National Cancer Institute
CARCINOGENESIS
Technical Report Series
NO. 191
1979

BIOASSAY OF TECHNICAL GRADE BIS(2-CHLORO-1-METHYLETHYL) ETHER FOR POSSIBLE CARCINOGENICITY

CAS No. 108-60-1

NCI-CG-TR-191

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

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NIH Publication No. 79-1747

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FOREWORD: This report presents the results of the bioassay of bis(2-chloro-1-methylethyl) ether conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that the test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS: This bioassay of bis(2-chloro-1-methylethyl) ether was conducted by Hazleton Laboratories America, Inc., Vienna, Virginia, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program.

The NCI project officers who were responsible for selecting the protocols used in this bioassay were Drs. N. P. Page (1,2) and C. Cueto (1). The principal investigators were Drs. M. B. Powers (3,9) and R. W. Voelker (3). Ms. K. J. Petrovics (3) was responsible for data management, and Mr. G. Najarian (3,4) for animal care. Histopathologic examinations were performed by Dr. B. M. Ulland (3), and the diagnoses included in this report represent his interpretation.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (5). Statistical analyses were

performed by Dr. J. R. Joiner (6), using methods selected for the bioassay program by Dr. J. J. Gart (7).

Chemicals used in this bioassay were analyzed at Midwest Research Institute (8), and gavage mixtures containing the test chemical were analyzed at Hazleton Laboratories by Dr. C. L. Guyton (3) and Mr. E. Missaghi (3). The results of these analyses were reviewed by Dr. C. W. Jameson (6,9).

This report was prepared at Tracor Jitco (6) in collaboration with Hazleton Laboratories and NCI. Those responsible for the report at Tracor Jitco were Dr. C. R. Angel, Acting Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens (10), toxicologist; Drs. R. L. Schueler, pathologist; Ms. L. A. Owen and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The following scientists at NCI (1) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Charles K. Grieshaber, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Morton H. Levitt, Dr. Harry Mahar, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. A. R. Patel, Dr. Marcelina B. Powers, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of technical-grade bis(2-chloro-1-methylethyl) ether for possible carcinogenicity was conducted by administering the test chemical by gavage to F344 rats.

Groups of 50 rats of each sex were administered a solution of bis(2-chloro-1-methylethyl) ether in corn oil 5 days per week at either 100 or 200 mg/kg/day for 103 weeks. Vehicle controls consisted of groups of 50 rats of each sex that were administered the corn oil alone. Untreated-control groups of the same size were also used. All surviving rats were killed at week 104 or 105.

Mean body weights of the dosed groups of male and female rats were lower than those of the corresponding vehicle-control groups throughout most of the study and were dose related. Similarly, survivals of the high-dose males and of both the high- and low-dose females were lower than those of the corresponding vehicle controls and were dose related. Almost all animals in the high-dose groups died by the end of the bioassay.

No tumors occurred in the dosed groups of rats of either sex at incidences that were significantly higher than those of the vehicle-control groups.

It is concluded that under the conditions of this bioassay, the technical-grade test material, bis(2-chloro-1-methlyethyl) ether, was not carcinogenic for F344 rats of either sex.

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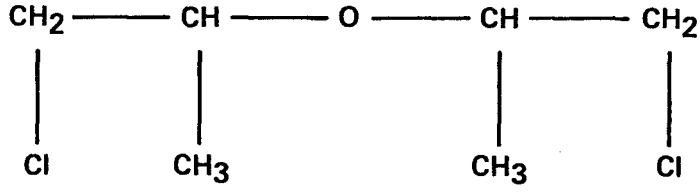
I. INTRODUCTION



Bis(2-chloro-1-methylethyl) ether

Bis(2-chloro-1-methylethyl) ether (CAS 108-60-1; NCI C50044) is a beta-haloether and a byproduct of propylene oxide and propylene glycol manufacture (Lapkin, 1966; Cook, 1971). It has been found in effluent from industrial plants, downstream from these plants in raw intake water, and in tap water from the Ohio River in Evansville, Indiana (Kleopfer and Fairless, 1972). Concentrations found in this river in 1971 ranged from 0.5 to 5 μg/l, and a concentration of 0.8 μg/l was found in the tap water of Evansville. There are also reports of its occurrence in the Kanawha River at Nitro, West Virginia (Rosen et al., 1963), in the Mississippi River at New Orleans (Mayes, 1971), and in the Rhine and Scheldt Rivers in the Netherlands (Piet et al., 1973). Bis(2-chloro-1-methylethyl) ether is considered by Kleopfer and Fairless (1972) to be practically nonbiodegradable. The chemical was formerly used in paint and varnish removers, in spotting agents and cleaning solutions, as an

intermediate in the manufacture of dyes, resins, and pharmaceuticals, and to assist the action of soap solutions in textile processes (Hake and Rowe, 1963). Five former manufacturers or distributors of bis(2-chloro-1-methylethyl) ether stated that they no longer carried the chemical for commercial use when contacted in 1978 (Jameson, 1978). One manufacturer stated that the process for manufacturing propylene oxide had been changed to eliminate bis(2-chloro-1-methylethyl) ether as a by-product (Dow Chemical Co., 1978).

Bis(2-chloro-1-methylethyl) ether has an acute oral LD_{50} of 240 mg/kg in rats of unspecified strain and sex (Smyth et al., 1951). Beta-haloethers are not as reactive chemically as alpha-haloethers. Van Duuren et al. (1972) considered them also to be less potent carcinogens than alpha-haloethers on the basis of studies performed by subcutaneous injection. However, the beta-haloether, bis(2-chloroethyl) ether, which is an analog of the test chemical, has been reported to induce hepatomas when administered in the diets to B6C3F1 or B6AKF1 mice (National Technical Information Service, 1968; Innes et al., 1969) and sarcomas at the site of subcutaneous injection in female ICR/Ha Swiss mice (Van Duuren et al., 1972). In comparison, alpha-haloethers such as chloromethyl methyl ether, bis(chloromethyl) ether, and bis(1-chloroethyl) ether were found to be highly carcinogenic. In particular, bis(chloromethyl) ether has been reported to induce, in addition to the sarcomas in female ICR/Ha Swiss mice (Van

Duuren et al., 1972), lung adenomas in subcutaneously injected newborn ICR Swiss mice (Gargus et al., 1969), lung adenomas in male A/Heston mice inhaling the vapors (Leong et al., 1971), lung squamous-cell carcinomas and olfactory esthesioneuroepitheliomas in Sprague-Dawley rats inhaling the vapors (Laskin et al., 1971), sarcomas in subcutaneously injected Sprague-Dawley rats (Van Duuren et al., 1969), and lung cancer in men exposed to the chemical in a manufacturing plant (Figueroa et al., 1973). Bis(2-chloro-1-methyl-ethyl) ether was selected by the NCI Carcinogenesis Testing Program because of its close chemical structural formula to that of known carcinogenic haloethers.

II. MATERIALS AND METHODS

A. Chemical

Bis(2-chloro-1-methylethyl) ether, also known as bis(2-chloroisopropyl) ether, was obtained in three batches from three different sources. The first batch (Lot No. 7) was obtained from MC&B Manufacturing Chemists, Cincinnati, Ohio, and was used during the first 46 weeks of the chronic study. The second batch (Lot No. PB41576) was obtained from Pfaltz and Bauer, Inc., Stanford, Connecticut, and was used during weeks 47 through 83. The third batch (Lot No. I62976) was obtained from I.C.N. Pharmaceuticals, Inc., Irvine, California, and was used from week 84 to the end of the study. Analysis of each batch at Midwest Research Institute included elemental analysis, boiling point, vapor-phase chromatography, and infrared and nuclear magnetic resonance spectrometry (Appendix C). The results indicated that each batch was a mixture of isopropyl and n-propyl ethers. Additional analysis by vapor-phase chromatography/mass spectrometry at Midwest Research Institute on Lot No. I62976 after completion of the bioassay indicated that this batch contained 69.4% bis(2-chloro-1-methylethyl) ether, 2.1% bis(2-chloro-n-propyl) ether, and 28.5% of the mixed iso and normal ether. These results were consistent with the amounts of the isomers

estimated in each batch by nuclear magnetic resonance spectrometry. This technical-grade test material is referred to as bis(2-chloro-1-methylethyl) ether in this report.

The test material was stored in its original glass containers at room temperature.

B. Dosage Preparation

Dosage mixtures of bis(2-chloro-1-methylethyl) ether were prepared fresh daily. The chemical was first dissolved completely in a small amount of corn oil (Duke's®, S.F. Sauer Co., Richmond, Va.). The stock solution was then diluted with additional corn oil to the desired final volume. The concentrations were made up based on the weight of chemical to volume of corn oil.

As a quality control check on the accuracy of preparation of the gavage solutions, the concentrations of bis(2-chloro-1-methylethyl) ether were determined in a random selection of different batches of gavage solutions during the chronic study. The results of these analyses indicated that all the gavage solutions were within a $\pm$ 10% limit of the theoretical concentration.

C. Animals

Male and female F344 (Fischer) rats, 3 to 4 weeks of age, were obtained from the NCI Frederick Cancer Research Center (Frederick, Md.). The animals were housed within the test facility for 2 to 3 weeks, and then assigned, five animals to a cage, on a weight basis to the various dosed or control groups.

