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AN SAB REPORT: REVIEW OF THE HEALTH RISK ASSESSMENT OF 1,3-BUTADIENE

REVIEW OF THE OFFICE OF RESEARC AND DEVELOPMENT'S DRAFT HEALTH RISK ASSESSMENT OF 1,3-BUTADIENE PREPARED BY THE ENVIRONMENTAL HEALTH COMMITTEE (EHC) OF THE SCIENCE ADVISORY BOARD (SAB)

November 19, 1998

EPA-SAB-EHC-99-003

Honorable Carol M. Browner
Administrator
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

Subject: Review of the Office of Research and Development's Draft Health Risk Assessment of 1,3-Butadiene (EPA/600P-98/001A).

Dear Ms. Browner:

At the request of the Office of Research and Development (ORD), the Environmental Health Committee (EHC) of the Environmental Protection Agency's Science Advisory Board (SAB) reviewed the Agency's Draft Health Risk Assessment of 1,3-Butadiene. The Committee met on April 30 and May 1, 1998 in Washington, DC.

The document reviewed by the Committee was developed by the EPA's Office of Research and Development (ORD). The ORD published its first risk assessment of 1,3-Butadiene in 1985. The first document covered cancer and mutagenicity and was prepared in response to a request from the Office of Air Quality Planning and Standards to support the classification of 1,3-Butadiene as a Hazardous Air Pollutant. The recent draft 1,3-Butadiene document was written in response to a request from the Agency's Office of Mobile Sources which, plans to use the final document to support a future Air Toxics Rule. The current draft 1,3- review document focuses on mutagenicity, carcinogenicity, and reproductive/developmental effects. The 1,3-Butadiene document reviewed by the Committee presents the Agency's first use of a benchmark dose analysis for reproductive/developmental factors, and incorporates many new studies published since the 1985 effort. The EPA concludes that this new information has changed the weight of evidence for its findings re cancer. In addition, there are exposure data available in an occupational study which are used to derive the cancer slope factor. The review document is not a comprehensive health assessment however, and contains only an overview of the ambient exposure and exposure of populations adjacent to emissions sources, without including any actual exposure assessment as such.

In addition to a general review of the document by the Committee, the Office of Research and Development specifically requested that the EHC provide comment on each of the following Charge issues:

a) Review the health risk assessment for technical quality, comprehensiveness and clarity.

b) Does the science support the classification of "known" human carcinogen?

c) Are the approaches taken to characterize plausible cancer risks reasonable given

the science?

d) Are the conclusions and quantitative estimations for reproductive/developmental effects adequately supported?

The EHC recognizes that preparing a health risk assessment (more correctly, a **hazard** assessment -- see below) of 1,3-Butadiene was a difficult and complicated task, given the large amount of information on 1,3-Butadiene toxicity, epidemiology, and mechanisms available, as well as the new and information being developed on a continual basis.

The Agency selected a cutoff date of January 31, 1997 for the inclusion of new information in the revised draft document. However, a significant amount of new and important information has been developed since then and is pertinent to a health risk assessment of 1,3-Butadiene. The Committee found that the report should reflect the most current research data possible, including the recent evaluations by the International Agency for Research on Cancer (IARC, 1998), and if finalized soon, the evaluation of Health Canada (Health Canada, 1997). Therefore, with respect to the first charge question on the technical quality, comprehensiveness and clarity of the document, the EHC found that the quality and comprehensiveness would be greatly improved by including research data published in the peer-reviewed literature since the cutoff date of January 31, 1997. The EHC notes that important research (such as the Delzell *et al.,* 1995) exposure re-estimation and pharmacokinetic modeling studies) are ongoing. Also, to improve the clarity of the document, the Committee recommended several editorial changes, such as the inclusion of summary tables in some of the chapters.

The majority of the Environmental Health Committee did not support classifying 1,3-Butadiene as a *known* human carcinogen, due to the lack of consistency between exposure response rates for leukemia or lymphosarcoma when both the styrene-Butadiene rubber (SBR) and monomer worker studies were considered in total. The majority opined that 1,3-Butadiene should be classified as a *probable* human carcinogen. This opinion was based on several lines of evidence: a) There was only one positive study on workers in the monomer process, and it showed only a small excess of lymphosarcoma; in addition, this study has not been replicated. (One would like to see at least a second independent confirmatory study before affirming that there is "sufficient evidence of human carcinogenicity" regarding Butadiene and leukemia. Instead a fairly large and reasonably sound second study shows no leukemia excess; while two smaller ones found no evidence of leukemia risk); b) In the monomer study, there was no evidence of an exposure response relationship for lymphosarcoma, nor was there evidence that those workers with longer-term exposure had a higher risk of lymphosarcoma; and c) A large study of the workers in the styrene-Butadiene rubber (SBR) industry showed an excess of leukemia. However, since these workers were exposed to several different chemicals, the cancer excess could not be attributed solely to 1,3-Butadiene. Furthermore, the findings concerning co-exposure to styrene from the reevaluation of the exposure estimates in the Delzell *et al.* (1995) study may also impact the risk assessment. The majority of the Committee felt that the finding of "known human carcinogen" should solely be based on observational studies in humans, without

regard to mechanistic or other information. Others on the Committee felt that Butadiene should be identified as a "known human carcinogen" using the cumulative evidence from epidemiology, animal cancer bioassays, and mechanistic studies as the basis for the judgment.

The Committee found the approaches taken to characterize plausible cancer risks to be reasonable, but points out specific data that may have been misinterpreted by the Agency. In particular, the discussion of metabolism and toxicokinetics failed to address critical differences in metabolism of Butadiene in different species which could account for differences in tumor susceptibility in different species. Inclusion of a discussion of state-of-the-art models for Butadiene metabolic pathways and kinetics would significantly strengthen the scientific quality of the document. Included in this discussion should be the strengths and weaknesses of the available models for risk prediction. This fuller discussion should replace the simple statements made about the inadequacy of the available models.

The Committee commends the Agency for looking at new approaches, such as the benchmark dose procedure, to improve quantitative assessment of non cancer endpoints. However, the Committee has submitted suggestions on how to further improve these approaches and how to make these new approaches more clear, accurate and concise, including the following recommendations:

a) Correct mathematical errors in the calculation of the benchmark concentration for reproductive and developmental effects.

b) Address new dominant lethal studies.

c) The different toxicokinetic hypotheses and the hypothesized role of the various metabolites should be discussed, at least qualitatively, with an indication of the degree to which the assessment would be impacted if some of the hypotheses were later proven true.

d) Inadequate justification is given for the application of the additional safety factor for the benchmark dose.

e) The rationale for the selection of the toxic non-cancer endpoint that is utilized in the derivation of the RfC is very important and should be more explicitly explained.

f) As noted above, the review document is not a comprehensive health assessment however, and contains only an overview of the ambient exposure and exposure of populations adjacent to emissions sources, without including any actual exposure assessment as such. Consequently, as the document now stands, it should be retitled as a "Hazard Assessment" until such time as an exposure assessment component can be incorporated. The document could be called "Health Risk

Assessment of 1,3-Butadiene: 1. Hazard Assessment."

The Committee appreciates the opportunity to review the draft Health Risk Assessment of 1,3-Butadiene and looks forward to receiving a written response from the Assistant Administrator, Office of Research and Development.

Sincerely,

/signed/
Dr. Joan M. Daisey, Chair
Science Advisory Board

Dr. Mark J. Utell, Chair,
Environmental Health Committee
Science Advisory Board

NOTICE

This report has been written as part of the activities of the Science Advisory Board, a public advisory group providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The Board is structured to provide balanced, expert assessment of scientific matters related to problems facing the Agency. This report has not been reviewed for approval by the Agency and, hence, the contents of this report do not necessarily represent the views and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

ABSTRACT

The Environmental Health Committee (EHC) reviewed the EPA's updated draft health risk assessment of 1,3-Butadiene, which had a cutoff date of January, 1997. A significant amount of new and important information has been developed since then, and the Committee felt that the report should reflect the most current research data.

The majority of the Environmental Health Committee did not support the proposed classification of 1,3-Butadiene as a known human carcinogen due to the lack of consistency between exposure response rates for leukemia or lymphosarcoma when both pertinent studies were considered. The majority opined that 1,3-Butadiene should be classified as a probable human carcinogen.

The Committee found the approaches taken to characterize plausible cancer risks to be reasonable but points out specific data that may have been misinterpreted by the Agency. The Committee supported the use of the benchmark dose procedure in developing Reference levels, and suggested how to further improve the approaches for quantitative assessment of non-cancer endpoints. Greater explanation is needed of the safety factors applied to the benchmark, and of the newly proposed models, especially those modeling time to impact. Also, the EHC recommends that the Agency explain, in more detail, the rationale for the selection of the toxic non-cancer endpoint that is utilized in the derivation of the RfC.

Keywords: 1,3-Butadiene, EPA's proposed Cancer Risk Assessment Guidelines, known human carcinogen, probable human carcinogen, lymphosarcoma, leukemia, reproductive/developmental effects, pharmacokinetics risk assessment.

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SCIENCE ADVISORY BOARD
ENVIRONMENTAL HEALTH COMMITTEE
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1. EXECUTIVE SUMMARY

The EPA Office of Research and Development prepared the draft *Health Risk Assessment of 1,3-Butadiene* (USEPA, 1998a) in response to a request from the Office of Mobile Sources (OMS). The document was requested by OMS in order to support a future Air Toxics Rule. The review document is not intended to be a comprehensive health assessment and therefore, does not contain any actual exposure assessment. The document focuses on mutagenicity, carcinogenicity, reproductive, and developmental effects and presents the Agency's first benchmark dose analysis for reproductive/developmental effects. In its document, the Agency states that the new studies published since 1985 change the weight of evidence for cancer. Based on the weight of overall evidence from human, animal, and mutagenicity studies, the Agency concludes that 1,3-Butadiene is a known human carcinogen.

On April 30 and May 1, 1998, the Environmental Health Committee met at the EPA's Waterside Mall complex in Washington, DC to review the Agency's draft *Health Risk Assessment of 1,3-Butadiene*. In addition to a general review of the document by the Committee, the Office of Research and Development specifically requested that the EHC provide comment on each of the following aspects of the document:

a) Review the health risk assessment for technical quality, comprehensiveness and clarity

b) Does the science support the classification of "known" human carcinogen?

c) Are the approaches taken to characterize plausible cancer risks reasonable given the science?

d) Are the conclusions and quantitative estimations for reproductive/developmental effects adequately supported?

The Committee acknowledges that this is an extremely difficult task given the large amount of information on 1,3-Butadiene toxicity, epidemiology, and mechanism available with new information being made available on a continual basis.

The Agency selected a cutoff date for the inclusion of new information in the revised, draft document of January 31, 1997. However, a significant amount of new and important information has been developed since then and is pertinent to a health risk assessment of 1,3-Butadiene. The Committee felt that the report should reflect the most current research data, including the recent evaluations by the International Agency for Research on Cancer (IARC, 1998) and on ongoing evaluation in Canada (Health Canada, 1997). Therefore, with respect to the first charge question on the technical quality, comprehensiveness and clarity of the document, the EHC found that the quality and comprehensiveness would be greatly improved by including research data published in

the peer-reviewed literature since the cutoff date of January 31, 1997. The EHC also notes that important research such as the Delzell *et al.* (1995) exposure re-estimation and the pharmacokinetic modeling studies is ongoing. To improve the clarity of the document, the Committee recommended several editorial changes, such as the inclusion of summary tables in some of the chapters.

The majority of the Environmental Health Committee did not support classifying 1,3-Butadiene as a *known* human carcinogen, due to the lack of consistency between exposure response rates for leukemia or lymphosarcoma when both the styrene-Butadiene rubber (SBR) and monomer worker studies were considered in total. The majority opined that 1,3-Butadiene should be classified as a **probable** human carcinogen. This opinion was based on several lines of evidence: a) There was only one positive study on workers in the monomer process, and it showed only a small excess of lymphosarcoma; in addition, this study has not been replicated. (One would like to see at least a second independent confirmatory study before affirming that there is "sufficient evidence of human carcinogenicity" regarding Butadiene and leukemia. Instead a fairly large and reasonably sound second study shows no leukemia excess; while two smaller ones found no evidence of leukemia risk); b) In the monomer study, there was no evidence of an exposure response relationship for lymphosarcoma, nor was there evidence that those workers with longer-term exposure had a higher risk of lymphosarcoma; and c) A large study of the workers in the styrene-Butadiene rubber (SBR) industry showed an excess of leukemia. However, since these workers were exposed to several different chemicals, the cancer excess could not be attributed solely to 1,3-Butadiene.

The Committee found the approaches taken to characterize plausible cancer risks to be reasonable but points out specific data that may have been misinterpreted by the Agency. In particular, the discussion of metabolism and toxicokinetics failed to address critical differences in metabolites of Butadiene in different species accounting for differences in tumor susceptibility. Inclusion of a discussion of state-of-the-art models for Butadiene kinetics would significantly strengthen the scientific quality oft he document. Included in this discussion should be the strengths and weaknesses of the available models for risk prediction. This fuller discussion should replace the simple statements made about the inadequacy of the available models.

The Committee commends the Agency for looking at new approaches, such as the benchmark dose procedure, to improve quantitative assessment of non cancer endpoints. However, the Committee has submitted suggestions on how to further improve these approaches and how to make these new approaches more clear, accurate and concise, including the following recommendations:

a) There was an apparent mathematical error in the calculation of the benchmark concentration for reproductive and developmental effects which must be addressed. All calculations should be easy to follow, and of course, carefully proofed.

b) There are new dominant lethal studies, which are not included in the risk assessment, that failed to replicate earlier findings.

c) A variety of viewpoints were expressed within the Committee over the extent to which toxicokinetic analyses should be incorporated into the assessment. At a minimum the different toxicokinetic hypotheses and the hypothesized role of the various metabolites should be discussed, at least qualitatively, with an indication of the degree to which the assessment would be impacted if some of the hypotheses were later proven true.

d) Inadequate justification is given for the application of the additional safety factor for the benchmark dose. Some of the Committee members could not understand the rationale for its conclusion.

e) The rationale for the selection of the toxic non-cancer endpoint that is utilized in the derivation of the RfC is very important and should be more explicitly explained.

f) The review document is not a comprehensive health assessment and contains only an overview of the ambient concentrations and exposure of those populations adjacent to emissions sources, without including any actual exposure assessment as such. Consequently, as the document now stands, it should be retitled as a "Hazard Assessment" until such time as an exposure assessment component can be incorporated. The document could be called "Health Risk Assessment of 1,3-Butadiene: 1. Hazard Assessment."

2. INTRODUCTION

2.1 Background

The draft *Health Risk Assessment of 1,3-Butadiene* (USEPA, 1998a) was developed by the EPA Office of Research and Development at the request of the Office of Mobile Sources. This document was requested to support a future Air Toxics Rule. The document was not intended to be a comprehensive health assessment. Consequently, an actual exposure assessment is not included in the document. The draft *Health Risk Assessment of 1,3-Butadiene* (USEPA, 1998a) focuses on carcinogenicity, mutagenicity, and reproductive/developmental effects. The document presents the Agency's first benchmark dose analysis for reproductive/developmental factors.

The draft document that was reviewed by the Committee updates a previously, published document (USEPA, 1995). In the current draft document, the Agency has included many new studies which have been published since 1985. Based on the weight of overall evidence from human, animal, and mutagenicity studies, the Agency concludes, in the current draft document, that 1,3-Butadiene is a known, human carcinogen.

The EHC subsequently met On April 30 and May 1, 1998, in Washington, DC to review the Agency's draft *Health Risk Assessment of 1,3- Butadiene* document.

2.2 Charge

The Committee was charged to provide comments on each of the following aspects of the document:

a) Review the health risk assessment for technical quality, comprehensiveness and clarity (Address each chapter, but with specific reference to Charges b, c, and d).

b) Does the science support the classification of "known" human carcinogen?

c) Are the approaches taken to characterize plausible cancer risks reasonable given the science?

d) Are the conclusions and quantitative estimations for reproductive and developmental effects adequately supported?

3. RESPONSE TO THE CHARGE

This report captures comments and recommendations reflecting a consensus by the Committee, as well as specific technical comments and recommendations provided by individual Committee Members (see Appendix A). It was not possible to review and achieve consensus for each and every technical point provided in this Committee report. On major points, however, an attempt has been made to reflect the consensus or range of views on the Committee.

3.1 Technical Quality, Comprehensiveness and Clarity

The Environmental Health Committee was asked to: *review the health risk assessment for technical quality, comprehensiveness, and clarity*. The Committee provided comments and recommendations for each chapter.

3.1.1 Chapter 1 - Introduction

The Introduction presents a clear and comprehensive summary of information on 1,3-Butadiene for the period from 1985 until January 31, 1997. It was concluded that apparent differences in assessments by different groups might be explained by the availability of studies at the time of evaluations, different cancer classification systems, and quantitative assessments done for different purposes. Does this mean that they are all equally valid and scientifically defensible? In other words can the statements made in Table 1-1 be restated in comparable terminology to show that they are all compatible? If they are, as implied, then the document should demonstrate this in subsequent chapters. If not, the Introduction should state so, and subsequent chapters should explain why. The EHC also recommends that concentrations be expressed in one constant unit throughout the document as well as in Table 1.

Every document must have a cut-off in time for completion. However, the International Agency for Research on Cancer (IARC, 1998) has recently re-evaluated the evidence on the carcinogenicity of 1,3-Butadiene, and Health Canada is completing a health evaluation on the compound. It will be important to include the IARC evaluation in an updated version of the health assessment, and should the Health Canada document be finalized soon, it should also be included. For example, Table 1-1 should include the IARC evaluation (IARC, 1998), and if available, the finalized Health Canada evaluation. Also, the Agency should clarify, in the Table, whether the OSHA classification listed 1,3-Butadiene as a "known" or "potential" carcinogen.

It is stated that the profile for setting the American Conference of Governmental Industrial Hygienists-Threshold Limit Value (ACGIH-TLV) for 1,3-Butadiene is not reviewed because it is not a risk assessment. This may be true for the EPA definition of a risk assessment, but the Agency should include some history of what has been used for the work environment, particularly because of the high values in the past and the importance of epidemiological studies on worker population.

There should be a comment in the final document on the accessibility of unpublished data that is referenced in Agency documents. This concern was based on the use of the Delzell (1995) study in the Agency's health risk assessment of 1,3-Butadiene even though the review document was not readily available to the public.

Finally, there is no mention in the Introduction of EPA's proposed Cancer Risk Assessment Guidelines. While these guidelines have not been completely finalized, they are sufficiently complete to be referenced and utilized as appropriate (EPA, 1996).

3.1.2 Chapter 2 - Overview of Exposure to 1,3-Butadiene

This chapter is intended to be an introductory review of possible sources of exposure to 1,3 Butadiene. It should be clearly indicated in the first paragraph that this chapter is not intended to be a comprehensive review of exposure so that this chapter is not mistakenly used as a source of data for enforcement purposes. An explanation should be included on how concentrations have been measured over the years and how this might impact a comparison of exposure levels measured in earlier years with more recent measurements. Ranges of concentrations are important to include along with averages. The Committee recommends that all of the exposure concentrations be expressed in one common unit throughout Chapter 2.

Additional, more specific, comments are provided in Appendix A.

3.1.3 Chapter 3 - Metabolism and Pharmacokinetics

Chapter 3 reviews the metabolism and pharmacokinetics of 1,3-Butadiene. This chapter is long, contains numerous incorrect or incomplete statements that need to be changed, and should be updated. The title of the chapter should be *Metabolism and Toxicokinetics*, as Butadiene is not used as a therapeutic agent. Also, the word, toxicokinetics, should replace pharmacokinetics throughout the document.

The Health Canada assessment of Butadiene (Health Canada, 1997) and comments submitted by the public during the open meeting (Himmelstein, 1998) will provide many of the new references that need to be incorporated into the revision. Specific issues that should be addressed in the revision of the risk assessment are provided below.

In evaluating the data on the metabolism of Butadiene and its epoxide metabolites across species, including humans, the EPA concluded that there are no clear species differences in Butadiene metabolism and that in many cases there is overlap in metabolic rates across species. In forming their conclusion, the EPA misinterpreted the *in vitro* data and ignored the major conclusion from the *in vivo* data. With respect to the *in vitro* data, the EPA compared maximal rates of metabolism (i.e., V_{max}) rather than basing their comparisons on a more appropriate measure of metabolic rates, the ratio of V_{max}/K_m. This ratio is more appropriate because: a)

species differences are observed in both metabolic parameters; and b) at non-metabolically saturating concentrations (i.e., those concentrations relevant for humans) it is the ratio, not the maximum rate, that dictates the rate of metabolism. With respect to the *in vivo* data, the EPA has not adequately summarized the data from two independent laboratories that clearly and unambiguously show large differences spanning orders of magnitude in the blood and tissue concentrations of the Butadiene metabolite diepoxybutane. In particular, Table 3-8 is misleading. This table includes data from the rat, mouse, and monkey for blood epoxybutene and diepoxybutane concentrations from studies conducted by Bond *et al.* (1986) and Dahl *et al.* (1991) which did not use gas chromatography-mass spectrometry (GC-MS) methods to quantitate these metabolites (Bond *et al.*, 1986; Dahl *et al.*, 1991). Therefore, the values reported by these investigators are unreliable. It would be preferable to, instead, compare the blood concentrations reported by Himmelstein *et al.* (1994), Bechtold *et al*. (1995), and Thorton-Manning *et al.* (1995a; 1995b; 1996) in one table. This comparison would serve two purposes. First, it would highlight the interlaboratory reproducibility in the values reported for these metabolites serving to increase the reliability of the data. Second, it would accentuate the dramatic species differences in the circulating levels of diepoxybutane, lending support to the hypothesis that this interspecies difference in metabolite formation underlies the differences in susceptibility observed in the chronic studies.

Mutagenicity studies strongly suggest that diepoxybutane is **a**, if not, **the** critical metabolite in Butadiene carcinogenicity. Thus, differences in levels of this metabolite formed *in vitro* and *in vivo* are highly consistent with the observed species differences in carcinogenicity. The Committee recommends that the Agency integrate and assess this information, develop conclusions based on the weight of all the information presented, and present these conclusions in the closing paragraphs of Chapter 3.

