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Mutagenicity and Carcinogenicity Assessment of 1,3-Butadiene

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MUTAGENICITY AND CARCINOGENICITY ASSESSMENT

OF

1,3-BUTADIENE

Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, D.C.

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PREFACE

The Mutagenicity and Carcinogenicity Assessment of 1,3-Butadiene was prepared to serve as a source document for Agency-wide use. This document was developed primarily for use by the U.S. Environmental Protection Agency's (EPA) Office of Air Quality Planning and Standards (OAQPS) to support decision-making regarding possible designation of 1,3-butadiene as a hazardous air pollutant. Because OAQPS requested that this assessment focus only on the mutagenicity and carcinogenicity of 1,3-butadiene, an evaluation of other health hazards has not been included herein. This document, therefore, is not a comprehensive health assessment document. The exposure information herein has not been rigorously reviewed, and is used for illustrative purposes only. An analysis of the ambient exposure and exposure to populations adjacent to emission sources will be carried out separately by OAQPS.

In the development of this assessment document, the relevant scientific literature through July 1, 1985, has been incorporated. Key studies have been evaluated and the summary and conclusions have been prepared so that the mutagenicity, carcinogenicity, and related characteristics of 1,3-butadiene are qualitatively identified. Measures of dose-risk relationships relevant to ambient exposures are also discussed so that the adverse health responses can be placed in perspective with possible exposure levels.

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1. SUMMARY AND CONCLUSIONS

1.1. SUMMARY

1,3-butadiene is a colorless gas with a slight aromatic odor at room temperature and pressure. It is used mainly by the styrene-butadiene rubber and polybutadiene rubber industries. No deaths and very few toxic effects have been reported from acute exposure to the vapor. The symptoms resulting from acute exposures are lethargy, drowsiness, and irritation to the mucous membranes of the eyes and the mouth.

The available information on the mutagenicity of 1,3-butadiene is quite limited in that only three studies have been reported. All three studies, however, indicate that 1,3-butadiene is a mutagen in *Salmonella typhimurium*. The mutagenicity is observed only in the presence of a liver S9 metabolic activation system. No whole animal studies have been reported. These results suggest that 1,3-butadiene is a promutagen in bacteria (i.e., its mutagenicity depends on metabolic activation).

There is no information on the metabolism of 1,3-butadiene in humans. *In vitro* data suggest that 1,3-butadiene is metabolized to 3,4-epoxybutene (epoxybutene) and then to 1,2:3,4-diepoxybutane (diepoxybutane). Evidence in rats and mice suggests that 1,3-butadiene is metabolized to 3,4-epoxybutene *in vivo*, indicating that the metabolic pathway outlined on the basis of *in vitro* data may occur *in vivo*.

3,4-Epoxybutene is a monofunctional alkylating agent, is a direct-acting mutagen in bacteria (*S. typhimurium*, *Klebsiella pneumoniae*, and *Escherichia coli*), and induces sister chromatid exchange and chromosomal aberrations in mice. Diepoxybutane is a bifunctional alkylating agent, and as such it can form cross-links between two strands of DNA. It is mutagenic in bacteria (*K.*

pneumoniae and S. typhimurium), fungi (yeast and Neurospora crassa), and the germ cells of Drosophila melanogaster. It also induces DNA damage in cultured hamster cells and in mice, is clastogenic in fungi and cultured rat cells, and produces chromosome damage/breakage in D. melanogaster germ cells. Therefore, the evidence indicates that 3,4-epoxybutene and diepoxybutane are mutagens/clastogens in microbes and animals.

Two lifetime inhalation carcinogenicity studies have been carried out in mice and rats. There was a marked increase in incidences of primary tumors among the exposed groups of mice in both sexes. These tumors included lymphomas, hemangiosarcomas, alveolar/bronchiolar adenomas (and carcinomas), acinar cell carcinomas, granulosa cell tumors or carcinomas, forestomach papillomas and carcinomas, and hepatocellular adenomas and carcinomas. The study had to be terminated at 60 to 61 weeks instead of the planned 104 weeks because of excessive deaths from the neoplasia among the exposed mice.

In female rats (Sprague-Dawley) exposed to 1,3-butadiene, increased incidences of mammary tumors, thyroid follicular cell adenomas, and uterine stromal sarcomas were observed. In the male rats, increases in tumor incidences were found in the exposed animals in the form of Leydig cell tumors and exocrine pancreatic adenomas. Zymbal gland tumors were increased in both sexes of exposed rats. The tumor sites involved were different in the mice and rats among the exposed groups. The severity of the cancers was also widely different; in the rats, no increase in mortality secondary to neoplasia was observed, and there was no early termination of the experiments. In addition to the differences found in the two sexes, rats were affected less than mice.

The epidemiologic studies evaluated in this review were of workers engaged in the production of synthetic rubber since synthetic rubber is produced from styrene and 1,3-butadiene. Three of the studies specifically identified their

study populations as being exposed to butadiene, as well as styrene. The concurrent exposure of the workers in these studies to styrene presents a potential problem since there is some limited evidence that styrene may be a carcinogen, and in particular, a leukemogen.

Of the three studies on workers specifically identified as being exposed to 1,3-butadiene, two were cohort studies, and one was a cross-sectional study designed to look at certain hematologic parameters. One of the cohort studies, a large study of almost 14,000 styrene-1,3-butadiene rubber (SBR) workers at eight plants, found none of the standardized mortality ratios (SMRs) for cancer to be significantly elevated. Some bias may have occurred, however, due to a possible underascertainment of total deaths and a possible overestimation of deaths among blacks. A second cohort study of workers, specifically identified as being exposed to SBR, found that the SMR for lymphatic and hematopoietic cancer was of a borderline significance for a subcohort of workers employed at a plant during the time when a batch production process was in operation. Solvent exposure may have been a confounding factor, however. In this study, exposure to 1,3-butadiene as well as styrene was actually measured, but the measurements were not historic; they were taken at the time of the study. The third study, in which workers were specifically identified by the authors as being exposed to 1,3-butadiene, was a cross-sectional investigation designed to look at certain hematologic parameters. It found no evidence of any hematologic abnormality in the study population. Exposure to 1,3-butadiene, as well as to styrene, toluene, and benzene was measured in this study.

Two studies found an association between employment in the synthetic rubber industry and an elevated risk of cancer. One of these studies, a case-control study of deaths among rubber plant workers from cancer of certain sites, diabetes mellitus, and ischemic heart disease, found workers in the synthetic rubber

area of the plant to have the highest risk ratio for deaths from lymphatic and hematopoietic cancer (ICD 200-209). There may have been some confounding due to organic solvent exposures however. The second study, a cohort mortality study of rubber plant workers, found that an excess of lung cancer deaths occurred among workers in the synthetic rubber area of the plant. This finding was based on only three deaths, however, and there was no control for smoking.

Given the inconsistency of results from different studies, the possible confounding due to exposure to solvents, styrene, and possibly other chemicals, and the potential biases in some of the studies, the epidemiologic data would have to be considered inadequate for evaluating whether a causal association exists between 1,3-butadiene exposure and cancer in humans.

Based on the linearized multistage model, and estimates of internal dose from external concentrations, a maximum likelihood estimate of incremental unit risk was calculated for 1,3-butadiene, using the geometric mean from the pooled male and pooled female significant tumor responses of the NTP mouse study. The mean value of $q_1 = 2.5 \times 10^{-2}$ $(ppm)^{-1}$ was then used to predict human cancer responses in several epidemiologic studies, and the predicted and actual responses were compared. The comparisons were hampered by a scarcity of information concerning actual exposures, age distributions, and work histories. In addition, because there was no consistent cancer response across all of the epidemiologic studies, the most predominant response, cancer of the lymphatic and hematopoietic tissues, was chosen as being the target for 1,3-butadiene. While the predicted and observed responses are consistent, in view of the uncertainties in the epidemiologic data, a fairly wide range of unit risk values and exposure estimates also predicts human response satisfactorily. Given the uncertainties, no better estimate of unit risk can be made.

1.2. CONCLUSIONS

1,3-Butadiene has been shown to be an indirect mutagen in bacteria. Two of its potential metabolites, 3,4-epoxybutene and diepoxybutane, are genotoxic in prokaryote as well as eukaryote test systems. Exposure of rodents to 1,3-butadiene results in ovarian tumors in mice and testicular tumors in rats, which offers suggestive evidence that 1,3-butadiene (or, more likely, a metabolite of 1,3-butadiene) may reach the germ cells. There is also evidence that the dimer of 1,3-butadiene, 4-vinyl-1-cyclohexene, causes ovarian tumors in mice.

The total body of evidence, including metabolism, mutagenicity, and carcinogenicity data, suggests that 1,3-butadiene may present a genetic risk to humans. However, mutagenicity studies in mammalian test systems, as outlined in the EPA's Proposed Guidelines for Mutagenicity Risk Assessment (1984a), should be conducted to further characterize the mutagenic potential of 1,3-butadiene.

On the basis of sufficient evidence from studies in two species of rodents, and inadequate epidemiologic data, 1,3-butadiene can be classified, according to EPA's Proposed Guidelines for Carcinogen Risk Assessment (1984b), as a "probable" human carcinogen, Group B2. Using the classification scheme of the International Agency for Research on Cancer, 1,3-butadiene would be classified as a "probable" human carcinogen, Group 2B.

A carcinogenic potency and related upper-bound estimate of incremental lifetime cancer risk can be estimated from the animal studies. These risk estimates are developed for the purpose of evaluating the possible magnitude of the public health impact if 1,3-butadiene is a human carcinogen.

Using a multistage model which is linear at low doses, a 95% upper-limit incremental unit risk for 1,3-butadiene is estimated on the basis of the NTP (1984) mouse study to be $q_1^* = 6.4 \times 10^{-1}$ $(ppm)^{-1}$. The upper-bound nature of

this estimate is such that the true risk is not likely to exceed this value and may be lower. In addition to a 95% upper-limit incremental unit risk, a measure of carcinogenic potency was determined for 1,3-butadiene. Among the 55 chemicals that the Carcinogen Assessment Group has evaluated as suspect carcinogens, 1,3-butadiene ranks in the third quartile.

2. INTRODUCTION

1,3-Butadiene (CAS No. 106-99-00) is a colorless gas produced as an ethylene co-product, by oxidative dehydrogenation of n-butenes, or by dehydrogenation of n-butanes. In 1977, between 2.1 and 7.3 billion pounds of 1,3-butadiene were produced or imported. 1,3-Butadiene ranked 36th in U.S. domestic chemical production in 1983. It is used as an intermediate in the production of polymers, elastomers, and other chemicals. The major use of 1,3-butadiene is in the manufacture of styrene-butadiene rubber (synthetic rubber). In addition, 1,3-butadiene is used as an intermediate to produce a variety of industrial chemicals, including the fungicides, captan and captofol. The U.S. Food and Drug Administration (FDA) has approved 1,3-butadiene for use in the production of adhesives used in certain types of food containers.

Although 1,3-butadiene has been found in U.S. drinking water, it is primarily an air contaminant. It has been detected in cigarette smoke, incineration products of fossil fuels, gasoline vapor, and automotive exhaust (Miller, 1978). Concentrations ranging from 1 to 9 ppb have been detected in urban air (Neligan, 1962). Higher concentrations, up to 45 ppm, have been reported in air samples and factory emissions at petrochemical plants (NTP, 1984).

Approximately 65,000 workers are potentially exposed to 1,3-butadiene, according to a report prepared by the National Institute for Occupational Safety and Health (NIOSH, 1984). The greatest occupational exposure is likely to occur in plants that manufacture 1,3-butadiene or use it to produce polymers or elastomers. Occupational hazards from exposure to 1,3-butadiene exist from inhalation of airborne concentrations and, to a lesser extent, by dermal contact. The current permissible exposure limit of 1,000 ppm as an 8-hour time-weighted average was adopted by the U.S. Occupational Safety and Health Admin-

istration (OSHA) from the 1968 Threshold Limit Values (TLV) set by the American Conference of Government Industrial Hygienists (ACGIH).

While 1,3-butadiene was introduced into commerce because of its use in the manufacture of synthetic rubber in the early 1940s, little information on the toxicity of this material is available except for scattered reports from eastern European countries. Indeed, it was not until 1981 that the first chronic toxicity study was conducted. To date, adequate long-term investigations in non-rodent experimental animals are not available. Both the International Agency for Research on Cancer (IARC, 1982) and the U.S. Department of Health and Human Services (DHHS, 1985) have recognized that occupational exposure in the rubber industry leads to an increased risk of cancer. Because many materials are used in the rubber industry, it may be impossible to identify any single causative agent, such as 1,3-butadiene, as a carcinogen, especially since other chemicals that are generally recognized as carcinogens (i.e., benzene and acrylonitrile) are also used in this industry segment.

Both IARC (1982) and DHHS (1985) recognize diepoxybutane, a metabolite of 1,3-butadiene, as a carcinogen. Based on reports of the carcinogenic potential of 1,3-butadiene, the ACGIH published a Notice of Intended Change (ACGIH, 1983) in their 1983-1984 Threshold Limit Values (TLV). They proposed to classify 1,3-butadiene as an industrial substance suspected of carcinogenic potential for man and assigned no numerical TLV. More recently, the ACGIH recommended a TLV of 10 ppm (ACGIH, 1984). Their previous TLV (the basis of OSHA's current permissible exposure limit) was 1,000 ppm based on mild irritation in man and limited effects in rats and guinea pigs at higher concentrations. Based on the same animal carcinogenicity studies, NIOSH issued a Current Intelligence Bulletin on 1,3-butadiene. They classified the chemical as a potential occupational carcinogen and recommended that worker exposure be reduced to the

fullest extent possible. In addition, NIOSH recommended that 1,3-butadiene be regarded as a potential occupational teratogen and possible reproductive hazard (NIOSH, 1984). The Office of Toxic Substances (OTS) of the U.S. Environmental Protection Agency assessed the risk to workers exposed during the production of 1,3-butadiene and during the use of 1,3-butadiene in the production of synthetic rubbers, plastics, and resins. They determined that 1,3-butadiene was a "probable" human carcinogen (category B2 according to EPA's Proposed Guidelines for Carcinogen Risk Assessment, 1984b). OTS concluded, using a one-stage model with pooled tumors from male mice, that the upper limits of carcinogenic risk for year-round exposure at 10, 1, and 0.1 ppm are 10^{-1}, 10^{-2}, and 10^{-3}, respectively (U.S. EPA, 1985).

The National Toxicology Program (NTP) is currently undertaking a series of investigations of 1,3-butadiene. These studies will provide additional information on the pharmacokinetics and toxicity of the chemical. In addition, the carcinogenic response at lower airborne concentrations may be established.

3. TOXICOLOGY

3.1. ACUTE TOXICITY

Information on the toxicity of 1,3-butadiene resulting from acute exposure is limited. For rats and mice, the median lethal concentrations of 1,3-butadiene for periods of exposure ranging from 2 to 4 hours are above 100,000 ppm. The oral LD_{50} values for rats and mice are 5.48 g/kg and 3.21 g/kg, respectively. The major acute toxic effects are irritation of the respiratory tract, mucous membranes, and eyes, and narcosis (NTP, 1984).

3.2. SUBCHRONIC TOXICITY

A 3-month toxicity study in rats preceded the 2-year chronic inhalation toxicity study conducted at Hazleton Laboratories Europe, Ltd. in England (1981a), and sponsored by the International Institute of Synthetic Rubber Producers, Inc. (IISRP). Further details of the chronic investigation as well as the results with regard to the carcinogenicity of 1,3-butadiene are presented in the carcinogenicity chapter of this document. The airborne concentrations of 1,3-butadiene used in the 3-month study were 1,000, 2,000, 4,000, and 8,000 ppm; a group exposed to filtered air (0 ppm) served as controls. The authors considered that there were no effects attributable to exposure to the test chemical on growth rate, food consumption, hemograms, blood biochemical investigations, or pathological evaluation. The only effect the investigators considered to be related to 1,3-butadiene exposure was a moderate increase in salivation, particularly among female rats during the last 6 to 8 weeks of exposure at the higher airborne concentrations (Crouch et al., 1979). These results are consistent with an earlier study by Carpenter et al. (1944) in which the investigators found only a slight reduction in body weight gain among rats and guinea pigs exposed for 8 months, 7.5 hours/day, 6 days/week,

to an airborne concentration of 8,000 ppm. These authors noted no effects among animals exposed at 600 and 2,300 ppm.

Preliminary inhalation toxicity studies in mice were used as a basis for dose selection for the chronic studies of B6C3F1 mice, conducted at Battelle Pacific Northwest Laboratories and sponsored by the NTP (1984). Further details of the chronic exposure and the results with regard to the carcinogenicity of 1,3-butadiene are presented in the carcinogenicity chapter of this document. Two range-finding studies, a 15-day and a 14-week study, were conducted at International Bio-Test Laboratories. In the 15-day study, weight loss at airborne concentrations of 1,250 ppm was observed. Even the mice exposed to 8,000 ppm, the highest airborne concentration, survived the exposure period. In the 14-week study, reduced body weight and death were observed among mice treated at 2,500 ppm or more. Necropsy findings were not reported (NTP, 1984).

Miller (1978) reviewed a series of papers from Russian investigators, particularly Ripp (1967), and summarized the subchronic toxic effects in rats. Ripp (1967) exposed rats to airborne 1,3-butadiene concentrations of 1, 3, and 30 mg/m^3 (1 mg/m^3 = 0.45 ppm). The highest concentration in this study (equivalent to 13.5 ppm) is only about 1/50 of the lowest concentration (600 ppm) in any of the other studies reported in this section. At 30 mg/m^3, blood cholinesterase was elevated, blood pressure was lowered, and motor activity was decreased to 60% of the pre-exposure rate. Histopathological evaluation revealed no changes at 1 mg/m^3 except for congestion in the spleen and hyperemia and leukocyte infiltration in the cardiac tissue. The changes in the cardiac tissues were more marked at the higher levels with hemmorhage and reduced cellular RNA reported at the highest concentration. At 3 and 30 mg/m^3, atelectasis, interstitial pneumonia, and emphysema were noted in the lung tissue. These results, showing an adverse response at such low levels, may

indicate that continuous exposure is more hazardous than intermittent exposure, the regimen used in all other investigations. An alternative explanation for these findings at such low levels is that Ripp (1967) examined more sensitive indicators of toxicity than other investigators.

3.3. CHRONIC TOXICITY

A 2-year chronic inhalation toxicity study of the effects of airborne concentrations (1,000 and 8,000 ppm) of 1,3-butadiene on rats was conducted at Hazleton Laboratories Europe, Ltd. in England (HLE, 1981a) and was sponsored by the IISRP. Among rats exposed to 8,000 ppm, clinical signs, consisting of excessive secretion of the eyes and nose plus slight ataxia, were observed between months 2 and 5 of the study. Variations in mean body weight suggested no consistent adverse effect. Review of the hemograms, blood chemistry, urine analysis, and behavioral testing was likewise not indicative of an adverse effect. In female rats exposed to either 1,000 or 8,000 ppm, subcutaneous masses appeared earlier and at higher incidences than in the control. A dose-related increase in liver weights was observed among rats at the necropsy performed at 52 weeks and among these killed at the termination of the study. This could indicate that the chemical induces liver enzymes. Otherwise, no significant changes were noted at the 52-week kill. Increased alveolar metaplasia and nephropathy were observed among males of the 8,000 ppm treatment groups at the termination of the study. Marked or severe nephropathy occurred in 27% of the male rats in the high-dose group as compared with 9% to 10% in the control and the low-dose groups. The authors considered nephropathy to be the cause of some of the early deaths in this study.

A lifetime chronic inhalation study in B6C3F1 mice at 1,3-butadiene concentrations of 625 and 1,250 ppm administered for 6 hours/day, 5 days per week, was sponsored by the NTP (1984). The exposures were prematurely terminated

after 61 weeks due to deaths resulting largely from the development of cancer. No increases in clinical signs could be associated with exposure to the test chemical. In addition to the neoplastic changes described in the carcinogenicity chapter, testicular atrophy (control-0/50, 625 ppm-19/47, 1,250 ppm-11/48) and ovarian atrophy (2/49, 40/45, 40/48) were elevated among mice at both doses. Furthermore, among male mice, there was a significant increase in liver necrosis at both doses. In female mice, liver necrosis was significantly elevated only at the higher airborne concentration. While neoplastic lesions of the nasal cavity were not found at any dose, there was an increase in non-neoplastic changes at the high dose. At 1,250 ppm, chronic inflammation of the nasal cavity (male, 33/50; female, 2/49), fibrosis (male, 35/50; female, 2/44), cartilaginous metaplasia (male, 16/50; female 1/49), osseous metaplasia (male, 11/50; female, 2/49), and atrophy of the sensory epithelium (male, 32/50) were observed. No non-neoplastic lesions of the nasal cavity were found in the controls (NTP, 1984). Huff et al. (1985) have suggested that the lack of neoplasms in the nasal cavity as compared to the lungs may reflect a requirement for biotransformation of 1,3-butadiene to a reactive epoxide intermediate. The nasal cavity changes then suggest that the intact molecule may have some adverse effect at 1,250 ppm. However, as discussed in the metabolism and carcinogenicity chapters, exposure to 1,3-butadiene did not decrease minute volume, as occurs with other respiratory irritants.

In summary, since a no-effect dose has not been established for the non-carcinogenic chronic toxicity, further investigations are warranted. These investigations should focus on the liver, testes, and ovaries because in mice these tissues are adversely altered at the lowest concentrations. In addition, the minimum effect dose for cardiac and respiratory tract changes needs to be further explored. For a complete understanding of the toxicity, non-rodent

species might be used.

3.4. REPRODUCTIVE TOXICITY

A teratogenicity study sponsored by the IISRP was conducted at Hazleton Laboratories Europe, Ltd. in England (HLE, 1981b). Pregnant female Charles River CD rats (Sprague-Dawley), obtained from Charles River Ltd., were exposed to airborne concentrations of 1,3-butadiene (200, 1,000, and 8,000 ppm), 6 hours/day, on days 6 to 15 of gestation. Each treatment group comprised 24 female rats; 40 were assigned to the control group (referred to as 0 ppm exposure) which was exposure to filtered air. A group of 26 pregnant rats was administered acetylsalicylic acid at a dose of 250 mg/kg by gavage and served as a positive control. The rats were observed daily and weighed at intervals during the study. On day 20 of gestation the females were killed and necropsied, and the uterine contents were inspected. One-third of each litter was examined for soft tissue abnormalities using a modified Wilson's technique (free hand sectioning of the heads). The remainder were examined for visceral and, after preparation, skeletal anomalies.

The positive control group had sufficient adverse reproductive and teratogenic effects to establish the responsiveness of this strain of rats in the hands of these investigators. The treated dams were not affected by exposure to 1,3-butadiene except for reduced weight gain in those exposed to 8,000 ppm. Selected data for other reproductive toxicity end points are summarized in Table 3-1. The authors concluded that there was embryonic growth retardation (fetal weight and length), and slight embryolethality (percentage implantation loss) in all dose groups as a result of the maternal toxicity and that the magnitude of the effect was dose related. The relationship between maternal toxicity and the fetal effects was a subjective judgment and not experimentally established. The authors further concluded that at the highest doses there was

TABLE 3-1. EFFECTS OF 1,3-BUTADIENE EXPOSURE ON PREGNANT RATS

Parameter	Control 0	200	1,000	8,000
% preimplantation loss	3.6	6.0	4.9	7.3
Mean fetal weight (g)	3.3	3.2	3.2	3.1[a]
Mean crown-rump length (mm)	37.8	37.2	37.2	35.9[b]
% of fetuses w/skeletal variants	86	90	86	98[c]
% of fetuses w/skeletal minor defects	23	27[a]	21	21
% of fetuses w/skeletal major defects	0.6	2.2	3.8[a]	5.9[b]

[a]Different from control ($p < 0.05$) using the Wilcoxon Test.
[b]Different from control ($p < 0.01$) using the Wilcoxon Test.
[c]Different from control ($p < 0.05$) using the Fisher Exact Test.

an indication of teratogenicity based on the presence of major fetal defects. Most of the major skeletal defects (marked or severe wavy ribs) were among fetuses of the 200 and 1,000 ppm groups. Some investigators might not consider such changes as major defects. There was a significant increase in "minor" skeletal defects at the lowest (200 ppm) airborne concentration. Furthermore, a statistically significant ($p < 0.05$) increase in minor external and visceral defects was noted among fetuses in the treatment groups. Subcutaneous hematomas were the most frequent finding in the lower exposure groups. The frequency of fetuses with lens opacities was increased at the highest exposure (HLE, 1981b). Whether these increases represent a qualitative dose-response change in view of the changes at the higher dose levels or are due to other factors, such as maternal toxicity, cannot be determined from the information available. Nevertheless, it would appear that the fetuses from dams exposed to concentrations of 200 to 8,000 ppm were adversely affected in a manner perhaps only related to growth.

In an earlier but inadequately reported study (Carpenter et al., 1944), decreased litter size was present among rats exposed to 6,700 and 2,300 ppm 1,3-butadiene, but not at 600 ppm. Carpenter et al. (1944) and the unpublished study sponsored by IISRP (HLE, 1981b) provide only suggestive evidence that 1,3-butadiene causes adverse reproductive effects in female rats. Further investigations using other species would be useful in evaluating this possible hazard. In view of the ovarian and testicular toxicity noted in the NTP (1984) chronic inhalation study, the scope of these investigations should not be limited only to exposure during pregnancy.

4. METABOLISM AND PHARMACOKINETICS

The major hazard with regard to the adverse effects of exposure to 1,3-butadiene is via inhalation. There is also a slight potential for dermal exposure. Absorption through the gastrointestinal tract is possible and might result from contamination of water or leaching of the compound into food.

Useful information concerning the pharmacokinetics of 1,3-butadiene has only recently become available. The older information is limited by the lack of sensitivity of the analytical techniques used, and also because the extremely high doses used frequently approached lethal doses.

Shugaev (1969) exposed rats for 2 hours to an airborne 1,3-butadiene concentration of 130,000 ppm and measured tissue concentrations. He found the highest concentration of the chemical in the perirenal fat (152 mg %) and lower concentrations, ranging from 36 to 50 mg %, in the liver, brain, spleen, and kidney. The tissue concentrations following exposure to the LC_{50} concentration in the brain tissue of rats and mice (only brain concentration was determined in mice) were 50.8 and 54.4 mg % for rats and mice, respectively.