D. Animal Maintenance

The rats were housed in solid-bottom polycarbonate cages (Maryland Plastic, Federalsburg, Md.) covered with stainless steel cage lids and nonwoven, spun-bonded Filtek fiber filter bonnets (Filtek, Appleton, Wis.). The rats were initially housed five per cage; however, at week 36 the males were divided into groups of two or three per cage.

All cages were furnished with heat-treated hardwood chip bedding (Sani-Chips®, Shurfire Products Corporation, Beltsville, Md.) that was changed twice per week. Diets of presterilized Wayne® Sterilizable Lab Meal (Allied Mills, Inc., Chicago, Ill.) and well water were provided ad libitum.

Feed hoppers and water bottles were refilled twice per week. Cages, water bottles, and sipper tubes were washed at 81°C twice per week, feed hoppers once per week, and cage racks once per month. An industrial dishwasher was used for the water bottles and sipper tubes; a cage and rack washer was used for the feed hoppers, cages, and racks. The detergent used was Super Soilax® (Economics Laboratory, Inc., St. Paul, Minn.). When racks were washed, clean racks containing cages of animals were randomly repositioned in the rooms.

Animal rooms were maintained at 20 to 24°C and 45 to 55% relative humidity. Incoming air for single-pass circulation was filtered through 2-inch-thick disposable fiberglass filters and supplied at a rate that allowed 12 changes of room air per hour. Lighting was provided on a 12-hour-per-day cycle. Food and tap water were available ad libitum.

Rats administered bis(2-chloro-1-methylethyl) ether by gavage and their corresponding controls were maintained in the same room as rats being administered the following chemicals:

Feed Studies

(CAS 119-53-9) benzoin
(CAS 120-61-6) dimethyl terephthalate
(CAS 89-78-1) dl-menthol
(CAS 13463-67-7) titanium dioxide

Gavage Studies

(CAS 127-69-5) sulfisoxazole
(CAS 7446-34-6) selenium sulfide

Drinking Water Studies

(CAS 108-95-2) phenol

At week 36, the cages of rats in the bioassay of bis(2-chloro-1-methylethyl) ether were moved to a separate room for the remainder of the bioassay.

E. Subchronic Studies

Subchronic gavage studies were conducted to determine the concentrations used in the chronic studies (referred to in this report as "low" and "high" doses). Groups of 10 males and 10 females were administered the test chemical by gavage once daily 7 days per week for a period of 13 weeks. Ten animals of each sex received only the corn oil (Duke's®) diluent. Table 1 shows doses given, the survival of animals in each dosed group at the end of the study, and the mean body weight of each dosed group at week 13, expressed as a percentage

Table 1. Survival and Mean Body Weights of Rats Gavaged with Bis(2-chloro-1-methylethyl) for 13 Weeks

Dose (mg/kg)	Male		Female	
	Survival(a)	Mean Weight at Week 13 as % of Control	Survival(a)	Mean Weight at Week 13 as % of Control
0	10/10	100	10/10	100
10	10/10	98	10/10	95
25	10/10	97	10/10	101
50	10/10	96	10/10	100
100	10/10	94	10/10	96
250(b)	10/10	80	10/10	92

(a) Number surviving/number in group.

(b) No abnormal gross or histopathologic findings were seen in any dosed groups. Occasional urine stains and a hunched or thin appearance were observed sporadically in the 250 mg/kg group during weeks 4 to 7.

of the mean body weight of the corresponding controls. At the end of the 13 weeks, the animals were killed and necropsied.

Survival of the rats was not affected by the test chemical at any dose used. An adverse effect on body weight was observed only at the 250 mg/kg/day dose. Based on these findings, the low and high doses for chronic studies using rats were set at 100 mg/kg/day and 200 mg/kg/day.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in table 2.

G. Clinical Examinations and Pathology

All animals were observed twice daily. Clinical signs and the presence of palpable masses were recorded every week. Mean body weights were recorded every 2 weeks for the first 12 weeks, then monthly until week 72, when the rats were weighed every 2 weeks for the duration of the study.

Table 2. Experimental Design for Chronic Gavage Studies with Bis(2-chloro-1-methylethyl) ether in Rats

Sex and Test Group	Initial No. of Animals(a)	Bis(2-chloro-1-methylethyl) ether Dose(b) mg/kg/day(c)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Untreated-Control	50	0		105
Vehicle-Control (d)	50	0		105
Low-Dose	50	100	103	1-2
High-Dose	50	200	103	1
Female				
Untreated-Control	50	0		105
Vehicle-Control (d)	50	0		105
Low-Dose	50	100	103	2
High-Dose	50	200	103	2

(a) Rats were approximately 5 weeks of age when placed on study.

(b) Dosed rats were administered a solution of the test chemical by gavage 5 days per week.

(c) Bis(2-chloro-1-methylethyl) ether was mixed with corn oil at appropriate concentrations to allow administration of 1 ml/kg of gavage mixtures containing the respective desired amounts of the test chemical.

(d) Vehicle controls received a volume of vehicle (corn oil) of 1 ml/kg of body weight by gavage 5 days per week.

Animals that were moribund and those that survived to the termination of the study were killed by exsanguination after they were anesthetized by intraperitoneal injections of 0.3-0.5 ml Diabutal® (Diamond Laboratories, Inc., Des Moines, Iowa) containing 60 mg/ml sodium pentobarbital.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Sections from the following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain. Occasionally, additional tissues were also examined microscopically. Special staining techniques were utilized as necessary.

Necropsies were also performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Data on this experiment were recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a

specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971).

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS

A. Body Weights and Clinical Signs

Mean body weights of the dosed groups of male and female rats were lower than those of corresponding control groups throughout the period of the bioassay and were dose related (figure 1). Weight loss and hunched appearance occurred in dosed groups.

B. Survival

Estimates of the probabilities of survival for male and female rats administered bis(2-chloro-1-methylethyl) ether by gavage at the doses of this bioassay, together with those of the vehicle and untreated controls, are shown by the Kaplan and Meier curves in figure 2. The untreated controls are not included in the statistical analysis because the test conditions of the vehicle controls resemble more closely those of the dosed groups. The result of the Tarone test for positive dose-related trend in mortality is significant (P less than 0.001) in each sex. An indicated departure from linear trend is also observed (P less than 0.001) in each sex