It is recognized that the EPA elected a cutoff date for inclusion of new information of January 31, 1997. However, there is no question that a significant amount of new and important information has been developed since this date that is particularly pertinent to Chapter 3 on Metabolism and Pharmacokinetics. In short, the chapter on Metabolism and Pharmacokinetics needs to be updated to include key references that have been published in the peer-reviewed literature since January 31, 1997. Many of the data sets that have been published since this date lend further support to the hypothesis that diepoxybutane is a critical metabolite involved in the carcinogenicity of 1,3-Butadiene.

Although this chapter references important literature relative to the *in vitro* and *in vivo* metabolism and Butadiene, it falls short in providing a comprehensive integration of the relatively diverse data sets. A critical concept that appears lacking in this chapter is the fact that diepoxybutane represents a critical metabolite of Butadiene and that there are significant species differences both *in vitro* and *in vivo* in the formation of this important Butadiene metabolite. This chapter neglects to note that metabolism and toxicokinetic studies of Butadiene conducted in whole animals and in rodent and human tissues provide important insights into the likely critical steps in the initiation of Butadiene carcinogenicity and importantly the identity of the most likely

chemical species responsible for the development of tumors. For example, dosimetry data on both epoxybutene and diepoxybutane following inhalation exposure to Butadiene clearly indicate that blood concentration of epoxybutene were up to 8-fold higher in mice compared with rats and that blood concentrations of diepoxybutane were nearly 40-fold higher in mice than in rats. Similarly, tissue concentrations of epoxybutene ranged from 3-10 times higher in mice compared with rats and tissue concentrations of diepoxybutane were up to 100 times higher in mice than rats. Importantly, therefore the correlation between measured circulating blood and tissue levels of the epoxides, particularly diepoxybutane and the observed development of tumors is clearly suggestive of a role of diepoxybutane in the initiation of cancer. This important concept is not presented in the chapter.

Moreover, *in vitro* data on the metabolism of Butadiene suggest that mice form epoxybutene and diepoxybutane at a faster rate than rats or humans. Studies on the *in vivo* metabolism and tissue concentrations of epoxybutene and diepoxybutane in mice and rats following inhalation exposure to Butadiene are consistent with the *in vitro* studies on metabolism of Butadiene. This is an important point and the chapter neglects to point out the close parallels between the observations from *in vitro* studies and *in vivo* studies.

The Agency notes that Butadiene is an animal carcinogen and that the mouse is more sensitive than the rat to Butadiene induced carcinogenicity. The Agency also notes that the reasons for these interspecies differences are not understood at this time. However, the available mechanistic data on the metabolism of Butadiene and its reactive epoxide metabolites supports the hypothesis that interspecies differences in metabolic rates form the underpinning for the increased sensitivity of the mouse compared with the rat. These differences in metabolic rates result in a faster production and slower detoxification of the diepoxybutane in mice compared with rats with resultant higher steady state levels of the diepoxybutane in blood and tissues in the mouse compared with the rat following exposure to Butadiene. This observation coupled with the demonstrated hundred-fold greater mutagenicity of the diepoxybutane compared with the epoxybutene points to interspecies differences in the formation of diepoxybutane being critical to interpretation of differences in response.

Additional, more specific, comments are provided in Appendix A.

3.1.4 Chapter 4 - Mutagenicity

This chapter summarizes the genotoxicity of 1,3 Butadiene (BD) and its metabolites for mice, rats and humans, considering both cytogenetic endpoints and gene mutations in somatic and germinal cells. The emphasis is on recent *in vivo* studies, although there is some mention of results from *in vitro* assays.

The chapter would be improved by adding text tables summarizing key animal and human findings derived from the entire body of information on BD. References to support key findings should be included in these tables. Separate tables should be included for *in vitro*, animal and

human findings, and similarities and species differences in response should be noted.

The chapter, as currently written, does not give sufficient emphasis and weight to the positive heritable translocation studies in mice (given their potential relevance for human heritable risks) (Adler *et al.,* 1998; 1995) and to several additional studies conducted in humans (Pacchierotti, 1998). This finding is also discussed in Section 3.1.5 of our report, which addresses the *Reproductive and Developmental Effects* chapter of the review document.

The conclusion section should be expanded to include what is known about the mutagenicity of BD and its metabolites from the extensive literature. Careful editing of the final document should be conducted to avoid missing dose units, units for mutant frequencies, and similar omissions.

The binding of BD metabolites to DNA should be discussed in greater detail. Evidence for reactivity with the DNA itself demonstrates that these BD intermediates reach their target molecule for genotoxicity causing pre-mutagenic DNA lesions.

Early studies showed that DEB binds to N-7 guanine and that it forms inter-strand crosslinks (Brooks and Lawley, 1961; Lawley and Brooks, 1967). Subsequently it was shown that B6C3F1 mice and Wistar rats exposed to ^{14}C labeled BD by inhalation have covalently bound reactivity in liver DNA, as noted in Chapter 3 (Kreiling *et al.*, 1986). The amounts bound in the two species were comparable. The nature of the bound residues was not determined. The complex kinds of adducts formed in DNA by BD metabolites are also under active investigation. Specific enantio- and regioisomeric EB adducts formations have been shown (Koivisto *et al.*, 1997; Tretyakova *et al.*, 1998). The N-7 position of guanine has been shown to be the most reactive with EB, followed by the N-3 and N-1 positions of adenine. EB adducts have also been found at the N-6 position of adenine but this may represent a rearrangement of the N-1 adenine adduct. Adenine adducts may be important for the genotoxicity of BD as shown by mutational spectral studies of this agent. (Leuratti *et al.,* 1994; Cochrane *et al.,* 1994).

The several nucleobase adducts in DNA formed by BD metabolites are being characterized further by several groups (Leuratti *et al.*, 1994; Neagu *et al.*, 1994, 1995; Selzer and Elfarra, 1996; Kumar *et al.*, 1996). Furthermore, attempts are being made to use high sensitivity methods for detecting DNA adducts in humans for biomonitoring purposes, including the detection of urinary DNA adducts. However, despite the statement made in Chapter 11, page 11-9, para. 1, line 2, the Committee is not aware that DNA adducts have even been observed *in vivo* in humans. The statement in this regard made on page 11-9 either needs a reference or should be corrected.

The extensive mutagenicity results covered in the several reviews cited in Chapter 4 should be discussed in more detail. These results include positive Salmonella assays in the presence of S9. It is important to note that human S9 also converts BD to mutagenic metabolites, as determined in the Salmonella system (Arce *et al.,* 1990). BD metabolites also have been

positive for mutagenicity in a variety of microorganisms with or without metabolic activation. Studies of mutation by BD at the tk locus in mouse lymphoma cells have been both positive (Sernau *et al.*, 1986) and negative (McGregor *et al.*, 1991). BD, as opposed to its metabolites, has not been found mutagenic *in vivo* in *Drosophila melanogaster*, as assessed by the sex-linked recessive lethal mutation assay (Foureman *et al.*, 1994) or by the Wing Spot Test (Victorin *et al.*, 1990).

The species comparisons should be considered in detail. There have been numerous cytogenetic studies *in vivo* in rodents. These are covered in the cited reviews and, as correctly stated in Chapter 4, support the dichotomy in carcinogenic response between these two species, where mice are more responsive than rats. However, it should be noted that, with regard to cytogenetic assays *in vivo* in these two species, there are no reports of positive results in rats exposed to BD (as exposed to BD metabolites) but there are several reports of positive results in mice.

It is important that the document reflect species differences in clastogenicity. The difference in BD's apparent mutagenic potencies for mice and rats are worthy of comparison. Studies can be considered as those measuring clastogenicity and those measuring specific gene mutations. In regards to clastogenicity, for mice there are numerous reports of chromosome aberrations, micronuclei and SCEs in somatic cells, i.e., in both lymphocytes and in bone marrow cells, several showing effects at low doses. However, in rats, neither chromosome aberrations nor micronuclei have been found in blood cells *in vivo* (i.e., in lymphocytes, bone marrow or peripheral red blood cells) after BD inhalation (Arce *et al.*, 1990; Autio *et al.*, 1994), although there is a report of a weak positive SCE response (Maki-Paakkanen *et al.*, 1993).

For germ cells, the contrast in clastogenicity results between these species is equally striking. In mice, there are reports of dominant lethal effects with inconsistent results (references given in the draft document), sperm head abnormalities (Morrissey *et al.*, 1990), micronuclei in spermatids (Xiao and Tates, 1995; Tommasi *et al.*, 1998) and a positive Comet assay in haploid and polyploid testicular cells (Brinkworth *et al.*, 1998). The dominant lethal studies discussed in Chapter 5 should be cross-referenced in Chapter 4 as they are important to the discussion of heritable chromosomal alterations induced by BD. Cytogenetic abnormalities in first-cleavage embryos sired by male mice treated by BD inhalation have also been reported recently (Pacchierotti *et al.*, 1998). By contrast, in the single study in rats, no dominant lethal mutations were found (BIBRA, 1996).

Of special importance among the studies of clastogenicity are those of heritable translocations in mice. The first study (Adler *et al.*, 1995) is discussed briefly in Chapter 4 . A second study by the same group has recently been reported (Adler *et al.*, 1998). These studies are particularly relevant to human health and risk assessments for human heritable damage and should be emphasized. In fact, a human risk estimate has recently been reported using a parallelogram approach that employed mouse somatic cell clastogenicity (micronuclei in bone marrow cells), mouse germ cell clastogenicity (the heritable translocations) and human somatic cell clastogenicity

(in lymphocytes from BD exposed workers) to estimate the heritable translocation risk for humans (Pacchierotti *et al.*, 1998). The estimated doubling dose for human heritable translocations was given as 1,100 ppmh (parts per million hours)

Species differences in mutagenicity should be detailed. Comparison of the specific locus mutagenicity of BD between mice and rats is not nearly as striking as is the comparison for clastogenicity. The studies summarized by Recio and Goldsworthy (1995) showing an increase in BD-induced lacI mutations at A:T base pairs in bone marrow stem cells from young transgenic B6C3F1 (BB) male mice are discussed in Chapter 4. However, it should be mentioned that an earlier study in young male Balb/c X DBA/2 (CD2F1) transgenic mice (MM) found lacZ BD induced mutations only in lung cells and not in liver or bone marrow stem cells (Recio *et al.*, 1992). There is also a report of a positive spot test in "T" stock mice that found an increase in *in vivo* mutations in embryonic melanoblasts (Adler *et al.*, 1994).

The several reports of positive hprt mutations in young B3C3F1 mice exposed to BD by inhalation should be noted in the revised document. The Cochrane and Skopek (1994b) study mentioned in Chapter 4 exposed pre-weanling mice and found dose-related mutational increases. Again, there was a bias for A:T changes. The Meng study mentioned in Chapter 4 (Meng *et al.,* 1996) also exposed young B6C3F1 male mice to BD and found increases in hprt mutations in both thymic and splenic lymphocytes that persisted for several weeks after the exposures. This work has now been accepted as a full publication and indicates that the hprt mutant frequency (MF) in thymic lymphocytes rises from 2.2×10^{-6} to 11.3×10^{-6} at two weeks (maximal) and, in splenic lymphocytes, from ~1.8×10^{-6} to 19.7×10^{-6} at five weeks (maximal). The mutagenic potency of BD in these mice, which is a measure of hprt mutant cell accumulation, was calculated to be 69.62. The negative reports for *in vivo* hprt mutations in other strains of mice that were older at the time of their BD exposures should also be noted. Tates *et al.* (1994) originally found low order hprt mutagenicity in splenic lymphocytes of 10-12-week-old (102/E1 X C3H/E1) F1 mice , but later failed to find an increase in such hprt mutations in a second study of this strain or in adult CD1 mice (Tates *et al.*, 1998).

In vivo mutations in rats should be noted in the document. In contrast to the clastogenicity studies, specific locus gene mutations have been found *in vivo* in rats exposed to BD by inhalation. Meng *et al.* (1996) exposed young rats with results as noted in Chapter 4, i.e., increases in hprt mutations in thymic lymphocytes from 2×10^{-6} to 4.9×10^{-6} at three weeks after exposure (maximal) and, in splenic T-cells, from ~1.9×10^{-6} to 10.1×10^{-6} at four weeks after exposure (maximal) (references given in chapter). The mutagenic potency of BD in rats was calculated to be 15.85. Thus, the ratio of mutagenic potencies between mice and rats (mice/rats) was calculated to be 4.4 or 5.0, depending on the weeks allowed for mutant cell accumulation. Although BD is five times more mutagenic in mice than in rats, the magnitude of this difference is not nearly as great as that reported for the carcinogenicity differential between these two species.

The many rodent studies of BD metabolites, some positive and some negative, show a similar pattern and should be discussed in the document. In general, younger animals tend to give

the positive results and, for mice, it is the B6C3F1 animals that are most often associated with positive studies. The Cochrane and Skopek studies of EB and DEB were noted in Chapter 4. Meng *et al.* (1996, 1997) have extended their studies in young mice (B6C3F1) and rats (Fischer) to EB and DEB by inhalation and have shown increased hprt mutations in splenic TÄcells in both species. In rats, however, the response to EB was equivocal. In contrast to these positive results, Tates *et al.* (1994) found no hprt mutation induction in older mice (102/E1 X C3H/E1) F1 and CD1 or rats (Lewis) administered EB or DEB by injection or in the drinking water. However, as noted in Chapter 4, rat germinal cells are at least as susceptible (or more so) to the clastogenicity of BD metabolites as are mouse germinal cells.

In summary, there are striking species differences between mice and rats in the reported studies as regards clastogenicity. There are numerous positive studies of this endpoint in mice, but BD induced clastogenicity has not been demonstrated *in vivo* in rats. Of note, heritable translocations have been demonstrated and confirmed following BD exposures to mice. Clastogenicity has been observed in mice and rats exposed to EB or DEB. For gene mutations, B6C3F1 mice appear to be more sensitive than other mouse strains to the mutagenic effects of BD, EB or DEB. In general, mice are also more sensitive than rats to specific gene mutations, although such mutations can be induced in younger rats. Mutations are most commonly seen in both species in young animals, indicating that cell proliferation may be required to produce these mutagenic effects.

Human Studies, because of their relevance to human health, are addressed in detail. Chapter 4 includes most of the relevant mutagenicity (clastogenicity and specific gene mutations) studies in human cells and/or *in vivo* in humans. In discussing the Cochrane and Skopek (1994) study of hprt and tk mutations *in vitro* in human TK6 cells described in page 4-1, the EPA document should note that mutation induction was assessed for EB, DEB and for Ebdiol (EBD). The mutagenic potencies of these BD metabolites were found to be DEB > EB > EBD. It is important to note that the metabolite EBdiol has been studied and has been found to be mutagenic. The importance of EBdiol lies in its abundance. Therefore, even though Ebdiol may be the least mutagenic of the metabolites, it may be the most abundant, and therefore may give the most mutations.

Some of the *in vivo* cytogenetic studies in humans are covered in Chapter 4. The positive challenge assay reported by Au *et al.* (1995) is noted, as is the negative report of chromosome aberrations, micronuclei and SCEs in exposed workers by Sorsa *et al*. (1994). Not noted, however, is the update by Sorsa *et al.* (1996) that reanalyzed these data according to GSTT1 status and found that the T1 null workers appeared to have increases in chromosome aberrations. Also, the increases in chromosome aberrations but not in frequency of micronuclei reported by Tates *et al.* (1996) and noted in Chapter 4 has been updated in a full manuscript that reports increases in both chromosome aberrations and SCEs in BD exposed Czech workers (Tates *et al.*, 1998). The frequencies of micronuclei were not increased and the Comet assay was negative. The *in vitro* findings of increased EB induced SCEs in lymphocytes from GST M null individuals and increased DEB induced SCEs in lymphocytes from GST T null individuals are reported in

Chapter 4 and are important, as is the observation that GST status does not appear to affect SCE frequencies induced by EBD. A recent *in vitro* study observed chromosome specific aneuploidy (for chromosomes 12 and X) in human lymphocytes *in vitro* following treatments with either EB or DEB (Xi *et al.*, 1997). The paper by Wiencke *et al*. (1995) demonstrating the affinity of the glutathione transferase theta enzyme for the diepoxide metabolite of BD should be added.

The status of the *in vivo* hprt mutation studies in humans has been accurately reported in Chapter 4. There are the three positive studies using the autoradiographic assay, as noted, and the two negative studies that employed the cloning assay. Chapter 4 discusses the discordance in these two groups of studies and concludes that, regardless of the reason for the difference, the positive results with the autoradiographic assay probably reflect a mutagenic effect of BD in the monitored workers. There was a difference in opinion among the Committee regarding the hprt results. Some of the Committee found the Agency's conclusions to be reasonable while other Members/Consultants were concerned that the autoradiographic results may be method-related.

The document's explanation as to possible reasons for the discordance between the positive effects obtained by autoradiography and the negative results found by cloning for human BD induced hprt mutations *in vivo* however should be rewritten. In considering the differences between the Ward *et al.* (1994; 1996) positive autoradiographic studies and the Hayes *et al.* (1996) and Tates *et al*. (1996) negative cloning assay studies, the draft document concludes that "a simple explanation would be that the increase in the autoradiographic assay was due to clones of mutants having arisen from earlier mutations." There was a diversity of opinion within the EHC regarding the discordance between the positive effects obtained by the autoradiography and the negative results found by cloning for human butadiene inducted hprt mutations *in vivo*. Some Members of the Committee found that the Agency's explanation is probably not correct for two reasons. First, the autoradiographic assay requires a technical step before the T-cells can be assessed for hprt mutations, i.e., cryopreservation or some measure to arrest those few cell that are cycling in human peripheral blood at the G1-S interphase. This is simply a technical means to insure that cycling non- mutant T-cells do not progress to their S-phase *in vitro* in the presence of 6-thioguanine and become labeled - even slightly labeled - and thereby become scored as mutants in the assay. Such cells are not mutants but would appear to be so by virtue of their labeling, i.e., they are phenocopies. This phenomenon, which elevates scored variant frequencies, can be eliminated by arresting the cycling cells at this phase of the cell cycle, from which they rapidly proceed into S *in vitro* before the label is added. Thus, they miss the scoring window and are not scored as variant cells.

The reason why this arresting or cryopreservation step is relevant to the issue of clonality is that T-cells that are undergoing clonal expansions *in vivo* tend to be among the cycling cells. If such large mutant clones were present, the cycling members of the clones would be eliminated from the scoring window by the cryopreservation step (even though these would be mutants). Therefore, if anything, clonal amplifications are less likely in the autoradiographic than in the cloning assay. Said the other way around, if *in vivo* clonality were the reason for the higher mutant frequencies in exposed vs. control workers, this phenomenon would have most likely

affected the results in the cloning assay. This is the exact opposite to what was observed, i.e., the increased mutant frequencies in exposed over controls were observed with the autoradiographic assay. Another reason why *in vivo* clonality cannot account for the differences in results between the assays is that molecular studies were not done. Such studies cannot be done for the autoradiographic assay but can when using the cloning assay. Molecular analyses allows detection of *in vivo* clonality using the cloning assay. Since no such studies were done, no "corrections" were made and the point is moot.

There are other differences between the autoradiographic and cloning assays that could account for the differences in results obtained with these two assays. The autoradiographic method is a phenotypic assay, meaning that mutants (or variants) are consumed by the assay and, therefore, cannot be analyzed at the molecular level. It is not, therefore, possible to demonstrate that the observed mutants scored by this assay are actually genotypic mutants. This, however, is not different from other phenotypic assays for mutations that are in wide use. To be scored in the autoradiographic assay, variant cells have simply to synthesize DNA in their first S *in vitro*. No cell division or growth is required. In addition, the cells must begin their DNA synthesis within 36-48 hours in culture. Therefore, a quite different T-cell subpopulation may be scored in the autoradiographic than in the cloning assay. It is possible that, in the former, only that subset of cells that are capable of division quickly *in vitro* is scored whereas, in the cloning assay, all T-cells are scored. Alternatively, what is being scored in the autoradiographic assay may not be fixed mutations but rather adduct-blocked RNA transcription. Another possibility is that the cell that would go into a G2 block, and therefore not be measured by the cloning assay, are scored in the autoradiographic assay because they are not required to go through G2 for scoring.

The autoradiographic assay requires that peripheral blood T-cells be cryopreserved in order to arrest cycling cells at the G1-S interphase. If all cycling cells are not eliminated, the autoradiographic assay might, because of artifacts, give an elevated reading because of the scoring of phenocopies. This is explained above. As for all mutation assays, the autoradiographic assay may have a scoring bias. However, the cloning assay too has an inherent observer bias and a tendency of some technicians to score only larger mutant colonies.

In addition, with respect to the BD studies, the differences between the positive and negative studies may be due, in part, to differences in study populations. The Ward *et al.* (1994; 1996) radiographic studies were conducted at a butadiene monomer production facility and an SBR production facility in Texas. The Tates *et al.* (1996) study was conducted in the Czech Republic (butadiene monomer production) and the Hayes *et al.* (1996) study in China (polybutadiene rubber production). Smoking and other lifestyle and confounding parameters would be different among these populations. In addition, the exposure assessment in these studies were not precise and no attempt was made to determine mutation susceptibility profiles of the monitored workers (It is of note, however, that one of the highest variant frequency values in the Ward *et al.* (1996) study was in a GST T null individual.).

Few conclusions are made at the end of Chapter 4. The quality of the conclusions would be improved by expanding this section and adding statements summarizing what we know about the mutagenicity of BD and its metabolites. The following list could serve as a guide:

a) Rodent studies indicate that some of the metabolites of BD are DNA binding agents.

b) There are numerous *in vitro* assays in several model systems that demonstrate the genotoxicity of BD and/or its metabolites.

c) *in vivo* studies in mice and rats show that the former is the more sensitive species for the genotoxicity that follows exposure to the parent BD.

d) Young animals (including mice), and perhaps certain strains within a species, are more susceptible to the genotoxicity of BD and/or its metabolites than are others as indicated by hprt results.

e) Clastogenicity has not been demonstrated for either somatic or germinal cells in rats exposed to the parent BD.

f) Heritable translocations are induced by exposure of male mice to BD by inhalation (This is an important point for estimating heritable risks.). Equivalent data are not available in the rat. Any estimation of human heritable risk must account for species differences in metabolism.

g) BD metabolites give genotoxic effects in both rats and mice with mice being the more sensitive to somatic effects but both species being equally susceptible (or rats more so) to the germinal effects.

h) Human studies have shown that BD metabolites cause gene mutations *in vitro* in human cells.

i) GST genotypes may affect susceptibility to the clastogenicity (and possibly mutagenicity) of BD metabolites.

j) There is evidence of clastogenicity *in vivo* in human lymphocytes from exposed workers.

k) Hprt mutations have been shown *in vivo* in human T-lymphocytes of exposed workers as documented by the autoradiographic but not by the cloning assay for these events.

