ATSDR Toxicological Profile for 1,3-Butadiene

Review of Minimal Risk Level (Withdrawn)

Reviewer #1

Question 1 Are the discussions of the withdrawal of the acute-duration MRL for 1,3- butadiene logical and clear?

The discussions of the withdrawal of the acute-duration MRL for 1,3-butadiene are logical and clear.

Question 2 Comment on the withdrawal of the acute-duration inhalation MRL and the justification for its withdrawal.

The comments appear to be correct and understandable regarding the decision to withdraw the acute-duration MRL. All the derived effects that have been addressed appear to be based on three developmental effects and one reproductive toxicity study in animals and no information was available that would identify the mechanism for these effects. The only question is whether another outcome may be more appropriate for acute duration effects in humans and whether the data exist to enable assessment of this outcome.

The authors have not defined what constitutes an acute duration inhalation exposure that would be used to consider effects in humans. The document notes that acute central nervous system (CNS) effects, namely narcosis, have been seen in humans and animals with acute high doses of 1,3,-butadiene. These effects were noted in an article by Carpenter 1944 and the doses to the subjects were very high. The document does mention other data indicating probable CNS effects such as vertigo in humans but without further elaboration of these findings or any animal data that might support CNS-mediated outcomes. The circumstances under which these events occur would suggest that the exposure to 1,3-butadiene itself and not its metabolites may be the damaging agent. Both the short duration of exposure to the agent and the rapidity of the onset of narcosis, for example, would suggest the CNS effects may be directly related to the parent chemical. These short term events may occur repeatedly with production of various types of CNS damage. The development of neurological tests has advanced rapidly in the past 65 years since 1944 but there are no comments on any newer literature reporting additional and less severe neurological effects studies that might be associated with 1,3-butadiene exposure in either animals or humans.

The literature on 1,3-butadiene has been focused on the toxicological effects in animals that might support or refute the risks of cancer following exposure. Therefore the reports of studies has emphasized metabolism of the agent and differences in the levels of these metabolites by species. This focus on differences in the risk of cancer based on differences in the levels of metabolites by species has been a major focus of the report. This point is important in cancer outcomes but possibly plays no role in acute duration exposures even if such exposures are repeated. As noted above, If one uses an outcome like narcosis, the timing of the effect is so rapid that the metabolism of the chemical may not play a role in these CNS effects. That should be investigated.

The document does mention episodes of frostbite in workers. That type of injury is usually occurs in maintenance or tank workers who are repairing leaks or opening valves. These activities not only are accompanied by 1,3-butadiene exposures but also inhalation of the vaporized chemical often with narcosis from air exposure and subsequent frostbite from exposure to the liquid which is rapidly volatilizing. As the document indicates, the dermal effects from exposure and the subsequent frostbite are associated with a physical effect from the chemical. The important point is that the circumstances surrounding these events would be accompanied by acute duration inhalation of the chemical and the latter exposures might produce acute and chronic effects on the individuals. Has there been a search of industrial accidents to see if there is information about these events to determine the inhalation level? Has anyone ever followed up these workers to determine whether they experienced any neurological or perhaps reproductive effects from single or repeated acute exposures? Such studies may only exist in the "gray" epidemiologic literature. As noted above, these workers are likely to have repeated exposures to perhaps high doses but the CNS damage is also likely to be cumulative and therefore may be manifest in various neurological or other effects. If data regarding these neurological effects from acute inhalation doses are not available then this is a serious missing piece of literature needed to define acute or chronic CNS or reproductive effects from single or repeated acute inhalation doses of this agent.

Question 3 Are you aware of other studies that would impact the MRL?

I am unaware of any neurological or psychological studies that have been conducted in humans following exposure to 1, 3-butadiene. The authors have reported only one study of neurological effects based on experimental delivery of the chemical to 2 men. (Carpenter 1944). If that truly is all that is available with the expertise that has developed in neurological testing over the past 65 years, neurological outcomes would be an important area for further study in the future.

Question 4 Do you agree with the withdrawal of the acute-duration inhalation MRL as documented?

I concur with the withdrawal of the acute-duration inhalation MRL based on reproductive outcomes.

The authors of the document should discuss what is defined as the timing of "acute duration inhalation", whether it can be single or repeated, and what is accepted as the timing of effects after such exposure. These considerations become important because the speed with which the metabolic products are produced in relation to time of onset of acute effects or the timing of suspected damage will make a major difference in whether mechanisms for metabolite production are relevant or the parent chemical itself may be related to any neurological outcomes of importance. The authors of this document are hampered in their conclusions because apparently very little data exists on the neurological outcomes and mechanisms that might be related to a true acute-duration inhalation effect. The toxicological data as well as the epidemiology data have generally focused on cancer as outcomes. Animal studies have added some information on developmental and reproductive studies as well but little information exists for humans. Therefore the literature does not exist perhaps to fully investigate this problem. Yet the importance of acute and perhaps repeated exposures to possibly high doses of 1,3-butadiene could be important. The chemical is somewhat unusual because of its high volatility and consequent rapid dispersion even after a very high exposure. The document should discuss the lack of information on acute duration inhalation exposures since this is apparently a major and potentially

important gap in our scientific knowledge of the effects of inhalation of 1,3-butadiene in humans as well as animals.