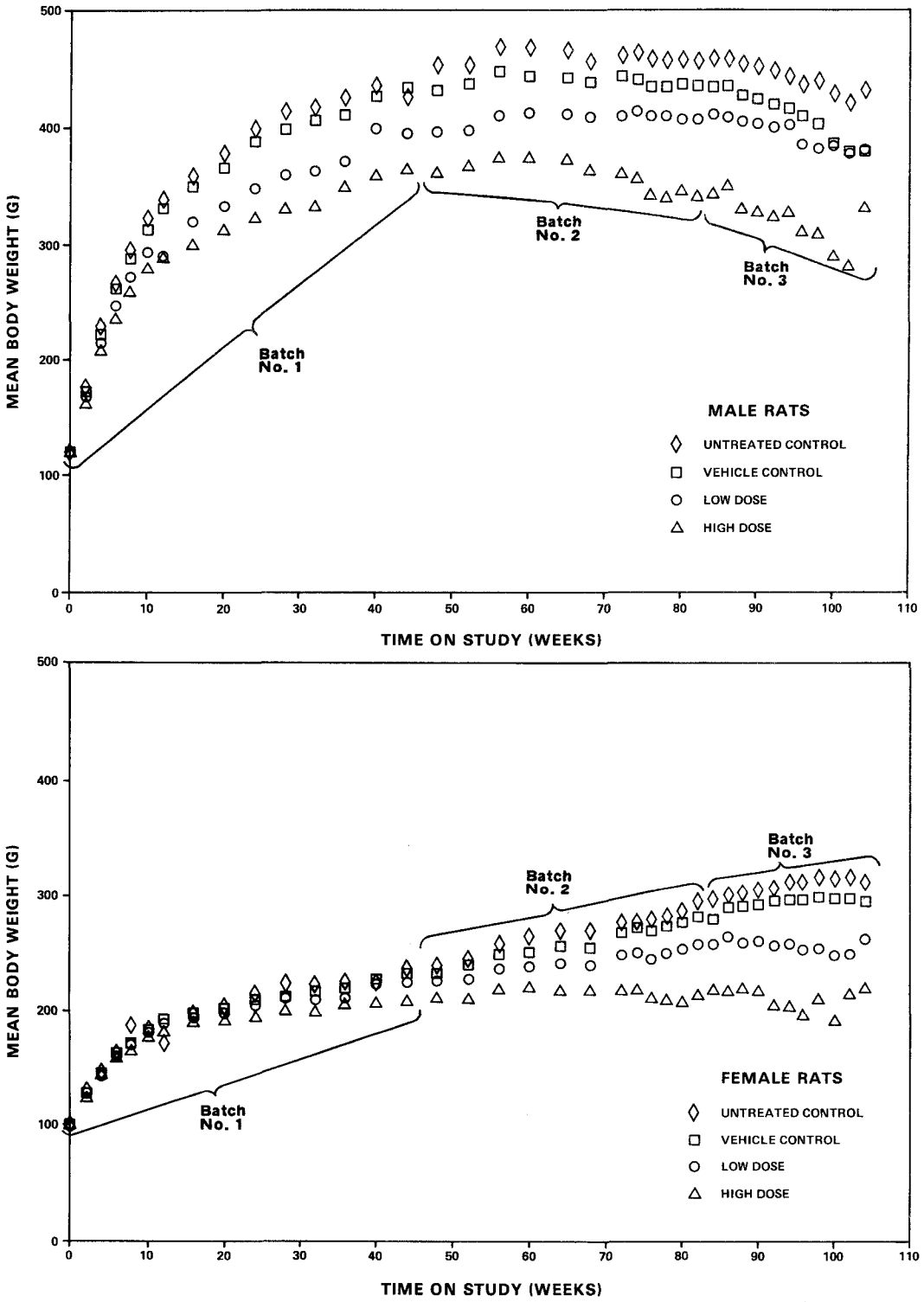


Figure 1. Growth for Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage

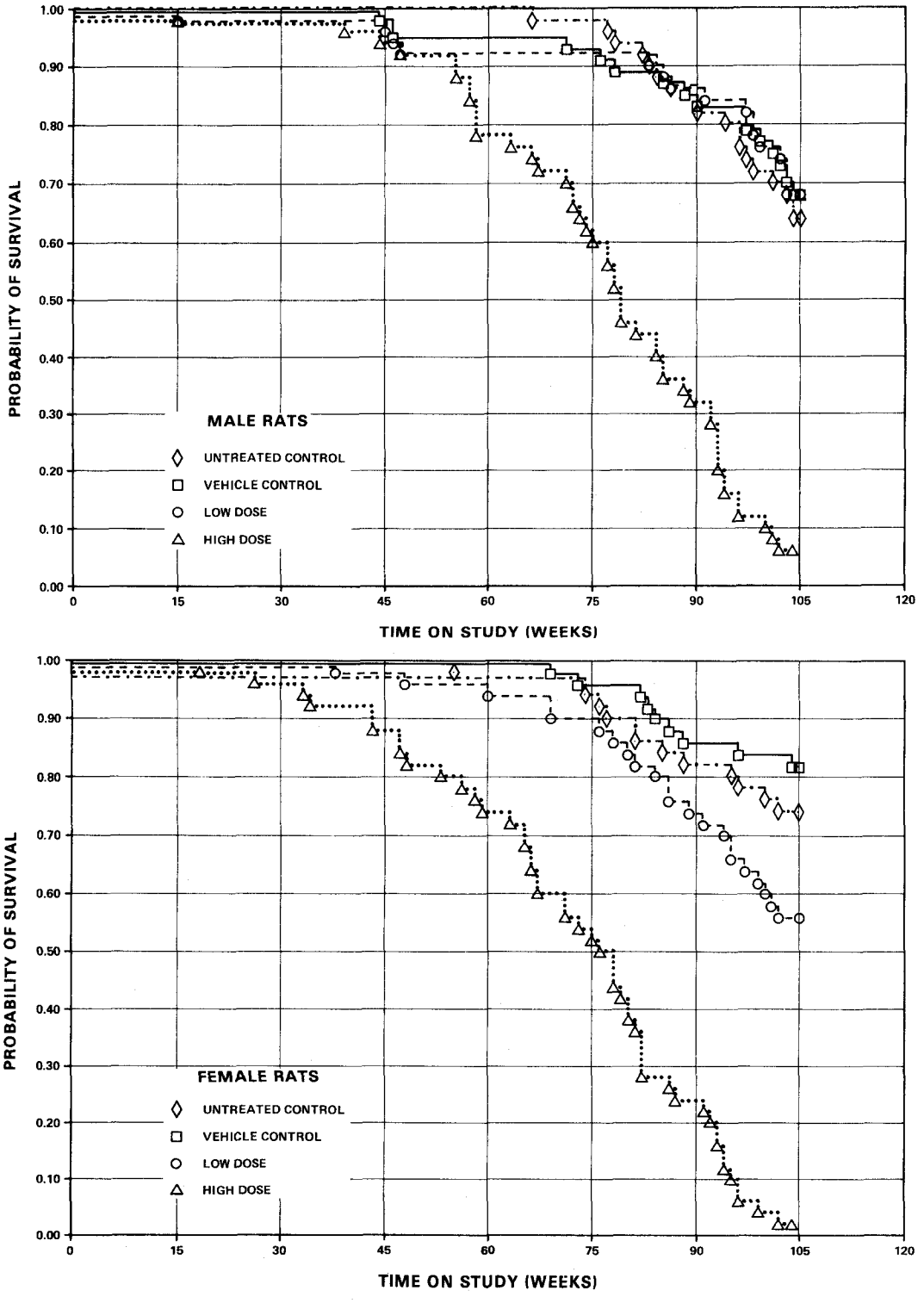


Figure 2. Survival Curves for Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage

due to the relatively steep decrease in survival in the high-dose group. In male rats, 28/50 (56%) of the high-dose group, 46/50 (92%) of the low-dose group, and 44/50 (88%) of the control group were alive at week 78 on study. In females, 25/50 (50%) of the high-dose group, 44/50 (88%) of the low-dose group, and 48/50 (96%) of the control group were alive at week 78 on study.

Except for the high-dose males and females, sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix B, tables B1 and B2.

The variety of tumors which occurred in both control and dosed groups of rats were of a type, incidence, and distribution commonly observed in aged F344 rats. These tumors, most of which occurred at higher incidences in controls than in tested animals, were not considered to be related to administration of the compound.

An increased incidence of esophageal hyperkeratosis was observed in high-dose male and female rats, as compared with their respective control groups. A small number of high-dose females also had esophageal acanthosis. The incidence of gastric hyperkeratosis was greater in vehicle controls than in dosed males and females, and the occurrence of gastric acanthosis was not appreciably different from the vehicle controls. These incidences are summarized in the following tabulation:

	MALE				FEMALE			
	Untreated Control	Vehicle Control	Low Dose	High Dose	Untreated Control	Vehicle Control	Low Dose	High Dose
No. of Tissues Examined Microscopically	47	50	50	49	49	50	49	48
Esophageal Hyperkeratosis	0(0%)	9(18%)	10(20%)	40(82%)	0(0%)	13(26%)	10(20%)	31(65%)
Esophageal Acanthosis	0(0%)	0(0%)	1(2%)	1(2%)	0(0%)	1(2%)	0(0%)	5(10%)
Gastric Hyperkeratosis	0(0%)	13(26%)	5(10%)	10(20%)	0(0%)	21(42%)	14(29%)	11(23%)
Gastric Acanthosis	1(2%)	6(12%)	4(8%)	9(18%)	0(0%)	8(16%)	5(10%)	9(19%)

A dose-related increased incidence of aspiration pneumonia was observed in low- and high-dose males and females.

The histopathologic examination provided no evidence that bis(2-chloro-1-methylethyl) ether was carcinogenic to F344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results

Tables 3 and 4 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group. The untreated controls are not included in this analysis because the test conditions of the vehicle controls resemble more closely those of the dosed groups.

The results of the Cochran-Armitage test for positive dose-related trend in the incidence of tumors and the results of the Fisher exact test comparing the incidence of tumors of the control group with that in each dosed group in the positive direction are not significant in either sex.

Significant results in the negative direction are observed in the

incidences of hematopoietic tumors and tumors of the adrenal, preputial gland, and testis in male rats, as well as tumors of the pituitary, uterus, and pancreatic islets in female rats. This significance in the negative direction may be accounted for by the relatively low survival of rats in the high-dose groups.

In each of the 95% confidence intervals for relative risk shown in the tables, the value of one or less than one is included: this indicates the absence of significant positive results. It should also be noted that some of the intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction by bis(2-chloro-1-methylethyl) ether, which could not be detected under the conditions of this test.