3.1.5 Chapter 5 - Reproductive and Developmental Effects

It was evident to the Committee that the EPA had a large amount of material to review since that there were over 20 animal bioassays on this topic area. In addition, many new studies have come in after the cut-off date for the assessment and, as noted below, this research will need to be included in the report.

Because of the volume and the complexity of the studies and reports for both endpoints, additional summary tables are necessary in Chapter 5. All pertinent studies that are discussed in this chapter should be introduced in a summary table at the start of each section for developmental and reproductive toxicity. A good example is Table 5-13 which was prepared for summarizing the structure-activity information.

Chapter 5 needs to include both positive and negative studies in the assessment. For example, text for Table 5-13 says that "since no non-neoplastic lesions were seen, then they were not included in the table." The report should include positive and negative data so the reviewer can develop a comprehensive understanding of the data base supporting the assessment. Also, the health assessment needs to include more current research. Every effort should be taken to extend the time for data inclusion to as close as possible to the release date of the assessment. For example, because of the cut-off date, several new dominant lethal studies were not included. These 2 new negative studies need to be included in the reassessment. The BIBRA Study III (BIBRA, 1998), which was a repeat of BIBRA I (BIBRA, 1995), was negative and needs to be considered in this health assessment especially since the BIBRA I study was used in one of the quantitative assessments (BIBRA III) (BIBRA, 1996; 1998). The written statement from Christian (1998) identifying and criticizing these studies should be considered in the revision of the document.

It was difficult for reviewers of this document to integrate the toxicological findings. A much greater emphasis on integrating findings across the chapters is needed. For example, information on toxicokinetics and metabolism needs to be fully integrated into this chapter. The information on toxicokinetics and metabolism in the review document appear to be almost an after thought. Interesting data on specific ovotoxic metabolites are available, and should be incorporated. The potential impact of including the PBPK model in the assessment of reproductive and developmental toxicity needs to be discussed. Does this help to explain dose response differences across species for ovotoxicity? The Agency should explain this in the assessment. It is important not only to show data but to discuss the implication of the data. Another example where integration is needed is with the general toxicity chapter with Chapter 5. Was there any evidence for neurotoxicity from general toxicity testing to suggest the need for developmental neurotoxicity assessment? What about effects in utero on ovarian or testicular development? The heritable translocation data from Chapter 3 should be integrated with Chapter 5.

Because of the potential interrelatedness of the reproductive tumors and reproductive organ atrophy data, the reproductive chapter should have some overlap with the chapter discussing tumor results for these reproductive organs. Also, Chapter 5 should include a discussion on the mechanisms responsible for atrophy and reproductive tumors, and provide discussion on the biological significance of ovarian and testicular atrophy. This is especially true as these endpoints are modeled in Chapter 9. How are exposed rodents different than control rodents? The Agency should explain its rationale for looking at ovarian atrophy in aged rodents.

The following issues should also be addressed in revising Chapter 5:

a) The reader needs to understand the rationale for modeling the dominant lethal data. The Agency should provide discussion that supports the use of these endpoints in Chapter 9. Since new studies on heritable translocations are available, these findings should be integrated with the chapter on mutagenicity. This will provide stronger evidence for the potential of this compound or its metabolites to produce heritable and multi-generational impact. The EPA should integrate the data so that the total picture of research supports its identification of critical endpoints and quantitative modeling.

b) For both the reproductive and developmental endpoints, it is necessary to list all assumptions, identify where the assessor is uncertain and identify agency action taken to respond to that uncertainty. The Agency should explain what the critical data needs are that will address this uncertainty and should be as specific and explicit as possible.

c) The Agency should avoid the use of speculative and editorial types of sentences. A specific example is on Page 5-28, lines 32-33; the assessment speculates that the compound does not cross the blood-testis barrier. Why does the Agency think that this is true? Is this consistent with the chemistry of the compound (i.e., solubility)? Is it consistent with the postulated effects of this compound on spermatozoa?

In summary, Chapter 5 does not prepare the reader for the discussion in Chapter 9 on the quantitative risk assessment for 1,3-butadiene. For example, how does the biology information affect your choice of models? In Chapter 5, the Agency briefly makes a statement about thresholds, but does not explain implications for quantitative risk assessment. How did the Agency decide to drop the highest dose levels? Was this because of excessive toxicity? If so, the EPA should explain the rationale. What criteria will the EPA apply to let the reader know when the Agency will drop the results on the basis of the doses?

3.1.6 Chapter 6 - Toxicity in Animals

This chapter provides detailed information about subchronic, chronic and carcinogenicity studies published from 1985 to present. Only three studies are mentioned in the subchronic

section. It is unclear why other repeat dose studies are not reviewed. Have they **all** been incorporated in other chapters? Repeat dose *in vivo* mammalian studies of 1,3 butadiene would be appropriate for inclusion in this section unless it is explicitly stated that they are covered elsewhere in the document. It is also important to insure that the data from *in vivo* repeat dose studies get thorough review because the Inhalation Reference Concentration (RfC) value should be derived on the most sensitive adverse endpoint that is meaningful for human health. The most sensitive non-cancer biological effect of 1,3 butadiene currently cannot be determined from the health risk assessment document, as comparative assessments are not made in any of the chapters.

In the chronic and carcinogenicity sections, the use of frequent text tables helps to simplify and to provide clarity to the presentation. Since the National Toxicology Program (NTP) chronic bioassay is a part of EPA's quantitative risk assessment, the detailed coverage is warranted. The NTP study (NTP, 1993) contains both chronic toxicity and carcinogenicity data and the study is discussed in both areas of EPA's draft. In order to make the chapter more understandable, the Committee recommends that the Agency present the study details presented in the first section (chronic) and then refer to them in the carcinogenicity section rather than repeat the material in slightly different words. As it reads now, the review document gives the impression that they are different studies. The Agency should also use the same categories of tumors for presenting data from the continuous treatment, 9 and 15-month interim sacrifices and stop-exposure study in order to permit comparisons. Most of the time this was done. However, for target lymphatic tumors the categories are different, preventing a direct comparison.

A table summarizing the positive oncogenic findings across all studies specifying the dose tested, and the type of tumor that was significantly elevated, should be added because of the numerous organs and tumor types involved. Data from the rat oncogenicity study should be included as this information is important and is only indirectly mentioned in the risk assessment.

For clarity, it would be preferable to separate the carcinogenic evidence on the mammalian metabolites of 1,3 butadiene from the data on related chemicals and place it in a subsection by itself. The section on related chemicals should be updated to include the results from studies on styrene, isoprene and any other relevant chemicals.

The quality of the chapter's Discussion and Conclusion section is good. The observation that concentration, not time is a critical determinant of potency is interesting but it is not supported by a comparison of the tumor data presented in Table 6-4 and Table 6-8 from the NTP study (NTP, 1993) for continuous lifetime treatment and stop-exposure study. It is rare that carcinogenic data are available from continuous, interim sacrifice and stop-exposure studies. Further analysis of a comparison of the data from continuous and lifetime treatment would be interesting to see if there is supporting evidence for a biological model of dose and time that could be used in the risk assessment.

3.1.7 Chapter 7 - Epidemiologic Studies of Carcinogenicity

It is unclear why this chapter presented details of all the historical reports for a given butadiene (BD) exposed group, since more recent reports supersede the previous ones by virtue of having longer follow-up and more numerous deaths. Chapter 7 should be restructured so that it is a detailed report on the latest follow-up of each epidemiologic study, with perhaps any additional analyses from previous reports that were not duplicated in the most recent report. More specific recommendations for Chapter 7 follow.

a) The case for an association of butadiene exposure with lymphosarcoma/ reticulosarcoma (ICD 200, a no-longer-used subset of non-Hodgkins lymphoma; hereafter called just "lymphosarcoma") is based on two studies of butadiene monomer plants which the EPA draft report indicated had excesses of lymphosarcoma. However, many of the findings did not support, or called into question, the purported association, specifically:

   1) Texaco Study: The largest study group was most recently reported by Divine and Hartman (1996) and consisted of 2,795 workers with an average of 32 years of follow-up. The overall observed/expected ratio (O/E) for lymphosarcoma was 9/4.7 = 1.91 (95% CI = 0.87-3.6) which was not statistically significant. The SMRs for those employed <5, 5-19 and 20+ years were 2.6, 1.8 and 0.8 respectively, which is largely counter to expectations. The lymphosarcomas were concentrated among those first employed during World War II (WWII)(O/E = 7/2.9 = 2.4, 95% Confidence Interval (CI) = 1.0-5.0). One feature that suggests it might be a real effect is that the WW II excess was limited to those in the group with jobs that entailed higher exposures ("varied exposure" group). However, this elevated risk among WW II workers showed an inverse association with length of employment, in spite of the fact that the authors indicate that high exposures still continued into the 1950's and 1960's-- which does not lend plausibility to the association. Although there was an overall excess of lymphosarcoma in the group with jobs that entailed higher exposures (O/E = 7/2.8 = 2.5, CI = 1.0-5.1) in the Divine-Hartman study, the subset of this group employed for 10+ years showed no excess (O/E = 1/1.0). Divine and Hartman created an index of cumulative butadiene exposure based on a job exposure matrix that considered job class and calendar time. Using a time-dependent Cox regression model for cumulative exposure vs. lymphosarcoma, there was not even suggestive evidence for an association between exposure and risk (Relative Risk (RR) = 1.00, 95% CI= 0.97, 1.04). On Page 7-32, Line 6 (and also Page 11-6, Line 22), in order to present a balanced summary of findings on lymphosarcoma and monomer production, it should be stated here that there was no indication of an exposure-response association, based on the latest follow-up by Divine and

Hartman (1996). Two other methods of analysis also reinforced the null association. Regression analyses that modeled the time spent in each of their six job classes as predictors were also conducted; again, there was no indication of an association. In summary, the case for an association between butadiene exposure and lymphosarcoma in this study is weak.

The EPA report of this study either fails to note or at least to consider the implications of a number of the findings noted above that go against the likelihood of a causal association between butadiene and lymphosarcoma. In the chapter summary (Pages 7-31 - 7-32) the Agency cites positive results from earlier follow-up studies of this cohort and one nominally positive result from this report as their summary of the study, without noting the important findings in the study that are not supportive of a positive association.

2) Union Carbide Study: Ward *et al.* (1996) studied 364 employees who worked at one of three units that had produced butadiene. For lymphosarcoma the ratio of O/E = 4/0.69 = 5.77 (95% CI = 1.6-14.8). These four cases had worked at a butadiene unit for 0.8, 2.9, 3.3 and 8.0 years, so the worktime of three of the four was relatively brief. Two of the four, with butadiene exposures of 2.9 years and 3.3 years, had worked in an acetaldehyde unit for 8 years and 29 years, and all four had exposure to a variety of other chemicals at the facilities. The fact that two cases had long-time exposure to acetaldehyde raises the possibility that those cases may have been associated with acetaldehyde rather than butadiene, and this possible confounder weakens the finding. The nature and extent of the possible confounding by acetaldehyde are glossed over in the EPA 1,3-butadiene report.

3) Shell Study: A third study of 614 employees at a butadiene monomer production plant yielded negative results (Cowles *et al.*, 1994). They were followed for an average of 12 years after entry to the study (which, because of the unusual criteria for study entry, was equivalent to 14-17 years of follow-up after first exposure) and had an average of 7.6 years of butadiene exposure. There were no deaths from lymphatic or hematopoietic cancer (1.2 expected). This fact was not even mentioned in the EPA report (Page 7-8). This cohort provides no support for an association between butadiene exposure and lymphosarcoma, although the fairly short follow-up time and relatively small sample size means the negative results should not receive a heavy weight. Nevertheless, the EPA statement that "this study failed to provide any negative evidence towards the causal association" (Page 7-32, Lines 15-16; also Page 7-9, Line 22-23 and Page 11-7, Lines 6-8) seems to be an overstatement; the study does provide

some, albeit not compelling, negative evidence. In fact, it is at least equivalent in size to the Ward *et al*. (1996) study that is highlighted as providing positive evidence.

4) Delzell Study: A large study by Delzell *et al.* (1996) of 15,649 styrene-butadiene rubber (SBR) workers found no association between butadiene exposure and lymphosarcoma. Overall the lymphosarcoma O/E = 11/13.8 = 0.8 (95% CI = 0.4-1.4). In those with the greatest exposure and latency (10+ years exposure and 20+ years since hire) there was no elevation in lymphosarcoma risk (O/E = 4/3.9 = 1.0, CI = 0.3-2.6). The other SBR studies are essentially earlier versions of this study, with minor differences in the cohorts, so this study summarizes the lymphosarcoma results for the SBR data.

   It is also of note that the coding of the ICD 200 category "lymphosarcoma and reticulosarcoma," as distinct from the ICD 202 category of other non-Hodgkins lymphomas (NHL) for which no butadiene-related excesses were found in the various studies, is very unreliable. In a review of medical records for death certificates coded as lymphomas (ICD 200 or 202), Matanoski *et al.*, 1993 found that two were not lymphomas at all "and the other 10 were so poorly classified into the 200 and 202 codes on the death certificates as compared with the hospital records that we combined these ICD categories" (Page 369) (Matanoski *et al.*, 1993). This calls into question the significance of the positive results reported above which were based on death certificate diagnoses of lymphosarcoma. Had the other categories of NHL (i.e., ICD 202) been put with this category, it is unclear whether there would be any excesses of NHL.

b) Delzell *et al.* (1996) conducted a study of 15,649 men who had worked for at least one year (during 1943-1991) at any of eight styrene-butadiene rubber (SBR) plants. The study included all but one small plant of the earlier Johns Hopkins study (a plant that did not begin SBR production until about 1970) and updated the mortality experience at all plants studied, along with conducting a much more detailed exposure reconstruction for these workers (Matanoski *et al.,* 1990). The cohorts differed slightly because of somewhat different definitions of eligibility, but, for all practical purposes, the Johns Hopkins cohort is subsumed by the Delzell cohort. Thus, there is essentially only one study of SBR workers, of which the Delzell *et al.* (1996) and Macaluso *et al.* (1996) reports represent the most recent follow-up -- for up to 49 years with a mean of 25 years.

c) The Committee did not feel it was appropriate to "lump" lymphohematopoietic tumors. Leukemia and lymphosarcoma are separate diseases.

d) The Criteria for Causal Inference are biased in their presentation. They should be revised to reflect the above points. It was generally agreed that the process used for SBR met the criteria, but the monomer did not.

Additional, more specific, comments are provided in Appendix A.:

3.1.8 Chapter 8 - Pharmacokinetic Modeling

There was consensus that this chapter is out of date. The chapter should be updated and should include the most recently published PBPK models. A major data gap is the lack of modeling of 3,4-epoxy-1,2-butanediol (EBD). A model describing the kinetics of this metabolite would be useful in relating external exposure concentrations to measures of internal dosimetry such as hemoglobin adducts. Such a linkage would facilitate reconstruction of BD exposure profile in exposed humans. A majority of the members were of the opinion that since DEB is very likely involved in BD-induced carcinogenesis, current models which describe this metabolite would be adequate for risk assessment. Other members felt that other metabolites, such as the diol, may play an important role in carcinogenesis at some sites, and that these metabolites needed to be addressed.

The technical quality and comprehensiveness of this chapter could be improved greatly by the inclusion of the most recent relevant literature on butadiene toxicokinetic modeling. Specific suggestions regarding a reorganization of Chapter 8 are offered below. Chapter 8 of the draft document on toxicokinetic modeling does not reflect the current state of knowledge regarding PBPK models for butadiene. Rather, this chapter describes and critiques the initial attempts of several laboratories to independently develop "first-generation" PBPK models for butadiene. These models were developed often without benefit of critical data such as solubility parameters and metabolic rate parameters. Additionally, definitive data on concentrations of butadiene and its metabolites in tissues of animals exposed to butadiene were largely lacking, precluding rigorous model validation. As such, some of the conclusions that the investigators drew from these early models are no longer relevant. It would be more appropriate to briefly acknowledge these early and important contributions. Then, attention could be focused on a serious critique of the more recent PBPK models (Sweeney *et al.*, 1996; Csanady *et al*., 1996; Reitz et al., 1996; and Sweeney *et al.,* 1997). These later models represent a significant advance over the previous generation PBPK models in that they describe the kinetics of diepoxybutane, a critical metabolite of 1,3-butadiene.

Inclusion of a discussion of state-of-the-art models for butadiene kinetics would significantly strengthen the scientific quality of the document. In particular, the numerous statements throughout the document indicating that an adequate PBPK model for butadiene risk assessment is not available should be revised to reflect the current state-of-the-art. At least two models that describe the kinetics of butadiene, epoxybutene and diepoxybutane were available to the EPA in 1996 (Csanady *et al.*, 1996; Reitz *et al*., 1996) and one was published in 1997 (Sweeney *et al.,* 1997). A majority of the Committee felt that any one of these models, combined

with available *in vitro* metabolic rate constants could be used to obtain more refined human dose estimates while other Committee members were concerned about the degree to which some models were able to describe the available data, the extent to which parameters had to be changed from measured values to obtain reasonable fits and the accuracy of the low dose extrapolation. As noted by the Agency, these refined dose estimates could be used in the animal based risk assessments for both cancer and reproductive toxicology endpoints. In accord with the revised EPA Cancer Risk Assessment Guidelines and given what is understood regarding the mechanisms of butadiene toxicity, a more scientifically based risk estimate for environmental exposure to butadiene is the most appropriate course of action. In chapter 8, concern is expressed that serious uncertainties exist pertaining to the model structure, parameter values and validation for the various PBPK models. It is true that each of the models differ somewhat in the details and that the parameter values chosen by various investigators are similar but not identical. However, these models are similar enough that any of the models is capable of predicting blood concentrations of butadiene, epoxybutene, and diepoxybutane following inhalation exposure to butadiene. This suggests that whatever differences in the underlying model structure exist, these differences are minor. Most importantly, each of these second generation PBPK models fully describes the kinetics of diepoxybutane. A majority of the Committee felt that since it is likely that it is diepoxybutane that is the critical metabolite for initiation of the carcinogenic effects following exposure to butadiene, inclusion of this metabolite makes these models especially useful for risk assessment. An alternative view on the Committee was that other metabolites, such as the diol, may play an important role and should be addressed in developing models predictive of human risk. Also, the models of the diepoxide require validation and further development to address parameter and structural uncertainty as well as interindividual variability in human metabolism.

The conclusions drawn in Chapter 8 need to be revisited in light of information on the kinetics and PBPK modeling of butadiene that is not presented in the draft report. In the conclusions, the Agency notes five areas in which more research is needed including:

a) evaluation of diepoxybutane kinetics;

b) investigation of the validity of *in vitro* metabolic data for extrapolating *in vivo* exposure;

c) clarification of values of various physiological parameters;

d) better characterization of the distribution values for the human metabolic rates; and

e) more measurement of tissue concentrations of metabolites for model validation.

Comments on each of these areas are noted below:

a) Revision of the draft risk assessment to include data published since January 31, 1997 will resolve this need. A paper by Valentine *et al*. (1997) reports on the

pharmacokinetics of diepoxybutane in rats after IV injection, the most appropriate route of administration for this non-volatile chemical. Sweeney *et al*. (1997) were able to successfully simulate this data using a PBPK model developed for butadiene, epoxybutene, and diepoxybutane. Given the high mutagenic potency of diepoxybutane, it is unlikely that kinetic measurements of this chemical will ever be made in humans *in vivo*. However, parallel studies using lung and liver tissue samples have quantitated the rates of hydrolysis and glutathione conjugation of diepoxybutane in rats, mice and humans (Boogaard *et al.,* 1996; Boogaard and Bond, 1996) and *in vitro* studies on the oxidation of epoxybutene have characterized the rate of diepoxybutane formation (Seaton *et al.*, 1995).

b) The validity of the *in vitro -in vivo* extrapolation for butadiene has been investigated in a manuscript by Sweeney *et al.* (1997), in which the *in vitro* data describing the rates of butadiene oxidation, epoxybutene oxidation, and hydrolysis and glutathione conjugation of epoxybutene and diepoxybutane determined *in vitro* were used directly in a PBPK model and the model predictions were validated against *in vivo* data obtained from butadiene exposure of mice and rats. The ability of the model to simulate both epoxybutene and diepoxybutane blood and tissue concentrations following exposure to butadiene points to the usefulness of *in vitro* parameters in i*in vivo* models. For carcinogenic or potentially carcinogenic chemicals it is unlikely that direct toxicokinetic measurements will ever be made in people. Therefore, the only viable option is to conduct *in vitro* experiments in which metabolic rates are determined in human and animal tissue samples and then use these metabolic rates in the context of the PBPK model to predict blood and tissue concentrations of both animal species and humans. Acceptance of this *in vitro -in vivo* extrapolation strategy would serve to encourage the collection of appropriate data with the ultimate goal of incorporating more mechanistic information into the risk assessment process.

c) Regarding the choice of model parameters, there is undue concern that all investigators have not used the same values for physiological parameters. All investigators developing butadiene PBPK models have selected specific parameter values from a distribution of widely accepted parameters values for various physiological parameters in these models. Selection of a single value for ventilation, perfusion, blood flow or organ volume is in itself a simplification of the biological system being modeled. Many of these parameters vary for a single individual throughout the day. All of these parameters vary among individuals. The important point is that the selected values are physiologically realistic.

d) In the characterization of human metabolic rates, the statement "more research is needed" appears to the reader to be gratuitous. As noted previously, V_{max} and K_m values have been determined for a number of human samples. The fact is that the data base for butadiene is particularly robust in terms of the number of samples

characterized. If the EPA has concerns regarding the quantitative and qualitative distribution of human metabolic rates, these concerns should be stated much more explicitly and in sufficient detail that specific research could be conducted to meet this need.

e) Finally, although more measurements of tissue concentrations of metabolites are always desirable for model validation, more than 13 individual inhalation exposures of mice and rats and monkeys to various concentrations of butadiene have been conducted. Measurements at steady state and post exposure for butadiene and its metabolites have been made in blood and tissues. These data have been summarized by Himmelstein *et al.* (1997). The data are remarkably consistent across laboratories and consistently point to dramatic species differences in metabolism of butadiene. It is really not clear what additional data the Agency might find useful for model validation.

Specific comments on Chapter 8 are displayed in Appendix A.

