Sperm-Head Morphology Study in B6C3F1 Mice Following Inhalation Exposure to 1,3-Butadiene

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MICE FOLLOWING INHALATION EXPOSURE TO
1,3-BUTADIENE

FINAL TECHNICAL REPORT
NIH No. NIH-Y01-ES-70153

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ABSTRACT

A series of studies conducted in this laboratory have evaluated the effects of whole-body inhalation exposures to 1,3-butadiene on the reproductive system. The present report describes the results of a study of the morphology of epididymal sperm heads of B6C3F1 mice that were exposed (6 hours/day for 5 consecutive days) to 0 (filtered air), 200, 1000 and 5000 ppm of 1,3-butadiene. During the fifth post-exposure week, the animals were killed and examined for gross lesions of the reproductive tract; suspensions of the epididymal sperm were prepared for morphologic evaluations. The mice were weighed prior to exposure and at 0, 1, 2, 3, 4, and 5 weeks after exposure and were observed for mortality, morbidity and signs of toxicity throughout the study.

No mortality was observed in any of the inhalation exposure groups. Transient toxic signs, including piloerection and dyspnea, were evident during a 20- to 30-minute period following exposure to 5000 ppm. Mean values for body weights and weight gains of the mice exposed to 1,3-butadiene were not significantly different from control values. A concentration-related increase in the incidence of sperm-head abnormalities was evident and the percentage of sperm heads that were morphologically abnormal was significantly higher in mice exposed to 1000 and 5000 ppm than in the controls. Since this sperm-head morphology assay was conducted only during the fifth postexposure week and was not designed to detect effects at all stages of gamete development, these findings of higher incidences of abnormalities suggest that at least the late spermatogonia or early primary spermatocytes may be sensitive to alteration by exposures of mice to 1000 ppm or higher concentrations of 1,3-butadiene.

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ABBREVIATIONS

ANOVA	= Analysis of variance
BNW	= Battelle, Pacific Northwest Laboratories; Battelle-Northwest
CDS	= Colorado Data Systems
CO ₂	= Carbon dioxide
EMS	= Ethyl methanesulfonate
FID	= Flame ionization detector
GC	= Gas chromatography
HP	= Hewlett-Packard
IIS	= Intelligent Interface System
MRI	= Midwest Research Institute
NIEHS	= National Institute of Environmental Health Sciences
NIOSH	= National Institute of Occupational Safety and Health
NTP	= National Toxicology Program
PNL	= Pacific Northwest Laboratory
ppm	= Parts per million
RH	= Relative humidity
v/v	= Volume to volume ratio

I. INTRODUCTION

The major uses of butadiene are in the manufacture of styrene-butadiene rubber, in the production of polymers, and as a chemical intermediate (SRI, 1979). The major manufacturers in the U.S. produced about 1.5 million tons in 1980 (SRI, 1981), and it has been estimated that about 62,000 workers were exposed (National Institute of Occupational Safety and Health [NIOSH], 1980). As with most chemicals, accidental release during their transportation poses a potential for exposure of the general population. From reviews of the literature, it appears that acute exposure to 1,3-butadiene is not highly toxic and that mice are more sensitive than rats when exposed by either oral or inhalation routes.

There are indications that chronic inhalation exposure to 1,3-butadiene alters the reproductive system. In a study using Sprague-Dawley rats (Hazelton, 1981), euthanasia was required for females with large mammary tumors and males with severe nephropathic changes after 24 months of exposure to 1000 to 8000 ppm of butadiene. In this study, there was an increased incidence of a number of tumor types, including those of the reproductive tract (Leydig cell tumors of the testes and uterine/vaginal stromal tumors). Another study, performed by Battelle, Pacific Northwest Laboratories (Battelle-Northwest; BNW) for the National Toxicology Program (NTP) in 1981 (BNW, 1984), in which mice were exposed to 625 and 1250 ppm of butadiene, was terminated at about 60 weeks because of high mortality associated with neoplasms at multiple sites, including ovarian granulosa cell tumors. There was also a significantly increased incidence of mice with testicular or ovarian atrophy.

There appear to be two reported studies in which the reproductive toxicity of inhaled 1,3-butadiene was specifically evaluated (Carpenter et al., 1944; Hazelton, 1982), and only the former involved exposure of males. In this study, exposure of rats to 600, 2300 and 6700 ppm of butadiene for 8 months resulted in a concentration-related depression of body weight (Carpenter et al., 1944). Reproductive studies of male and female animals were performed at unstated times during these exposures, but no data were given concerning the number of matings, or of barren females. The number of litters per female rat in all exposed groups tended to be lower than in controls, although litter sizes were stated to slightly

exceed the "expected norm" of six/litter. Limited breeding tests of the offspring in the 2300- and 6700-ppm exposure groups suggested that there was reduced fecundity, but it was not determined whether the deficit was associated with the males or with the females. Although details were not provided, 12 guinea pigs and 4 rabbits of each exposure group were also used for reproductive studies. Animals of all groups evidently produced progeny, except for rabbits exposed to 600 and 2300 ppm. The data are not supportive of the authors' conclusion that butadiene exposure did not affect reproductive performance and that any apparent reduction in fertility was due to hereditary characteristics.

A review of the limited data available in the literature indicated a need for further investigations concerned with the reproductive toxicity of 1,3-butadiene, and specific evaluation of the potential of this chemical for producing genetic damage. Bioassays for genetic toxicology in male rodents, as exemplified by the dominant lethal assay, are usually conducted using experimental regimens that will detect damage to gametes at specific stages of development. For these studies, one exposure (acute), five successive exposures (subacute) or exposure over the entire spermatogenic cycle may be employed. Following acute or subacute exposures, the males are mated at weekly intervals with unexposed females, which are subsequently necropsied to determine the status of the conceptuses (Green et al., 1985; Bateman and Epstein, 1971). Following exposures over the entire spermatogenic cycle, biweekly matings are performed sequentially to determine the effect of the agent. Obviously, these experimental regimens to detect the mutagenic or toxic properties of chemicals are time-consuming and require the acquisition and care of a large number of test animals. Therefore, Wyrobek and Bruce (1975) have proposed a rapid screening method that utilizes the detection of sperm abnormalities in mice. They studied the incidence of these abnormalities at various intervals following exposure to 25 chemicals that were previously implicated as agents with mutagenic, teratogenic or carcinogenic activity. They found that increased incidences of epididymal sperm abnormalities were observed following exposure to all of these chemicals after 4 weeks, to some of the chemicals after 1 week, and to most of the chemicals after 10 weeks. They demonstrated that a variety of mutagens (ethyl or methyl methanesulfonate, METEPA, THIOTEPA, mitomycin C and myleran) induced marked elevations in sperm abnormalities that can be observed at 4 weeks after exposure to the chemical and often persist as long as 10 weeks after exposure. Certain antimetabolites (hydroxyurea, Imuran, IUdR and aminopterin), which are

putative teratogens, also produced sperm abnormalities, but they were less severe and did not persist as long. Although the mechanism of abnormality induction has not been identified, Wyrobek and Bruce have suggested that an agent that produces sperm abnormalities may interfere with the integrity of the sperm DNA or with the expression of this genetic material.

Soares et al. (1979) reported that an insult to late spermatogonia or early spermatocytes by certain agents can induce disruption of normal differentiation of the sperm-cell morphology, which is polygenically determined and has a high heritability. Studies by Hugenholtz and Bruce (1976) and Topham (1980) reported that increased incidences of sperm abnormalities were transmitted from exposed males to their male offspring. This evidence also suggested that interference with the mechanism controlling sperm-head morphology in exposed male mice may be associated with the induction of transmissible mutations. On the other hand, Komatsu et al. (1982) observed an increased incidence of sperm abnormalities in mice on moderately and severely restricted food intake. These results suggested that the abnormalities observed following the administration of certain toxic chemicals might be due to reduced food intake rather than to a mutagenic effect.

