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CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT

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October 29, 1985

CERTIFIED MAIL - RETURN RECEIPT REQUESTED

**Document Control Officer (WH-557)
Information Management Division
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460**

Attention: Mr. Frank Kover

Dear Mr. Kover:

Re: 8EHQ-0985-0567

This is written in response to your letter of September 27, 1985, requesting certain information concerning 1,4-dichlorobutene-2.

As requested, a complete copy of the final report of the second inhalation study mentioned in my letter of September 5, 1985 is attached (Attachment 1). We have not written a final report for the first inhalation study, but plan to prepare such report by the first Quarter of 1986 and we will provide a copy of the final report when it has been completed.

Also, as requested, we are enclosing a copy of the study of Mortality and Cancer Incidence among workers exposed to 1,4-dichlorobutene-2 at the Victoria Plant (Attachment 2).

In addition to the above, Mr. David R. Williams of your office has asked that we provide copies of correspondence with the Interagency Testing Committee (ITC) or the International Agency for Research on Cancer (IARC) relative to 1,4-dichlorobutene-2.

October 29, 1985

IARC in cooperation with the U.S. National Cancer Institute conducts surveys of institutes throughout the world that undertake long-term testing of chemicals for carcinogenicity. IARC publishes its survey results in an information bulletin so as to enable such institutes to avoid unnecessary duplication of research and to increase communication among scientists. The Haskeil Laboratory is one of the institutes polled by IARC and we have provided information to assist IARC in its efforts. We are providing a copy of the title page and page 187 of the 1984 IARC Information Bulletin which include an entry on 1,4-DCB for your files (Attachment 3).

With regard to the ITC review of 2,4-dichlorodioxin-2, we are enclosing as Attachment 4 the following:

A letter from D. Modi, dated November 24, 1981, transmitting information on 2,4-DCB to the ITC contractor which included the following information relating to 1,4-DCB:

- An April 1981 Haskeil Laboratory literature review which included the following:
 - A summary of the findings from the first 1,4-DCB inhalation study.
 - Information that a second chronic inhalation study on 1,4-DCB at levels of 0, 0.1, 0.5, and 1.0 ppm was starting.
 - A summary of the epidemiology study of the Victoria plant workers.

We trust that the above documents will be of assistance as you continue your review of our reports and, as indicated above, we will provide a copy of the report of the first inhalation study upon completion.

Sincerely,

Charles F. Reinhardt

Charles F. Reinhardt, M. D.,
Director

CFR/rlm
Enclosures
3.10

(10/29/85)

ATTACHMENT 1

**LONG-TERM INHALATION STUDY WITH
1,4-DICHLOROBUTENE-2 (DCB) IN RATS**

**FINAL REPORT ON A STUDY CONDUCTED
8/25/80 - 9/23/82
HASKELL LABORATORY REPORT NO. 477-85**

LONG-TERM INHALATION STUDY WITH
1,4-DICHLOROBUTENE-2 (DCB) IN RATS
FINAL REPORT ON A STUDY CONDUCTED
8/25/80 - 9/23/82

MEDICAL RESEARCH NOS. 3616 AND 5272-001

HASKELL LABORATORY REPORT NO. 477-85

cc: J. B. Armitage (6)

LONG-TERM TOXICITY STUDY WITH
1,4-DICHLOROBENZENE (DCB) IN RATS

FINAL REPORT ON A STUDY CONDUCTED
9/25/80 - 9/23/82

MEDICAL RESEARCH PROJECT NOS. 3616-001 AND 5272-001

MASKELL LABORATORY REPORT NO. 477-85

Date

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Date Issued: September 24, 1985

This report contains 568 pages.

Notebook Nos.: E-23072, E-23073, E-23074, E-23075, E-23076, E-23077,
E-23077-AA, E-23077-AB, E-23077-AC, E-23077-BA, E-23077-BB,
E-23077-BC, E-23077-CA, E-23077-DA, E-23077-EA, E-28251,
E-28299

QUALITY ASSURANCE DOCUMENTATION

STUDY: MR 3616 AND 5272 LONG-TERM INHALATION STUDY WITH
1,4-DICHLOROBUTENE-2 (DCB) IN RATS

AUDITS:

<u>Interval</u>	<u>Audit Dates</u>	<u>Audit Report#</u>	<u>Date Findings Reported to Management & Study Director</u>
3 months	11/20, 24, 25/80	221	11/26/80
6 months	2/25/81	236	3/2/81
9 months	5/28, 29; 6/1, 2/81	253	6/3/81
12 months	8/21 & 24/81	270	2/24/81
15 months	11/12, 13, 16/81	285	11/16/81
18 months	2/26; 3/1, 3, 4, 5/82	301	3/5/82
21 months	5/25, 26, 27/82	311	5/28/82
24 months	10/4-8/82	329	10/11/82

Reported by: Christiann Barba
Christiann Barba
Quality Assurance Auditor

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Acknowledgments

David P. Kelly developed the generation system and analytical methods for this study. The study was conducted by Mr. Terry S. Sperr with the assistance of Messrs. Lewis R. Jewell, Gerald L. King, James L. Loving, Richard P. Mathena, Charles W. Miller, Alan L. Olsen, Frank L. Russell, Jeffrey T. Turner, and James W. Wetzel, and Ms. Elizabeth Cunningham, Nancy C. Goodman, H. Denise Hall, Carla A. Lyman, and Deborah L. Tyler. Mr. John W. Sarver supervised the study under study directors Drs. Phillip W. Schneider, Jr. and Craig K. Wood. Necropsies were conducted by Messrs. W. Troy Baxter, Gerald M. Hickman, Lewis A. Peterson, August H. Stenholm, William T. Swan, and Russell O. Zuendel. Preparation of slides was conducted by Messrs. John L. Agnew, Jeffrey L. Arthur, John H. Cooper, Odel T. Cooper, James H. Peterson, and Ms. Florence F. Watson and Joan A. Wolfe. Histopathological evaluation of the slides was conducted and the pathology reports were prepared by Dr. Taisan Chiu at the direction of Dr. William C. Krauss. Statistical analyses were conducted by Dr. G. Jay Graepel and Ms. Linda S. Mullin. The final report for this study was prepared by Ms. Linda S. Mullin with assistance from Messrs. Harry W. Furness and John D. Oldham and Ms. Helen P. Spencer under the direction of Drs. Craig K. Wood and Robert W. Rickard.

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8/25/80 - 9/23/82

MEDICAL RESEARCH PROJECT NOS. 3616-001 AND 5272-001

MASKELL LABORATORY REPORT NO. 477-85

Summary

This study was conducted to elucidate the time- and dose-response relationships of long-term low-level 1,4-dichlorobutene-2 (DCB) inhalation exposure in male rats. Groups of male CrI:CD⁰(SD)BR rats were exposed approximately 6 hours per day, 5 days per week to DCB vapors at concentrations of 0 (Group I, 160 rats), 0.10 ± 0.01 (Group II, 150 rats), 0.31 ± 0.01 (Group III, 150 rats), or 1.05 ± 0.06 ppm (Group IV, 128 rats). Exposure durations ranged from 3 to 19 months (86 weeks). Rats that survived 19 months of exposure were held without treatment for an additional 5 months.

During the study, rats from the various groups were sacrificed according to the following schedule and necropsied:

Test Month	Number of Rats Sacrificed			
	Group I 0 ppm	Group II 0.1 ppm	Group III 0.3 ppm	Group IV 1.0 ppm
3	10	0	0	10
6	0	0	0	10
9	0	0	0	10
10	0	0	0	10
11	0	0	0	10
12	10	10	10	10
15	10	10	10	10
18	0	0	0	10
19	10	10	10	0
24	All	All	All	All
	Surviving	Surviving	Surviving	Surviving

Tissues from the respiratory tract, lymph nodes, and brain were evaluated microscopically.

During this study, rats in all groups exhibited infection by Corynebacterium kutscheri which was manifested as necrotizing bronchopneumonia. The infection was first observed in rats from the control group during the sixth month, in rats from the low-exposure group during month eight, in rats from the intermediate group during month 15, and in a few rats from the high-exposure group during month 16. Despite the presence of the infection and associated pulmonary pathology, the infection was not considered to have adversely affected achievement of the study's objectives, since DCB induced oncogenic effects in nasal tissue while the necrotizing bronchopneumonia associated with C. kutscheri infection occurred in the lung. Exclusion of data from rats that exhibited pathological evidence of infection substantiates this conclusion.