Table 3. Analyses of the Incidence of Primary Tumors in Male Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage (a)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma or Leukemia (b)	14/50(28)	9/50(18)	3/50(6)
P Values (c,d)	P = 0.003 (N)	N.S.	P = 0.003 (N)
Relative Risk (f)		0.643	0.214
Lower Limit		0.271	0.042
Upper Limit		1.441	0.709
Weeks to First Observed Tumor	88	15	15
Pituitary: Chromophobe			
Adenoma (b)	4/43(9)	4/50(8)	3/41(7)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.860	0.787
Lower Limit		0.170	0.122
Upper Limit		4.360	4.361
Weeks to First Observed Tumor	105	85	79

Table 3. Analyses of the Incidence of Primary Tumors in Male Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage (a)

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Adrenal: Pheochromocytoma (b)	8/50 (16)	3/50(6)	2/50(4)
P Values (c,d)	P = 0.025 (N)	N.S.	P = 0.046 (N)
Relative Risk (f)		0.375	0.250
Lower Limit		0.067	0.027
Upper Limit		1.460	1.176
Weeks to First Observed Tumor	105	104	78
Pancreatic Islets:			
Islet-cell Adenoma	0/49(0)	3/50(6)	0/50(0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend(e)	P = 0.014		
Relative Risk (f)		Infinite	--
Lower Limit		0.590	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	98	--

Table 3. Analyses of the Incidence of Primary Tumors in Male Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage (a)

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma (b)	0/50(0)	3/50(6)	0/50(0)
P Values (c,d)	N.S.	N.S.	--
Departure From Linear Trend (e)	P = 0.014		
Relative Risk (f)		Infinite	--
Lower Limit		0.601	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	103	--
Preputial Gland:			
Carcinoma, NOS (b)	7/50(14)	1/50(2)	0/50(0)
P Values (c,d)	P = 0.002 (N)	P = 0.030 (N)	P = 0.006 (N)
Relative Risk (f)		0.143	0.000
Lower Limit		0.003	0.000
Upper Limit		1.052	0.515
Weeks to First Observed Tumor	78	104	--

Table 3. Analyses of the Incidence of Primary Tumors in Male Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage (a)

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor (b)	42/49(86)	43/50(86)	27/49(55)
P Values (c,d)	P less than 0.001 (N)	N.S.	P = 0.001 (N)
Departure From Linear Trend (e)	P = 0.035		
Relative Risk (f)		1.003	0.643
Lower Limit		0.848	0.510
Upper Limit		1.188	0.856
Weeks to First Observed Tumor	86	86	55

(a) Dosed groups received 100 or 200 mg/kg/day, 5 days/week.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05, otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table 4. Analyses of the Incidence of Primary Tumors in Female Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage (a)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Integumentary System: Fibroma of the Subcutaneous Tissue (b)	3/50(6)	3/49(6)	0/48(0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.020	0.000
Lower Limit		0.143	0.000
Upper Limit		7.273	1.730
Weeks to First Observed Tumor	104	97	--
Hematopoietic System: Lymphoma or Leukemia (b)	3/50(6)	7/49(14)	2/48(4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.381	0.694
Lower Limit		0.581	0.060
Upper Limit		13.550	5.794
Weeks to First Observed Tumor	83	84	18

Table 4. Analyses of the Incidence of Primary Tumors in Female Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage (a)

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe			
Adenoma (b)	14/50(28)	8/49(16)	3/48(6)
P Values (c,d)	P = 0.003 (N)	N.S.	P = 0.004 (N)
Relative Risk (f)		0.583	0.223
Lower Limit		0.233	0.044
Upper Limit		1.348	0.737
Weeks to First Observed Tumor	82	60	78
Thyroid: C-cell			
Carcinoma (b)	4/50(8)	1/46(2)	1/48(2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.272	0.260
Lower Limit		0.006	0.005
Upper Limit		2.613	2.508
Weeks to First Observed Tumor	105	105	104

Table 4. Analyses of the Incidence of Primary Tumors in Female Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage (a)

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma or Adenoma (b)	4/50(8)	3/46(7)	1/48(2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.815	0.260
Lower Limit		0.125	0.005
Upper Limit		4.556	2.508
Weeks to First Observed Tumor	105	69	104
Pancreatic Islets: Islet-cell Adenoma (b)	4/50(8)	1/49(2)	0/48(0)
P Values (c,d)	P = 0.028 (N)	N.S.	N.S.
Relative Risk (f)		0.255	0.000
Lower Limit		0.005	0.000
Upper Limit		2.459	1.122
Weeks to First Observed Tumor	105	105	--

Table 4. Analyses of the Incidence of Primary Tumors in Female Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage (a)

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Mammary Gland:			
Fibroadenoma (b)	8/50(16)	7/49(14)	3/48(6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.893	0.391
Lower Limit		0.298	0.070
Upper Limit		2.598	1.517
Weeks to First Observed Tumor	73	76	71
Uterus: Endometrial			
Stromal Polyp (b)	16/48(33)	13/49(27)	7/47(15)
P Values (c,d)	P = 0.025 (N)	N.S.	P = 0.031 (N)
Relative Risk (f)		0.796	0.447
Lower Limit		0.398	0.172
Upper Limit		1.565	1.033
Weeks to First Observed Tumor	82	69	66

Table 4. Analyses of the Incidence of Primary Tumors in Female Rats Administered Bis(2-chloro-1-methylethyl) ether by Gavage (a)

(continued)

(a) Dosed groups received 100 or 200 mg/kg/day, 5 days/week.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05, otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

IV. DISCUSSION

Mean body weights of the dosed groups of male and female rats were lower than those of the corresponding vehicle-control groups throughout most of the bioassay and were dose related. Similarly, survivals of the high-dose males and of both the high- and low-dose females were lower than those of the corresponding vehicle controls and were dose related. Almost all animals in the high-dose groups died by the end of the bioassay.

No tumors occurred in the dosed groups of rats of either sex at incidences that were significantly higher than those of the vehicle-control groups. Several kinds of tumors occurred, however, at lower incidences in dosed groups of the males and females than in the corresponding control groups; this may have been due, in part at least, to the low survival of animals in the high-dose groups.

Two male rats, one high-dose and one low-dose, died during week 15 with malignant lymphoma affecting multiple organs. These early deaths with tumors were not considered to be related to the test compounds. F344 rats are known to have juvenile lymphoid tumors and these deaths were considered as isolated events.

The occurrence of nonneoplastic lesions indicates the toxicity of the test substance and possible effects of the gavage treatment. An increased incidence of esophageal hyperkeratosis was observed in high-dose male and female rats, as compared with their respective control groups. A small number of high-dose females also had esophageal acanthosis. The incidence of gastric hyperkeratosis and acanthosis was greater in vehicle controls and in low- and high-dose male and female rats than in the untreated controls.

Several related haloethers have been found to be carcinogenic, but tests with bis(2-chloro-1-methylethyl) ether for possible carcinogenicity have not previously been reported.

Under the conditions of this bioassay, technical-grade bis(2-chloro-1-methylethyl) ether was not carcinogenic for F344 rats of either sex. The test material can not be considered to have been adequately tested, however, until bioassays are conducted in other animal species.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED BIS(2-CHLORO-1-METHYLETHYL) ETHER BY GAVAGE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED BIS(2-CHLORO-1-METHYLETHYL) ETHER BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	50	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(50)	(50)	(50)	(50)
PAPILLOMA, NOS			1 (2%)	
SQUAMOUS CELL CARCINOMA		1 (2%)	1 (2%)	1 (2%)
BASAL-CELL CARCINOMA		1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)	(50)
FIBROMA	3 (6%)	2 (4%)	1 (2%)	2 (4%)
FIBROSARCOMA	1 (2%)	1 (2%)	1 (2%)	
HEMANGIOSARCOMA		1 (2%)		
RESPIRATORY SYSTEM				
#LUNG	(50)	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA				1 (2%)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (4%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)			
LIPOSARCOMA, METASTATIC			1 (2%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	1 (2%)	1 (2%)	1 (2%)
MONOCYTIC LEUKEMIA	15 (30%)	12 (24%)	8 (16%)	2 (4%)
#SPLEEN	(50)	(50)	(50)	(50)
HEMANGIOSARCOMA	1 (2%)			
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)		
#THYMUS	(35)	(34)	(29)	(36)
THYMOMA	1 (3%)			

