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A Brief Survey of Butadiene Health Effects: A Role for Metabolic Differences

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1,3-Butadiene is a major monomer in the rubber and plastics industry and is one of the highest-production industrial chemicals in the United States. Although not highly acutely toxic to rodents, inhalation of concentrations as low as 6.25 ppm causes tumors in mice. Butadiene is oncogenic in rats, but much higher exposure concentrations are required than in mice. Chronic toxicity targets the gonads and hematopoetic system. Butadiene is also a potent mutagen and clastogen. Differences in the absorption, distribution, and elimination of butadiene appear to be relatively minor between rats and mice, although mice do retain more butadiene and its metabolites after exposure to the same concentration and have a higher rate of metabolic elimination. Recent studies have demonstrated that major species differences appear to occur in the rate of detoxication of the primary metabolite, 3-epoxybutene (butadiene monoepoxide [BDMO]). Mice have the greatest rate of production of BDMO as compared to other species, but the rate of removal of BDMO appears to be less than in other species. Mice have low levels of epoxide hydrolase; rats have intermediate levels; monkeys and humans appear to have high levels of this detoxifying enzyme. Thus, while only low levels of butadiene exposure may result in an accumulation of BDMO in the mouse, much higher levels would be required to result in an elevation of circulating BDMO in other species. The level of this reactive metabolite may be correlated with the species differences in butadiene sensitivity.

Introduction

1,3-Butadiene is a colorless gas used mainly in the production of synthetic rubber and plastics. Annual production of more than 3 billion pounds in the United States accounts for approximately one-fourth of the world's production (*1*). Occupational exposure can occur during its production and use, as well as during storage and transport. Although such exposure is generally well below 20 ppm, much higher exposures have been reported (*2*). Environmental exposure occurs due to the production of butadiene in cigarette smoke, fossil fuel incineration, and automotive emissions (*3*). Production and use of butadiene resulted in the emission of 6.4 million pounds into the air in 1988 (*4*). Low levels of butadiene are found in gasoline (*5*).

Because of the low acute toxicity, the Occupational Safety and Health Administration (OSHA) set the permissible exposure limit (PEL) for butadiene at 1000 ppm (*6*). However, reports of the rodent carcinogenicity of butadiene caused the American Conference of Governmental Industrial Hygienists to lower the threshold limit value (TLV) to 10 ppm (*7*). OSHA has recently proposed lowering the PEL to 2 ppm (*8*). Butadiene was identified as a hazardous air pollutant in the 1991 reauthorization of the Clean Air Act (*9*). The International Agency for Research on Cancer (IARC) has classified butadiene as a "IIB" carcinogen, i.e., possibly carcinogenic in humans (*10*); the U.S. Environmental Protection Agency classifies it similarly as a B2 carcinogen (*11*).

Toxicity

Acute toxicity to butadiene requires high exposure concentrations. Shugaev (*12*) reported that the LC_{50} in rats after a 4-hr exposure was 129,000 ppm, whereas a concentration of 122,000 ppm resulted in 50% lethality in mice after only 2 hr of exposure. Exposure to 250,000 ppm for approximately 30 min resulted in the death of exposed rabbits (*13*). Exposure of men to 2000 ppm for 6–8 hr resulted only in minor eye irritation (*13*).

Few adverse effects result from exposures of experimental animals to concentrations below 4000 ppm. Exposure of various experimental animal species to 6700 ppm for 8 months (7.5 hr/day, 6 days/week) revealed only a mild growth retardation and reversible liver degeneration (*13*). More recent studies in rats showed essentially no effects in rats after exposure for 13 weeks (6 hr/day, 5 days/week) to 8000 ppm butadiene (*14*).