4.1. METABOLISM

4.1.1. In vitro Metabolism

In light of the implication that the mutagenic effect of 1,3-butadiene towards *S. typhimurium* is due to the formation of reactive metabolites (de Meester et al., 1978, 1980), the formation of these metabolites in mammalian species is of vital interest. In addition, the metabolite, diepoxybutane, has also been demonstrated to be carcinogenic by dermal application and subcutaneous injection (IARC, 1982). Malvoisin and Roberfroid (1982) demonstrated that 1,3-butadiene is metabolized to epoxybutene, 3-butene-1,2-diol, diepoxybutane, and 3,4-epoxy-1,2-butanediol by rat liver microsomes. The pathway proposed

by these authors for 1,3-butadiene metabolism, based on data obtained from *in vitro* experiments, is that epoxybutene is formed from 1,3-butadiene via microsomal oxidase (P450) and that it is then further oxidized to diepoxybutane by the same enzyme system or converted to 3-butene-1,2-diol by epoxide hydrolase. The 3-butene-1,2-diol then reacts with molecular oxygen to form the epoxide, 3,4-epoxy-1,2-butanediol, again via the microsomal oxidase system. Figure 5-1 depicts the proposed pathway of 1,3-butadiene metabolism. Liver microsomes prepared from animals pretreated with phenobarbital metabolized more 1,3-butadiene to 3,4-epoxy-1,2-butanediol and diepoxybutane than microsomes from animals not pretreated. Microsomes prepared from animals pretreated with Metyrapone and SKF 525A metabolized less 1,3-butadiene to the diepoxide but not the diol. Styrene oxide and vinylcyclohexene oxide almost completely inhibited the formation of the diol. Both were observed to have substantially greater affinity than 1,3-butadiene as substrates for the epoxide hydrolase enzyme. The authors stated that epoxybutene is a poor substrate for epoxide hydrolase relative to styrene oxide. They suggested that this may account for the fact that 1,3-butadiene is a more potent mutagen than styrene (Malvoisin et al., 1980; Malvoisin and Roberfroid, 1982).

The *in vitro* formation of epoxybutene (vinyl oxirane) by rat liver microsomal preparations has been confirmed by Bolt et al. (1983). These investigations also found that microsomes prepared from animals pretreated with phenobarbital or 3-methylcholanthrene metabolized more 1,3-butadiene to epoxybutene, and that microsomes prepared from animals pretreated with SKF 525A or diethyldithiocarbamate metabolized less 1,3-butadiene than microsomes from animals not pretreated.

Malvoisin and Roberfroid (1982) found that epoxybutene reacts both chemically and enzymatically with glutathione-S-transferase to form a glutathione

conjugate. The significance of this observation with respect to the toxicity of epoxybutene awaits further investigation. Citti et al. (1984) demonstrated the formation of an N-7 guanine adduct of epoxybutene after incubation with either deoxyguanosine or DNA. The authors suggested that the formation of these adducts may account for the mutagenic effect of 1,3-butadiene and its reactive epoxide metabolites.

4.1.2. In vivo Metabolism

Bolt et al. (1983) exposed rats (Wistar) to 1,3-butadiene by inhalation and found epoxybutene in the exhaled air, thus confirming the formation of this mutagen in *in vivo* experiments. These results were corroborated by Filser and Bolt (1984) and Bolt et al. (1984), who also found epoxybutene in expired air of rats after exposure to 1,3-butadiene. Their results are described in more detail in section 4.2.

Laib et al. (1985) reported on binding of ^{14}C-1,3-butadiene to liver and nucleoproteins and DNA. Four rats (Wistar) and 24 B6C3F1 mice were exposed to labeled (1,4-C^{14}) 1,3-butadiene at an air concentration of about 700 ppm for 6.6 or 4 hours, respectively. More than 98% of the total activity could be accounted for when the animals were sacrificed 30 minutes after exposure. The radioactivity expressed as cpm/mg in the liver of early nucleoproteins and DNA has been tabulated in Table 4-1. The late-eluting nucleoproteins had the same rat/mouse ratio. These results show that mice incorporate 1,3-butadiene or its metabolites into the nucleoproteins and DNA at a rate greater than the rats.

All four of the *in vivo* studies cited above reported that the metabolism of 1,3-butadiene to epoxybutene was at a maximum. In rats this maximum conversion appears to take place at atmospheric concentrations of 1,3-butadiene greater than 1,000 ppm (Bolt et al., 1983, 1984; Filser and Bolt, 1984; Laib et al., 1985). Pretreatment of rats with Aroclor 1254 (polychlorinated biphenyl)

TABLE 4-1. MEAN RADIOACTIVITY IN LIVER NUCLEOPROTEINS AND DNA OF MOUSE AND RAT AFTER EXPOSURE TO ^{14}C-1,3-BUTADIENE

cpm/mg		cpm/mg/hour	
rat	mouse	rat	mouse
nucleoprotein			
323	613	48.9	153
DNA			
720	628	109	157

SOURCE: Laib et al., 1985.

rendered the metabolism of 1,3-butadiene less complete at atmospheric concentrations up to 12,000 ppm (Bolt et al., 1984). The metabolism of 1,3-butadiene in mice approaches maximum conversion at 1,800 ppm (Laib et al., 1985).

4.2. PHARMACOKINETICS

The pharmacokinetics of 1,3-butadiene have been studied using two different methods of exposure. The first involved the exposure of rats (Bolt et al., 1983, 1984; Filser and Bolt, 1984; Laib et al., 1985) and mice (Laib et al., 1985) to various atmospheric concentrations of 1,3-butadiene in a sealed chamber. 1,3-Butadiene was introduced into the chamber at selected concentrations, and the declines in these concentrations were measured over time. The pharmacokinetics of this system were described by Filser and Bolt (1981). Filser and Bolt (1981) assumed that the decline in the concentration of 1,3-butadiene in the chamber was a result of uptake and metabolism of the compound by the animal. Based on these assumptions, certain pharmacokinetic parameters may be estimated. By

inhibiting the metabolism of the compound, the relative contributions of uptake and metabolism may also be assessed. In addition, the results from such experiments have been used to estimate the kinetics of the compound under the assumption that the volume of the chamber is infinite (Filser and Bolt, 1981). The resulting parameter estimates were reported to apply to environmental exposure in which the concentration of the compound in the atmosphere is constant and unaffected by the animal.

Initial experiments in rats (Wistar) by Bolt et al. (1983) indicated that, under the conditions described above, the decline in the concentration of 1,3-butadiene in a closed system follows zero-order kinetics at atmospheric concentration above 1,000 ppm and that below that concentration, the decline in the concentration of 1,3-butadiene follows first-order kinetics. When the concentration of 1,3-butadiene in the chamber was above 1,000 ppm, there was a constant accumulation of the primary metabolite epoxybutene. When the concentration of 1,3-butadiene was 1,000 ppm or less, the concentration of epoxybutene declined in approximately first-order fashion, which suggests that epoxybutene was reabsorbed and further metabolized. This observation was confirmed by Filser and Bolt (1984) in experiments in which rats were administered epoxybutene by inhalation in a closed system. In these experiments, a first-order decline of epoxybutene was observed at concentrations as high as ~3,500 ppm. The same rate of decline was observed when epoxybutene was administered intraperitoneally and then exhaled.

A kinetic analysis of 1,3-butadiene in rats using the closed-system technique was conducted and reported by Bolt et al. (1984). As observed in their previous studies, the decline in the concentration of 1,3-butadiene in the chamber exhibited zero-order kinetics at atmospheric concentrations above 1,000 ppm and first-order kinetics below this concentration. About 20% of the initial

content of the chamber remained after 6 hours. Using equations reported in an earlier article (Filser and Bolt, 1981) the pharmacokinetic parameters shown in Table 4-2 were calculated. Using these parameters, Filser and Bolt (1984) found that only 30% of epoxybutene that had been predicted to be formed from the metabolism of 1,3-butadiene under saturated conditions was expired by the animals under these conditions. The remaining 70% apparently was either retained in the body, further metabolized, or excreted via other routes.

Laib et al. (1985) conducted experiments with mice (B6C3F1) that were similar to the rat (Sprague-Dawley) experiments of Bolt et al. (1984). The pharmacokinetic parameters obtained for mice are compared to those of rats in Table 4-1. Of particular interest is that the rate of uptake of 1,3-butadiene ($k_{12}V_1$), the static and dynamic equilibrium constants (K_{eq} and K_{st}, respectively), the total clearance (Cl_{tot}), the maximum rate of metabolism (V_{max}), and the atmospheric concentration of 1,3-butadiene resulting in the saturation of uptake and/or metabolism are greater in mice than in rats. Using the pharmacokinetic parameters calculated for rats (Bolt et al., 1984) and for mice, Laib et al. (1985) concluded that the rate of metabolism in mice was approximately twice that in rats at any given atmospheric concentration of 1,3-butadiene.

In investigations in progress for the National Toxicology Program (NTP) at Lovelace Inhalation Toxicology Research Institute (LITRI) (NTP, 1985a), rats (Sprague-Dawley) and mice (B6C3F1) were exposed to 1,3-butadiene via inhalation using a different exposure paradigm. In the initial studies, animals were exposed nose-only to constant atmospheric concentrations of ^{14}C-1,3-butadiene for 6 hours and then placed in metabolism cages which allowed for the separate collection of urine, feces, and expired air. There is one important difference between this method of exposure and the closed-chamber method used by Bolt and coworkers. In the LITRI studies, animals were exposed to a constant

TABLE 4-2. PHARMACOKINETIC PARAMETERS FOR 1,3-BUTADIENE

Parameter	Bolt et al. (1984) rats	Laib et al. (1985) mice	Units
$k_{12}V_1$	5,750	10,280	$mL \cdot hr^{-1}$
K_{eq}	2.3	3.2	--
K_{st}[a]	0.5	1.0	--
Cl_{tot}[a]	4,490	6,750	$mL \cdot hr^{-1}$
V_{max}[b]	220	400	$\mu moles \cdot hr^{-1} \cdot kg^{-1}$
Metabolic saturation	1,000	1,800	ppm

[a]K_{st} and Cl_{tot} calculated for $V_1 \rightarrow \infty$.

[b]V_{max} valid for saturation range.

SOURCE: Laib et al., 1985.

atmospheric concentration of 1,3-butadiene for a fixed length of time, and then exposure was discontinued. In contrast, using the closed-chamber method, animals are exposed to a constantly decreasing concentration of 1,3-butadiene. The latter procedure allowed for rebreathing of the parent compound and exhaled metabolites.

In the preliminary studies conducted at LITRI, rats were exposed for 6 hours to atmospheric concentrations of ^{14}C-1,3-butadiene of 125, 1,700, and 12,800 μg/L. Mice were exposed to concentrations of 13, 145, and 1,900 μg/L for 6 hours. These concentrations correspond to 70, 930, and 7,100 ppm, respectively, for rats and 7, 80, and 1,040 ppm, respectively, for mice under the conditions at LITRI (25°C, 620 mm). Preliminary experiments were also conducted in which rats and mice were exposed to 1,3-butadiene for 6 hours and their respiratory parameters were measured by plethysmographs. No significant concentrated-related differences were reported for minute volume in rats. In mice, respiratory irritants decreased the minute volume.

The fraction of the inhaled compound that was absorbed and retained was calculated (see Table 4-3) based on the respiratory parameters obtained from the plethysmographic studies and the amount of radioactivity recovered in another study from animals that were similarly exposed and placed in metabolism cages. Dose-dependent retention was observed in both species. This is in general agreement with Bolt et al. (1984) and Laib et al. (1985). However, using the calculated areas under the curves of exposure concentration versus time from 0 to 6 hours as a measure of relative exposure, the levels at which dose-dependent uptake occurred are lower in the LITRI report than in the Bolt studies. This is most likely due to the differences in methods of exposure. The LITRI study also indicates that at similar concentrations mice received 3 to 4 times the dose (μmol/kg) of that received by rats (Table 4-3).

TABLE 4-3. ABSORPTION OF 1,3-BUTADIENE BY INHALATION

Species	Exposure concentration ppm	Exposure concentration (μg/L)	1,3-Butadiene inhaled (μmol)	1,3-Butadiene retained (μmol)	1,3-Butadiene retained (μmol/kg)	Percent retained
Rats	70	125	235	16.3	40	7.1
	930	1,700	3,100	64.7	160	3.1
	7,100	12,800	17,000	243	660	1.5
Mice	7	13	1.7	0.9	33	54
	80	145	34.7	3.2	120	9.6
	1,040	1,900	435	19.1	660	4.7

SOURCE: NTP, 1985a.

The proportions of the absorbed radioactivity accounted for in the breath, urine, feces, and body of rats and mice after exposure to ^{14}C-1,3-butadiene in the LITRI study are presented in Table 4-4. As can be seen from the table, the data suggest that the rats displayed a dose-related increase in the proportion of the absorbed radiolabel exhaled during the 65 hours after exposure. The rat data also suggest that a concomitant dose-related decrease in the proportion of the absorbed radiolabel excreted in urine was also observed. The data from mice showed no dose-related trend in the pattern of excretion at the exposure levels used. The proportion of the absorbed radiolabel excreted in feces and retained in the body after 65 hours was similar over the range of exposures examined. Although these results are expressed in terms of total radioactivity and the radioactive species in any of these media were not identified, dose-dependent metabolism of 1,3-butadiene can be inferred.

In summary, the work of Bolt and coworkers (Bolt et al., 1983, 1984; Filser and Bolt, 1984; Laib et al., 1985) and LITRI (NTP, 1985a) provide information on the metabolism and pharmacokinetics of 1,3-butadiene. The apparent differences between the results from these two laboratories are probably due to different methods of exposure. The published papers from Bolt's laboratory and the preliminary reports from LITRI are in general agreement that the uptake of 1,3-butadiene in rats and mice is concentration-dependent and that its dose-dependent metabolism is observed at relatively higher atmospheric concentrations in mice than in rats.

TABLE 4-4. PERCENTAGE OF ABSORBED RADIOLABEL EXCRETED VIA BREATH, URINE, AND FECES AND REMAINING IN THE BODY 65 HOURS AFTER EXPOSURE TO ^{14}C-1,3-BUTADIENE[a]

Species	Exposure concentration (μg/L)	Percent excreted in			Percent remaining in the body
		Breath	Urine	Feces	
Rats	125	27	48	5	20
	1,700	46	32	2	20
	12,800	76	8	2	14
Mice	13	42	40	7	11
	145	24	49	11	16
	1,900	48	37	3	12

[a]Percentages estimated from Figures 5 and 6 of the NTP report.

SOURCE: NTP, 1985a.

5. MUTAGENICITY OF 1,3-BUTADIENE AND ITS REACTIVE METABOLITES

This chapter is concerned with the mutagenicity of 1,3-butadiene, which is a gas at room temperature, and also includes a discussion of the mutagenicity of its reactive metabolites (3,4-epoxybutene and 1,2:3,4-diepoxybutane). The available evidence suggests that 1,3-butadiene is mutagenic by virtue of its metabolism to mutagenic intermediates.

5.1. MUTAGENICITY OF 1,3-BUTADIENE

1,3-Butadiene was tested for its mutagenic potential in the Salmonella typhimurium histidine reversion assay by de Meester et al. (1978). The sample of 1,3-butadiene studied was 99.5% pure and was obtained from Matheson Gas Products, Belgium. Salmonella strains TA1530, TA1535, TA1537, TA1538, TA98, and TA100 were exposed to 1,3-butadiene vapors for 20 hours in closed desiccators. Mutagenic activity was observed in strains TA1530 and TA1535 both in the presence and absence of S9 prepared from Aroclor-pretreated rats. The bacteria were exposed to only one dose of 1,3-butadiene and that dose was not clearly specified. This study suggests that 1,3-butadiene is a direct-acting, base-pair substitution mutagen in bacteria.

In a subsequent study, de Meester et al. (1980) exposed strain TA1530 to 1,3-butadiene vapors for 24 hours at 0, 4, 8, 16, 24, and 32% (vol/vol) in closed desiccators. In the absence of S9 mix or in the presence of S9 prepared from untreated rats, no increase in the revertant frequency was observed. However, when the bacteria were exposed to 1,3-butadiene in the presence of S9 mix prepared from phenobarbitone- or Aroclor 1254-pretreated rats, mutagenic activity was observed. The number of histidine revertants increased in a dose-related fashion from 17 per plate in the absence of 1,3-butadiene up to 255 per plate at 16% (vol/vol) 1,3-butadiene. These results suggest that

1,3-butadiene itself is not a mutagen, and that it is metabolized to mutagenic intermediates that cause base-pair substitutions.

Data suggesting that the mutagenic metabolites are volatile were also reported by de Meester et al. (1980). Plates containing Salmonella but no S9 mix were coincubated in 1,3-butadiene atmospheres (4%-32%) with plates containing Salmonella plus S9 mix from chemically pretreated animals. The reversion rates were enhanced in both sets of plates, indicating that 1,3-butadiene was metabolized in the plates containing the S9 mix to volatile mutagens which were active in the plates without the S9 mix. The number of mutant colonies was proportional to the level of 1,3-butadiene in the desiccators up to 16%.

This second study by de Meester and coworkers (de Meester et al., 1980) contradicts their earlier observation (de Meester et al., 1978) that 1,3-butadiene is mutagenic in the absence of S9 mix. If, in the earlier study, plates containing bacteria plus S9 mix were incubated in the same dessicators with plates containing bacteria but no S9 mix, volatile mutagenic metabolites of 1,3-butadiene generated in the first set of plates may have been responsible for the mutagenic effects observed in the second set of plates.

A study by Poncelet et al. (1980) supports the conclusion of de Meester et al. (1980) on the mutagenic potential of 1,3-butadiene in Salmonella strain TA1530. Mutagenic effects were observed when the assays were performed in a 16% gaseous atmosphere of 1,3-butadiene in the presence of Aroclor-induced S9 mix. When the bacteria were exposed to 1,3-butadiene under the conditions of the plate incorporation method or preincubation in liquid medium, mutagenicity was not observed. The 1,3-butadiene sample was 99.5% pure and was obtained from Matheson Gas Products, Belgium.

In summary, the weight of the available evidence suggests that 1,3-butadiene is a promutagen in bacteria; its mutagenicity depends on metabolic activa-

tion by S9 mix prepared from chemically induced animals. No whole-animal mutagenicity studies have been reported.

5.2. METABOLISM OF 1,3-BUTADIENE AND REACTION OF METABOLITES WITH DNA

As described in the previous section, 1,3-butadiene itself does not appear to be mutagenic. Mutagenic activity is observed only when 1,3-butadiene has been metabolized to reactive intermediates. A scheme for the metabolism of 1,3-butadiene and the alkylating ability of the probable metabolites are briefly discussed in this section.

Malvoisin et al. (1979) and Malvoisin and Roberfroid (1982) studied the metabolism of 1,3-butadiene *in vitro* using rat liver microsomes. They reported that the metabolism proceeds via a mixed-function oxidase-catalyzed oxidation to 3,4-epoxybutene, and they suggest that this compound is subsequently metabolized to 1,2:3,4-diepoxybutane (diepoxybutane) and 3-butene-1,2-diol (Figure 5-1). Both 3,4-epoxybutene and diepoxybutane are probably reactive intermediates, whereas 3-butene-1,2-diol and its metabolite 3,4-epoxy-1,2-butanediol are probably detoxification products. Preliminary evidence in rats suggests that 1,3-butadiene is metabolized to 3,4-epoxybutene *in vivo* (Bolt et al., 1983), indicating that the metabolic pathway outlined in Figure 5-1 on the basis of *in vitro* data may occur *in vivo*. Both 3,4-epoxybutene and diepoxybutane are mutagenic, as described more fully below. Malvoisin and Roberfroid (1982) state that their unpublished results indicate that 3-butene-1,2-diol and 3,4-epoxy-1,2-butanediol are not mutagenic.

The alkylating activity of the two reactive metabolites of 1,3-butadiene (3,4-epoxybutene and diepoxybutane) has been investigated, 3,4-epoxybutene in two studies and diepoxybutane in one study. Hemminki et al. (1980) found that 3,4-epoxybutene alkylated 4-(p-nitro-benzyl)-pyridine (NBP) and deoxyguanosine, which are nucleophiles that were used as models for DNA. The NBP reaction was

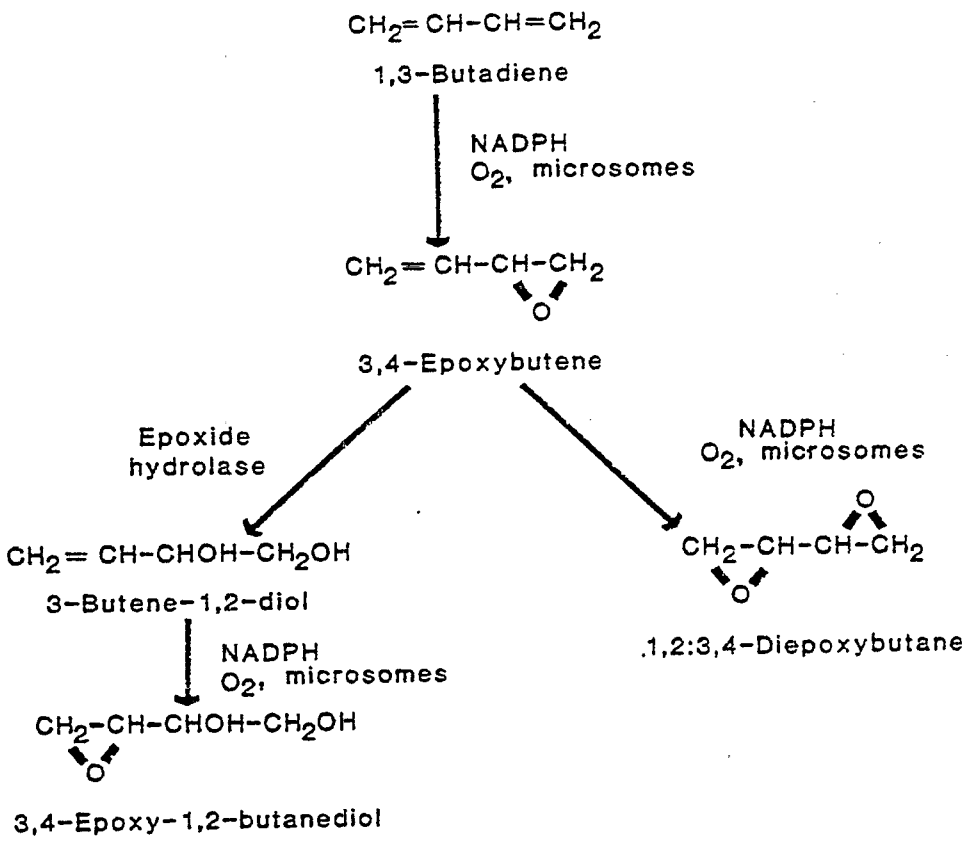

Figure 5-1. A hypothetical scheme for the metabolism of 1,3-butadiene.

SOURCE: Malvoisin and Roberfroid, 1982.

carried out at 37°C using the test compound at 0.286 μM. The deoxyguanosine reaction was carried out at 37°C using the test compound at 0.1 M. In both cases, aliquots of the reaction mixture were assayed for alkylation at 0 minutes, 20 minutes, 1 hour, 3 hours, and 5 hours. The reaction rates were determined from the initial rates, and the results were reported using epichlorohydrin as a reference. 3,4-Epoxybutene alkylated NBP and deoxyguanosine at rates that were 31% and 14%, respectively, of the alkylation by epichlorohydrin. In agreement with Hemminki et al. (1980), Citti et al. (1984) published data suggesting that 3,4-epoxybutene alkylates deoxyguanosine and DNA in vitro. The main products formed for both deoxyguanosine and DNA were 7-(2-hydroxy-3-buten-1-yl)deoxyguanosine and 7-(1-hydroxy-3-buten-2-yl)deoxyguanosine.

Lawley and Brookes (1967) reported that diepoxybutane reacts with DNA in a manner typical of bifunctional alkylating agents and causes interstrand cross-linking in DNA. Salmon sperm DNA was dissolved in 0.5 mM sodium citrate at 2 mg/mL (5.4 mM DNA phosphorus) and 25 mL was treated with redistilled diepoxybutane (2.4 mg/mL, 28 mM) at 37°C. Samples were withdrawn after 2, 5, 7, 24, 48, 72, 120, 168, and 193 hours. Ultraviolet spectroscopy was used to measure the reaction of diepoxybutane with DNA. At 37°C, diepoxybutane reacted with DNA slowly, as shown by changes in ultraviolet absorption of the reaction mixtures.

The extent of interstrand cross-linking (i.e., covalent linkage of the two DNA strands by the reaction of diepoxybutane with a nucleotide base in each DNA strand) was studied by measuring the reversible denaturation (renaturation) of diepoxybutane-treated DNA. In this experiment, diepoxybutane-treated DNA was first incubated at 60°C for various time periods and then rapidly cooled. At 60°C, untreated DNA denatures when it is dissolved in a solution of low ionic strength (i.e., the two strands separate). When cooled, the DNA renatures (i.e., the two strands rejoin to reform the typical double-stranded DNA

molecule). Diepoxybutane-treated DNA renatured to a greater extent than did normal untreated DNA, suggesting that diepoxybutane-treated DNA was cross-linked by a diepoxybutane bridge covalently joining the two DNA strands.

In summary, the above three in vitro studies indicate that 3,4-epoxybutene and diepoxybutane can alkylate DNA and that diepoxybutane causes interstrand cross-links in DNA. In addition, preliminary in vivo data suggest that 1,3-butadiene alkylates DNA in rats and mice after inhalation exposure (Laib et al., 1985), presumably via epoxide intermediates.

5.3. MUTAGENICITY OF 3,4-EPOXYBUTENE

In addition to studying the mutagenicity of 1,3-butadiene, de Meester et al. (1978) tested its monoepoxide metabolite, 3,4-epoxybutene, by the plate incorporation method in Salmonella. Strains TA1537, TA1538, and TA98 exhibited a negative response. Reversion to histidine prototrophy was observed with strains TA1530, TA1535, and TA100, with maximal numbers of revertants occurring at a 3,4-epoxybutene concentration of 100 μmol/plate.

