rightarrow \text{GS - OH} + \text{GS - OH}^* \]

Figure 7. Potential interaction of p-benzoquinone with glutathione (1) anaerobic [1:1 stoichiometry; rapid]; (2) aerobic (slow autooxidation).

Figure 8. The formation of p-benzoquinone 2,3-oxide from p-benzoquinone and its metabolic fate. Step 1. p-Benzoquinone is converted to its epoxide by the addition of HOOH. Step 2A. p-Benzoquinone is reduced to 1,2,4-benzenetriol by either one-electron reduction (1) catalyzed by P450 reductase or two-electron reduction (2) catalyzed by DT diaphorase. Step 2B. Reaction of 1,2,4-benzenetriol with glutathione to yield glutathionyl 1,2,4-benzenetriol.
modifications in these cells, their individual impacts on oxidative stress and antioxidant factors in these cells are different.

Potency of Benzene and Its Metabolites in Producing Toxicity

In attempting to sort out the role played by the various benzene metabolites in the production of benzene toxicity, it is helpful to examine the potency with which each of the metabolites, alone or in combination, causes toxic effects. There are many stages in bone marrow cell maturation and amplification and there are a number of functions peculiar to the stromal cells that provide targets for attack by benzene and its metabolites. It would be helpful to review a range of potencies of each. Table 5 shows the relative potency with which benzene and its metabolites inhibit erythropoiesis, as measured by the method of Lee et al. (43) using the [59Fe] uptake technique. The numbers are not absolute values but are rounded off to demonstrate the range of doses at which significant depressions in red cell production were observed. The data demonstrate that when administered to mice in a defined dosing regimen, benzene is the least potent member of the series and the combination of hydroquinone plus muconaldehyde provides the greatest potency. The doses of each chemical used in the latter case were too low for either muconaldehyde or hydroquinone to produce bone marrow depression given independently, but they were highly effective when given in combination. Many of the other benzene metabolites were effective at decreasing iron uptake, albeit with different potencies, except for phenol, which was clearly ineffective. 1,2,4-Benzenetriol was ineffective in preliminary studies, but further work is needed to establish the significance of these observations, since a decrease in lymphocytes has been observed in the bone marrow of animals treated with 1,2,4-benzenetriol (CC Hedli and RS Snyder, unpublished observations). In addition, the recent demonstration of 1,2,4-benzenetriol as a microsomal metabolite of benzene in isolated mouse but not rat hepatocytes (44) suggests that the production of this metabolite might correlate with susceptibility to toxicity. Further experiments are in progress to determine the effect of 1,2,4-benzenetriol treatment on circulating blood cells and other hematopoietic tissue.

Summary and Conclusions

Benzene toxicity, which involves both bone marrow depression and leukemogenesis, appears to require metabolites of benzene that impinge on several cell types and on a variety of functions. We must continue to study the metabolism of benzene with the intent of understanding which bone marrow cells metabolize benzene and its metabolites, identifying the specific array of metabolites responsible for the disease processes, and ultimately understanding the relationship between the events in bone marrow toxicity and the generation of specific metabolites.

| Table 4. Impact of hydroquinone, p-benzoquinone, and 1,2,4-benzenetriol on oxidative stress and antioxidant factors in HL-60 cells activated by 12-O-tetradecanoylphorbol-13-acetate. |
| Factor | HQ | BQ | BT | Method |
| --- | --- | --- | --- | --- |
| Superoxide | Increase | Increase | Increase | Reid and Loeb (45) |
| Nitric oxide | Increase | Increase | No change | Green et al. (46) |
| Hydrogen peroxide | Increase | Increase | Increase | Bass et al. (47) |
| Myeloperoxidase | Decrease | Decrease | No change | Himmelhoch et al. (48) |
| Catalase | Decrease | Decrease | No change | Aebi (49) |
| SOD | Decrease | No change | Decrease | Marklund and Marklund (50) |
| Vitamin C | No change | No change | No change | Attwood et al. (51) |
| Thiols (GSH) | Decrease | No change | Decrease | Beutler et al. (52) |

| Table 5. Relative potencies of benzene and benzene metabolites in short-term tests of erythropoiesis. |
| Compound | Dose, mg/kg (3 doses, 2 days) |
| --- | --- |
| Benzene | 1000 |
| Phenol | 100 |
| Hydroquinone sulfate | 300 |
| Hydroquinone | 100 |
| Catechol | 10 |
| 1,2,3-Benzenetriol | 1 |
| p-Benzoquinone | 1 |
| Muconaldehyde | 1 |
| Muconaldehyde + hydroquinone | 0.05–0.01 (est) |

*Measured using the [59Fe] uptake assay (42). |

*Estimated value.

