Future directions in butadiene risk assessment and the role of cross-species internal dosimetry

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Abstract

The 2005 International Symposium on the evaluation of butadiene and chloroprene health risks provided the opportunity to consider the past, present and future state of research issues for 1,3-butadiene. Considerable advancements have been made in our knowledge of exposure, metabolism, biomarkers of exposure and effect, and epidemiology. Despite this, uncertainties remain which will impact the human health risk assessment for current worker and environmental exposures. This paper reviews key aspects of recent studies and the role that biomarkers of internal dosimetry can play in addressing low to high exposure, gender, and cross-species differences in butadiene toxicity and metabolism. Considerable information is now available on the detection and quantification of protein adducts formed from the mono-, di- and dihydroxy-epoxide metabolites of butadiene. The diepoxide metabolite appears to play a key role in mutagenesis. Species differences in production of this critical metabolite are reflected by the diepoxide-specific hemoglobin adduct, pry-Val. To date, the pry-Val adduct has not been quantifiable in human blood samples from workers with cumulative occupational exposures up to 6.3 ppm-weeks; whereas, the pry-Val was quantifiable in the blood of mice and rats with similar cumulative exposures. Levels in mice were much higher than in rats. Further improvements in analytical sensitivity for the pry-Val adduct are on the horizon. Epidemiology studies are also described and ongoing efforts promise to help bridge our understanding of past and future risks.

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1. Introduction

The 2005 International Symposium on the Evaluation of Butadiene and Chloroprene Health Risks provided a truly outstanding update on current knowledge related to exposure, metabolism, biomarkers of exposure and effect, and epidemiology of butadiene (BD). This international symposium was preceded by four previous meetings on butadiene health effects [1–4]. Future research directions proposed at the 2000 international meeting related to butadiene risk assessment are summarized in Table 1.
Table 1
Outstanding butadiene health effect research issues, 2000 International Symposium on Butadiene and Chloroprene Health Risks

- Resolve species differences in metabolism, including differences in origin of carbon dioxide
- Understand relative roles/binding properties of CYP 2E1 and epoxide hydrolase of different species
- Use sensitivity analysis to optimize predictive ability of toxicokinetic models
- Resolve discrepancy between human Hprt study findings
- Conduct additional study of altered metabolism of BD due to DMDTC exposure
- Quantify historical monomer exposure in the Divine and Hartman [19] butadiene monomer worker cohort using methods from SBR worker study
- Leukemia risk in other SBR & monomer worker cohorts (including women)
- Consider reproductive-related outcomes
- Analyze epidemiologic data using WHO lymphopoietic cancer classification

Many of the research questions in Table 1 have been addressed in studies conducted over the last 30 years, when potential adverse health effects of butadiene were first raised in the 1970s. Key studies conducted over this period are described in the time line provided in Fig. 1. These studies have been interdisciplinary in nature and included epidemiology, toxicology, and molecular biology. Despite extensive progress made and reported in this current symposium, several key aspects remain uncertain and require additional research. This paper reviews key aspects of recent studies and the role that biomarkers of internal dosimetry can play in addressing low to high exposure, gender and cross-species differences in butadiene toxicity and metabolism.

2. Applications of internal dosimetry

Detailed molecular studies of DNA and protein adducts suggest that these areas of research will continue to contribute to our knowledge of metabolic and stereochemical effects that drive biological events such as mutagenesis [5,6]. The session on Risk Assessment stressed that we clearly need in vivo data on metabolism and key events such as mutagenesis in animals and humans. Recent molecular epidemiologic studies by Albertini et al. [7,8] have collected excellent exposure data in the same individuals who were being evaluated for a variety of genotoxic endpoints, as well as biomarkers of exposure and metabolism. Thus, we are positioned to make major advances in our understanding of butadiene’s mode of action in rodents and humans, as well as its application to more accurate risk assessment for current worker and environmental exposures.

In late 2004, the development of a new immunoaffinity LC-MS/MS assay for the N-terminal peptide adduct