The mutagenic potential of 3,4-epoxybutene was studied in the fluctuation test with Klebsiella pneumoniae as the test organism (Voogd et al., 1981). The compound was obtained from K and N (ICN, Life Sciences Division, New York), was analytical grade, and was not further purified. The chemical was dissolved and diluted in dimethylsulfoxide and subsequently added to broth which was inoculated with the test organism. The genetic characteristic studied was streptomycin resistance. The average spontaneous mutation rate for streptomycin resistance was 0.1676×10^{-9}. Triplicate experiments were averaged, and the results were expressed as the quotient of the observed and spontaneous mutation rates. At 1 and 2 mM 3,4-epoxybutene, the quotients were 1.7 and 2.5, respectively, providing evidence of a dose-related positive response.

In addition to studying the alkylating activity of 3,4-epoxybutene,

Hemminki et al. (1980) studied its mutagenicity (reversion to tryptophan prototrophy) in E. coli strain WP2 uvrA. The concentrations of 3,4-epoxybutene used were not specified but were based on toxicity determinations. Although the study suggests that 3,4-epoxybutene is mutagenic in E. coli WP2 uvrA, there was no indication of a dose-related response; the result for only one dose was reported and that dose was unspecified. Although this study is of limited use for risk assessment purposes, it supports the study of Voogd et al. (1981) in suggesting that 3,4-epoxybutene is mutagenic in bacteria.

Unpublished data suggest that 3,4-epoxybutene injected intraperitoneally induces sister chromatid exchange (SCE) and chromosomal aberrations in mouse bone marrow cells (Allen, personal communication, 1985). SCE involves the reciprocal exchange of DNA segments between sister chromatids and is considered an indication of DNA damage. A dose-related increase in SCEs was observed at 3,4-epoxybutene doses ranging from 10 mg/kg to 150 mg/kg (Table 5-1). A dose-related increase in chromosomal aberrations at 25-150 mg/kg was also observed (Table 5-2). The types of aberrations observed included chromatid and chromosome/isochromatid breaks, chromatid deletions, fragments, acentric fragments, and polyploidy. It is possible that the 3,4-epoxybutene was biotransformed after intraperitoneal injection and that the positive results for SCE and chromosomal aberrations in bone marrow cells were due to a metabolite of 3,4-epoxybutene.

In summary, 3,4-epoxybutene is mutagenic in bacteria and induces SCE and chromosomal aberrations in mice.

5.4. GENOTOXICITY OF 1,2:3,4-DIEPOXYBUTANE

Information on the genotoxicity of diepoxybutane can be found in a review on the genotoxicity of several epoxides by Ehrenberg and Hussain (1981). This review covers much of the early literature on diepoxybutane that is not discussed

TABLE 5-1. SCE IN MOUSE BONE MARROW CELLS AFTER *IN VIVO* EXPOSURE TO 3,4-EPOXYBUTENE

Treatment	Number of animals	SCE/cell[a]
Control	4	4.65 ± 1.250
Corn oil	4	4.43 ± 0.499
Cyclophosphamide (15 mg/kg)	4	28.65 ± 3.496
3,4-Epoxybutene		
10 mg/kg	4	6.68 ± 0.737
25 mg/kg	4	17.85 ± 3.793
50 mg/kg	4	18.78 ± 4.117
100 mg/kg	3	36.83 ± 6.585
150 mg/kg[b]	1	47.63 ± 8.42

[a]Mean ± SCE of 3-4 animals/group, except at 150 mg/kg where three out of four animals died due to toxicity; 30 cells per animal were assayed for SCE.
[b]Inhibition of cell cycling occurred.

SOURCE: Allen, personal communication, 1985.

TABLE 5-2. CHROMOSOME ABERRATIONS IN MOUSE BONE MARROW AFTER *IN VIVO* EXPOSURE TO 3,4-EXPOXYBUTENE

Dose	Number of animals	Total number of cells scored	Gaps[a]	Total aberrations scored	Aberration per cell
Control					
No treatment	4	400	11	1	0.0025
BrdU only	4	400	2	3	0.0075
BrdU + corn oil	4	400	1	3	0.0075
3,4-Epoxybutene					
25 mg/kg	4	400	6	12	0.0300
50 mg/kg	4[b]	400	9	20	0.0500
100 mg/kg	4	400	18	49	0.1225
150 mg/kg	1[c]	100	16	20	0.2000
Cyclophosphamide (positive control)					
15 mg/kg	4	400	9	14	0.0350

[a]Chromatid and chromosome gaps are not included as aberrations.
[b]One mouse in this dose group was a mosaic (39/40).
[c]Three animals died before harvest.

SOURCE: Allen, personal communication, 1985.

below, including testing in plants. This section on the genotoxicity of diepoxybutane covers more recent information and provides an indication of the types of genotoxic effects rather than an exhaustive survey of the literature.

5.4.1. Studies in Bacteria

Voogd et al. (1981) investigated the mutagenic potential of diepoxybutane in *K. pneumoniae* in the same paper cited previously for the mutagenicity of 3,4-epoxybutene. The source of the diepoxybutane was Merck (Darmstadt, F.R.G.); it was of analytical grade and was not further purified. At an equal chemical concentration (1 mM), diepoxybutane was approximately 16 times as mutagenic as 3,4-epoxybutene. The quotients of observed and spontaneous rates of mutation to streptomycin resistance for 0.05, 0.1, 0.2, 0.5, and 1 mM diepoxybutane were 1.7, 3.1, 6.2, 15.7, and 27, respectively. These results clearly indicate that diepoxybutane is mutagenic in *K. pneumoniae* and provide strong evidence of a dose-related response as well.

Diepoxybutane is also mutagenic in the *S. typhimurium* histidine reversion assay (Wade et al., 1979). Plate incorporation assays were performed with strains TA98 and TA100, and averages of two to five determinations were reported. At 0.02, 0.10, and 0.50 mg of diepoxybutane per plate, there were 196, 325, and 663 revertant colonies per plate with strain TA100 and 32, 22, and 29 revertant colonies per plate with strain TA98. These results suggest that diepoxybutane is a base-pair substitution mutagen in *S. typhimurium* because it produced a dose-related positive response in strain TA100. Although strain TA100 is not specific for mutagens that induce base-pair substitutions, it responds well to such mutagens, and the result in strain TA98, which detects many frameshift mutagens, was negative.

5.4.2. Studies in Fungi

The mutagenic potential of diepoxybutane in the yeast *Saccharomyces*

cerevisiae was studied by Olszewska and Kilbey (1975). They used a diploid yeast strain that is homozygous for the ilv mutation and therefore requires isoleucine and valine to grow. Kinetic studies of the induction of revertants were carried out by treating cells for various times with 0.1 M diepoxybutane at 25°C. About 1.2×10^6 to 1.5×10^6 cells were plated per petri dish. Diepoxybutane induced an increase in ilv reversions with increasing time of exposure up to 20 minutes. At 5, 10, 15, and 20 minutes, the number of ilv^+ revertants per 10^6 cells was about 3, 10, 20, and 38, respectively. Reversion of the ilv mutation indicates that diepoxybutane induces point mutations in yeast.

Zaborowska et al. (1983) have shown that diepoxybutane induces mitotic crossing-over and mitotic gene conversion in the SBTD and D7 strains of S. cerevisiae. Stationary-phase cells were treated with 0.4% (vol/vol) diepoxybutane (Merck, purity not specified) at 30°C for 15, 30, and 45 minutes. The results of these experiments are shown in Table 5-3. The frequency of mitotic crossing-over in the SBTD strain was dose (exposure time) related. Dose-dependence of mitotic crossing-over in strain D7 is less clear. Dose-related increases in mitotic gene conversion were obtained in both strains. Taken together, these results indicate that diepoxybutane is recombinogenic in yeast, and recombinogenicity is an indication of DNA damage.

Luker and Kilbey (1982) reported that diepoxybutane causes point mutations and multigenic deletions in Neurospora crassa. They developed a Neurospora heterokaryon in which both point mutations and deletions can be detected by the use of selective techniques. Point mutations were scored by reversion to adenine independence. Deletions were detected by first assaying for resistance to p-fluorophenylalanine (pFPA) and then testing for sensitivity to cycloheximide. These two genes are closely linked on chromosome V.

Information on the source and purity of the sample of diepoxybutane studied

TABLE 5-3. INDUCTION OF MITOTIC GENE CONVERSION AND MITOTIC CROSSING-OVER IN SBTD AND D7 STRAINS OF *S. cerevisiae* BY DIEPOXYBUTANE

Exposure to 0.4% diepoxybutane (min)	Survival	Mitotic crossovers (%)	Convertants[a]
SBTD strain			
0	100	0	0.7
15	100	1.4	86.6
30	93.2	2.8	167.1
45	41.3	6.1	515.2
D7 strain			
0	100	0	0
15	100	1.5	40.4
30	78.4	1.7	278.0

[a]Convertants calculated per 10^7 survivors in the SBTD strain and per 10^6 survivors in the D7 strain.

SOURCE: Zaborowska et al., 1983.

was not provided. Suspensions of Neurospora conidia were treated for various times with 0.1 M diepoxybutane in 0.067 M phosphate buffer (pH 7.0) at 27°C. The treatments were terminated by filtering off the mutagenic solution and washing the cells with 10% sodium thiosulfate solution. Diepoxybutane induced dose (exposure time) related increases in both adenine reversions and pFPA resistance, as shown in Table 5-4. The results shown in Table 5-5 suggest that about one-fourth of the pFPA-resistant mutants were deletions rather than point mutations because pFPA resistance was associated with sensitivity to cycloheximide 26.3% of the time. The evidence therefore shows that diepoxybutane induces both point mutations and multigenic deletions in Neurospora.

5.4.3. Studies in Mammalian Cells

Dean and Hodson-Walker (1979) tested diepoxybutane for the induction of chromosomal aberrations in cultured rat liver epithelial-like cells. The sample of diepoxybutane studied was obtained from Fluka A.G., Switzerland. Its purity was not described. The epithelial-like cell line used, designated RL_1, is neardiploid, having a chromosome number of 44 or 45 (compared to the normal number of 42 in the rat karyotype). The appropriate concentrations of diepoxybutane for testing were determined from cytotoxicity studies. Because diepoxybutane is volatile, sealed flask cultures of the rat liver cells were used. The cell cultures were exposed to diepoxybutane for 24 hours, and colcemid at 0.3 μg/mL was added 2 hours before harvesting the cells with 0.25% trypsin. Distilled water was added to the harvested cells to produce hypotonic conditions. The cells were fixed in methanol/acetic acid (3:1). Chromosome preparations were made by air-drying the cells on microscope slides and staining with Giemsa. The slides were randomly coded, and 100 metaphases from each slide were analyzed for structural chromosome changes. The results (Table 5-6) suggest that diepoxybutane is clastogenic in rat liver cells, producing significant chromatid

TABLE 5-4. INDUCTION OF ADENINE REVERSIONS AND pFPA RESISTANCE IN *N. crassa* BY 0.1 M DIEPOXYBUTANE

Treatment (min)	Survival (%)	Heterokaryotic conidia screened ($\times 10^6$) for ad^+	Heterokaryotic conidia screened ($\times 10^6$) for pFPA resistant	Number of mutants scored ad^+	Number of mutants scored fpr^+	Mutation frequency $\times 10^{-6}$ ad^+	Mutation frequency $\times 10^{-6}$ fpr^r
0	100.0	15.64	4.17	5	2	0.32	0.48
10	78.1	6.90	1.38	22	12	3.19	8.70
20	60.4	5.54	1.13	34	21	6.03	18.58
30	40.8	2.53	0.51	53	21	20.95	41.18

SOURCE: Luker and Kilbey, 1982.

TABLE 5-5. ANALYSIS OF $pFPA^r$ MUTANTS AS PUTATIVE DELETIONS

Treatment with 0.1 M diepoxybutane (min)	Number of pFPA-resistant mutants tested	Number acquiring sensitivity to cycloheximide	Putative deletions (%)
10	12	5	42
15	7	2	29
20	41	10	24
30	94	21	22
45	25	10	40
60	11	2	18
Total	190	50	26.3

SOURCE: Luker and Kilbey, 1982.

TABLE 5-6. EFFECTS OF DIEPOXYBUTANE ON CHROMOSOMES OF RAT LIVER CELLS

Diepoxybutane (μg/mL)	Number of cells analyzed	Chromatid gaps (%)	Chromatid deletions (%)	Chromatid exchanges (%)	Chromosome aberrations (%)
0	269	2.6	0.4	0	0.4
0.1	117	11.1	4.3	8.5	0.9
0.5	24	8.3	20.8	33.3	0
1.0	29	0	0	3.4	0

SOURCE: Dean and Hodson-Walker, 1979.

lesions at 0.1 μg/mL. At the higher concentrations, fewer metaphases were apparently available for cytogenetic analysis, perhaps because of cytotoxic effects at these doses.

Perry and Evans (1975) reported that exposure of cultured Chinese hamster ovary cells to diepoxybutane resulted in a dose-related induction in SCEs. The cells were treated with 10 μM bromodeoxyuridene and 0.3 μM or 3 μM diepoxybutane for two cycles of DNA replication before treatment with colcemid (2 hours at 0.2 μM), collection by mitotic shakeoff, pretreatment with 75 mM KCl, and fixation in methanol/acetic acid (3:1). Twenty mitotic cells were scored for each dose. SCEs/cell at 0, 0.3, and 3 μM diepoxybutane were 12.2, 20.2, and 90.9, respectively.

5.4.4. In vivo Studies

Studies of the mutagenic potential of diepoxybutane in whole animals have been carried out in mice and *Drosophila melanogaster* (fruit flies).

Conner et al. (1983) studied the ability of diepoxybutane to induce *in vivo* SCE in bone marrow, alveolar macrophages, and regenerating liver cells in mice. The sample of diepoxybutane studied was 97% pure and was obtained from Aldrich Chemical Co. It was dissolved in phosphate-buffered saline just prior to injection. The dose-response studies were performed in three intact and three partially hepatectomized Swiss Webster mice (mean weight of 27 g). Diepoxybutane (10-291 μmol/kg) was injected intraperitoneally just prior to bromodeoxyuridine infusion (10 mg/mL; intravenous flow rate of 3.6 mL/24 hours). Colchicine (3.3 mg/kg) was then injected intraperitoneally. Bone marrow and alveolar macrophage cells from intact mice and regenerating liver cells from hepatectomized mice were harvested 4 hours later and analyzed for SCEs. As shown in Table 5-7, diepoxybutane produced similar dose-dependent responses for SCE in bone marrow, alveolar macrophages, and regenerating liver cells. Conner

TABLE 5-7. SCE FREQUENCIES INDUCED IN BONE MARROW, ALVEOLAR MACROPHAGES, AND REGENERATING LIVER CELLS OF SWISS WEBSTER MICE FOLLOWING INJECTION OF DIEPOXYBUTANE

Diepoxybutane (μmol/kg)	Bone marrow		Alveolar macrophages		Regenerating liver	
	A[a]	B[b]	A	B	A	B
Hepatectomized mice						
0	4.2 ± 0.6	66	3.5 ± 0.6	77	3.7 ± 0.9	80
10	7.2 ± 0.9	63	7.9 ± 0.4	71	7.0 ± 1.4	73
39	8.1 ± 1.4	72	9.8 ± 1.0	70	11.9 ± 2.6	69
97	10.9 ± 1.5	63	13.4 ± 3.4	75	14.9 ± 4.0	69
193	22.3 ± 3.5	44	23.6 ± 4.5	59	28.9 ± 6.1	43
291	32.0 ± 6.6	22	30.4 ± 4.9	23	31.1 ± 5.9	29
Intact mice						
0	3.0 ± 0.8	64	3.6 ± 0.5	63		
10	5.4 ± 0.6	58	6.7 ± 1.2	54		
39	8.8 ± 0.8	63	10.2 ± 0.3	57		
97	9.7 ± 1.5	63	12.8 ± 2.3	62		
193	14.6 ± 2.1	43	17.1 ± 2.2	41		
291	27.3 ± 3.7	46	28.6 ± 3.8	42		

[a]Mean SCE/cell ± S.D. of three mice at each dose. Individual animal means were calculated from SCEs scored in 20 cells of each type.
[b]Mean number of second division cells observed in 100 consecutive metaphases.

SOURCE: Conner et al., 1983.

et al. (1983) also reported that the DNA lesions did not persist beyond one cell cycle. These results show that diepoxybutane is very effective in producing a dose-dependent SCE response, but that the initially induced lesions disappear in subsequent division cycles.

The sex-linked recessive lethal test in Drosophila, which tests the mutagenic potential of chemicals in the germ line of an intact animal, was positive for diepoxybutane in two studies from the same laboratory. In the first study, Sankaranarayanan (1983) exposed wild-type Berlin-K adult 3- or 4-day-old male flies to 2 mM diepoxybutane in 5% sucrose by feeding for 48 hours. The diepoxybutane sample studied was obtained from Fluka, A.G., Switzerland. Information on its purity was not reported. The males were mated to Oster females to raise three successive 2-day broods (A, B, and C), and the F_1 female progeny were used in the tests for lethals. Brood A tests mature spermatozoa, brood B tests late spermatids, and brood C tests early spermatids. The results are shown in Table 5-8. A concurrent no-exposure control was not done in this study or in the subsequent study described below. However, a historical control value for Drosophila of 0.18% has been established in the same laboratory from the evaluation of 13,151 chromosomes (Vogel, 1976). The results suggest that diepoxybutane is a strong inducer of sex-linked recessive lethal mutations (6.5%, 4.8%, and 4.6% compared to the historical control value of 0.18%). The results also indicate that mature spermatozoa (brood A) respond with higher frequencies of recessive lethals than late and early spermatids (broods B and C).

Similar positive results (Table 5-8) were obtained in a later study in Drosophila (Sankaranarayanan et al., 1983). The experimental details in this study were identical to those described above, except that Canton-S and ebony males were exposed to diepoxybutane and mated to Muller-5 females. These results demonstrate that the strong mutagenic response is independent of the

TABLE 5-8. FREQUENCIES OF SEX-LINKED RECESSIVE LETHALS INDUCED BY 2 mM DIEPOXYBUTANE IN POSTMEIOTIC MALE GERM CELLS OF *D. melanogaster*

Experimental strain	Brood[a]	Number of chromosomes	Lethals	
			Number	%
Berlin-K[b]	A	800	42	6.5
	B	914	32	4.8
	C	840	30	4.6
Canton-S[c]				
Experiment 1	A	934	88	9.4
	B	949	68	7.2
	C	960	66	6.9
Experiment 2	A	938	64	6.8
	B	951	54	5.7
	C	350	14	4.0
Ebony[c]				
Experiment 1	A	932	88	9.4
	B	912	65	7.1
	C	925	56	6.1
Experiment 2	A	876	57	6.5
	B	924	41	4.4
	C	885	33	3.7

[a]Brood A corresponds to treatment of mature spermatozoa; Brood B corresponds to late spermatids; and Brood C corresponds to early spermatids.
[b]Taken from Sankaranarayanan, 1983.
[c]Taken from Sankaranarayanan et al., 1983.

strains employed. The strongly positive results in the sex-linked recessive lethal test provide clear evidence that diepoxybutane reaches the gonads and is strongly mutagenic in germ cells of Drosophila.

There is evidence that diepoxybutane induces chromosome damage in germ cells of Drosophila (Zimmering, 1983). Treated males carried an X chromosome in the form of a closed ring and a Y chromosome carrying dominant markers, one at the end of long arm of the Y and one at the end of the short arm. The males were permitted to feed on a solution of 1.25 mM diepoxybutane in 5% sucrose for 24 hours and then mated with repair-proficient (ordinary) females or to repair-deficient females. There was no evidence of toxicity in the treated males. The F_1 offspring were scored for complete loss of the X or Y chromosomes (in ring-X males, virtually all complete loss is attributable to ring loss) and for partial loss of the Y chromosome, indicated by the loss of one but not both of the Y chromosome markers. Complete loss indicates chromosome breakage and/or sister chromatid exchange. Partial loss of the Y chromosome is a consequence of breakage. Results shown in Table 5-9 provide evidence of a relatively strong effect on complete loss (5-6%) and a significant increase in partial loss which is most apparent from matings with the repair-deficient females (approx. 3%).

In summary, the results of the Drosophila experiments assaying for sex-linked recessive lethals and chromosome loss provide strong evidence that diepoxybutane is a mutagen and a chromosome damaging agent in germ cells of Drosophila. In addition, the results of the SCE assay in mice suggest that diepoxybutane is a DNA damaging agent in mice.

The diepoxybutane was injected intraperitoneally in the mice and fed to the Drosophila. It is possible that the diepoxybutane was biotransformed before reaching mouse bone marrow or Drosophila gonads. Therefore, the mutagenic, clastogenic, or DNA-damaging effects described in these studies may be

TABLE 5-9. CHROMOSOME LOSS IN *D. melanogaster* FROM MATINGS OF MALES WITH REPAIR-PROFICIENT (RP) AND REPAIR-DEFICIENT (RD) FEMALES

Series	Female	N	Complete loss	Partial loss	Percent induced Complete loss	Percent induced Partial loss
Control	RP	8551	51	0		
Treated		7390	515	8	6.37	0.11
Control	RD	3178	30	2		
Treated		1285	82	39	5.44	2.97

All induced frequencies are statistically significant at or below the 0.01 level. The repair-deficient mutant was *mei-9*[a], which is deficient in excision repair.

SOURCE: Zimmering, 1983.

due to a metabolite of diepoxybutane.

5.5. MUTAGENICITY OF 4-VINYL-1-CYCLOHEXENE AND ITS METABOLITES

Under certain conditions, such as during rubber curing, 1,3-butadiene can dimerize to form 4-vinyl-1-cyclohexene (Rappaport and Fraser, 1976). Unpublished data from the National Toxicology Program indicate that 4-vinyl-1-cyclohexene was not mutagenic in the *Salmonella* preincubation assay (strains TA100, TA1535, TA1537, and TA98) in the presence or absence of liver S9 mix prepared from chemically induced rats or hamsters (NTP, 1985b).

In contrast with the negative mutagenicity response of 4-vinyl-1-cyclohexene, its potential mono- and diepoxide metabolites (including 4-vinyl-1,2-epoxycyclohexane, 4-epoxyethyl-1,2-dihydrocyclohexane, and 4-vinyl-1-cyclohexene diepoxide) are mutagenic or clastogenic in various *in vitro* prokaryotic and eukaryotic test systems (Murray and Cummins, 1979; Simmon and Baden, 1980; Turchi et al., 1981; Voogd et al., 1981). These compounds are base-pair substitution mutagens, in agreement with the data for 1,3-butadiene and its potential epoxide metabolites.

5.6. SUMMARY OF MUTAGENICITY STUDIES

The available information on the mutagenicity of 1,3-butadiene is quite limited in that only three studies have been reported. All three studies, however, indicate that 1,3-butadiene is a mutagen in *S. typhimurium*. The weight of the available evidence suggests that 1,3-butadiene is mutagenic only in the presence of a liver S9 metabolic activation system. No whole-animal studies have been reported. These results suggest that 1,3-butadiene is a promutagen in bacteria (i.e., its mutagenicity depends on metabolic activation).

In vitro data suggest that 1,3-butadiene is metabolized to 3,4-epoxybutene and then to diepoxybutane. Preliminary evidence in rats suggests that 1,3-butadiene is metabolized to 3,4-epoxybutene *in vivo*, indicating that the

metabolic pathway outlined on the basis of *in vitro* data may occur *in vivo*.

There is no information on the metabolism of 1,3-butadiene in humans. However, a scientific basis for the extrapolation from animal metabolism data to humans for 1,3-butadiene is provided by the similarities in the epoxidation of the isolated double bond in benzo(a)pyrene by organ and tissue cultures from animal and human sources (Autrup et al., 1980).

3,4-Epoxybutene is a monofunctional alkylating agent, is a direct-acting mutagen in bacteria (*S. typhimurium*, *K. pneumoniae*, and *E. coli*), and induces SCE and chromosomal aberrations in mice. Diepoxybutane is a bifunctional alkylating agent, and as such it can form cross-links between the two strands of DNA. It is mutagenic in bacteria (*K. pneumoniae* and *S. typhimurium*), fungi (yeast and *Neurospora*), and the germ cells of *Drosophila*. It also induces DNA damage in cultured hamster cells and in mice, is clastogenic in fungi and cultured rat cells, and produces chromosome damage/breakage in *Drosophila* germ cells. Therefore, the evidence indicates that 3,4-epoxybutene and diepoxybutane are mutagens/clastogens in microbes and animals.

Exposure of rodents to 1,3-butadiene results in ovarian tumors in mice (Huff et al., 1985) and testicular tumors in rats (Hazleton Laboratories Europe, Ltd., 1981a), which offers suggestive (not sufficient) evidence that 1,3-butadiene (or, more likely, a metabolite of 1,3-butadiene) may reach the germ cells. There is also evidence that the dimer of 1,3-butadiene, 4-vinyl-1-cyclohexene, causes ovarian tumors in mice (NTP, 1985b).

The total body of evidence from the metabolism, mutagenicity, and carcinogenicity data suggests that 1,3-butadiene may present a genetic risk to humans. However, mutagenicity studies in mammalian test systems, as outlined in the U.S. EPA's Proposed Guidelines for Mutagenicity Risk Assessment (1984a), should be conducted to further characterize the mutagenic potential of 1,3-butadiene.

6. CARCINOGENICITY

The purpose of this chapter is to provide an evaluation of the likelihood that 1,3-butadiene is a human carcinogen and, on the assumption that it is a human carcinogen, to provide a basis for estimating its public health impact and evaluating its potency in relation to other carcinogens. The evaluation of carcinogenicity depends heavily on animal bioassays and epidemiologic evidence. However, additional factors, including mutagenicity, pharmacokinetics, and other toxicological characteristics have an important bearing on both the qualitative and quantitative assessment of carcinogenicity. This section presents an evaluation of the animal bioassays, the epidemiologic evidence, and the qualitative and quantitative aspects of risk assessment.

6.1. ANIMAL STUDIES

6.1.1. Chronic Toxicity and Carcinogenicity Studies in Mice

A chronic toxicity and carcinogenicity inhalation study of 1,3-butadiene in B6C3F1 mice, sponsored by the NTP, was conducted at Battelle Pacific Northwest Laboratories. Preliminary inhalation toxicity studies in mice were used as a basis for dose selection for a chronic study. A 15-day study and a 14-week study were conducted at International Bio-Test Laboratories. In the 15-day study, weight loss at airborne concentrations of 1,250 ppm was observed. The mice exposed to 8,000 ppm, the highest airborne concentration, survived the exposure period. In the 14-week study, reduced body weight and death were observed among mice treated at 2,500 ppm or more. Necropsy findings were not reported (NTP, 1984). The changes in the 14-week study at 2,500 ppm indicated that 1,250 ppm is probably a maximum tolerated dose.