Despite these limitations, the sperm morphology assay in B6C3F1 mice was selected as one of the studies to detect potential impairment of reproductive function by exposure to 1,3-butadiene. Body weights were measured at frequent intervals to provide an indicator of toxic effects. In addition, a separate study to evaluate the induction of dominant lethality in CD-1 mice by exposure to 1,3-butadiene was performed in this laboratory. This latter study was designed to determine reproductive integrity and viability of the conceptus by serial matings performed for 8 weeks following exposure to the chemical. Exposures of mice for sperm morphology and dominant lethal assays were performed sequentially using identical exposure regimens (atmospheric concentrations of 0, 200, 1000 and 5000 ppm for 6 hours/day during 5 successive days).

II. MATERIALS AND METHODS

A. PROCUREMENT AND CHARACTERIZATION OF 1,3-BUTADIENE AND ETHYL METHANESULFONATE

1. 1,3-Butadiene

The procurement of 1,3-butadiene from Phillips Chemical Company (Borger, TX) and its initial chemical characterization by Midwest Research Institute (MRI; Kansas City, MO) were arranged by the National Institute of Environmental Health Sciences (NIEHS). The chemical composition of Lot Number F858, which was analyzed by Phillips Chemical Company, was reported by MRI on July 23, 1985 to be 99.84% 1,3-butadiene, 0.13% cis(butene)2, 0.03% heavies (residues remaining following evaporation of 1,3-butadiene at room temperature), and 13 ppm of the peroxide inhibitor, t-butyl catechol.

For the study reported here, a single lot (F909) of 1,3-butadiene was packaged in three 28-gal steel cylinders and shipped directly from Phillips Chemical Company to Pacific Northwest Laboratory (PNL). The cylinders were fitted with double-entry valves, which permitted sampling of liquid 1,3-butadiene through an eductor tube or gaseous material through a valve from the cylinder headspace. As received, the test-material cylinders contained variable amounts of nitrogen, which had been used by the manufacturer to dispense the 1,3-butadiene from bulk storage into appropriate containers for off-site shipment. The nitrogen was released from the cylinders prior to the withdrawal of 1,3-butadiene for analyses or animal exposures. A single cylinder (BNW 50846-36-1) was used for the animal exposures of 5 days' duration in the study described in this report.

Procedures for reanalysis of bulk chemicals were provided by MRI, and the materials were analyzed in our laboratory prior to the initiation of the animal exposures. The infrared spectrum of Lot Number F909 of 1,3-butadiene (4000 to 600 cm^{-1} , using a gas cell with NaCl windows) confirmed its identity and did not detect major impurities (Appendix A). Data were consistent with the spectrum of Lot Number F850 and with values obtained from the literature (Erley and Blake).

Gas chromatographic analysis (2 m x 2 mm Porapak QS 100/120, 100°C isothermal, flame ionization detector; FID) for area percent purity of 1,3-butadiene for this study determined purity to be 99.88% (Appendix A). An unidentified minor peak, which eluted from the column 2 minutes later than the 1,3-butadiene, represented 0.11% of the total peak area. Analyses for an impurity, 4-vinyl-1-cyclohexene, were also performed by gas chromatography (GC; 1 m x 2 mm Porapak QS 80/100, 150°C isothermal, FID). This impurity results from the thermally catalyzed dimerization of 1,3-butadiene, which occurs continuously during storage. Since the rate of "dimer" formation is temperature-dependent, care was taken to assure constant storage temperatures of approximately 72°F. The dimer is considerably less volatile than 1,3-butadiene and therefore concentrates in the cylinder "heel" as the contents of the cylinder are exhausted. To prevent the appearance of high concentrations of dimer in the 1,3-butadiene atmospheres used for the exposures, cylinder usage was limited to 80% of the net contents, and the acceptable dimer concentration in material sampled from the headspace was specified to be <500 ppm. Prior to the study, the headspace dimer concentration of Cylinder 1 was 161 ppm (Appendix A); periodic analyses performed during animal exposures yielded a mean headspace dimer concentration of 174 ± 13 ppm (Appendix A).

2. Ethyl Methanesulfonate (EMS)

The EMS (CAS# 62-50-0; Lot # Kodak A11E) was obtained from Eastman Organic Chemical Company, Rochester, NY. The clear, colorless liquid, which was used as a positive-control material, was stored at room temperature. Infrared spectroscopy was used to confirm the identity and gross purity of the chemical. Gas chromatographic analyses (4 mm x 6' glass column packed with 10% Carbowax 20 M on 80/100 chromosorb W AW; 150°C injection temperature; FID) were performed on the aqueous injection solutions, which were determined to be stable during a 2-hour period. With this system, which employed a helium carrier at ~66 ml/minute, the retention time of EMS was ~7.6 minutes.

B. INHALATION EXPOSURE SYSTEM

The animals were exposed to the test atmospheres within stainless-steel chambers designed at BNW (U.S. Patent #4,216,741; Brown and Moss, 1981; Moss et al., 1982) and fabricated by Hazelton Systems, Inc. (Aberdeen, MD). The total volume of

the chamber was 2.3 m³, and the active mixing volume was 1.7 m³. There were three levels of caging in each chamber; each level was split into two tiers, which were offset from each other and from the chamber walls. Drawer-like stainless-steel cage units, consisting of individual animal cages, were suspended in the space above each tier. Stainless-steel catch pans for collection of urine and feces were positioned below each cage unit. Mice were exposed in individual cages (60/each cage unit) equipped with feed troughs and automatic watering. During exposure, the feed troughs were removed from each cage unit. The chamber was designed so that uniform atmospheric concentrations of the test material could be maintained throughout the chamber when the catch pans were in position (Moss et al., 1982; Griffis et al., 1981).

Environmental conditions of the exposure chambers and the room were monitored throughout the study. These data included temperature, which was measured by means of resistance temperature devices, and relative humidity (RH), which was determined using an EG&G Model 910 chilled-mirror dewpoint hygrometer. A multiplexed orifice meter system, in which calibrated flow orifices were installed at the inlet and exhaust outlet of each chamber, measured chamber air flow. A Validyne pressure transducer system was used to measure chamber air flow and chamber vacuum. Data for all environmental parameters are summarized in Appendix B.

All data-acquisition and automated system controls originated from an executive computer (Hewlett-Packard [HP] Model 9816), located in the Suite Control Center. Data from GC analyses were collected and preconditioned by an HP Model 85B computer; data from all other monitoring equipment were interfaced through a Colorado Data Systems (CDS) Model 53A-IBX Intelligent Interface System (IIS). System control, by means of devices such as valves, drive motors, audible alarms, and indicator lamps, was provided from the computer by means of control relays in the CDS IIS.

For generation of chamber atmospheres, the 1,3-butadiene was withdrawn directly from the gas cylinder through a solenoid valve and, subsequently, through a check-valve, filter-flow-limit switch and a flow meter, which accurately metered gas to a distribution manifold, where it was initially diluted with filtered air. An air-

driven vacuum pump delivered the butadiene-air mixture to the exposure chamber inlet for final dilution to the desired concentration.