3.1.9 Chapter 9 - Quantitative Risk Assessment for 1,3-Butadiene

The Agency is to be commended for its efforts to develop a clear, well-reasoned quantitative assessment of 1,3-butadiene cancer risk. The assessment is made particularly difficult, by the continuing release of new and relevant scientific data, including toxicokinetic, epidemiological and mechanistic data. Furthermore the analyses were undertaken after the release and SAB review of the proposed EPA carcinogen guidelines but before they have been finalized. The Agency is to be commended for looking at new approaches, such as the benchmark dose procedures, to improve quantitative assessment on non-cancer endpoints. However, the Committee has submitted suggestions on how to further improve these approaches and how to make these new approaches more clear, accurate and consistent. Additional comments on chapter 9 are given below.

a) *Dose response analysis of the human carcinogenesis data*

1) Quality of human data: The Committee agrees that the Agency's risk assessment of 1,3-butadiene should include the latest follow-up of each of the epidemiologic studies, including Delzell *et al.* (1995) and Matanoski *et al.* (1997). Delzell fits a variety of dose response models, accommodating a wide range of dose response curves. The models fit equally well, and as noted by EPA as a whole were consistent with a considerable range of low dose risk predictions. Thus the Delzell study does not permit one to discriminate among models for the purpose of low dose prediction. In this sense the data can be viewed as limited for the purpose of low dose risk prediction. The reason why equivalent model fits are obtained requires further explanation in order to convey a greater appreciation of the nature

of the dose response data. For example, is this due to a relatively narrow range of dose response data in the exposed cohort or to considerable scatter?

The assessment notes that few subjects were exposed to benzene, but benzene was not seen to confound the relationship between butadiene or styrene and leukemia. Because benzene is widely recognized as a human leukemiogen, further discussion of this point is needed (e.g., an indication of whether the benzene exposures were insufficient to cause effects; consistency with expectations based on a Chinese cohort).

In the section on uncertainties, the potential importance of exposure misclassification in the Delzell study is discussed, but further discussion of the uncertainties of dose reconstruction and the potential magnitude of impact on the risk predictions is desirable.

2) Presentation of multiple model fits and low dose extrapolations: The results of the multiple model fits derived by Delzell and colleagues are tabulated, along with low dose risk predictions. While it is reasonable to compare results to make the point that the data do not provide the basis for discriminating among models of quite different shapes, once it is established that this is the case the further presentation of low dose analyses is not needed and unnecessarily complicates the presentation (e.g., in Table 9-3 and Table 9-4). Presentation of results for the linear model would suffice, along with perhaps effect concentration estimates, within but not outside the range of observation. The other point to be made, that the exposure concentration at 1% risk level (EC_{01}) results for the final versus initial square root model differ by roughly 40 - 50% could also be made, for example, in the text. It motivates the need for obtaining comparable estimates for the linear model, by omitting styrene and race (as was done for the "final" square root model).

The results for the square root model and the power model (which is apparently best fit by a coefficient less than unity) are included. Superlinear models such as these are not biologically plausible, and should therefore not be used for low dose risk predictions. Synergy between genotoxicity and cell proliferation frequently occurs as does saturation of detoxification and metabolic activation. These factors result in sublinear data sets. Metabolic data for butadiene clearly show saturation of activation and the molecular dosimetry data are supralinear. An analogous point was made by the Committee in commenting on proposed EPA carcinogen guidelines regarding the use of the Weibull-in-dose model without constraints for exponents less than unity for risk prediction. Lack

of biological plausibility is another reason for not presenting low dose risk predictions for the square root and power models.

The measure of deviance for the models seem to be within 0.4, while normally one would say models differ if their difference in deviance is about 4.0. Also, the best fit for the multiplicative model is sublinear; for the power model it is superlinear and as noted by Delzell and colleagues, the square root model fits best (although marginally so) - again, this reinforces the notion that the data provide limited information on dose response. Perhaps replacing the dose response figures with one figure showing the data points with their confidence intervals, and superimposing on the figure the various models fit would provide a better understanding of why this is the case. The inclusion of data should clearly illustrate why the data are equally explained by the wide range of models tried.

3) Dose rate, duration and timing: With benzene induced leukemia and lymphoma the recency of exposure is an important feature of the dose -time-response, with recent exposures most important for leukemia, and distant, high exposure most important for lymphosarcoma. On Page 9-2, the assessment notes the finding of Delzell and colleagues that excluding exposures within 5-10 years of death slightly increased the exposure response relationship but excluding exposures within the last 20 years almost eliminated the relationship. It would be desirable for the Agency to explore in detail the possible dose time relationships. On a related point, Delzell and colleagues have recently reported on the possible importance of peak exposures to 1,3-butadiene on leukemia risk (Delzell *et al.*, 1995). It is important to explore the dose rate/time/response issues through careful analyses of the Delzell data set.

4) Extrapolation of occupational results to general population: he available data for risk assessment is for males, exposed as young and middle-aged adults, and for a selected group in that they were workers in the jobs studied. The extent to which this group can be used to represent the general population should be addressed more carefully, with an attempt to quantitatively address, where possible, differences between cancer potency for the occupationally exposed and the general population. Such an analysis should consider the following:

b) *potential increased sensitivity for women and girls*: No dose response information appears to be available for women. The mammary gland was a sensitive site in both rats and mice. Given the large public health concern for breast cancer in women, and the findings in the bioassay, the issue should be explicitly addressed. In terms of overall risk, female rats in the Hazelton Laboratories (Hazelton, 1981)

study were clearly more sensitive than male rat, at least by an order of magnitude. Although given the uncertainties in the quantitative analysis, this is less clear for the mouse, cancer potency estimates were greater in the female compared to the male by about a factor of 2. Although toxicokinetic differences are not apparent in hemoglobin data for men and women with similar exposure, the potential for increased susceptibility of mammary tissue cannot be excluded.

c) *healthy worker effect*: The extent to which a presumably small adjustment may be appropriate should be addressed. The extent to which restrictions on smoking in occupational settings with butadiene exposure may have contributed to the healthy worker effect should be addressed by the Agency. Internal comparisons done by Delzell *et al*. (1995) that were used to derive the human cancer potency estimate are not influenced by the healthy worker effect since no external comparison population is being used. However, the healthy worker effect is an important consideration in extrapolating from the occupational setting to the general population, and this could not be assessed through an internal comparison.

d) *exposure during a working life versus other life stages*: Issues falling under this category to address include the potential for inherent increased susceptibilities at different ages (e.g., *in utero*, and during infancy, childhood and old age), and those related to the stages and mechanisms of carcinogenesis (e.g., time of exposure versus observation; after a point, diminution of risk with time since exposure). Analyses of the Delzell *et al*. (1995) and perhaps Matanowski *et al.* (1997) data, and observations from the large Chinese benzene/leukemia cohort may shed light on the second issue. The available data on butadiene obviously does not provide direct information on the first issue, but data on leukemogenesis from other agents may fill this gap.

e) *other potentially susceptible subpopulations:* The potential range of susceptibility within the general population should be explored quantitatively, by for example, considering the impact of polymorphisms on low dose risk, and addressing the extent to which certain potentially susceptible groups are contained within the Delzell cohort.

The document would be improved by presenting greater detail on the derivation of lifetime risks from continuous exposure from the fits to the occupational data. Perhaps this could be done in an appendix.

1) <u>Choice of maximum likelihood estimate over Upper Confidence Limit (UCL) on potency/LEC (95% lower confidence interval)</u>: Maximum likelihood estimates (MLEs) rather than lower confidence bound on the effective concentration (EC) (or upper confidence bound on potency) are used in the final characterization of the dose response derived from the

human data. It has been the practice of the Agency to use maximum likelihood estimates in potency derivations from human data and upper confidence bounds on derivations from animal data. The rationale provided in the 1,3-butadiene document is that the simple linear model fit to the human data does not have the instabilities that can be associated with polynomial fits, and that there is far less uncertainty in the potency estimate derived from human data. In this particular case there are a number of unaddressed issues suggesting that, at least, the EC_{01} may be underestimated for the general population. These include exposure misclassification in the Delzell cohort, the lack of availability of data on all but males exposed occupationally during adulthood, and the lack of availability of a fit of a "final" linear model (comparable to the model for the square root model [omitting styrene and race]). In addition, as previously stated in this report, there is a possibility that there was an overestimation due to exposures being underestimated in the Delzell *et al.* (1995) study. It would be preferable to explicitly take these factors into account, to the extent possible; it is unlikely that application of a confidence bound will provide adequate correction.

2) Availability of Delzell study: Although the Delzell study has been published (Delzell *et al.*, 1996; Macaluso *et al.*, 1996), some of the key analyses that aid in interpreting the data are available only in an unpublished technical report and thus is of limited availability to the public. At the meeting the Committee heard that there was peer review of the unpublished technical report. A report so critical to the dose response analysis and so heavily cited in the document should be made widely available to the public, along with description of the peer review conducted by the Agency.

f) *Dose response analysis of animal cancer bioassay data*:

1) Time dependent analyses: The default, time dependent analyses of the cancer bioassay data were carefully done and are well presented.

Because the cancer incidence rates are large for some tumor sites, errors are introduced if it is assumed the polynomial represents a true model of multistage carcinogenesis, as Moolgavkar (1994) has pointed out. This should be acknowledged in discussions which infer number of stages on the basis of modeling results (e.g., Page 9-20). The errors may be relatively small given the availability of data at relatively low doses; it would be desirable to attempt to gain an understanding of the magnitude of the error for these fits.

The method used to develop the upper 95% confidence bound for the sum of the incremental lifetime unit cancer risk for humans (q_1) parameter across sites is reasonable. An alternative approach for future assessments would be to derive the distribution for the q_1 parameter for each tumor site and use Monte Carlo simulation or numerical techniques to obtain the distribution of the sum. The result should differ marginally from the one presented, and thus it is not been suggested that the analysis be redone for this case.

2) Lack of site concordance across species: While leukemia is the observed endpoint in humans, leukemias are not observed in the animal bioassay in either rats or mice, in either sex. The rationale for the default inferences regarding site concordance, and the inclusion of sites such as the Harderian gland and forestomach should be clearly stated. Ovarian tumors are observed, along with ovarian atrophy in the animal bioassay. The degree to which the toxicity may have played a role in certain sites observed in the bioassay and the extent of relevance to humans exposed at lower doses should be discussed.

3) Toxicokinetics: A variety of viewpoints were expressed within the Committee over the extent to which toxicokinetic analyses should be incorporated into the assessment for use in interspecies and high to low dose extrapolation. The Agency was criticized by some for not incorporating the results of the recent models. Others noted significant deficiencies in the proposed models and the very recent developments suggesting the field was undergoing rapid development, and found the models speculative. Nonetheless, the different toxicokinetic hypotheses and the hypothesized role of the various metabolites should be discussed, and at least qualitatively, with an indication of the degree to which the assessment would be impacted if some of the hypotheses were later proven true. The discussion should acknowledge the variety of competingview-points and hypotheses of researchers in the field. Where information on toxicokinetics and mechanism provide adequate understanding of findings at certain sites within one rodent and not the other, this should be given.

g) *The assessment of the developmental and reproductive toxicity endpoints*: Improvement in non cancer risk assessment, such as use of effect levels as suggested in the benchmark dose procedures, was supported by the Committee. However, since these are relatively new procedures, the Agency must meet a high standard of clarity and transparency in their initial applications as presented in this this assessment. The Committee encourages the EPA to review the accuracy of their calculations. The Committee also requests that additional discussion be added to the document the to explain the use of EPA's newly proposed models,

especially those modeling time to impact, and to provide additional explanation of the safety factors applied to the benchmark calculations resulting from the modeling exercises:

1) A clear rationale for the selection of one particular point of departure over another is needed -- for each case for which the default is applied and for cases deviating from the default.

2) Where it is not obvious the biological significance of particular endpoints for the assessment should be conveyed.

3) When applying novel approaches, such as the time-to-response modeling of atrophy, the advantages and limitations in utilizing the approach in the context of reference concentration estimation should be clearly understood and presented.

4) The incorporation of information on toxicokinetics (e.g., ovotoxicity of diepoxide) should be used where possible on at least a qualitative if not quantitative basis to inform the choice of endpoints, modeling and uncertainty factors.

5) The exclusion of points at high doses contributing to a poor fit is a reasonable approach, and typically can be explained, for example, in terms of toxicities other than the one being modeled, or toxicokinetics. When the approach is applied where possible the specific rationale for the case at hand should be presented. The use of the procedure and the rationale provided for should be consistent across endpoints and across assessments for the same scenarios.

6) The EHC did not come to a consensus about the propriety of using a risk reduction factor, when reference concentrations are based on benchmarks associated with risk of effect. There was agreement that if this factor is used, the rationale for such use needs to be clearly stated.

7) Analyses of this type applied to these endpoints may be unfamiliar to others working in the general area. Confusion over the presentation of some results (e.g., logarithm versus linear scales) was obvious in some of the comments received. Also, calculations should be easily followed. Some of the Committee noted, as did several consultants presenting comments to the Committee, that mathematical errors were evident. EPA should carefully review this section of the document. Tables and figures should be free standing, with statistics referenced.

Additional comments and recommendations for Chapter 9 appear in Appendix A.

3.1.10 Chapter 10 - Weight of Evidence

The Committee did not reach a unanimous opinion about whether one can conclude that 1,3- butadiene is a *known* human carcinogen. There was a consensus that there is sufficient evidence to say that working in synthetic rubber production is causally associated with leukemia. The majority felt that there were conflicting results among SBR and monomer workers for leukemia and a lack of compelling evidence for a relationship between lymphosarcoma and butadiene exposure. There was concern that an increased risk of leukemia was not seen in the epidemiology studies of workers in the butadiene monomer industry. To most Committee members, this lack of association significantly reduced confidence in the assumption that 1,3-butadiene was the causative agent for leukemia in the SBR industry study. In addition, confounding or coexposure to other chemicals could not be ruled out with confidence.

There were a variety of views amongst the EHC as to how the call should be made on whether a substance can be considered a "known human carcinogen." One view was that the human data - from observational studies of cancer in humans - must stand on its own to make the finding of "known human carcinogen," without regard to mechanistic or other information. A second view was that the cumulative evidence, from human and animal studies, as well as mechanistic data, particularly as it relates to human findings, should be used as the basis for the judgment. The majority of the Committee felt that the judgment should be made on the basis of human cancer observations alone and the evidence was not sufficient for 1,3-butadiene. A few of the Committee members considered the human evidence in and of itself sufficient or that the cumulative evidence was sufficient to make a finding of "known human carcinogen." Other Committee members considered the body of mechanistic data to be indicative of the fact that significant interspecies differences in response to 1,3-butadiene exist between rodents and humans. For these members, the mechanistic data were consistent with 1,3-butadiene not being classified as a known human carcinogen.

With respect to the narrative discussion in the evaluation, the Committee felt that it should more reflect the range of opinion on the matter when discussing the human findings. Clearly 1,3-butadiene, when mutagenic or clastogenic, is so through its metabolism. This finding should be provided in the evaluation, as well as a statement regarding the mechanistic studies most relevant to humans.

The weight of evidence is confined to addressing cancer endpoints. The reproductive endpoint is the basis for RfC calculations and, therefore, also should be addressed.

3.1.11 Chapter 11 - Risk Characterization

The majority of the Environmental Health Committee did not consider 1,3-butadiene to be a known human carcinogen due to the lack of consistency between exposure and leukemia or

lymphosarcoma data when the styrene-butadiene rubber (SBR) and monomer worker studies were considered in total. The majority opined that 1,3-butadiene should be classified as a probable human carcinogen whereas a minority felt that the science supported the classification of 1,3-butadiene as a known human carcinogen. Most of the Committee felt that occupational exposure to the SBR process was a known cause of cancer.

The majority of the EHC was reluctant to classify 1,3-butadiene as a known human carcinogen because it was felt that there was no consistent relationship between exposure and leukemia or lymphosarcoma when the SBR and monomer studies were considered in total. Only one study population (SBR industry) had credible leukemia excess related to exposure. Leukemia was not elevated or related to estimated exposure in butadiene monomer industry. Lymphosarcoma was only elevated in short term, not long term workers in the monomer industry and also not elevated in the SBR industry. Hence, there was no dose response for the lymphosarcoma. The lymphopoietic cancers should be considered separately when assessing consistency across studies. The majority of the Committee felt that the finding of "known human carcinogen" for the SBR process could be based solely on observational studies in humans, without regard to mechanistic or other information. A minority of the Committee held that 1,3-butadiene should be identified as a "known human carcinogen" because the cumulative evidence, from epidemiology, animal cancer bioassays, and mechanistic studies should be used as the basis for the judgment.

Finally, the Committee was unable to follow the logic in the use of the "risk reduction factor" that was applied to the benchmark dose based-RfC. This section of the EPA document should be rewritten to make the adjustment process clear.

Futher details and recommendations will be found in Appendix A.

3.2 Classification of 1,3-Butadiene as a Known Human Carcinogen

There was a majority opinion within the Committee that the extant science supports the SBR process exposures as a known human carcinogen, but does not support such a finding for exposures to butadiene monomer. The lack of positive findings regarding lymphosarcoma in the large Delzell cohort and the lack of positive findings regarding leukemia in the three butadiene monomer studies weaken the case for a straightforward causal association between these endpoints and 1,3-butadiene exposure. The EPA report suggests these discrepancies may be due to: a) butadiene "dose rate,"; b) different confounding factors; or c) different "co/modifying" factors (Page 11-7). The first reason seems very implausible. There is no known example of a chemical or substance that causes different cancers depending on dose rate, especially at the relatively low dose rates in these studies (unlike the rodent studies with hundreds of ppm). The second reason raises the possibility that one or both of the putative associations may not be real but may be caused by confounding by some unknown factor(s). This reason weakens the case for causality. The third reason, that there may be some cofactor or modifying factor, suggests that even though butadiene might induce cancer in the presence of some cofactor or modifying factor,

it would not do so in the absence of that factor. If this is the case, then it would be inappropriate to generalize to other exposure scenarios for the general public since they would likely not have concomitant exposure to the cofactor. There is some evidence to support this later point in the studies of Leavens *et al.* (1997) in which mice were exposed to mixtures of 1,3-butadiene and styrene as well as 1,3-butadiene or styrene alone. Genotoxicity was noted in mice exposed to 1,3-butadiene; cytotoxicity was noted in mice exposed to styrene; both cytotoxicity and genotoxicity were noted in mice exposed to the mixture. This study provides some mechanistic support for the observation of leukemia in SBR workers but not in butadiene monomer workers as it is consistent with biologically-based models that demonstrate the requirement for both cytotoxic and genotoxic events for the development of cancer. In short, this inconsistency in findings, whatever its basis, weakens the case for causal associations and/or that such associations can be generalized to exposure of the public to butadiene.

The case for an association between lymphosarcoma and butadiene exposure is weakened by the fact that in the main study (Divine *et al.*, 1996) that purportedly showed the association, there was no indication of an exposure-response relationship nor was there evidence that those with longer-term exposure had a higher risk of lymphosarcoma.

The report claims (on page 11-3, lines 11-13) that "'sufficient evidence' of human carcinogenicity is based on more than 10 epidemiological studies examining five different groups of workers" and summarizes them in Table 11-1. But there are effectively only 4 groups of workers, not 5, since the Matanoski and Delzell cohorts have a high degree of overlap (i.e., about 95% of the Matanoski cohort is included in the Delzell cohort). In addition, the "more than 10" studies are just earlier reports of the same cohorts and do not add anything to an inference of causality beyond that seen in the most recent follow-ups. Of the four independent studies, one small one (Cowles *et al.*, 1994) is completely negative for lymphosarcoma and leukemia; another small one was positive for lymphosarcoma, but based on only 4 cases, and showed no excess of leukemia (Ward *et al.*, 1996); a relatively large study was mostly negative, but somewhat suggestive of a lymphosarcoma excess, and provided no support for a leukemia excess (Divine *et al.*, 1996); and one large study was positive for leukemia but not for lymphosarcoma (Delzell *et al.*, 1996).

In summary, the weight of epidemiological evidence does not support an association between butadiene exposure and lymphosarcoma/reticulosarcoma. While the Delzell *et al.* (1996) and Macaluso *et al.* (1996) are large and methodologically sound studies, one would like to see at least a second independent confirmatory study before affirming there is "sufficient evidence of human carcinogenicity" regarding butadiene and leukemia. Instead, one sees a fairly large and reasonably sound study that shows no leukemia excess (Divine and Hartman, 1996) plus two smaller ones with no evidence of leukemia risk, and these weaken the case.

A majority of the EHC members felt that the body of mechanistic data on butadiene does not support the classification of known human carcinogen. The Agency notes (page 9-52) that there are large unexplained differences in the response of rats and mice to butadiene and states

that the specific mechanisms of 1,3-butadiene induced carcinogenesis are unknown. However, there is strong evidence from both metabolism and genetic toxicology studies that diepoxybutane is a critical metabolite in the carcinogenic process. The extensive capability of mice to form the diepoxide metabolite and the extensively higher levels of the diepoxide metabolite in blood and tissues of mice compared with rats, most likely forms the basis for this dramatic species difference in carcinogenic response. This is an important point because it suggests that the diepoxide metabolite may be the best dosimeter for assessing risks for humans exposed to butadiene.

Toxicokinetic modeling cannot prove or disprove the relationship between chemical exposure and toxic or carcinogenic effect. However, these models can be developed to test quantitative hypotheses regarding proposed mechanisms of toxicity or carcinogenicity of chemicals. The Agency notes that "pharmacokinetic modeling of 1,3-butadiene has not elucidated the reasons for interspecies differences in carcinogenic response between rats and mice" and "mice and rats also exhibit substantial quantitative differences in their metabolism of 1,3-butadiene to potentially reactive metabolites. Unfortunately, existing pharmacokinetic models have been unable to explain the species differences in carcinogenic response." These statements are not true if one considers the current PBPK models that describe the disposition of butadiene, epoxybutene and diepoxybutane. These PBPK models clearly simulate the dramatic species differences in tissue and blood concentrations of diepoxybutane between rats and mice observed *in vivo*. The models suggest that species differences in response to butadiene are most likely related to differences in rates of formation and removal of diepoxybutane. Experimental data and PBPK model simulations indicate that mice produce far greater concentrations of this reactive metabolite compared with rats.