Fig. 1. Butadiene research time line.
of butadiene diepoxide (DEB), \(N,N\)-(2,3-dihydroxy-1,4-butadiyl)valine (pyr-Val) in rats and mice was reported [9]. This assay provided the first measurements of pyr-Val in animals exposed by inhalation to BD and demonstrated that mice formed much higher amounts of the adduct than rats under identical exposure conditions ranging from 3 to 62.5 ppm BD. Pyr-Val was formed in mice (48.7 ± 3.2 pmol pyr-Val/g globin), and in rats (3.9 ± 0.8 pmol pyr-Val/g globin) exposed to 3 ppm BD for 6 h/day for 10 days. When mice and rats were exposed to 62.5 ppm BD for 10 days, 130.4 ± 64 pmol pyr-Val/g globin was formed in mice, while 38.3 ± 1.2 pmol pyr-Val/g globin was formed in rats. Mice were also exposed to 1250 ppm for 10 days, which resulted in 2487 ± 426 pmol pyr-Val/g globin, demonstrating that they continue to metabolize BD to DEB even at these high concentrations. In contrast, this metabolic step appeared to be saturated in rats, based on samples from animals exposed to 1000 ppm BD for 90 days, where only 58.1 ± 17.3 pmol pyr-Val/g globin was formed. As reported by Meng et al. [10], 62.5 ppm BD for 10 days is the lowest exposure in rats associated with an increase in mutagenicity in the \(Hprt\) assay, while 3 ppm is the lowest exposure exhibiting increased mutagenicity in mice. These data, combined with the potent mutagenicity of DEB, suggest strongly that DEB is a critical metabolite associated with the induction of mutations.

In collaboration with Vernon Walker, at Lovelace Respiratory Research Institute, we have recently completed additional exposures to male and female rats and mice, including 20-day exposures to 1.0, 6.25 or 62.5 ppm BD. Globin from this study has been analyzed for pyr-Val using the method of Boysen et al. [9]. Fig. 2 demonstrates the marked species difference in pyr-Val formation, with mice forming over 30 times more of the DEB-specific adduct than rats. Furthermore, following 20 days exposure, there was no difference between male and female rats, or male and female mice. Thus, following 4 weeks of inhalation exposure, no gender differences were observed in the metabolism of BD to DEB and the subsequent formation of pyr-Val.

In view of the ready detection of pyr-Val in rats and mice exposed to 1 ppm BD for 20 days, we proceeded to evaluate all of the blinded globin samples from the second study of Czech BD workers reported in this issue by Albertini et al. [8]. For these comparative studies, 50 mg of globin from the workers, rats and mice were analyzed by the method of Boysen et al. [9]. Whereas all rat and mouse samples had quantifiable amounts of pyr-Val, none of the human samples did (Table 2). While it is true that the average human exposures were lower than 1 ppm BD, it must be recognized that the period of exposure was six times longer than for the rodents. Since globin adducts form over the lifespan of the erythrocyte (humans, 120 days; rats, 63 days; and mice, 43 days), the human globin had a cumulative exposure similar to that of both rats and mice: Human females (0.18 ppm BD × 17.1 weeks = 3.1 ppm-weeks, versus

Table 2
Butadiene diepoxide-specific globin adducts in mice, rats and humans exposed to butadiene

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Exposure</th>
<th>Globin (mg)</th>
<th>pyr-Val (pmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Female</td>
<td>1 ppm 6 h/day</td>
<td>50</td>
<td>23.5 ± 3.1</td>
</tr>
<tr>
<td>Mouse</td>
<td>Male</td>
<td>1 ppm 6 h/day</td>
<td>50</td>
<td>30.8 ± 4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks (4.0 ppm-weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Female</td>
<td>1 ppm 6 h/day</td>
<td>50</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks (4.0 ppm-weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Male</td>
<td>1 ppm 6 h/day</td>
<td>50</td>
<td>0.9 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks (4.0 ppm-weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>Female</td>
<td>Mean 0.18 ppm for 4 months (3.1 ppm-weeks)</td>
<td>50</td>
<td>N.D.</td>
</tr>
<tr>
<td>Human</td>
<td>Male</td>
<td>Mean 0.37 ppm for 4 months (6.3 ppm-weeks)</td>
<td>50</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., below limits of detection (<0.3 pmol/g).
1.0 ppm BD × 4 weeks = 4.0 ppm-weeks in the rodents); human males (0.37 ppm BD × 17.1 weeks = 6.3 ppm-weeks, versus 1.0 ppm BD × 4 weeks = 4.0 ppm-weeks in the rats and mice). The peaks in rats exposed to 1 ppm BD were large enough that quantitative measurements could have been made with 1/3 of the globin, suggesting that humans form at least 3-times less pyr-Val than similarly exposed rats and 100-times less than similarly exposed mice. The new rodent data do not support a gender difference in BD metabolism to DEB following 4 weeks exposure.

These issues will be further clarified in the near future, through the use of even more sensitive mass spectrometers. Our laboratory is acquiring two new state-of-the-art instruments over the next year. Based on pilot runs of analytical standards for pyr-Val, we anticipate gaining an additional factor of five in sensitivity over the present instrument used for these analyses. This offers the exciting potential to be able to measure pyr-Val in at least some of the human globin samples from the first study in Czech workers reported by Albertini et al. [7] and in the second study in Czech BD workers [8]. If measurable amounts of pyr-Val are found, quantitative species differences can be assessed. Furthermore, if individuals with similar exposures to BD show differences in pyr-Val, it will become possible to explore the genetic differences associated with greater and lesser formation of pyr-Val. In addition, similar peptide methods for the other two BD epoxides are under development that offer the promise of developing individual profiles of BD metabolism in rats, mice, and humans. This could provide important information relevant to human susceptibility issues and species differences for BD. Since the assay for the diepoxide biomarker is relatively new, it may be beneficial to examine blood from patients treated with treosulfan (which is metabolized in vivo to butadiene diepoxide) to further validate the new diepoxide assay methodology and to assess genotoxicity.

