@CTITLE = Species Differences in Metabolism of 1,3-Butadiene

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@H1 = INTRODUCTION

1,3-Butadiene (BD) is a 4-carbon gaseous compound with two double bonds. Used in high tonnage to make styrene-butadiene polymers in the rubber industry, BD is one of the top 20 chemicals in amount manufactured in the USA (Agency of Toxic Substances and Disease Registry, 1992). BD has also been detected in ppb levels in urban air, gasoline exhaust, and cigarette smoke. Because of large amounts used, BD was tested for toxicity in 2-year inhalation exposures of both Sprague-Dawley rats (Owen et al., 1987) and B6C3F₁ mice (Huff et al., 1985; Melnick et al., 1990).

@ = The results of the two-species studies were dramatically different. In the initial study in mice (Huff et al., 1985), BD was shown to be a potent multiple-site carcinogen at exposure levels of 625 and 1250 ppm. There were increased incidences of neoplasia in the heart, lung, mammary gland, and ovary; malignant lymphomas resulted in early deaths of the mice so that the planned 2-year study was stopped after only 61 weeks of exposure. The second study in mice (Melnick et al., 1990) was conducted at much lower exposure concentrations (6.25, 20, 62.5, 200, and 625 ppm) and lasted 104 weeks. Increased incidences of hemangiosarcomas of the heart and lung neoplasia were observed in males exposed to 62.5 ppm BD, while females had increased lung neoplasia even at the 6.25 ppm exposure level. Early deaths from lymphomas were again observed at the high exposure concentration (625 ppm). A noncancer toxicity observed in mice was a macrocytic, megaloblastic anemia.

@ = In contrast, the results of exposures of rats to 1000 or 8000 ppm BD over a 2-year period indicated that BD is a weak carcinogen (Owen et al., 1987). Some increase in mammary tumors was observed in females exposed to 1000 ppm BD; after exposure to 8000 ppm BD, there were also increases in thyroid and testicular tumors. Lymphomas nor anemia were observed. The species differences in response to BD caused several laboratories to begin studies on species differences in metabolism of BD as a potential cause for the different responses.

@H1 = PATHWAYS OF METABOLISM OF BD

A simplified summary of the metabolism of BD to reactive intermediates is shown in Figure 1. Three epoxides can be formed: the monoepoxide (BDO), which is the initial metabolic product; the diepoxide (BDO₂), which can be formed from the monoepoxide; and the diolepoxide, which can be formed from either the partial hydrolysis of the diepoxide or the oxidation of the butene diol formed from hydrolysis of BDO. The mutagenicity of these epoxides has been studied in several laboratories, but recently Cochrane and Skopek (1994) compared the mutagenicity of all three epoxides in the same two test systems. They used TK6 human lymphoblastoid cells and assayed for mutations at the *tk* and *hprt* loci. BDO₂ was an approximately 100-fold more potent mutagen than the other two epoxides. At the *hprt* locus, half of the mutations induced by the diepoxide involved loss of wild-type *hprt* restriction fragments, suggesting cross-linking. Such cross-linking is a characteristic expected from a molecule with two reactive sites, like the diepoxide. Such deletions were not observed in mutations induced by the monoepoxide.

@FIGURE = Figure 1. Major metabolic pathways of BD. Cyt P450 = cytochrome P₄₅₀; EH = epoxide hydrolase; GSH = glutathione; M-I = 1,2-dihydroxy-4-(N-acetylcysteinyl-S-)butane;

MASTER

M-II = 1-hydroxy-2-(N-acetylcysteinyl-S-)-3-butene. The three epoxide metabolites are enclosed in rectangles.

@H1 = *IN VITRO* STUDIES OF SPECIES DIFFERENCES IN METABOLISM OF BD

Early studies by Schmidt and Loeser (1986) compared the metabolism of BD and BDO in liver S-9 preparations from mice, rats, monkeys, and humans. The conversion of BD to BDO was greatest in the mouse and lowest in the monkey, with a 5-fold difference between the two species. Rat and human liver were approximately equal and intermediate between the mouse and human preparations. The hydrolysis of BDO to form the butene diol was high in the two primate species and more than 10-fold higher than in the rodents.

