UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

June 10, 1985

OFFICE OF
THE ADMINISTRATOR

Honorable Lee M. Thomas
Administrator
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Dear Mr. Thomas:

The Environmental Health Committee of EPA's Science Advisory Board has completed its review of the Agency's draft Mutagenicity and Carcinogenicity Assessment of 1,3-Butadiene. The stated purpose of the document is primarily to support decision making by the Office of Air Quality Planning and Standards regarding possible regulation of butadiene as a hazardous air pollutant.

The draft document is an improvement over some recent drafts for other substances and is a well written review of the current literature for butadiene. The final version should explain, however, the reasons for restricting this document to mutagenicity and carcinogenicity and why little or no exposure information is presented. The final document also should contain additional discussion to put the issue of reproductive effects into perspective. The Committee recommends that a separate chapter on pharmacokinetics be written from information already in the draft document and that recent information on the pharmacokinetics of butadiene be incorporated into the revised draft.

The Committee concurs with the general conclusion that butadiene is mutagenic for microbes and lower animals. However, the evidence for submammalian mutagenicity is not compelling, given the lack of data from whole animal studies. The Committee agrees that the animal evidence of carcinogenicity is "sufficient" and that the epidemiological evidence for carcinogenicity is "inadequate," according to the criteria of the International Agency for Research on Cancer (IARC). This information places butadiene into IARC category 2B. It is thought prudent for a regulatory Agency to presume a category 2B substance to be a probable human

carcinogen. The Committee recommends that the quantitative estimate of carcinogenicity be revised, as detailed in the attached technical report. We commend the comparison of epidemiological data with the quantitative estimates derived from animal data for its creative approach.

We appreciate the opportunity to comment on this public health issue and stand ready to provide any further scientific advice. We request a written response to our advice.

Sincerely,

Richard A. Griesemer

Richard A. Griesemer, D.V.M., Ph.D.
Chair, Environmental Health Committee

Norton Nelson

Norton Nelson, Ph.D.
Chair, Executive Committee

Enclosure

cc: A. James Barnes
Assistant Administrators

TECHNICAL REPORT OF THE ENVIRONMENTAL HEALTH COMMITTEE OF EPA'S SCIENCE ADVISORY BOARD REGARDING A DRAFT MUTAGENICITY AND CARCINOGENICITY ASSESSMENT OF 1,3 BUTADIENE

INTRODUCTION

On April 10-11, 1985, the Environmental Health Committee of the Science Advisory Board reviewed a draft Mutagenicity and Carcinogenicity Assessment of 1,3-Butadiene [EPA-600/8-85-004A; February, 1985; Review Draft]. The document was prepared by the Office of Health and Environmental Assessment (OHEA) in the Office of Research and Development. The Committee's major conclusions and technical comments are presented below.

FORMAT AND CONTENT

The draft document, like the recent Health Assessment Document for Chloroform, is an improvement over some previous documents for other substances, in that (1) attention is given to the limitations of positive data; (2) the quantitative assessment makes an attempt to compare the predictions of animal studies with the available human data, while pointing out the inherent uncertainties and limitations; (3) the quantitative assessment is described and interpreted more completely; and (4) in general, the document is well written.

Although the draft document adequately reviews the current information on the carcinogenicity and mutagenicity of butadiene, given the few, scattered data on which it had to rely, it is not surprising that the authors had a difficult time integrating these data into a coherent conclusion. The reasons for restricting this document to mutagenicity and carcinogenicity should be stated in the preface. The preface does not make clear why the objective of the document limits the review. The preface also should explain why little or no exposure information is presented and, in general terms, what exposure information was reviewed by the staff.

Acknowledgment that butadiene produces non-neoplastic organ systems' damage (for example, alveolar metaplasia, nephropathy, and testicular atrophy) belongs either in the preface or in a separate chapter. The Committee disagrees with the statement (in Section 4.2.3) which attempts to equate cardiac tumors, cardiac disease and cardiac malformations, as these are unrelated pathological processes.