Chamber monitoring of 1,3-butadiene concentrations was performed using an HP 5840 gas chromatograph equipped with an FID, a Valco (1-ml loop) sampling valve and a Valco stream-select valve capable of sampling eight different sites. Both valves were mounted in the GC oven, which was maintained at 120°C. The 1/8- x 12-in. nickel column was packed with 1% SP-1000 on 60/80 mesh Carbopack B. The gas chromatograph was calibrated with volumetric standards prepared with calibrated syringes and gas-sampling bags. These calibrations were in good agreement with certified gas standards, the concentrations of which had been selected to bracket the range of exposure concentrations. Eight sites were sampled every 16 minutes: four exposure chambers, two "holding" chambers, the exposure room and the GC standard. Data from the monitor were accumulated by the HP 85B computer and compared with limit values for the specific sampling location. When the value exceeded the control limits, the HP 85B computer transmitted the information to the executive computer, which initiated an appropriate action to correct the concentration. A summary of atmospheric concentrations of 1,3-butadiene for each of the three exposure chambers is shown in Table 1.

TABLE 1. Summary of Chamber Atmospheric Concentrations of 1,3-Butadiene During Exposure of Mice for the Sperm-Head Morphology Study

Observation	Chamber Concentrations (ppm)		
	200	1000	5000
Mean chamber concentration $\pm$ standard deviation (ppm)	199 $\pm$ 6.12	999 $\pm$ 22.6	4980 $\pm$ 130
Maximum-minimum (ppm)	229-174	1020-811	5180-3870
Relative standard deviation (%)	3	2	3
Mean of target concentrations (%)	100	100	100

Periodic measurements of the chamber atmosphere for the dimer of 1,3-butadiene (4-vinyl-1-cyclohexene) were performed using an HP 5790 gas chromatograph equipped with an FID, a Valco sampling valve with a 5-ml loop and a column similar to that previously described for butadiene monitoring. Column temperature was programmed to 190°C at 30°C/minute, following an 0.5-minute hold at 120°C. With this system, which employed a nitrogen carrier at 20 ml/minute, 1,3-butadiene eluted at 0.33 minutes, and the dimer at 3.25 minutes. A 100 ppm v/v certified stan-

dard of dimer in nitrogen was used to calibrate the gas chromatograph. Previous analyses for dimer concentrations in the atmosphere of the 1000-ppm chamber had shown that these values were consistent with the analyses of dimer concentrations in the cylinder headspace and indicated that additional dimerization did not occur during generation of the test material (Appendix A).

Determinations of chamber characteristics were obtained in the presence and absence of animals. These included chamber balance measurements, which demonstrated the uniformity of chamber mixing (Appendix C) and chamber buildup and decay information. During buildup, the chamber concentration was within 5% of the target concentration. During decay, the concentration was less than 5% of the target concentration within 15 minutes after cessation of exposure, with and without animals in the chamber (Figure 1).

FIGURE 1. Buildup and Decay of the Atmosphere of the 1000-ppm 1,3-Butadiene Exposure Chamber in the Presence and Absence of Animals

C. ANIMAL HUSBANDRY AND EXPERIMENTAL PROCEDURES

Salient features of the experimental design for this study are summarized in Table 2. Since many of the studies concerning the effects of various agents on sperm-head morphology have been performed using the B6C3F1 strain, these hybrid mice (C57BL6 x C3H/He), were purchased from the Charles River Laboratory,

Portage, MI Facility. One hundred males (7 to 8 weeks old; >20 g) were isolated for 5 weeks before exposure. After 2 weeks of isolation, five mice were killed and examined for internal and external parasites and bacterial pathogens; their sera were tested for antibodies to selected pathogens, and histopathologic examinations of lung, liver, kidney, ileum, colon, and heart were performed (Appendix D). At terminal sacrifice, serum from five males of the control group and five of the high dose group was tested for antibodies to selected pathogens; negative results were obtained.

TABLE 2. Experimental Design for the Sperm Morphology Study of Mice Exposed to 1,3-Butadiene or Ethyl Methanesulfonate

1,3-Butadiene Exposure Groups	Chamber concentrations (ppm): 0, 200, 1000 and 5000 Exposure regimen: 5 successive days; 6 hours/day Number of males/group: 20
Positive-Control Group	Ethyl methanesulfonate (EMS) concentration: 167 mg/kg Administration regimen: 0.5 ml (10 mg EMS/ml)/30 g mouse administered intraperitoneally for 5 successive days Number of males: 14
Time of Sacrifice	During the fifth postexposure week
Observations	Mortality and signs of toxicity: body weights prior to experimental treatment and at 0, 1, 2, 3, 4, and 5 weeks after treatment; examination of epididymal preparations for sperm-head abnormalities

Animal facilities and procedures at PNL were inspected and certified by the American Association of Laboratory Animal Care. During the isolation period, the animals were housed by sex, 10 mice/wire-mesh cage and provided (ad libitum) NIH-07 open formula diet; water was supplied by an automatic watering system. Temperatures and RH in the animal rooms were maintained in ranges of $72 \pm 3^\circ\text{F}$ and $50 \pm 15\%$, respectively. Following the isolation period, the males were individually identified by numbered eartags, weighed and assigned to exposure groups by means of a computer-assisted randomization program, which is based on a single blocking factor, body weight. In addition to individual eartag numbers, animals in each exposure group were identified by means of a distinctive toeclip on the forepaw.

During the 3 days prior to the initiation of exposure, the animals were housed in the exposure chambers in the exposure room. Environmental data were collected during the entire interval that the animals were housed in the exposure chambers. Each animal was exposed to its assigned treatment (atmospheric concentrations of 0

[filtered air], 200, 1000 and 5000 ppm of 1,3-butadiene) for 6 hours/day for 5 consecutive days (01/06/86 through 01/10/86). The positive-control animals, which were housed in an animal room separate from the inhalation exposure room, received daily intraperitoneal injections of 167 mg/kg of EMS (10 mg EMS/ml of distilled water, 0.5 ml/30 g mouse) on each of the 5 days that the butadiene-treated groups were being exposed. The EMS solutions administered to the animals were analyzed daily and compared with a gravimetrically prepared standard (Table 3). Following termination of the exposure or EMS treatment, the animals were held in individual wire-mesh cages during the 4-week interval between treatment and sacrifice. This EMS regimen was selected to reduce the mortality observed in studies (Wyrobek and Bruce, 1980) which used a dose level of 200 mg EMS/kg to induce 25% abnormalities in mouse sperm heads.

TABLE 3. Ethyl Methanesulfonate (EMS) Concentrations in Aqueous Solutions Administered to Male Mice in the Positive-Control Group of the Sperm Morphology Assay

Day of Administration	EMS (mg/ml) ^a
1	9.5
2	8.9
3	9.8
4	9.5
5	10.2

^aTarget concentration: 10 mg/ml

D. EXPERIMENTAL OBSERVATIONS

The animals were observed twice daily for mortality, morbidity and signs of toxicity; body weights were determined at weekly intervals. During the fifth post-exposure week, the mice were euthanized with CO₂, weighed and examined for lesions of the reproductive tract and for gross tissue abnormalities. The cauda of the right epididymis was removed and processed to obtain the sperm suspensions (NTP, 1983). Sperm suspensions were stained with 1% Eosin Y and two to three drops were transferred to microscope slides (four slides/mouse). The suspensions were dried and mounted under coverslips with mounting media. The slides were labeled with the animal number so that the identity of the treatment group would not be known to the examiner at the time of evaluation. The morphology of at

least 500 sperm heads from each mouse was categorized as normal or abnormal (blunt-hook, banana, amorphous, pinhead, two heads/two tails, short, and any other types of variants encountered during the examinations).

E. STATISTICAL METHODS

For body weight measurements, which were repeated over time, a randomization test (Zerbe, 1979) analyzed for differences among growth curves. Values for normal and abnormal sperm heads were expressed as a percentage of the total number of cells examined for each animal. These data were subjected to an arcsin transformation and evaluated by an analysis of variance (ANOVA). If the ANOVA F statistic was significant, Duncan's multiple range test was used to delineate inter-group differences. Dose-response trends were determined by means of orthogonal contrasts (Winer, 1971).