Mean body weights and weight gains of rats in the DCB-exposed groups were generally greater than those of rats in the control group during the study. This was most likely the result of lower body weight gain of controls due to early infection with C. kutscheri.

Mortality and clinical observations made during the study were generally considered related to the C. kutscheri infection. Clinical observations included respiratory abnormalities and colored discharge from the nose and/or eyes. After mortality was adjusted to eliminate scheduled sacrifices and the

incidence of S. Lutscheri mortality in the high-exposure group was statistically greater than in the control group.

Organ weights and relative organ-to-body-weight ratios were statistically analyzed only for those sacrifices at which a control group was available for comparison (3, 12, 15, 19, and 24 months). There were no statistically significant differences in mean final body weights or mean absolute or relative organ weights which were considered attributable to DCB exposure.

Two distinctly different types of lesions were noted in this study. The first were pulmonary lesions which occurred in those rats infected with S. Lutscheri. These lesions coincided with the occurrence of respiratory abnormalities and an increased incidence of mortality. The pulmonary lesions were characterized as pulmonary abscesses, pleural fibrous or fibrinous adhesions, bronche-bronchiolar luminal exudate, pleuritis, and suppurative or necrotizing pneumonia.

The second type of lesion occurred in the nasal region and was considered, based on a dose-response relationship, to be attributable to DCB exposure. After three months of exposure, mucosal atrophy and basal cell hyperplasia of the mid-dorsal nasal cavity was found in Group IV rats. The incidence and severity of these lesions were greater in subsequent sacrifices. At ten months, several Group IV rats had clusters of epithelial-like cells at the base of the epithelial lining in the olfactory region.

At the 12 month sacrifice, rats from all DCB-exposed groups (but not control rats) had slight basal cell flattening/hyperplasia, mucosal atrophy, and clusters of epithelial-like cells in the basilar epithelium of the dorso-anterior olfactory region. Some of the Group IV rats had clusters of atypical cells in the same area. These changes were observed at subsequent sacrifices.

Benign tumors (adenomas) were observed in Group IV rats starting at 10 months, in Group III at 12 months, and in Group II at 19 months. Benign tumors occurred in the respiratory region of the nasal cavity.

Malignant tumors occurred in the olfactory region of the nasal cavity. These tumors were observed in Group IV rats sacrificed in extremis starting in the period between 12 and 15 months, in Group III rats that died or were sacrificed after 19 months, and in a Group II rat during month 17. The malignant nasal tumor in the group II rat was not clearly attributable to DCB exposure. One control rat, sacrificed in extremis in the period between 6 and 9 months had an unclassified sarcoma of the nasal cavity.

Tumor incidence data were adjusted for survival because of the occurrence of the respiratory infection in some rats. Estimates of lifetime tumor incidence of benign tumors, malignant tumors, as well as all types of tumors combined were analyzed by the method of Peto et al. When analyzed by this method, there were statistically significant dose-related trends in the incidences of both benign and malignant nasal tumors when looked at independently or when both types of tumors were combined. The increase in tumor incidence was statistically significant at all exposure concentrations for

benign tumors, at 1.0 ppm for malignant tumors and at 0.3 and 1.0 ppm for both tumor types combined. The presence of C. fetus infection did not appear to affect tumor incidence although the infection reduced the numbers of rats at risk due to an increased mortality.

LONG-TERM INHALATION STUDY WITH
1,4-DICHLOROBUTENE-2 (DCB) IN RATS

FINAL REPORT ON A STUDY CONDUCTED
8/25/80 - 9/23/82

MEDICAL RESEARCH PROJECT NOS. 3616-001 AND 5272-001

HASKELL LABORATORY REPORT NO. 477-85

Introduction

The subject compound, 1,4-dichlorobutene-2 (DCB), is a colorless liquid found as a by-product in unrefined chloroprene at a concentration of approximately 0.02%.¹ DCB readily penetrates the skin, hydrolyzing to hydrochloric acid thereby producing severe tissue damage.^{1,2} DCB vapors produce eye irritation and lacrimation.^{1,2} DCB is highly toxic on an acute basis when administered to rats by the inhalation route of exposure. The median lethal concentration (LC50) for adult male rats exposed for four hours to trans-1,4-dichlorobutene-2 was 86 ppm.²

Histopathologic evaluation of male and female rats exposed to 8 or 12 ppm DCB, six hours a day, five days a week for four weeks, revealed inflammation of the respiratory tract tissues and eyes.² The ocular effects of both exposure groups were reversible after a two-week recovery period; the respiratory inflammation was reversible only in rats exposed to 8 ppm. These effects were not observed in rats exposed to 0.5 or 2 ppm DCB.

In a previous long-term study, groups of 140 rats/sex/exposure group were exposed to 0, 0.5, or 5 ppm (decreased to 2.5 ppm) DCB for six hours a day,

five days a week. Rats in the control and low concentration groups were exposed for two years. Due to unexpected toxicity, rats in the high concentration groups were exposed to 5 ppm for 30 weeks followed by 2.5 ppm for 23 weeks at which time exposures were terminated and the rats held for an additional 52 weeks. An increased incidence of tumors (adenomas and adenocarcinomas) of the nasal epithelium occurred in all groups exposed to DCB. These tumors were grossly visible in the high-exposure group rats and microscopically visible in the 0.5 ppm exposed rats.²

Study Objective

The objective of this study was to supplement data generated during a previous study relative to the oncogenicity and chronic toxicity of DCB. The study was designed to elucidate the time- and dose-response relationships for occurrence of nasal tumors in rats exposed to low exposure concentrations of DCB vapors. The inhalation route of exposure was selected as the route which most appropriately simulates the route of potential human exposure.

Sponsor and Test Facility

This study was cosponsored by the Polymer Products Department (formerly Elastomer Chemicals Department) and the Central Research and Development Department and was conducted in the inhalation facilities at Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, located in Newark, Delaware.

Test Material

The test material, 1,4-dichlorobutene-2 (DCB), was supplied by the Central Research and Development Department, E. I. du Pont de Nemours and Company, as a liquid mixture of cis- and trans-isomers which met the following specifications:

cis-1,4-dichlorobutene-2	35 ± 10%
trans-1,4-dichlorobutene-2	65 ± 10%
3,4-dichlorobutene-1	<0.5%
1,3,4-trichlorobutene-2	<500 ppm
high boilers	<500 ppm

The samples were prepared by distillation of 3,4-dichlorobutene-1 (3,4-DCB-1) side stream to remove 3,4-DCB-1. Distillation was performed by the Central Research and Development Department from samples of 3,4-DCB-1 supplied by the Polymer Products Department. Approximately monthly shipments of DCB were received by Haskell Laboratory in acid-washed borosilicate glass and stored at < 0°C until used. The test materials were assigned a Haskell Laboratory identification number (H #13,601) and were used for the generation of chamber atmospheres from 8/25/80 to 4/14/82. Stability of the test sample was not analyzed on this study since DCB was prepared fresh monthly and fresh and since chamber concentrations were monitored for DCB each day.

Records Retention

All original toxicology data and the original of this report will be maintained at Haskell Laboratory for Toxicology and Industrial Medicine or at the Hall of Records E. I. Du Pont de Nemours and Company, Wilmington,

Belmore. Preserved rat tissues, paraffin blocks, and histopathology slides will be maintained at Nicholl Laboratory.

Methods

The protocol for this study with all amendments and addenda is contained in Appendix A and is described in the following section.

A. Test Animals

For the study, six hundred eight male Cr1:CD⁰(SD)BR rats, born 7/16/80, were received from Charles River Breeding Laboratories, Wilmington, MA, on 8/7/80. The Cr1:CD⁰(SD)BR rat was selected because of extensive experience with this strain with respect to hardiness, longevity, and sensitivity, and because nasal tumors occurred in this strain in a previous study with DCB.