Another important area of uncertainty to be addressed relates to human susceptibility issues with regard to gender. The influence of BD metabolism on this can be evaluated in the ongoing study of male and female styrene-butadiene rubber (SBR) workers [8]. The University of Alabama (UAB) investigators reported on their ongoing efforts to examine mortality patterns among female SBR workers [11]. Once these data have been fully analyzed, it will be possible to evaluate whether there are mortality risk differences by gender. Additionally, a combined analysis of male and female SBR worker mortality may be possible with increased numbers of leukemia cases for analysis. However, the application of the World Health Organization’s (WHO) disease classification system discussed below in such a combined analysis would require obtaining pathology reports for the female cohort, since current plans are to collect only death certificate information for the female cohort.

Another health effect question raised in the 2000 symposium related to reproductive outcomes in female BD exposed subjects. While the female SBR mortality study mentioned above will not address reproductive effects, Albertini et al. [8] reported results from a survey of reproductive outcomes among female workers in the Czech Republic molecular epidemiologic study. While the Albertini et al. [8] study has limitations, the results suggested no difference in pregnancy outcomes (e.g., miscarriage, still birth, ectopic pregnancies) between exposed subjects and controls. Animal studies only reported reproductive toxicity in mice and these have been associated with DEB formation and ovarian toxicity [12]. As shown by the pyr-Val data, high amounts of DEB were formed in mice only. Thus, future research on mouse, rat and human formation of DEB may shed considerable light on the mode of action and species specificity of adverse reproductive outcomes.

The effects of BD exposures on Hprt mutagenesis have been evaluated in numerous studies in mice, rats and humans. Furthermore, neither chromosome aberrations nor micronuclei have been induced in rats following inhalation exposure to the parent compound (BD). In contrast, BD exposures have induced micronuclei and chromosome aberrations in mice [13]. However, if rats are exposed to DEB, they do develop chromosomal aberrations and micronuclei [13]. In this issue, Meng et al. [5] present new data on lower BD exposures in rats, demonstrating that 62.5 ppm BD for 2 weeks caused a small, but significant increase in Hprt mutagenesis (Control: 1.06 ± 0.28 × 10⁻⁶ MF versus 62.5 ppm BD: 1.82 ± 1.82 × 10⁻⁶ MF, p = 0.03). Likewise, those investigators had previously reported a small, but statistically significant increase in Hprt mutagenesis in female B6C3F1 mice exposed to 3 ppm BD for 2 weeks (Control: 0.96 ± 0.51 × 10⁻⁶ MF versus 3 ppm BD: 1.54 ± 0.82 × 10⁻⁶ MF, p = 0.004) [10]. These data on mutagenesis can be compared with the pyr-Val data from Boysen et al. [9]. While mice produce much more pyr-Val than rats at comparable exposures, it is of interest to note that rats exposed to 62.5 ppm BD for 10 days had 38.3 ± 0.6 pmol pyr-Val/g globin, while mice exposed to 3 ppm BD for 10 days had 48.7 ± 3.23 pmol pyr-Val/g globin. Thus, the present LOAEL for Hprt mutagenesis in rats and mice is highly correlated with similar concentrations of the DEB biomarker of exposure. Considering the high mutagenic potency of DEB, these data suggest that pyr-Val may be a useful biomarker for species com-
parisons of mutagenesis at low exposures. Future studies will examine this relationship between mice and rats in much greater detail, including the delineation of NOAEL exposures for Hprt mutagenesis, as well as pyr-Val formation at non-mutagenic exposures to rats and mice.

Of potentially great importance are the three recent human biomarker studies on BD workers that examined Hprt mutagenesis using blinded clonogenic assays, as well as chromosomal aberrations [7,8,14]. Of these three studies, Hayes et al. [14] evaluated workers with the highest exposures, averaging exposures to 1.0 or 3.5 ppm BD, depending on job classification. The initial study of Albertini et al. [7] had average exposures to 0.8 ppm BD, while the second study of Albertini et al. [8] had exposures of 0.18 ppm BD in female workers and 0.37 ppm BD for male workers. Some of the same males were studied in both of the studies of Czech BD workers, so it may be possible to examine exposure–response data in the same individuals. None of these three studies showed evidence of an increase in Hprt mutagenesis or chromosomal aberrations, with the Albertini et al. studies using both conventional methods and fluorescent in situ hybridization (FISH). This again can be viewed in the context of the pyr-Val data presented in Table 2. Pyr-Val analyses were below the level of quantitation when 50 mg of globin was used, suggesting that the amount of pyr-Val was <0.3 pmol pyr-Val/g globin. This is nearly 50-fold less than the pyr-Val values at the rodent Hprt LOAELs.