Group	Mean body weight (g)		Standard deviation (g)	Significance (p)
	At birth	At 14 days		
Control	1.85 ± 0.05	21.5 ± 0.5	0.5	
Low dose	1.85 ± 0.05	21.5 ± 0.5	0.5	
Medium dose	1.85 ± 0.05	21.5 ± 0.5	0.5	
High dose	1.85 ± 0.05	21.5 ± 0.5	0.5	
Very high dose	1.85 ± 0.05	21.5 ± 0.5	0.5	
Abnormal sperm	1.85 ± 0.05	21.5 ± 0.5	0.5	
Normal sperm	1.85 ± 0.05	21.5 ± 0.5	0.5	

III. RESULTS

No deaths occurred in any of the four inhalation exposure groups or in the positive-control group. When the mice were examined immediately after the third, fourth and fifth days of exposure to 1,3-butadiene, piloerection and dyspnea were observed in the mice exposed to 5000 ppm, but no toxic signs were evident in the animals exposed to the lower concentrations of the chemical. The appearance and behavior of the mice in the 5000-ppm group were normal within 20 to 30 minutes of the termination of the exposure. Evidently these toxic effects may have been present, but were not observed during the first 2 exposure days because the animals were not inspected immediately after the cessation of exposure.

Mean values for body weights of 1,3-butadiene- or of EMS-treated mice were not significantly different from those of the control mice (Table 4). However, when these data were expressed in terms of weight gain, based on the weight measured before the first exposure or injection, the values for the EMS-treated group were significantly lower than control values of the inhalation-exposure mice during all postexposure intervals until sacrifice (Figure 2). This depressed weight gain was attributable to a loss of weight during the week of treatment with the positive-control material.

TABLE 4. Body Weights (g, Mean $\pm$ Standard Error) of Male Mice Exposed to 1,3-Butadiene or Ethyl Methanesulfonate (EMS)

Weeks After Initiation of Exposure	1,3-Butadiene Concentration (ppm)				EMS ^a
	0	200	1000	5000	
0	29.4 $\pm$ 0.4	29.8 $\pm$ 0.4	29.6 $\pm$ 0.3	29.0 $\pm$ 0.4	29.8 $\pm$ 0.5
1	30.1 $\pm$ 0.4	30.9 $\pm$ 0.4	30.1 $\pm$ 0.3	29.9 $\pm$ 0.4	29.2 $\pm$ 0.5
2	30.6 $\pm$ 0.3	30.8 $\pm$ 0.4	30.5 $\pm$ 0.3	29.4 $\pm$ 0.4	29.7 $\pm$ 0.6
3	31.5 $\pm$ 0.4	31.4 $\pm$ 0.4	31.3 $\pm$ 0.4	30.7 $\pm$ 0.4	30.2 $\pm$ 0.5
4	32.5 $\pm$ 0.4	32.2 $\pm$ 0.4	32.4 $\pm$ 0.5	31.8 $\pm$ 0.5	31.7 $\pm$ 0.6
5	31.5 $\pm$ 0.4	31.7 $\pm$ 0.4	32.1 $\pm$ 0.5	31.5 $\pm$ 0.5	31.9 $\pm$ 0.6

^aPositive control (5 daily doses of 167 mg EMS/kg)

At necropsy, lesions were noted in the reproductive tracts of two males that had been exposed to 1,3-butadiene. An infected preputial gland was observed in mouse #459 of the 200-ppm exposure group. No unusual clinical observations were

recorded for this animal and subsequent morphologic evaluation of epididymal sperm detected only 1.1% abnormalities. In the second mouse (animal #458 of the 1000-ppm exposure group), an injury in the scrotal area was observed 8 days prior to the initiation of exposure and was subsequently noted for 6 days. At necropsy, the cauda of the left epididymis was adherent to the scrotal sac. Because of the evidence for complicating traumatic injury and the presence of 36.4% sperm-head abnormalities in this animal, all data for mouse #458 were excluded from statistical evaluations.

FIGURE 2. Weight Gain (Mean Percent) in B6C3F1 Mice Exposed to 1,3-Butadiene (BD) or Ethyl Methanesulfonate (EMS)

Analyses of the morphologic data demonstrated that values for sperm with normal morphology were significantly lower in mice exposed to 1000 to 5000 ppm of 1,3-butadiene than in control mice (Table 5). This depression in the percentage of normal sperm in males exposed to the two highest concentrations of the chemical could be attributed to significant increases in values for three categories of sperm-head abnormalities (blunt hook, banana and pinhead). Analyses for significant linear trends in these data also demonstrated that the decreased incidence of normal sperm and the increased incidence of three of the abnormalities (blunt hook, banana and amorphous) were concentration-related. No significant differences were observed in values for any sperm-head categories in the mice injected with EMS when they were compared with results from control mice. In addition, there were no significant differences among classes of abnormalities in mice exposed to increasing concentrations of 1,3-butadiene or to the positive-control agent (Table 6). Observations included in the category of "other abnormalities" were limited to a midpiece abnormality. In the few sperm with this anomaly, the characteristic midpiece organization was replaced by a fine, thread-like structure; a small spherical enlargement was apparent at the distal end of this structure.

TABLE 5. Morphologic Classification of Sperm Heads (Mean Percentage of Total Examined $\pm$ Standard Error) of Mice Exposed to 1,3-Butadiene or Ethyl Methanesulfonate (EMS)

Classification of Sperm Heads	1,3-Butadiene Concentration (ppm)				EMS ^a
	0	200	1000	5000	
Normal ^b	98.40 $\pm$ 0.37 ^c	98.08 $\pm$ 0.12 ^{cd}	97.23 $\pm$ 0.19 ^d	96.34 $\pm$ 0.42 ^e	97.98 $\pm$ 0.18
Blunt hook ^b	0.49 $\pm$ 0.11 ^c	0.58 $\pm$ 0.07 ^c	0.74 $\pm$ 0.07 ^c	1.10 $\pm$ 0.14 ^d	0.65 $\pm$ 0.11
Banana ^b	0.64 $\pm$ 0.11 ^c	0.85 $\pm$ 0.10 ^{cd}	1.18 $\pm$ 0.13 ^d	1.72 $\pm$ 0.27 ^e	0.66 $\pm$ 0.07
Amorphous ^b	0.39 $\pm$ 0.18	0.39 $\pm$ 0.08	0.61 $\pm$ 0.07	0.75 $\pm$ 0.11	0.49 $\pm$ 0.08
Pinhead	0.06 $\pm$ 0.03 ^c	0.08 $\pm$ 0.03 ^{cd}	0.17 $\pm$ 0.05 ^d	0.08 $\pm$ 0.03 ^{cd}	0.14 $\pm$ 0.06
Two heads/two tails	0	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0	0.01 $\pm$ 0.01
Short	0.03 $\pm$ 0.02	0.04 $\pm$ 0.02	0.02 $\pm$ 0.02	0.03 $\pm$ 0.02	0.02 $\pm$ 0.02
Other abnormalities	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	0.06 $\pm$ 0.04	0	0.01 $\pm$ 0.01
Total abnormal ^b	1.61 $\pm$ 0.38 ^c	1.95 $\pm$ 0.11 ^{cd}	2.79 $\pm$ 0.18 ^d	3.69 $\pm$ 0.42 ^e	1.98 $\pm$ 0.18

^aValues for the positive-control group (5 daily doses of 167 mg EMS/kg) are not significantly different ($P \leq 0.05$) from control values.

^bSignificant ($P \leq 0.05$) linear trend

^{c-e}Values that do not share a common superscript letter are significantly different ($P \leq 0.05$) from one another following arcsin transformation of the data.