REFERENCES

1. Parke DV, Williams RT. Studies in detoxication 49. The metabolism of benzene containing (14C) benzene. Biochem J 54:231–238 (1953).
2. Snyder R, Kocsis JJ. Current concepts of chronic benzene toxicity. Crit Rev Toxicol 3:265–288 (1975).
3. Snyder R, Witz G, Goldstein BD. The toxicityology of benzene. Environ Health Perspect 100:293–306 (1993).
4. Sammet D, Lee EW, Kocsis JJ, Snyder R. Partial hepatocytosis reduces both metabolism and toxicity of benzene. J Toxicol Environ Health 5:785–792 (1979).
5. Andrews LS, Lee EW, Wittert CM, Kocsis JJ, Snyder R. Effects of toluene on the metabolism, disposition, and hemopoietic toxicity of 3H benzene. Biochem Pharmacol 26:293–300 (1977).
6. Andrews LS, Sassahe HA, Gillette JR. 3H Benzene metabolism in rabbit bone marrow. Life Sci 25:567–572 (1979).
7. Irons R, Dent J, Baker T, Rickert D. Benzene is metabolized and covalently bound in bone marrow in situ. Chem Biol Interact 30:241–245 (1980).
8. Post G, Snyder R, Kalf GF. Metabolism of benzene and phenol in macrophages in vitro and inhibition of RNA synthesis by benzene metabolites. Cell Biol Toxicol 2:231–246 (1986).
9. Schlosser MJ, Kalf GF. Metabolic activation of hydroquinone by macrophage peroxidase. Chem Biol Interac 72:191–207 (1989).
10. Subrahmanyam VV, Doane-Setzer P, Steinmetz KL, Ross D, Smith MT. Phenol-induced stimulation of hydroquinone bioactivation in mouse bone marrow in vivo: possible implications in benzene myelotoxicity. Toxicology 62:107–116 (1990).
11. Subrahmanyam VV, Kolanchana P, Smith MT. Hydroxylation of phenol to hydroquinone catalyzed by a human myeloperoxidase-superoxide complex: possible implications in benzene myelotoxicity. Free Radic Res Comm 15:285–296 (1991).
12. Witz G, Zhang Z, Goldstein BD. Reactive ring-opened aldehyde metabolites in bone marrow hematoxicity. Environ Health Perspect 104(Suppl 6):1195–1199 (1996).
13. Ioue O, Seiji K, Nakatsuka H, Watanabe T, Yin S-N, Li G-L, Cai S-X, Jin C, Ikeda M. Excretion of 1,2,4-benzenetriol in the
Toxicol Appl Pharmacol 46:559-565 (1989).
14. Sabourin PJ, Bechtold WE, Birnbaum LS, Lucier G, Henderson RF. Differences in the metabolism and disposition of inhaled [3H] benzene by F344/N rats and B6C3F1 mice. Toxicol Appl Pharmacol 94:128-140 (1988).
15. Sabourin PJ, Bechtold WE, Henderson RF. A high pressure liquid chromatographic method for the separation and quantitation of water-soluble radiolabeled benzene metabolites. Anal Biochem 170:316-327 (1988).
16. Neerland DE, Pierce WM. Identification of N-acetyl-S(2,5-dihydroxyphenyl)-L-cysteine as a urinary metabolite of benzene, phenol and hydroquinone. Drug Metab Dispos 18(6):958-961 (1990).
17. Drummond JC, Finar IL. Muconic acid as a metabolic product of benzene. Biochim Biophys Acta 32:79-84 (1938).
18. Kline SA, Robertson JF, Grotz VL, Goldstein BD, Witl G. Identification of 6-hydroxy-trans,trans-2,4-hexadienoic acid, a novel ring-opened urinary metabolite of benzene. Environ Health Perspect 101:310-312 (1993).
19. Muller G, Koebl M, Hegar M, Norpoh K. Urinary S-phenyl mercapturic acid and phenylguanine as indicators of benzene exposure. In: Biological Monitoring of Exposure to Chemicals, Organic Compounds (Ho MH, Dillion HK, eds), New York: John Wiley & Sons, 1987, 91-98.
20. Norpoh K, Stucker W, Krewet E, Muller G. Biomonitoring of benzene exposure by trace analysis of phenylguanine. Int Arch Occup Environ Health 60:163-168 (1988).
21. Snyder R, Chepiga T, Yang CS, Thomas H, Platt K, Oesch F. Benzene metabolism by reconstituted cytochromes P450 2B1 and 2E1 and its modulation by cytochrome b5, microsomal epoxide hydrolase, and glutathione transferase: evidence for an important role of microsomal epoxide hydrolase in the formation of hydroquinone. Toxicol Appl Pharmacol 122:172-181 (1993).
22. Irons RD, Neptun DA. Effects of principle hydroxy-metabolites of benzene on microtubule polymerization. Arch Toxicol 45:297-305 (1980).
23. Pfeifer RW, Irons RD. Alteration of lymphocyte function by quinines through sulphhydryl-dependent disruption of microtubule assembly. Int J Immunopharmacol 5:463-470 (1983).
24. Snyder R, Kalf GF. A perspective on benzene leukemogenesis. Crit Rev Toxicol 24(3):177-209 (1994).
25. Snyder R, Lee EW, Kocsis JJ. Binding of labeled benzene metabolites in mouse liver and bone marrow. Res Commun Chem Pathol Pharmacol 20:191-194 (1978).