These PBPK models can be, and have been, extended to humans. *In vitro* data on rates of butadiene, epoxybutene, and diepoxybutane metabolism obtained in human tissue samples can also be used to predict blood and tissue concentrations of both epoxide metabolites in humans. When these simulations are conducted using average values for human metabolic rates results indicate that human diepoxide concentrations would be orders of magnitude less than those of mice and lower but much more similar to concentrations predicted for rats. Nonetheless, 1,3-butadiene produces cancer in rats, albeit at higher dose levels than for mice, so similarity of human and rat metabolism would still contribute to the weight of evidence. Some Committee Members pointed out limitations in the models and the underlying assumptions regarding choice of human parameters and activity of metabolites. These Members also point to the considerable other mechanistic data (which are described in comments on Chapter 4).

3.3 Approaches Taken to Characterize Plausible Cancer Risks

The database for butadiene is very challenging and robust, but all of the important issues are not settled. The EPA must state the facts in proper perspective and should not be afraid to say that more information is needed. The Agency must clearly address known issues, search for (currently) unknown issues, and separate the important data from the unimportant.

Risk estimates use external butadiene exposures as the dose estimate given the sophisticated PBPK models currently developed and the availability of *in vitro* metabolic parameters in rodents and humans an approach that used an estimate of internal dose . The Agency notes (Page 11-12, Lines 1-3) that a review of the available pharmacokinetic data and models reveal that the state of this science is currently inadequate for either explaining interspecies differences or improving on default dosimetry assumptions. As noted previously in this report, this statement should be revised to reflect the available scientific data and pharmacokinetic models. The fact that human variability in metabolic response is noted should not be viewed as a limitation for these models, but instead as an opportunity to reduce uncertainty and characterize variability. If metabolic activation of butadiene is necessary to produce genetic damage, and ultimately carcinogenicity, which is not an unreasonable hypothesis, then individuals with the highest metabolic capacity for activation and the lowest metabolic capacity for deactivation would be at most risk for exposure to butadiene. The PBPK model could be used to determine the extent to which metabolic differences among humans is the dominant process controlling the magnitude of the effective dose at the target site.

The Agency states (page 9-16, lines 1-7) that risk assessments based on rat carcinogenicity data "are not considered the most appropriate estimates of human risks .... EPA believes that the mouse is likely to represent a better rodent model for human cancer risk assessment from 1,3-butadiene." By contrast, the EHC believes that available mechanistic data on the formation of epoxybutene and diepoxybutane obtained in rat, mouse and human tissue samples suggest that the rat is a more appropriate model for assessing risks for humans than is the mouse. Choosing a rat versus a mouse as the most appropriate animal for assessing risks in humans may, in fact, be an oversimplification. As noted above, the development of PBPK models that are capable of predicting concentrations of the reactive metabolites in the target tissues and availability of distributions of human metabolic rate constants would allow one to not rely on either mouse or rat *per se*. Instead one can use all of the available mechanistic data within the context of a PBPK model to predict the butadiene doses in humans necessary to yield epoxide concentrations in tissues similar to those predicted for rats or mice exposed to carcinogenic concentrations of butadiene.

The Agency notes (page 9-17, lines 6-11) that no attempt was made to adjust for internal doses of reactive 1,3-butadiene metabolites because the PBPK data were inadequate to develop a reliable PBPK model. As noted previously PBPK models not reviewed by the Agency in this draft document have been developed that are capable of predicting the butadiene epoxybutene and diepoxybutane blood and tissue concentrations in rats, mice and humans following exposure to butadiene. In this case, a human PBPK model could be readily used to obtain either point estimates using average human values or a range of estimates by conducting Monte Carlo simulations to sample from a distribution of available parameter values for humans for both metabolic rate parameters and physiological parameters. A plausible assumption is that for a given diepoxybutane tissue concentration the human response would be equivalent to the rodent response. Then, concentrations of butadiene necessary to elicit tissue doses of diepoxybutane in humans equivalent to diepoxybutane concentrations in mice or rats at doses which yielded tumors

could be calculated. PBPK models of these and the diepoxybutane and other active metabolites can then be applied to human risks predictions. The advantage of using a PBPK model is that mechanisms underlying absorption, distribution, metabolism and elimination of butadiene, epoxybutene, epoxybutanediol and diepoxybutane which are either similar or different across species and across doses would be accounted for. Given the availability of these PBPK models, this approach would be far superior to using a simple arithmetic adjustment for continuous daily exposure versus exposure under bioassay conditions.

On page 9-25, lines 1-7 the EPA notes that "mice and rats also exhibit substantial quantitative differences in their metabolism of 1,3-butadiene to potentially reactive metabolites. Unfortunately, existing pharmacokinetic models have been unable to explain the species differences in carcinogenic response." A majority of the Committee assert that there are several published PBPK models that have been able to successfully explain species differences in the carcinogenic response exposure to 1,3-butadiene based on differences in the metabolism of 1,3-butadiene to reactive metabolites while other Committee members were concerned about the degree to which some models were able to describe the available data, the extent to which parameters had to be changed from measured values to obtain reasonable fits and the accuracy of the low dose extrapolation. In particular, the highly mutagenic diepoxybutane is formed to a much greater extent in mouse tissues compared with rats.

Again on page 9-25, lines 15-19, the EPA notes that "ideally a PBPK model for the internal dose of the reactive metabolites would decrease some of the quantitative uncertainty in interspecies extrapolation. However, current PBPK models are inadequate for this purpose." The available pharmacokinetic models and emerging data on human pharmacokinetics should be further explored for use in risk assessment. As noted in the previous comments, some of the Committee members assert that PBPK models that are capable of interspecies and low dose extrapolation for this purpose do exist. Other Committee members assert that although the PBPK models require validation and development, they may prove useful in the near term. In either case, models should be further developed to address parameter and structural uncertainty as well as interindividual variability in human metabolism.

On page 9-51 the Agency asserts that NOAEL, LOAEL, EC10, or LEC10 should be converted to appropriate human equivalent exposures before using these exposure levels as points of departure and that theoretically this is best accomplished using a PBPK model. The Agency also notes that the current PBPK models are inadequate for use in risk assessment. However, as noted previously, some of the Members felt that there are several available PBPK models that are capable of predicting diepoxide concentrations in target tissues for rats, mice and humans and that any one of these models could be used to obtain a more appropriate human equivalent exposure. Other Committee members felt that, at a minimum, the different toxicokinetic hypotheses and the hypothesized role of the various metabolites should be discussed, at least qualitatively, with an indication of the degree to which the assessment would be impacted if some of the hypotheses were later proven true. In the 1,3-butadiene health risk assessment, the Agency should address the variety of viewpoints and hypotheses of research in the field of PBPK modelling.

The EPA report pays relatively little attention to the issue of how much peak exposures to butadiene may have influenced leukemia risk in the Delzell study. It indicates (page. 7-22, lines 30-33) that there was an association of peak exposures (defined as >100 ppm) with leukemia but dismisses it as an "irregular" association. The Acquavella (1998) commentary on the EPA draft that was sent to the EHC presents a table showing how much peak exposures affect the association of butadiene ppm-years with leukemia risk (Page 10). The formal linear regression estimates with and without adjustment for peak exposures are not presented, but it is apparent that the regression estimate would be appreciably less when peak exposure was adjusted for. Specifically, for the three highest cumulative exposure groups, the excess relative risks with and without control for peak exposures were 0.0 and 1.0 respectively for 20-99 ppm-years, 0.3 and 1.4 respectively for 100-199 ppm-years, and 1.5 and 3.6 respectively for 200+ ppm-years. In each dose group, adjustment for peak exposures reduced the leukemia risk substantially. Since butadiene exposures to the public will almost never approach the peak exposure range, a more appropriate model for risk would factor out the peak-exposure component.

Regarding the Delzell analysis of butadiene exposure vs. leukemia, (on Page 9-2, Lines 24-25) it is noted that "excluding exposures within 20 years of death weakened and almost eliminated the relationship...." This indicates that in modeling lifetime risk, a model that assumes a limited effect time (i.e., that leukemia risk during a given year of age is affected largely by the butadiene exposures received during the previous, say, 20 years, and only slightly or not at all by more distant ones) should be considered. This "windows of exposure" model has precedents, e.g., lung cancer risk from radon has been modeled in this way in a National Academy of Sciences report (NAS, 1988) because lung cancer risk was little affected by radon exposures in the distant past; leukemia risk from radiation is highly elevated at 5-10 years after irradiation but there is little elevation by 20-30 years after irradiation (NAS, 1990). If this model were considered for projecting lifetime risk, it would show appreciably less risk from chronic exposures than does the present one, which assumes that excess relative risk at, say, age 70 is an additive function of all the exposure accumulated in the previous 69 years.

3.4 Conclusions and Quantitative Estimations for Reproductive/Developmental Effects

The Committee supports the Agency's use of benchmark dose procedures and the modeling of reproductive toxicity endpoints. The EHC also supports the continuing attempts to develop new strategies, such as those presented in the report to address ovarian, uterine and testicular atrophy, to quantitatively address reproductive endpoints in risk assessment documents. This is one of the first such assessments and some general suggestions are made regarding conduct of the analyses and the presentation of results for the analyses:

a) A clear rationale for the selection of one particular point of departure over another is needed -- for each case for which the default is applied and for cases deviating from the default.

b) Where it is not obvious the biological significance of particular endpoints for the assessment should be conveyed.

c) When applying novel approaches, such as the time-to-response modeling of atrophy, the advantages and limitations in utilizing the approach in the context of reference concentration estimation should be clearly understood and presented.

d) The incorporation of information on toxicokinetics (e.g., ovotoxicity of diepoxide) should be used where possible on at least a qualitative if not quantitative basis to inform the choice of endpoints and modeling.

e) The exclusion of points at high doses contributing to a poor fit is a reasonable approach, and typically can be explained, for example, in terms of toxicities other than the one being modeled, or toxicokinetics. When the approach is applied where possible the specific rationale for the case at hand should be presented. The use of the procedure and the rationale provided for should be consistent across endpoints and across assessments for the same scenarios.

f) The Committee did not agree about the propriety of using a risk reduction factor, when reference concentrations are based on benchmarks associated with risk of effect. The rationale for use of such a factor needs to be clearly laid out.

g) Analyses of this type applied to these endpoints may be unfamiliar to others working in the general area. Confusion over the presentation of some results (e.g., logarithmic versus linear scales) was obvious in some of the comments received. Also, calculations should be easily followed, and of course need to be carefully proofed by someone other than the one making them. Tables and figures should be free standing, with statistics referenced.

Some of the Committee members are of the opinion that the quantitative estimations for reproductive/developmental effects could benefit greatly from the application of PBPK modeling to estimate the effective dose at the target site. The Agency notes (Page 9-46, lines 6-11) that "ovarian atrophy has been shown to be related to the amount of the diepoxide metabolite in the tissue. Modeling of the ovarian atrophy and uterine atrophy data was considered based on internal dose of the diepoxide metabolite, however an adequate model was not available to estimate levels of the diepoxybutane." There are several PBPK models that are currently published that allow calculation of the dose of the epoxide metabolite in target tissue. Any one of these models could be used to determine the internal dose of the diepoxide metabolite and provide a more refined estimate of risks for this reproductive endpoint.

4. SUMMARY OF RECOMMENDATIONS

The Committee's principal findings and recomendations are:

a) The EHC recommends that the Agency's updated, draft health risk assessment of 1,3-butadiene reflect the new information that has been published in the peer-reviewed literature since the Agency's cut-off date of January 31, 1997. The inclusion of this new information would greatly improve the technical quality and comprehensiveness of the report.

b) Since critical research like the Delzell *et al.* (1995) exposure reestimation and the pharmacokinetic modeling has not yet been completed, the Committee recommends that the Agency's risk assessment of 1,3-butadiene be labeled as an interim hazard assessment (The document could then be called "Health Risk Asssessmment of 1,3-Butadiene: 1. Hazard Assessment").

c) The majority of the Committee recommends that the Agency classify 1,3-butadiene as a "probable human carcinogen" (rather than a "known human carcinogen") because there was not consistency between the exposure-response rates for leukemia or lymphosarcoma when both the SBR and monomer worker studies were considered in total. Also, the role of confounders and cofactors was unclear.

d) The Committee found the approaches taken to characterize plausible cancer risks to be reasonable but points out specific improvements that can be made.

e) The Committee supports the Agency's use of benchmark dose procedures and the development of mathematical models for reproductive endpoints. To improve the analysis and the clarity of the results, the EHC offered specific recommendations focused on:

   (1) providing a clear rationale for selecting one particular point of departure over another;

   (2) conveying the biological significance of particular endpoints of the assessment;

   (3) presenting the advantages and limitations of novel approaches such as the time-to-response modeling of atrophy;

   (4) incorporating information on toxicokinetics on at least a qualitative, if not quantitative basis to inform the choice of endpoints;

(5) excluding points at high doses that contribute to a poor fit;

(6) explaining the rationale for using a risk reduction factor; and

(7) proofing calculations and correcting them where needed.

f) In Chapter 1, Introduction, the Agency should state whether the different cancer classification systems and quantitative assessments are equally valid and scientifically defensible, and explain the rationale for these judgements. In addition, the Agency should comment on its Cancer Risk Assessment Guidelines.

g) In Chapter 2, Overview of Exposure to 1,3-Butadiene, the Agency should clearly indicate that the chapter is not intended to be a comprehensive review of exposure. The chapter should include an explanation on how concentrations have been measured over the years and how this might affect a comparison of exposure levels measured in earlier years with those from more recent years. Specific recommendations for improvements for chapter 2 are included in Section 3.1.2.

h) There are several recommendations regarding Chapter 3, Metabolism and Pharmacokinetics. The main recommendations include the following with additional comments included in Section 3.1.3.

(1) The title of Chapter 3, Metabolism and Pharmacokinetics, should be changed to *Metabolism and Toxicokinetics* because 1,3-butadiene is not used as a therapeutic agent.

(2) The word, *toxicokinetics*, should replace the word, *pharmacokinetics*, throughout the document.

(3) Many new studies are not incorporated into the chapter. The Health Canada assessment and the comments that were submitted by Dr. Himmelstein provide many of those new references (Himmelstein, 1998).

(4) The Agency should revisit its statement regarding species differences in butadiene metabolism, taking into account the most recent information on species differences in the production of diepoxybutane and other reactive metabolites.

(5) The chapter should point out the close parallels between the observations in i*in vitro* studies on metabolism and tissue concentrations of epoxybutene and diepoxybutane in mice and rats and *in vivo* studies on the metabolism of butadiene.

i) The Committee's recommendations for Chapter 4, Mutagenicity, are numerous. Some of the recommendations are given below and additional recommendations are included in Section 3.1.4:

(1) The statement on Page 11-1 regarding the lack of sufficient data to determine if children or other subpopulations are afffected differently by exposure to 1,3-butadiene should be revisited in light of the studies by Nelson *et al.* (1995), Wiencke *et al*. (1995) and Kelsey *et al*. (1995). Nelson *et al.* (1995) found the glutathione transerase theta (GSTT1) to be highly polymorphic, due to wide variation in its ethnic distribution.

(2) Most of the extensive work on mutagenicity prior to 1994 should be included.

(3) The chapter should include text tables that summarize key animal and human findings derived from the entire body of information on butadiene.

(4) The tables should include references to support key findings.

(5) There should be separate tables for *in vitro*, animal and human findings.

(6) The similarities and species differences in response should be noted.

(7) The chapter should include more emphasis on the positive heritable translocation studies in mice because of their potential relevance for human heritable risks, and to several additional studies conducted in humans.

(8) The conclusion section should be expanded to include what is known about the mutagenicity of 1,3-butadiene and its metabolites.

(9) The missing dose units, missing units for mutant frequencies and other similar omissions should be added.

(10) The explanation as to possible reasons for the discordance between the positive effects obtained by autoradiography and the negative results found by cloning for human 1,3-butadiene induced hprt mutations *in vivo* may be incorrect and should be reconsidered.

(11) The conclusion section should be expanded by adding statements summarizing what is known about mutagenicity of 1,3-butadiene and its metabolites.

j) Some of the recommendations for Chapter 5, Reproductive and Developmental Effects, are included in recommendations cited in (e). Additional recommendations are provided in detail in Section 3.1.5 and are summarized below.

(1) All of the pertinent studies should be introduced in a summary table at the beginning of each section.

(2) Both positive and negative studies should be included so the reader can develop a comprehensive understanding of the data base supporting the assessment.

(3) More current research should be included, especially the new dominant lethal studies.

(4) Chapter 5 should be integrated with the other chapters, especially the chapters on pharmacokinetics and metabolism, animal toxicity, and quantitative risk assessment.

(5) The Agency should identify where it is uncertain with regard to conclusions about the reproductive and developmental endpoints, identify the action it will take to respond to the uncertainty, and should include all of the assumptions regarding uncertainty in the respective chapter.

k) The Committee's recommendations on Chapter 6, Toxicity in Animals, are listed in Section 3.1.6 and include the following:

(1) The rationale for the selection of the toxic non-cancer endpoint that is utilized in the derivation of the RfC is very important and should be more explicitly explained.

(2) It is unclear whether all of the repeat dose studies have been reviewed by the Agency. The EPA should incorporate the repeat dose *in vivo* mammalian studies of 1,3-butadiene in Chapter 6 unless these are covered elsewhere in the document and the Agency explicitedly so states.

(3) The Agency should present the NTP study details in the first section (chronic) of Chapter 6 and then refer to them in the carcinogenicity section.

(4) The EPA should use the same categories of tumors for presenting data from the continuous treatment, 9 and 15-month interim sacrifices and stop-exposure study in order to permit comparisons.

(5) A table summarizing the positive oncogenic findings across all studies specifying the dose tested, and the type of tumor that was significantly elevated, should be added since there were numerous organs and tumors involved.

(6) The carcinogenic evidence on the mammalian metabolites of 1,3- butadiene should be separated from the data on related chemicals and placed in a subsection by itself.

(7) The rat carcinogenicity data should be presented in similar detail to the NTP data so that the reader does not have to find the old risk assessment to see the data.

(8) The observation that concentration, not time is a critical determinant of potency is not supported by a comparison of the tumor data presented in Table 6-4 and Table 6-8 from the NTP (1993) study for continuous lifetime treatment and stop-exposure study.

l) The Committee recommendations for Chapter 7 include:

(1) A statement that, for the Delzell *et al*. (1996) study, there was no excess among those hired before 1950 (Observed/Expected = 17/16.4 = 1.04) when one would expect the highest exposures, but there was an excess among those hired during 1950-59 (Observed/Expected = 20/10=2.0, Confidence Interval = 1.2-3.1)

(2) A statement regarding the inappropriateness of "lumping" the lymphohematopoietic tumors should be added.

(3) A statement regarding the possible role of confounding should be included in the document.

m) The recommendations for Chapter 8 are provided in Section 3.1.8 and make the following revisions:

(1) The most recent relevant literature on butadiene toxicokinetic modeling should be included.

(2) The Agency should revisit its conclusion in Chapter 8 once it includes the recent PBPK models as explained in Section 3.1.8.

n) The recommendations for Chapter 9 are provided in Section 3.1.9 and include the following:

(1) The data of Matanowski *et al.* (1997) should be considered,

2) The Agency should explain or provide further explanation on the following:

(i) why equivalent model fits for the Delzell data are obtained in order to convey a greater appreciation of the nature of the dose response data.

(ii) the role of benzene as a confounder.

(iii) the potential importance of the uncertainties of dose reconstruction and the potential magnitude of the impact on the risk predictions,

(iv) where possible, the rationale for the exclusion of points at high doses contributing to a poor fit.

(v) information on toxicokinetics (e.g., ovotoxicity of diepoxide).

o) Chapter 10, Weight of Evidence, should be rewritten to reflect the range of opinion regarding the human findings. In addition, the finding that 1,3-butadiene, when mutagenic or clastogenic, is so through its metabolism should be provided in both the evaluation and in the statement regarding the mechanistic studies that are most relevant to humans.

p) Specific recommendations for Chapter 11, Risk Characterization, are provided in Section 3.1.11 and include the following:

(1) The statement that the conclusion of "sufficient evidence" of human carcinogenicity is based on more than 10 epidemiologic studies examining five different groups of workers should be rewritten since it is misleading.

(2) In discussing the excess leukemia risk in the nested case-control study in the Matanoski *et al.* (1990) study, it is important to indicate that there was no excess of leukemia observed in the cohort study.

(3) The chapter should clearly state that the leukemia excess observed in the Delzell *et al*. (1996) study has not been replicated in a completely different study population. In addition, a weight of evidence approach should incorporate all three studies rather than to emphasize the one positive study.

(4) The strength of the association evaluation for the lymphosarcomas and the leukemias should focus on these different cancers separately.

(5) The EPA should incorporate the PBPK modeling into Chapter 11 if it has time to do so or should at a minimum, discuss future possible directions and consider alternative ways (e.g., reduced safety factor) to account for the species differences.

(6) Some members felt that the additional safety factor of 3 to move from an effect dose to a no effect dose should be removed because it is inappropriate.

(7) The document should point out important data gaps in our knowledge and research needs.

APPENDIX A--TECHNICAL ISSUES

Detailed Comments on Specific Aspects of the Draft Document

Chapter 2

a) *Section 2.2.1*. Butadiene monomer production facilities need to be discussed especially since the epidemiology section discusses exposure in these facilities

b) *Section 2.2.1*. The impact of compliance with the 1994 Hazardous National Emissions Standard for Hazardous Air Pollutants and the polymers and resins Maximum Achievable Control Technology (MACT) on air emissions should be discussed.

c) *Section 2.3.3*. This section discusses open burning of tires. Data on controlled burning of tires should also be included and distinguished from open burning. Specifically, results from a pilot study conducted by the U.S. EPA (USEPA, 1994) should be included. In this study, 1,3 butadiene could not be detected from controlled combustion of tire-derived materials.

d) *Section 2.4*. For a better perspective, where possible, tables should indicate the number of samples that were below the detection limit as well as the ranges of exposure concentrations. More recent data on emissions, such as the 1995 and 1996 Toxics Release Inventory, (USEPA, 1997; 1998b), and the EPA's 1996 nationwide emissions inventory for butadiene (when released) should be included. If available, exposure concentrations inside automobiles should be added.

e) *Section 2.5*. In this discussion of exposures, similarities and differences of general population and occupational cohort exposures should be discussed so that the limitations of extrapolation from occupational setting to exposure of the general population are understood. For example, short peak exposures in industrial setting vs. chronic low levels of exposure to general population may impact the validity of an extrapolation of occupational data on exposure-response relationships.