TABLE 6. Distribution of Sperm-Head Abnormalities^a (Mean Percentage of Total Examined $\pm$ Standard Error) of Mice Exposed to 1,3-Butadiene or Ethyl Methanesulfonate (EMS)

Classification of Sperm-Head Abnormality	1,3-Butadiene Concentration (ppm)				EMS ^b
	0	200	1000	5000	
Blunt hook	30.34 $\pm$ 4.78	28.26 $\pm$ 2.69	27.68 $\pm$ 2.78	32.14 $\pm$ 4.06	31.33 $\pm$ 3.50
Banana	46.56 $\pm$ 5.62	43.14 $\pm$ 4.55	40.59 $\pm$ 2.25	43.62 $\pm$ 3.74	36.95 $\pm$ 4.47
Amorphous	17.23 $\pm$ 3.51	21.06 $\pm$ 4.27	23.39 $\pm$ 2.76	20.64 $\pm$ 3.16	24.29 $\pm$ 3.85
Pinhead	3.16 $\pm$ 1.47	3.73 $\pm$ 1.81	5.37 $\pm$ 1.48	2.23 $\pm$ 0.88	5.83 $\pm$ 2.24
Two heads/two tails	0	0.38 $\pm$ 0.38	0.43 $\pm$ 0.43	0	0.32 $\pm$ 0.32
Short	1.46 $\pm$ 1.04	2.30 $\pm$ 1.17	0.64 $\pm$ 0.44	1.37 $\pm$ 1.02	0.80 $\pm$ 0.56
Other abnormalities	1.25 $\pm$ 1.25	1.13 $\pm$ 0.81	1.90 $\pm$ 1.33	0	0.48 $\pm$ 0.48

^a(Percentage of individual classes of abnormalities $\div$ percentage of sum of abnormal sperm heads) $\times$ 100

^bPositive control (5 daily doses of 167 mg EMS/kg)

IV. DISCUSSION

Although a toxic response to the positive-control material, EMS, as measured by depressed body weight gains, was detected in the mice, no increase in the incidence of sperm-head abnormalities was observed. Previous studies by Wyrobek and Bruce (1980) reported that five daily injections of 200 mg EMS/kg produced mortality and 25% abnormalities of sperm heads in mouse epididymal samples examined 4 weeks after treatment. Accordingly, a lower dose of EMS (167 mg/kg) was used in the present study to avoid excessive mortality, but evidently this dose level, which produced signs of toxicity, was not sufficient to induce a significant increase in the incidence of sperm-head abnormalities. In another study performed in this laboratory, EMS was administered on 5 successive days to B6C3F1 mice (from the same source as the animals in the present study) at dose levels of 200 and 250 mg/kg. Mortality was only 5 and 15%, respectively, in the mice that received 200 and 250 mg/kg. Mean percentages of abnormal sperm were: 0.99 ± 0.99 for control mice, 3.62 ± 1.40 for mice injected with 200 mg/kg of EMS and 13.04 ± 2.39 for mice receiving 250 mg/kg (PNL, 1988).

The percentage of sperm-head abnormalities in control mice of this study (1.6 ± 0.4) is in good agreement with a value of 1.5% reported by Wyrobek and Bruce (1980) and $1.5 \pm 0.3\%$ (Komatsu et al., 1982) for B6C3F1 mice. The protocol for the present study was based on the rapid-screening method of Wyrobek and Bruce (1975). Therefore, the study was designed to determine the effects of chemical exposure by obtaining sperm samples during the one interval in which there was the highest probability for detecting sperm abnormalities. Since the sperm were examined at only one time period, no conclusions can be drawn concerning cellular sensitivity to 1,3-butadiene during all phases of gamete development. In the 1,3-butadiene-exposed males assayed during the fifth postexposure week of this study, a significant concentration-related increase in the frequency of sperm-head abnormalities was observed. These significant differences in the percentage of abnormalities between control mice and males exposed to 1000 to 5000 ppm indicated that their late spermatogonia or early primary spermatocytes were sensitive to this chemical. These findings were detected in animals that evidenced no depression in body weight gain and exhibited only transient signs of toxicity immediately following exposure to the highest atmospheric concentration of 1,3-butadiene.

Although speculations concerning the reproductive consequences of these findings cannot be made, verification of these results by an independent study is indicated. A separate study to evaluate the induction of dominant lethality in CD-1 mice, using an identical exposure regimen, has been conducted in this laboratory. This study should provide additional information regarding the reproductive potential of 1,3-butadiene-exposed males; however, the comparison will not be equivalent because variations among strains in sensitivity to mutagenic agents (Wyrobek and Bruce, 1980) and in control levels of abnormal sperm have been detected.

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APPENDIX A
ANALYTICAL DATA FOR 1,3-BUTADIENE (LOT NUMBER F909)

FIGURE A1. Infrared Spectrum of 1,3-Butadiene

BULK CHEMICAL REANALYSIS FOR 1,3-BUTADIENE

Compound: 1,3-Butadiene
 NTP number: 10514-J
 CAS number: 106-99-0
 Lot: Phillips F909; BNW notebook number 50846-36-(2&3)
 Appearance: NA
 Receipt date: 06/28/85
 Analysis period: 4 months after receipt
 Storage temperature: Room temperature
 Sample submittal date: NA
 Sample analysis date: November 13 and 14, 1985
 Analysis procedure: Method #ØB-AC-3AØ3-Ø3
 Notebook reference: BNW 50846-60 through 63

Assay: Gas chromatography, using a 2-m x 2-mm glass column packed with Porapak QS 100/120 for purity by area percent and 1-m x 2-mm glass column packed with Porapak PS 80/100 for the dimer analysis, dimer content is determined from the ratio of the area of the dimer peak versus the area of the 1,3-butadiene peak. Instrument: Hewlett-Packard 5840A

Results:

Date	Sample ID	Bulk
11/85	BNW 50846-36-2	99.88
	BNW 50846-36-3	99.88

1,3-Butadiene eluted at ~6.7 minutes. A minor peak eluted 2 minutes later; it represented 0.11% of the total peak area.

Date	Sample ID	Dimer (ppm)
11/85	BNW 50846-36-2	332 ^a
	BNW 50846-36-3	189

1,3-Butadiene eluted at ~0.4 minutes, the dimer at ~4.7 minutes. The dimer retention time was confirmed by running a dimer standard.

^aDifferences in values for dimer concentrations in individual cylinders reflect the thermal and usage history of each cylinder (see p. 5 of report).

HP RUN # 372
AREA %

NOV/14/85

TIME 12:46:23

RT	AREA	AREA %
0.25	74	0.000
0.32	1315	0.001
2.09	3874	0.004
2.33	2006	0.002
6.68	87700000	99.878
8.48	99500	0.113

DIL FACTOR: 1.0000 E+0

HP RUN # 373
AREA %

NOV/14/85

TIME 12:00:33

RT	AREA	AREA %
0.33	47	0.000
2.00	1949	0.002
2.51	652	0.001
4.49	20	0.000
6.69	84740000	99.888
8.48	99000	0.117

DIL FACTOR: 1.0000 E+0

FIGURE A2. Gas Chromatogram of 1,3-Butadiene

HEADSPACE DIMER CONTENT OF THE 1,3-
BUTADIENE CYLINDER (BNW 50846-36-1)
DURING ANIMAL EXPOSURES

Date	Dimer Concentration (ppm v/v)
01/03/86	161
01/06/86	163
01/09/86	188
01/10/86	170

APPENDIX B

**CONCENTRATION, TEMPERATURE AND RELATIVE
HUMIDITY MEASUREMENTS IN THE EXPOSURE CHAMBERS**

TABLE B1. Exposure Atmospheric Concentrations (Mean Daily Concentration $\pm$ Standard Deviation) of 1,3-Butadiene during the Sperm Morphology Study in Mice

Exposure Day	Exposure Chamber (ppm)		
	200	1000	5000
1	197 $\pm$ 11.4	989 $\pm$ 45.3	4920 $\pm$ 268 ^a
2	199 $\pm$ 3.0	999 $\pm$ 1.5	5000 $\pm$ 21.6
3	200 $\pm$ 5.1	1000 $\pm$ 7.8	5000 $\pm$ 27.7
4	201 $\pm$ 2.4	1000 $\pm$ 7.7	5010 $\pm$ 26.3
5	199 $\pm$ 2.0	1010 $\pm$ 7.9	4990 $\pm$ 43.4

^aThe last gas chromatographic reading (3865 ppm) before termination of the exposure for the day was less than the lower critical operating limit of 4000 ppm. The cause of this low value could not be determined.