The mice used in the chronic study were obtained from Charles River Laboratories and were exposed to graded concentrations of 625 and 1,250 ppm

of 1,3-butadiene for 6 hours/day, 5 days/week. The exposures were conducted in dynamic negative-pressure exposure chambers, and the chamber concentrations were generated by mixing the test gas with filtered air. The chamber concentrations were measured 7 to 12 times a day for the first 150 days with a photoionization detector, and thereafter by gas chromatography. It was intended that the dimer (4-vinyl-1-cyclohexene) concentration in the test material before use should be controlled to less than 100 ppm. However, three cylinders with slightly more than 100 ppm of dimer were used because replacements were not available. The mice were 8 to 9 weeks of age when the exposures began, and were housed individually throughout the study. There were 50 mice per sex per dose group.

The mice were weighed weekly for the first 12 weeks of the study, and monthly thereafter. They were examined for subcutaneous masses beginning after the 12th week. Clinical signs were recorded weekly. Histopathological evaluation (32 tissues) was performed on all mice.

While the original plan was for this to be a 2-year study, all surviving mice were killed after week 60 to 61 because of excessive deaths among the treated mice. Many of these deaths were caused primarily by the developing neoplasia. The survival (mice at risk; corrected for mice that were missing or were accidentally killed) at this early termination was as follows:

	Airborne concentration (ppm)		
	0	625	1,250
Males	49/49	11/50	7/46
Females	46/46	14/47	30/48

There were no increases in clinical signs that could be associated with exposure to 1,3-butadiene except those related to tumor development and death.

The body weights were not affected by inhalation exposure to the test chemical.

There was a marked increase in the overall frequency of mice with primary tumors, as indicated below:

	Airborne concentration (ppm)		
	0	625	1,250
Males	10/50	44/50	40/50
Females	6/50	40/50	46/49

In addition to a marked increase in the number of animals with primary tumors, there was also an increase in the number of animals with multiple primary tumors. Among the tumor-bearing male mice, there were 11, 73, and 61 such tumors in the control, low-, and high-exposure groups, respectively. In the females, there were 6, 66, and 100 tumors in the tumor-bearing animals of the control, low-, and high-exposure groups, respectively.

The histopathologic evaluation indicated significant increases in tumors of various types, as shown in Table 6-1. These tumors began to appear remarkably early in the course of the study. Lymphomas were diagnosed in mice dead at 22 and 20 weeks of exposure for males and females, respectively, of the high-exposure group. The first tumors of this type were found in low-dose mice at 24 and 29 weeks, respectively. Of the survivors, two males in the low-dose group and one in the high-dose group had lymphomas. In the females, one lymphoma was found among the surviving control mice, three in the low-dose group, and one in the high-dose group. Many of the early deaths were judged to be caused by this type of tumor. While this tumor type is sometimes associated with immunosuppression, no evidence of immunosuppression was reported in the histopathologic evaluation of the lymphoid and hematopoietic tissues.

TABLE 6-1. SUMMARY OF THE STATISTICALLY SIGNIFICANT INCIDENCE OF TUMORS IN MICE EXPOSED FOR 60-61 WEEKS TO 1,3-BUTADIENE

Tumor type and site	Sex	Airborne concentration (ppm)		
		0[a]	625[b]	1,250[b]
Hemangiosarcomas (heart)	M	0/50 p=0.032	16/49 p<0.001	7/49 p=0.006
	F	0/49 p=0.001	11/48 p<0.001	18/49 p<0.001
Malignant lymphomas (hematopoietic system)	M	0/50 p<0.001	23/50 p=0.001	29/50 p=0.001
	F	1/50 p<0.006	10/49 p=0.003	10/49 p=0.003
Alveolar/bronchiolar adenoma	M	2/50 p=0.10	12/49 p=0.003	11/49 p=0.007
	F	3/49 p<0.001	9/48 p=0.056	20/49 p<0.001
adenoma/carcinoma	M	2/50 p<0.001	14/49 p<0.001	15/49 p<0.001
	F	3/49 p<0.001	12/48 p=0.010	23/49 p<0.001
Acinar cell carcinoma (mammary)	F	0/50 p=0.007	2/49 p=0.242	6/49 p=0.012
Granulosa cell tumor or carcinoma (ovary)	F	0/49 p<0.001	6/45 p=0.010	13/48 p<0.001
Forestomach (All papilloma and carcinoma)	M	0/49 p=0.354	7/40 p=0.037	1/44 p=0.473
	F	0/49 p<0.001	5/42 p=0.018	10/49 p<0.001
Hepatocellular adenoma	F	0/50 p=0.025	1/47 p=0.485	4/49 p=0.056
adenoma/carcinoma	F	0/50 p=0.016	2/47 p=0.232	5/49 p=0.015

[a]The p-values were calculated with the Cochran-Armitage Trend Test.
[b]The p-values were calculated with the Fisher Exact Test.

The heart was the principal organ in which hemangiosarcomas occurred. The first hemangiosarcomas were diagnosed at 32 and 42 weeks in the low- and high-dose males and at 41 and 43 weeks among the females. The cardiac hemangiosarcomas may have caused some of the mice to die early. Atypical cardiac endothelial hyperplasia, a likely preneoplastic lesion, was not observed among the controls but was present in treated males (625 ppm - 10%, 1,250 ppm - 4%) and females (625 ppm - 10%, 1,250 ppm - 16%).

Alveolar/bronchiolar adenomas and carcinomas occurred (both separately and combined) at increased frequency in both male and female mice. In the high-dose groups the first such lesions appeared at week 42 for males and week 50 for females. Neoplastic changes in the lungs of the controls were not detected until the termination of the study.

Among the 10 control male mice with primary tumors, eight had hepatocellular adenomas and/or carcinomas. This type of tumor is normally observed among male mice of this strain in 2-year bioassays. It may be that the inclusion of mice with this type of tumor in considering the number of tumor-bearing animals tends to deemphasize the frequency of compound-induced neoplasia. On the other hand, among the females, the frequency of hepatocellular adenomas and/or carcinomas was increased. The occurrence of this type of tumor among females of this strain is more suggestive of adverse chemical-related effects. Among the male mice, there was a significant increase in liver necrosis at both doses. In the female mice, liver necrosis was significantly elevated only at the higher airborne concentration.

In addition to the neoplastic changes in the ovary and forestomach, ovarian atrophy and forestomach epithelial hyperplasia were elevated among the mice at both doses. Since Zymbal gland tumors have been reported in the chronic rat study to be discussed, it is worth noting that in this study, one

also occurred in high-dose female mice and two occurred in high-dose male mice. This tumor is not normally found in control mice, even at the termination of a 2-year study. Testicular atrophy was observed in male mice at both dose levels; however, the increase in tumors of the testes that had occurred in the rats did not occur in the mice.

An audit of this chronic study was conducted by the NTP. Potential discrepancies that might have significantly influenced the interpretation of this study were resolved. It should be noted that in 1981 some genetic variation was observed in the male C3H parents of B6C3F1 mice used as test animals. NTP (1984) has noted that the effect of genetic nonuniformity in the hybrid mice on the results is unknown, but that the results were valid because of the use of matched concurrent controls. The NTP considered that this study provided clear evidence of carcinogenicity, which is the highest classification in their system of categorizing evidence of carcinogenicity.

6.1.2. Chronic Toxicity Studies in Rats

A 2-year chronic inhalation toxicity study using rats as the experimental animals was conducted by Hazleton Laboratories Europe, Ltd. (1981a) in England. The study was sponsored by the International Institute of Synthetic Rubber Producers, Inc. As previously discussed, the chronic study was preceded by a 3-month toxicity study. The highest concentration used in that study was 8,000 ppm, which produced only minimal signs of toxicity and moderate salivation in the female rats. Thus, the highest dose in the chronic rat inhalation study was not established as a maximum tolerated dose. In addition, no explanation of the eightfold difference between the low and high dose was offered.

For the chronic investigation (Hazleton Laboratories Europe, Ltd., 1981a), Charles River CD rats (Sprague-Dawley rats obtained from Charles River Ltd.)

were exposed to graded concentrations of 1,000 and 8,000 ppm of 1,3-butadiene. The exposures (6 hours/day, 5 days/week) for 111 and 105 weeks for males and females, respectively, were conducted in a dynamic negative-pressure exposure chamber. The chamber concentrations were generated by mixing the test gas with filtered air. The concencentrations were measured with an infrared gas analyzer. The dimer (4-vinyl-1-cyclohexene) concentrations in the test material before use were less than 1,000 ppm, but samples in the 700 to 800 ppm range were used, and the dimer concentration of these averaged 413 ppm. The rats were 4 1/2 weeks of age when the exposures began, and were housed five to a cage throughout the study. There were 110 rats per sex per dose group, and a similar number of rats exposed to filtered air served as a control group.

The rats were weighed weekly and examined for subcutaneous masses and other clinical signs. Blood chemistries, hemograms, and urine analyses were evaluated at 3, 6, and 12 months. Neuromuscular function was evaluated periodically through week 77 of the study. Ten rats per sex per dose were killed and necropsied at week 52. Histopathological evaluations were performed on all rats from the high-dose group and the control group. Tissues from the low-dose group that were deemed to be of toxicological significance were also examined.

Variations in mean body weight suggested no consistent adverse effect. Review of the hemograms, blood chemistry, urine analysis, and behavioral testing was likewise not indicative of an adverse effect.

In the females of the treated groups, subcutaneous masses appeared earlier and at a higher incidence than in the control group. A dose-related increase in liver weights was observed at the necropsy performed at 52 weeks and at the termination of the study. This could indicate that the chemical induces liver enzymes. Otherwise, no significant changes were noted at the 52-week kill.

In the control group, 45% of the males and 46% of the females survived

until the end of the study (note: corrected for interim kill). In the high-dose group, survival was 32% and 24%, while in the low-dose group 50% and 32% survived. The decreased survival in the high-dose group was statistically significant.

Increased alveolar metaplasia and nephropathy were observed among males of the 8,000-ppm treatment groups at the termination of the study. Marked or severe nephropathy occurred in 27% of the male rats in the high-dose group, as compared with 9 to 10% in the control and the low-dose groups. The authors considered nephropathy to be the cause of some of the early deaths in this study. The frequency of metaplasia was 5/44 in the surviving male rats (8,000 ppm) as compared to 5/45 in the controls.

With regard to the carcinogenic potential of 1,3-butadiene, the authors of this study concluded that exposure of male and female rats under the conditions of this investigation was associated with significant increases in both common and uncommon tumors. Furthermore, they stated that the results of this 2-year inhalation study supported the premise that 1,3-butadiene is a suspect weak oncogen.

The incidence of selected neoplasms is shown in Table 6-2. In the females there was an increase in mammary carcinoma tumors (control - 8%, 1,000 ppm - 42%, 8,000 ppm - 38%). Also, in the females follicular thyroid adenomas were encountered more frequently among the treated females than among the controls (control - 0%, 1,000 ppm - 2%, 8,000 ppm - 8%).

In the males there was an increase in Leydig cell adenomas (control - 0%, 1,000 ppm - 2%, 8,000 ppm - 7%). A single Leydig cell tumor (unspecified) was observed in one male of each exposed group. Exocrine pancreatic adenomas were increased in the male rats of the high-dose group (control - 3%, 1,000 ppm - 1%, 8,000 ppm - 10%). One carcinoma was observed in this tissue in the males of the

TABLE 6-2. SUMMARY OF THE INCIDENCE[a] OF TUMORS IN RATS EXPOSED TO 1,3-BUTADIENE (100 RATS PER SEX PER DOSE GROUP)

Tumor type and site	Sex	Airborne concentration (ppm)		
		0[b]	1,000[c]	8,000[c]
Multiple mammary gland tumors	F	50 p<0.001	79 p<0.001	84 p<0.001
Thyroid follicular (adenoma and carcinoma)	F	0 p<0.001	4 p=0.06	11 p<0.001
Uterine cervical/ stromal sarcoma	F	1 p=0.115	4 p=0.184	5 p=0.106
Leydig cell (adenoma and carcinoma)	M	0 p<0.003	3 p=0.12	8 p<0.001
Pancreatic exocrine				
Carcinoma	M	0	0	1
Adenoma	M	3 p=0.019	1 p=0.879	10 p=0.041
Zymbal gland (carcinoma)	M	0 p=0.384	1 p=0.5	1 p=0.5
	F	0 p=0.037	0	4 p=0.061

[a]Complete information on the number of animals examined was not available. In calculating incidences, it is assumed that 100 animals per sex per group survived the first year and were histologically examined.
[b]The p-values were calculated using the Cochran-Armitage Trend Test.
[c]The p-values were calculated using the Fisher Exact Test.

high-dose group.

Zymbal gland tumors were increased in the high-dose group when male and female rats were combined. However, the increase in Zymbal gland tumor incidence is based on the assumption that all glands were examined, and this is not likely to have been the case. While not statistically significant, there were four gliomas among the high-level males versus one in the controls. Gliomas were present among the exposed mice. Thus, the incidence of Zymbal gland tumors could have been higher than reported. Except for Zymbal gland tumors, the increase in tumors in this investigation was limited to those developed in hormonal-dependent tissues.

The results of the Hazleton (1981a) study have not been published, and the final unpublished report does not include detailed individual histopathological evaluations. While the U.S. EPA's Carcinogen Assessment Group has requested these particular data, they have not been made available. Furthermore, because the report is unpublished, no independent data quality evaluation was performed, and requested data were not available (July 1, 1985). Qualitatively, this investigation shows a positive finding of the carcinogenicity of 1,3-butadiene in the rat. Quantitatively, however, the uncertainty about the numbers of tissues actually examined severely limits its usefulness in animal-to-human risk extrapolation (see section 6.3.).

6.1.3. Carcinogenicity of Related Compounds

Recently, a draft report from the NTP (1985b) on the toxicology and carcinogenicity of 4-vinyl-1-cyclohexene, a dimer of 1,3-butadiene encountered in the offgassing during tire curing, has become available. The test material was administered in corn oil by gavage 5 days/week for 103 weeks to groups of 50 F344/N rats and B6C3F1 mice of each sex. The doses were 200 and 400 mg/kg, and corn oil alone served as a vehicle treatment for control (0 ppm) rats. The

doses were selected on the basis of 14-day and 13-week studies. The end points of survival, body weight gains, and histopathologic effects were the basis for determination of 400 mg/kg as a maximum tolerated dose.

The NTP concluded that there was clear evidence of carcinogenicity of 4-vinyl-1-cyclohexene in female mice as shown by increased ovarian neoplasms at both doses. The incidences of mixed benign tumors among the female mice were 0/49, 25/48 (52%), and 11/47 (23%) in the control, 200, and 400 mg/kg groups, respectively. The incidence of granulosa cell tumor or carcinoma combined was 1/49 (2%), 10/48 (21%), and 13/47 (13%) for the 0, 200, and 400 mg/kg treatments, respectively. In addition, the increased incidence (vehicle control, 0/50; 200 mg/kg, 3/49; and 400 mg/kg, 4/48) of adrenal gland adenomas was judged to be probably related to the test material. With regard to the effects among the male mice, it was concluded that the evidence for carcinogenicity was equivocal based on marginal increases of malignant lymphomas (vehicle control, 3/37; 200 mg/kg, 5/39; 400 mg/kg, 4/7) and alveolar/bronchiolar adenomas combined (vehicle control, 3/37; 200 mg/kg, 5/39; and 400 mg/kg, 3/7). The sensitivity for detecting the carcinogenic response may have been limited, however, by the poor survival at the high dose. The response in the male rats was considered to provide inadequate evidence of carcinogenicity, at least in part because of excessive mortality. Among the female rats, the increased incidence of adenomas or squamous cell carcinomas (combined) of the clitoral gland at the low dose (vehicle control, 1/50; 200 mg/kg, 5/50) despite excessive mortality at the high dose, was considered to provide equivocal evidence of carcinogenicity (NTP, 1985b).

Van Duuren et al. (1963) applied epoxybutene (butadiene monoxide), dl-1,2;3,4-diepoxybutane and meso-1,2;3,4-diepoxybutane three times a week for life to the skin of 30 male Swiss-Millerton mice. The diepoxybutanes were in 10%

acetone. The monoxide was applied undiluted. Ninety mice served as solvent controls, and 207 mice were used as untreated controls. While the combined tumor incidence (papillomas and squamous cell cancer) was elevated only for meso-1,2;3,4-diepoxybutane as compared to the controls (20% vs. 8.9%), the cancer incidence was higher for all three compounds as compared to the controls (butadiene monoxide, 3.3% vs. 0.5%; dl-1,2;3,4-diepoxybutane, 3.3% vs. 0%; and meso-1,2;3,4-diepoxybutane, 13.3% vs. 0%).

In another study, these investigators (Van Duuren et al., 1966) injected dl-1,2;3,4-diepoxybutane in tricaprylin once a week subcutaneously into female Swiss-Millerton mice at doses of 0.1 and 1.1 mg per mouse. No local sarcomas were observed in the controls, while 5/50 and 5/30 mice at the low and high doses, respectively, developed fibrosarcomas. In the same investigation, female Sprague-Dawley rats were injected intraperitoneally with 1 mg of dl-1,2;3,4-diepoxybutane in tricaprylin once a week, and 9/50 developed local fibrosarcomas.

6.1.4. Discussion of Carcinogenicity Studies

The two chronic studies available at this time are compared in Table 6-3. There is an obvious difference between the mice and the rats with regard to carcinogenic response. The mice might be expected to respond more than rats for several reasons. For example, if the carcinogenic response is elicited by a metabolite, as has been suggested (de Meester et al., 1978, 1980), mice, because of their higher rate of metabolism, might be expected to yield a greater response than rats. Furthermore, some of the rats were exposed at the airborne concentrations expected to produce metabolic saturation. In addition, the mice could have had less activity because of group housing, thereby reducing respiration rate.

TABLE 6-3. SIGNIFICANT EFFECTS OF EXPOSURE TO 1,3-BUTADIENE ON SPRAGUE-DAWLEY RATS AND B6C3F1 MICE IN INHALATION STUDIES

	Rats[a] (Hazleton Laboratories Europe, Ltd., 1981a)	
	1,000 ppm	8,000 ppm
Neoplasms:		
Males	Leydig cell adenoma[b]	Leydig cell adenoma[b] Pancreas: exocrine tumors[b] Brain: glioma
Females	Mammary gland: fibroadenoma/carcinoma[b] Thyroid: follicular cell adenoma[b] Uterus: stromal sarcoma[b]	Mammary gland:fibroadenoma/carcinoma[b] Thyroid:follicular cell adenoma[b] Uterus: stromal sarcoma[b] Zymbal gland: carcinoma[b]
Nonneoplastic lesions:		
Males		Increased focal alveolar epithelialization Nephropathy

	Mice[c] (National Toxicology Program, 1984)	
	625 ppm	1,250 ppm
Neoplasms:		
Males	Heart: hemangiosarcoma[b] Malignant lymphoma[b] Lung: alveolar/bronchiolar adenoma and carcinoma[b] Forestomach: papilloma[b] Preputial gland: squamous cell carcinoma[d] Brain: glioma[d]	Heart: hemangiosarcoma[b] Malignant lymphoma[b] Lung: alveolar/bronchiolar adenoma and carcinoma[b] Preputial gland: squamous cell carcinoma[d] Zymbal gland: carcinoma[d] Brain: glioma[d]
Females	Heart: hemangiosarcoma[b] Malignant lymphoma[b] Lung: alveolar/bronchiolar adenoma and carcinoma[b] Forestomach: papilloma[b] Ovary: granulosa cell tumor[b]	Heart: hemangiosarcoma[b] Malignant lymphoma[b] Lung: alveolar/bronchiolar adenoma and carcinoma[b] Forestomach: papilloma[b] Mammary gland: acinar cell carcinoma[b] Ovary granulosa cell tumor[b] Liver: hepatocellular adenoma or carcinoma (combined)[b]
Nonneoplastic lesions:		
Males	Forestomach: epithelial hyperplasia[b] Liver necrosis[b] Testicular atrophy[b]	Forestomach: epithelial hyperplasia[b] Liver necrosis[b] Nasal cavity lesions (chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, atrophy of sensory epithelium[b] Testicular atrophy[b]
Females	Liver necrosis[b] Forestomach: epithelial hyperplasia[b] Ovary: atrophy[b] Uterus: involution[b]	Forestomach: epithelial hyperplasia[b] Ovary: atrophy[b] Uterus: involution[b]

[a]Groups of 100 male and female Sprague-Dawley rats were exposed to air containing 0, 1,000 or 8,000 ppm 1,3-butadiene 6 hours/day, 5 days/week for 105 weeks (female), or 111 weeks (male); survival in dosed groups decreased.
[b]Statistically significant ($p < 0.05$).
[c]Groups of 50 male and female B6C3F1 mice were exposed to air containing 0, 625 or 1,250 ppm 1,3-butadiene 6 hours/day, 5 days/week for 60 weeks (male) or 61 weeks (female); survival in dosed groups decreased and was the reason for early termination.
[d]Considered uncommon at 60 weeks.

SOURCE: National Toxicology Program, 1984.

The higher dimer content of the material to which the rats were exposed might also be expected to contribute to the difference in the effective dose of 1,3-butadiene. In addition, intralaboratory variations in dimer formation in the test atmospheres might be a contributing factor.

The role that the presence of the dimer at higher concentrations in the rat study might have played is unknown. The dimer could have inhibited tumor response by inducing metabolizing enzymes, by competing at active sites, or by inducing competing tumors. The recent draft NTP report (1985b) indicates that in mice the incidence of granulosa cell tumors was elevated in the gavage study with 4-vinyl-1-cyclohexene, as was observed in the mouse inhalation bioassay by NTP (1984). The dimer also induced a marginal increase in malignant lymphomas, and increased alveolar/bronchiolar tumors were present in the mouse inhalation study. The gavaged dimer induced tumors of the clitoral gland. While not reported in the inhalation study, this is a hormonally sensitive tumor, as were most of the tumors in the rat inhalation bioassay.

The tumors in the rats exposed to airborne concentrations of 1,3-butadiene are largely characterized as occurring in hormonal-dependent tissues. Some suggestion of this is observed in the mice, but not to so great an extent. The occurrence of a similar array of tumors in the mice could have been masked by the early deaths from more rapidly developing neoplasia. It is worth noting that Zymbal gland tumors developed in both species, but were not as marked in the mice. Likewise, brain gliomas were found in both test species.

In the NTP mouse inhalation study, hemangiosarcomas of the heart, a very rare tumor, were markedly elevated in both groups exposed to 1,3-butadiene. While other carcinogenic agents have been reported to induce hemangiosarcomas, in most instances these tumors have been found in the spleen or liver.

The positive response of the monoepoxybutene and diepoxybutane metabolites after dermal, subcutaneous, and intraperitoneal administration, supplemented by short-term tests and metabolic information, strongly indicates that 1,3-butadiene acts as a carcinogen by means of metabolic activation.

In summary, the two positive rodent inhalation bioassays provide sufficient evidence that 1,3-butadiene is an animal carcinogen. In addition, related compounds, the dimer and the metabolites, are positive in animal carcinogenic bioassays. This adds to the weight of evidence with regard to the classification of 1,3-butadiene as a carcinogen.

6.2. EPIDEMIOLOGIC STUDIES

The manufacture of styrene-butadiene rubber (SBR) involves the use of, and hence exposures to, several different chemicals. The two major components of SBR polymers are styrene and butadiene. In a typical recipe for the production of SBR, butadiene and styrene account for 26% and 9%, respectively, of the total ingredients. It should be pointed out that water accounts for 63% of the volume. At room temperature styrene is a clear, colorless liquid, while butadiene is a gas.

Two other agents, toluene and benzene, need to be considered, although they are not used directly in the manufacture of SBR. Toluene exposures result from its periodic use as a tank-cleaning agent; it may also exist as an impurity of styrene. Benzene exposures may occur as an impurity of styrene or toluene.

Occupational epidemiologic studies investigating the potential health hazards associated with the production of synthetic rubber have been very limited in number. Because styrene and butadiene are the two basic materials used in the manufacture of SBR, with benzene and toluene as byproducts, it is at best difficult to assess the singular contribution of each. Benzene exposure has been identified with excessive risk, particularly acute leukemia (Linet, 1985).

Styrene also may be a leukemogen in humans (Ott et al., 1980; Lilis and Nicholson, 1976). Although many studies of rubber production workers have been conducted, only a few of those studies are relevant to butadiene exposure. Those studies, which are reviewed here, include studies of workers specifically identified as working in styrene-butadiene production or the manufacture of synthetic rubber. A study was also included if it was a preliminary study to one in which the workers were identified as SBR or synthetic rubber workers, or if it added to the interpretation of one of the studies of SBR or synthetic rubber workers.

6.2.1. McMichael et al. (1974, 1976)

In 1974, McMichael et al. identified, through company records, a historic prospective cohort of 6,678 hourly paid male workers in a rubber tire manufacturing plant in Akron, Ohio. The cohort was composed of all active and retired male employees aged 40 to 84 years as of January 1, 1964.

During the 9-year follow-up period from 1964 through 1972, 1,783 workers died. Death certificates were obtained for 99.5% of these workers, and the causes of death were coded according to the 8th revision of the International Classification of Diseases (ICD), by a National Center for Health Statistics nosologist.

SMRs were calculated for all males using the 1968 U.S. male population as a standard population. SMRs for all causes for the active age range of 40-64 and the full age range of 40-84 were 93 and 99, respectively. In cause-specific SMRs, statistically significant excesses were observed for stomach cancer (SMR = 219, observed = 12, expected = 5.5, $p < 0.01$), lymphosarcoma (SMR = 251, observed = 6, expected = 2.4, $p < 0.05$), and leukemia (SMR = 315, observed = 11, expected = 3.5, $p < 0.001$), in the active age range of 40-64. For the full age range of 40-84, significant SMR increases were observed for cancers of the

stomach (SMR = 187, observed = 39, expected = 20.9, $p < 0.001$), prostate, (SMR = 142, observed = 49, expected = 34.4, $p < 0.05$), lymphosarcoma (SMR = 226, observed = 14, expected = 6.2, $p < 0.01$), diabetes mellitus (SMR = 143, observed = 43, expected = 30, $p < 0.05$), and arteriosclerosis (SMR = 154, observed = 34, expected = 22.1, $p < 0.05$).