Future studies will take the present estimate of pyr-Val even lower through the application of a new, more sensitive mass spectrometer. Likewise, exposures of mice and rats to BD concentrations as low as 0.1 ppm will clearly establish the exposure–response for pyr-Val and Hprt mutagenesis in rodents.

It is anticipated that Hprt mutagenesis will reach a threshold that cannot be distinguished from that found in control animals at BD exposures between 0.1 and 1.5 ppm BD and between 1.0 and 20 ppm BD in rats. In contrast, it is anticipated that pyr-Val will be measurable at exposures below the threshold for Hprt mutagenesis and that pyr-Val will be linear over the entire range of low exposures. Such data will strongly call into question the default that genotoxic risk is linear over the entire range of low exposures. These negative mutagenicity data can be viewed in context of current scientific knowledge, where a strong rationale can be made for nonlinear mutagenic responses at very low exposures. All mutagenic assays have measurable numbers of mutations in control cells, i.e., mutations do not go to zero. It is also well established that all species and cells studied have endogenous (background) DNA damage, such as that arising from oxidative processes. When mutagenesis assays reach background levels, rather than continuing to go down to zero, the most plausible explanation for this threshold is that endogenous DNA damage is then driving the biology of mutagenesis and that very low numbers of specific exogenous DNA adducts no longer result in biologically significant numbers of mutations.

3. Implications for human epidemiology studies

Since cancer risk is thought to be the result of one or more mutations in critical genes, strong data sets in experimental animals and humans on nonlinear inflection points in the exposure–response for mutagenesis in the presence of linear DNA or protein adducts will also bring into question the linear default for cancer risk assessment. Thus, establishment of nonlinear exposure–response data for mutagenesis with linear DNA or protein adducts will be able to improve the scientific validity of low dose cancer risk assessment. Such information does not negate positive epidemiologic studies, such as the Delzell observations for BD, as workers were exposed to much higher concentrations of BD in the past. Tsai et al. [15] reported that OSHA lowered the PEL from 1000 ppm BD to 1 ppm in 1996, and that the Shell Butadiene Medical Surveillance Program found a mean TWA exposure of 4.55 ppm BD from 1979–1996, but 0.25 ppm BD from 1997–2003. Likewise, Sathiakumar et al. [16] reported that the earlier estimates of BD exposure in the Delzell [17] study had underestimated exposure. Knowledge of the exposure response for mutagenesis and biomarkers such as pyr-Val will, however, strongly challenge estimates of risk at much lower exposures to BD.

The UAB investigators reported updated results for the male SBR worker cohort based on an additional 10 years of mortality follow up (through 1998). This update utilized the WHO disease classification for lymphopoietic cancers, which is in line with research recommendations from the 2000 symposium (see Fig. 1). The most recent UAB findings suggest that myeloid neoplasms are more strongly associated with peak BD exposures, while lymphoid neoplasms show an opposite pattern (i.e., stronger association with cumulative versus peak exposure [18]).

While data on the butadiene monomer cohort studied by Divine and Hartman [19] were presented at the previous symposium, questions remain about historical quantitative exposure levels in this cohort (see Table 1). However, Sathiakumar et al. [16] reported on their work to validate exposure estimates in the SBR worker cohort. Efforts to validate historical exposure estimates in the
SBR cohort are notable in that these data are the basis for several cancer risk estimate calculations (e.g., see Sielken et al. presented at this symposium [20]).

Finally, the most recent UAB epidemiology results have not completely resolved the issue of other SBR exposures such as dimethylidithiocarbamate (DMDTC) and their relation to leukemia risk in SBR workers. The 2000 symposium noted the need for additional research on the potential for DMDTC to alter BD metabolism in relation to hematopoietic effects, and on the effects of different exposure scenarios (e.g., discontinuous exposure—see Table 1). Future studies could include rodent in vivo studies with co-exposures to butadiene and other SBR chemicals (e.g., DMDTC, styrene) using a discontinuous (peaks) exposure protocol to simulate actual worker exposure patterns. Inclusion of additional metabolic parameters such as glutathione depletion may also be informative.

In conclusion, exciting advances have been achieved in the past 5 years that further clarified many of the uncertainties outlined in the 2000 symposium. What is even more exciting, however, is the high likelihood that many of the remaining gaps in our knowledge on butadiene mechanisms of carcinogenesis and associated risks will be minimized in the near future. This will permit the application of sound new scientific data to further reduce uncertainty in the assessments of butadiene risks.

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