Upon receipt, all rats were removed from shipping cartons and housed in pairs in suspended stainless steel, wire-mesh cages. During an eighteen-day pretest period, the rats were temporarily identified by means of cage carding and colored tail marks and were observed with respect to eating habits, weight gain, and any clinical signs of disease or injury. At the end of the pretest period and on the basis of weight gain and freedom from any clinical signs of disease or injury, 588 rats were divided by computerized stratified distribution into four groups. Following assignment to the treatment groups, the order of rats in each group was randomized and the rats were individually

identified by means of toe- and ear-clips. The rats were pair-housed in suspended, stainless steel, wire-mesh cages. Each group of rats was designated to be exposed to atmospheres which contained the following concentrations of DCB:

<u>Group Designation</u>	<u>Number of Animals</u>	<u>Nominal DCB Concentration (ppm v/v)</u>
I	160	Control (0 ppm)
II	150	0.1 ppm DCB
III	150	0.3 ppm DCB
IV	128	1.0 ppm DCB

B. Diet and Housing

Throughout the pretest and test periods, except during exposures, all rats received Purina® Laboratory Chow #5002 Checkers and tap water ad libitum.

During non-exposure periods, all study rats were housed in rooms separate from exposure rooms. Relative humidity levels and temperatures were targeted at 50 ± 10 % and 76 ± 4 °F, respectively. Control rats were not housed with rats exposed to any other test material. Initially, rats were housed in single-pass, flow-through ventilated animal rooms with control and low-dose groups each in separate rooms and intermediate- and high-dose groups in the same room. Housing procedures following the outbreak of a respiratory infection are described in the following section.

C. Intercurrent Infection

Approximately six months after initiation of the study, signs of a respiratory infection by Corynebacterium kutscheri were first observed among rats in the control group. Since the infection was not considered highly fatal, it was decided to continue the study and implement procedures to minimize the spread of the disease to other study rats. The initial step involved moving the control-group rats into a laminar-flow, filtered/recirculated, air-ventilated animal room for housing during nonexposure periods. For approximately one week following this change, the control group's treatment regimen was maintained. However, mortalities due to the infection persisted during this period. Because the stress of the treatment regimen was considered an antagonistic factor in controlling the infection, the control-group exposures were terminated for three weeks (study days 206 through 227; 16 exposures were missed). During this time, the death of rats due to infection ceased. Because the infection was considered under control, treatment was reinstated and the rats were returned to their original housing in a single-pass, flow-through, air-ventilated animal room.

At about the same time that treatment of the control-group rats was reinstated, during the seventh month of the study, signs of C. kutscheri infection were observed among rats in the low-dose group. These rats were therefore moved into a laminar-flow, filtered/recirculated, air-ventilated animal room. However, the DCB-exposure regimen for this group of rats was not interrupted. During test month eight, rats from the other three groups were similarly housed and all rats were thus housed until termination of the DCB exposures during the nineteenth month of the study.

After C. kutscheri infection spread to rats in the low-dose group, all rats in the study were treated with 643 mg tetracycline-HCl/l. in the drinking water. The treatment extended for two weeks (study days 243 through 257) and the approximate daily intake of tetracycline was 9 mg tetracycline/100 g of body weight. Mortalities were again observed during the ninth month of the study, five weeks after completion of the tetracycline treatment. Therefore, second and third administrations of tetracycline of eight and four weeks duration, respectively, were initiated during the ninth month (study days 292 through 345) and seventeenth month (study days 526 through 554) of the study, respectively.

Despite the hygienic and chemotherapeutic efforts, the C. kutscheri infection persisted. Because the continued presence of the infection in Haskell Laboratory threatened the health status of Haskell's entire rat population, the DCB exposures were terminated approximately 19 months after initiation of the study and all rats were transported to Stine Laboratory Building No. 10, a Du Pont isolation facility located on the Stine-Haskell Laboratory site. Relocation consisted of transporting cage racks containing the rats 0.75 miles in a truck with a heated cargo area (25°C). Each cage rack was exposed to the outdoor environment for a total of approximately one minute (30 seconds each during loading and unloading). Weather conditions prevailing at the time of the study's relocation were mostly sunny, temperature 62°F, and 31% relative humidity. Total time to load, transport and unload all study rats was approximately 20 minutes. At Stine Laboratory, all rats from the study were housed in a single animal room.

D. Test Chambers and Conditions of Exposure

For the DCB exposures, rats were placed into one of four inhalation chambers. Each of the 4.6 m³ chambers were identically constructed of stainless steel and glass and were quadrangular in shape with pyramidal tops and bottoms (see Figure 1). Each chamber and all surfaces that DCB contacted prior to exhausting from the chamber were constructed of materials that neither reacted with nor absorbed DCB and were free of contaminants from any previous study. All chambers were housed in a single room.

The chambers were operated in a one-pass, flow-through mode with an air flow rate of approximately 600 L/min. Chamber temperatures and relative humidities ranged from 65-80°F and 30-65%, respectively. Rats were exposed six hours per day, five days a week (except holidays) for approximately 86 weeks. All exposures were conducted during the same eight-hour period of the day to minimize the potential effects of diurnal physiological variations. At the end of each exposure, test groups were left in their respective exposure chambers for approximately 20 minutes to allow clearance of test material from chamber atmospheres before transfer of rats to housing facilities. Cage position within housing facilities and exposure chambers were randomized daily during unloading and loading procedures. Control rats were treated exactly as test rats, except that the control chamber did not contain test material.

E. Generation and Monitoring of DCB Atmospheres

DCB chamber atmospheres were generated by bubbling nitrogen through liquid DCB maintained between 8 and 10°C in an Exacol Constant Temperature Bath, Model #EX200. Brooks flow meters, models R-2-15-AAA, R-2-15-A, and R-2-15B were used to generate the low, intermediate and high exposures, respectively. Chamber atmospheres were analyzed for DCB at least hourly using a Hewlett Packard Model 5880A gas chromatograph (G.C.) equipped with a 15 mL sampling loop and a flame ionization detector. Chamber atmospheres were collected for analysis by drawing chamber air through the G.C. sampling loop with a Neptune Dyna vacuum pump Model #2. The G.C. column was 5% OV-7 on Chromosorb® G-HP 100/120. G.C. oven temperature was approximately 110°C and detector temperature was 250°C. Peak areas were measured and compared with those produced from known standards. Standards were prepared daily by quantitatively injecting liquid DCB into glass sampling bottles and then preparing gas dilutions in Teflon® sampling bags.

F. Body Weights and Clinical Observations

All rats were observed at least twice daily on exposure days for gross signs of toxicity and changes in appearance and behavior. In addition, each rat was individually examined for abnormalities in appearance or behavior during each weighing session. The date of appearance, location, and size of all observed masses were recorded. On weekends and holidays cage-site examinations for moribund or dead rats were conducted. When possible, the cause

of morbidity or mortality was determined. From the termination of exposures during the eighty-sixth week of the study, through completion of the final sacrifice on 9/23/82, all rats were observed twice daily.

All rats were individually weighed weekly for the first three months of the study and twice monthly thereafter. Rats which lost $\geq 10\%$ of their body weight between weighings were observed daily until health status returned to normal or the rat was sacrificed. Groups III and IV were weighed on the day following the weighing days for Groups I and II. However, for the purpose of this report, the body weights are adjusted to represent the same study day.

G. Evaluation of Serum Proteins

Nine times during the study, approximately 1 mL of peripheral (tail) blood was collected from five control and five Group IV rats and sent to the Central Research and Development Department at the Experimental Station, for evaluation of serum protein characteristics. Results of these evaluations are contained in separate Experimental Station records.