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM				
#ENDOCARDIUM	(50)	(50)	(50)	(50)
SARCOMA, NOS	1 (2%)			
DIGESTIVE SYSTEM				
#LIVER	(50)	(49)	(50)	(50)
NEOPLASTIC NODULE		1 (2%)		
HEPATOCELLULAR CARCINOMA			1 (2%)	
ANGIOSARCOMA				1 (2%)
#STOMACH	(50)	(50)	(50)	(50)
ANGIOMA	1 (2%)			
#SMALL INTESTINE	(50)	(50)	(49)	(49)
MUCINOUS ADENOCARCINOMA		1 (2%)		
URINARY SYSTEM				
#KIDNEY	(50)	(50)	(50)	(50)
TRANSITIONAL-CELL CARCINOMA	1 (2%)			
TUBULAR-CELL ADENOMA	1 (2%)			
#KIDNEY/TUBULE	(50)	(50)	(50)	(50)
PAPILLARY CYSTADENOMA, NOS	1 (2%)			
#KIDNEY/PELVIS	(50)	(50)	(50)	(50)
CARCINOSARCOMA	1 (2%)			
ENDOCRINE SYSTEM				
#PITUITARY	(44)	(43)	(50)	(41)
CHROMOPHOBE ADENOMA	5 (11%)	4 (9%)	4 (8%)	3 (7%)
#ADRENAL	(50)	(50)	(50)	(50)
PHEOCHROMOCYTOMA	5 (10%)	8 (16%)	3 (6%)	2 (4%)
#THYROID	(49)	(49)	(49)	(50)
FOLLICULAR-CELL ADENOMA	1 (2%)		2 (4%)	2 (4%)
FOLLICULAR-CELL CARCINOMA	1 (2%)	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
C-CELL ADENOMA	1 (2%)		1 (2%)	
C-CELL CARCINOMA	4 (8%)		1 (2%)	
#PANCREATIC ISLETS	(50)	(49)	(50)	(50)
ISLET-CELL ADENOMA	2 (4%)		3 (6%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(50)	(50)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)	
FIBROADENOMA			3 (6%)	
*PREPUTIAL GLAND	(50)	(50)	(50)	(50)
CARCINOMA,NOS	6 (12%)	7 (14%)	1 (2%)	
SQUAMOUS CELL CARCINOMA				1 (2%)
#TESTIS	(50)	(49)	(50)	(49)
INTERSTITIAL-CELL TUMOR	46 (92%)	42 (86%)	43 (86%)	27 (55%)
*SCROTUM	(50)	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA				1 (2%)
MESOTHELIOMA, MALIGNANT	1 (2%)			
MESOTHELIOMA, METASTATIC			1 (2%)	
NERVOUS SYSTEM				
#CEREBRUM	(50)	(50)	(48)	(49)
ASTROCYTOMA			1 (2%)	
SPECIAL SENSE ORGANS				
*ZYMBAL'S GLAND	(50)	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA				1 (2%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MEDIASTINUM	(50)	(50)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
*PERITONEUM	(50)	(50)	(50)	(50)
MESOTHELIOMA, NOS				1 (2%)
*MESENTERY	(50)	(50)	(50)	(50)
HEMANGIOSARCOMA	1 (2%)			
*TUNICA VAGINALIS	(50)	(50)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)	1 (2%)
MESOTHELIOMA, MALIGNANT			2 (4%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(50)	(50)	(50)	(50)
MESOTHELIOMA, METASTATIC	1 (2%)		1 (2%)	
DIAPHRAGM				
ADENOCARCINOMA, NOS, METASTATIC			1	
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	15	14	15	47
MORIBUND SACRIFICE	3	1	1	
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED		2		
TERMINAL SACRIFICE	32	33	34	3
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	50	45	47	34
TOTAL PRIMARY TUMORS	102	84	82	48
TOTAL ANIMALS WITH BENIGN TUMORS	47	43	46	30
TOTAL BENIGN TUMORS	67	56	63	38
TOTAL ANIMALS WITH MALIGNANT TUMORS	29	22	17	8
TOTAL MALIGNANT TUMORS	35	27	18	8
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		4	1
TOTAL SECONDARY TUMORS	1		6	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	1	2
TOTAL UNCERTAIN TUMORS		1	1	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED BIS(2-CHLORO-1-METHYLETHYL) ETHER BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	50	50	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	49	48
INTEGUMENTARY SYSTEM				
*SKIN	(50)	(50)	(49)	(48)
SQUAMOUS CELL CARCINOMA	1 (2%)			
KERATOACANTHOMA			1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(49)	(48)
FIBROMA	1 (2%)	3 (6%)	3 (6%)	
FIBROSARCOMA		2 (4%)	1 (2%)	
RESPIRATORY SYSTEM				
*NASAL SEPTUM	(50)	(50)	(49)	(48)
SQUAMOUS CELL CARCINOMA, METASTA	1 (2%)			
#LUNG	(49)	(50)	(49)	(48)
SQUAMOUS CELL CARCINOMA, METASTA	1 (2%)			
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)			
PHEOCHROMOCYTOMA, METASTATIC	1 (2%)		1 (2%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(50)	(50)	(49)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)		1 (2%)
MONOCYTIC LEUKEMIA	7 (14%)	2 (4%)	6 (12%)	1 (2%)
#BRONCHIAL LYMPH NODE	(49)	(50)	(49)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)	
CIRCULATORY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(49)	(50)	(49)	(48)
HEPATOCELLULAR CARCINOMA	1 (2%)			
#PANCREAS	(49)	(50)	(49)	(48)
INFILTRATING DUCT CARCINOMA		1 (2%)		
#ESOPHAGUS	(49)	(50)	(49)	(48)
SQUAMOUS CELL PAPILLOMA				1 (2%)
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(49)	(50)	(49)	(48)
CHROMOPHOBE ADENOMA	18 (37%)	14 (28%)	8 (16%)	3 (6%)
#ADRENAL	(49)	(50)	(49)	(48)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)		1 (2%)	
#THYROID	(49)	(50)	(46)	(48)
FOLLICULAR-CELL ADENOMA			1 (2%)	
C-CELL ADENOMA			2 (4%)	
C-CELL CARCINOMA	1 (2%)	4 (8%)	1 (2%)	1 (2%)
#PANCREATIC ISLETS	(49)	(50)	(49)	(48)
ISLET-CELL ADENOMA		4 (8%)	1 (2%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(50)	(50)	(49)	(48)
ADENOMA, NOS	1 (2%)	1 (2%)	2 (4%)	
ADENOCARCINOMA, NOS				1 (2%)
PAPILLARY ADENOCARCINOMA		1 (2%)		
FIBROADENOMA	10 (20%)	8 (16%)	7 (14%)	3 (6%)
*PREPUTIAL GLAND	(50)	(50)	(49)	(48)
CARCINOMA, NOS	3 (6%)	1 (2%)	1 (2%)	1 (2%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
*VAGINA	(50)	(50)	(49)	(48)
FIBROSARCOMA	1 (2%)			
ENDOMETRIAL STROMAL SARCOMA, INV	1 (2%)			
#UTERUS	(49)	(48)	(49)	(47)
CARCINOMA,NOS		1 (2%)		
PAPILLARY CYSTADENOMA, NOS			1 (2%)	
FIBROSARCOMA				1 (2%)
LEIOMYOMA	1 (2%)			1 (2%)
ENDOMETRIAL STROMAL POLYP	11 (22%)	16 (33%)	13 (27%)	7 (15%)
ENDOMETRIAL STROMAL SARCOMA	1 (2%)	1 (2%)	1 (2%)	
#UTERUS/ENDOMETRIUM	(49)	(48)	(49)	(47)
PAPILLARY ADENOMA		1 (2%)		
#OVARY	(49)	(48)	(49)	(46)
GRANULOSA-CELL CARCINOMA				1 (2%)
NERVOUS SYSTEM				
#CEREBELLUM	(49)	(50)	(49)	(48)
ASTROCYTOMA		1 (2%)		
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(50)	(50)	(49)	(48)
INFILTRATING DUCT CARCINOMA, MET		1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
FIBROSARCOMA, METASTATIC				1 (2%)
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	11	8	20	48
MORIBUND SACRIFICE	2	1	2	1
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	37	41	28	1
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	36	39	32	15
TOTAL PRIMARY TUMORS	59	62	51	22
TOTAL ANIMALS WITH BENIGN TUMORS	29	31	28	11
TOTAL BENIGN TUMORS	43	47	39	15
TOTAL ANIMALS WITH MALIGNANT TUMORS	14	13	12	7
TOTAL MALIGNANT TUMORS	16	15	12	7
TOTAL ANIMALS WITH SECONDARY TUMORS#	3	1	1	1
TOTAL SECONDARY TUMORS	4	1	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED BIS(2-CHLORO-1-METHYLETHYL) ETHER BY GAVAGE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED BIS(2-CHLORO-1-METHYLETHYL) ETHER BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	50	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(50)	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)	2 (4%)	1 (2%)	
ULCER, NOS				1 (2%)
ABSCESS, NOS		1 (2%)		
NECROSIS, NOS	1 (2%)			
HYPERKERATOSIS	5 (10%)			
ACANTHOSIS	2 (4%)			
*SUBCUT TISSUE	(50)	(50)	(50)	(50)
HEMATOMA, ORGANIZED				1 (2%)
ABSCESS, NOS				1 (2%)
RESPIRATORY SYSTEM				
*NOSE	(50)	(50)	(50)	(50)
INFLAMMATION, ACUTE				1 (2%)
#TRACHEA	(50)	(50)	(50)	(49)
INFLAMMATION, NECROTIZING				1 (2%)
INFLAMMATION, ACUTE			2 (4%)	
#LUNG	(50)	(50)	(50)	(50)
THROMBOSIS, NOS		2 (4%)		
CONGESTION, NOS	1 (2%)	1 (2%)		7 (14%)
EDEMA, NOS		1 (2%)		2 (4%)
HEMORRHAGE	7 (14%)	2 (4%)	3 (6%)	2 (4%)
PNEUMONIA, ASPIRATION		2 (4%)	7 (14%)	12 (24%)
ABSCESS, NOS				2 (4%)
PNEUMONIA, CHRONIC MURINE	42 (84%)	48 (96%)	44 (88%)	36 (72%)
INFARCT, NOS		1 (2%)		
HEMOSIDEROSIS		1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(48)	(50)	(50)	(50)
MYELOFIBROSIS			1 (2%)	
HYPERPLASIA, HEMATOPOIETIC			2 (4%)	2 (4%)
#SPLEEN	(50)	(50)	(50)	(50)
CONGESTION, NOS	1 (2%)	1 (2%)		
HEMORRHAGE		1 (2%)		
INFLAMMATION, CHRONIC		1 (2%)		
FIBROSIS		2 (4%)	1 (2%)	
FIBROSIS, FOCAL		1 (2%)		
HEMOSIDEROSIS		8 (16%)	13 (26%)	12 (24%)
ANGIECTASIS		1 (2%)		
HEMATOPOIESIS	20 (40%)	15 (30%)	14 (28%)	10 (20%)
#SPLENIC CAPSULE	(50)	(50)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)		
#SPLENIC FOLLICLES	(50)	(50)	(50)	(50)
ATROPHY, NOS				2 (4%)
#CERVICAL LYMPH NODE	(50)	(50)	(50)	(50)
LYMPHANGIECTASIS	1 (2%)			
ABSCESS, NOS		1 (2%)		
HYPERPLASIA, LYMPHOID		1 (2%)	3 (6%)	
#LYMPH NODE OF THORAX	(50)	(50)	(50)	(50)
ATROPHY, NOS	1 (2%)			
#BRONCHIAL LYMPH NODE	(50)	(50)	(50)	(50)
LYMPHANGIECTASIS		1 (2%)		
HEMORRHAGE		1 (2%)		
#THYMUS	(35)	(34)	(29)	(36)
VEGETABLE FOREIGN BODY		1 (3%)		
HEMORRHAGE		2 (6%)		
ABSCESS, NOS		1 (3%)		
CIRCULATORY SYSTEM				
#HEART	(50)	(50)	(50)	(50)
THROMBOSIS, NOS		1 (2%)		