Developmental toxicity has been examined in both rats and mice using a standard Segment II protocol. Studies conducted at Battelle Northwest Laboratories (*15–17*) have demonstrated that butadiene is maternally toxic to Sprague-Dawley rats at 1000 ppm. However, there is no developmental toxicity observed in the rat fetuses even at doses where maternal toxicity is observed. In contrast, both maternal and developmental toxicity is observed at

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200 ppm in CD-1 mice. A reduction in the weight of male mouse fetuses was also observed at 40 ppm, but no maternal toxicity was evident. After exposure to 200, 1000, or 5000 ppm for 5 days, there was a concentration-related increase in abnormal sperm in male $B6C3F_1$ mice (*17*). The immunotoxicity of butadiene was examined in male $B6C3F_1$ mice exposed to 1250 ppm (6 hr/day, 5 days/week) for 12 weeks (*18*). No effects were noted in either humoral or cell-mediated immunity.

Chronic toxicity studies have been conducted by the National Toxicology Program (NTP) in $B6C3F_1$ mice (*19*). Testicular atrophy was noted at 625 ppm in male mice, and ovarian atrophy occurred at doses as low as 6.25 ppm in female mice exposed for 40 weeks (6 hr/day, 5 days/week). Bone marrow toxicity was also observed at this time point with a decrease in red blood cells, hemoglobin, and packed red cell volume at $\geq$ 62.5 ppm. An increase in mean corpuscular volume was noted at 625 ppm. These changes are consistent with poorly regenerative anemia at doses 62.5 ppm. Earlier studies conducted by Irons and co-workers (*20*) in $B6C3F_1$ mice exposed to 1250 ppm for 6 weeks had demonstrated a macrocytic-megaloblastic anemia characterized by an increase in micronuclei and leukopenia due mainly to neutropenia. These changes may result from interference with normal bone cell differentiation. There is also some indication of altered hematological parameters in humans occupationally exposed to 20 ppm butadiene (*21*).

Butadiene is a potent mutagen both *in vivo* and *in vitro* (*22*). In studies involving bacterial mutagenicity, butadiene has been shown to be a base-pair mutagen in *Salmonella typhimurium* strains TA1530 and 1535 (*23*). Its mutagenicity requires metabolic activation (*24*). Butadiene also causes cytogenetic effects in the whole animal. Tice and coworkers (*25*) examined the effects in bone marrow after a 2-week exposure to male $B6C3F_1$ mice (6 hr/day, 5 days/week). Significant increases were noted in sister chromatid exchanges at 6.25 ppm, in circulating micronuclei at 62.5 ppm, and in chromosome aberrations at 625 ppm. Recent studies by Goodrow et al. (*26*) have demonstrated that proto-oncogenes are activated in mouse liver and lung tumors and lymphomas after exposure to butadiene. The activating mutation is in codon 13 of the K-*ras* oncogene, a mutation never seen in spontaneously arising liver tumors. The inactivation of suppressor genes has also been noted in tumors from butadiene-exposed mice (*27*). On-going studies using transgenic mice have demonstrated *in vivo* activation of transgenes after butadiene exposure (*28*). *In vitro* work involving TK-6, a human lymphoid cell line, has demonstrated mutation in the *HGPRT* locus (*29*).

Carcinogenicity

The carcinogenicity of butadiene in rats has been examined by a study conducted under the auspices of the International Institute of Synthetic Rubber Producers. This study was first reported in 1981 (*30*) and in the peer-reviewed literature in 1987 (*31*). Male and female Sprague-Dawley rats, 100/group, were exposed to concentrations of 0, 1000, and 8000 ppm butadiene for 2 years (6 hr/day, 5 days/week). Butadiene caused significant increases at the high dose in pancreatic and testicular tumors in males. In females, significant increases occurred at both the low and high dose in mammary gland tumors and at the high dose in thyroid gland tumors. There was also an increasing trend observed in females for uterine and Zymbal gland tumors. Thus, butadiene is a multisite carcinogen in both sexes of rats. It is important to note that survival was not compromised at the low concentration.