H. Pathological Evaluations

Approximately three months after the start of exposures, ten rats each from the control and high-exposure (1.0 ppm) groups were serially selected, sacrificed by chloroform anesthesia and exsanguination, and necropsied. Ten rats from the high-exposure group were sacrificed at each of the following time intervals: 6, 9, 10, and 11 months. At 12 and 15 months after the study

start, ten rats from each exposure group and the control group were sacrificed. After 18 months of exposure, ten rats from the high-exposure group were sacrificed, but the scheduled sacrifice of the control, low- and intermediate-exposure groups was postponed until approximately 19 months when exposures were terminated. All surviving rats were sacrificed approximately 24 months after the start of exposures. All rats included in the scheduled sacrifices and those found dead or sacrificed in extremis during the study were subjected to a complete gross pathological examination and a histopathological examination of the entire respiratory tract (nasal turbinates, trachea, lungs), cervical lymph nodes and brain except when tissue autolysis prevented evaluations as noted in the pathology reports. The following tissues from rats in the scheduled sacrifices were weighed: brain, heart, lungs, liver, spleen, kidneys, testes, thymus, adrenals, and pituitary.

I. Statistical Methods

Daily exposure concentrations were estimated by calculation of the averages of the analytical data for each chamber.

Body weight and relative and absolute organ weight data were analyzed by a one-way analysis of variance. When the test for differences among test group means (F test) was significant, pairwise comparisons were made between test and control groups. For body weights, these comparisons were made with the least significant difference (LSD) test. For organ weights, the

comparisons were made with both LSD and Dunnett's test. Bartlett's test for homogeneity of variances and a test for linear trend were conducted on the organ weight data. Significance was judged at the $p < 0.05$ level.

Because C. kutscheri affected survival in some groups, mortality data were adjusted to exclude those rats that exhibited histopathological evidence of infection. The following designations of lung lesions as noted in the pathology reports were considered to be indicative of the presence of C. kutscheri: abscesses, pleural fibrinous or fibrous adhesions, broncho-bronchiolar luminal exudate, pleuritis, and suppurative or necrotizing pneumonia. The probability of survival was estimated by the Kaplan-Meier method³ for all rats in the study as well as for all rats not displaying lesions indicative of C. kutscheri. Fisher's Exact test was used to compare control and test group mortality at the final sacrifice after excluding all rats with lesions indicative of C. kutscheri. Significance was judged at the $p < 0.05$ level.

Estimates of lifetime tumor incidence were made by the Kaplan-Meier procedure. Tumor rates were analyzed by the method of Peto et al.⁴, where all tumors were assumed to be incidental. For purposes of analysis, adenomas and papillary adenomas were combined into the category of benign nasal tumors. The following designations of nasal neoplasms were combined into the category of malignant nasal tumors: unclassified sarcoma, spindle cell sarcoma, rhabdomyosarcoma, adenocarcinoma, carcinosarcoma, squamoadenocarcinoma, and mixed carcinoma. Incidences of pulmonary tumors were also analyzed by the method of Peto et al.

Results

A. Analytical Concentrations and Purity

DCB was analyzed at the Experimental Station prior to shipping and was within the specifications described in the procedures section.

Daily average chamber concentrations of DCB are tabulated in Appendix B. Mean weekly concentrations are contained in Table 1 and Figure 2. Over the 86-week exposure period the daily average concentrations were 0.10 ± 0.01 , 0.31 ± 0.01 , and 1.05 ± 0.06 ppm for the low-, intermediate-, and high-exposure groups, respectively. The average daily chamber concentrations ranged from 0.08 to 0.15 ppm, 0.25 to 0.39 ppm, and 0.79 to 1.30 ppm for the low-, intermediate- and high-exposure groups, respectively.

B. Body Weights and Weight Gains

Individual body weights are contained in Appendix C. The group mean body weights and body weight gains are contained in Tables 2 and 3, respectively. Group mean body weights are shown in Figure 3. The body weights reported in Table 2 and Figure 3 are adjusted to represent the same study day, although not every group was weighed on the same day of the week.

The mean body weights of rats in all DCB-exposed groups were often significantly higher than those of the controls. The effects were especially pronounced during study weeks 30 through 40 and at weeks 90 through the end

of the study. It can be seen from Figure 3 that the mean weights of control rats plateaued or decreased during those time periods. The lack of weight gain in the control group coincides with the appearance of C. kutscheri infection in the control rats at those time periods.

C. Clinical Observations and Mortality

Clinical observations are summarized in Table 4. The clinical signs and individual rats affected in each study group are listed in Appendix D. Other than skin sores and grossly visible tissue masses, clinical observations were generally consistent with the presence of C. kutscheri infection. Clinical observations associated with the infection were colored discharges from the eyes and nose and respiratory abnormalities such as lung noise or irregular respiration.

Individual mortality data for each group are contained in Appendix E. Cumulative mortality is summarized in Table 5. Survival curves for each group are shown in Figure 4. Groups I (control) and II (0.1 ppm) exhibited an early high incidence of mortality which coincided with the observation of C. kutscheri infection among rats in these groups. The first incidence of C. kutscheri infection was observed in the control group during the sixth month on test, in Group II during the eighth month, and in Groups III and IV near the end of the study. A summary of cumulative incidence of rats with lesions associated with C. kutscheri is contained in Table 6. Figure 5 shows survival after elimination of those rats which had evidence of respiratory

infection upon pathological examination. The cumulative incidence of mortality at the end of the study period when adjusted to eliminate scheduled sacrifices and those rats with C. kutschera infection was 54 of 72, 62 of 85, 44 of 61, and 37 of 40 in groups I, II, III, and IV, respectively. The incidence was significantly higher in group IV compared to the control group.

D. Pathology

Individual organ and final body weights are tabulated in Appendix F. Results of all gross and microscopic findings for the sacrifices at 3, 6, 9, 10, 11, 12, 15, 18, 19, and 24 months are contained in Appendix G.

Three-Month Sacrifice. Ten rats from Group I (0 ppm) and ten rats from Group IV (1.0 ppm) were sacrificed by design on study day 93. There were no apparent differences in macroscopic observations between Group IV and control rats. Histopathologically, focal mucosal atrophy manifested by shortening or disruption of the nasal mucosal epithelial cells and slight basal cell squamous hyperplasia were observed in several Group IV rats but not in any control rats. The changes were located in the mid-dorsal area of the nasal cavity.

One Group III rat was found dead prior to and one group IV rat was found dead just after the three-month sacrifice. Tissue autolysis prevented complete examination, but both rats had heavy, dark lungs.

Mean final body and absolute organ weights and mean relative organ weights for rats examined at the three-month sacrifice are contained in Tables 7 and 8, respectively. No statistically significant differences were observed in mean final body or absolute or relative organ weights of rats in Group IV when compared to those of the control rats.

Six-Month Sacrifice. Ten rats from Group IV (1.0 ppm) were sacrificed by design on study day 185. Atrophy of the mid-dorsal area of the nasal cavity was found in several of these rats. In addition, all of the ten rats had varying degrees of basal cell hyperplasia or metaplasia in the nasal cavity.

Three control and two Group II (0.1 ppm) rats were found dead or were sacrificed in extremis during the period from three to six months. Two of the control rats had severe necrotizing pneumonia associated with infection by C. kutschera.

Individual and mean organ and final body weights for Group IV rats examined at the six-month sacrifice are contained in Appendix F. These data were not analyzed statistically since no control rats were sacrificed for comparison at this time period.

Nine-Month Sacrifice. Ten rats from Group IV (1.0 ppm) were sacrificed by design on study day 275. Abnormalities in nasal mucosa consisted of basal cell flattening/hyperplasia and mucosal atrophy and disorganization. The changes were slightly more pronounced than at the six-month sacrifice.

Sixteen control, 17 Group II (0.1 ppm), 2 Group III (0.5 ppm) and 2 Group IV (1.0 ppm) rats were found dead or were sacrificed in extremis during the period from six to nine months. A majority of the rats in the control and low-exposure groups that were found dead or sacrificed in extremis had pulmonary lesions associated with C. kutscherae. The lesions observed at this and/or later pathologic examinations included pulmonary abscesses, pleural fibrous or fibrinous adhesions, pleuritis, broncho-bronchiolar luminal exudate, and suppurative or necrotizing pneumonia. One control rat had a tumor of the nasal cavity which was categorized as an unclassified sarcoma. No compound-induced changes were apparent in the rats from the low- or intermediate-exposure groups that were found dead or sacrificed in extremis.

Individual and group mean organ and final body weights for Group IV rats examined at the nine-month sacrifice are contained in Appendix F. Since no control rats were sacrificed, the data for Group IV were not subjected to statistical analysis.