26. Lurz WK, Schlatter CH. Mechanism of the carcinogenic action of benzene: irreversible binding to rat liver DNA. Chem Biol Interact 18:241-245 (1979).
27. Longacre S, Kocsis JJ, Snyder R. Influence of strain differences in mice in the metabolism and toxicity of benzene. Toxicol Appl Pharmacol 60:398-409 (1981).
28. Rushmore T, Kalf G, Snyder R. Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow microdria in vivo. Chem Biol Interact 49:133-154 (1984).
29. Iwa L, Witl G, Snyder R, Winkle S, Kalf GF. Synthesis and characterization of deoxyguanosine-benziquinone adducts. J Appl Toxicol 10:47-54 (1990).
30. Pongracz K, Kaur S, Burlingame AL, Bodell WJ. Detection of 2'-hydroxy-2'-deoxyadenosine 3'-phosphate by 32P-postlabeling of DNA reacted with p-benzoquinone. Carcinogenesis 11:1469-1472 (1990).
31. Pongracz K, Bodell WJ. Detection of 3'-hydroxy-1,46-benzetheno-dioxycytidine 3'-phosphate by 32P-postlabeling of DNA reacted with p-benzoquinone. Chem Res Toxicol 4:199-202 (1991).
32. Levay G, Pongraz K, Bodell WJ. Detection of DNA adducts in HL-60 cells treated with hydroquinone and p-benzoquinone by 32P-postlabeling. Carcinogenesis 12:1181-1186 (1991).
33. Arfellini G, Grilli S, Colaci A, Mazzullo M, Parodi G. In vivo and in vitro binding of benzene to nucleic acids and proteins of various rat and mouse organs. Cancer Lett 28:159-168 (1985).
34. Mazzullo M, Bartoli S, Bonora B, Colacci A, Grilli S, Lattanzi G, Niero A, Turina MP. Benzene adducts with rat nucleic acids and proteins: dose-response relationship after treatment in vivo. Environ Health Perspect 82:259-266 (1989).
35. Collins SJ. The HL-60 promyelocytic leukemia cell line: proliferation, differentiation and cellular oncogene expression. Blood 70:1233-1244 (1987).
36. Levay G, Bodell WJ. Potentiation of DNA adduct formation in HL-60 cells by combinations of benzene metabolites. Proc Natl Acad Sci USA 89:7105-7109 (1992).
37. Levay G, Ross D, Bodell WJ. Peroxidase activation of hydroquinone results in the formation of DNA adducts in HL-60 cells, mouse bone marrow macrophages and human bone marrow. Carcinogenesis 14:2329-2334 (1993).
38. Hedli CC, Rao NR, Reuhl KR, Wittmer CM, Snyder R. Effects of benzene metabolite treatment on DNA adduct formation and granulocytic differentiation in HL-60 cells. Arch Toxicol 70:135-145 (1996).
39. Williams RJP. An introduction to the biological chemistry of oxygen. In: The Biology and Chemistry of Oxygen (Banister JV, Bannister WH, eds). New York: Elsevier, 1984.
40. Boersma GM, Balvers WG, Boeren S, Vervoort J, Rietjens IMCM. NADPH-cytochrome reductase catalyzed redox cycling of 1,4-benzoquinone; hampered at physiological conditions, initiated at increased pH values. Biochem Pharmacol 47:1994-1995 (1994).
41. Brunmark A, Cadenas E. Reductive addition of glutathione to p-benzoquinone, 2-hydroxy-p-benzoquinone, and p-benzoquinone epoxides. Effect of hydroxy- and glutathionyl substituents on p-benzoquinone autooxidation. Chem Biol Interact 86:273-298 (1988).
42. Rao NR, Snyder R. Oxidative modifications produced in HL-60 cells on exposure to benzene metabolites. J Appl Toxicol 15:403-409 (1995).
43. Lee EW, Kocsis JJ, Snyder R. The use of ferrokinetics in the study of experimental anemia. Environ Health Perspect 39:29-37 (1981).
44. Orzechowski A, Schwarz LR, Schwegler U, Bock KW, Snyder R, Schrenk D. Benzene metabolism in rodent hepatocytes: role of sulphate conjugation. Xenobiota 25(10):1093-1102 (1995).
45. Reid TM, Loeb AL. Mutagenic specificity of oxygen radicals produced by human leukemia cells. Cancer Res 52:1082-1086 (1992).
46. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and (15N)nitrate in biological fluids. Anal Biochem 126:131-138 (1982).
47. Bass DA, Parce JW, Dechatlot LR, Szejda P, Seeds MC, Thomas M. Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation. J Immunol 130:1910-1917 (1988).
48. Himmelhoch HR, Evans WH, Mage MG, Peterson EA. Purification of myeloperoxidase from the bone marrow of the guinea pig. Biochemistry 8:9194-9197 (1969).
49. Aebi H. Catalase in vitro. Methods Enzymol 105:121-126 (1984).
50. Marklund S, Marklund G. Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469-474 (1974).
51. Attwood KC, Ruley EE, Ross J, Bradley F, Kramer JJ. Determination of platelet and leukocyte vitamin C and the levels found in normal subjects. Clin Chim Acta 54:95-105 (1974).
52. Beutler E, Duron D, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 61:882-886 (1963).