TABLE B2. Exposure Chamber Temperatures ($^{\circ}$ F, Daily Mean $\pm$ Standard Deviation) during the Sperm Morphology Study of 1,3-Butadiene in Mice

Exposure Day	Exposure Chamber (ppm)			
	0	200	1000	5000
1	74.1 $\pm$ 0.40	73.3 $\pm$ 0.37	73.2 $\pm$ 0.33	74.2 $\pm$ 0.29
2	74.2 $\pm$ 0.21	73.4 $\pm$ 0.36	73.5 $\pm$ 0.28	74.5 $\pm$ 0.40
3	74.2 $\pm$ 0.37	73.3 $\pm$ 0.42	73.3 $\pm$ 0.45	74.5 $\pm$ 0.43
4	74.4 $\pm$ 0.38	73.4 $\pm$ 0.39	73.6 $\pm$ 0.43	74.6 $\pm$ 0.45
5	74.0 $\pm$ 0.45	73.1 $\pm$ 0.40	73.1 $\pm$ 0.41	74.2 $\pm$ 0.40

TABLE B3. Exposure Chamber Relative Humidity (RH; %, Daily Mean $\pm$ Standard Deviation) during the Sperm Morphology Study of 1,3-Butadiene in Mice

Exposure Day	Exposure Chamber (ppm)			
	0	200	1000	5000
1	47.8 $\pm$ 3.2	44.7 $\pm$ 5.7	40.0 $\pm$ 3.0	61.5 $\pm$ 9.9
2	45.3 $\pm$ 3.2	47.0 $\pm$ 1.5	39.6 $\pm$ 1.4 ^a	64.0 $\pm$ 3.6
3	48.1 $\pm$ 3.9	45.1 $\pm$ 4.6	54.0 $\pm$ 9.0	56.7 $\pm$ 3.4
4	49.7 $\pm$ 3.6	46.3 $\pm$ 4.8	56.9 $\pm$ 5.4	56.2 $\pm$ 5.0
5	48.9 $\pm$ 3.3	50.5 $\pm$ 1.8	61.4 $\pm$ 4.5	60.3 $\pm$ 5.3

^aThe mean daily RH (mean = 39.6%; minimum = 37.0%) was less than the lower operating limit (40%) specified in the protocol.

APPENDIX C
CHAMBER UNIFORMITY TESTS FOR 1,3-BUTADIENE CONCENTRATIONS

COMPOUND: IRT 1,3 BUTADIENEEXPOSURE ROOM NUMBER: 436

TPV MEASUREMENTS

CHAMBER:		200 ppm		1000 ppm		5000 ppm			
DATE:		12/27/85		12/27/85		12/27/85			
SAMPLE PORT		MONITOR READING	%RSD	MONITOR READING	%RSD	MONITOR READING	%RSD	MONITOR READING	%RSD
BACK:	1B	188	99.6%	938	100.2%	4993	99.9%		
	2B	190	100.7%	940	100.4%	4967	99.3%		
	3B	189	100.1%	939	100.3%	4997	99.9%		
	4B	190	100.7%	941	100.5%	4972	99.4%		
	5B	187	99.1%	933	99.9%	4967	99.3%		
FRONT:	6B	190	100.7%	939	100.3%	5002	100.0%		
	1F	189	100.1%	931	99.5%	4984	99.7%		
	2F	188	99.6%	932	99.6%	5003	100.0%		
	3F	189	100.1%	933	99.7%	5012	100.2%		
	4F	189	100.1%	934	99.8%	5035	100.7%		
	5F	188	99.6%	933	99.7%	5038	100.7%		
	6F	188	99.6%	936	100.0%	5036	100.7%		
MEAN:		188.8	100.0%	935.9	100.0%	5000.7	100.0%		
TPV:		0.97	0.5%	3.40	0.4%	25.88	0.5%		
BPV:		//////////	≤0%	//////////	0.2%	//////////	0.2%	//////////	//////////

WPV MEASUREMENTS

IN-LINE	1st	190	100.7%	940	100.2%	4967	100.0%		
	2nd	188	99.6%	940	100.2%	4994	100.5%		
	3rd	188	99.6%	933	99.6%	4945	99.5%		
MEAN:		188.7	100.0%	938.3	100.0%	4968.7	100.0%		
WPV:		1.15	0.6%	2.89	0.3%	24.54	0.5%		

MONITOR TYPE: GC 809569

SERIAL #: _____

MONITOR DATA LOCATION: _____

COMMENTS: Pre-exposure chamber balance without animalsENTERED BY: ML ClarkDATE: 12/31/85REVIEWED BY: MJCLDATE: 1/3/86

FIGURE C1. Chamber Uniformity Data Sheet--Without Animals

COMPOUND: IRT 1,3 Butadiene, Sperm Head Morphology StudyEXPOSURE ROOM NUMBER: 436

TPV MEASUREMENTS

CHAMBER: #6 200 ppm		#7 1000 ppm		#8 5000 ppm			
DATE: 1/10/86		1/10/86		1/10/86			
SAMPLE PORT	MONITOR READING	%	MONITOR READING	%	MONITOR READING	%	MONITOR READING
BACK: 1B	177500	100.5%	886000	100.0%	4367000	100.0%	
2B							
3B							
4B	176100	99.7%	886200	100.0%	4365000	99.9%	
5B							
6B							
FRONT: 1F							
2F							
3F							
4F	176200	99.8%	886000	100.0%	4374000	100.1%	
5F							
6F							
MEAN:	176600.0	100.0%	886066.7	100.0%	4368000.0	100.0%	
WV:	781.02	0.4%	115.47	0.0%	5567.76	0.1%	
WV:		≤0%		≤0%		0.1%	

WPV MEASUREMENTS

IN-LINE 1st	177500	100.5%	886000	100.1%	4367000	99.9%	
2nd	176400	99.9%	886200	100.0%	4374000	100.1%	
3rd	173700	99.5%	886600	99.8%	4368000	100.0%	
MEAN:	176533.3	100.0%	884933.3	100.0%	4369666.7	100.0%	
WV:	907.38	0.5%	1222.02	0.1%	3785.94	0.1%	

MONITOR TYPE: GC type 5840

SERIAL #: N809569

MONITOR DATA LOCATION:

COMMENTS: Chambers housing animalsENTERED BY: ML ClarkDATE: 1/11/86REVIEWED BY: M. S. CHLDATE: 1/31/86

FIGURE C2. Chamber Uniformity Data Sheet--With Animals

APPENDIX D
HEALTH EVALUATIONS OF EXPERIMENTAL ANIMALS

Battelle

Pacific Northwest Laboratories

Project Number _____

Internal Distribution

MG Brown
w/o attachment
File/LB

Date December 20, 1985
To P. L. Hackett
From S. E. Rowe *SEP*
Subject Pre-Exposure Health Evaluation of B6C3F1 Mice
for 1,3 Butadiene Study

These B6C3F1 mice were received in good condition on 12-3-85 from Charles River's Portage, MI facility, area 02. On 12-18, 5 males were randomly selected for health evaluation. Examination included culture of nasopharyngeal wash for bacterial pathogens, serologic tests for viral antibodies, gross necropsy and microscopic examination of selected tissues.