McMichael et al. (1976) attempted to evaluate the relationship of these mortality excesses to specific jobs within this plant by designing a nested case-control study. Out of a total of 1,983 deaths observed during the 10-year follow-up period of 1964 through 1973, 455 individuals who had died from certain specific causes were selected as cases. The specific causes of death included stomach, colorectal, respiratory, prostate, and bladder cancers; cancers of the lymphatic and hematopoietic systems; lymphatic leukemias; ischemic heart disease; and diabetes mellitus. Out of these 455 cases, 353 deaths were attributed to cancers and 102 to noncancer causes. From the remainder of the plant population of male workers, an age-stratified random sample of 1,500 individuals, with 500 individuals in each age group of 40-54, 55-64 and 65-84 was obtained. Complete work histories were obtained for 99% (1,482) of this age-stratified sample drawn as a control group.

As of January 1, 1964, the plant population of male workers had a racial composition of 86% white and 14% black. Thirty-eight percent, 30%, and 32% were in the 40-54, 55-64, and 65-84 age ranges, respectively. Forty-eight percent had begun work in the plant at least 25 years prior to 1964, and 99% had worked for at least 10 years by 1964.

Work exposure histories were restricted to the period from 1940 through 1960. Cumulative job exposures of less than 2 years were excluded from the analyses. Because follow-up extended to 1972, the period between first exposure and death could range from 12 to 32 years, which should allow for the observation

of occupationally induced cancers in adults.

For each of the cause-specific mortality groups, as well as the group of controls, rates of exposure for minimum duration of 2 years and 5 years were calculated for each of 16 occupational title groups (OTGs) in order to ascertain any dose-response relationships. The exposure rates in each case group were age-adjusted by the direct method of adjustment to the age distribution of the controls. For the nine cause-specific mortality groups, the ratios of their age-adjusted job classification exposure rates to the rates within the sample of controls were calculated in order to provide an approximation for the more conventional odds ratios.

For all of the causes of death under investigation, there were statistically significant ($p < 0.001$) associations with many of the work areas in which workers had had at least 5 years of exposure. In the synthetic plant area, the significant ($p < 0.001$) risk ratios were 6.2 for lymphatic and hematopoietic cancer, 3.9 for lymphatic leukemia, 3.0 for ischemic heart disease, and 2.2 for stomach cancer. Among the various work areas, the risk ratios for lymphatic leukemia and for lymphatic and hematopoietic cancer were the highest in the synthetic plant. Spirtas (1976) reported, however, that the risk ratio for lymphatic and hematopoietic cancer dropped from 6.2 to 2.4 when a smaller matched control group was used; the statistical significance of the 2.4 risk ratio was not indicated.

McMichael et al. (1976) reported that a case-control study (McMichael et al., 1975) had found an association between lymphatic leukemia and solvent exposure in the rubber industry. Many of the lymphatic leukemia deaths were the same as those reported in the McMichael et al. (1976) study. Further analysis by Checkoway et al. (1984) of 11 of the lymphatic leukemia cases studied by McMichael et al. also found an association between lymphatic leukemia and solvent

exposure. Spirtas (1976) reported that of the six deaths from neoplasms of lymphatic and hematopoietic tissue of individuals who had worked in the synthetic rubber area of the plant in the McMichael et al. (1976) study, three were due to leukemia. Of these three individuals, two had had experience with solvents other than in the synthetic plant. Thus the role of the transfer of individuals from one work area into another needs to be investigated. Also, racial factors could not be accounted for in exposure calculations because data on race were not available for much of the study population at the time of sampling.

6.2.2. Andjelkovich et al. (1976, 1977)

The mortality experience during the period from January 1, 1964 through December 31, 1973 of a historic prospective cohort of 8,938 male rubber workers (known as the "1964 cohort") from a single plant located in Akron, Ohio, was observed by Andjelkovich et al. in 1976. Any person who was 40 years of age or more on January 1, 1964, and was an active or living retired hourly worker from the plant under study, was included in the 1964 cohort.

Data were collected from company records, life insurance death claims, and bureaus of vital statistics of several states. A trained nosologist coded the causes of death according to the 8th revision of the ICD. Follow-up was achieved for 96.7% of the cohort. Out of 8,938 males, 94% (8,418) were white males and were equally distributed in the age groups 40-54, 55-64, and 65-84. Although 6% of the cohort consisted of black males, the major analyses were done on white males. During the 10-year observation period 2,373 (28%) of the white males died. SMRs were calculated using the age-, race-, and sex-specific rates of the 1968 U.S. population. SMRs for deaths from all causes in the 40-64, 65-84, and 40-84 age groups were, respectively, 92 (observed = 619), 95 (observed = 1,754, $p < 0.05$) and 94, (observed = 2,373, $p < 0.01$).

Many cause-specific SMRs showed increases, but only two disease SMRs,

those for neoplasms of lymphatic and hematopoietic tissue (SMR = 138, observed = 40) and chronic rheumatic heart disease (SMR = 170, observed = 16) for the age group 65-84 were statistically significant ($p < 0.05$). The only statistically significant ($p < 0.05$) SMR for the age group 40-64 was for cerebrovascular disease (SMR = 138, observed = 48). On further detailed examination of neoplasms of lymphatic and hematopoietic tissue, statistically significant excesses were found for monocytic leukemia (SMR = 441, observed = 3, $p < 0.01$) and other neoplasms of lymphatic and hematopoietic tissue (SMR = 276, observed = 10, $p < 0.001$) in the age group 65-84. There were no deaths by either of these causes in the age group 40-64.

An important finding of this study is that it found a high mortality rate in workers who had retired between the ages of 40 and 64, the mandatory retirement age being 65. The SMR for all causes for this group was 202, which is highly statistically significant (observed = 299, $p < 0.001$). The SMR for almost every cause analyzed was elevated, and out of 26 categories, 13 of them were statistically significant. For malignant diseases, the authors found significant elevations in SMRs for malignant neoplasms of the prostate (SMR = 278, observed = 4, $p < 0.05$); large intestine (SMR = 231, observed = 6, $p < 0.05$); trachea, bronchus, and lung (SMR = 241, observed = 28, $p < 0.001$); and brain and central nervous system (SMR = 323, observed = 3, $p < 0.05$). In non-malignant diseases, SMRs were statistically significant for 1) benign neoplasms and neoplasms of unspecified nature (SMR = 541, observed = 2, $p < 0.01$); 2) endocrine, nutritional, and metabolic diseases (SMR = 396, observed = 11, $p < 0.001$); 3) diseases of the nervous system and sense organs (SMR = 577, observed = 6, $p < 0.001$); 4) chronic rheumatic heart disease (SMR = 440, observed = 7, $p < 0.001$); 5) ischemic heart disease (SMR = 180, observed = 112, $p < 0.001$); 6) cerebrovascular disease (SMR = 258, observed = 22, $p < 0.001$);

7) other respiratory disease (SMR = 309, observed = 5, $p < 0.01$); 8) diseases of the digestive system (SMR = 357, observed = 16, $p < 0.01$); and 9) symptoms and ill-defined conditions (SMR = 263, observed = 4, $p < 0.05$).

As opposed to these increases, the SMR for deaths from all causes for active workers in the 40-64 age group was 61 (observed = 320), substantially lower than the SMR of 202 for retired workers in this age group. The overall SMR for active and retired workers combined was 92 (observed = 619), showing a dilution effect by the active workers and confirming the "healthy worker" effect. Some cause-specific SMRs were elevated slightly in this active group, but none of them were statistically significant.

In an attempt to evaluate the relationship between the mortality increases and occupational exposures, Andjelkovich et al. (1977) re-analyzed the same data for 28 work areas within the plant under study in 1977. The OTG of each person was decided on the basis of the most representative department (obtained from personnel folders) in which the individual had worked. The closing date for the active workers was December 31, 1973, while for the retired or terminated workers the period of study was from the date of hire to the last date worked.

All causes of death and cause-specific SMRs were calculated by using the experience of the entire cohort as a reference group. Marginal increases in SMRs for all causes were observed for many OTGs for all three of the age groups considered: 40-64, 65-84, and 40-84 years. The only statistically significant excess observed was for cast film manufacture (SMR = 230, observed = 7, $p < 0.05$) in age group 65-84. Statistically significant ($p < 0.05$) SMR deficits from all causes were observed for OTGs: 1) product fabrication (tire and beads), 2) product fabrication (valves, tubes, and flaps), and 3) bulk chemicals and metal products, for at least one or more of the three age groups.

SMR increases were statistically significant for all neoplasms in the following four OTGs: 1) cast film manufacture for age group 40-84, 2) special products manufacture for age group 40-84, 3) milling for age groups 65-84 and 40-84, and 4) miscellaneous for age groups 40-64 and 40-84. Out of these four OTGs, the first three departments dealt with the manufacture of industrial products.

For selected cancers, the SMRs were significantly ($p < 0.05$) elevated in age group 40-84 in certain OTGs, namely, cancer of the stomach in compounding and mixing (SMR = 479, observed = 3), and milling (SMR = 369, observed = 6); cancer of the large intestine in special products (SMR = 629, observed = 4); cancers of the trachea, bronchus, and lung in synthetic latex (SMR = 434, observed = 3); cancer of the prostate (SMR = 212, observed = 10); and leukemia (SMR = 246, observed = 6) in general services. All SMRs, except the one for the compounding and mixing department, showed statistically significant excesses of deaths in more than one age group.

For non-malignant diseases, Andjelkovich et al. (1977) calculated significantly ($p < 0.05$) elevated SMRs for diabetes mellitus, acute myocardial infarction, arteriosclerosis, and suicide in various OTGs for various age groups. Significant excesses of deaths in the general service department and arteriosclerosis in the shipping and receiving department were observed in more than one age group. Statistically significant deficits were observed for ischemic heart disease in the industrial chemicals department and acute myocardial infarction in the stock preparation department, both in the 40-84 age group.

Since the authors were aware that the job transfer patterns of the deceased workers were not necessarily representative of the job transfer patterns of the entire cohort, they used the available information on deceased workers to estimate the length of time spent in the most representative department. A simple

random sample of 50 deceased workers was chosen, and detailed work histories were reviewed for them. The 50 workers had spent an average of 28.3 years in the industry. On an average, each worker had spent 50% of his work time in his most representative OTG. However, the fraction of time spent in the most representative OTG ranged from less than 10% up to 100% of total employment duration.

The Andjelkovich et al. studies have a number of limitations. First, their use of the most representative departments should be questioned in view of the fact that the people under study could have worked in these departments from 10% to 100% of their total employment duration. The only elevated SMR in synthetic latex was for cancer of the trachea, bronchus, and lung, based on only three deaths, while there was no control for cigarette smoking, which is a potential confounder. Another limitation was the use of 1968 mortality data for trachea, bronchus, and lung cancer to calculate the expected number of deaths. Mortality for trachea, bronchus, and lung cancer was rising sharply during the period 1964 to 1973, the follow-up period of this study. The use of 1968 data may have underestimated the expected number of deaths and thus overestimated the SMR. Although a statistically significant excess of deaths for cancers of lymphatic and hematopoietic tissue was observed for persons whose most representative department was general services, this job category does not necessarily involve contact with SBR production. With regard to the questionable exposure, Taulbee et al. (1976) reported that in an analysis of the work histories of 37 leukemia cases and four matched controls per case from the 1964 cohort of Andjelkovich et al., none of the cases was found to have worked in the OTG "synthetic plant." Some of the cases had worked in departments in which there may have been exposure to the synthetic process, but this association was not statistically significant ($p < 0.05$), nor was the association

found to increase by duration of exposure.

One positive aspect of the Andjelkovich et al. (1977) study is that the entire cohort is used as a reference group, which should reduce the "healthy worker effect" and allow for a more unbiased evaluation. It would have been interesting, however, to see the comparison of SMRs calculated from the U.S. population (1968), which was used as a standard population in the 1976 study, with the SMRs calculated from the internal cohort as a reference group.

Both the McMichael et al. and the Andjelkovich et al. studies are suggestive of some health problems in the synthetic plant, indicating that specific exposure investigations should be undertaken.

6.2.3. Checkoway and Williams (1982)

Since the study of McMichael et al. (1976) indicated the potential presence of carcinogens in SBR plants, Checkoway and Williams conducted a combined industrial hygiene and hematology cross-sectional survey at the same plant studied by McMichael et al. The objectives of the Checkoway and Williams study were to quantify workplace exposures to styrene, butadiene, benzene, and toluene, and to relate exposure levels to hematologic measurements.

During the week of May 15-19, 1979, personal breathing-zone air samples were collected with both a charcoal tube and a passive diffusion dosimeter during the day and evening shifts for seven different departments. The departments were: 1) tank farm, 2) reactor and recovery, 3) latex blending and solution, 4) shipping and receiving, 5) storeroom, 6) factory service, and 7) maintenance areas. Sampling periods ranged from 4 to 6 hours. Charcoal tubes were changed at intervals of 1 to 2 hours during the sampling period to avoid overloading.

Blood samples of male hourly production workers for the same departments were obtained on 4 separate days, May 15-18. Of the 163 workers (26-65 years

of age with a median age of 45 years; 144 whites and 19 blacks) who participated in the industrial hygiene survey, 154 (135 whites and 19 blacks) also participated in the blood survey. Because of work scheduling demands, blood samples were collected from participants from each of the departments on all 4 days, thereby minimizing any bias due to day effect. The hematological parameters measured included red cell count (RBC), hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration, reticulocyte count, platelets, total leukocytes (WBC), and differential distributions of neutrophils, neutrophil band forms, eosinophils, basophils, monocytes, and lymphocytes. Medical histories were obtained by means of questionnaires. Data from persons who reported positive histories of either malignant disease, radiation therapy, or current anemia of known etiology were excluded from the analyses. Only one individual, who reported a history of leukemia, was excluded from the study.

The mean 8-hour time-weighted averages and ranges show that all four chemical exposures were well below the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs) recommended at that time. The TLVs in parts per million (ppm) for butadiene, styrene, benzene, and toluene are 1,000, 100, 10, and 100, respectively. With the exception of butadiene and styrene, for which time-weighted averages of 20.03 ppm and 13.67 ppm, respectively, were observed in the tank farm area, the mean levels for the four chemicals in all other departments were less than 2 ppm. Even the maximum concentration of benzene, the most strongly suspected leukemogen of the four chemicals analyzed, was less than 1 ppm in all plant departments.

With regard to the hematologic survey, there were generally no associations ($p > 0.05$) of hematologic values with chemical exposures. Red blood cell count was negatively associated with butadiene and styrene exposure, while basophil

count was positively related to the aforementioned chemicals, as measured by Pearson product moment analysis. The negative association of styrene with erythrocyte counts and the positive association of the basophil proportions with butadiene persisted after controlling for age and medical status in stepwise multiple linear regression analyses. However, there were curious opposing findings for mean corpuscular hemoglobin concentration--a positive relationship to butadiene and a negative association with styrene.

Mean hematologic mesurements adjusted for age and medical status were compared for tank farm area workers and all other workers. The tank farm workers had slightly lower levels of circulating erythrocytes, hemoglobin, platelets, and neutrophils, in addition to mean corpuscular red cell volumes and neutrophil band circuits that were slightly higher than those of the other workers.

This study was undertaken to quantify exposure levels and to find out if there is any evidence of hematopoietic toxicity in relation to these exposure levels. With the exception of the tank farm area, the average exposures to the four chemicals assayed were uniformly less than 2 ppm; even in the tank farm area, the styrene and butadiene concentrations were considerably lower than the recommended ACGIH TLVs, although they were considerably higher than in other workplaces studied.

Because this study is cross-sectional in design, it is very limited with regard to determining whether styrene-butadiene exposure is carcinogenic. Individuals in the plant who may have developed cancer probably left the work force and hence were not available for blood sampling. The industrial hygiene survey findings cannot be generalized to the past, since the concentrations may have differed quantitatively as well as qualitatively. It can be concluded that there was no pronounced evidence of hematologic abnormality in this study population.

6.2.4. Meinhardt et al. (1982)

Meinhardt et al. (1982) reported on a retrospective cohort mortality study conducted by NIOSH at two adjacent SBR facilities in eastern Texas. This study was motivated by the report of two men who had worked at both plants, and who had died of leukemia in January 1976.

Personnel employment records documenting the employment of 3,494 workers from plant A and 2,015 from plant B were available since January 1943 and January 1950, respectively, to the study cutoff date of March 31, 1976. The study cohorts from plants A and B consisted of 1,662 and 1,094 white males who had had at least 6 months of non-management and non-administrative employment, respectively. The average lengths of employment for the study cohort in plants A and B were, respectively, 9.48 and 10.78 years.

At the time of the study, environmental samples were obtained at each plant. At plant A, time-weighted average exposures of styrene, butadiene, and benzene were 0.94 ppm (0.03-6.46), 1.24 ppm (0.11-4.17) and 0.10 ppm (0.08-0.14) respectively. For plant B, time-weighted average exposures for styrene and butadiene were 1.99 ppm (0.05-12.3) and 13.5 ppm (0.34-174.0), respectively (benzene was not measured). No historical monitoring data were available for either plant.

The study cohorts from plants A and B accounted for 34,187 and 19,742 person-years at risk of dying. It is also important to note that the survival status of 54 individuals (3.25%) from cohort A and 34 individuals (3.11%) from cohort B was unable to be determined. In subsequent analyses, these individuals were considered to be alive.

The age, race, sex, calendar time, and cause-specific mortality rates of the U.S. population were applied to the appropriate strata of person-years at risk in order to obtain the expected number of cause-specific deaths in the

study populations. Differences in observed and expected numbers of deaths were evaluated by a test statistic based on the Poisson distribution.

In cohorts from plants A and B, observed and expected numbers of deaths were compared for the following cause-specific categories: tuberculosis; malignant neoplams (including cancers of the lymphatic and hematopoietic tissues); all other cancers; diseases of the nervous, circulatory, respiratory, and digestive systems; accidents; and all other causes. With the exception of mortalities from cancers of the lymphatic and hematopoietic tissues, there were deficits (in some instances, striking deficits) in the cause-specific SMRs for the study cohorts in both the plants.

With regard to the total number of deaths due to all causes, cohorts A and B had observed numbers of deaths of 252 (SMR = 80) and 80 (SMR = 66), respectively. Although it is possible that the "healthy worker" effect may, in part, explain these deficits, the relative magnitudes of the deficits, particularly for plant B, suggest that there may have been an underreporting of deaths or that selection factors in the choice of the study groups reduced the mortality rate.

Meinhardt et al. observed that all five of the individuals from plant A whose underlying cause of death was leukemia began employment before the end of December 1945. This date corresponds to the time when the batch process for SBR production was converted to a continuous-feed operation. The decision was made, therefore, to evaluate the mortality experience of those 600 white male employees who had had at least 6 months of employment in plant A between January 1, 1943 and December 31, 1945. This subgroup was employed for an average of 11.9 years, and had an accumulation of 17,086 person-years at risk of dying. The survival status of 34 individuals (5.7%) was unknown. As in the previous mortality analyses, with the exception of deaths from cancers of the lymphatic and hematopoietic tissues, there generally were large deficits in the cause-

specific observed number of deaths. For malignant neoplasms of lymphatic and hematopoietic tissues, the SMR was 212 (9 observed, 4.25 expected, $0.05 < p < 0.1$); for lymphosarcoma and reticulosarcoma the SMR was 224 (3 cases, 1.34 expected, $p > 0.05$); for Hodgkin's disease the SMR was 213 (1 case, 0.47 expected, $p > 0.05$); and for leukemia and aleukemia the SMR was 278 (5 cases, 1.80 expected, $0.05 < p < 0.1$). The total number of observed deaths due to all causes was 201 (SMR = 83, 242.09 expected, $p < 0.05$). For cohort B, there were no significant ($p > 0.05$) excesses of mortality from any cause. Deaths from all malignant neoplasms (SMR = 53, observed = 11, expected = 20.78, $p < 0.05$) and "all other causes" (SMR = 54, observed = 9, expected = 16.80, $p < 0.05$) were significantly decreased, however.

The authors calculated the likelihood of detecting a doubling and a quadrupling of the expected occurrence of leukemia for cohort A, subcohort A, and cohort B. These likelihoods were 26% and 88% for cohort A, 20% and 77% for subcohort A, and 13% and 51% for cohort B. Thus, this study suggests that some component of the SBR manufacturing process may be a leukemogen.

6.2.5. Matanoski et al. (1982)

This study was performed to determine whether there are health risks associated with the production of synthetic rubber, specifically styrene-butadiene rubber (SBR). The populations studied were obtained from seven U.S. and one Canadian rubber plants. The study population consisted of males who had worked for more than one year, and whose records contained birth dates and employment dates. In addition, workers selected had been employed by the facility from the date of the facility's first SBR production to December 1976. The total number of people available from the eight plants surveyed was 29,179, of whom 13,920 (48%) met the criteria for selection.

Data obtained from the personnel records of each facility included employee

name, Social Security number, job history, date(s) of employment, birth information, death information, and limited data on retirees. Individual workers' jobs were coded according to first job, last job, and job held longest during the period of employment. For the analyses, jobs were categorized in four general work areas: production, utilities, maintenance, and other. In three plants (plants 3, 4, and 5), race classification was unknown for 176 (85%), 329 (50%), and 4,540 (98%) of the cohort populations, respectively. Plants 3 and 4 were expected to have employed black workers; plant 5 had employed few or no blacks at any time in its history. In all, 7,209 (52%) of the study population were unable to be classified racially. If race was not specified, individuals were assumed to be white males.

Follow-up activities to determine vital status of the study population in the seven U.S. plants included searching by the Social Security Administration, tracing through motor vehicle administration records, and contacts by telephone. Through these follow-up activities, 42% of the study population were traced. For the Canadian plant, follow-up was performed by searching the company insurance plan records for death benefit information. Determination of vital status for the study populations revealed 10,899 workers alive, 2,097 known dead, and 924 lost to follow-up. It was determined from a 10% sample in each plant that about 4% of the study population assumed to be living was actually dead. Thus, as many as 440 deaths, or 17% of the total possible deaths, might have been missed. For the populations who were known dead, 90% of the death certificates requested were received. Death certificates were coded according to the 8th revision of the ICD.

Most of the statistical analyses were done using the worker records from the time when record-keeping systems became complete, or any time thereafter through December 1976. Females, workers employed less than one year, and those

with unknown birth dates or employment dates were omitted. The total eligible population numbered 13,608. SMRs for the workers as compared to the general population were calculated by using a modified version of U.S. Death Rates Programs (Monson, 1979). SMRs were calculated separately for the white population and the black population, and the ratios were combined to correct for differences in age, race, and calendar time. The total number of deaths occurring among the eligible study population was 1,995, with an SMR of 81. The study cohort accounted for 250,000 person-years.

Power calculations were performed to test the ability of the data to determine increases in risk of 0.1, 0.25, 0.5, and 2 times greater than the U.S. population. The calculations showed a 100% probability of detecting a twofold increase in all causes of death and in all cancers, and an 89% probability of detecting a 25% increase in lung cancer.

The average period of follow-up was 19 to 20 years. The average age at death was 61 years. The overall SMR for all causes of death was 81, with SMRs of 98 for blacks and 78 for whites. The low mortality among members of this population is, in part, a reflection of the "healthy worker" effect. However, a question must also be raised as to the effect of the 440 possible deaths that may have been missed. Furthermore, the large difference in SMRs for blacks and whites may be due to an undercounting of blacks and an overcounting of whites. This is further exemplified by similar patterns in SMRs for all accidents, motor vehicle accidents, and suicides. The SMRs for all causes of death do not appear to increase with duration of employment but do appear to increase with increasing follow-up period.

The leukemias found in the population included nine acute, five chronic, and three unspecified on the death certificate, with a median latent period of 17 years from time of first employment. The types found were not considered

to have a distribution remarkably different from that found in the general population. The study population as a whole did not demonstrate leukemia in excess.

None of the analyses demonstrated significant increases in SMRs for other specific causes in the total study population. Black males appear to have a significantly elevated risk of arteriosclerotic heart disease ($p < 0.05$), but this value may be artificially inflated due to undercounting of the blacks, resulting in a smaller denominator. Vascular lesions of the central nervous system were also in excess in black males, but they were not statistically significant.

Increases in mortality according to job classifications are noteworthy, but only two of them are statistically significant ($p < 0.05$). The SMR for testicular cancer among maintenance workers is 294 (observed = 3, $p < 0.05$). SMRs for esophagus, stomach and large intestine, and larynx cancers are elevated in the utilities and maintenance work areas. The SMR for larynx cancer in utilities workers is statistically significant (SMR = 476, observed = 4, $p < 0.05$). Hodgkin's disease is associated with high SMRs in all work areas. Very few high SMRs are found for the production area.

It should be further pointed out that, with the exceptions of Hodgkin's disease and stomach cancer, all of the cancers had relatively long latent periods. For all cancers, half of the individuals had had 12 years of employment or more.

In addition to complete ascertainment of deaths and racial distribution, further investigation is needed in order to obtain information specific to various jobs and the associated exposures of individuals in those jobs. It would also be useful to separate the number of years employed within each job category, in order to determine the periods of possible exposure in the work-

place. Moreover, changes in the SBR process, in plant design, and in worker practices should be given greater attention in the evaluation of mortality for the four worker categories studied.

Other methodologic limitations of this study include the fact that less than 50% of the total population of eight plants was studied. This raises questions about the population that was excluded due to lack of birth dates or employment dates. This may have been an older population, which probably had longer exposure and was therefore more likely to suffer from occupational diseases. Out of eight cohorts, only 50% were followed from 1943, whereas in the rest of the plants, follow-up starting dates ranged from 1953 to 1970. It is probable that the employees from the latter plants were not followed long enough for malignancies to develop.

6.2.6. Summary of Epidemiologic Studies

McMichael et al. (1974) found significant ($p < 0.05$) excess mortality from cancers of the stomach, prostate, and lymphatic and hematopoietic system as well as from diabetes mellitus, arteriosclerosis, and ischemic heart disease in their historic prospective cohort study. To evaluate these excesses, McMichael et al. (1976), in a case-control study, investigated exposure rates for various jobs within the same rubber plant for several cause-specific deaths. The data indicated that the most probable health risks were of prostate cancer in janitoring and trucking; bladder cancer in milling and reclaiming operations; lymphatic and hematopoietic cancers in the synthetic plant; and lymphatic leukemia in the synthetic plant, inspection-finishing-repair, and tread cementing. Nonmalignant mortality excesses included ischemic heart disease in the synthetic plant and tread cementing and diabetes mellitus in janitoring and trucking and inspection-finishing-repair.