@ = More extensive studies have been conducted by Csanàdy et al. (1992, 1993) in which the metabolism of BD and BDO was measured in lung and liver homogenates from mice, rats, and humans; the conversions of BDO to BDO₂ and to the glutathione (GSH) conjugate were measured in addition to BDO hydrolysis. The conversion of BD to BDO was highest in the mouse liver, as was observed in the earlier studies, and lowest in the rat (4-fold lower than mouse). However, the metabolism of BD to BDO in the lung tissue was 10-fold higher in the mouse than in the human or rat tissue and equaled the metabolism found in mouse liver. The conversion of BDO to BDO₂ was detected only in the mouse liver. As was observed in the studies of Schmidt and Loeser (1986), the hydrolysis of BDO to the diol was 10 times higher in human liver preparations than in either rodent samples. On the other hand, the conversion of BDO to a GSH conjugate was 10-fold higher in the mouse preparations than in the human. The data suggest that the operative pathway for eliminating BDO in the mouse is by GSH conjugation; in primates, it is the hydrolysis to the butene diol that is important.

@H1 = *IN VIVO* STUDIES OF SPECIES DIFFERENCES IN BD METABOLISM

Concurrent with the *in vitro* studies on BD metabolism, studies were conducted on metabolism of BD in whole animals. In a series of studies at the Inhalation Toxicology Research Institute, B6C3F₁ mice, Sprague-Dawley rats, and cynomolgus monkeys were exposed to varying levels of ¹⁴C-BD, and metabolites in blood and urine were measured (Bond et al., 1986; Dahl et al., 1991; Sabourin et al., 1992). The studies addressed the question of species differences in uptake of inhaled BD, excretion routes, and blood and urine levels of BD metabolites. At low exposure concentrations, the percent of inhaled BD that was retained in the body was 20% in mice and 3–4% in rats or monkeys (Bond et al., 1986; Dahl et al., 1991). This 5-fold difference between the uptake in mice versus the other species is similar to the 5-fold difference in the metabolism of BD to BDO in mouse liver preparations compared to liver preparations from other species. Excretion patterns were similar in mice and rats with half of the internal dose of BD being excreted in the urine, 15–20% being exhaled as volatile metabolites or small amounts of BD, 15–10% retained bound in the body, and small fractions excreted in feces or exhaled as CO₂. The monkey exhaled almost twice as much of the internal dose of BD as CO₂ as was excreted in urine. The high conversion of BD to CO₂ in the monkey may reflect the high level of epoxide hydrolase activity in primate livers resulting in conversion of BD to the tetrol and further oxidation to CO₂. Further experimental work is required to support this hypothesis. The ¹⁴C content of the blood of the exposed mice, rats, and monkeys indicated that for equivalent exposure concentrations, the mouse has the highest amount of BD-derived ¹⁴C, rats have half the amount in mice, and monkeys have less than 7% of that of mice. Vacuum distillation of the blood followed by trapping of volatile metabolites by cold traps at the appropriate temperatures suggested the

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presence of BDO in mouse blood at 5-fold higher levels than in the rat and at 15-fold higher levels than in the monkey (Dahl et al., 1991).

@ = Characterization of the urine from mice, rats, or monkeys exposed to ^{14}C -BD indicated two major urinary metabolites, both mercapturic acids (Sabourin et al., 1992; Bechtold et al., 1994). One mercapturic acid (called M-II) was formed from the GSH conjugation with BDO and was highest in amount relative to the total urinary metabolites in the mouse compared to the rat and the monkey. The other mercapturic acid (called M-I) was identified as 1,2-dihydroxy-4-(N-acetylcysteinyl)butane, the mercapturic acid of butane-1,2-diol, the hydrolysis product of BDO. If fully deuterated BD was administered to rodents, the M-I metabolite lost one deuterium atom, suggesting that an oxidation/reduction reaction occurred in the process (Fig. 2). M-I was the major urinary metabolite in the urine of exposed monkeys with only trace amounts of M-II present. In analyses of urine from humans exposed to low levels of BD in the workplace, using nonradioactive methods of analysis, only M-I, and not M-II, could be detected (Bechtold et al., 1994). The rat urine had approximately equal amounts of M-I and M-II. The ratio of M-I to the total M-I + M-II in the urine of the different species was proportional to the epoxide hydrolase activity (measured using BDO as a substrate) in livers of the different species (Sabourin et al., 1992; Bechtold et al., 1994). The data are in agreement with the *in vitro* data and suggest that a major pathway for removal of BDO in the mouse is via conjugation with GSH, while a major route in primates is by hydrolysis to the butenediol followed by conjugation with GSH.

@FIGURE = Figure 2. Possible metabolic pathway to form M-I. Used by permission from Bechtold et al., 1994.