The information on reproductive effects raises concerns, but the major study on this subject is not published and will not be easily available to the general public. Therefore, the document should contain additional discussion to put reproductive concerns into perspective as much as possible. The information on teratogenicity should either be placed in the preface or enlarged into a separate chapter. In particular, the brief description of teratogenicity data was ambiguous, difficult to interpret and is inappropriately located in the carcinogenicity chapter. A consultant to the Committee, Dr. Ronald Hood, undertook a fuller review [1] of reproductive effects issues. His comments have been communicated separately to OHEA.

The Agency should place the information on metabolism (found in the mutagenicity chapter) and the information on elimination rates (found in the carcinogenicity chapter) into a separate pharmacokinetics chapter. In the current draft the discussion of pharmacokinetics is fragmented and partially duplicated.

EXPOSURE

In the recent past the Committee has relied on separate memoranda from the Office of Air Quality Planning and Standards (OAQPS) for summary information on exposure. However, OAQPS did not supply exposure information on butadiene. The exposure information in the draft document is inadequate to form an impression of either risk or critical hazards.

The draft should review the dimerization of butadiene to 4-vinylcyclohexene, because the variable presence of dimer in the material administered to laboratory animals may affect the results of toxicology studies. The National Toxicology Program (NTP) Technical Reports Review Subcommittee found that a recent NTP study of the carcinogenesis of 4-vinylcyclohexene (Report No. 303) was inadequate to draw conclusions regarding effects on rats or male mice because extensive mortality occurred in the treated groups.[2] A majority of the Subcommittee concluded that 4-vinylcyclohexene caused cancer in female mice. The health assessment document also should provide a reference to support the statement that butadiene has been detected in cigarette smoke, fossil fuels, and the incineration products of fossil fuels. The statement that "concentrations ranging from 1 to 5 ppb have been detected in urban air" is in error. The concentration range is actually 1 to 9 ppb.[3]

PHARMACOKINETICS

It is essential that recent information on the pharmacokinetics of butadiene be incorporated into the revised draft since they indicate that rats can metabolize butadiene to butadiene monoxide and that this route of metabolism is saturable. Studies by Bolt and co-workers[4] and by Filser and Bolt[5] are particularly important in this regard.

The section on metabolism is incomplete. The document adequately discusses the in vitro aspects of butadiene metabolism in mammalian tissues, but a report by Malvoisin and co-workers was not included in this discussion.[6] The paper discusses the enzymatic hydration of butadiene monoxide and its importance in the overall metabolism of butadiene. This report should be cited and the results incorporated into the discussion of the metabolic activation and detoxication of butadiene metabolites. The draft document states that there is no information available on the mutagenicity of 3-butene-1,2-diol and 3,4-epoxy-1,2-butanediol. However, Malvosin and Roberfroid indicate that their unpublished results show that these two metabolites are not mutagenic.[7]

The potential role of glutathione in the metabolic inactivation of butadiene monoxide should be mentioned. While there are no data at present to support the hypothesis that glutathione is involved in the metabolic detoxication of butadiene monoxide, Malvoisin and co-workers indicate that a glutathione conjugate of butadiene monoxide is formed both chemically and enzymatically.[6] (This was mentioned in the discussion of the paper as an unpublished result).

A recent abstract presented at the Society of Toxicology annual meeting by Bolt and coworkers showed that B6C3F1 mice are capable of metabolizing butadiene at rates approximately twice that observed in Sprague Dawley rats, and that this metabolism was saturated at high exposure concentrations.[8] Furthermore, this study provided data on binding of radiolabel to DNA and total protein following inhalation exposure. These observations suggest a possible explanation for some of the species difference in the incidence of cancer following inhalation of butadiene.

Recent studies sponsored by the National Toxicology Program (NTP) should confirm and extend the quantitative observations of Bolt and his collegues. In both rats and mice the absorption of butadiene decreases significantly as the exposure concentration increases. These studies also show that metabolism of butadiene by the rat is saturated at concentrations that were used in the chronic toxicity study (8000 ppm). The specific details of this study should be available soon in the NTP quarterly report.