Andjelkovich et al. (1976) carried out a similar kind of study (historic

prospective cohort) in white males in a single rubber plant, and observed significantly ($p < 0.05$) increased SMRs for malignant neoplasms of lymphatic and hematopoietic tissues (monocytic leukemia and other neoplasms of lymphatic and hematopoietic tissue), chronic rheumatic heart disease, and cerebrovascular disease. They also observed high SMRs ($p < 0.001$) for all causes and for most of the cause-specific deaths for a group of workers who had retired between the ages of 40 and 64. Andjelkovich et al. (1977) evaluated these excesses in mortality ratios in relation to various work areas by using the entire cohort as a reference group, and found that only malignant neoplasms of the trachea, bronchus, and lung were associated with the synthetic latex department. This finding was based on only three observed deaths, however, and no smoking data were taken.

Checkoway and Williams (1982) carried out an industrial hygiene and hematology cross-sectional survey at the same plant in which McMichael et al. had conducted their case-control study. With the exception of the tank farm area, in which 8-hour time-weighted averages for butadiene and styrene were observed to be 20.3 ppm and 13.67 ppm, respectively, all other departments had mean exposure levels of less than 2.0 ppm. No evidence of hematologic abnormality was noted. Because of its cross-sectional design, however, this study could not be expected to identify an excess cancer risk.

Meinhardt et al. (1982), conducted a retrospective cohort mortality study at two plants in Texas. Male rubber workers in one of the plants (plant A) were followed from January 1, 1943 through March 31, 1975; employees in the other plant (plant B) were followed from January 1, 1950 through March 31, 1976. These intervals of observation are important to note because the two plants changed from a batch process for SBR production to a continuous-feed operation in 1946. With regard to cancers of the lymphatic and hematopoietic

system and lymphatic leukemia, plant A exhibited excess mortalities, although these were not statistically significant ($p > 0.05$); plant B did not show any mortality excesses. When the mortality experience in plant A was analyzed further for those workers who had had at least 6 months of employment between January 1, 1943, and December 31, 1945 (the interval for which the batch process was used), excess mortalities for the above-mentioned cancers were shown to be of borderline statistical significance ($0.05 < p < 0.01$, two-sided). It is also of interest to note that all of the employees from the total cohort of plant A whose causes of death were cancers of the lymphatic and hematopoietic tissues had been employed between 1943 and 1945. Had the analysis in plant A commenced with the date of first employment in 1946, the SMRs in question would have been reduced to zero, and the lack of excess mortality would have been similar to plant B.

Matanoski et al. (1982) also conducted a retrospective cohort mortality study in which eight SBR plants were involved. As with the cohort in plant B investigated by Meinhardt et al., there was a general lack of excess mortalities. It should also be noted that half of the cohort was followed from 1943 to 1979. The date of entry for the remaining half of the cohort ranged from 1953 to 1976, with follow-up terminating in 1979.

Although both the McMichael et al. (1976) and the Meinhardt et al. (1982) studies found some evidence of an association between styrene-butadiene exposure and lymphatic and hematopoietic cancer, confounding due to exposures to solvents cannot be ruled out for either study. In addition, the results from the Meinhardt et al. study for the subcohort employed during the batch process of production were only of borderline ($0.05 < p < 0.1$, two-sided) significance, and a possibly serious underascertainment of deaths, and/or selection factors in the choice of the study group may have biased the results.

The Andjelkovich et al. (1977) study found an association between employment in the synthetic part of the plant with mortality from cancer of the trachea, bronchus, and lung. The association was based on only three deaths, however, and there was no control for smoking.

The study by Matanoski et al. of almost 14,000 styrene-butadiene production workers found no excesses of cancer mortality that were statistically significant ($p < 0.05$). Again, however, a possibly serious underascertainment of deaths may have biased the results. An undercounting of blacks in the study population may also have resulted in a potential bias.

The epidemiologic evaluation of SBR workers with regard to the carcinogenicity of 1,3-butadiene is particularly difficult because styrene may also be a carcinogen and, in particular, a leukemogen (Ott et al., 1980; Lilis and Nicholson, 1976). Because of the inconsistency of the results from different studies, the possible confounding due to solvent and styrene exposures, and the potential for bias in some of the studies, the epidemiologic data are considered inadequate for determining a causal association between 1,3-butadiene exposure and cancer in humans.

6.3. QUANTITATIVE ESTIMATION

This section deals with the incremental unit risk for 1,3-butadiene in air, and the potency of 1,3-butadiene relative to other carcinogens that the CAG has evaluated. The incremental unit risk estimate for an air pollutant is defined as the excess lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 ppm or 1 $\mu g/m^3$ of the agent in the air they breathe. This calculation estimates in quantitative terms the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other, and 2) to

give a crude indication of the population risk that might be associated with air exposure to these agents if the actual exposures were known. Hereafter, the term "unit risk" will refer to incremental unit risk.

The development of the quantitative estimation section will be to first describe the procedures, assumptions, and uncertainties involved in animal-to-human extrapolation (section 6.3.1.), and then to use these procedures in the actual calculation of unit-risk estimates, (section 6.3.2.). These estimates will then be used with the available human data to determine if the extrapolated animal values actually predict the human response (section 6.3.3.). Finally, the carcinogenic potency of 1,3-butadiene will be compared with the potencies of other compounds that the EPA has evaluated as known or suspect human carcinogens.

6.3.1. Procedures for Determination of Unit Risk

The data used for quantitative estimation are taken from one or both of the following: 1) lifetime animal studies, and 2) human studies where excess cancer risk has been associated with exposure to the agent. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses, with an incidence determined by an extrapolation model.

There is no solid scientific basis for any mathematical extrapolation model that relates carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. For practical reasons, such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies.

The linear nonthreshold model has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship (U.S. EPA,

1984b). The incremental risk estimates made with this model, and the corresponding 95% upper-limit incremental unit risks, should be regarded as conservative, representing the most plausible upper limits for the risk, i.e., the true risk is not likely to be higher than the estimates, but it could be lower.

The mathematical formulation chosen to describe the linear nonthreshold dose-response relationship at low doses is the linearized multistage model. The multistage model employs enough arbitrary constants to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment. This procedure effectively linearizes the model at low doses. Thus, the multistage model, described below, is fitted to the data in the observational or experimental range. The fit of the curve allows for a linear term which dominates the risk estimate at low doses. The 95% upper limit, q_1^*, described below, is technically an upper-limit estimate on the linear term, but, practically, functions as the upper-limit low dose-response function.

6.3.1.1. Description of the Low-Dose Extrapolation Model--Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_1 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0,\ i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[-(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d.

The point estimate of the coefficients q_i, $i = 0, 1, 2, \ldots, k$, and consequently, the extra risk function, $P_t(d)$, at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL83, Howe (1983). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^*, on parameter q_1. Whenever $q_1 > 0$ at low doses, the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1 \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk, and R/q_1^* is a 95% lower confidence limit on the dose, producing an extra risk of R. Both the model and the curve-fitting methodology used, including the step-down procedure for eliminating the highest dose groups, are discussed in detail by Anderson et al. (1983). For the Hazleton female rat inhalation study, the multistage model did not fit the full data set and the highest dose group was dropped.

When all of the higher-order terms in the multistage model are zero except for the linear term, the multistage model reduces to the one-hit model, which

is a true low-dose linear nonthreshold model. As will be seen with the NTP male mouse data and the Hazleton female rat data, this is the case with 1,3-butadiene.

For cases of partial lifetime exposure where time-to-tumor or time-to-tumor death is known, Crump and Howe (1983a) have developed the multistage model to include a time-dependent dose pattern. The form of this model is one which is linear in dose and in which time has a power and form determined by both the number of assumed stages and the stage affected by the carcinogen. A best fit is determined by the method of maximum likelihood in the ADOLL183 computer program (Crump and Howe, 1983b). Application of this program to the NTP mouse data was unsuccessful because of lack of convergence when attempting to extrapolate from the 60-61 week study to the normal 104-week treatment period.

6.3.1.2. Calculation of Human Equivalent Dosages from Animal Data--Following the suggestion of Mantel and Schneiderman (1975), it is assumed that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, surface area is proportional to the two-thirds power of weight, as would be the case for a perfect sphere, the dose in mg/day per two-thirds power of the weight is also considered to be equivalent dose. In an animal experiment, this equivalent dose is computed in the following manner:

Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e), and

W = average weight of the experimental animal

The lifetime dose is then

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

1,3-Butadiene is slightly soluble in water and can be considered a partially soluble vapor. The dose in m = mg/day of partially soluble vapors is proportional to the O_2 consumption, which in turn is proportional to $W^{2/3}$ and is also proportional to the solubility of the gas in body fluids, which can be expressed as an absorption coefficient, r, for the gas. Therefore, expressing the O_2 consumption as $O_2 = k\ W^{2/3}$, where k is a constant independent of species and V = mg/m^3 of the agent in air, it follows that:

$$m = k\ W^{2/3} \times v \times r$$

or

$$d = \frac{m}{W^{2/3}} = kvr$$

In the absence of experimental information or a sound theoretical argument to the contrary, the absorption fraction, r, is assumed to be the same for all species. Therefore, for these substances (including 1,3-butadiene) a certain concentration in ppm or μg/m^3 in experimental animals is assumed equivalent to the same concentration in humans. This is supported by the observation that the minimum alveolar concentration necessary to produce a given "stage" of anesthesia is similar in man and animals (Dripps et al., 1977). It is further

supported by the results of the NTP absorption study in mice and rats (1985a) discussed in Chapter 4.

As shown previously, since both the NTP mouse and Hazleton rat cancer bioassays used inhalation exposure in terms of 1,3-butadiene concentrations in the air, the direct ppm-animal-to-ppm-human exposures are appropriate for low exposure concentrations. However, as shown in section 4.1., the absorption fraction, r, is not constant for the high external concentrations presented to the animals in the bioassays, but decreases with increasing concentration. Thus, the procedure for determining animal-to-equivalent-human dose must be adjusted to account for the fact that at high concentrations the internal dose (in mg/kg) is not directly proportional to the external concentration. The method of adjustment used is to first calculate the function for the incremental cancer risk to the animal based on internal dose (in mg/kg for within-species standardization), then convert back to risk for low-dose ppm equivalents in the animal. Since low-dose conversions from animals to humans for 1,3-butadiene are on a ppm equivalence in the air, the final animal risk in units of ppm will also be that for humans. This is done because there are no human data on external concentration to internal dose.

In order to convert to internal dose in animals, the results of the NTP absorption study of butadiene in male rats and mice via inhalation (1985a) are used. These results, presented in Table 4-3 as μmol/kg of butadiene retained, are first transformed to μg/kg retained for each of the exposure concentrations--70, 930, and 7,100 ppm for the rats and 7, 80, and 1,040 ppm for the male mice. Responses for the high-dose rat group and middle-dose mouse group were adjusted upward by the factor 12/11 to standardize to a 6-hour exposure. Log μg/kg retained vs. log ppm exposure concentration curves were fitted and these curves were used to estimate the internal (retained) dose of the

mouse and rat cancer bioassays. The estimated internal doses for the mice are 25.7 mg/kg (474 μmol/kg) at 625 ppm and 38.9 mg/kg (719 μmol/kg) at 1,250 ppm exposure concentration for 6 hours. For the rat data the estimated internal doses are 10.5 mg/kg (195 μmol/kg) at 1,000 ppm and 37.1 mg/kg (685 μmol/kg) at 8,000 ppm exposure concentration for 6 hours. Thus, the mice exposed to 625 ppm actually received more than twice the internal dose, on a mg/kg basis, than did the rats exposed to 1,000 ppm. Even the mice exposed to 1,250 ppm received a larger internal dose than the rats exposed to 8,000 ppm.

6.3.1.2.1. Adjustments for less than lifetime duration of experiment. When analyzing quantal data, if the duration of experiment L_e is less than the natural lifespan of the test animal L, the slope q_1^*, or more generally the exponent g(d), is increased by multiplying by a factor $(L/L_e)^3$. We assume that if the average dose d had been continued, the age-specific rate of cancer would have continued to increase as a constant function of the background rate. The age-specific rates for humans increase at least by the second power of the age and often by a considerably higher power, as demonstrated by Doll (1971). Thus, it is expected that the cumulative tumor rate would increase by at least the third power of age. Using this fact, it is assumed that the slope q_1^*, or more generally the exponent g(d), would also increase by at least the third power of age. As a result, if the slope q_1^* [or g(d)] is calculated at age L_e, we expect that if the experiment had been continued for the full lifespan, L, at the given average exposure, the slope q_1^* [or g(d)] would have been increased by at least $(L/L_e)^3$. This correction is used for extrapolation from the NTP mouse study, which was terminated after 60-61 weeks instead of running for a full 2 years.

For time-to-tumor data, this adjustment is also conceptually consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor

model considered by Crump (1979), where the probability of cancer by age t and at dose d is given by

$$P(d,t) = 1 - \exp[-f(t) \times g(d)]$$

It is also consistent with the partial lifetime exposure extension of the multistage model developed by Crump and Howe (1983a), which as discussed above is linear in dose, but has a power function of time.

6.3.1.3. Interpretation of Quantitative Estimates--For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification regarding carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent as compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex, and by the same route of exposure, preferably inhalation.

The quantitative aspect of carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, e.g., setting regulatory priorities, evaluating the adequacy of technology-based

controls, etc. However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. Because of the limited data available from animal bioassays, which is at the high dose levels required for testing, almost nothing is known about the true shape of the dose-response curve at low environmental levels. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk; i.e., it is not likely that the true risk would be much more than the estimated risk, but it could be considerably lower. The risk estimates presented in subsequent sections should not be regarded as accurate representations of the true cancer risk even when the exposures are accurately defined. The estimates presented may, however, be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

6.3.1.4. Alternative Models--The methods used by the CAG for quantitative extrapolation from animal to man are generally conservative, i.e., tending toward high estimates of risk. The most important part of the methodology contributing to this conservatism is the CAG's use of the linearized multistage nonthreshold extrapolation model. There are a variety of other extrapolation models that could be used, most of which would give lower risk estimates. Among these alternative models, two which are currently popular and which often tend to give different low-dose extrapolations from the multistage model are the log-Probit and Weibull models. These models have not been used in the following analyses because the data do not warrant it. As discussed below, all models are of limited value for predicting low-dose risks for 1,3-butadiene based on the mouse responses, which were greater than 60% at the lowest dose tested. With respect to the rat study, even though low-dose response was less than that of the mouse, the quality of the study, and its results, have not been peer-reviewed or published. Furthermore, complete

individual pathology information was not available. Thus, the results of the extrapolation procedure are limited by the actual data bases.

6.3.1.5. Internal Dose vs. External Concentration--Pharmacokinetic modeling would be a very useful way to predict the internal concentration of the compounds (parent and metabolites) of interest in this case. To do such modeling, certain basic *in vivo* data are necessary. From the present data outlined here, it is not possible to do such modeling.

Even a simple one-compartment classical model would need data regarding blood/plasma concentrations of at least the parent compound. At this time these data are not available. Several other pieces of data are also required to construct such a model with confidence. For example, elimination of the unmetabolized parent compound via the kidneys and lungs would need to be established.

However, it appears after reviewing possible metabolic paths of this 1,3-butadiene that a physiologically based pharmacokinetic model is in order. Such a model could adequately describe the disposition of the parent compound and its metabolites in several organs of interest. It would consider many physiological parameters and would rationally consider scale-up to humans so that the internal concentrations predicted could be used in risk assessment.

Further, risk assessment based on the internal concentration of dose retained, as is done here, does not consider the possibility that more than one metabolite is being formed. It is possible that not all of these metabolites are toxic and that relative concentrations may be changing in a manner different from the total labeled materials. Therefore, while the total retained labeled materials may be increasing, the amount of toxic metabolite may increase only to some saturated level. Thus, such a scenario may wrongly estimate the amount of toxic metabolite(s) within the animal tissues. A physiologically

based pharmacokinetic model that could consider metabolites and differentiate mobile metabolites from incorporated metabolites would be extremely useful.

To develop such a physiologically based model would require much more *in vivo* data than are currently available. Examples of needed information include organ concentrations at various times during and after exposure, arterial and venous blood concentrations, renal and pulmonary clearance rates, and binding information. Also, the labeled materials would have to be characterized to discriminate between the parent compound and its metabolites. It might not be necessary to identify the metabolites, but it would be useful to determine which portion of metabolites is mobile and which portion is incorporated into the biomolecules of the various organs and tissues.

The formulation of such a model would improve the data base used for bettering risk assessment. These types of models can, with proper verification, provide reliable estimates of internal concentrations, which may also be resolved at organ level.

6.3.2. Calculation of Quantitative Estimates

Human studies have provided inadequate evidence for the carcinogenicity of 1,3-butadiene. The major weakness is lack of good 1,3-butadiene exposure information. Additionally, concurrent exposure to several other possible carcinogens also limits the use of these studies as primary sources for calculating quantitative risk estimates. For animal-to-human extrapolation, there are two suitable animal bioassays, the NTP mouse study and the Hazleton rat study, both showing significant carcinogenic response. As discussed above and in previous sections, the rat bioassay has deficiencies limiting its use as the primary data set for animal-to-man extrapolation. Nevertheless, it will be compared with the results of the mouse bioassay for the purposes of sensitivity analysis. The mouse study will be considered as the primary study.

6.3.2.1. Mouse-to-Human Extrapolation--The NTP (1984) mouse inhalation study showed highly statistically significant increases ($p < 0.01$) both in hemangiosarcomas of the heart and malignant lymphomas and in both males and females at 625 ppm and 1,250 ppm. Both of these tumor types are life-threatening and appeared quite early in the study. As discussed at length in the qualitative section and shown in Table 6-1, several other tumor sites were also significantly increased in the study, which was stopped at 60-61 weeks due to high mortality from tumors in the treated groups.

Using the continuous equivalent dosages, maximum likelihood and 95% upper-limit incremental, unit risk estimates were calculated from both the male and female mouse data. For the male mice, the fractions of animals either with tumors at significantly increased sites, or with tumors considered unusual for 60 weeks (preputial gland squamous cell carcinomas and Zymbal gland carcinomas, see Tables 6-1 and 6-3) were 2/50, 43/49, and 40/45 for the control, 625-ppm, and 1,250-ppm groups, respectively. For the females, the fractions of animals with significantly increased tumors or brain gliomas were 4/48, 31/48, and 45/49. Animals that died before the first tumor was seen (at 20 weeks) were eliminated. These results are presented in Table 6-4, with internal doses multiplied by 5/7 to determine an average daily dose. Also shown are the maximum likelihood estimates (MLE) and the 95% upper-limit incremental unit risk estimates (q_1^*) based on these data. The initial upper-limit estimates based on the 60-week (male) and 61-week (female) studies are then adjusted to project for natural lifetime risk (see section 6.3.1.2.1.). The final estimates are $q_1^* = 6.1 \times 10^{-1}$ (mg/kg/ day)$^{-1}$ for the males and $q_1^* = 3.0 \times 10^{-1}$ (mg/kg/day)$^{-1}$ for the females.

TABLE 6-4. MOUSE: CALCULATION OF CANCER RISK BASED ON INTERNAL DOSES AND CONVERSION TO LOW PPM CONCENTRATIONS. FRACTIONS (%) OF MICE (NTP, 1984) WITH AT LEAST ONE OF THE STATISTICALLY SIGNIFICANT INCREASED TUMORS OR TUMORS CONSIDERED UNUSUAL AT TIME OF TERMINAL SACRIFICE.[a] ALSO, MLE AND 95% UPPER-LIMIT INCREMENTAL UNIT RISK ESTIMATES BASED ON THE LINEARIZED MULTISTAGE MODEL

	Males		Females	
Nominal exposure (ppm)	Equivalent continuous internal dose[b] (mg/kg/day)	Number with tumors/ number examined[c]	Equivalent continuous internal dose (mg/kg/day)	Number with tumors/ number examined[c]
0	0	2/50 (4%)	0	4/48 (8%)
625	18.4	43/49 (88%)	18.4	31/48 (65%)
1,250	27.8	40/45 (89%)	27.8	45/49 (92%)

[a]See Tables 6-1 and 6-3 for tumor sites.
[b]Interpolated daily internal doses x 5/7 since treatment was 5 days per week for a lifetime.
[c]Examined for either hemangiosarcomas or lymphomas, eliminating animals that died prior to 20 weeks.

Initial maximum likelihood estimates:

Males

$q_0 = 0.042$
$q_1 = 9.35 \times 10^{-2}$ (mg/kg/day)$^{-1}$

Females

$q_0 = 0.086$
$q_1 = 0$
$q_2 = 3.0 \times 10^{-3}$ (mg/kg/day)$^{-2}$

Initial estimates of 95% upper-limit:

$q_1^* = 1.17 \times 10^{-1}$ (mg/kg/day)$^{-1}$

$q_1^* = 6.03 \times 10^{-2}$ (mg/kg/day)$^{-1}$

Adjustment factor for early sacrifice:

$(104/60)^3 = 5.21$

$(104/61)^3 = 4.96$

Final estimate of 95% upper-limit in (mg/kg/day)$^{-1}$:

$q_1^* = 6.1 \times 10^{-1}$ (mg/kg/day)$^{-1}$

$q_1^* = 3.0 \times 10^{-1}$ (mg/kg/day)$^{-1}$

Final estimate of 95% upper limit in (ppm)$^{-1}$: 1 ppm = 1.5 mg/kg/day

$q_1^* = 9.2 \times 10^{-1}$ (ppm)$^{-1}$

$q_1^* = 4.5 \times 10^{-1}$ (ppm)$^{-1}$

Geometric mean of 95% upper-limit:

$$q_1^* = 6.4 \times 10^{-1} \text{ ppm}^{-1}$$

This risk estimate from animal internal dose must be converted back to units of $\mu g/m^3$ and ppm. The conversion factor at 25°C and atmospheric pressure is

$$1 \text{ ppm} = \frac{\text{M.W. 1,3-butadiene}}{1.2 \times \text{M.W. air}} = 1.2 \times \frac{54.1}{28.8} \text{ mg/m}^3$$

To convert from mg/kg/day internal dose in the mouse to low exposure ppm in the mouse, the following additional information is needed: (1) the air volume intake of a mouse per day, and (2) the percentage of 1,3-butadiene absorbed by the mouse at low exposure levels. For a 35-g mouse, the volume intake is estimated as

$$I = 0.0345\ (0.035/0.025)^{2/3}\ m^3/\text{day} = 4.3 \times 10^{-2}\ m^3/\text{day}$$

In order to determine the percentage of 1,3-butadiene absorbed at low levels, the NTP (1985a) absorption study must be used (see Table 4-3). For mice exposed to 13 $\mu g/L$ (7 ppm equivalent atmospheric exposure), the absorption rate was estimated as 54%. Assuming that linear kinetics hold under unsaturated conditions, the mice at lower concentrations will also absorb 54%. In any event, use of this figure will not cause a large underestimate of the risk.

Putting this information together, the conversion for the mouse for 1 ppm continuous exposure is: 1 ppm = 2.25 $(mg/m^3) \times 0.54 \times 4.3 \times 10^{-2}\ (m^3/\text{day}) \times 1/0.035$ kg = 1.5 mg/kg/day as the internal dose. The final risk conversion for the male mouse is

$$q_1^* = 6.1 \times 10^{-1}\ (\text{mg/kg/day})^{-1} \times 1.5\ \frac{\text{mg/kg/day}}{\text{ppm}} = 9.1 \times 10^{-1}\ (\text{ppm})^{-1}$$

and for the female mouse is

$$q_1^* = 3.0 \times 10^{-2}\ (\text{mg/kg/day})^{-1} \times 1.5\ \frac{\text{mg/kg/day}}{\text{ppm}} = 4.5 \times 10^{-1}\ (\text{ppm})^{-1}$$

Since these male and female mouse data sets are so comparable, the geometric mean, $q_1^* = 6.4 \times 10^{-1}\ (\text{ppm})^{-1}$, was chosen as the final 95% upper-limit incremental unit risk estimate.

6.3.2.2. Rat-to-Human Extrapolation--Rats exposed to 1,3-butadiene in the Hazleton (1981a) inhalation study also developed tumors at multiple sites (see Table 6-2). Among animals surviving the first year, the fractions of male rats with at least one of Leydig cell tumors, pancreatic exocrine tumors, and/or Zymbal gland carcinomas were 4/100, 4/100, and 21/100 for control, low-, and high-exposure groups. For females with mammary gland carcinomas only, thyroid follicular tumors, and/or Zymbal gland carcinomas, the corresponding fractions were 8/100, 46/100, and 50/100. All of the increase in mammary gland tumors were carcinomas, not adenomas. These figures are shown in Table 6-5 which presents the corresponding extrapolation results from the rat to those produced from the mouse.

The internal doses for the rat estimated from the radiolabeled material collected in the NTP absorption study (1985a) is estimated as 10.5 mg/kg and 37.1 mg/kg for the low and high exposure concentrations, respectively. These doses were multiplied by 5/7 to determine daily equivalent continuous doses of 7.5 mg/kg/day and 26.5 mg/kg/day. Other calculations are similar to those for the mouse data. The final 95% upper-limit incremental unit cancer risk estimate for the male rat is $q_1^* = 7.0 \times 10^{-3}\ (\text{mg/kg/day})^{-1}$, and for the female rat is $q_1^* = 9.4 \times 10^{-2}\ (\text{mg/kg/day})^{-1}$. The 95% upper-limit estimates for the female mouse and female rat based on internal dose are within a factor of three of

TABLE 6-5. RAT: CALCULATION OF CANCER RISK BASED ON INTERNAL DOSES AND CONVERSION TO LOW PPM CONCENTRATIONS. FRACTION OF RATS (HLE, 1981a) WITH AT LEAST ONE OF THE SIGNIFICANT TUMORS.[a] ALSO, MLE AND 95% UPPER-LIMIT INCREMENTAL UNIT RISK ESTIMATES BASED ON THE LINEARIZED MULTISTAGE MODEL

	Males		Females	
Nominal exposure (ppm)	Equivalent continuous internal dose[b] (mg/kg/day)	Number with tumors/ number examined[c]	Equivalent continuous internal dose[b] (mg/kg/day)	Number with tumors/ number examined[c]
0	0	4/100	0	8/100
1,000	7.5	4/100	7.5	46/100
8,000	26.5	21/100	26.5	50/100[d]

[a]See Table 6-2, Table 6-3, and text for tumor sites.
[b]Interpolated daily internal doses x 5/7 since treatment was 5 days per week for a lifetime.
[c]Approximate number surviving first year and examined histologically.
[d]The highest dose group was dropped because of the poor fit of the model.