Chapter 3

a) *Page 3-1, Line 15*: Much of the literature used the abbreviation BD for 1,3-butadiene. It should be less confusing if BDiol is used for the 3-butene-1,2-diol. This section does not even mention the 3,4-epoxy-1,2-butanediol (EBD). The latest research on molecular dosimetry strongly suggests that EBD is the major electrophile that binds to DNA and hemoglobin. EBD should be readdressed in the final version of this risk assessment.

b) *Page 3-2, Line 14*: Recent data shows that the trihydroxybutane (THB) adducts are clearly the predominant adducts in DNA and hemoglobin. These can arise from either 3,4-epoxy-1,2-butanediol or 1,2:3,4-diepoxybutane (DEB). Based on tissue measurements of DEB and EB, the ratios of epoxybutene (EB) to DEB/EBD(THB) adducts strongly suggest that EBD is the primary source of the THB adducts. The EBD metabolite has not been quantitated following *in vivo* exposure. This represents a major gap in our knowledge that needs to be acknowledged

c) *Page 3-2, Line 14-18*: The toxicokinetic data clearly does not support the statement that the possible crotonaldehyde metabolites are "Of greater significance." This editorial comment should be removed from the document, since no causal role of these metabolites in butadiene mutagenicity or carcinogenicity has been shown.

d) *Page 3-39, Line 18-25*: The toxicokinetics of butadiene are much more complicated than are discussed. The molecular dosimetry of DNA adducts following 4 weeks of exposure clearly shows that the first oxidation to EB and its subsequent binding to DNA is linear over a range of 20-625 ppm in rats and mice (Swenberg, *et al.*, 1998). It is rapidly converted to BDiol and then to EBD. To a lesser extent, EB is oxidized to DEB. Both EBD and DEB form THB adducts, although most of these come from EBD. The formation of THB adducts is saturated at 62.5 ppm in the rat, so that exposure to higher amounts such as 1000-8000 ppm results in little more THB adducts than does exposure to 62.5 ppm. In contrast, the mouse shows a biphasic response for the formation of THB adducts, with a steep slope between 0 and 62.5 ppm and a lesser slope from 62.5-625 ppm. The mouse does not show total saturation of the formation of THB adducts, suggesting that it has a second enzymatic pathway that is still active at high exposures. These data were presented at the SAB meeting, were presented at the Society of Toxicology (SOT) and Health Effects Institutes (HEI) annual meetings in 1998, and will be submitted for publication this summer (Tretyakova *et al.*, 1998; Swenberg *et al.*, 1998).

e) *Page 3-40, Line 13-14*: This sentence is unclear. What is metabolic capacity of EB? Is this formation or further metabolism? Available DNA data suggests that formation continues to occur in an exposure related manner.

f) *Page 3-45*: The section on Discussion and Conclusions should be revised. Lines 8-10 are oversimplified. Line 12 is wrong -- 1,3-butadiene epoxide should be identified as butene diol. Lines 19-23 clearly do not reflect molecular dosimetry data and actually appear to be reversed. Line 27 is wrong. Butene diol is not toxic. EBD needs to be added to this section, as it is likely to be a major metabolite.

g) *Page 3-3*: This chart has several errors that need correction. 3-Butene-1,2-diol is not reactive and should not have a box around it. Furthermore, the arrow for Reaction 11 is going in the wrong direction. Crotonaldehyde and acrolein are reactive and should have boxes around them. The urinary metabolites M-I and M-II should be shown.

h) *Page 3-27*: (9) This reaction seems to be mislabeled. It appears that this refers to BDiol GSH-BDiol.

i) *Page 3-38, Line 21-22*: It is stated that rat-excreted 1,3-dihydroxypropanone may be derived from hydroysis of diepoxybutane. However, since most 1,3-dihydroxypropanone probably comes from EBD this statement gives a wrong impression.

k) *Page 3-41, Line 8*: Butene diol is not a hydrolysis product of *DEB*. This should be *EB*.

l) *Page 3, Line 41-42*: The sections on DNA and hemoglobin adducts are seriously out of date. Data from several laboratories have shown that the THB adducts are predominant over EB adducts. At high concentrations of butadiene (625-1000 ppm), the ratio of THB/EB is 1.5-4 for rats and 3-9 for mice. This drastically changes at nonsaturating exposures of 20 ppm and 62.5 ppm, where rats have 27.5 and mice have 43-47 times more THB adducts. The same finding has been demonstrated for human hemoglobin adducts. THB-Valine adducts are formed about 40 times more frequently than EB-valine adducts. The EPA document only mentions EB adducts. A molecular epidemiology study of hemoglobin adducts was recently completed at the National Cancer Institute (Swenberg *et al.*, 1998) in a Chinese butadiene worker study. Nearly all blood samples examined, whether from exposed or unexposed individuals, had measurable THB-Valine adducts. The number of adducts in unexposed individuals averaged ~40 pmol/g globin. There are similar data for U.S. research workers. With exposure to butadiene estimated to be 1-3.5 ppm, the number of adducts increased 2-3-fold ($R^2 = 0.33$). Of interest is the finding that glutathione S-transferase theta (GSTT1) genotype had no effect on THB-Valine adducts. Since the GSTT1 null genotype has clearly been associated with increased susceptibility to DEB-induced sister chromatid exchanges (SCEs) and is primarily located in erythrocytes, this observation supports EBD as the primary human electrophile forming THB-Valine adducts.

m) *Page 3-45*: The Discussion/Conclusions section of the risk assessment should also address what is known about genetic polymorphisms that are likely to affect individual susceptibility to butadiene and its metabolites. Several genes appear to be important. Inherent susceptibilities have been shown for both DEB and EB

(Weincke and Kelsey, 1993), which may be due to glutathione S-transferase theta (GSTT1) status. Also, glutathione S-transferase μ(GSTM1) appears to be an important detoxifying factor for EB, so that GSTM1 null individuals would be expected to have greater effects following formation of EB. Unfortunately, no data have been published on the effects of GST polymorphisms on EBD. This is a gap in our knowledge. Genetic polymorphisms have also been identified for epoxide hydrolase (EH) and CYP 2E1 that would be expected to affect susceptibility to butadiene and its metabolites. The role of these proteins in the toxicokinetics of numerous chemicals is reasonably well known. Three studies (Csanday *et al.*, 1992; Seaton *et al.*, 1995; and Duescher and Elfarra, 1994) have shown *in vitro* using rodent and human tissue samples that CYP2E1 plays a role in the oxidation of both BD and EB. It is possible to expect that polymorphisms that reduce EH activity will increase susceptibility to butadiene. Likewise, rapid CYP 2E1 metabolizers would be expected to be at greater risk.

Chapter 5

a) *Pages 5-1, lines 29-31:* Reword this sentence as shown below (changes in italics) to make it neutral: "Because the results were not analyzed statistically and other details regarding the duration of the mating periods were not present, it is not possible to conclude that 1,3-butadiene *either* had *or did not have* an effect on fertility in rats."

b) *Pages 5-9, lines 27-29*: Under what condition is 108± ppm 4 vinyl-1-cyclohexene identified? What is the stock solution? The missing information must be added.

c) *Pages 5-25, tables 5-13*: Why are only some of the studies of structurally related compounds listed on this table? Why are negative observations not included? The explanation given on pp.5-24, lines 17-19 was weak. Since neoplastic lesions in reproductive organs were seen, these findings should be added here, albeit identified as neoplastic or non neoplastic. The title of this table does not limit effects on neoplastic status. However, the table should not just give positive studies but also include negative observations, like those in rats. The observations of decreased Graafian follicules should be included if significant (note that significance status was not given in lines 25-27).

d) *Pages 5-28, lines 15-17*: The summary introduces new structure-activity information from Maronpot, 1987. This data should be introduced and integrated with Chapter 5 prior to the summary. See also summary lines 7-33.

e) *Pages 5-28*: The summary speculates about reduced steroidogenesis, has any study measured steroid levels?

f) *Pages 5-28, lines 32-33*: The Committee did not see any data presented to support this speculation, in fact the observed effects of 1,3-butadiene on spermatozoa and spermatids (discussed in the next paragraph) argues against this "protective" effect of this barrier. The Committee recommends the removal of this sentence.

g) *Table 5-1*: Table 5-1 has numbers in parenthesis that are not defined.

h) *Section 5.1.6*: Sections such as this would be clearer if a data table would accompany the text. For example, see Tables 5-4 through 5-12.

i) *Page 5-28, lines 11-17*: The paragraph on ovarian lesions fails to present the well established mechanism for ovarian toxicity and carcinogenicity. DEB is highly toxic to the ovary, which makes it non-responsive to FSH. Continual elevation of FSH results in the carcinogenicity.

Chapter 7

a) *Page 7-3, Line. 33*: The latency period of 10-19 years was left out.

b) *Page 7-5, Line. 14-15*: Singling out an intermediate subgroup that gave a suggestive elevation in risk, when subgroups with more exposure did not, is a questionable scientific procedure (i.e., picking and choosing the ad hoc results that support a particular point of view).

c) *Page. 7-20, Line. 1-5 & Page 7-33, Lines 9-12*: It seems curious to report subgroup analyses based on just 3 of the 8 plants, especially when the reason given for choosing them ("three plants who had geometric means of exposure" out of the 7 plants with measurements) seems irrelevant.

d) *Page 7-21, Line 30-32*: The document states at this point that "When this subcohort was further restricted..." Please define the subcohort that is discussed, and also state the endpoint that is being discussed.

e) *Page 7-23, Line 25-26*: One important feature was that the job- exposure matrices (JEM) estimates were specific for calendar time. This should be noted.

f) *Page 7-31, Line 32-33*: It is inappropriate to cite positive findings from some earlier follow-up of the cohort when these were not confirmed by the latest follow-up.

g) *Page 7-33, Line 1-8 and Page 11-5, Line 12-25*: In order to present a balanced review of the Matanoski *et al*. (1990) case-control study, the re-analysis by Cole *et*

al. (1993) that yielded a null RR should also be reported, with an indication of how highly sensitive the results were to the particular cutpoints chosen

The Cole-Acquavella (Acquavella *et al.*, 1994) results are mentioned only obliquely, and the discrepancy is not articulated and its implication are not considered.

h) *Page 7-34, Line 33,35*: To be more balanced, the report should indicate that the Meinhardt (1982) results were not statistically significant.

i) *Page 7-36, Line 23:* This sentence should be deleted because it suggests that the stop-exposure studies conducted by Melnick *et al*. (1990) confirm the findings of excess lymphosarcoma among short-term monomer workers in the Divine *et al*. (1996) study. This statement is incorrect.

j) *Page 7-36, Line 23:* There is no way that the stop exposure studied in mice "confirm" the short term worker effect. Long term workers employed at the same time and later did not develop increased lymphosarcomas.

j) In the total cohort the leukemia rate was somewhat elevated (O/E = 48/36.6 = 1.31, 95% CI = 1.0-1.7) with some 11 excess leukemias (Delzell *et al*., 1996). Surprisingly, there was no excess among those hired before 1950 (O/E = 17/16.4= 1.04) when one would expect the highest exposures, but there was an excess among those hired during 1950-59 (O/E= 20/10.0= 2.0, CI= 1.2-3.1). Research is ongoing as to whether the risk elevation beginning in 1950 is a function of concomitant exposure to DMDTC (Dimethyldithiocarbamate) in the SBR process beginning in about 1950, but it is premature to judge that hypothesis at this time.

k) There should be a table to present the actual exposure-response data for the Macaluso *et al*. (1996) study of butadiene exposure and leukemia risk, rather than just burying the values in the text (e.g., Page 7-22, Line 22 and Page 7-25, Line 7). These are among the most important numbers in the report, so they should be prominently displayed.

l) According to Macaluso *et al. (*1996), the 0-dose group contained a substantial number of salaried workers but the other groups apparently did not. This could potentially bias the exposure-response risk estimate, and it should be mentioned.

m) There seems to be a substantial discrepancy between two sets of risk estimates given for this study in the report on Page 7-22 (Line 22) and Page 7-25 (Line 7), as shown in the table below. One wonders if the second set (the lower half of the table), which appear to be the one used by the EPA, may be incorrect. Notice that in the upper half of the table, the RR for >0-19 ppm-years is 1.1, whereas in the

lower half, the two cells that cover the range of >0-19 ppm-years have RRs of 2.0 and 2.1 -- rather implausible values, especially the RR of 2.0 for <1 ppm-year. The middle range is likewise higher in the lower half of the table (RR = 2.4 for 20-79, vs. 1.8 for 20-99 ppm-years in the upper half). The RRs in the high exposure range also differed notably: RR = 4.5 for 80+ ppm-years in the lower half of the table, but in the upper half the RRs for 100-199 and 200+ ppm-years are 2.1 and 3.6. The reason for the discrepancies is not clear; the only difference noted in the derivation of the two sets of estimates is that the upper set adjusted for years since hire and calendar period, whereas the lower one did not. If one of these two sets is not in error, then the discrepant results suggest that the results must be very sensitive to the particular cutpoints, confounders used, etc., which would argue for observing caution so as not to extrapolate from the most extreme results. It is of note that most of the analyses reported by Delzell *et al*. (1996) in their technical report, e.g., Tables 60-62, are similar to the upper half of the table below, rather than the lower half.

Table 1, below, displays the two sets of estimates of leukemia relative risks (RR) (in relation to butadiene exposure) reported from the Macaluso *et al.* (1996) study.

Exposure range (ppm-years)	**0**	**>0-19**	**20 - 99**	**100 - 199**	**200+**
RR	1.0	1.1	1.8	2.1	3.6
Exposure range (ppm/years)	0	<1	1-19	20-79	80+
RR	1.0	2.0	2.1	2.4	4.5

Table 1 (from Delzell *et al*., 1996)

It is unclear from the EPA report as to how high the correlation between butadiene and styrene exposure levels was in this study. However, one would expect the correlation to be fairly large. Since there is also some indication that styrene may be associated with leukemia in this study, partialling out the effect that is attributable to butadiene is problematic, particularly if there is more reliability or accuracy in assigning butadiene exposures than in assigning styrene exposures, or vice versa, since variations in reliability/accuracy could drive the proportion of variance attributed to one chemical vs. the other in regression analyses.

Chapter 8

a) *Page 8-1, lines 6-7:* The PBPK models cited by the EPA in this report should be updated. More mechanistic and sophisticated models are now available including

Reitz *et al*. (1996), Csanady *et al*. (1996), Sweeney *et al.* (1996; 1997), and Kohn (1997).

b) *Page 8-2, lines 1-32:* The model of Hattis and Wasson (1987) was developed before there were any experimental data on the rates of 1,3-butadiene activation and detoxication in experimental animals and before there were any experimentally determined data for partition coefficients, estimates of solubility that are used in PBPK models. Given the acknowledged limitation of this model, it would be more appropriate to simply mention that this model is one of the first PBPK models to be developed without going into details regarding the model predictions. This is particularly important given the conclusion of Hattis and Wasson that "differences in pharmacokinetics failed to account for differences in carcinogenesis between mice and rats and that with respect to risk assessment, uncertainties in PBPK modeling are trivial compared with the differences in apparent sensitivities between these species" (Page 8-2, lines 18-32). The overwhelming body of experimental data on the toxicokinetics of 1,3-butadiene and its metabolites collected in rats and mice since the publication of the Hattis and Wasson model have shown that differences in pharmacokinetics between these two species can account for species differences in carcinogenesis. It is also significant that the Hattis and Wasson model was an unpublished report and never benefited from peer-review. It would be more appropriate for the EPA to devote a paragraph to the presentation of the Hattis and Wasson model similar to what was done for the Hallenbeck (1992) model (page 8-4, lines 3-9).

c) *Page 8-4, lines 10-33:* It might be appropriate for the EPA to cite the Kohn (1996) model instead of the 1993 model primarily because the model of Kohn and Melnick (1993) relied on theoretically derived partition coefficients rather than experimentally determined partition coefficients. The use of these calculated partition coefficients resulted in overpredictions of the concentrations of 1,3-butadiene in tissues, especially fat.

d) *Page 8-7, lines 27-34:* As noted above, the use of empirically derived calculated partition coefficients rather than experimentally determined values led Kohn and Melnick in their 1993 model to conclude that storage in fat is a significant fraction of the retained 1,3-butadiene, especially in rats and humans. It is generally recognized that this conclusion is based on the use of calculated rather than experimentally measured partition coefficients. Inclusion of this paragraph in the chapter may lead readers not knowledgeable in 1,3-butadiene toxicokinetics to believe that this model prediction is accurate. While the EPA does discuss this limitation (page 8-8, lines 1-13), it is still misleading for the EPA to devote significant discussion to models in which theoretical values are used when later experiments determined the values to be inaccurate. It would be much more prudent for the EPA to report the most recent PBPK models noting when

necessary that these laboratories had also participated in the development of earlier models.

e) *Page 8-12, lines 21-23:* The EPA notes that "Johanson and Filser are reportedly working on a corresponding PBPK model for humans but it has not yet been published." At this point it would be most appropriate for the EPA to cite the Csanady *et al.* (1996) PBPK model for 1,3-butadiene that includes model predictions for man.

f) *Page 8-15, lines 9-11:* The EPA notes that a limitation of the model of Evelo *et al*. (1993) is that metabolism of butadiene is limited to the lung and the liver. This should not be viewed as a limitation. The objective of PBPK models is to account for the most significant mechanistic steps in the disposition of chemicals with the goal of predicting the concentration time profile of the toxic agent in either the target tissue or a suitable surrogate for the target tissue such as blood. It is not practical nor is it necessarily advantageous to develop a model that incorporates all pathways in the disposition of a chemical however minor. The simplest models that are the most useful for risk assessment are most likely to have the greatest value since these models will have the fewest number of parameters that require independent experimental determination in animals and humans. Thus, it is not clear why the Agency feels it is necessary in the case of butadiene to account for metabolism of this chemical in all tissues of the body. Extensive modeling efforts have determined that inclusion of metabolic activation in the liver, the major organ for metabolism of butadiene, and in the lung, a target organ for mice, are the most appropriate from a mechanistic standpoint.

g) *Page 8-15, line 15:* The EPA notes that the most recent PBPK model published for 1,3-butadiene is the model of Medinsky *et al*. (1994). This statement is correct only in the context of the January 31, 1997 cutoff date for consideration of reports in this document. However, the statement is misleading as there are a number of other PBPK models that have been published since 1994, including models published in 1996 and 1997. The EPA is urged to revise this chapter to include these more recent models. These models not only consider the metabolism of 1,3-butadiene, but also include the disposition of its two epoxide metabolites, epoxybutene and diepoxybutane, thereby making the models more appropriate for use in risk assessment.

h) *Page 8-16, lines 6-14:* The EPA notes that "in the model of Medinsky *et al.*, the microsomal concentrations reported by Csanady *et al*. (1996) were not used to scale metabolic rates. Instead, literature values for microsomal concentrations were used." While the Agency correctly summarizes the approach taken, the rationale for taking this approach is not appropriately presented. The objective of the study reported by Csanady *et al*. (1992) was to determine the rates of

butadiene oxidation in microsomes from rodents and humans. To achieve this objective it is essential that purified microsomes be obtained from liver, but it is not essential that all of the microsomal protein in the liver be accounted for. Thus, Csanady *et al.*, 1992 report microsomal yield which is less than the total microsomal protein content of liver or lung. Other investigators, in contrast, have sought to determine the total amount of microsomal protein in liver or lung. These investigators have used other techniques for this assessment. Extrapolating *in vitro* results expressed per milligram of /microsomal protein to the entire organ requires knowledge of the total amount of microsomal protein in an organ not simply the yield of microsomal protein obtained in the biochemical experiment. Thus, the appropriate approach for scaling *in vitro* rates to the whole animal is to use total microsomal total protein content rather than yield. The literature values used by Medinsky *et al*. (1994) for total microsomal protein content were similar to those used by Johanson and Filser (1993) and Kohn (1997). In contrast, Kohn and Melnick (1993) used the values of microsomal yield reported by Csanady *et al*. (1992).

i) *Page 8-19, Section 8.3, Summary:* The EPA notes that "pharmacokinetic modeling of 1,3-butadiene has not elucidated the reasons for interspecies differences in carcinogenic response between rats and mice." This statement is not true if one evaluates the current PBPK models that describe the disposition of 1,3-butadiene, epoxybutene and diepoxybutane. These PBPK models clearly demonstrate that the dramatic species differences between rats and mice in response to 1,3-butadiene are most likely related to species differences in rates of formation and removal of the diepoxybutane metabolite. Experimental data and PBPK model simulations indicate that mice produce far greater concentrations of this reactive metabolite compared with rats. Sweeney *et al*. (1997) have used *in vitro* metabolism data collected in tissues from rats, mice, and humans directly into a PBPK model to make predictions regarding the epoxide concentrations in blood and tissues following exposure to 1,3-butadiene. Using *in vitro* derived parameters they were able to adequately simulate the pharmacokinetics of 1,3-butadiene, epoxybutene, and diepoxybutane. This ability to use *in vitro* data to make *in vivo* predictions suggests that *in vitro* data on rates of 1,3-butadiene, epoxybutene, and diepoxybutane metabolism obtained in human tissue samples can also be used to predict blood and tissue concentrations of both epoxide metabolites in humans. When these simulations are conducted, using average values for human metabolic rates, the results indicate that diepoxybutane concentrations in humans would be orders of magnitude less than those of mice and lower than, but much more similar to, concentrations predicted for rats. Thus, the EPA should revise this chapter to include a discussion of the toxicokinetic models for 1,3-butadiene that are now capable of simulating not only the disposition of 1,3-butadiene, but also its two most important epoxide metabolites.

j) *Page 8-19, lines 33-34:* The EPA statement that uncertainties in the existing PBPK models and data make them unreliable for use in risk assessment must be revisited. Given that several investigators have been able to use these models and the underlying metabolic data to predict butadiene, epoxybutene, and diepoxybutane concentrations in rodents exposed to butadiene, it is not clear what additional data and what uncertainties need to be resolved prior to the use of these models in risk assessment. For example, Sweeney *et al*. (1997) have used *in vitro* metabolism data obtained from rat, mouse, and human tissues directly in a PBPK model to make predictions regarding the epoxide concentrations in blood and tissues following exposure to 1,3-butadiene. Using *in vitro* derived parameters they were able to adequately simulate the toxicokinetics of butadiene, epoxybutene, and diepoxybutane.

k) *Page 8-20, line 5-21:* In this paragraph the EPA presents a number of criticisms regarding the parameters used in the PBPK models that are not necessarily accurate. For example, the EPA notes that "with respect to parameter values, there are disagreements about the ventilation rate and about metabolic parameters." As noted above, while it is true that each investigator uses a different value for ventilation rate or metabolic parameter, the values used by all investigators are within the normal range associated with these parameters. It is also not unreasonable to expect that if the EPA were to use a PBPK model in risk assessment that other point estimates also within the range of reported values for these parameters would be chosen by the EPA.