Ten-Month Sacrifice. Ten Group IV (1.0 ppm) rats were sacrificed by design on study day 305. The nasal mucosal changes were similar to those of the nine-month sacrifice. Four Group IV rats had clusters of epithelial-like cells at the base of the epithelial lining in the olfactory region. This was the first observation of this nature. A benign tumor (adenoma or papillary adenoma) located between the nasal and maxillary turbinates was observed in one rat from Group IV.

Four control and four Group II (0.1 ppm) rats were found dead or were sacrificed in extremis during the period from nine to ten months. Pulmonary lesions observed in these rats were similar to those that occurred previously and were considered to be due to C. kutschera.

Individual and mean organ and final body weights for the Group IV rats examined at the ten-month sacrifice are contained in Appendix F. Since no control rats were sacrificed at this time period, the data were not analyzed statistically.

Eleven-Month Sacrifice. Ten rats from Group IV (1.0 ppm) were sacrificed by design on study day 334. Nasal adenomas were found in two of these rats. Both tumors were found on the maxilloturbinates. Other changes observed in the rats from the high-exposure group included basal cell flattening/hyperplasia, mucosal atrophy, and clusters of cells in the basal epithelium and were similar to those observed at the ten-month sacrifice.

One Group II (0.1 ppm) rat and one Group III (0.3 ppm) rat were sacrificed in extremis during the period from 10 to 11 months. The Group II rat displayed lesions indicative of C. kutschera infection.

Individual and group mean organ and final body weights for the Group IV rats examined at the eleven-month sacrifice are contained in Appendix F. Since no control rats were sacrificed at this time period, the data were not analyzed statistically.

Twelve-Month Sacrifice. Ten rats from the control and each of the DCB-exposed groups were sacrificed by design on study day 372. Seven Group IV (1.0 ppm) rats, one Group III (0.3 ppm) and one Group II (0.1 ppm) had clusters of epithelioid cells in the basilar epithelium of the dorso-anterior olfactory region. Two of the seven Group IV rats had clusters of atypical cells in the same area. Slight basal cell flattening/hyperplasia and mucosal atrophy of the dorso-anterior olfactory epithelium were observed in all exposed groups, but not in control rats.

Five control and five Group III (0.3 ppm) rats were found dead or were sacrificed in extremis in the period from 11 to 12 months. A nasal adenoma was present in one of the Group III rats which was found dead. The tumor was located at the nasoturbinates and was morphologically similar to those that occurred in rats in the high-exposure group at the eleven-month sacrifice. All other lesions observed were considered to be unremarkable.

Mean absolute organ and final body weights and mean relative organ weights for rats examined at the twelve-month sacrifice are contained in Tables 9 and 10, respectively. No statistically significant differences in final body weights or in absolute or relative organ weights of rats in any of the test groups were observed when compared to those of rats in the control group. However, weights of lungs and adrenal glands show significant dose-related trends (decreases in weight with increasing concentration).

Fifteen-month Sacrifice. Ten rats from the control and each of the exposure groups were sacrificed by design on study day 463. In addition, nine control, ten Group II (0.1 ppm), five Group III (0.3 ppm) and four Group IV (1.0 ppm) rats were found dead or were sacrificed in extremis in the period from 12 to 16 months. Two of the Group IV rats sacrificed in extremis had malignant nasal tumors. One had a rhabdomyosarcoma which nearly occluded one nasal chamber and partially invaded the surrounding tissue. The other rat had a mixed carcinoma (squamousadenocarcinoma) of the nasal cavity. In addition, five other Group IV rats in the scheduled sacrifice had benign tumors (adenomas) of the anterior nasal cavity. One of these rats had three adenomas and another, two. Two Group III rats (one sacrificed in extremis and one sacrificed by design) had small adenomatous foci in the anterior nasal cavity. These findings were considered to represent either early adenomas or sections of tissue cut at the edge of existing tumors. No tumors were observed in Group II.

Basal cell flattening/hyperplasia and atrophy/disorganization of the dorso-anterior olfactory mucosa were seen in all DCB-exposed groups, but not in the control. Clusters of epithelioid cells with atypical cells were observed in the dorso-anterior olfactory mucosa of Group III and Group IV rats. Other changes were interpreted to be unrelated to DCB exposure.

Mean absolute organ and final body weights and mean relative organ weights for rats examined at the fifteen-month sacrifice are contained in Tables 11 and 12, respectively. The mean absolute lung weights of all three

DCB-exposed groups were significantly less than that of the control group and showed a dose-related trend. No other statistically significant differences in organ or body weights occurred.

Eighteen-Month Sacrifice. Ten rats from Group IV (1.0 ppm) were sacrificed by design on study day 549. The scheduled sacrifice of rats in the other three groups was postponed until 19 months. Seventeen control, 15 Group II (0.1 ppm), 59 Group III (0.3 ppm) and 18 Group IV (1.0 ppm) rats were found dead or sacrificed in extremis during the period from fifteen to eighteen months. Nasal tumors were detected in 10 of the 28 Group IV rats. Seven were malignant tumors (four adenocarcinomas, three carcinosarcomas) of the olfactory region. Three of the rats with adenocarcinomas also had adenomas of the respiratory region of the nasal cavity. Similar adenomas of the respiratory region of the nasal cavity were observed in three other Group IV rats. Two of 59 Group III rats had similar benign nasal tumors (adenomas). One of 15 Group II rats had a spindle cell sarcoma in the olfactory region of the nasal cavity. None of the 17 control rats had nasal tumors.

Exposure-related changes reported for the fifteen-month sacrifice were also present at this time. A number of rats which were found dead during the period from 15 to 18 months, particularly in Group III (0.3 ppm), had pulmonary lesions associated with C. kutscheri infection. Pituitary tumors were the other main cause of deaths or unscheduled sacrifices.

Individual and group mean organ and final body weights for Group IV rats examined at the eighteen-month sacrifice are contained in Appendix F. The values were not statistically compared to controls since no control rats were sacrificed at this time period.

Nineteen-Month Sacrifice. Ten rats from the control group and Groups II (0.1 ppm) and III (0.3 ppm) were sacrificed by design on study day 599. In addition, seven control, ten Group II (0.1 ppm), six Group III (0.3 ppm) and nine Group IV (1.0 ppm) rats were found dead or were sacrificed in extremis during the period from 18 to 19 months. Nasal tumors were detected in four of the nine Group IV rats. One was a malignant tumor (adenocarcinoma) of the olfactory region; the other three were benign tumors (adenomas) of the respiratory region. A similar adenoma of the respiratory region of the nasal cavity was detected in one of the twenty Group II rats. No tumors were detected in the 17 control or 16 Group III rats.

Clusters of cells in the basal epithelium and mucosal atrophy/disorganization were observed in the olfactory region of the nasal cavity of several rats in all three DCB-exposed groups.

Mean absolute organ and final body weights and mean relative organ weights for rats examined at the nineteen-month sacrifice are contained in Tables 13 and 14, respectively. No statistically significant differences occurred in final body weights or in mean absolute or relative organ weights of rats in Groups II or III when compared to control rats sacrificed at this time period.

Final Sacrifice. Eighteen control, 23 Group II (0.1 ppm), 17 Group III (0.3 ppm), and 3 Group IV (1.0 ppm) rats were sacrificed at the termination of the study on study days 758 through 760. They had been exposed to DCB for approximately 19 months and were held an additional five months. In addition, 41 control, 38 Group II, 24 Group III, and 11 Group IV rats were found dead or were sacrificed in extremis in the period from 19 months to the time of the final sacrifice.

No gross changes attributable to DCB exposure were observed at necropsy. Thirteen of the 14 rats in Group IV were subjected to histopathologic examination. Eleven had nasal tumors. Three of these rats had only benign tumors, five had only malignant tumors and three had concurrent benign and malignant tumors. Nine of 40 Group III rats examined had nasal tumors; two had malignant and seven had benign. Two rats in Group II had benign nasal tumors. All these tumors are considered exposure-related. No nasal tumors were observed in the control rats.