ABSORPTION FRACTION

The statement that the absorption fraction is "assumed the same for all species" for butadiene is not warranted, and the attempt to support the assumption of equivalent absorption fraction by a citation to the textbook by Dripps and coworkers is not justified. The fact that the minimum alveolar concentration necessary to produce a given stage of anesthesia is similar in man and animals does not mean that all chemicals will have similar absorption characteristics across species.

MUTAGENICITY AND CLASTOGENICITY

The Committee agrees with the general conclusion of this chapter that butadiene is mutagenic for microbes and animals. However, the evidence for sub-mammalian mutagenicity is not compelling, given the lack of data from whole animal studies. The emphasis of the chapter has been placed on studies with presumed animal metabolites because of the lack of data on the parent compound, and this supporting information from mutagenesis studies on the major metabolites has been well-developed.

The draft document suggests that butadiene is a mutagen by virtue of its metabolism to diepoxybutane. One study develops the hypothesis that butadiene is metabolized to diepoxybutane, but this pathway is not proven.[7] The data do not show that this metabolite is responsible for observed mutagenicity with butadiene. The statements in the text therefore require some qualification. Also diepoxybutane also is unlikely to reach the gonads of mammals as such following inhalation. While it increased the rate of sex-linked recessive lethal mutations in Drosophila, in the experiment the diepoxide was administered "by feeding." Following absorption from the gut, diepoxybutane probably was biotransformed to other metabolites.

The conclusion that diepoxybutane is a powerful clastogenic agent is questionable. Fewer cells were examined at the mid-dose and high-dose levels, suggesting that correction for cytotoxicity and non-specific toxic damage might be in order. At the dose of one microgram per milliliter of diepoxybutane, chromosomes were not affected. Diepoxybutane is clastogenic, but if comparative adjectives such as "powerful" are applied, the basis for the comparison must be given. The draft document also concludes that diepoxybutane is responsible for producing a dose-dependent, sister chromatid exchange response. Again, in mammals, the diepoxide is reactive and unlikely to be transported to bone marrow following intraperitoneal injections without undergoing further modification.

Several additional reports should be discussed in the document. A study by de Meester and coworkers indicates that butadiene is a direct mutagen in several strains of S. typhimurium.[9] However, a study published by the same group in 1980 contradicts this observation and suggests that the observations reported earlier regarding the direct mutagenic aspects may have been due to an "artifact."[10] Analysis of the data from both studies suggest that the results from the 1978 study may have been due to a volatile metabolite of butadiene. A paper by Citti and coworkers has not been incorporated into the discussion of DNA alkylation.[11] In that paper, the authors demonstrate that butadiene monoxide can react with DNA in vitro. This paper also characterizes the DNA adducts formed with butadiene monoxide. The draft document states that "alkylation activity correlated with mutagenicity in E. Coli WP2 uvrA," but the discussion is not clear, since no data are presented on which to base this statement. A paper by De Meester and co-workers describes the mutagenicity of butadiene monoxide in bacteria.[9]

ANIMAL STUDIES OF CARCINOGENICITY

The Committee agrees with the apparent conclusion of the document, which is not clearly stated, that the animal evidence of carcinogenicity is "sufficient" according to the criteria of the International Agency for Research on Cancer (IARC). An increased incidence of malignant tumors of several histological types and one rare type occurred in more than one species. The incidence of malignant tumors was especially high for mice. Genotoxicity information (see above) is consistent with this categorization.

The unusual multiplicity of tumor types and the production of extremely rare heart tumors deserve repeated emphasis here and in the Summary and Conclusions Section. The draft document states that the heart was the only organ in which hemangiosarcomas occurred. A report prepared for EPA could be cited appropriately at this point.[12] This report summarizes a study by Ripp of several tissues, including the heart, following subchronic exposure of rats to inhaled butadiene. Some of the early changes described in that report may relate to the hemangiosarcomas observed in mice following long-term exposure.