The EPA also notes that there is a paucity of human *in vitro* data for extension of the PPBK model to humans and that the few measurements that have been made on a few metabolic parameters show a high amount of variability. Relative to other chemicals there is an extensive amount of experimental data on the rates of metabolism of 1,3-butadiene by human tissue samples. Each of the metabolic pathways important in the disposition of 1,3-butadiene and its metabolites have been quantitated with V_{max} and K_m values obtained from multiple human samples. These pathways include oxidation of 1,3-butadiene, oxidation of epoxybutane, hydrolysis of epoxybutane, glutathione conjugation of epoxybutane, glutathione conjugation of diepoxybutane, and hydrolysis of diepoxybutane. Means and standard deviations for these parameters have been calculated because multiple human samples have been used. Thus, sample distributions can be generated from which population values can be obtained. A PBPK model applied to human risk assessment could employ either average values to obtain deterministic predictions or Monte Carlo simulation techniques to get probabalistic estimates of the range of responses of hundreds of simulated humans. The latter approach using Monte Carlo simulation would provide some estimate of the potential variability in human response to inhaled 1,3-butadiene in addition to an estimate of the response of the most sensitive humans. Regarding the large amount of variability associated with

these metabolic parameters when measured in humans, this reflects the inherent variability in the expression of various xenobiotic metabolizing enzymes in the human population, a fact that has been well documented in the literature. Thus, it is not unexpected that assessment of metabolic capacity from tissues from multiple humans should yield a range of outcomes.

l) *Page 8-20, lines 22-34:* The EPA notes that the existing models have been subjected to a very limited validation mostly by comparison of simulation results with chamber uptake data. This statement is true regarding the PBPK models that were published in 1994 and earlier (e.g., the models reviewed in this current document). However, since the publication of these first generation models, multiple inhalation toxicokenetic studies have been conducted in rats and mice where blood and tissue concentrations of 1,3-butadiene, epoxybutene, and diepoxybutane have been quantitated following inhalation exposure to 1,3-butadiene. The second generation models that include the prediction of not only epoxybutene but also diepoxybutane have utilized this recent *in vivo* toxicokenetic data for model validation.

The EPA also notes that "for PBPK models to be more reliable, they should also be validated against tissue concentration data for various metabolites and various tissues. More recently these data have become available although they must be interpreted with caution because it appears that metabolites in some of the tissues are subject to further metabolism during the lag time between termination of exposure and measurement of tissue concentrations." This statement is true and ironically the implications of post exposure metabolism were first recognized when one of these second generation PBPK models (Sweeney *et al.*, 1996) failed to adequately simulate tissue concentrations of epoxide metabolites. When the authors modified the initial conditions of the model to reflect the time lag between termination of exposure and measurement of tissue concentrations and the capacity of the tissues to metabolize 1,3-butadiene post exposure, they were successful in predicting the actual measured epoxide tissue concentrations.

m) *Page 8-21, lines 4-23:* The EPA concludes that the existing PBPK models and data cannot explain the interspecies differences in 1,3-butadiene carcinogenicity. As noted previously, the first PBPK models discussed in this report did not include the toxicokinetics of diepoxybutane. More recent second generation PBPK models that include the formation and elimination of this metabolite are successful in simulating *in vivo* data in both rats and mice for 1,3-butadiene for epoxybutene and diepoxybutane concentrations in blood and tissues. Both model predictions and experimental data indicate that the dramatic interspecies differences in carcinogenic response to 1,3-butadiene can, in fact, be explained by the dramatic interspecies differences in circulating concentrations of the diepoxybutane.

Chapter 9

a) *Page 9-4:* Since previous EPA lifetime risk estimates have used a 70-year time-frame, it would seem appropriate to follow this precedent for the sake of comparability, rather than use an 85-year estimate.

b) *Figures 9-9 to 9-14:* These figures should be prepared so that the axis are understandable without reading the full text.

c) *Page 9-27, line 33:* Why does this sentence say 600 ppm and above? Why doesn't it say 625 ppm, a dose that was tested?

d) *Page 9-5 & 9-7:* There is a discrepancy in the 95% lower confidence interval with a 1% level of risk, LEC_1, value between the text and the figure (0.12 ppm vs. 0.066 ppm). Two of the figures appear to be mislabeled.

e) *Page 9-13, lines 18-19:* It was difficult to follow the logic in the last sentence. This sentence should be modified to provide necessary rationale to follow this choice.

f) *Page 9-36, lines 13,14:* What is the rationale for this statement? Either the explanation should be expanded or the statement should be deleted.

g) *Tables 9-13 thru 9-15:* Statistics should be included for these summary tables.

h) *Figures 9-9 thru 9-14:* For clarity, the axis should be labeled so that reader can easily convert dose to ppm without going back to the text. Also figures, like tables, should indicate when exposures were adjusted to 24 hour daily exposures.

i) *Section 9.3:* Although the 95% lower confidence interval with a 10% level of risk (LEC_{10}) values are taken for the "point of departure" for the RfC's calculated for the benchmark based approach, minimal discussion was given as to why the LEC_{10} was chosen over the 95% lower confidence interval with a 5% level of risk (LEC_5) nor why the 95% lower confidence interval with a x% level of risk (LEC_x) versus ED_x values are chosen. Justification and rationale for this issue for all modeled endpoints should be provided.

j) *Pages 9-49, lines 33-34*: Is this statement true for dominant lethal effects as well as fetal weight reduction? This Committee urges caution in such general statements or authors should provide specific justification of these statements.

k) *Pages 9-42, lines 5-7:* Text cites Allen *et al*. (1994b) as source of information supporting the use of LEC_{10} as being "at or below the range of detectable

responses." Since this paper dealt only with developmental toxicity data, this statement as well as those later in this section are extrapolations from that research. The following text should be reworded, "Other studies are supportive of this statement. For example, the statistical power of detection of this study design supports this statement."

l) *Pages 9-44, Table 9-19:* A footnote should be added to describe what the Z statistic is and how Q O-2 are obtained. This section was very unclear.

m) *Pages 9-40 thru 9-44, Section 9.3.4, pp. 5-3 thru 5.5 and Section 5.1.4:* The Agency needs to address more fully the statistical and biological significance of the testicular atrophy. The footnote on page 5-4, Table 5-1 states that statistics were not conducted on the testicular lesions yet in Section 9.3.4 this endpoint becomes a study for modeling. Is the background rate for this lesion in the control B6C3 F1 mice low compared to historical mouse population statistics?

n) *Pages 9-46, line 1-2:* In this example, the text provides a reason for discarding the top doses in the modeling however, this decision is inconsistently applied in the modeling Section 9.3 as evidenced in Table 9-16. The text should provide some common guidance on what will be done regarding dropping higher dose levels from modeling calculations.

o) *Pages 9-46, line 10-11:* Statement in lines 10-11 appear to differ from more recent research published on PBPK models. Can this health risk assessment go further in using the data about known ovotoxicity of the diepoxide? See examples provided in testimony from the Chemical Manufacturers Associations's documents. (CMA, 1998a; 1998b)

p) *Pages 9-51, lines 2-4:* The Committee was unconvinced about the superior nature of the time-to-response modeling that was conducted for the LEC_{10} determination for atrophy. Additional discussion is needed to support this statement especially given the limitations of the biological time-to-response data for this endpoint.

q) *Pages 9-51, lines 16-26:* The EHC agrees with these limitations but would then use these points to justify using PBPK modeling to improve the target organ and time concentration curves relative to these specific reproductive versus developmental endpoints. The text has provided the justification but the assessment falls short of acting on these suggestions.

r) *Pages 9-51, lines 27-31:* The Committee agrees on these issues. Please see the earlier comments on how to constructively address these points.

s) *Mathematical errors need to be corrected:*. It is absolutely necessary that all calculations in Section 9 be carefully reviewed. The Committee noted numerous problems in this section and refer USEPA to the written statement of R. Seilken (1998) that is especially relevant to this issue. There may be confusion with some numbers presented as natural logs versus non-log numbers. Please proof very carefully. All calculations must be easy to follow by a general scientific audience. Chapter 9 did not meet this goal.

Chapter 11

a) *Page 11-1:* The statement regarding the lack of sufficient data to determine if children or other subpopulations are affected differently by exposure to 1,3-butadiene should be revisited in light of the studies by Nelson *et al.* (1995), and Wiencke *et al.* (1995). Nelson et al. (1995) found the glutathione transferase theta (GSTT1) to be highly polymorphic with wide variation in its ethnic distribution (Nelson *et al.*, 1995). Wiencke *et al.* (1995) have shown the association between the genotype for the production of glutathione transferase theta and genetic anomalies in sister-chromatidic exchange induced by metabolites of 1,3- butadiene: epoxybutene and diepoxybutane. This implies that the protein produced by the gene is important in conjugating both of these metabolites. GSTT1 only affects DEB and GSTM1 only affects EB. These are two proteins from two genes. Given the profound racial distribution of the polymorphism, it is important to note that this may account again for the significant portion of the alterations in the metabolism of 1,3-butadiene. Consequently, the enzymes responsibilities for the metabolic conversion of 1,3-butadiene to its mono- and di-epoxide forms as well as its diol form are highly polymorphic. This implies that there may be differential susceptibility to the genotoxic effects of exposure to butadiene. Investigations of this are rapidly moving ahead. There is some indication from field studies that these polymorphisms may contribute to directly-measurable genetic effects (Sorsa *et al.*, 1994). Hence, risk assessment and future studies of this compound should take note of this and adjust as is appropriate.

b) *Page 11-3, line 11-13 and Table 11-1*: EPA states that the conclusion of "sufficient evidence" of human carcinogenicity is based on more than 10 epidemiologic studies examining five different groups of workers. This statement is misleading because it implies that there is a consistency of results across several studies of equal caliber examining completely different populations. The predominant emphasis should be on the methods and findings of the two latest studies of butadiene monomer and SBR workers. The emphasis on the early epidemiologic studies is misleading to the extent that it gives the impression of consistency across several study populations in the SBR and buta diene monomer industries. There is really only one study population in each industry that provides appreciable information. Each of these studies consolidated and/or more

accurately refined the populations used in the earlier studies. This needs to be clarified because it is central to the understanding of how consistent the relationship is between 1,3 butadiene and cancer. For example, EPA should explain that the Delzell *et al*. (1995; 1996) studies included all of the eligible population from the Matanoski *et al*. (1990) study and the Meinhardt *et al*. (1982) study. The leukemia finding in the Matanoski *et al*. (1990) study should not be presented as if it were a separate finding in a completely different population from the Delzell study. In addition, the Delzell study supersedes this and other previous studies and rectifies many of the limitations and errors of the earlier studies.

c) *Page 11-5, line 12-34:* The Matanoski study is presented as if it were a study on a completely different population from the Delzell studies. In discussing the excess leukemia risk in the nested case-control study in the Matonoski study, it is important to indicate in line 14 that there was no excess of leukemia observed in the cohort study (standardized mortality ratio was 1.0 representing 22 observed, 22.9 expected). This is an important major point to be made up-front in this section because it helps explain the scientific debate later referred to in lines 26-28. The evidence linking butadiene exposure and cancer is still strongest for leukemia based on one large, high quality cohort study of SBR workers (Delzell studies) which supercedes the Matanoski study.

d) *Pages 11-6 to 11-7:* It is never clearly stated that the leukemia excess seen in the Delzell study has not been replicated in a completely different study population. The studies of butadiene monomer workers and of other butadiene exposed workers report null results for leukemia.

e) *Page 11-7, line 17:* The lymphosarcomas and leukemias are discussed as if they could be considered as the same type of cancer. It is implied that two different populations have an excess in lymphohematopoietic cancers thereby demonstrating consistency across studies. The strength of association evaluation should focus on these different cancers separately.

The EPA review states on page 11-7, lines 20-26 that the monomer workers exposed to shorter periods of time probably had higher exposures than workers exposed for longer periods of time. This statement needs to be removed as there is no evidence for this. The available evidence is insufficient for a causal relationship between butadiene and lymphosarcoma. Lymphosarcoma was elevated for short term exposed workers but not among long term exposed workers indicating a lack of dose-response. Of equal importance, lymphosarcoma/non-Hodgkins lymphoma (NHL) was not elevated in the SBR cohort which had excess leukemia. These points must be discussed in the EPA document. The possibility that it is the SBR process and not butadiene alone that may explain excess leukemia should be discussed regardless of final decision on cancer classification. Delzell's finding that

the leukemia excess concentrated among workers who began employment in the 1950's and not those that worked exclusively in 1940's led to hypothesis by Irons and Pyatt (1998) that DMDTC might be a contributing factor in the leukemia excess. This hypothesis needs to be discussed as it is part of the scientific literature.

f) *Page 11-8, Table 11-2*: Some of the entries in this table seem unbalanced.

(1) Matanoski (1993) is cited as showing "7 to 9 times higher relative odds for leukemia" without mentioning that others analyzed the same data using a slightly different cutpoint and found an odds ratio of <1.0.

(2) Most of the dose-response tabulations by Delzell *et al*. (1996) do not show mortality ratios in the highest dose groups as high as the ones cited.

(3) It states that a dose-response relation for monomer plant workers "Cannot be demonstrated due to lack of quantitative exposure data," when in fact several dose-response analyses were performed (albeit with an imperfect dose metric) and showed not even a hint of an association.

g) *Page 11-9, Line 24*: The Committee classified the SBR process as a known human carcinogen.

h) *Section 11.3.3, Page 11-9*: This section needs to more accurately reflect the scientific literature that demonstrates a clear difference in metabolic activation between humans and rodents and should discuss how PBPK modeling can refine the risk assessment process. If EPA cannot incorporate PBPK modeling into risk assessment within the mandated time-contraints, EPA should discuss future possible directions and consider alternative ways (reduced safety factor) to account for the species differences.

i) *Page 11-10, line 36 to Page 11-11, lines 1-2*: The EPA states that the tumor type in rodents most analogous to the lymphohematopoietic cancers is the lymphocytic lymphomas. To properly discuss the strengths and weaknesses of this statement, EPA should discuss the data generated by Irons *et al*. (1996) which shows that T-cell lymphoma in mice is due to a specific population of stem cells in the mouse bone marrow that is not present for humans or rat bone marrow cells. EPA should also point out that the link between 1,3-butadiene exposure and lymphoma is weak as there was no consistent dose-response relationship. Additionally, the Agency should show consistency in combining different tumor types across documents. For example, in the revised cancer risk assessment guidelines a case study for an aromatic hydrocarbon (presumably benzene) is presented where mice are shown to develop lymphomas following exposure whereas humans develop acute

myelogenous leukemia. In this case, the Agency did make the distinction that the response in the animal models and the response in the humans was different. The Agency should acknowledge that there is considerable disagreement as to whether these two tumor types, lymphomas and leukemias, derive from the same origin.

The potential impact of task specific peak exposures must be addressed. The primary metric for SBR workers is based on time weighted average exposure. But SBR workers frequently get the majority of their exposures during a small fraction of the work day during the conduct of specific tasks. The leukemogenic effect was associated with those jobs that involved high peak exposures in latex sampling (laboratory workers and in vessel cleaning for maintenance laborers). The general population is typically exposed to lower ambient levels. An additional uncertainty that should be discussed is the uncertainty of extrapolating from an occupational setting where peak exposures occurred to the generally low ambient levels of exposure.

j) *Page 11, Lines 11-13*: The statement that the evidence regarding human carcinogenicity is based on ten studies is deceptive, because the reports are not independent, but most reports are updates of previous ones. In addition, there are basically only four independent cohorts, not five, because the Delzell study included about 95% of the workers in the Matanoski studies.

k) *Section 11.5, Pages 11-13-11-14:* This section needs to be re-evaluated in light of the two more recent negative dominant lethal studies. A weight of evidence approach should be taken looking at all 3 studies rather than to emphasize the one positive study.

l) *Section 11.5 Pages 11-14, lines 1-3:* This sentence should be rewritten as fact not as speculation.

m) *Pages 11-14, lines 10-16:* These two sentences should be rewritten or removed. Text discusses concept of "meaningful increases in risk." What is the definition of this phrase? Is this in the USEPA guidelines? This phrase appears to be an editorial. For this reviewer to understand that "...effects are not expected in humans exposed to low environmental exposures.", the assessment must provide a detailed rationale that follows from Chapters 5 and 9. This summary statement was not supported in the document.

n) *Section 11.6, Pages 11-15*: This summary section needs to be integrated with the biology discussed in Chapter 5 and quantitative assessments in Chapter 9. No new "surprise," non-justified statements should appear in this section.

o) *Section 11.6*: The importance of the positive heritable translocation studies in mice should be more heavily emphasized.

p) *Section 11.6, Page 11-15, line 10-17*: The additional safety factor of 3 to extrapolate from a LOAEL to NOAEL is inappropriate and should be removed. Inadequate justification is given for the application of the additional safety factor for the benchmark dose. The EHC could not understand the rationale for its inclusion. This section must discuss the rationale for using the "hybrid model" for continuous data analysis and exactly what the hybrid model is so that the risk assessment can be completely transparent. On the surface it appears that the approach yielding the lowest LEC was selected. Some of the EHC Members/Consultants recommend that the Agency use the EC_{10} (central estimate) as the point of departure rather than the LEC_{10}. Other EHC Members/Consultants did not agree with the recommendation.

q) *Section 11.8 on Page 11.16:* The Agency should expand on future research needs to fill gaps in knowledge. The list is inadequate.

GLOSSARY - ACRONYMS AND ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
TLV	Threshold Limit Value
Bdiol	3-butene-1,2-diol
C	confidence interval
DEB	1,2,3,4-diepoxybutane
DMDTC	Dimethyldithiocarbamate
EB	1,2 epoxy-3-butene
EBD	3,4-epoxy-1,2-butanediol
Ec_x	effective concentration at x% risk, ranging from 0.1% to 10%
EHC	Environmental Health Committee
ED	effective dose
EBdiol	1,2 dihydroxy-3-4 epoxybutane
GC-MS	gas chromatography - mass spectrometry
GSTT1	glutathione S-transferase theta
GSTM1	glutathione S-transferase μ
HEI	Health Effects Institute
ICD	International Classification of Diseases
JEM	job-exposure matrices
K_m	substrate concentration at one-half maximum velocity
LEC_p	95% lower confidence intervals associated with a risk (p), ranging from 1% to 10%
LOAEL	lowest-observed-adverse effect
MACT	maximum achievable control technology
mg	milligram
MLE	maximum likelihood estimate
NCI	National Cancer Institute
NHL	non-Hodgkins lymphoma
NOAEL	no-observed-adverse effect
NTP	National Toxicology Program
O/E	observed/expected
OMS	Office of Mobile Sources
ORD	Office of Research and Development
PBPK	physiologically-based pharmacokinetic
ppm	parts per million
ppmh	parts per million per hours
q_1	the incremental unit cancer risk for humans
RfC	Inhalation Reference Concentration
RR	relative risk
SBR	styrene butadiene rubber
SCE	sister chromatid exchange

SOT	Society of Toxicology
THB	trihydroxybutane
TRI	Toxics Release Inventory
UAB	University of Alabama at Birmingham
V_{mzx}	maximum velocity for an enzyme-mediated reaction
WWII	World War II

REFERENCES CITED

Acquavella J. F. Delzell E., and P. Cole. 1994. Butadiene and lymphatic and hematopoietic cancer. *Epidemiology* 5:262-263.

Acquavella, J.F. 1998. Comments on EPA Health Risk Assessment for 1,3-butadiene: external review draft (EPA/600/P-98/001A, January 1998), April 17, 1998 submitted to the EPA on behalf of the International Institute of Synthetic Rubber Institute.[2]

Adler, I.D., Cao, J., and P. Gasner. 1994. Mutagenicity of 1,3-butadiene inhalation in somatic and germinal cells of mice. *Mutation Research* 309(2):307-314.

Adler, I.D., Filser, J.G., and P. Gassner. 1995. Heritable translocations induced by inhalation exposure of male mice to 1,3-butadiene. *Mutation Research* 347(3,4):121-127.

Adler, I.D., Filser, J., Gonda, H., and G. Schriever-Schwemmer. 1998. Dose response study for 1,3-butadiene-induced dominant lethal mutations and heritable translocations in germ cells of male mice. *Mutation Research*, Special Issue 397:85-92.

Allen, B.C., Kavlock, R.J., and C.A. Kimmel. 1994. Dose-response assessment for developmental toxicity, II. Comparison of generic benchmark dose estimates with NOAELs. *Fundamentals of Applied Toxicology* 23:487-495.

Arce, G.T., Vincent, D.R., and M.J. Cunningham. 1990. *In vitro* and *in vivo* genotoxicity of 1,3-butadiene and metabolites. *Environmental Health Perspectives* 86:75-78.

Au, W.W., Bechtold, W.E., and E.B. Wharton, Jr. 1995. Chromosome aberrations and response to gamma-ray challenge in lymphocytes of workers exposed to 1,3-butadiene. *Mutation Research* 334:125-130.

Autio, K., Renzi, L., and J. Catalan. 1994. Induction of micronuclei in peripheral blood and bone marrow erthyrocytes of rats and mice exposed to 1,3-butadiene by inhalation. *Mutation Research* 309(2):315-320.

Bechtold, W.E., Strunk, and M.R. Thornton-Manning, Jr. 1995. Analysis of butadiene, butadiene monoxide and butadiene diepoxide in blood by gas chromatography/gas chromatography/mass spectroscopy. *Chemical Research Toxicology,* 8:182-187.

[2]Not published in the peer reviewed literature

BIBRA. 1998. Brinkworth, M.H., Anderson, D., and J.A. Hughes. Genetic effects of 1,3-butadiene on the mouse testis. [BIBRA Mouse Study No. 3] *Mutation Research - Special Issue* 397(1):67-75.

BIBRA. 1995. The detection of dominant lethal mutations and foetal malformations, tumours and chromosome damage in the offspring of male mice treated sub-chronically with butadiene. BIBRA Report No. 1060/5/95 (Draft, April, 1995). [BIBRA Mouse Study 1] BIBRA International, Surrey, Great Britain.

BIBRA. 1996. The detection of dominant lethal mutations and foetal malformations and chromosome damage in the offspring of male mice treated sub-chronically with butadiene by inhalation - second study. BIBRA Report No. 1542/1/96. [BIBRA Mouse Study 2] BIBRA International, Surrey, Great Britain.