The carcinogenicity of butadiene was examined in male and female $B6C3F_1$ mice, 50/group, exposed to 0, 625, or 1250 ppm butadiene for 60 (male) or 61 (female) weeks (6 hr/day, 5 days/week (*32,33*). The study had been intended to last for 2 years, but early mortality due to the induction of lethal tumors resulted in study termination. For example, by 60 weeks, less than 20% of the males exposed to the high concentration and 25% exposed to the low concentration were still alive. Survival of the controls was greater than 95% at this time. Malignant T-cell lymphomas were present in both males and females at the low concentration (625 ppm) and were the cause of early death in most cases. These thymic lymphomas are distinct from the spontaneous lymphomas occurring in this strain of mouse, which are derived from B-cells. Hemangiosarcomas of the heart, an extremely rare tumor, were also found in both sexes, as were lung and forestomach tumors. The incidence of hemangiosarcomas, lymphomas, and lung tumors was similar at the two concentrations. The decrease in forestomach tumors at the high concentration may well have been due to the excessive early mortality. In addition, tumors were found in the mammary gland, ovary, and liver of female mice at 1250 ppm. Thus, as in the rat, butadiene was a multisite carcinogen in both sexes of $B6C3F_1$ mice, but at significantly lower exposure concentrations.

In order to better understand better the induction of tumors in the mouse by butadiene, a second carcinogenicity study was conducted by the NTP (*19*). This study focused on both dose–response relationships and the issue of time to tumor, with the inclusion of stop studies. Male and female $B6C3F_1$ mice, 70/group, were exposed to 0, 6.25, 20, 62.5, 200, and 625 ppm butadiene for 40 (10 mice), 65 (10 mice), or 103 (50 mice) weeks (6 hr/day, 5 days/week). In addition, mice (50/group/sex) were exposed to 200 ppm for 40 weeks, 625 ppm for 13 weeks, 312 ppm for 52 weeks, or 625 ppm for 26 weeks, resulting in a total exposure of approximately 8000 or 16,250 ppm-weeks. As in the earlier study, survival was severely compromised in both sexes at 625 ppm; all the mice died before 70 weeks of exposure. Survival was also decreased at 200 ppm, but this effect was much less severe as the concentration decreased. For example, in females the thymic lymphomas led to reduced survival at 200 ppm. The only concentration at which survival was not affected by exposure to butadiene was 6.25 ppm.

As in the earlier study, tumors appeared at multiple sites in both sexes of mice. A significant increase in malignant tumors was detected at 20 ppm in males and 6.25 ppm in female mice. In addition to the lymphomas, hemangiosarcomas, and lung and forestomach tumors seen previously, tumors were now observed in the Harderian gland, prepu-

tial gland, and liver in males and in the Harderian gland, liver, ovary, and mammary gland in females. The detection of these additional sites appears to be due to the disappearance of the thymic lymphomas at the low exposure concentrations. The lymphomas are early-appearing tumors, which can occur in less than 26 weeks after the start of exposure. In contrast, the hemangiosarcomas do not appear until 40 weeks. When the concentration of butadiene is lower, the lymphomas do not occur, allowing for the development of malignancies in other tissues. A similar pattern is apparent for the induction of lung tumors, which also do not appear until approximately 40 weeks of exposure. This tumor is the most sensitive tumor end point in the female mouse, occurring with a statistically significant increase at the lowest dose used in this study, 6.25 ppm. Examination of the concentration–response curves for the induction of tumors in both male and female mice confirms the observation that the T-cell response is a high-concentration effect. In contrast, a linear relationship is observed between incidence and exposure concentration for the other major tumors (lung, Harderian gland, heart, forestomach).

Studies that maintained a constant concentration-time relationship demonstrated that the high exposure concentration was responsible for the induction of lymphomas. Only 13 weeks of exposure to 625 ppm resulted in a 47% incidence of thymic lymphomas in male mice, whereas no significant increase occurred when mice were exposed to 200 ppm for 40 weeks. In contrast, the total exposure (ppm-weeks) appears to determine the response in the other tissues.

Thus, butadiene causes tumors at multiple sites in both sexes of rats and mice. However, while tumors occur at 6 ppm in the mouse, the lowest concentration at which tumors were noted in rats was at 1000 ppm. The only tumor site in common between the two species was the mammary gland in the female. The rat tumors are all in endocrine tissues. Nevertheless, butadiene is clearly a potent animal carcinogen.