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC				2 (4%)
#BASE OF HEART	(50)	(50)	(50)	(50)
ABSCESS, NOS		1 (2%)		
#HEART/ATRIUM	(50)	(50)	(50)	(50)
THROMBOSIS, NOS	2 (4%)	1 (2%)		2 (4%)
#MYOCARDIUM	(50)	(50)	(50)	(50)
INFLAMMATION, CHRONIC	19 (38%)	8 (16%)	34 (68%)	29 (58%)
FIBROSIS		25 (50%)	8 (16%)	
DEGENERATION, NOS	18 (36%)	6 (12%)	1 (2%)	2 (4%)
CALCIFICATION, NOS	2 (4%)			
*AORTA	(50)	(50)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)	
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(50)	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE				1 (2%)
HYPERPLASIA, CYSTIC			1 (2%)	
#LIVER	(50)	(49)	(50)	(50)
THROMBOSIS, NOS	1 (2%)	1 (2%)		
CONGESTION, NOS	1 (2%)	2 (4%)		
INFLAMMATION, NOS				1 (2%)
HEPATITIS, TOXIC	3 (6%)	3 (6%)		1 (2%)
PELIOSIS HEPATIS	1 (2%)			
NECROSIS, NOS	2 (4%)	1 (2%)	1 (2%)	
NECROSIS, FOCAL			2 (4%)	2 (4%)
INFARCT, NOS	1 (2%)		1 (2%)	1 (2%)
METAMORPHOSIS FATTY	6 (12%)		1 (2%)	2 (4%)
FOCAL CELLULAR CHANGE	20 (40%)	20 (41%)	15 (30%)	8 (16%)
ANGIECTASIS				1 (2%)
HEMATOPOIESIS			1 (2%)	
#LIVER/CENTRILOBULAR	(50)	(49)	(50)	(50)
NECROSIS, NOS	4 (8%)	5 (10%)	2 (4%)	11 (22%)
#BILE DUCT	(50)	(49)	(50)	(50)
INFLAMMATION, CHRONIC	26 (52%)	2 (4%)	1 (2%)	
FIBROSIS	17 (34%)	1 (2%)	3 (6%)	3 (6%)
HYPERPLASIA, NOS	34 (68%)	29 (59%)	24 (48%)	3 (6%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#PANCREAS	(50)	(49)	(50)	(50)
INFLAMMATION, CHRONIC	2 (4%)	1 (2%)	2 (4%)	
PIGMENTATION, NOS			1 (2%)	
ATROPHY, NOS	13 (26%)	1 (2%)	1 (2%)	
ATROPHY, FOCAL		10 (20%)	2 (4%)	
#ESOPHAGUS	(47)	(50)	(50)	(49)
INFLAMMATION, CHRONIC			1 (2%)	2 (4%)
HYPERKERATOSIS		9 (18%)	10 (20%)	40 (82%)
ACANTHOSIS			1 (2%)	1 (2%)
#STOMACH	(50)	(50)	(50)	(50)
MINERALIZATION	2 (4%)			
HEMORRHAGE	2 (4%)	3 (6%)	2 (4%)	3 (6%)
ULCER, FOCAL				1 (2%)
INFLAMMATION, ACUTE		1 (2%)		
INFLAMMATION, CHRONIC	3 (6%)		1 (2%)	
NECROSIS, NOS	5 (10%)		1 (2%)	1 (2%)
NECROSIS, FOCAL	1 (2%)		1 (2%)	3 (6%)
CALCIFICATION, METASTATIC		1 (2%)		
HYPERKERATOSIS		13 (26%)	5 (10%)	10 (20%)
ACANTHOSIS	1 (2%)	6 (12%)	4 (8%)	9 (18%)
#LARGE INTESTINE	(49)	(50)	(50)	(49)
PARASITISM	1 (2%)	3 (6%)	2 (4%)	4 (8%)
#CECUM	(49)	(50)	(50)	(49)
INFLAMMATION, ACUTE				1 (2%)
URINARY SYSTEM				
#KIDNEY	(50)	(50)	(50)	(50)
HYDRONEPHROSIS		1 (2%)		
INFLAMMATION, CHRONIC	49 (98%)	44 (88%)	45 (90%)	41 (82%)
CALCIFICATION, NOS	1 (2%)			1 (2%)
PIGMENTATION, NOS	2 (4%)			
HYPERPLASIA, TUBULAR CELL		1 (2%)		
#KIDNEY/CORTEX	(50)	(50)	(50)	(50)
CYST, NOS	1 (2%)			
ABSCESS, NOS				1 (2%)
#RENAL PAPILLA	(50)	(50)	(50)	(50)
NECROSIS, NOS		1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#KIDNEY/TUBULE	(50)	(50)	(50)	(50)
NECROSIS, CYTODEGENERATIVE				1 (2%)
PIGMENTATION, NOS		1 (2%)	3 (6%)	
#URINARY BLADDER	(47)	(50)	(49)	(46)
HEMORRHAGE		1 (2%)		
INFLAMMATION, ACUTE			1 (2%)	1 (2%)
INFLAMMATION, CHRONIC		2 (4%)		
NECROSIS, NOS		2 (4%)		
HYPERPLASIA, EPITHELIAL		1 (2%)		
METAPLASIA, OSSEOUS				1 (2%)
ENDOCRINE SYSTEM				
#PITUITARY	(44)	(43)	(50)	(41)
CYST, NOS	1 (2%)	1 (2%)	2 (4%)	1 (2%)
HEMORRHAGE		2 (5%)	1 (2%)	
HYPERPLASIA, FOCAL	2 (5%)	2 (5%)	1 (2%)	
#ADRENAL	(50)	(50)	(50)	(50)
THROMBOSIS, NOS		1 (2%)		
METAMORPHOSIS FATTY	2 (4%)			
ANGIECTASIS		2 (4%)		
#ADRENAL/CAPSULE	(50)	(50)	(50)	(50)
FIBROSIS	1 (2%)			
#ADRENAL CORTEX	(50)	(50)	(50)	(50)
DEGENERATION, NOS	1 (2%)	1 (2%)		
ANGIECTASIS		1 (2%)	1 (2%)	
#ADRENAL MEDULLA	(50)	(50)	(50)	(50)
HYPERPLASIA, NOS	4 (8%)	3 (6%)		
#THYROID	(49)	(49)	(49)	(50)
FOLLICULAR CYST, NOS	2 (4%)			
INFLAMMATION, CHRONIC				2 (4%)
HYPERPLASIA, C-CELL	2 (4%)	5 (10%)	2 (4%)	
#PARATHYROID	(41)	(37)	(40)	(40)
HYPERPLASIA, NOS		2 (5%)		
#PANCREATIC ISLETS	(50)	(49)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, FOCAL			1 (2%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(50)	(50)	(50)	(50)
GALACTOCELE		1 (2%)		
CYST, NOS	3 (6%)			
CYSTIC DUCTS	2 (4%)	1 (2%)	3 (6%)	
INFLAMMATION, CHRONIC		1 (2%)		
HYPERPLASIA, CYSTIC			1 (2%)	
*PREPUTIAL GLAND	(50)	(50)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)	
HYPERPLASIA, CYSTIC			1 (2%)	
#PROSTATE	(49)	(49)	(49)	(45)
INFLAMMATION, SUPPURATIVE			2 (4%)	
INFLAMMATION, ACUTE	13 (27%)	11 (22%)	9 (18%)	4 (9%)
ABSCESS, NOS		3 (6%)		2 (4%)
INFLAMMATION, CHRONIC	7 (14%)	7 (14%)	2 (4%)	2 (4%)
*SEMINAL VESICLE	(50)	(50)	(50)	(50)
INFLAMMATION, NOS				1 (2%)
INFLAMMATION, ACUTE				2 (4%)
#TESTIS	(50)	(49)	(50)	(49)
HEMORRHAGE		1 (2%)		
PERIARTERITIS	1 (2%)			
DEGENERATION, NOS	2 (4%)	3 (6%)	2 (4%)	5 (10%)
HYPOSPERMATOGENESIS				1 (2%)
HYPERPLASIA, INTERSTITIAL CELL	29 (58%)	38 (78%)	14 (28%)	18 (37%)
*EPIDIDYMIS	(50)	(50)	(50)	(50)
STEATITIS	2 (4%)		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)	2 (4%)	1 (2%)	
GRANULOMA, SPERMATIC		1 (2%)		1 (2%)
NECROSIS, FAT		1 (2%)	2 (4%)	1 (2%)
*SCROTUM	(50)	(50)	(50)	(50)
NECROSIS, FAT			1 (2%)	
NERVOUS SYSTEM				
#CEREBRUM	(50)	(50)	(48)	(49)
HEMORRHAGE		1 (2%)	2 (4%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, NOS		1 (2%)		
#CEREBELLUM	(50)	(50)	(48)	(49)
HEMORRHAGE			1 (2%)	1 (2%)
SPECIAL SENSE ORGANS				
*EYE	(50)	(50)	(50)	(50)
HEMORRHAGE				1 (2%)
SYNECHIA, POSTERIOR	1 (2%)	1 (2%)	1 (2%)	1 (2%)
CATARACT	1 (2%)			
PHTHISIS BULBI		2 (4%)		
*EYE/RETINA	(50)	(50)	(50)	(50)
INFLAMMATION, CHRONIC			2 (4%)	
DEGENERATION, NOS	1 (2%)	2 (4%)		1 (2%)
*EAR CANAL	(50)	(50)	(50)	(50)
HYPERKERATOSIS				1 (2%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(50)	(50)	(50)	(50)
STEATITIS	3 (6%)	2 (4%)	2 (4%)	
NECROSIS, FAT	1 (2%)	8 (16%)	2 (4%)	3 (6%)
*MESENTERY	(50)	(50)	(50)	(50)
NECROSIS, FAT			1 (2%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED BIS(2-CHLORO-1-METHYLETHYL) ETHER BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	50	50	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	49	48
INTEGUMENTARY SYSTEM				
*SKIN	(50)	(50)	(49)	(48)
EPIDERMAL INCLUSION CYST			1 (2%)	
ACANTHOSIS			1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(49)	(48)
ABSCESS, NOS				1 (2%)
RESPIRATORY SYSTEM				
#TRACHEA	(49)	(49)	(49)	(48)