Two lifetime inhalational bioassays establish the carcinogenicity of butadiene for rodents. The two studies also show that rats and mice respond differently. Although this difference is indicated in the document, a fuller review, particularly of quality assurance aspects of the two studies, seems justified. The absence of evidence of immunosuppression based on histologic examination of lymphoid and hematopoietic tissues also could be mentioned. For each bioassay, additional paragraphs should note the limitations or special considerations in the tests, as recognized by NTP reviewers (such as early deaths, and individual housing of mice, and for rats the eight-fold interval in gas concentrations, the large proportion of dimer, the problem of sampling mammary and zymbal glands, the lack of peer review, the high incidence of rhinitis that occurred in male mice at high doses and so forth).

The discussion of diepoxybutane carcinogenicity could be expanded to give enough details of the Van Durren and Shimkin experiments so that the reader has a sense of the extent of the experimentation and the weight of the evidence.[13] As expected for epoxides, dermal or subcutaneous application of diepoxybutane produced local tumors at the sites of application. Intraperitoneal injection produced lung tumors in the typical lung tumor assay (but not leukemias or angiosarcomas in the short experiment).

EPIDEMIOLOGICAL STUDIES OF CANCER

The Committee agrees with the conclusion in the draft document that the epidemiological evidence for carcinogenicity is "inadequate," according to the IARC criteria.

The review of the epidemiology of butadiene is thoughtful, systematic, analytical and thorough. The draft document describes study design, the composition of the study groups, the methods of ascertainment and analysis, the role of bias and confounding factors, and most importantly, the nature of knowledge about the exposure. Exposure is "most important," because the exposure usually occurs at a job site or within an occupational category.

Multiple study designs were used, and several occupational environments were chosen. No strong, consistent or specific carcinogenesis results could be documented. The review objectively analyzes the strengths and limitations of each study, and concludes "Given the inconsistency of results from different studies, the possible confounding due to exposure to sol-

vents, styrene, and possibly other chemicals, and the potential biases in some of the studies, the epidemiological data would have to be considered inadequate for evaluating whether a causal association exists between butadiene exposure and cancer in humans." The analysis provides a valid basis for this conclusion.

Since the data available for humans all deal with workplace exposures to the multiple substances found there, and since no data exist for human exposure to butadiene alone, this chapter might well be based on the IARC review of the rubber industry in which 12 types of cancer are evaluated and tied to the presumed agent or job category.[14] If one wished to go further, one could review separately the experimental and epidemiological evidence of styrene, benzene, etc. and estimate their contributions, if any, to the workplace risks. The conclusion will still be reached, however, that the epidemiologic data do not permit an evaluation of the carcinogenicity of butadiene.

QUALITATIVE CARCINOGENICITY CONCLUSIONS

The Committee agrees with the conclusion in the draft document that butadiene can be classified in IARC category 2B. The document also describes a ranking of B2 under the proposed EPA Guidelines for Carcinogen Risk Assessment.[15]

The terminology of "inadequate for determining a causal association" derives from the IARC definitions of the "degrees of evidence for carcinogenicity from studies in humans." They are categorized as:

i. Sufficient evidence.....

ii. Limited evidence.....

iii. Inadequate evidence, which indicates that one of three conditions prevailed: (a) there were few pertinent data; (b) the available studies, while showing evidence of association, did not exclude chance, bias or confounding; (c) studies were available which do not show evidence of carcinogencity.

The evidence under consideration does not address the issue of inferring the lack of carcinogenicity. Further, it may be noted that the IARC Working Group was unable to define criteria for "negative" evidence. Thus, the IARC carcinogen assessment system does not contain a Group iv, which might have been defined as "noncarcinogenic in humans." Thus, the term "inadequate" does not have the common meaning of unsuitable. "Inadequate" can mean iii (c) above, merely that the available studies do not show evidence of carcinogenicity in humans. The judgment that it is prudent for regulatory agencies to regard butadiene as a "probable" human carcinogen rests on the evidence from studies in two species of rodents.