The exquisite sensitivity of the $B6C3F_1$ mouse to butadiene-induced tumors has been hypothesized to relate to the presence of an endogenous ecotropic retrovirus in these mice (*34*). Exposure of NIH Swiss mice, which do not have the retrovirus, results in a much lower lymphoma incidence. However, these studies demonstrate that the virus is not required for tumor induction, although it may play a modulatory role. Other strain differences could also explain the different lymphoma incidence.

Although butadiene is clearly a potent rodent carcinogen, the question of its human carcinogenicity is not clear from epidemiological studies. Three mortality cohort studies have been conducted (*35–37*). In all cases, the total cancer mortality was low. Although there was a significant increase in the standardized morality ratio (SMR) for hematopoietic cancers, the SMRs were lower for long-terms workers in the butadiene industry than for short-term workers. The lymphopoietic case–control study of Matanoski et al. (*38*), which was nested within the largest of the three cohort studies, does suggest an increase in leukemia as well as in lymphosarcoma and other lymphatic tumors. However, these increases are essentially only in the black production workers and involve very small numbers of workers. An exposure-based cohort study is clearly needed to resolve the issue of whether butadiene is a human carcinogen.

Pharmacokinetics

The dramatic differences in species sensitivity to butadiene carcinogenicity, as well as the observed varying susceptibilities for developmental toxicity end points, suggest that pharmacokinetic differences among species may exist. Pharmacokinetic studies involve analysis of the uptake, absorption, distribution, metabolism, and elimination of butadiene and its metabolites, both experimentally and by mathematical modeling. The major approaches used to study the pharmacokinetic behavior of butadiene in experimental animals and humans have involved gas uptake, chemical disposition, and *in vitro* metabolism studies. In the early 1980s, the laboratories of Filser and Bolt (*39*) pioneered the use of closed-chamber systems to study the pharmacokinetics of butadiene. They placed Sprague-Dawley rats (*40*) or $B6C3F_1$ mice (*41*) in air-tight chambers filled with a known concentration of butadiene and then monitored the disappearance of butadiene from the chamber. In this system, the loss of butadiene was due to its uptake and metabolism by the animals. The disappearance of butadiene was nonlinear at exposure concentrations greater than 1000 ppm, with metabolic saturation occuring at concentrations $\geq$ 1500 ppm in both rats and mice. The major difference between mice and rats was that the rate of metabolic elimination (V_{max}) in mice was approximately twice that of rats. These investigators (*40*) also observed that the metabolic elimination of butadiene could be blocked by pretreatment of the rats with dithiocarb, indicating that metabolism was mediated by the cytochrome P450 monoxygenase system. Metabolism was also inducible by Aroclor 1254. Such pretreatment resulted in a dramatic increase in the V_{max} and no evidence of saturation at exposure concentrations as high as 12,000 ppm butadiene.

Metabolism of butadiene occurs via epoxidation of one of the double bonds to produce 1,2-epoxybutene-3, also known as vinyl oxirane or butadiene monoepoxide (BDMO). Malvoisen and co-workers (*42*) demonstrated that this microsomal reaction was NADPH dependent and was inducible by phenobarbital but not by 3-methylcholanthrene. It could be inhibited by SKF525A. Such behavior is characteristic of a cytochrome P450 mediated reaction. The production of this metabolite *in vivo* could be demonstrated in the closed-chamber system (*43*).

There are multiple pathways that can lead to the disappearance of the monoepoxide. Malvoisen and Roberfroid (*44*) used an *in vitro* system to demonstrate that BDMO could be hydrated via epoxide hydrolase to form the 3,4-dihydroxy-1,2-butene (butenediol). Using microsomes from Wistar rats, they observed that the K_m for this pathway was relatively high compared to that of the initial epoxidation step, suggesting that the activation of butadiene to BDMO is favored over hydration of BDMO to the diol.