Bond, J.A., Dahl, A.R., and R.F. Henderson. 1986. Species differences in the disposition of inhaled butadiene. *Toxicology and Applied Pharmacology* 84:617-627.

Boogaard, P.J., and J.A. Bond. 1996. The role of hydrolysis in the detoxification of 1,2:3,4-diepoxybutane by human, rat, and mouse liver and lung *in vitro*. *Toxicology and Applied Pharmacology* 141: 617-627.

Boogaard, P.J., Sumner, S.C.J., and J.A. Bond. 1996. Glutathione conjugation of 1,2:3,4-diepoxybutane in human liver and rat and mouse liver and lung. *In vitro*. *Toxicology and Applied Pharmacology* 136:307-316.

Brooks, P. and P.D. Lawley. 1961. The reaction of mustard gas with nucleic acids *in vitro* and *in vivo*. *Biochemical Journal*, 77: 478-484.

CMA. 1998a. Comments of the Olefins Panel of the Chemical Manufacturers Association on EPA's Draft Health Risk Assessment for 1,3-Butadiene, April 17, 1998 submitted to the National Center for Environmental Assessment, USEPA, Washington, DC.

CMA. 1998b. Comments of the Olefins Panel of the Chemical Manufacturers Association on EPA's Draft Health Risk Assessment for 1,3-Butadiene, April 29, 1998 submitted to the National Center for Environmental Assessment, USEPA, Washington, DC.

Christian, M. 1998. Excerpts of M. Christian's Testimony to OSHA, Appendix H *In* Comments of the Olefins Panel of the Chemical Manufacturers Association on EPA's Draft Health Risk Assessment for 1,3-Butadiene, Chemical Manufacturers Association, April 17, 1998, submitted to the National Center for Environmental Assessment, USEPA, Washington, DC.

Cochrane, J.E., and T.R. Skopeck. 1994. Mutagenicity of butadiene and its epoxide metabolites: II. Mutational spectra of butadiene, 1,2-epoxybutene, and diepoxybutane at the hprt locus in splenic T cells from exposed $B6C3F_1$ mice. *Carcinogenesis* 15:719-723.

Cole, P., Delzell, E., and J. Acquavella. 1993. Exposure to butadiene and lymphatic and hematopoietic cancer. *Epidemiology* 4:96-103.

Cowles S.R., Tsai, S.P., Snyder P, and C. Ross. 1994. From a cohort of long term workers involved in 1,3-butadiene monomer production. *Occupational and Environmental Medicine*, 51:323-329.

Csanady, G.A., Guengerich, F.P., and J.A. Bond. 1992. Comparison of the biotransformation of 1,3-butadiene and its metabolite, butadiene monoepoxide, by hepatic and pulmonary tissue from humans, rats, and mice. *Carcinogenesis* 13:1143-1153.

Csanady G.A., *et al*. 1996. A physiological toxicokinetic model for 1,3-butadiene in rodents and man: blood concentration of 1,3-butadiene, its metabolically formed epoxides and hemoglobin adducts - relevance of glutathione depletion. *Toxicology* 113:300-305.

Dahl, A.R., Sun, D.J., and L.S. Birnbaum. 1991. Toxicokinetics of inhaled 1,3-butadiene in monkeys: comparison to toxicokinetics in rats and mice. *Toxicological and Applied Pharmacology*, 110:9-19.

Delzell, E., Sathiakumar, N., Macaluso, M., Hovinga, M., Larson, R., Barbone, F., Beall, C., and P. Cole. 1995. A follow-up study of synthetic rubber workers. Final report prepared under contract to International Institute of Synthetic Rubber Producers, October 2, 1995.

Delzell E, Sathiakumar N., and M. Hovinga. 1996. A follow-up study of synthetic rubber workers. *Toxicology*, 113:182-189.

Divine B.J., and C.M. Hartman. 1996. Mortality update of butadiene production workers. *Toxicology* 113:169-181.

Evelo, C.T.A., Oostendorp, J.G.M., and W.F. ten Berge. 1993. Physiologically based toxicokinetic modeling of 1,3-butadiene lung metabolism in mice becomes more important at low doses. *Environmental Health Perspectives* 101:496-502.

Foureman, P., Mason, J.M., and R. Valencia. 1994. Chemical mutagenesis testing in Drosophila. *Environmental and Molecular Mutagenesis* 23:51-63.

Hallenbeck, W. 1992. Cancer risk assessment for the inhalation of 1,3-butadiene using physiologically based pharmacokinetic modelling. *Bulletin of Environmental Contamination and Toxicology* 4:66-70.

Hattis, D. and J. Wasson. 1987. Pharmacokinetic/mechanism-based analysis of the carcinogentic risk of butadiene, PB88-20817, Rockville, MD: National Institute for Occupational Safety and Health, 1-122.

Hayes, R.B., Xi, L, and W. Bechte. 1996. Hprt mutation frequency among workers exposed to 1,3-butadiene in China. *Toxicology* 113:100-105.

Hazelton. 1981. 1,3-butadiene: inhalation tetratogenicity study in the rat (final report), Harrowgate, England: Hazelton Laboratory, addendum no. 2788-522/3

Health Canada. 1997. Canadian Environmental Protection Act Priority Substances List, Supporting Documentation Health-Related Sections, 1,3-Butadiene, Draft, December 1997.

Himmelstein, M.W., Turner, M.J., and B. Asgharian. 1994. Comparison of blood concentrations of 1,3-butadiene and butadiene epoxides in mice and rats exposed to 1,3- butadiene by inhalation. *Carcinogenesis*, 15:1479-1486.

Himmelstein, M.W., Acquavella, J.F., Recio, L., Medinsky, M.A., and J.A. Bond. 1997. Toxicology and Epidemiology of 1,3-Butadiene, *CRC Reviews in Toxicology*. 27:1-108.

Himmelstein, M. 1998. Comments on Chapter 3 -- Metabolism and Pharmacokinetics, Health Risk Assessment for 1,3-Butadiene (External Review Document), submitted by the Chemical Manufacturers Association to Dr. Aparna M. Koppikar, Project Manager for 1,3-Butadiene, Technical Information Staff (8623-D), National Center for Environmental Assesment - Washington Office, Washington, DC, April 29, 1998

IARC. 1998. IARC Monographs on the evaluation of carcinogenic risks to humans, re-evaluation of some industrial chemicals. 71 [106-99-0] in preparation. The IARC Working Group that evaluated 1,3-butadiene met on February 17-24, 1998 in Lyons, France. The findings were presented at the EHC's April 30-May 1, 1998 meeting by Dr. James Bond of the Chemical Industry Institute of Toxicology.

Irons, R., Colagiovanni, D., and W. Stillman. 1996. Murine thymic lymphoma is associated with a species-specific hematopoietic progenitor cell subpopulation. *Toxicology* 113:59-67
Irons, R. and Pyatt, D. 1998. Dithiocarbamates as potential confounders in butadiene epidemiology. *Carcinogenesis* 19, 4:101-104.

Johanson, G. and J.G. Filse. 1993. A physiologically based pharmacokinetic model for butadiene and its metabolite butadiene monoxide in rat and mouse and its significance for risk extrapolation. *Archives of Toxicology*, 67:151-163.

Koivisto, P., Sorsa, M., and F. Pacchierotti. 1997. 32P-postlabelling/HPLC assay reveals an enantioselective adduct formation in N7 guanine residues *in vivo* after 1,3-butadiene inhalation exposure. *Carcinogenesis* 18(2):439-443.

Kohn M.C. and R.L. Melnick. 1996. Effects of the structure of a toxicokinetic model of butadiene inhalation exposure on computed production of carcinogenic intermediates. *Toxicology* 113:31-39.

Kohn, M.C. and R.L. Melnick. 1993. Species differences in the production and clearance of 1,3-butadiene metabolites: a mechanistic model indicates predominantly physiological, not biochemical, control. *Carcinogenesis*, 14:619-628.

Kreiling, R., Laib, RJ., and H.M. Bolt. 1986. Alkylation of nuclear proteins and DNA after exposure of rats and mice to (1,4-super(14C)1,3-butadiene). *Toxicology Letters* 30(2):131-136.

Kumar, R., Vodicka, P., and P. Koivisto. 1996. 32-P-postlabelling of diastereomeric 7-alkyl-guanine adducts of butadiene monoepoxide. *Carcinogenesis* 17:1297-1303.

Lawley, P.D.,and P.B. Brooks. 1967. Interstrand cross-linking of DNA by difunctional alkylating agents. *Journal of Molecular Biology* 25:143-160.

Leavens, T.L., Farris, G.M., and R.A. James. 1997. Genotoxicity and cytotoxicity in male B6C3F1 mice following exposure to mixtures of 1,3-butadiene and styrene. *Environmental Molecular Mutagenesis* 29:335-345.

Leuratti, C., Jones, N.J., and E. Marafante. 1994. DNA damage induced by the environmental carcinogen butadiene: Identification of a diepoxybutane-adenine adduct and its detection by super (32) P-postlabelling. *Carcinogenesis* 15(9):1903-1910.

McGregor, D., Brown, A., and P. Cattanach. 1991. Responses of the L5178Y mouse lymphoma forward mutation assay: v. gases and vapors. *Environmental and Molecular Mutagenesis* 17:122-129.

Macaluso M., Larson R., and E. Delzell. 1996. Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. *Toxicology* 113:190-202.

Maki-Paakkanen *et al*. 1993. Cited in: Norppa, H. and M. Sorsa. 1993. Genetic toxicity of 1,3-butadiene and styrene. *IARC Sci*. Publication No. 127:185-193.

Matanoski G.M., Santos-Burgoa, C., and L. Schwartz. 1990. Mortality of a cohort of workers in the styrene-butadiene polymer manufacturing industry (1943-1982). *Environmental Health Perspective* 86:107-117.

Matanoski G., Francis M., Correa-Villasenor A., Elliot E., Santos-Burgoa, C., and L. Schwartz. 1993. Cancer epidemiology among styrene-butadiene rubber workers. *In*: Sorsa M, Peltonen K, Vainio H, Hemminki K, eds. Butadiene and Styrene: Assessment of Health Hazards. Baltimore: IARC Scientific Publications No. 127:363-374.

Matanoski, G., Elliot, E., and X. Tao. 1997. *Lymphohematopoietic cancers and butadiene and styrene exposure in synthetic rubber manufacture*. Ann NY Acad Sci pp. 157-168.

Matanoski, G., Elliot, E., Tao, X., Francis, M., Correa-Villasenor, A., and C. Santos-Burgoa. 1998. *Lymphohematopoietic Cancers and Butadiene and Styrene Exposure in Synthetic Rubber Manufacture*, unpublished. Johns Hopkins University, Department of Epidemiology, Baltimore, Maryland.

Medinsky, M.A., Leavens, T.L., and G.A. Csanady. 1994. *in vivo* metabolism of butadiene by mice and rats: a comparison of physiological model predictions and experimental data. *Carcinogenesis* 15:1329-1340.

Meinhardt, T.J., Lemen, R.A., and M.S. Crandall. 1982. Environmental epidemiologic investigations of the styrene-butadiene rubber industry: mortality patterns with discussion of the hematopoietic and lymphatic malignancies. *The Scandinavian Journal of Work, Environment, and Health* 8: 250-259.

Melnick, R.I., Huff, J.E., and B.J. Chou. 1990. Carcinogenicity of 1,3-butadiene in C57BL/6xC3HF1 mice at low exposure concentrations. *Cancer Research* 50:6592-6599.

Meng, Q., Recio, L., and A. Reilly. 1996. The *in vivo* mutagenic potential of 1,3-butadiene, evaluated at the hprt locus of T-cells, is greater in mice than in rats exposed concurrently by inhalation. Abstract presented at the Twelfth Health Effects Institute Annual Conference.

Meng, Q., Henderson, R., Dahl, A., and V.E. Walker. 1997. Species comparisons of the mutagenic potency of epoxybutane and diepoxybutane in hprt of splenic T-cells following exposures of female F344 rats and B6C3F1 mice. 13th Health Effects Institute Conference, May 4-6, 1997, Annapolis, MD (Abstract p. 37).

Moolgavkar, S.H. 1994. Biological models of carcinogenesis and quantitative cancer risk assessment. *Risk Analysis* 14(6):879-882.

Morrissey, R.E., Schwetz, B.Z., and P. Hackett. 1990. Overview of reproductive and developmental toxicity studies of 1,3-butadiene in rodents. *Environmental Health Perspectives* 86:79-84.

NAS. 1988. Health Risks of Radon and Other Internally Deposited Alpha-Emitters (BEIR IV). National Academy of Sciences/National Research Council. Washington, DC. National Academy Press.

NAS. 1990. Health Effects of Exposure to Low Levels of Ionizing Radiation (BEIR V). National Academy of Sciences/National Research Council. Washington, DC. National Academy Press.

Neagu, I., Koivisto, P., aand C. Neagu. 1994. Alkylation of purine bases with 3,4-expoxy-1-butene, a reactive metabolite of 1,3-butadiene. *IARC Sci*. Publication No. 125:419-422.

Neagu, I., Koivisto, P., and C. Neagu. 1995. Butadiene monoxide and deoxyguanosine alkylation products at the N7-position. *Carcinogenesis* 16:1809-1813.

Nelson, H., Wiencke, J., and D. Christiani. 1995. Ethnic differences in the prevalence of the homozygous deleted genotype of glutathione S-transferase theta. *Carcinogenesis* 16(5):1243-1245.

NTP. 1993. National Toxicology Program (NTP). U.S. Public Health Service, U.S. Department of Health and Human Services. Toxicology and carcinogenesis studies of 1,3-butadiene (CAS No. 106-99-0) in $B6C3F_1$ mice (inhalation studies). NTP TR 434, ResearchTriangle Park, North Carolina.

Pacchierotti, F. 1998. Genetic effects of 1,3-butadiene and associated risk for heritable damage. *Mutation Research*. - Special Issue 397(1):93-115.

Pacchierotti, F., Tiveron, C., and R. Ranald. 1998. Reproductive toxicity of 1,3-butadiene in the mouse:cytogenetic analysis of chromosome aberrations in first-cleavage embryos and flow cytometric evaluation of spermatogonial cell killing. *Mutation Research* - Special Issue, 397(1):55-66.

Recio, L., Osterman-Golkar, S., and G. Csnady. 1992. Determination of mutagenicity in tissues of transgenic mice following exposure to 1,3-butadiene and N-ethyl-N- nitrosourea. *Toxicology and Applied Pharmacology* 117:58-64.

Recio, L. and T. Goldsworthy. 1995. The use of transgenic mice for studying mutagenicity induced by 1,3-butadiene. *Toxicology Letters* 82:607-12

Reitz, R.H., Gargas, M.L., and S.M. Hays. 1996. Expansion of the CIIT PBPK model for butadiene (Description of diepoxide kinetics). Research Report submitted to EPA May 29, 1996.

Seaton, M.J., Follansbee, M.H., and J.A. Bond. 1995. Oxidation of 1,2-epoxy-3-butene to 1,2:3,4-diepoxybutane by cDNA-expressed human cytochrome P450 2E1 and 3A4 and human, mouse and rate liver microsomes. *Carcinogenesis* 16:2287-2292.

Seilken, R. 1998. Sielken Report-II: Corrected Calculations of the Benchmark Concentrations (BMCs) for Male-Mediated Developmental Toxicity, Appendix E in Comments of the Olefins Panel of the Chemical Manufacturers Association on EPA's Draft Health Risk Assessment for 1,3-Butadiene. Chemical Manufacturers Association, April 17, 1998.

Selzer, R. and A. Elfarra. 1996. Characterization of N super(1)- and N super(6)-adenosine adducts and N super(1)-inosine adducts formed by the reaction of butadiene monoxide with adenosine: Evidence for the N super(1)-adenosine adducts as major initial products. *Toxicology*, 9(5):875-881.

Sernau, J., Cavagnaro, J., and P. Kehn. 1986. 1,3-butadiene as an S9 activation-dependent gaseous positive control substance in L5178Y cell mutation assays. *Environmental Mutagenesis, Suppl*. 8:75-76 (Abstract 203).

Sorsa, M., Osterman-Golkar, S., and K. Peltonen. 1996. Assessment of exposure to butadiene in the process industry. *Toxicology* 113:77-83.

Sorsa, M., Autio, K., and N.A. Demopoulous. 1994. Human cytogenic biomonitoring of occupational exposure to 1,3-butadiene. *Mutat. Res*. 309:321-326.

Sweeney L.M. 1996. Physiologically-based pharmacokinetic modeling of blood: tissue epoxide measurements for butadiene. *Toxicology* 113:318-321.

Sweeney L.M. 1997. Physiologically-based pharmacokinetic modeling of 1,3-butadiene, 1,2-epoxybutene and 1,2,3,4-diepoxybutane toxicokinetics in mice and rats. *Carcinogenesis* 18:611-625.

Swenberg, J.A., Christova-Gueorguieva, N.I., Scheller, N., and A. Ranasinghe. 1998. Quantitation of expoxybutene and trihydroxybutane hemoglobin adducts within exposed and control mice, rats and humans. Presented at the Health Effects Institute Annual Conference, Boston, MA, April 5-7, 1998.

Tates, A.D., van Dam, F.J., and A.T. Natarajan. 1994. Frequencies of hprt mutants and micronuclei in lymphocytes of cancer patients under chemotherapy: A prospective study. Mutation Research, 309(1):299-306.

Tates, A.D., van Dam, F.J., and F.A. de Zwart. 1996. Biological effect monitoring in industrial workers from the Czech Republic exposed to low levels of butadiene. *Toxicology* 113:91-99.

Tates, A.D., and F.J. van Dam. 1998. Comparison of induction of hprt mutations by 1,3-butadiene and/or its metabolites 1,2-epoxybutene and 1,2,3,4- diepoxybutane in lymphocytes from spleen of adult male mice and rats *in vivo*. *Mutation Research - Special Issue* 397(1):21-36.

Tretyakova, N., Lin, Y., and R. Sangaiah. 1997. Identification and quantitation of DNA adducts from calf thymus DNA exposed to 3,4-epoxy-1-butene. *Carcinogenesis* 18(1):137

Tretyakova, N.Y., Ealker, V.E., and J.A. Swenberg. 1998. Formation and persistence of DNA adducts in liver of rats and mice exposed to 1,3-butadiene. *Toxicological Science* 42:180.

Thornton-Manning, J.R., Dahl, A.R., and W.E. Bechtold. 1995a. Disposition of butadiene monoepoxide and butadiene diepoxide in various tissues of rats and mice following a low-level inhalation exposure to 1,3-butadiene. *Carcinogenesis* 16:1723-1731.

Thornton-Manning, J.R., Dahl, A.R., and W.E. Bechtold. 1995b. Gender differences in the metabolism of 1,3-butadiene in Sprague-Dawley rats following low-level inhalation exposure. *Carcinogenesis* 16:2875-2878.

Thornton-Manning, J.R., Bechtold, W.E., and R.F., and R.F. Griffith. 1996. Tissue concentrations of butadiene epoxides in rodents following multiple and single exposures to 1,3-butadiene (BD) by inhalation (Abstract). *Toxicologist* 30:215.

Tommasi, A.M., deConti, S., and M.M. Dobrzynska. 1998. Evaluation and characterization of micronuclei in early spermatids of mice exposed to 1,3-butadiene. *Mutation Research - Special Issue* 387(1):45-53.

USEPA. 1985. Mutagenicity and carcinogenicity assessment of 1,3-butadiene. Office of Health and Environmental Assessment, Office of Research and Development, Washington, DC. EPA/600/8-85/004F.

USEPA. 1996. EPA's proposed guidelines for carcinogen risk assessment. USEPA, Office of Research and Development, Washington, DC. Federal Register 61(79):17960-18011.

USEPA. 1994. Pilot-Scale Evaluation of the Potential for Emissions of Hazardous Air Pollutants from Combustion of Tire-Derived Fuel, U.S. Environmental Protection Agency, Control Technology Center, EPA-600/R-94-070 (NTIS PB-94-169463), April 1994.

USEPA. 1997. 1995 Toxics Release Inventory (TRI) - Public Data Release Book. USEPA, Washington, DC, April 1997. EPA745-R-97-005. [The TRI is available on the TOXNET computer system. Registered National Library of Medicine online services users can access the TOXNET system via the modem using the FTS Toll-free number (1-800-525-0216) or through the Internet (Telnet directly @ toxnet.nlm.nih.gov).

USEPA. 1998a. Health Risk Assessment of 1,3-Butadiene, External Review Draft, USEPA Office of Research and Development, EPA/600/P-98/001A, January 1998.

USEPA. 1998b. 1996 Toxic Release Inventory - Public Data Release - 10 Years of Right to Know. USEPA, Washington, DC. EPA745-R-98-005, May 1998.

Valentine, J.L., Boogaard, P.J., Sweeney, L.M., Turner, M.J,. Bond, J.A., and M.A. Medinsky. 1997. Disposition of butadiene epoxides in Sprague Dawley rats. *Chemico.-Biological Interactions*103-115.

Victorin, K., Busk, L., and H. Cederberg. 1990. Genotoxic activity of 1,3-butadiene and nitrogen dioxide and their photochemical reaction products in Drosophila and in the mouse bone marrow micronucleus assay. *Mutation Research* 228:203-209.

Ward, J.B., Jr., Au, W.W., and M.M. Ammenheuser. 1994. Hprt Mutant lymphocyte frequencies in workers at a 1,3-butadiene production plant. *Environmental Health Perspectives* 102:79-85.

Ward E.M., Fajen J.M., Ruder A.M., Rinsky R., Halperin W., and C. Fessler-Flesch . 1996. Mortality study of workers employed in 1,3-butadiene production units identified from a large chemical workers cohort. *Toxicology* 113:157-168.

Wiencke, J.K., Pemble, S., Ketterer, B., and K.T. Kelsey. 1995. Gene deletion of glutathione S-transferase theta: correlation with induced genetic damage and potential role in endogenous mutagenesis. *Cancer Epidem Biomarkers and Prev* 4:253-259.

Xi, L., Zhang, L., and Y. Wange. 1997. Induction of chromosome-specific aneuploidy and micronuclei in human lymphocytes by metabolites of 1,3-butadiene. *Carcinogenesis* 18(9):1687-1693.

Xiao, Y., and A.D. Tates. 1995. Clastogenic effects of 1,3-butadiene and its metabolites 1,2-epoxybutene and 1,2,3,4-diepoxybutane in splenocytes and germ cells of rats and mice in vivo. *Environmental and Molecular Mutagenesis*, 26(2):97-108.

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