Mean absolute organ and final body weights and mean relative organ weights for rats examined at the final sacrifice are contained in Tables 15 and 16, respectively. There were no statistically significant differences observed in final body weights or in mean absolute organ weights in any of the exposed groups when compared to those of the control group at the final sacrifice. The mean relative testes weight of the Group II rats was significantly less than that of the controls. No other differences in relative organ weights occurred in any of the exposed groups.

Tumor Incidence. Because C. kutscheri infection resulted in early mortality in the control- and low-exposure groups, a statistical method was used that took varying mortality rates between groups into account. The incidence of pulmonary tumors was unrelated to DCB exposure. Overall nasal tumor incidence and nasal tumor incidence adjusted for mortality are contained in Table 17. There were statistically significant increases in the incidence of benign tumors at all three exposure concentrations and in the incidence of malignant tumors at 1.0 ppm. There was a statistically significant increase in the total number of rats bearing nasal tumors at 0.3 and 1.0 ppm. The increases in both benign and malignant nasal tumors showed significant dose-related trends, when these two tumor types were analyzed either independently or combined for total tumor incidence. Table 18 presents nasal tumor incidence after rats with lesions indicative of C. kutscheri are eliminated. The same statistically significant dose-related trend was observed after tumor incidence was adjusted for the presence of C. kutscheri and the tumor incidence rate in disease-free rats was basically similar to the overall rate presented in Table 17.

Discussion and Conclusions

Groups of male Cri:CD⁰(SD)BR rats were exposed to 0, 0.1, 0.3, or 1.0 ppm 1,4-dichlorobutene-2 (DCB), 65% trans-isomer, 35% cis-isomer for up to 19 months (86 weeks). Surviving rats were held without treatment for an additional five months.

The mean body weights of all DCB-exposed rats were often significantly higher than that of the controls at the same time period. The effect was especially pronounced during study weeks 30 through 40 and weeks 90 through the end of the study. This was due to a lower rate of weight gain in the control rats that coincided with the occurrence of a respiratory infection, C. kutscheri, in the controls. The effect therefore is not attributed to DCB exposure.

The most common clinical observations made during the study and the high incidence of mortality are considered related to C. kutscheri. The infection first occurred in the control group rats after 6 months on test, followed shortly thereafter by its appearance in rats in the 0.1 ppm exposure group, and in rats in the 0.3 ppm and 1.0 ppm exposure groups near the end of the study. Clinical observations included colored discharge from the nose and/or eyes and respiratory abnormalities such as labored breathing and lung noise. When mortality was adjusted to eliminate scheduled sacrifices and the incidence of C. kutscheri, the survival rate for rats exposed to 1.0 ppm DCB was lower than that of control and other treatment groups.

At three months after the start of exposure, ten rats each from the control and high-exposure group were sacrificed. Ten Group IV rats were also sacrificed at 6, 9, 10, and 11 months. At 12 and 15 months, ten rats from each group were sacrificed. At 18 months, ten rats from the high exposure group were sacrificed, but the scheduled sacrifice of ten rats from each of the other three groups was postponed until approximately 19 months when exposures were terminated. All surviving rats were sacrificed five months after termination of exposures.

Organ weight and relative organ to body weight ratios were statistically analyzed only for those sacrifices at which a control group was available for comparison (3, 12, 15, 19, and 24 months). There were no statistically significant differences in mean final body weights or mean absolute or relative organ weights between rats in any of the groups examined and control rats after three or twelve months of exposure.

At fifteen months, the mean absolute lung weights of all three DCB-exposed groups were significantly less than those of the control group. However, these differences may be related to differences in body weights between the control and test groups. Although not statistically significant, the mean body weights of the control rats in this sacrifice were greater than both the mean body weights of the other sacrificed groups and the mean body weight of the surviving control group rats at this period (week 66). That this effect may be related to body weight difference is supported by the comparable relative lung weights between control and DCB-exposed groups. Also contributory to the difference in lung weights is the presence of C. kutscheri in five of the control rats sacrificed by design, while only one rat sacrificed by design from the low-exposure group and none from the intermediate- or high-exposure groups were infected. After exclusion of those rats with C. kutscheri, the mean lung weights of the control (3.278 ± 0.496) and test groups were not significantly different. Therefore significant differences in lung weights are attributed to body weight differences and C. kutscheri infection rather than DCB exposure. Although no DCB-exposed groups were statistically different from controls, a similar linear trend in lung weights was observed at twelve months, but not at nineteen months or at the final sacrifice.

At nineteen months, when exposures were terminated, no statistically significant differences were observed for final body weights or for absolute or relative organ weights of rats in Groups II or III when compared to controls. All surviving rats (18 control, 23 Group II, 17 Group III, and 3 Group IV) were sacrificed 24 months after the start of the study. These rats had been exposed to DCB for approximately 19 months and held without further treatment for five months. The mean relative testes weight of the rats in Group II was significantly less than that of the controls. There was also a nonstatistically significant trend to lower testes weights in the higher exposure groups. However, this is not attributed to DCB exposure. The lower mean testes weight in Group IV is attributed to the small group size (3 rats) with one outlying value, a testes weight of 0.8 g in a rat with a pituitary tumor and low body weight. Further since there were no significant differences in organ weights between Groups III or IV and controls, the significantly lower testes weight in Group II is not attributed to DCB exposure.

Two distinctly different types of lesions were detected on histopathologic examination of the rats in this study. Pulmonary lesions were associated with the respiratory infection and coincided with increased incidences of respiratory abnormalities and mortality. The pulmonary lesions indicative of C. kutscheri infection were: pulmonary abscesses, pleural fibrous or fibrinous adhesions, broncho-bronchiolar luminal exudate, pleuritis, and suppurative or necrotizing pneumonia.

The second type of lesions occurred in nasal tissue and are considered to be attributable to DCB. The time course for the development of these lesions can be followed in Group IV rats. Mucosal atrophy and basal cell hyperplasia of the mid-dorsal area of the nasal cavity was found after three months of exposure. Basal cell metaplasia or squamous metaplasia was observed after six months. These findings became more pronounced and occurred more frequently on subsequent sacrifices. At ten months, several Group IV rats had clusters of epithelial-like cells at the base of the epithelial lining in the olfactory region. At twelve months, some of the Group IV rats had clusters of atypical cells in the same area. Rats from the other DCB-exposed groups sacrificed at twelve months had mucosal atrophy and basal cell hyperplasia and one each from Group II and III had clusters of cells in the basilar epithelium. These effects were similar to those reported at earlier sacrifices in Group IV.

Benign tumors (adenomas) were observed in Group IV rats starting at 10 months, in Group III at 12 months, and in Group II at 19 months. Benign tumors occurred in the respiratory region of the nasal cavity.

Malignant nasal tumors occurred in the olfactory region of the nasal cavity. Malignant nasal tumors were observed in Group IV rats sacrificed in extremis starting in the period between 12 and 15 months, in Group III rats that died or were sacrificed after 19 months, and in a Group II rat during month 17. It is not clear whether the malignant tumor in the Group II rat, a spindle cell sarcoma, was attributable to DCB-exposure since it is an extremely rare tumor and therefore comparison data is not available.

The presence of C. kutschert infection did not affect tumor incidence since tumor incidence rates are virtually the same whether or not rats with C. kutschert lesions are included. The only effect of the infection appeared to be a reduction of the numbers of rats at risk due to an increased early mortality in groups of infected rats.

Aspects of this study which had to be considered in interpretation of the data included the concurrent presence of C. kutschert infection, a malignant nasal tumor (unclassified sarcoma) in a control group rat which also had C. kutschert infection, and the unequal group sizes and sacrifices at various time intervals. After considering these factors, the following conclusions were made:

- Non-neoplastic lesions were first observed in the nasal cavity of Group IV rats after three months of exposure and in the other two DCB-exposed groups at the twelve month sacrifice. They were progressive in severity and frequency and are considered compound related.
- Compound-related benign nasal tumors (adenomas) occurred in all three DCB-exposed groups, starting in Group IV at study month 10. They were significantly increased in all DCB-exposed groups compared to the controls and showed an exposure-related trend.
- Malignant nasal tumors occurred in all three DCB-exposed groups as well as one in the control group and showed an exposure-related trend. The malignant nasal tumors observed at 0.3 and 1.0 ppm are considered compound-related but are statistically increased only at 1.0 ppm. The observation of a spindle cell sarcoma in one Group II rat is not clearly attributable to DCB exposure.