Maximum likelihood estimates:

Males

$q_0 = 0.035$

$q_1 = 0$

$q_2 = 2.8 \times 10^{-3}$ (mg/kg/day)$^{-2}$

Females

$q_0 = 0.083$

$q_1 = 7.1 \times 10^{-2}$ (mg/kg/day)$^{-1}$

95% upper-limit estimate in (mg/kg/day)$^{-1}$:

$q_1^* = 7.0 \times 10^{-3}$ (mg/kg/day)$^{-1}$ $\qquad$ $q_1^* = 9.4 \times 10^{-2}$ (mg/kg/day)$^{-1}$

Final estimate of 95% upper-limit in (ppm)$^{-1}$: 1 ppm = 0.6 mg/kg/day

$q_1^* = 4.2 \times 10^{-3}$ (ppm)$^{-1}$ $\qquad$ $q_1^* = 5.6 \times 10^{-2}$ (ppm)$^{-1}$

each other; the 95% upper-limit estimate based on the male rat is significantly less than the other estimates.

The final 95% upper-limit estimates, q_1^*, in units of $(ppm)^{-1}$, are converted similarly to those for the mouse with the exception that the daily volume for a 700 g rat must be estimated. This is

$$I = 0.105\ (0.700/0.113)^{2/3}\ m^3/day = 0.354\ m^3/day.$$

In order to determine the percentage absorbed at low levels, the same 54% used for the mouse was used for the rat. Table 4-3 shows that mice and rats exposed to similar concentrations (in $\mu g/L$) retained very similar percentages of the inhaled material. Therefore, it seems reasonable to assume that if the lowest rat exposure was as low as the lowest mouse exposure, the rat would have retained about the same 54% of the dose as did the mouse.

The final conversion from internal to low exposure external concentration then becomes

$$1\ ppm = 2.25\ (mg/m^3) \times 0.54 \times 0.345\ m^3/day \times 1/0.700\ kg = 6.0 \times 10^{-1}\ mg/kg/day$$

and the final 95% upper-limit incremental unit risk conversion based on the male rat is

$$q_1^* = 7.0 \times 10^{-3}\ (mg/kg/day)^{-1} \times \frac{6.0 \times 10^{-1}\ mg/kg/day}{ppm} = 4.2 \times 10^{-3}\ (ppm)^{-1}$$

and for the female rat is

$$q_1^* = 9.4 \times 10^{-2}\ (mg/kg/day)^{-1} \times \frac{6.0 \times 10^{-1}\ mg/kg/day}{ppm} = 5.6 \times 10^{-2}\ (ppm)^{-1}$$

In comparison with the mouse data, Table 6-6 shows estimates based on the female mouse is about eight times higher than those for the female rat. For the males, the corresponding estimates were some 200 times higher for the mouse than for the rat.

TABLE 6-6. COMPARISON OF THE ANIMAL-TO-HUMAN UPPER-LIMIT INCREMENTAL UNIT CANCER RISK ESTIMATES FOR THE MOUSE AND THE RAT BASED ON EXTERNAL CONCENTRATIONS

	Upper-limit q_1^* $(ppm)^{-1}$	
Sex	Mouse[a]	Rat[b]
Male	9.2×10^{-1} $(ppm)^{-1}$	4.2×10^{-3} $(ppm)^{-1}$
Female	4.5×10^{-1} $(ppm)^{-1}$	5.6×10^{-2} $(ppm)^{-1}$
Geometric mean	6.4×10^{-1} $(ppm)^{-1}$	---[c]

[a]Final estimates, Table 6-4.
[b]Final estimates, Table 6-5.
[c]Geometric mean not taken because male and female rat tumor responses were not similar.

6.3.3. Comparison of Human and Animal Inhalation Studies

The purpose of this section is to evaluate whether or not the animal-to-man extrapolated estimate of 1,3-butadiene-caused cancer is reasonably borne out by human data. The section considers the limited human data base and determines to what extent extrapolation from the positive animal data might overestimate the human response.

While rat and especially mouse exposures to 1,3-butadiene caused a broad spectrum of cancers, human response associated with the SBR process was neither extensive nor consistent across studies. Various cohorts displayed excess

mortality from cancers of the stomach or intestine, prostate, and/or respiratory system. The most consistent excesses (and, therefore, the focus of this section) appear to be restricted to cancers of the lymphatic and hematopoietic systems, cancers which include leukemias, Hodgkin's disease, and lymphosarcomas.

It must be emphasized that exposure to 1,3-butadiene alone cannot be isolated from exposure to several other potential carcinogens. Always associated with the SBR process is concurrent exposure to styrene, a compound for which there is limited evidence of carcinogenicity in animals and inadequate evidence in humans (IARC, 1982). The small amount of human evidence associated with styrene exposure and cancer suggests an association with leukemia and, possibly, lymphomas. Styrene, like 1,3-butadiene, metabolizes to an epoxide; both epoxides are the suspected carcinogens. (Ethylene oxide, also an epoxide, is also associated with leukemias.) In addition to styrene, the SBR process involves numerous other exposures concurrent with 1,3-butadiene. These concurrent exposures will not be dealt with in the following analysis, because if the animal risk extrapolation based on 1,3-butadiene alone overestimates the human risk, then the animal risk extrapolation will most likely be too high.

Probably the strongest evidence for human cancer associated with the SBR process is that of Meinhardt et al. (1982), in which workers exposed to the high-temperature batch polymerization process from 1943 through 1945 showed a marginally significant increase in cancers of the lymphatic and hematopoietic tissues, with an SMR of 212 from 9 deaths out of 600 study members. For workers first exposed after the process was changed to continuous feed in 1946, with correspondingly less exposure, no deaths from lymphopoietic system cancers occurred among more than 1,000 study members. Unfortunately, no exposure estimates are available for the pre-1946 cohort. For the cohort exposed after 1946, only 1,3-butadiene measurements taken after 1975 are available. They

show an 8-hour time-weighted average mean concentration of 1.24 ppm butadiene (± 1.20 standard deviation [SD]), 0.10 ppm benzene (± 0.035 SD), and 0.94 ppm styrene (± 1.23 SD). (Benzene is not used in SBR manufacturing, but may be present as an impurity of styrene or toluene.) The Meinhardt et al. (1982) study also contained an analysis from a second plant whose workers were first exposed in 1950. Based on a cohort of 1,094, the SMR for cancers of the lymphatic and hematopoietic tissues was 78, slightly higher than the overall SMR of 66, the latter being significantly ($p < 0.05$) less than that of the comparable general population. Meinhardt et al. reported average 1,3-butadiene exposure levels in 1977 of 13.5 ppm.

The next strongest evidence for cancer associated with the SBR process is based on the case-control study of McMichael et al. (1976). These authors estimated an age-standardized risk ratio of 6.2 for lymphatic and hematopoietic cancers among workers with at least 5 years of exposure in the synthetic plant, relative to all other workers as controls. (This ratio decreased to 2.4 when a matched control analysis was used.) The synthetic plant is where the SBR process is located. McMichael et al. also found a dose-related risk ratio in the synthetic plant by number of years worked there.

Estimates of exposures in the McMichael et al. study are based upon a later paper. Checkoway and Williams (1982) measured 1,3-butadiene, styrene, benzene, and toluene levels at the same synthetic plant in which McMichael et al. (1976) found that "leukemia and lymphoma (cases) among hourly paid rubber workers from one company were 6 times more likely than controls to have worked at jobs in the SBR plant." Exposure levels of 1,3-butadiene typically averaged below 1 ppm, but exposure levels in the tank farm area averaged 20 ppm.

The most extensive investigation specifically designed to study the health effects of the SBR process shows very little association of 1,3-butadiene with

lymphatic and hematopoietic tissue cancer (ICD 200-207). The Matanoski et al. (1982) study of one Canadian and seven U.S. synthetic rubber plants showed, possibly, a trend with more exposure as defined by production, maintenance, utilities, or other jobs, but none of the SMRs (Table 6-7) are statistically significant. Only Hodgkin's disease (ICD 201), shows a consistently high SMR in all three cohorts, but the numbers of cases were small. No exposure estimates were presented in the Matanoski et al. report, but the plants studied were of the same type as those studied by Meinhardt et al. (1982). Some workers in seven of the eight plants might have started as early as 1943, and Matanoski states (personal communication) that the batch process in several of these plants was continued into the early 1970s. Based on these observations, the estimates of 4 ppm for production workers, 3 ppm for maintenance workers, and 1 ppm for utility workers have been used for calculation purposes. It is emphasized, however, that none of the estimates are based on contemporary measurements.

A February 1985 review draft of this document presented exposure estimates for the Meinhardt early (1943-45) Plant A cohort and for the Matanoski study that were five times as high as the present estimates. These exposure estimates have been reduced based on comments by industry representatives to EPA's Science Advisory Board that actual human exposures in the epidemiologic studies were considerably lower, both because of the olfactory threshold and 1,3-butadiene's explosive properties. Accordingly, estimates for these two study groups were reduced to be more in line with these comments. Estimates for the other Meinhardt groups remain the same since these were measured concentrations in the plants. The uncertainty of all these estimates should be stressed. Any increase or decrease in exposure estimates causes a porportional change in predicted cancers.

TABLE 6-7. SUMMARY OF CANCER OF LYMPHATIC AND HEMATOPOIETIC TISSUES ASSOCIATED WITH THE STYRENE-BUTADIENE SYNTHETIC RUBBER PROCESS. CONFIDENCE LIMITS ON ACTUAL SMRs PREDICTED EXCESS DEATHS BASED ON INITIAL MLE INCREMENTAL UNIT RISKS OF THE MOUSE DATA

Study	Sample size	Cancer type	Observed	Expected	SMR	95% Confidence intervals	Excess deaths from exposure predicted from mouse data (observed-expected)[a]	Power to detect predicted deaths (α=.0.05)[b]	Comments
Meinhardt et al. (1982) Plant A 1943-1945	600	Lymphatic and hematopoietic tissues ICD (200-205)	9	4.25	2.12	0.96-4.02	0.9 (4.75)*[c]	6%	Estimates from 1943-1945: 4 ppm from 1946-1952: 2 ppm
Plant A 1946-1976	1,062	Same as above	0	1.54	0	0-2.34	0.6 (-1.54)	7%	Average exposure = 1.24
Plant B 1950-1976	1,094	Same as above	2	2.55	0.78	0.09-2.83	6.3 (-0.55)**	96%	Average exposure = 13.5
Matanoski et al. (1982) (by job last held) Production	3,269	All lympho-poietic ICD (200-207)	11	10.6	1.04	0.52-1.86	10.0 (0.4)*	72%	Same types of plants as those studied by Meinhardt et al. Assume: 4 ppm (estimate)
Maintenance	3,683	Same as above	13	16.2	0.80	0.47-1.37	5.2 (-3.2)*	22%	2 ppm (estimate)
Utilities	550	Same as above	4	6.5	0.62	0.17-1.58	0.3 (-2.5)	2%	1.0 ppm (estimate)
McMichael et al. (1976)		All lym-phatic and hematopoietic (200-209)	--	--	6.2 (risk ratio in synthetic plants 2.4 in matched controls	No further estimates possible	--	--	Increases asso-ciated with com-pounding, mixing, cement mixing, inspection-finishing-repair, synthetic plant. Average exposure < 1 ppm except for tank farm area (20 ppm)

[a]Test for observed cancers not significantly different from predicted (Poisson test or normal approximation to the Poisson).
[b]α = 0.05, power determined by formula $Z_{1-\beta} = Z_{\alpha} - 2\,(SMR^{0.5} - 1)E^{0.5}$ (Beaumont and Breslow, 1981).
[c]More deaths predicted than observed.
*$p < 0.05$.
**$p < 0.01$

Is the unit risk estimate based on the mouse tumor data a reasonable extrapolation? To answer this question we must be able to estimate the predicted effect based on actual (or estimated) exposure. (We must also assume the effect on these cancers from other exposures to be nil.) For illustration, we choose the Meinhardt et al. (1982) study plant B, with estimated exposure of 13.5 ppm. Although this exposure was measured in 1977, we will consider it to be representative of exposures from 1950 through 1976. We must also know that the 1,094 study members converted to 19,742 person-years at risk for an average of 18 years per person. Since average employment or exposure is given as 10.78 years, we can estimate the expected contribution as follows:

1. The continuous lifetime equivalent exposure based on 10 working years out of about 50 possible remaining years is:

$$13.5 \text{ ppm} \times \frac{10.78}{50} \text{ years} \times \frac{240}{365} \text{ day} \times \frac{8}{24} \text{ hours} = 0.64 \text{ ppm}$$

2. The inital best estimate (MLE) of risk based on the average male and female mouse data for a 1 ppm continuous exposure for approximately 40/70 of a lifetime is $q_1 = 2.5 \times 10^{-2}$ $(\text{ppm})^{-1}$.* This geometric mean MLE incremental risk estimate will be used to predict human excess deaths for the present purpose of deciding whether the number of deaths among industrial workers is consistent with the expected deaths derived using the animal extrapolation procedure. The initial estimate (Table 6-4) is used since it projects the limited animal

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*From Table 6-4: $[(9.35 \times 10^{-2}) \times (3.0 \times 10^{-3})]^{1/2} \times 1.5 = 2.5 \times 10^{-2}$ $(\text{ppm})^{-1}$. This estimate is valid only in the 1 ppm exposure range. (An arithmetic mean yields an estimate nearly three times as high.)

observation period (60-61 weeks out of a 2-year lifetime) to the limited human observation period (average of up to 29 years out of 50 years remaining lifetime).

3. Based on the MLE* unit risk factor $q_1 = 2.5 \times 10^{-2}$ $(ppm)^{-1}$, each of the 1,094 workers would be expected to have an additional lifetime risk of

$$R = 2.5 \times 10^{-2} (ppm)^{-1} \times 0.64 = 1.6 \times 10^{-2}$$

4. Based on 1,094 workers at risk for 18 of their 50 remaining years, this converts to the following expected excess number of cancers:

$$1.6 \times 10^{-2} \times 1{,}094 \times \frac{18}{50} = 6.3$$

5. Adding 6.3 to the 2.55 cancer deaths expected based on no exposure (Table 6-7), we could expect, with the exposure, to observe 8.8 deaths from cancers of the lymphatic and hematopoietic tissues. The probability of observing two or fewer deaths with 8.8 expected is

$$P\ (\text{deaths} \leq 2 | \lambda = 8.8) = \sum_{x=0}^{2} \frac{e^{-\lambda} \lambda^{x}}{x!} = 0.0073$$

or $p < 0.01$.

The statistical power to detect a predicted SMR of (8.8/2.55) or 3.5 is given by Beaumont and Breslow (1981) as $Z_{1-\beta} = Z_{\alpha} - 2\ (SMR^{0.5} - 1)E^{0.5}$. For

plant B this is

$$Z_{1-\beta} = 1.96 - 2\ (3.5^{0.5} - 1)\ (2.55)^{0.5} = -1.75$$

which corresponds to a power of 0.96, or 96% at $\alpha = 0.05$. This means that the study was powerful enough ($p = 0.96$) to detect the 6.3 predicted deaths at the $p = 0.05$ level.

Results based on similar calculations are presented in Table 6-7 for the other two Meinhardt cohorts and for three Matanoski cohorts. They show inconsistent results. For the 1943-1945 plant A Meinhardt cohort, the deaths predicted from animal extrapolation actually underpredict the observed human response ($p < 0.5$). For one other Meinhardt cohort and the Matanoski utilities cohort, the predicted and observed results are not significantly different, although the power to detect the predicted difference in these three cases is low--7% or less. For the two larger Matanoski cohorts, the extrapolated deaths do significantly ($p < 0.05$ in both cases) overpredict response. However, if as suggested in the review of this study, that underascertainment might have missed approximately 17% of the deaths, then neither of these results from the two larger Matanoski cohorts would have been statistically significant.

The interpretation of these results, if we dismiss momentarily the large uncertainties in the exposure estimates, is that the predicted deaths are consistent with the observations. In fact, no predicted results will satisfy the observed results in all six cohorts. Were we to lower the risk estimates to try to better accommodate the Meinhardt Plant B cohort, we would further underpredict the observed results for the Meinhardt early plant A cohort. Based on the information we have, no single extrapolated value can predict the human response. Considering the uncertainties in the human exposure data, and the

ascertainment in the Matanoski study cohort, the estimate based on animal extrapolation is consistent and the best that can be achieved at present.

Finally, the same analysis as computed for lymphatic and hematopoietic cancers in Table 6-7, can be done for all cancers on the theory that 1,3-butadiene might be a broad-spectrum carcinogen in humans as it is in mice. This analysis is presented in Table 6-8. Since we have used the same extrapolation from the mouse data, the same number of excess deaths as predicted in Table 6-7 will result, but these excess deaths are spread out over all cancers. It should be noted that only one of the SMRs is statistically significant and that all, in fact, are less than one. Based on the predicted excess deaths extrapolated from animal data and estimated exposures, two of the cohorts experienced significantly fewer deaths than predicted. For one of these cohorts, the Meinhardt plant B, the deficit in observed versus expected deaths was significant even if there were no predicted deaths. For the other, the Matanoski production workers, an underascertainment of 16 deaths (17%) would more than explain the statistical significance at the $p = 0.05$ level (one-sided).

Comparing Tables 6-7 and 6-8, we see fairly similar results: weak, if any, evidence of a human carcinogenic risk from 1,3-butadiene, but also no strong evidence that the unit risk extrapolation from animal to human results is unreasonable, or that it seriously overpredicts a potential risk.

6.3.4. Relative Potency

One of the uses of quantitative estimation is to compare the relative potencies of different carcinogens. To estimate relative potency, the unit risk slope factor is multiplied by the molecular weight, and the resulting number is expressed in terms of $(\text{mmol/kg/day})^{-1}$. This is called the relative potency index.

TABLE 6-8. SUMMARY OF ALL CANCERS (ICD 140-205) ASSOCIATED WITH THE STYRENE-BUTADIENE SYNTHETIC RUBBER PROCESS. CONFIDENCE LIMITS ON ACTUAL SMRs PREDICTED EXCESS DEATHS BASED ON INITIAL MLE INCREMENTAL UNIT RISKS OF THE MOUSE DATA

Study	Sample size	All cancers (ICD 140-205) Observed	Expected	SMR	95% confidence intervals	Excess deaths from exposure predicted from mouse data (observed-expected)[a]	Power to detect predicted deaths (α= 0.05)[b]	Comments
Meinhardt et al. (1982)								Estimates from 1943-1945: 4 ppm from 1946-1952: 2 ppm
Plant A 1943-1945	600	39	45.14	0.86	0.63-1.18	0.9 (-6.14)	3%	
Plant A 1946-1976	1,062	10	12.19	0.82	0.45-1.51	0.6 (-2.19)	4%	Average exposure = 1.24
Plant B 1950-1976	1,094	11	20.78	0.58	30-0.95*	6.3 (-9.78)**	22%	Average exposure = 13.5
Matanoski et al. (1982) (by job last held)								Same types of plants as those studied by Meinhardt et al. Assume: 4 ppm (estimate)
Production	3,269	94	105.6	0.89	0.73-1.09	10.0 (-9.6)*	16%	
Maintenance	3,683	168	176.8	0.95	0.82-1.11	5.2 (-8.8)	6%	2 ppm (estimate)
Utilities	550	25	26.3	0.95	0.58-2.07	0.3 (-1.3)	2%	1 ppm (estimate)

[a]Test for observed cancers not significantly different from predicted (Poisson test or normal approximation to the Poisson).
[b]α = 0.05, power determined by formula $Z_{1-\beta} = Z_{\alpha} - 2\,(SMR^{0.5} - 1)E^{0.5}$ (Beaumont and Breslow, 1981).
[c]More deaths predicted than observed.
*$p < 0.05$.
**$p < 0.01$.

Figure 6-1 is a histogram representing the frequency distribution of potency indices of 55 suspect carcinogens evaluated by the CAG. The actual data summarized by the histogram are presented in Table 6-9. Where positive human data are available for a compound, they have been used to calculate the index. Where no human data are available, animal oral studies and animal inhalation studies have been used, in that order. In the present case, only the animal inhalation studies provide sufficient evidence of carcinogenicity and have sufficient exposure information.

The potency index for 1,3-butadiene based on the NTP mouse inhalation study (NTP, 1984) is 9.9×10^{-2} (mmol/kg/day)$^{-1}$. This is derived as follows: the upper-limit incremental unit risk estimate from the inhalation study is $q_1^* = 6.4 \times 10^{-1}$ (ppm)$^{-1}$. Transforming this to mg/kg/day, the conversion factor for 1,3-butadiene in humans (assuming 54% absorption at low concentrations) is

$$1 \text{ ppm} = 2.25 \text{ mg/m}^3 \times 20 \text{ m}^3\text{/day} \times 0.54 \times 1/70 \text{ kg} = 0.35 \text{ mg/kg/day}$$

Then

$$q_1^* = 6.4 \times 10^{-1} \text{ (ppm)}^{-1} \times 1 \text{ ppm}/0.35 \text{ mg/kg/day} = 1.8 \times 10^{0} \text{ (mg/kg/day)}^{-1}$$

Multiplying by the molecular weight of 54.1 gives a potency index of 9.9×10^1. Rounding off to the nearest order of magnitude gives a value of 10^2, which is the scale presented on the horizontal axis of Figure 6-1. The index of 9.9×10^1 lies in the third quartile of the 55 chemicals that the CAG has evaluated as known or suspect human carcinogens. Thus, in terms of potency alone, 1,3-butadiene would place in the lower half of these carcinogens. However, the fact that 1,3-butadiene causes so many fatal tumors in animals and sharply

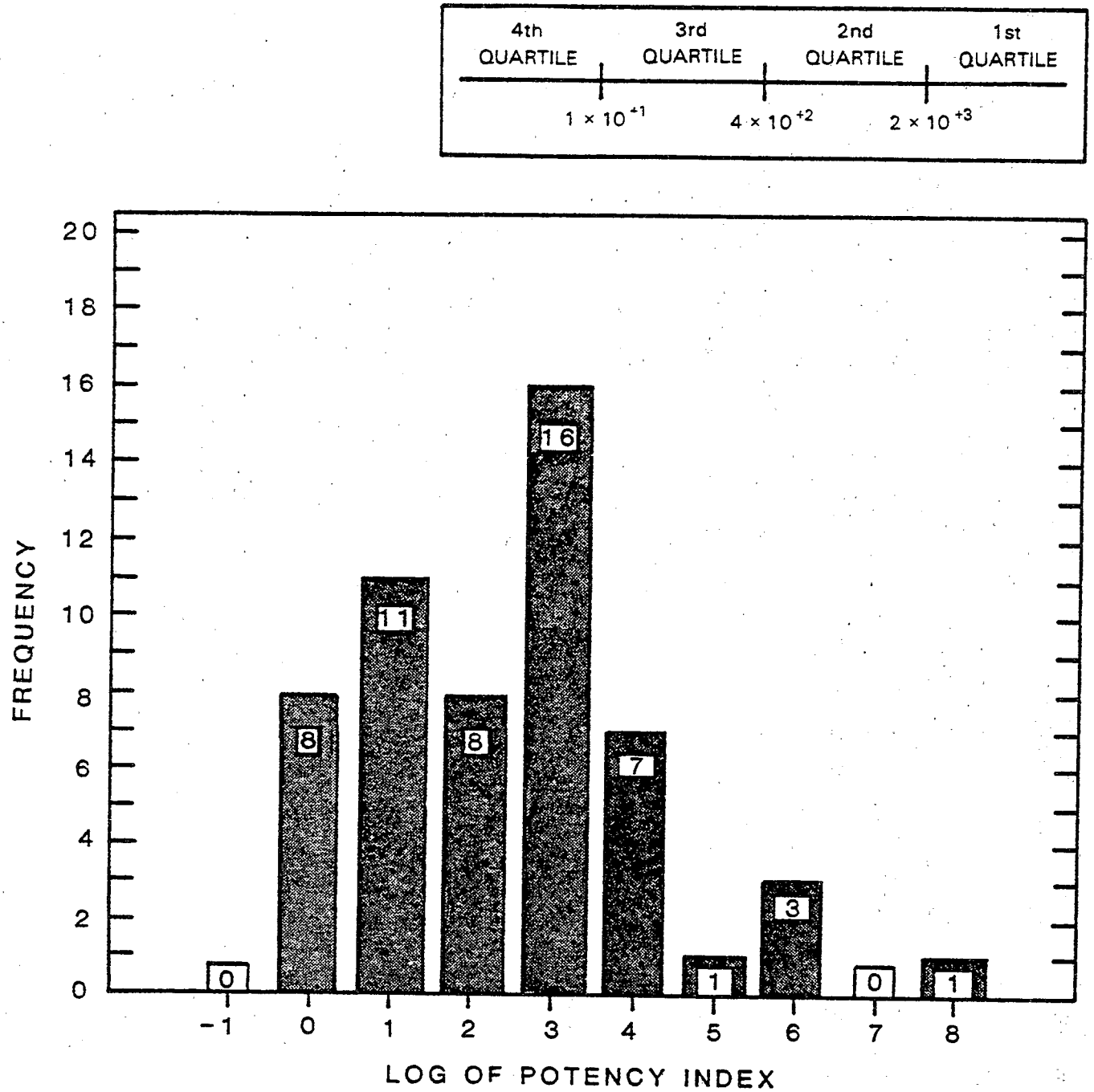

Figure 6-1. Histogram representing the frequency distribution of the potency indices of 55 suspect carcinogens evaluated by the Carcinogen Assessment Group.