BDMO can also serve as a substrate for a further round of epoxidation, leading to the production of diepoxybutane, which is more highly reactive and carcinogenic than the monoepoxide (*22*). Whether this metabolite is actually produced *in vivo* is unsure given the lack of detection of diepoxybutane-induced DNA crosslinks *in vivo* in either rats or mice (*45*). Both the mono- and diepoxide can also conjugate with glutathione, a reaction catalyzed by a soluble glutathione-*S*-transferase. Recent studies have indicated that this reaction results in detoxication (*46*). The butenediol can undergo another round of epoxidation and hydration to form the tetrol, erythritol, which can also be produced by action of epoxide hydrolase on the diepoxybutane.

The metabolic elimination of BDMO can be followed in a closed-chamber system. If rats or mice are exposed in a closed chamber to BDMO, the disappearance of BDMO is linear up to 1000 ppm in rats, while saturation of metabolic elimination is observed at 500 ppm in mice. In fact, in the linear range (<500 ppm), the steady-state concentration of BDMO in mice is approximately 10 times that in rats. Rats are able to eliminate BDMO faster than mice. The V_{max} for metabolic elimination in rats is 8 times greater than observed in mice (*47*). Thus, mice are able to generate more BDMO than rats when exposed to a similar concentration of butadiene; rats more readily eliminate this reactive metabolite.

The suggestion that differences in the rate of metabolic elimination of butadiene and its reactive metabolite, BDMO, could play a role in the differential species sensitivity observed has been supported by a series of studies of the disposition of butadiene in mice, rats, and monkeys (*48*). These studies involved dynamic, nose-only exposures to radiolabeled butadiene. The retention of the radioactivity and its tissue distribution was examined in Sprague-Dawley rats and B6C3F$_1$ mice at multiple time points following cessation of exposure to butadiene concentrations ranging from 0.08 to 8000 ppm. The elimination of butadiene-derived radioactivity was examined in the exhaled air, urine, and feces after exposure. At the same exposure concentration, mice retained more of the butadiene-derived radioactivity than rats (*49*), resulting in a higher dose. This may be due to the greater minute volume in mice as compared to rats. However, recent studies in cynomolgus monkeys have indicated that retention in monkeys is similar to that observed in rats (*50*). The disposition studies using radioactivity also indicated that nonlinearities in metabolism were observed in the mice at exposure concentrations above 100 ppm, as indicated by an increase in the percentage of radioactivity exhaled as CO_2. This shift occurred at higher concentrations in the rats in good agreement with the results from the closed-chamber studies.

The distribution of butadiene-derived radioactivity was also examined in the tissues of mice and rats (*51*). Few differences were observed. Fat was the major tissue depot. The concentration of radioactivity in the blood was greater in mice than in rats. This labeled material has been tentatively identified as butadiene monoepoxide; it is clearly not the parent compound (*48*). The concentration of butadiene-derived radioactivity is lowest in the blood of exposed monkeys (*50*). In all three species, routes of elimination of the butadiene-derived radioactivity were similar, with most of the metabolites appearing in the expired air and urine.

Although there are some differences in the uptake and metabolic rate between rats and mice, these alone are not sufficient to explain the dramatic differences in species sensitivity to butadiene carcinogenesis. One possibility that requires examination is the species differences in metabolic pathways, as suggested by the differential ability to remove the monoepoxide observed in the closed-chamber studies. To investigate different metabolic pathways as well as the role of tissues other than the liver in the metabolic activation and detoxication of butadiene, *in vitro* systems have been used. Malvoisen and Roberfroid (*44*), as mentioned above, demonstrated that when using rat hepatic microsomes, activation of butadiene was favored over hydration of the monoepoxide. The critical role of epoxide hydrolase in the detoxication of BDMO was demonstrated by Bolt et al. (*43*), who demonstrated that incubation of microsomes with trichloropropylene oxide, a specific inhibitor of epoxide hydrolase, increased the levels of BDMO. These investigators observed that addition of glutathione (GSH) and soluble enzymes to the microsomes resulted in a decrease in the level of the monoepoxide. This ability of GSH to detoxify BDMO was further examined using *in vivo* exposures in which the depletion of glutathione was observed to be more pronounced in mice than in either Sprague-Dawley or Wistar rats (*52*). Such depletion is often correlated with enhanced toxicity. In fact, the levels of BDMO appear to build up once the GSH is depleted. Deutschmann and Laib (*53*) demonstrated a dose-dependent decrease in GSH concentrations starting between 100 and 250 ppm in the mouse. Higher exposure concentrations also led to depletion of GSH in the lung and heart of the mouse. In contrast, a major reduction in hepatic glutathione was not observed in the rat until exposure to 2000 ppm butadiene. Few changes were observed in the rat heart or lungs.