Rare tiny foci of hepatocellular necrosis and/or inflammation were found in the livers of 3 mice. These lesions are very common in recently shipped mice and I believe they are caused by shipping stress without intercurrent infection. Serologic tests for mouse hepatitis virus were negative.

No other significant lesions or pathogens were found and the mice were verbally released from quarantine on 12-20-85. Detailed results can be found in the attached lab report (N-135).

APPENDIX E
MORPHOLOGIC CLASSIFICATION OF SPERM HEADS OF INDIVIDUAL MICE

TABLE E1. Morphologic Classification of Sperm Heads of Individual Mice^a

aColumn Headings:

CASE = Sequential number of samples
ID# = Eartag number of male mouse

TREATMNT = Exposure concentration

1 = 0 ppm 1,3-butadiene
2 = 200 ppm 1,3-butadiene
3 = 1000 ppm 1,3-butadiene
4 = 5000 ppm 1,3-butadiene
5 = 167 mg/kg ethyl methanesulfonate

Morphologic Classifications of Sperm Heads

%NORMAL = Normal
%BLNT HK = Blunt hook
%BANANA = Banana
%AMORPHO = Amorphous
%PINHEAD = Pinhead
%2HD2TL = Two heads/two tails
%SHORT = Short
%OTHER = Other abnormalities
%ABNORM = Total abnormalities

CASE	ID#	TREATMNT	%NORMAL	%BLNT HK	%BANANA	%AMORPHO	%PINHEAD	%2HD2TL	%SHORT	%OTHER	%ABNORM
1	450.	1.	99.2	0.4	0.0	0.0	0.2	0.0	0.0	0.2	0.0
2	452.	1.	98.4	1.0	0.2	0.4	0.0	0.0	0.0	0.0	1.0
3	460.	1.	98.0	0.4	0.0	0.2	0.0	0.0	0.0	0.0	1.4
4	464.	1.	99.1	0.2	0.5	0.2	0.0	0.0	0.0	0.0	0.9
5	471.	1.	98.2	0.4	0.6	0.6	0.2	0.0	0.0	0.0	1.0
6	477.	1.	98.5	0.2	1.0	0.4	0.0	0.0	0.0	0.0	1.0
7	479.	1.	99.4	0.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0
8	482.	1.	99.0	0.2	0.4	0.0	0.0	0.0	0.0	0.0	1.0
9	490.	1.	99.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	1.0
10	492.	1.	98.8	0.2	0.0	0.2	0.0	0.0	0.0	0.0	1.2
11	496.	1.	99.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
12	497.	1.	99.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.4
13	501.	1.	99.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.4
14	503.	1.	97.0	0.0	1.0	0.0	0.0	0.0	0.2	0.0	3.0
15	511.	1.	98.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4
16	515.	1.	99.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.4
17	522.	1.	98.2	0.4	1.0	0.2	0.2	0.0	0.0	0.0	1.0
18	530.	1.	91.9	2.2	1.0	3.0	0.4	0.0	0.2	0.0	0.2
19	531.	1.	98.2	0.0	0.0	0.2	0.2	0.0	0.0	0.0	1.0
20	540.	1.	97.9	0.9	0.9	0.2	0.0	0.0	0.0	0.0	2.0
21	453.	2.	99.0	0.2	0.4	0.2	0.0	0.0	0.2	0.0	1.0
22	458.	2.	98.0	1.0	1.0	0.4	0.2	0.0	0.0	0.0	3.2
23	459.	2.	98.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	1.2
24	461.	2.	98.2	0.2	1.2	0.4	0.0	0.0	0.0	0.0	1.0
25	462.	2.	98.0	0.4	0.5	0.4	0.0	0.0	0.0	0.0	1.3
26	463.	2.	97.9	0.7	0.7	0.1	0.5	0.1	0.0	0.0	2.1
27	466.	2.	98.0	0.0	0.2	1.0	0.0	0.0	0.0	0.0	2.0
28	468.	2.	97.4	1.0	0.4	1.0	0.0	0.0	0.2	0.0	2.0
29	472.	2.	97.7	0.4	1.2	0.4	0.2	0.0	0.0	0.2	2.4
30	475.	2.	98.0	0.4	0.0	1.0	0.0	0.0	0.0	0.0	2.0
31	491.	2.	98.0	1.0	0.0	0.2	0.0	0.0	0.2	0.0	2.0
32	496.	2.	98.0	0.0	0.5	0.3	0.2	0.0	0.2	0.0	2.0
33	509.	2.	97.0	1.2	1.0	0.2	0.0	0.0	0.0	0.0	2.4
34	519.	2.	97.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	2.4
35	520.	2.	98.4	0.2	1.4	0.0	0.0	0.0	0.0	0.0	1.0
36	521.	2.	98.1	0.2	1.0	0.0	0.0	0.0	0.0	0.0	2.0
37	523.	2.	98.0	0.4	0.4	0.0	0.4	0.0	0.0	0.2	1.4
38	526.	2.	98.2	0.4	1.0	0.4	0.0	0.0	0.0	0.0	1.0
39	528.	2.	98.3	0.0	1.0	0.2	0.0	0.0	0.0	0.0	1.0
40	532.	2.	98.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	2.0
41	446.	3.	96.0	0.0	1.4	1.2	0.0	0.0	0.0	0.0	3.4
42	451.	3.	97.3	0.4	1.0	0.0	0.2	0.0	0.0	0.4	2.0
43	454.	3.	98.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0	1.0
44	457.	3.	98.4	0.0	1.0	0.0	0.2	0.0	0.2	0.0	3.0
45	459.	3.	85.2	22.5	5.3	0.3	0.0	0.0	0.0	0.0	34.0