TABLE 6-9. RELATIVE CARCINOGENIC POTENCIES AMONG 55 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS

Compounds	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope[b] $(mg/kg/day)^{-1}$	Molecular weight	Potency index[c]	Order of magnitude (log_{10} index)
		Humans	Animals					
Acrylonitrile	107-13-1	L	S	2A	0.24(W)	53.1	$1x10^{+1}$	+1
Aflatoxin B_1	1162-65-8	L	S	2A	2900	312.3	$9x10^{+5}$	+6
Aldrin	309-00-2	I	L	3	11.4	369.4	$4x10^{+3}$	+4
Allyl chloride	107-05-1				$1.19x10^{-2}$	76.5	$9x10^{-1}$	0
Arsenic	7440-38-2	S	I	1	15(H)	149.8	$2x10^{+3}$	+3
B[a]P	50-32-8	I	S	2B	11.5	252.3	$3x10^{+3}$	+3
Benzene	71-43-2	S	S	1	$2.9x10^{-2}$(W)	78	$2x10^{0}$	0
Benzidene	92-87-5	S	S	1	234(W)	184.2	$4x10^{+4}$	+5
Beryllium	7440-41-7	L	S	2A	2.6(W)	9	$2x10^{+1}$	+1
1,3-Butadiene	106-99-0	I	S	2B	1.8(I)	54.1	$1x10^{+2}$	+2
Cadmium	7440-43-9	L	S	2A	6.1(W)	112.4	$7x10^{+2}$	+3
Carbon tetrachloride	56-23-5	I	S	2B	$1.30x10^{-1}$	153.8	$2x10^{+1}$	+1
Chlordane	57-74-9	I	L	3	1.61	409.8	$7x10^{+2}$	+3

(continued on the following page)

TABLE 6-9. (continued)

Compounds	CAS Number	Level of evidence[a] Humans	Level of evidence[a] Animals	Grouping based on IARC criteria	Slope[b] $(mg/kg/day)^{-1}$	Molecular weight	Potency index[c]	Order of magnitude (log_{10} index)
Chlorinated ethanes								
1,2-Dichloroethane	107-06-2	I	S	2B	9.1×10^{-2}	98.9	9×10^{0}	+1
hexachloroethane	67-72-1	I	L	3	1.42×10^{-2}	236.7	3×10^{0}	0
1,1,2,2-Tetrachloroethane	79-34-5	I	L	3	0.20	167.9	$3 \times 10^{+1}$	+1
1,1,2-Trichloroethane	79-00-5	I	L	3	5.73×10^{-2}	133.4	8×10^{0}	+1
Chloroform	67-66-3	I	S	2B	8.1×10^{-2}	119.4	1×10^{1}	+1
Chromium VI	7440-47-3	S	S	1	41(W)	100	$4 \times 10^{+3}$	+4
DDT	50-29-3	I	S	2B	0.34	354.5	$1 \times 10^{+2}$	+2
Dichlorobenzidine	91-94-1	I	S	2B	1.69	253.1	$4 \times 10^{+2}$	+3
1,1-Dichloroethylene (Vinylidene chloride)	75-35-4	I	L	3	1.16(I)	97	$1 \times 10^{+2}$	+2
Dichloromethane (Methylene chloride)	75-09-2	I	S	2B	1.4×10^{-2}(I)	84.9	1×10^{0}	0
Dieldrin	60-57-1	I	S	2B	30.4	380.9	$1 \times 10^{+4}$	+4
2,4-Dinitrotoluene	121-14-2	I	S	2B	0.31	182	$6 \times 10^{+1}$	+2
Diphenylhydrazine	122-66-7	I	S	2B	0.77	180	$1 \times 10^{+2}$	+2
Epichlorohydrin	106-89-8	I	S	2B	9.9×10^{-3}	92.5	9×10^{-1}	0
Bis(2-chloroethyl)ether	111-44-4	I	S	2B	1.14	143	$2 \times 10^{+2}$	+2

(continued on the following page)

TABLE 6-9. (continued)

Compounds	CAS Number	Level of evidence[a] Humans	Level of evidence[a] Animals	Grouping based on IARC criteria	Slope[b] $(mg/kg/day)^{-1}$	Molecular weight	Potency index[c]	Order of magnitude (log_{10} index)
Bis(chloromethyl)ether	542-88-1	S	S	1	9300(I)	115	$1x10^{+6}$	+6
Ethylene dibromide (EDB)	106-93-4	I	S	2B	41	187.9	$8x10^{+3}$	+4
Ethylene oxide	75-21-8	L	S	2A	$3.5x10^{-1}$(I)	44.1	$2x10^{+1}$	+1
Heptachlor	76-44-8	I	S	2B	3.37	373.3	$1x10^{+3}$	+3
Hexachlorobenzene	118-74-1	I	S	2B	1.67	284.4	$5x10^{+2}$	+3
Hexachlorobutadiene	87-68-3	I	L	3	$7.75x10^{-2}$	261	$2x10^{+1}$	+1
Hexachlorocyclohexane								
technical grade					4.75	290.9	$1x10^{+3}$	+3
alpha isomer	319-84-6	I	S	2B	11.12	290.9	$3x10^{+3}$	+3
beta isomer	319-85-7	I	L	3	1.84	290.9	$5x10^{+2}$	+3
gamma isomer	58-89-9	I	L	3	1.33	290.9	$4x10^{+2}$	+3
Hexachlorodibenzodioxin	34465-46-8	I	S	2B	$6.2x10^{+3}$	391	$2x10^{+6}$	+6
Nickel refinery dust		S	S	1	1.05(W)	240.2	$2.5x10^{+2}$	+2
Nickel subsulfide	0120-35-722	S	S	1	2.1(W)	240.2	$5.0x10^{+2}$	+3
Nitrosamines								
Dimethylnitrosamine	62-75-9	I	S	2B	25.9(not by q_1^*)	74.1	$2x10^{+3}$	+3
Diethylnitrosamine	55-18-5	I	S	2B	43.5(not by q_1^*)	102.1	$4x10^{+3}$	+4
Dibutylnitrosamine	924-16-3	I	S	2B	5.43	158.2	$9x10^{+2}$	+3
N-nitrosopyrrolidine	930-55-2	I	S	2B	2.13	100.2	$2x10^{+2}$	+2
N-nitroso-N-ethylurea	759-73-9	I	S	2B	32.9	117.1	$4x10^{+3}$	+4

(continued on the following page)

TABLE 6-9. (continued)

Compounds	CAS Number	Level of evidence[a] Humans	Level of evidence[a] Animals	Grouping based on IARC criteria	Slope[b] $(mg/kg/day)^{-1}$	Molecular weight	Potency index[c]	Order of magnitude (log_{10} index)
N-nitroso-N-methylurea	684-93-5	I	S	2B	302.6	103.1	$3x10^{+4}$	+4
N-nitroso-diphenylamine	86-30-6	I	S	2B	$4.92x10^{-3}$	198	$1x10^{0}$	0
PCBs	1336-36-3	I	S	2B	4.34	324	$1x10^{+3}$	+3
Phenols								
2,4,6-Trichlorophenol	88-06-2	I	S	2B	$1.99x10^{-2}$	197.4	$4x10^{0}$	+1
Tetrachlorodibenzo-p-dioxin (TCDD)	1746-01-6	I	S	2B	$1.56x10^{+5}$	322	$5x10^{+7}$	+8
Tetrachloroethylene	127-18-4	I	L	3	$5.1x10^{-2}$	165.8	$8x10^{0}$	+1
Toxaphene	8001-35-2	I	S	2B	1.13	414	$5x10^{+2}$	+3
Trichloroethylene	79-01-6	I	L/S	3/2B	$1.1x10^{-2}$	131.4	$1x10^{0}$	0
Vinyl chloride	75-01-4	S	S	1	$1.75x10^{-2}$(I)	62.5	$1x10^{0}$	0

[a]S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.
[b]Animal slopes are 95% upper-bound slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear nonthreshold model. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available. The slope value is an upper bound in the sense that the true value (which is unknown) is not likely to exceed the upper bound and may be much lower, with a lower bound approaching zero. Thus, the use of the slope estimate in risk evaluations requires an appreciation for the implication of the upper bound concept as well as the "weight of evidence" for the likelihood that the substance is a human carcinogen.
[c]The potency index is a rounded-off slope in $(mmol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

decreases the latency period increases concern beyond that based simply on relative potency.

Ranking of the relative potency indices is subject to the uncertainties of comparing potency estimates for a number of chemicals based on different routes of exposure in different species, using studies whose quality varies widely. Furthermore, all of the indices are based on estimates of low-dose risk using linear extrapolation from the observational range. Thus, these indices are not valid to compare potencies in the experimental or observational range if linearity does not exist there. The uncertainty of the estimate of low exposure potency for 1,3-butadiene is increased because the response at 625 ppm for the mouse is greater than 60%, which is probably not on the linear part of the dose-response curve.

6.3.5. Summary of Quantitative Estimation

Based on the linearized multistage model and an external concentration to internal dose conversion, a 95% upper-limit incremental unit cancer risk of $q_1^* = 6.4 \times 10^{-1}$ $(ppm)^{-1}$ was calculated for 1,3-butadiene using the geometric mean of the 95% upper-limit incremental risk estimates from the pooled male and pooled female significant tumor responses of the NTP mouse study. The quantitative estimates based on mouse-to-man extrapolation were then used to predict human responses in several epidemiologic studies, and the predicted and actual responses were then compared. The comparisons were hampered by a scarcity of information in the epidemiologic data concerning actual exposures, age distributions, and work histories. In addition, because there was no consistent cancer response across all of the studies, the most predominant response, cancer of the lymphatic and hematopoietic tissues, was chosen as being the target for 1,3-butadiene. Based on the comparisons between the predicted and observed human response, the extrapolated value from the mouse

data was consistent with human response, but in view of all the uncertainties and apparent inconsistencies in the epidemiologic data, a fairly wide range of potency estimates and exposure scenarios would also be satisfactory.

In addition to a 95% upper-limit incremental unit risk, a measure of carcinogenic potency was determined for 1,3-butadiene. Among the 55 chemicals that the CAG has evaluated as known or suspect human carcinogens, 1,3-butadiene ranks in the third quartile. Based on the wide spectrum of cancers and sharply decreased latency associated with these cancers, however, 1,3-butadiene should evoke more concern than the potency numbers alone indicate.

7. REFERENCES

Allen, J.W. 1985. Unpublished data from FDA-EPA Interagency Agreement on Cytogenetic Effects of Medical Device Chemicals (Monomers). Personal communication.

American Conference of Governmental Industrial Hygienists (ACGIH). 1983. Threshold limit values for chemical substances and physical agents in the work environment, with intended changes for 1983-84. Cincinnati, OH.

American Conference of Governmental Industrial Hygienists (ACGIH). 1984. Threshold limit values for chemical substances and physical agents in the work environment, with intended changes for 1984-85. Cincinnati, OH.

Anderson, E.L., and the Carcinogen Assessment Group. 1983. Quantitative approaches in use to assess cancer risk. Risk Analysis 3:277-295.

Andjelkovich, D., J. Taulbee, and M. Symons. 1976. Mortality experience of a cohort of rubber workers, 1964-1973. J. Occup. Med. 18:387-394.

Andjelkovich, D., J. Taulbee, M. Symons, and T. Williams. 1977. Mortality of rubber workers with reference to work experience. J. Occup. Med. 18:387-394.

Autrup, A., F.C. Wefald, A.M. Jeffrey, H. Tate, R.D. Schwartz, B.F. Trump, and C.C. Harris. 1980. Metabolism of benzo(a)pyrene by cultured tracheobronchial tissues from mice, rats, hamsters, bovines, and humans. Int. J. Cancer 25:293-300.

Beaumont, J.I. and N.E. Breslow. 1981. Power consideration in epidemiologic studies of vinyl chloride workers. Am. J. Epidemiol. 114:725-734.

Bolt, H.M., J.G. Filser, and F. Stoermer. 1984. Inhalation pharmacokinetics based on gas uptake studies. V. Comparative pharmacokinetics of ethylene and 1,3-butadiene in rats. Arch. Toxicol. 55:213-218.

Bolt, H.M., G. Schmiedel, J.G. Filser, H.P. Rolzhauser, K. Lieser, D. Wistuba, and V. Schurig. 1983. Biological activation of 1,3-butadiene to vinyl oxirane by rat liver microsomes and expiration of the reactive metabolites by exposed rats. J. Cancer Res. Clin. Oncol. 106:112-116.

Carpenter, C., C. Shaffer, C. Weil, and H. Smith. 1944. Studies on the inhalation of 1,3-butadiene; with a comparison of its narcotic effect with benzol, toluol, and styrene, and a note on the elimination of styrene by the human. J. Ind. Hyg. Toxicol. 26:69-78.

Checkoway, H., and T.M. Williams. 1982. A hematology survey of workers at a styrene-butadiene synthetic rubber manufacturing plant. Am. Ind. Hyg. Assoc. J. 43:164-169.

Checkoway, H., T. Wilcosky, P. Wolf, and H. Tyroler. 1984. An evaluation of the association of leukemia and rubber industry solvent exposures. Am. J. Ind. Med. 5:239-249.

Citti, L., P.G. Gervasi, G. Turchi, G. Bellucci, and R. Bianchini. 1984. The reaction of 3,4-epoxybutene with deoxyguanosine and DNA in vitro: synthesis and characterization of the main adducts. Carcinogenesis 5:47-52.

Conner, M., J. Luo, and O. Gutierrez de Gotera. 1983. Induction and rapid repair of sister-chromatid exchanges in multiple murine tissues in vitro by diepoxybutane. Mutat. Res. 108:251-263.

Cox, C.R. 1972. Regression model and life tables. J. Roy. Stat. Soc. B 34:187-220.

Crouch, C., D. Pullinger, and I. Gaunt. 1979. Inhalation toxicity studies with 1,3-butadiene. Part 2: Three-month toxicity study in rats. Am. Ind. Hyg. Assoc. J. 40:796-802.

Crump, K.S. 1979. Dose-response problems in carcinogenesis. Biometrics 35:157-167.

Crump, K.S., and Watson, W.W. 1979. GLOBAL79. A FORTRAN program to extrapolate dichotomous animal carcinogenicity data to low dose. Natl. Inst. of Environ. Health Science Contract No. 1-ES-2123.

Crump, K.S., and R.B. Howe. 1983a. The multistage model with a time-dependent dose pattern: applications to carcinogenic risk assessment. EPA Contract No. 68-01-3111. Unpublished.

Crump, K.S., and R.B. Howe. 1983b. ADOLL1-83 and ADOLL2-83, FORTRAN programs for implementing a multistage model with a time-dependent dose pattern. Unpublished instructions for use of computer programs.

Dean, B.J., and G. Hodson-Walker. 1979. An in vitro chromosome assay using cultured rat-liver cells. Mutat. Res. 64:329-337.

de Meester, C., F. Poncelet, F. Roberfroid, and M. Mercier. 1978. Mutagenicity of butadiene and butadiene monoxide. Biochem. Biophys. Res. Commun. 80:298-305.

de Meester, C., F. Poncelet, F. Roberfroid, and M. Mercier. 1980. The mutagenicity of butadiene towards Salmonella typhimurium. Toxicol. Lett. 6:125-130.

Doll, R. 1971. Weibull distribution of cancer. Implications for models of carcinogenesis. J. Roy. Statistical Soc. A 13:133-136.

Dripps, R.D., Eckenhoff, J.E., and Vandam, L.D. 1977. Introduction to anesthesia, the principles of safe practice. 5th ed. Philadelphia, PA: W.B. Saunders Company, pp. 121-123.

Ehrenberg, L., and S. Hussain. 1981. Genotoxicity of some important epoxides. Mutat. Res. 86:1-113.

Filser, J.G., and H.M. Bolt. 1981. Inhalation pharmacokinetics based on gas uptake studies. I. Improvement of kinetic model. Arch. Toxicol. 47: 279-292.

Filser, J.G., and H.M. Bolt. 1984. Inhalation pharmacokinetics based on gas uptake studies. VI. Comparative evaluation of ethylene oxide and butadiene monoxide as exhaled reactive metabolites of ethylene and 1,3-butadiene in rats. Arch. Toxicol. 55:219-223.

Hazleton Laboratories Europe, Ltd. (HLE). 1981a. The toxicity and carcinogenicity of butadiene gas administered to rats by inhalation for approximately 24 months. Prepared for the International Institute of Synthetic Rubber Producers, New York, NY. Unpublished.

Hazleton Laboratories Europe, Ltd. (HLE). 1981b. 1,3-Butadiene: Inhalation teratogenicity study No. 2788 522/3. Prepared for the International Institute of Synthetic Rubber Producers, New York, NY. Unpublished.

Hemminki, K., K. Falck, and H. Vainio. 1980. Comparison of alkylation rates and mutagenicity of directly acting industrial and laboratory chemicals. Arch. Toxicol. 46:277-285.

Howe, R.B. 1983. GLOBAL83: an experimental program developed for the U.S. Environmental Protection Agency as an update to GLOBAL82: a computer program to extrapolate quantal animal toxicity data to low doses (May 1982). K.S. Crump and Co., Inc., Ruston, LA. Unpublished.

Huff, J.E., R.L. Melnick, H.A. Solleveld, J.K. Haseman, M. Powers, and R.A. Miller. 1985. Multiple organ carcinogenicity of 1,3-butadiene in B6C3F1 mice after 60 weeks of inhalation exposure. Science 227:548-549.

International Agency for Research on Cancer (IARC). 1982. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Supplement 4. Lyon, France.

Laib, R.J., R. Kreiling, and H.M. Bolt. 1985. Species differences in butadiene metabolism between mouse and rat as evaluated by inhalation pharmacokinetics and covalent binding of reactive butadiene metabolites. The Toxicologist: Abstracts of the 1985 Annual Meeting of the Society of Toxicology 5:131.

Lawley, P.D., and P. Brookes. 1967. Interstrand cross-linking of DNA by difunctional alkylating agents. J. Mol. Biol. 25:143-160.

Lilis, R., and W.J. Nicholson. 1976. Cancer experience among workers in a chemical plant producing styrene monomers. Proceedings of Styrene-Butadiene Briefing, Covington, KY, April 30, 1976. DHEW (NIOSH) Pub. No. 77-129. Cincinnati, OH, pp. 22-27.

Linet, M.S. 1985. The leukemias: epidemiologic aspects. Oxford, England: Oxford University Press.

Luker, M.A., and B.J. Kilbey. 1982. A simplified method for the simultaneous detection of intragenic and intergenic mutations (deletions) in Neurospora crassa. Mutat. Res. 92:63-68.

Malvoisin, E., G. Lhoest, F. Poncelet, M. Roberfroid, and M. Mercier. 1979. Identification and quantitation of 1,2-epoxybutene-3 as the primary metabolite of 1,3-butadiene. J. Chrom. 178:419-429.

Malvoisin, E., and M. Roberfroid. 1982. Hepatic microsomal metabolism of 1,3-butadiene. Xenobiotica 12:137-144.

Malvoisin, E., M. Mercier, and M. Roberfroid. 1980. Enzymic hydration of butadiene monoxide and its importance in the metabolism of butadiene. Adv. Exp. Med. Biol. 136A:437-444.

Mantel, N., and Schneiderman, M.A. 1975. Estimating "safe" levels, a hazardous undertaking. Cancer Res. 35:1379-1386.

Matanoski, G.M., L. Schwartz, J. Sperrazza, and J. Tonascia. 1982. Mortality of workers in the styrene-butadiene rubber polymer manufacturing industry. Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD. Unpublished.

McMichael, A.J., R. Spirtas, and L.L. Kupper. 1974. An epidemiologic study of mortality within a cohort of rubber workers, 1964-72. J. Occup. Med. 16:458-464.

McMichael, A.J., R. Spirtas, L.L. Kupper, and J.F. Gamble. 1975. Solvent exposure and leukemia among rubber workers: an epidemiologic study. J. Occup. Med. 17:234-239.

McMichael, A.J., R. Spirtas, J.F. Gamble, and P.M. Tousey. 1976. Mortality among rubber workers: Relationship to specific jobs. J. Occup. Med. 18:178-185.

Meinhardt, T.J., R.A. Lemen, M.S. Crandall, and R.J. Young. 1982. Environmental epidemiologic investigation of the styrene-butadiene rubber industry. Scand. J. Work Environ. Health 8:250-259.

Miller, L.M. 1978. Investigation of selected potential environmental contaminants: butadiene and its oligomers. EPA-560/2-78-008. U.S. Environmental Protection Agency, Office of Toxic Substances, Washington, DC.

Monson, R.R. 1979. U.S. death rates programs, versions USDR 27 and USDR 38. Harvard University School of Public Health, Boston, MA. Unpublished.

Murray, M.P., and J.E. Cummins. 1979. Mutagenic activity of epoxy embedding reagents employed in electron microscopy. Environ. Mutagen. 1:307-313.

National Institute for Occupational Safety and Health (NIOSH). 1984. Current Intelligence Bulletin 41: 1,3-butadiene. Washington, DC.

National Toxicology Program (NTP). 1984. Toxicology and carcinogenesis studies of 1,3-butadiene (CAS 106-99-0) in B6C3F1 mice (inhalation studies).

National Toxicology Program (NTP). 1985a. Quarterly report from Lovelace Research Institute, January 1 through March 31, 1985. Interagency agreement 22-Y01-ES-0092. (L. Birnbaum, NTP Project Officer).

National Toxicology Program (NTP). 1985b. Draft report on the toxicology and carcinogenesis studies of 4-vinylcyclohexene in F344/N rats and B6C3F1 mice. NIH Publication No. 85-2559.

Neligan, R.E. 1962. Hydrocarbons in the Los Angeles atmosphere. Arch. Environ. Health 5:67-77.

Olszewska, E., and B.J. Kilbey. 1975. The mutagenic activity of diepoxybutane in yeast. Mutat. Res. 33:383-390.

Ott, M.G., R.C. Kolesar, H.C. Scharnweber, E.J. Schneider, and J.R. Venable. 1980. A mortality survey of employees engaged in the development or manufacture of styrene-based products. J. Occup. Med. 22(7):445-460.

Perry, P., and H.J. Evans. 1975. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. Nature 258:121-125.

Poncelet, F., C. de Meester, M. Duverger-van Bogaert, M. Lambotte-Vandepaer, M. Roberfroid, and M. Mercier. 1980. Influence of experimental factors on the mutagenicity of vinylic monomers. Arch. Toxicol. Suppl. 4:63-66.

Rappaport, S.M., and D.A. Fraser. 1976. Gas chromatographic - mass spectrometric identification of volatiles released from a rubber stock during simulated vulcanization. Anal. Chem. 48:476-481.

Ripp, G.K. 1967. Hygienic basis for the permissible concentration of butadiene in the atmosphere. Biol. Deislvie Gig. Znachenis Atmos. Zagryazneini 10:33-54 (Russian). As cited in Miller, 1978.

Sankaranarayanan, K. 1983. The effects of butylated hydroxytoluene on radiation and chemically-induced genetic damage in Drosophila melanogaster. Mutat. Res. 108:203-223.

Sankaranarayanan, K., W. Ferro, and J.A. Ziljlstra. 1983. Studies on mutagen-sensitive strains of Drosophila melanogaster. III. A comparison of the mutagenic sensitivities of the ebony (UV and X-ray sensitive) and Canton-S (wild-type) strains to MMS, ENU, DEB, DEN, and 2,3,6-Cl_3-PDMT. Mutat. Res. 110:59-70.

Shugaev, L. 1969. Concentration of hydrocarbons in tissues as a measure of toxicity. Arch. Environ. Health 18:878-882.

Simmon, V.F., and J.M. Baden. 1980. Mutagenic activity of vinyl compounds and derived epoxides. Mutat. Res. 78:227-231.

Spirtas, R. 1976. Mortality among rubber workers: relationship to jobs with styrene-butadiene exposures. Proceedings of NIOSH Styrene-Butadiene Briefing, Covington, KY, April 30, 1976. DHEW (NIOSH) Pub. No. 77-129. Cincinnati, OH, pp. 9-21.

Taulbee, J., D. Adjelkovich, T. Williams, J.F. Gamble, and P. Wolf. 1976. A study of possible associations between exposure to SBR processes and mortality from leukemia and related diseases based on toxicologic, industrial hygiene and epidemiologic considerations (for workers in the 1951 and 1964 cohorts and deaths 1964-1973). Proceedings of NIOSH Styrene-Butadiene Briefing, Covington, KY, April 30, 1976. DHEW (NIOSH) Pub. No. 77-129. Cincinnati, OH, pp. 113-129.

Turchi, G., S. Bonatti, L. Citti, P.G. Gervasi, and A. Abbondandolo. 1981. Alkylating properties and genetic activity of 4-vinylcyclohexene metabolites and structurally related epoxides. Mutat. Res. 83:419-430.

U.S. Department of Health and Human Services (DHHS). 1985. Fourth annual report on carcinogens. Washington, DC.

U.S. Environmental Protection Agency. 1984a. Proposed guidelines for mutagenicity risk assessment: request for comments. Federal Register 49: 46314-46321.

U.S. Environmental Protection Agency. 1984b. Proposed guidelines for carcinogen risk assessment. Federal Register 49(227):46294-46301.

U.S. Environmental Protection Agency. 1985. Assessment of cancer risks to workers exposed to 1,3-butadiene during production of 1,3-butadiene monomer and production of synthetic rubbers, plastics, and resins. Office of Toxic Substances, Washington, DC.

Van Duuren, B.L., N. Nelson, L. Orris, E.D. Palmes, and F.L. Schmitt. 1963. Carcinogenicity of epoxides, lactones, and peroxy compounds. J. Natl. Cancer Inst. 31:4-55.

Van Duuren, B.L., L. Langsteth, L. Orris, G. Teebor, N. Nelson, and M. Kuschner. 1966. Carcinogenicity in epoxides, lactones, and peroxy compounds. IV. Tumor response in epithelial and connective tissue in mice and rats. J. Natl. Cancer Inst. 37:825-838.

Vogel, E. 1976. Mutagenicity of carcinogens in Drosophila as a function of genotype-controlled metabolism. In: F.J. de Serres, J.R. Fouts, J.R. Bend, and R.M. Philpot (eds.). In vitro metabolic activation in mutagenesis testing. Amsterdam: Elsevier/North-Holland Biomedical Press, pp. 63-79.

Voogd, C.E., J.J. van de Stel, and J.J.J.A.A. Jacobs. 1981. The mutagenic action of aliphatic epoxides. Mutat. Res. 89:269-282.

Wade, M.J., J.W. Moyer, and C.H. Hine. 1979. Mutagenic action of a series of epoxides. Mutat. Res. 66:367-371.

Zaborowska, D., Z. Swietlinska, and J. Zuk. 1983. Induction of mitotic recombination by UV and diepoxybutane and its enhancement by hydroxyurea in Saccharomyces cerevisiae. Mutat. Res. 120:21-26.

Zimmering, S. 1983. The mei-9[a] test for chromosome loss in Drosophila: a review of assays of 21 chemicals for chromosome breakage. Environ. Mutagen. 5:907-921.

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