Butadiene can be metabolized in tissues in addition to the liver. Bond and co-workers (*54*) compared the *in vitro* metabolism of butadiene in microsomes from liver and lung of rats and mice. The rate of butadiene disappearance was similar using rat and mouse liver microsomes, but appeared to be higher in rat than in mouse lung. Recent studies in which BDMO formation was directly measured *in vitro* revealed that mouse lung microsomes activate butadiene at rates nearly 15 times greater than in rats (*55*). Lorenz and co-workers (*56*) also reported that the relative activation of butadiene by mouse lung microsomes was greater than that observed in rat lung, in agreement with studies by Schmidt and Loeser (*57*). *In vitro* hepatic activities observed from all of these groups has been greater in the mouse than in the rat. Pretreatment of the rats and mice for 5 days with butadiene failed to induce its own metabolism in either tissue (*54*). The metabolic rate in rat nasal mucosa appeared similar to that observed in liver and lung (*54*). Recent studies have indicated that myeloperoxidase-mediated metabolism of butadiene may occur in the bone marrow (*58*).

Several investigators have attempted to compare the rate of butadiene activation *in vitro* in humans with that observed in experimental animals. Using liver microsomes from Wistar and Sprague Dawley rats, NMR and B6C3F$_1$ mice, rhesus monkeys, and human liver, Schmidt and Loeser (*57*) observed the following rate of formation of BDMO: mouse > rat > man > monkey. Csanady and coworkers (*55*) noted a lower rate of formation of BDMO in rat liver microsomes than in humans or mice. Rates of butadiene activation in human lung microsomes were similar to rats (*55*).

The critical issue is not the rate of formation of BDMO, but the steady-state levels in the organism. This has not yet been experimentally determined. However, hints at species differences are apparent from comparison of the relative rates for BDMO formation and its further biotransformation to other metabolites. As discussed above, removal of BDMO appears to be catalyzed by two major enzymes: epoxide hydrolase and glutathione-*S*-transferase. The *in vivo* studies suggest that rats may have greater epoxide hydrolase activity than mice, resulting in lower utilization of the glutathione transferase pathway, and hence, lower probability of causing glutathione depletion. Lorenz et al. (*56*) observed that although rats had greater hepatic epoxide hydrolase activity, mice had higher levels of the transferase. Csanady et al. (*55*) noted that mouse liver microsomes had approximately twice the rate of diol formation from BDMO than rats, but that this was much lower than that observed in human liver microsomes. This is in agreement with the earlier observation of Schmidt and Loeser (*57*) that epoxide hydrolase activity was highest in monkeys and humans but very low in rodents. Recent studies by Kreuzer et al. (*59*) noted that the relative epoxide hydrolase activity using BDMO as a substrate was man > rat > mouse. This relative activity profile was reversed for glutathione-*S*-transferase-catalyzed conjugation of GSH with BDMO. In fact, the ability of human liver microsomes to form the diol from the monoepoxide was about 25 times greater than the activation of butadiene to the monoepoxide. Overall, the ability of rats to generate the monoepoxide and then eliminate it is not as great as that of the human but better than the mouse, which is very active at activating butadiene but has only limited ability to remove the monoepoxide.