INFLAMMATION, ACUTE			1 (2%)	
METAPLASIA, SQUAMOUS				1 (2%)
#LUNG/BRONCHIOLE	(49)	(50)	(49)	(48)
HYPERPLASIA, NOS		1 (2%)		
#LUNG	(49)	(50)	(49)	(48)
CONGESTION, NOS			1 (2%)	7 (15%)
EDEMA, NOS			1 (2%)	
HEMORRHAGE	1 (2%)	2 (4%)	8 (16%)	1 (2%)
PNEUMONIA, ASPIRATION		1 (2%)	16 (33%)	22 (46%)
INFLAMMATION, ACUTE				2 (4%)
ABSCESS, NOS				3 (6%)
PNEUMONIA, CHRONIC MURINE	48 (98%)	45 (90%)	42 (86%)	24 (50%)
FOREIGN MATERIAL, NOS				1 (2%)
HEMOSIDEROSIS			1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)		
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(48)	(50)	(48)	(48)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#SPLEEN	(48)	(49)	(49)	(48)
HEMOSIDEROSIS	33 (69%)	38 (78%)	26 (53%)	19 (40%)
HYPERPLASIA, LYMPHOID		1 (2%)		
HEMATOPOIESIS	28 (58%)	27 (55%)	19 (39%)	11 (23%)
#SPLENIC FOLLICLES	(48)	(49)	(49)	(48)
ATROPHY, NOS			2 (4%)	1 (2%)
#CERVICAL LYMPH NODE	(49)	(50)	(49)	(48)
HEMOSIDEROSIS			1 (2%)	
HYPERPLASIA, LYMPHOID			2 (4%)	
#MESENTERIC L. NODE	(49)	(50)	(49)	(48)
LYMPHANGIECTASIS		1 (2%)		
ATROPHY, NOS	2 (4%)			
#THYMUS	(20)	(35)	(18)	(26)
HEMORRHAGE	1 (5%)			
CIRCULATORY SYSTEM				
#HEART/ATRIUM	(49)	(50)	(49)	(48)
THROMBOSIS, NOS	1 (2%)			
#MYOCARDIUM	(49)	(50)	(49)	(48)
INFLAMMATION, CHRONIC	29 (59%)	9 (18%)	12 (24%)	5 (10%)
FIBROSIS	4 (8%)	8 (16%)	3 (6%)	1 (2%)
DEGENERATION, NOS	2 (4%)	19 (38%)	13 (27%)	12 (25%)
CALCIFICATION, NOS	1 (2%)			
#ENDOCARDIUM	(49)	(50)	(49)	(48)
FIBROSIS			1 (2%)	
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(48)	(50)	(49)	(48)
INFLAMMATION, CHRONIC	1 (2%)			
#LIVER	(49)	(50)	(49)	(48)
CONGESTION, NOS				1 (2%)
INFLAMMATION, NOS				2 (4%)
INFLAMMATION, FOCAL			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HEPATITIS, TOXIC	3 (6%)		2 (4%)	
NECROSIS, NOS		1 (2%)		
NECROSIS, FOCAL	2 (4%)	1 (2%)	2 (4%)	2 (4%)
METAMORPHOSIS FATTY		1 (2%)	2 (4%)	3 (6%)
HEMOSIDEROSIS	1 (2%)			
FOCAL CELLULAR CHANGE	24 (49%)	37 (74%)	23 (47%)	9 (19%)
#LIVER/CENTRILOBULAR	(49)	(50)	(49)	(48)
NECROSIS, NOS		1 (2%)	1 (2%)	7 (15%)
#LIVER/HEPATOCYTES	(49)	(50)	(49)	(48)
NECROSIS, NOS				1 (2%)
#BILE DUCT	(49)	(50)	(49)	(48)
INFLAMMATION, CHRONIC	5 (10%)	3 (6%)	4 (8%)	
FIBROSIS	4 (8%)	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, NOS	14 (29%)	15 (30%)	1 (2%)	2 (4%)
#PANCREAS	(49)	(50)	(49)	(48)
EMBRYONAL REST				2 (4%)
ATROPHY, NOS	2 (4%)			
ATROPHY, FOCAL	3 (6%)	7 (14%)	3 (6%)	2 (4%)
#ESOPHAGUS	(49)	(50)	(49)	(48)
HYPERKERATOSIS		13 (26%)	10 (20%)	31 (65%)
ACANTHOSIS		1 (2%)		5 (10%)
#STOMACH	(48)	(50)	(49)	(48)
HEMORRHAGE	1 (2%)	1 (2%)		2 (4%)
ULCER, FOCAL				1 (2%)
INFLAMMATION, ACUTE		2 (4%)		
INFLAMMATION, CHRONIC	1 (2%)			
NECROSIS, NOS		1 (2%)		
NECROSIS, FOCAL		3 (6%)	1 (2%)	2 (4%)
HYPERKERATOSIS		21 (42%)	14 (29%)	11 (23%)
ACANTHOSIS		8 (16%)	5 (10%)	9 (19%)
#LARGE INTESTINE	(47)	(50)	(49)	(48)
INFLAMMATION, ACUTE	1 (2%)			
PARASITISM	3 (6%)	1 (2%)	3 (6%)	4 (8%)
URINARY SYSTEM				
#KIDNEY	(49)	(49)	(49)	(48)
ABSCESS, NOS		1 (2%)	1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC	37 (76%)	27 (55%)	15 (31%)	7 (15%)
#KIDNEY/CORTEX	(49)	(49)	(49)	(48)
CYST, NOS	1 (2%)	2 (4%)	2 (4%)	
#KIDNEY/TUBULE	(49)	(49)	(49)	(48)
NECROSIS, CYTODEGENERATIVE				1 (2%)
PIGMENTATION, NOS	8 (16%)	6 (12%)	2 (4%)	1 (2%)
#KIDNEY/PELVIS	(49)	(49)	(49)	(48)
CALCIFICATION, NOS				1 (2%)
#URINARY BLADDER	(48)	(45)	(47)	(44)
HYPERPLASIA, PAPILLARY			1 (2%)	
ENDOCRINE SYSTEM				
#PITUITARY	(49)	(50)	(49)	(48)
CYST, NOS	5 (10%)	11 (22%)	5 (10%)	7 (15%)
HEMORRHAGE		5 (10%)	5 (10%)	
HEMORRHAGIC CYST	1 (2%)			
HYPERPLASIA, FOCAL	3 (6%)	6 (12%)	3 (6%)	1 (2%)
ANGIECTASIS	4 (8%)	3 (6%)	1 (2%)	2 (4%)
#ADRENAL	(49)	(50)	(49)	(48)
FIBROSIS				1 (2%)
METAMORPHOSIS FATTY	3 (6%)			
ANGIECTASIS	2 (4%)	5 (10%)	1 (2%)	2 (4%)
#ADRENAL CORTEX	(49)	(50)	(49)	(48)
DEGENERATION, NOS	5 (10%)	4 (8%)	10 (20%)	1 (2%)
ANGIECTASIS	5 (10%)	2 (4%)	3 (6%)	13 (27%)
#ADRENAL MEDULLA	(49)	(50)	(49)	(48)
NECROSIS, NOS			1 (2%)	
HYPERPLASIA, NOS			1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)	
#THYROID	(49)	(50)	(46)	(48)
HYPERPLASIA, C-CELL	5 (10%)	3 (6%)	4 (9%)	1 (2%)
#PANCREATIC ISLETS	(49)	(50)	(49)	(48)
HYPERPLASIA, NOS		1 (2%)	1 (2%)	
HYPERPLASIA, FOCAL	1 (2%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(50)	(50)	(49)	(48)
GALACTOCELE	1 (2%)	4 (8%)	1 (2%)	1 (2%)
CYSTIC DUCTS	23 (46%)	15 (30%)	9 (18%)	1 (2%)
HYPERPLASIA, NOS			1 (2%)	
HYPERPLASIA, CYSTIC	14 (28%)	22 (44%)	11 (22%)	5 (10%)
#UTERUS	(49)	(48)	(49)	(47)
HYDROMETRA	3 (6%)	4 (8%)	1 (2%)	3 (6%)
HEMORRHAGE	1 (2%)	3 (6%)	1 (2%)	
#CERVIX UTERI	(49)	(48)	(49)	(47)
INFLAMMATION, CHRONIC	1 (2%)			
#UTERUS/ENDOMETRIUM	(49)	(48)	(49)	(47)
CYST, NOS		1 (2%)		
INFLAMMATION, VESICULAR			1 (2%)	
#OVARY	(49)	(48)	(49)	(46)
FOLLICULAR CYST, NOS	1 (2%)	2 (4%)	1 (2%)	
PAROVARIAN CYST			1 (2%)	
NERVOUS SYSTEM				
#BRAIN/MENINGES	(49)	(50)	(49)	(48)
INFLAMMATION, SUPPURATIVE				1 (2%)
INFLAMMATION, ACUTE	1 (2%)			
#CEREBRUM	(49)	(50)	(49)	(48)
HEMORRHAGE	1 (2%)			
#CEREBELLUM	(49)	(50)	(49)	(48)
INFLAMMATION, ACUTE	1 (2%)			
SPECIAL SENSE ORGANS				
*EYE	(50)	(50)	(49)	(48)
HEMORRHAGE		1 (2%)		
CATARACT		1 (2%)		
PHTHISIS BULBI	1 (2%)	1 (2%)		
*EYE/RETINA	(50)	(50)	(49)	(48)
INFLAMMATION, CHRONIC				1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DEGENERATION, NOS		1 (2%)		
*EYE/CONJUNCTIVA	(50)	(50)	(49)	(48)
INFLAMMATION, CHRONIC				1 (2%)
ACANTHOSIS				1 (2%)
MUSCULOSKELETAL SYSTEM				
*RIB	(50)	(50)	(49)	(48)
HEALED FRACTURE			1 (2%)	
*MUSCLE OF NECK	(50)	(50)	(49)	(48)
INFLAMMATION, CHRONIC		1 (2%)		
BODY CAVITIES				
*ABDOMINAL CAVITY	(50)	(50)	(49)	(48)
STEATITIS	1 (2%)		1 (2%)	
NECROSIS, FAT	1 (2%)	1 (2%)	3 (6%)	
*MESENTERY	(50)	(50)	(49)	(48)
INFLAMMATION, ACUTE			1 (2%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(50)	(50)	(49)	(48)
HEMATOPOIESIS				1 (2%)
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED				1
AUTO/NECROPSY/NO HISTO	1			
AUTOLYSIS/NO NECROPSY			1	2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX C