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MM-13,601
PW-3616
NC-15

TABLE I

MEAN WEEKLY CHAMBER CONCENTRATIONS OF 1,4-DICHLOROBUTENE-2

WEEK NUMBER	CHAMBER CONCENTRATION (ppm)		
	GROUP: NOMINAL CONCENTRATION: <u>0.1</u>	<u>0.3</u>	<u>1.0</u>
1	0.10	0.32	0.96
2	0.10	0.32	1.05
3	0.10	0.31	1.06
4	0.11	0.31	1.03
5	0.11	0.31	1.05
6	0.09	0.31	0.98
7	0.10	0.31	1.02
8	0.11	0.28	1.06
9	0.11	0.29	1.01
10	0.10	0.31	0.98
11	0.10	0.30	0.99
12	0.10	0.31	1.04
13	0.11	0.30	1.01
14	0.12	0.31	1.02
15	0.10	0.31	0.97
16	0.10	0.31	0.97
17	0.10	0.30	1.01
18	0.10	0.30	1.09
19	0.10	0.30	1.17
20	0.10	0.29	1.01
21	0.10	0.31	1.08
22	0.09	0.29	1.10
23	0.10	0.30	1.08
24	0.10	0.29	1.02
25	0.10	0.30	1.04
26	0.11	0.31	1.08
27	0.11	0.32	1.16
28	0.10	0.31	1.11
29	0.10	0.30	1.20
30	0.10	0.33	1.08
31	0.11	0.31	1.04
32	0.10	0.34	1.11
33	0.10	0.31	1.11
34	0.11	0.31	1.05
35	0.10	0.32	1.04

ATTACHMENT 2

**MORTALITY AND CANCER INCIDENCE
AMONG WORKERS EXPOSED
TO 1,4-DICHLOROBUTENE-2
AT THE VICTORIA PLANT**

JANUARY 1985

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CONFIDENTIAL

*does not pertain to
TSCA CBI per submitter
11-5 pg*

**MORTALITY AND CANCER INCIDENCE AMONG WORKERS
EXPOSED TO 1,4-DICHLOROBUTENE-2
AT THE VICTORIA PLANT**

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January 1985

**Mortality and Cancer Incidence Among Workers
Exposed to 1,4-Dichlorobutene-2
at the Victoria Plant**

Summary

Cancer incidence, and mortality from all causes and from cancer, among a cohort of males exposed to 1,4-Dichlorobutene-2 at the Victoria Plant in Texas, were compared to that expected based on Du Pont and U.S. rates. Data through 1980, including mortality follow-up from the Social Security Administration, did not show significant excesses of cancer deaths among this group. The incidence of cancer through 1983 was also not significantly elevated.

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**Mortality and Cancer Incidence Among Workers
Exposed to 1,4-Dichlorobutene-2
at the Victoria Plant**

Introduction

Dichlorobutene (DCB) is a highly corrosive formed by the reaction of chlorine and butadiene. Produced and used at the Victoria, Texas site from startup in 1951 through December 1982, 1,4-Dichlorobutene-2 has been handled with great care in closed systems. Although there has been no evidence that DCB is a human carcinogen, Van Duuren at New York University reported in 1975 that subcutaneous injection of DCB resulted in an increased incidence of tumors in mice. This epidemiologic study was initially undertaken in 1976 to determine if workers exposed to DCB at the Victoria Plant have experienced excess cancer mortality or incidence. Preliminary analyses of data through 1978 did not reveal any significant elevations of cancer deaths or cases among this cohort.

Methods

This is a retrospective cohort study in which persons with a common past exposure to DCB were identified from records and traced through December 31, 1980, for vital status. Several data sources were used to identify the cohort:

- **Shift Schedules** - These contained the names of all production foremen and operators assigned to each Plant Area. They began in 1953 and consisted of at least one schedule per year.

- Maintenance Field Reports (Nylon) - These contained the names of all maintenance supervisors, foremen, and mechanics assigned to specific sections of the Nylon Area. They began in 1951 and were issued once every six months.
- Electrical and Instrument (E&I) Field Reports (Nylon) - These contained the names of all E&I supervisors, foremen, and mechanics assigned to specific sections of the Nylon Area. They began in 1951 and contained one to two reports per year. These records were combined with the Maintenance Field Reports in 1965.
- Payroll Records - These records of wage roll employees were maintained by the foreman's code.
- Organization Charts - These were not available on any regular frequency. They contained only exempt personnel and generally listed only areas assigned rather than specific operations.

The following information was retrieved by plant personnel for persons identified from the search of the above records:

- name
- social security number
- sex
- payroll classification
- race
- birth date

- termination date and reason
- occupation and dates for each assignment in DCB areas

These data were then keypunched and entered onto a computer tape. Employment status has been updated from computer files in Wilmington. Medical data from several sources have been added to the tape:

- The Du Pont Company Mortality File: This file contains information on all deaths among active employees and pensioners from 1957-1980. Cause, coded according to the International Classification of Diseases, and date of death are included.
- Death certificates obtained upon identification of additional deaths by the Social Security Administration (SSA): The names of persons who terminated employment at Du Pont were provided to the SSA, who notified us, where applicable, of date of death and last known residence. We then sought death certificates from individual state health departments.
- The Du Pont Company Cancer Registry: This file contains cancer diagnoses and deaths among active employees from 1956-1983. Diagnoses are derived primarily from Accident and Health Insurance Claims for disabilities of at least 8 days. About 95% of the Company participates in this voluntary insurance program. Deaths are taken from the life

insurance program, which is provided free to all employees.

The actual numbers of deaths and cancer cases found from the sources described above were compared with the numbers that would have been expected based on Company and national experience, adjusted for age and calendar time period. In computing the expected numbers, person-years of risk were tallied for each cohort member, starting at his date of first exposure, but not earlier than 1957 for mortality, and 1956 for cancer incidence. For mortality analyses, person-years were counted until date of death for deceased persons, date of termination or last date known to be alive for persons who were lost to follow-up, or until December 31, 1980, for persons known to be alive at that date. For incidence analyses, the tally continued until the earliest of the following events: date of cancer diagnosis, date of termination from the Company (due to death, pension, or other reasons), or December 31, 1983 (for active employees).

Cancer mortality and incidence are analyzed separately, since Company rates used in the calculation of expected numbers are based on different, but not mutually exclusive, populations. The Cancer Registry includes information only on persons who were actively employed at the time of diagnosis, since we are unable to identify all cancer cases diagnosed after termination from the Company. Mortality rates for the entire Company include both active and pensioned employees. Some persons who were diagnosed with cancer may

also be deceased, and these cases would be included in both incidence and mortality analyses.

Mortality and incidence comparisons were made using Du Pont Company-wide rates as well as national rates. Since statistics derived from the general population include persons hospitalized or otherwise too ill to work, expected numbers based on U.S. rates will be higher than those determined using a healthier, employed population. For this reason, internal comparisons using Du Pont rates provide more sensitive indicators of excess mortality or cancer incidence. The Du Pont comparison for incidence is also more appropriate than using U.S. rates since cases were ascertained in a similar manner for the cohort and the entire Company.

Age, sex, and time-specific rates were applied to the appropriate accumulated person-years, yielding age-specific expected numbers, which were then summed over all age strata to give an overall age-adjusted expected number. Company-wide rates were calculated for wage and salary rolls separately, and analyses were performed according to payclass at first exposure to DCB. National rates used were for white males, and were applied to the wage and salary groups separately, regardless of race. Only a very small percentage of the cohort was not Caucasian. National average annual incidence rates were available for the period 1973-1977, obtained from selected areas in the U.S. These age-specific rates were applied to the accumulated person-years over all study years (i.e. 1956-1983);

although this comparison is not ideal, it provides a rough estimate of the number of cancer cases expected based on U.S. rates.

Observed numbers were compared to those expected by use of the Poisson probability distribution ($p < .05$, two-sided). Statistical testing was performed only when the number of observed deaths or cases was 4 or larger.