In vivo studies examining the profile of urinary metabolites after butadiene exposure have lent support to the hypothesis that while mice preferentially metabolize BDMO via conjugation with glutathione, rats use this pathway, as well as epoxide hydrolase, and monkeys favor diol formation almost exclusively (*60*). Inhalation exposure of monkeys to butadiene resulted in the presence of only one major metabolite in the urine, which has been identified as the *N*-acetylcysteine conjugate of butanediol. This metabolite is a product of the sequential activity of epoxide hydrolase on BDMO, generating the butenediol, which then becomes a substrate for the glutathione-*S*-transferase. In contrast, in mice, although there are low amounts of this butenediol conjugate, the major metabolite is the *N*-acetylcysteine conjugate of butene-2-ol, the product of direct conjugation of BDMO with GSH. There are nearly equivalent amounts of the two metabolites in the rat. Thus, in rodents the levels of circulating epoxide are high enough to be available for conjugation with glutathione, but in primates the high levels of epoxide hydrolase activity ensure that the monoepoxide is rapidly converted to the diol.

The weight of evidence suggests that the mouse has the greatest ability to create the active metabolite of butadiene, BDMO, but the least capacity to remove it. While proof of elevated steady-state levels of BDMO has not yet been obtained, there is additional indirect *in vivo* data in support of this hypothesis. Butadiene exposure to mice and rats resulted in approximately 2-fold higher binding to nucleoproteins in mice than in rats (*61*). Measurement of hydroxybutenylvaline in hemoglobin, which is a product of the reaction of BDMO with the N-terminal valine, reveals that mice produce more than 10 times the level of this adduct following exposure to 2000 ppm butadiene, as do rats (J. Bond, personal communication). Hemoglobin adducts are a reflection of the circulating levels of the monoepoxide; they are a function of the balance between butadiene activation and detoxication. DNA adducts are found after inhalation exposure only in the livers of mice, not rats (*62*). This adduct was tentatively identified as 7-(1-hydroxy-3-buten-2-yl)guanine (*63*). The generation of this adduct in the mouse at exposure concentrations that cause tumors in mice but not in rats supports the central role for BDMO in butadiene carcinogenesis.

A physiologically based pharmacokinetic model was developed by Hattis and Wasson (*64*) to describe the behavior of butadiene. This model predicted that butadiene metabolism scaled with the general metabolic rate, in good agreement with the experimental data. Using a Michaelis-Menten-type equation to describe the conversion of butadiene to the monoepoxide, the model successfully predicted the observed nonlinearities in metabolic elimination with exposure concentrations. Extrahepatic metabolism also appeared to play a role in the overall pharmacokinetic behavior. Csanady et al. (*65*) have recently developed a physiologically based pharmacokinetic model to describe the disposition of butadiene. The model predictions provide an excellent fit to the experimentally determined data using the metabolic constants determined *in vitro*. Further development and validation of this model to predict the levels of hemoglobin adducts formed from the monoepoxide in rodents should enable predictions concerning the levels of such adducts in occupationally exposed workers.

Conclusions

Differential species sensitivity has been demonstrated not only for the carcinogenic effects of butadiene but for the developmental effects as well. The mouse appears to be extremely sensitive to the toxic effects of this chemical. Although rates of uptake and elimination may play a role in this differential species sensitivity, it is highly unlikely that these form the basis for the observed differences. However, there do appear to be significant differences in the metabolic pathways that are preferentially used

between different species. The results to date suggest that the mouse has the greatest ability to activate butadiene to the highly reactive monoepoxide BDMO; however, the mouse has only limited ability to remove this metabolite. Rats form less of the monoepoxide but have a greater ability to remove it by diol formation than mice. The ratio of activation to inactivation, i.e., the formation of monoepoxide versus its removal, appears to continue to decrease when one considers primates. If steady-state levels of the monoepoxide after inhalation exposure are in fact lower in monkeys than in rats and the level in rats are lower than those in mice, this might suggest that monkeys and humans would be less sensitive to the toxic and carcinogenic actions of butadiene than are rodents.

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