QUANTITATIVE ESTIMATION OF CANCER INCIDENCE

The Committee recommends that the quantitative estimate of carcinogenic potency for humans be revised beyond the changes recommended by OHEA staff at the meeting.

In brief, the situation is unusual in several respects. These include (1) markedly different quantitative results for incidence and tumor site were obtained in rats than in mice; (2) the epidemiological studies show very little, if any, evidence of a carcinogenic effect; (3) the mortality rates for mice were greater than expected from the subchronic study used to estimate the maximally tolerated dose; and (4) the incidence data for carcinogenicity in mice suggests a "plateau effect" for the two exposure concentrations of butadiene.

The latter observation and the results outlined above on pharmacokinetics suggest that nonlinear kinetics may play a role in the tumor incidence in mice. The information about kinetics was not utilized in the potency estimate in extrapolating between animals and man. As data are available which relate absorbed dose to administered dose for the mouse, this information should be factored into the potency assessment.

As a first step, the dose levels in the mouse experiments should be adjusted to reflect "effective dose" before being used in the statistical models. Since the Agency's objective is to use animal data at higher butadiene exposures to infer possible human effects at low exposures, it is not necessary to have data on the pharmacokinetics of humans at high butadiene exposures for this exercise. The available mouse data, for example, are adequate to correct for internal dose at high exposures. The internal doses can be extrapolated to low doses, and then the same standard factors as those used in the draft document can be applied to achieve an estimate for humans.

Some comment could be made on the lower frequency of mice with tumors at the higher dose. This may have certain implications regarding metabolism (i.e. metabolic saturation) of butadiene at these high concentrations. Similar observations are found in the data presented in Tables 8 and 9 of the draft document. The data from Bolt and his collegues also suggest that dose-dependent kinetics comes into play at these higher concentrations.

As a second step, a time to tumor approach should be employed. A multistage model that includes time of death is available. The adjustment factor used in the draft document is based on the assumption that time-of-tumor increases as the third power of time. This adjustment is in the range observed for many cancers in humans, but in those cases the exposure levels are not so high as to produce a carcinogenic response in about half of the expected lifespan. The third power of time is observed because incidence increases gradually over time and then much more markedly in the latter part of life. The situation with butadiene is reminiscent of a study of mammary carcinomas induced in female mice by vinylidene chloride.

In this study more susceptible mice die sooner. Apparently, the mice in the butadiene study were not authentic hybrids of inbred strains, so that a heterogeneous population response is a distinct possibility.

As a third step, the Committee recommends that an upper bound calculation be made using the rat data and that this estimate be presented as a sensitivity analysis case for comparison with the estimate based on mouse data. The conceptual approach suggested here differs only slightly from that used to compare the estimate based on mouse data to the human epidemiological data. In addition, absorption and elimination studies suggest marked species differences in pharmacokinetics, as do the metabolism studies by Bolt and his colleagues. It would appear that metabolism of butadiene may be the key to interpreting the tumor response. This is particularly noteworthy since the non-linear kinetics noted above for butadiene may have a profound impact on the relative carcinogenic response in rats and mice.

Since carcinogenicity results are available for rats and mice with varying results, it would be desirable to have comparative pharmacokinetic data to know which species are more relevant to man. In the absence of human data, the Agency probably will have to use the higher incidence mouse data for an upper bound estimate of potency because of policy and quality assurance considerations. However, EPA should not ignore the rat to mouse difference as it reflects on the uncertainty inherent in the extrapolation from the mouse to humans.

Data regarding human metabolism would enhance our ability to extrapolate from the animal data, and it is unfortunate that none are available to EPA. Although such human data may be difficult or impossible to obtain in the U.S., due to low domestic exposures, this may not be true of all areas of the world. It is likely that there are countries where human exposure to butadiene is such that adequate samples could be obtained and analyzed for metabolites. The draft document should cite this and other research needs.