REVIEW REPORT OF ATSDR'S TOXICOLOGICAL PROFILE OF 1,3-BUTADIENE
Ref: Review of Minimal Risk Level section (withdrawn)

Reviewer #2

Introduction:

As per ATSDR definition, a minimal risk level (MRL) is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure (acute:1-14 days; intermediate: 15-364 days and chronic 365 days and longer). In the 2009 public comment draft of the toxicological profile for 1,3-butadiene, ATSDR had derived an acute-duration inhalation MRL of 0.1 ppm based on reduced male fetal body weight gain in offspring of mice exposed to 40 ppm to 1,3-butadiene 6 h/day on gestation days 6-15 (DOE/NTP 1987). The LOAEL of 40 ppm was adjusted for intermittent exposure (6 hours/day) resulting in a duration-adjusted LOAEL of 10 ppm. A $LOAEL_{HEC}$ (human equivalent concentration) of 10 ppm was derived, which was divided by an uncertainty factor of 90 (3 for use of a minimally adverse effect, 3 for extrapolation from animals to humans, and 10 for human variability). It is my understanding that ATSDR has reevaluated the MRL for 1,3- butadiene and determined that the acute MRL be withdrawn based on lack of mechanistic data that could be used to identify relevant internal metrics. The following describes my review on the MRL discussions and includes my comments on the MRLs as requested in the Charge to Peer Reviewers document through the following 4 questions:

1. Are the discussions of the withdrawal of the acute MRL for 1,3-butadiene logical and clear?

The MRL Rationale Statement starts with a presentation of the industrial and environmental sources for 1,3-butadiene exposure. The section leads to the conclusion that the main sources of exposure to this chemical is through inhalation. In fact, because 1,3-butadiene is a gas, the hazard by ingestion is unlikely since 1,3-butadiene is poorly soluble in water and when released in water it would rapidly evaporates. The Inhalation MRLs section provide a succinct account of the studies conducted to evaluate the toxicity of 1,3-butadiene following inhalation exposure, including epidemiological, acute, intermediate and chronic duration animal studies performed in rats and mice. A comprehensive list of observed effects is provided in each subheading discussing acute-duration, intermediate duration and chronic duration inhalation. The section also provides an accurate discussion regarding the well-known interspecies differences observed in sensitivity to 1,3- butadiene, leading to the now well-accepted conclusion that mice is more sensitive than rats or humans to the toxic effects of butadiene. While it is widely accepted that the metabolic pathway is generally the same in the different species, this interspecies difference is attributed to differences in the rates of formation and detoxification of the toxic metabolites between the species. The section also describes the different studies conducted showing differences in metabolism between the species. This leads to the conclusion that the use of rodent data to derive MRLs may not be appropriate as it would require the use of an internal dose metric to account for the interspecies difference in 1,3- butadiene toxicokinetics. Hence, a clear and logic discussion is provided for the justification of withdrawal of the acute MRL for 1,3-butadiene.

2. Please comment on the withdrawal of the acute-duration inhalation MRL and the justification for its withdrawal:

The ATSDR approach for deriving MRLs is analogous to the derivation of reference doses/reference concentrations (RfDs/RfCs) developed by EPA. Traditionally, MRLs have been derived from either epidemiological studies, controlled human studies (when possible), or controlled studies in animals that serve as surrogates for human populations. In the case of 1,3-butadiene, extrapolation from rodent studies, and in particular mice studies, may not be appropriate given the well-documented interspecies differences observed in the toxicokinetics of this chemical, with mice being highly sensitive to the genotoxic effects of 1,3-butadiene than rat or humans. Major species differences are clear for the complex metabolism of BD and these differences do impact risk for the general human population. Furthermore, it is unclear how 1,3-butadiene caused the observed non-carcinogenic effects in exposed animals, as the toxicodynamic mechanisms (e.g. interaction with specific receptors, membrane sites, other proteins, or epigenetic mechansims) has not been investigated, and whether the same mechanisms occur in humans is not known. After initial chemical insult, subsequent biochemical reactions may lead to toxicologically significant changes that could be different across species. As correctly mentioned in the review, although PBPK models for 1,3-butadiene have been developed in rodents and humans, the models are limited in their ability to predict internal doses for key metabolites, especially those responsible for non-carcinogenic effects. While an alternative would be the use of biomarkers of exposure to probe the levels of these metabolites, the major focus of 1,3-butadiene biomarkers of exposure research has been so far directed toward identifying biomarkers reflecting the genotoxic metabolites of butadiene, specifically, EB, EBD and DEB. Therefore, even with the use of hemoglobin adducts, which have been shown to be good surrogate biomarkers for the reactive butadiene metabolites (EB, EBD and DEB), there is still limited mechanistic data that would allow the identification of the metabolite(s) that would be responsible for the non-neoplastic effects of 1,3-butadiene following acute exposure, which is necessary for determining the acute-duration MRL. The conclusion that there is a lack of

mechanistic data that can be used to identify relevant dose metrics precluding the derivation of acute-inhalation MRLs at this time is therefore appropriate.