Although the etiology of cancer is largely unknown, it is thought that the disease usually develops many years after initial contact with an agent. Latency periods of fifteen years or more have frequently been associated with chemical carcinogenesis. The DCB operation began in 1950. Therefore, cancer cases diagnosed during the early years of plant operation are unlikely to be related to DCB exposure. Inclusion of such cases could mask a true relationship between DCB and cancer.

To adjust for latency, the data were reanalyzed, considering a person to begin to be at risk fifteen years after his initial exposure. Only person-years which accrued and cases/deaths which occurred fifteen years after first exposure were used in these determinations of observed and expected numbers.

Results

The cohort consisted of 525 males on the wage roll and 73 males on the salary roll as of first exposure to DCB. An additional 34 males held jobs in DCB areas, but were not

included since their birthdates were not available. Of these 34, 28 were known from the SSA search to be alive as of December 31, 1980, and 6 were lost to follow-up. Almost all were short-term employees (less than 1 year) at the Victoria Plant. Two females with exposure to DCB were not included in these analyses.

The status of the cohort as of December 31, 1980 is shown in Table 1. Vital status was unknown for only 8 of the 598 employees (1%). The person-years of observation contributed by the cohort is shown in Table 2 by payclass and time period, for mortality and cancer incidence analyses.

There were 23 deaths among the wage group, with 29.6 expected based on Du Pont rates, and 44.1 expected based on U.S. rates (Table 3). Cause of death was unknown for one male. Seven deaths due to malignant neoplasms occurred: 4 lung, 2 pancreas, and 1 rectum. There were 6.6 deaths due to cancer expected based on Du Pont rates, and 8.4 expected based on U.S. rates (Table 3). No specific cause of death or type of cancer death was significantly in excess in either analysis.

There were 6 deaths among the salary group, with 4.8 expected based on Du Pont rates, and 8.5 expected based on U.S. rates (Table 3). Only 1 death was attributed to a malignant neoplasm (cancer of the prostate). No specific cause of death was significantly in excess in either analysis.

Thirteen cases of cancer among active wage roll employees were recorded in the Cancer Registry, as compared to 12.7 expected based on Du Pont rates, and 15.0 expected based

on U.S. rates (Table 4). Primary sites included 2 lung, 3 pancreas, 2 malignant melanoma, and 1 each large intestine, rectum, kidney, testis, leukemia, and Hodgkin's disease. Four of these cases (1 lung, 1 rectum, and 2 pancreas) were also included in the mortality analyses. The 3 cases of pancreatic cancer were higher than the 0.3 expected based on Du Pont rates and the 0.4 expected based on U.S. rates. Two cases of cancer (prostate and kidney) occurred among the salary group. The prostate cancer case was also included in the mortality analyses.

The person-years of observation corresponding to a latent period of at least 15 years since first exposure to DCB are shown in Table 5 by time period for the wage group. Some persons left employment before 15 years elapsed or were too recently hired to have 15 years of service, resulting in a decrease in person-years and observed and expected numbers. The population at risk numbered 302 for mortality and 314 for incidence. The results of these analyses are shown in Table 6. Again, no statistically significant differences were found. Seven cancer deaths (4 lung, 2 pancreas, and 1 rectum) were observed versus 4.6 expected based on Du Pont rates, and 5.7 expected based on U.S. rates, and 8 cancer cases (2 lung, 2 pancreas, and 1 each rectum, kidney, leukemia, and Hodgkin's disease) were seen versus expected numbers of 8.3 (Du Pont rates) and 9.4 (U.S. rates). Pancreatic cancer was still slightly high; 2 cases (both of whom died) were observed as compared to less than 0.5 expected using Du Pont or U.S. rates

in incidence or mortality analyses. Latent-period analyses were not performed for the salary roll, since only 2 cancer cases and 1 death due to cancer occurred in this group.

Conclusions

These data do not indicate that persons exposed to DCB at Victoria are at increased risk of developing or dying from cancer. Effects may not be seen because the time since initial exposure is too short or because the cohort is too small to yield statistically meaningful data. Assuming a 15-year latent period for cancer deaths and cases, the study at this point would have only a 40-60% chance of detecting a two-fold excess in risk among the DCB-exposed wage group compared to the Company wage population. Pancreatic cancer appears to be slightly in excess, but small numbers prohibit the drawing of any conclusions.

Follow-up of the cohort will continue. Results from periodic updates of the study will be reported when available.

TABLE 1

Status as of 12/31/80
Victoria DCB Cohort, Males

	<u>Wage</u>	<u>Salary</u>	
Actively Employed	374	41	
Died while Employed	8	5	
Resigned, Terminated	92	8	
Living	76	7	
Deceased	9	0	
Unknown	7	1	
Pensioned	51	19	
Living	45	16	
Deceased	6	3	
Total	525	73	

TABLE 2

**Person-Years of Observation
Victoria DCB Cohort, Males**

<u>Time Period</u>	<u>Wage</u>	<u>Person-Years Mortality</u>	<u>Salary</u>
1957-59	431.7		94.8
1960-64	1140.9		177.2
1965-69	1785.7		216.1
1970-74	2262.2		269.0
1975-80	3030.0		398.1
Total	8700.5		1155.2

<u>Time Period</u>	<u>Wage</u>	<u>Person-Years Cancer Incidence</u>	<u>Salary</u>
1956-59	546.0		120.7
1960-64	990.2		164.9
1965-69	1532.8		198.2
1970-74	1918.9		237.3
1975-83	3500.4		419.7
Total	8488.3		1140.8

TABLE 2**Observed and Expected Deaths
1957-1980, No Latency
Victoria DCB Cohort, Males
(Selected Causes)**

<u>Cause of Death</u>	<u>Observed</u>	<u>Expected Based on Du Pont Rates</u>	<u>Expected Based on U.S. Rates</u>
<u>Wage Roll</u>			
All Causes	23	29.6	44.1
All Malignant Neoplasms	7	6.6	8.4
Cancer of the:			
Lung	4	2.5	2.9
Pancreas	2	0.4	0.4
<u>Salary Roll</u>			
All Causes	8	4.8	8.5
All Malignant Neoplasms	1	1.3	1.8

TABLE 4

**Observed and Expected Cancer Cases
1956-1983, No Latency
Victoria DCB Cohort, Males
(All Cancer¹ and Selected Sites)**

<u>Cancer Site</u>	<u>Observed</u>	<u>Expected Based on Du Pont Rates</u>	<u>Expected Based on U.S. Rates</u>
<u>Wage Roll</u>			
All Malignant Neoplasms	13	12.7	15.0
Cancer of the:			
Lung	2	2.2	3.3
Pancreas	3	0.3	0.4
Malignant Melanoma	2	0.6	0.8
<u>Salary Roll</u>			
All Malignant Neoplasms	2	2.2	2.8

¹Excludes non-melanoma skin cancer

TABLE 3

**Person-Years of Observation
15-Year Latency
Victoria DCB Cohort, Males, Wage Roll**

<u>Time Period</u>	<u>Person-Years Mortality</u>
1957-59	0
1960-64	0
1965-69	377.3
1970-74	752.8
1975-79	356.3
Total	2486.4

<u>Time Period</u>	<u>Person-Years Cancer Incidence</u>
1956-59	0
1960-64	0
1965-69	302.5
1970-74	586.1
1975-83	1626.3
Total	2514.9

TABLE 6

**Observed and Expected Events
15-Year Latency
Victoria DCB Cohort, Males, Wage Roll
(All Cancer and Selected Sites)**

**Cancer Deaths
1957-1980**

<u>Cause of Death</u>	<u>Observed</u>	<u>Expected Based on Du Pont Rates</u>	<u>Expected Based on U.S. Rates</u>
All Malignant Neoplasms	7	4.6	5.7
Cancer of the: Lung	4	1.9	2.2
Pancreas	2	0.3	0.3

**Cancer Cases¹
1956-1983**

<u>Cancer Site</u>			
All Malignant Neoplasms	8	8.3	9.4
Cancer of the: Lung	2	1.7	2.4
Pancreas	2	0.2	0.3

¹Excluding non-melanoma skin cancer