The possible quantitative effects from the presence of the dimers in the bioassay material on the experimental outcomes should be discussed because complete conversion to dimers would halve the effective concentration of active material, and the potency of 4-vinylcyclohexene also may differ from the potency of monomer.

Quantitative estimates of risk for non-carcinogenic effects should be displayed in terms of an "acceptable daily intake" or "margin of safety." Quantitative results are displayed in the draft document for carcinogenic incidence at exposures from 10 ppb to 10 ppm in comparing female mouse data with three different models. When these documents are used by different regulatory agencies, "safe" levels are derived from q_1^* or q_1 by the use of a preselected risk level. Important information is provided by knowing both the cancer risk and the margin of safety for noncarcinogenic effects at different exposure levels. However, butadiene may be difficult

to address in this manner because of the apparent limitations of the toxicological data. The document also will be more useful if the models used to derive q_1 for butadiene are given, as well as the results obtained from the models at different exposures. Then the user will not have to interpolate between exposure levels at higher exposures.

Relative potency comparisons should either be avoided or their uncertainties explained. The relative potency table in the draft document is calculated from data sets of various quality through the use of different methods, and it reflects different degrees of uncertainty. However, the table does not reflect important qualifying biological information, such as time to tumor, degree of malignancy, and so forth. Such a summary statistic can be greatly misinterpreted. The upper bound nature of the potency estimates should be clearly stated. One improvement might be the presentation of a range of estimates of potency for each chemical. Another might be the addition of information on the weight of evidence, and so forth. The exposure cited in the text for a unit risk of one gram per cubic meter is in error. The appropriate value is one microgram per cubic meter.

COMPARISON OF ANIMAL ESTIMATES AND EPIDEMIOLOGY

The Committee commends the Agency staff for comparing the epidemiological data with the quantitative estimates derived from animal data. It is an imaginative endeavor to integrate all available data, and it tends to validate the animal-to-man extrapolation of potency for doses in the range of human exposures. Further, the calculation of "power to detect predicted deaths" makes the argument more cogent. This effort is the best seen by the Committee so far to check the consistency of human and animal data.

The method used in the draft document has the potential to overcome the problem of relying exclusively on animal data for potency estimates. For butadiene, the poor exposure data and confounding factors severely limit the extent to which the upper bound potency estimate from the animal data can be compared to an upper bound estimate from the epidemiological data. Yet, the comparison of animal and epidemiological data does provide feedback on the reasonableness of the animal models and the uncertainty in the estimate.

Some problems with the application of the comparison should be noted, however. These include: (1) the calculations need to be explained more clearly in the document; (2) as much as possible, the comparison should be extended to the rat data; (3) the comparison should attempt to further explain inconsistent results by reviewing individual epidemiology studies for their alignment with the animal estimate. Confounding exposures, latency, mistaken estimates of exposure, and so forth, may provide a basis to understand discrepancies. (4) The epidemiological investigations are, as the document notes, "inadequate for determining a causal association between butadiene exposure and cancer in humans." This statement is super-

ficially inconsistent with the comparison of epidemiologic data with the potency estimate from the mouse experiments. The text should anticipate and respond to this issue. (5) It diminishes the scientific cogency of the document to assert that, "based on the comparisons between the predicted and observed human response, the extrapolated value from the animal data appeared high by a factor of about 3..." The document would carry greater impact if it pointed out the implications of the comparison in general terms, since emphasis on a point estimate is unwarrented, in view of the poor quality of the exposure data. Rather, a wide range of uncertainty exists.

Industry representatives have stated that the actual human exposures encountered in the epidemiological studies were lower than those estimated in the document because of both the olfactory threshold and the possibility of an explosion. Thus, the human potency estimate derived with more realistic exposure values will be closer to the potency estimate for humans derived from the mouse data. A question remains as to whether many of the workers included in the epidemiological studies received enough exposure to warrant their study.

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