CASE	ID#	TREATMNT	%NORMAL	%BLNT HK	%BANANA	%AMORPHO	%PINHEAD	%2HD2TL	%SHORT	%OTHER	%ABNORM
46	478.	3.	97.8	0.8	0.8	0.8	0.0	0.0	0.0	0.0	2.2
47	480.	3.	97.8	0.8	0.8	0.8	0.0	0.0	0.0	0.0	2.2
48	484.	3.	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
49	487.	3.	98.8	0.4	1.8	0.8	0.2	0.0	0.0	0.0	3.2
50	502.	3.	95.8	0.6	2.4	1.0	0.4	0.0	0.0	0.0	4.4
51	507.	3.	97.8	0.8	0.8	0.4	0.2	0.0	0.0	0.0	2.2
52	510.	3.	97.2	1.4	1.0	0.2	0.2	0.0	0.0	0.0	2.8
53	512.	3.	97.1	0.8	0.8	0.8	0.0	0.0	0.0	0.6	3.0
54	514.	3.	98.3	0.4	0.6	0.8	0.0	0.0	0.0	0.0	1.8
55	516.	3.	98.6	1.0	1.4	0.2	0.8	0.0	0.0	0.0	3.4
56	518.	3.	98.4	0.4	0.4	0.8	0.2	0.0	0.0	0.0	1.8
57	527.	3.	98.7	0.8	1.6	0.6	0.2	0.0	0.2	0.0	3.3
58	529.	3.	97.4	0.8	1.2	0.4	0.0	0.2	0.0	0.0	2.6
59	534.	3.	98.3	0.8	2.1	0.4	0.4	0.0	0.0	0.0	3.7
60	537.	3.	97.8	1.4	0.8	0.2	0.0	0.0	0.0	0.0	2.4
61	448.	4.	98.0	0.4	0.8	1.0	0.0	0.0	0.0	0.0	2.0
62	449.	4.	98.1	0.2	1.3	0.6	0.0	0.0	0.0	0.0	2.0
63	465.	4.	95.7	1.4	1.2	1.6	0.0	0.0	0.2	0.0	4.4
64	467.	4.	98.1	0.6	2.3	1.1	0.0	0.0	0.0	0.0	4.0
65	473.	4.	97.8	1.4	0.6	0.2	0.0	0.0	0.0	0.0	2.2
66	481.	4.	98.2	1.0	1.8	1.0	0.0	0.0	0.0	0.0	3.8
67	483.	4.	98.6	0.8	1.8	0.4	0.4	0.0	0.0	0.0	3.4
68	485.	4.	93.2	0.8	4.4	1.4	0.2	0.0	0.0	0.0	6.8
69	488.	4.	92.4	1.7	4.6	1.3	0.0	0.0	0.0	0.0	7.8
70	488.	4.	97.7	1.7	0.6	0.0	0.2	0.0	0.0	0.0	2.4
71	494.	4.	95.8	1.4	1.8	1.0	0.0	0.0	0.0	0.0	4.2
72	500.	4.	94.4	2.0	1.8	1.7	0.2	0.0	0.0	0.0	5.7
73	504.	4.	98.4	0.8	0.2	0.6	0.2	0.0	0.0	0.0	1.8
74	505.	4.	98.8	1.2	2.0	0.0	0.0	0.0	0.0	0.0	3.2
75	506.	4.	93.2	2.2	3.4	0.8	0.2	0.0	0.2	0.0	6.8
76	508.	4.	98.9	0.4	2.0	0.8	0.0	0.0	0.0	0.0	3.2
77	517.	4.	95.4	2.2	1.6	0.8	0.2	0.0	0.0	0.0	4.8
78	524.	4.	99.0	0.6	0.2	0.0	0.0	0.0	0.2	0.0	1.0
79	538.	4.	98.0	0.2	1.0	0.8	0.0	0.0	0.0	0.0	2.0
80	539.	4.	97.2	1.2	1.4	0.2	0.0	0.0	0.0	0.0	2.8
81	447.	5.	98.6	0.2	1.0	0.0	0.2	0.0	0.0	0.0	1.4
82	455.	5.	98.6	0.2	0.6	0.8	0.0	0.0	0.0	0.0	1.6
83	469.	5.	98.3	0.7	0.8	0.1	0.0	0.0	0.0	0.0	1.8
84	470.	5.	98.0	0.6	0.6	0.2	0.0	0.0	0.0	0.0	1.4
85	474.	5.	97.2	1.6	0.2	1.0	0.0	0.0	0.0	0.0	2.8
86	476.	5.	97.4	1.0	1.0	0.3	0.2	0.0	0.0	0.0	2.5
87	489.	5.	98.9	0.6	1.0	0.8	0.7	0.1	0.1	0.0	3.1
88	493.	5.	97.0	1.2	0.6	0.6	0.2	0.0	0.2	0.2	3.0
89	498.	5.	98.2	0.6	0.2	0.6	0.4	0.0	0.0	0.0	1.8
90	499.	5.	98.5	0.6	0.8	0.2	0.0	0.0	0.0	0.0	1.6

CASE	ID#	TREATMNT	%NORMAL	%BLNT HK	%BANANA	%AMORPHO	%PINHEAD	%2HD2TL	%SHORT	%OTHER	%ABNORM
91	513.	5.	99.2	0.2	0.4	0.2	0.0	0.0	0.0	0.0	0.8
92	533.	5.	98.4	0.4	0.5	0.7	0.0	0.0	0.0	0.0	1.8
93	535.	5.	97.7	0.8	0.8	0.7	0.0	0.0	0.0	0.0	2.3
94	536.	5.	97.8	0.4	0.8	0.8	0.2	0.0	0.0	0.0	2.2

APPENDIX F

QUALITY ASSURANCE STATEMENT, SAMPLE AND RECORD DISPOSITION, CALENDAR OF EVENTS FOR THE SPERM-HEAD MORPHOLOGY STUDY, AND LIST OF PERSONNEL FOR THE SPERM-HEAD MORPHOLOGY STUDY

SPERM-HEAD MORPHOLOGY ASSAY OF 1,3-BUTADIENE IN MICE

QUALITY ASSURANCE STATEMENT

Listed below are the phases and/or procedures included in the study described in this report which were reviewed by the Quality Assurance Unit during the period, 12/1/86 - 2/15/86, specifically for this study and the dates the reviews were performed and findings reported to management. (All findings were reported to the study director or his designee at the time of the review.)

Phase/Procedure Reviewed	Review Date	Date Findings Submitted in Writing to Study Director/Management
Data	12/4/85*	12/18/85
Health Screen	12/18/85*	12/19/85
Body Weights	12/30/85*	1/03/86
Animal Identification	12/30/85*	1/03/86
Dosing	1/07/86*	1/07/86
Dosing (positive controls)	1/07/86*	1/07/86
Animal Receipt	1/08/86	1/08/86
Necropsy	2/11&12/86*	2/12/86
Data	2/5-7/86*	2/11/86
Data	4/27,28 & 12/14/87*	1/19/88
Final Report	12/14/87 & 1/18/88*	1/19/88

* Reviewed specifically for this study.

Quality Assurance Specialist

1 / 19 / 88

SAMPLE AND RECORD DISPOSITION

Upon completion of the 1,3-butadiene studies, all slides and tissue specimens will be shipped to the National Toxicology Program (NTP) Archives. Records generated in the conduct of the study will be microfiched. Computer tapes of biological data, the original and two copies of the microfiche, and the microfiche index will be sent to Dr. Schwetz (National Institute of Environmental Health Sciences) for storage in NTP Archives.

The Quality Assurance Unit at Pacific Northwest Laboratory (PNL) will retain all documents and records associated with the conduct of the study for a period of at least 2 years following completion of the study. These materials will be placed in Room 1433 of the Life Sciences Laboratory-II building at PNL according to 21 CFR 58.195. NTP will be notified of the completion of the storage period so that they may specify the terms of the disposition of these records.

CALENDAR OF EVENTS FOR THE SPERM-HEAD MORPHOLOGY STUDY IN MICE

Animal order:	10/10/85 (B6C3F ₁ mice from Charles River Laboratories, Portage Facility, MI)
Receipt of animals:	12/02/85 (ARC #860014; 95 males; date of birth, 10/10/85)
Initial health screen:	12/18/85
Inhalation exposure:	01/06/86 through 01/10/86 (6 hours/day)
Body weight measurements:	Week 0 01/06/86 Week 1 01/13/86 Week 2 01/20/86 Week 3 01/27/86 Week 4 02/03/86 Week 5 At sacrifice
Sacrifice:	02/10/86 through 02/12/86
Completion of sample evaluations:	03/14/86
Completion of statistical evaluations:	05/09/86
Submission of Draft Report:	May 1987
Submission of Final Report:	April 1988

LIST OF PERSONNEL FOR SPERM-HEAD
MORPHOLOGY STUDY OF 1,3-BUTADIENE

Responsibility	Name
Principal Investigators	P.L. Hackett
	B.J. McClanahan
Exposure System	M.L. Clark
	J.R. Decker
	G.R. Ell
	L.G. Florek
	D.K. Hammerberg
	E.M. Milliman
	E.J. Rossignol
	R.D. Swanneck
Monitoring/Analytical Chemistry	M. McCulloch
	N.S. Stamps
	K.H. Stoney
	R.B. Westerberg
Animal Resources Center	M.G. Brown
	S.E. Rowe
	A.E. Jarrell
Morphologic Evaluations	B.J. McClanahan
	J.J. Evanoff
	R.L. Rommereim
	M.L. Sours
Statistical Analyses	R.L. Buschbom

P.L. Hackett 4/14/88

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