@ = Recently, there has been increased interest in BDO_2 as the potential potent carcinogen in mice. Two laboratories reported finding no BDO_2 in the blood of rats exposed to BD, while the mouse had blood BDO_2 levels approximately equal to that of BDO (Table 1) (Himmelstein et al., 1994; Bechtold et al., 1995). Subsequently, a method was developed to increase the ability to detect low levels of metabolites (Bechtold et al., 1995). The method used multidimensional gas chromatography/gas chromatography/mass spectroscopy (GC/GC/MS) in which an initial, large-bore GC column was used to increase the volume of samples that could be applied, a cryofocusing unit was used to collect peaks coming off the large column, and the peaks were then reinjected on a small-bore column for resolution. The peaks were identified and quantitated by isotope dilution MS. Even this method did not result in detection of BDO_2 in rat blood. However, when blood or tissues from three rats were combined, and the volatile metabolites (including BDO_2) were vacuum distilled into cold traps, BDO_2 could be detected in rat blood, albeit at levels 100-fold less than in the mouse (J. R. Thornton-Manning, personal communication).

@TABLE = Table 1. Blood levels of BD metabolites (nmole/ml; $\bar{X} \pm \text{SE}$)

@H1 = DISCUSSION

The data from both *in vitro* and *in vivo* studies suggest that species differences in the metabolism of BD, particularly in the formation of the BDO_2 , can help explain the dramatic species differences in the toxicity of inhaled BD. The qualitative nature of metabolism of BD is similar in the different species, to the extent the pathways have been studied. The quantitative nature of the metabolism along the different pathways is, however, quite different in the different species. All species studied thus far form BDO as the primary metabolite of BD. The major differences lie in how the different species further metabolize the BDO, to form BDO_2 or to clear BDO via excretion as one of two types of mercapturic acid.

@ = The quantitative differences in clearance via the excretion pathways to form either M-I or M-II parallel the quantitative differences in epoxide hydrolase activities in the livers of the different species. In the mouse, more of the BDO is cleared by conjugation of the BDO with GSH, followed by excretion of the mercapturic acid (M-II) in the urine than is cleared by hydrolysis of BDO followed by conjugation with GSH. Knowing that 50% of the internal dose of BD in the mouse is excreted in the urine (Bond et al., 1986), and 62% or 185 of the urinary metabolites are M-II or M-I, respectively (Sabourin et al., 1992; Bechtold et al., 1994), one can calculate that approximately one-third of the internal dose of BD is cleared via direct conjugation of BDO with GSH and only 9% by hydrolysis followed by conjugation with GSH.

@ = In the rat, the amount of M-I and M-II excreted in the urine suggests that approximately 40% of the internal dose of BD is also excreted via the pathways leading to formation of M-I and M-II, but about 20% is excreted by each pathway. However, either very little BDO₂ is formed in the rat, or what is formed is rapidly removed. In primates, in which 31% of the internal dose is excreted in urine, 53% of urinary metabolites is M-I, and 4% is M-II. This suggests that 16% of the BDO is cleared via hydrolysis, then conjugation with GSH, and 1% by direct conjugation with GSH.

@ = The greatest quantitative differences among the species in the metabolism of BD are the formation and clearance of the highly mutagenic BDO₂. In the mouse, a large fraction of BDO is converted to the highly mutagenic BDO₂, based on the findings of two laboratories that blood levels of BDO₂ equal those of BDO. In the rat, levels of BDO₂ are barely detectable. Either the rat does not make much BDO₂, or the rat can rapidly clear the compound. No toxicity studies have been reported in the monkey, so the blood levels of BDO₂ following BD exposure are unknown.

@ = In conclusion, by far the major quantitative difference observed between metabolic products of BD in the sensitive species (mouse) compared to the nonsensitive species (rat) is the 100-fold higher blood concentrations of BDO₂ in exposed mice compared to rats. The evidence suggests that BDO₂ may be of primary importance in the carcinogenicity of BD in mice.

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Table 1
Blood Levels of BD Metabolites
(nmole/ml; $\bar{X} \pm \text{SE}$)

	62.5 ppm BD, 6 hr CIIT ^a	100 ppm BD, 4 hr ITRI ^b
Rat		
BDO	0.07 $\pm$ 0.01	0.1 $\pm$ 0.06
BDO ₂	ND	ND
Mouse		
BDO	0.56 $\pm$ 0.04	0.38 $\pm$ 0.14
BDO ₂	0.65 $\pm$ 0.10	0.33 $\pm$ 0.19

^aHimmelstein et al., *Carcinogenesis* 15:1479, 1994.

^bBechtold et al., *Chem. Res. Toxicol.* (1995, in press).

1,2-Dihydroxy-4-(N-Acetylcysteine-S)-Butane

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