3. Are you aware of other studies that would impact the MRL?

There is a recent study by Boysen et al (Toxicological Sciences, 125(1), 30-40, 2012) that reports the determination of the DEB-specific *pyr*-Val adduct in butadiene –exposed workers. For a long time, there were no biomarkers available to measure the production of the highly mutagenic butadiene metabolite DEB in humans. The *pyr*-Val hemoglobin adduct was developed and used successfully before, however, for the determination of DEB production in mice and rats exposed to various concentrations of 1,3-butadiene. This study extends this work to humans since measurement of protein adducts provides useful information on exposure-specific internal formation of individual epoxide metabolites. In contrast to DNA adducts, protein adducts are not repaired. It should be pointed out, however, that this study targeted the determination of a mutagenic butadiene metabolite (DEB). The conclusion from the study is consistent with the literature indicating that humans are much less efficient in forming DEB than mice or rats at similar exposure levels. In fact, the study reported that the formation of *pyr*-Val was more than 50-fold lower that has been associated with increased mutagenesis in rodents. This study adds to the already existing weight of evidence of interspecies differences in butadiene toxicokinetics, particularly with respect to the generation of its reactive intermediates.

4. Do you concur with the withdrawal of the acute duration inhalation MRL as documented?

As discussed by Kirman et al (Critical Rev. Toxicol., 40(S1):1-11, 2010), in human risk assessment, it is generally assumed that humans are as sensitive as, if not more sensitive than, the most sensitive test species. This assumption may not hold true for 1,3-butadiene given the large interspecies variability in its toxicokinetics, making it difficult to extrapolate data from

animal models to humans. Therefore, in light of the data available, I concur with the withdrawal of the acute duration inhalation MRL as documented.

1,3 Butadiene

Toxicological Profile

Reviewer #3

An extensive body of literature has documented that mice are much more sensitive than rats with regard to butadiene toxicity. Although the metabolic scheme looks qualitatively similar, closer examination has revealed significant quantitative differences (by an order of magnitude) in the extent to which the more genotoxic metabolites of butadiene (diepoxide and diolepoxides) are produced by mice compared to rats and humans. Studies have also suggested that the carcinogenesis of butadiene may be mediated through these epoxy metabolites which may explain the observed differences in sensitivity between mice and rats. If one follows the conventional approach and assumes that "humans are at least as sensitive as the most sensitive test species", it may lead to significant overestimation of risk associated with butadiene exposure.

In the revised Toxicological Profile for 1,3-Butadiene, ATSDR has elected to withdraw the inhalation MRL citing the lack of mechanistic data linking the "relevant internal dose metric" (i.e. specific metabolites) with the endpoint of interest. The document is well written and provides an excellent overview of the literature documenting the species differences in butadiene toxicity.

However, the withdrawal of the inhalation MRL based on the aforementioned rational poses additional dilemma because the majority of MRLs (and RfDs/RfCs for that matter) are derived based on the relationship between an external or administered dose and resulting adverse outcomes, NOT by relating specific intermediate metabolites with the adverse health outcomes. It is also important to acknowledge that the epoxides, which are the focus of discussion, are known to play a role in the carcinogenesis, but their role in the non-cancer outcomes – which is the focus of MRL – are less clear.

Further, we may not even know the specific individual or combination of intermediate metabolites responsible for the observed adverse outcomes in the vast majority of the cases for

which MRLs are derived, as there is always a possibility that an additional “unmeasured” metabolite may be at play. Similarly, there is a lack of data that unequivocally links the observed ovarian atrophy in mice is in fact associated with diepoxide, and not the other metabolites including the monoepoxides. Therefore, rather than completely withdrawing the MRL, considerations should be given to revising the MRL including:

- Additional approach would be to use the mice data applying a value of 1 for the uncertainty factor for animal to human extrapolation. Since an extensive body of literature on butadiene has documented that mice are much more sensitive than humans, an argument could be made that there is no need for an additional interspecies safety factor.

- Using a different principal study focused in rats/monkeys to calculate MRL, since mice may not be the most appropriate species in this particular case, and the use of mice data may overestimate the risk in humans. The EPA default assumption about treating humans as the most sensitive test species should be invoked only when there is lack of data, which is not the case here.