ANALYSIS OF BIS(2-CHLORO-1-METHYLETHYL) ETHER

APPENDIX C

Analysis of Bis(2-chloro-1-methylethyl) ether

Midwest Research Institute

A. Elemental Analysis

Element:		C	H	Cl
Theory:		42.12	7.07	41.45
Found:	Lot No. 7	41.92	6.93	41.78
		41.85	7.05	41.74
	Lot No. PB41576	41.48	6.93	42.67
		41.60	7.09	42.91
	Lot No. I62976	42.14	7.05	41.37
		42.28	7.12	41.43

B. Boiling Point

Literature:		187 to 188°C at 761 mm Hg (Dewael et al., 1930)
Found:	Lot No. 7	181.5 to 184°C at 767 mm Hg
	Lot No. PB41576	184°C at 742.4 mm Hg
	Lot No. I62976	181.8 to 186.6°C at 765 mm Hg

C. Vapor-Phase Chromatography

Lot No. 7

System 1:

1. Instrument: Varian Aerograph 1400
2. Detector: Thermal conductivity
3. Column: Chromosorb 102, 2 mm x 1.8 m
4. Program: 100 to 250°C at 10°C/min
5. Results: Major peak and seven impurities

Peak	Retention Time*	Area*
1	0.01	<0.1
2	0.11	0.3
3	0.21	<0.1
4	0.40	<0.1
5	0.42	<0.1
6	0.75	0.4
7	0.96	6.5
8(major)	1.00(13.0 min)	100

System 2:

1. Instrument: Tracor MT 220
2. Detector: Flame ionization
3. Column: 3% Dexsil 400, 2 mm x 1.8 m
4. Program: 50°C, 14 min: 50-200°C at 10°C/min
5. Results: Major peak and four impurities

Peak	Retention Time*	Area*
1	0.32	1.1
2	0.40	0.8
3	0.76	0.2
4(major)	1.00(5.0 min)	100
5	2.76	0.04

Lot No. PB41576

System 1:

Instrument: Tracor MT 220
Detector: Flame ionization
Inlet temperature: 200°C
Detector temperature: 260°C
Column: 10% Carbowax 20 M on 80/100 Chromosorb W AW, 1.8 m x 4 mm ID, glass
Oven temperature program: 5 min at 75°C, then 75 to 200°C at 10°C/min
Results: Major peak and 23 impurities

* Retention times and areas normalized to major peak.

Peak	Retention Time (min)	Retention Time (Relative to Bis(2-chloro-1-methylethyl) ether)	Relative Areas (Compared to Major Peak)
1	0.9	0.08	trace, < 0.01
2	1.0	0.09	trace, < 0.01
3	1.1	0.10	trace, < 0.01
4	1.8	0.16	trace, < 0.01
5	2.4	0.21	trace, < 0.01
6	2.8	0.24	0.3
7	3.0	0.26	shoulder, < 0.03
8	3.3	0.29	0.03
9	4.8	0.43	0.04
10	6.0	0.58	0.4
11	8.4	0.74	0.6
12	9.4	0.83	0.2
13	9.6	0.84	shoulder
14	10.2	0.90	trace, < 0.01
15	10.8	0.95	shoulder
16	11.4	1.00	100
17	12.4	1.09	0.04
18	12.6	1.11	0.02
19	13.2	1.16	shoulder
20	13.4	1.18	0.8
21	14.2	1.26	2.0
22	14.9	1.30	0.03
23	15.2	1.34	0.04
24	15.6	1.37	0.3

System 2:

Instrument: Tracor MT 220
Detector: Flame ionization
Inlet temperature: 200°C
Detector temperature: 270°C
Column: Chromosorb 102, 100/120, 1.8 m x 4 mm ID, glass
Oven temperature program: 100 to 200°C at 10°C/min
Results: Major peak and nine impurities

Peak	Retention Time (min)	Retention Time (Relative to Bis(2-chloro-1-methylethyl) ether)	Relative Areas (Compared to Major Peak)
1	3.1	0.09	0.001
2	3.9	0.12	0.001
3	6.2	0.19	0.006
4	9.6	0.29	0.002
5	10.7	0.32	0.3
6	11.0	0.33	0.3
7	18.8	0.56	1.0
8	20.3	0.61	1.4
9	22.4	0.68	0.6
10	33.3	1.00	100

System 3:

Instrument: Varian Aerograph 2400
Detector: Thermal conductivity
Inlet temperature: 200°C
Detector temperature: 210°C
Column: Chromosorb 102, 100/120, 1.8 m x 2 mm ID, stainless steel
Oven temperature program: 100 to 250°C at 10°C/min
Results: Major peak and 20 impurities

Peak	Retention Time (min)	Retention Time (Relative to Bis(2-chloro-1-methylethyl) ether)	Relative Areas (Compared to Major Peak)
1	0.3	0.01	trace, <0.01
2	0.5	0.02	trace, <0.01
3	1.3	0.05	0.12
4	2.6	0.10	trace, <0.01
5	3.6	0.15	trace, <0.01
6	7.8	0.33	0.02
7	8.9	0.38	trace, <0.01
8	9.8	0.41	trace, <0.01
9	13.1	0.55	trace, <0.01
10	14.2	0.60	0.06
11	15.4	0.64	0.02
12	16.2	0.68	0.39
13	16.8	0.70	0.19
14	18.4	0.77	trace, <0.01
15	19.0	0.80	trace, <0.01

Peak	Retention Time (min)	Retention Time (Relative to Bis(2-chloro-1-methylethyl) ether)	Relative Areas (Compared to Major Peak)
(continued)			
16	20.0	0.84	trace, <0.01
17	20.8	0.87	0.03
18	22.1	0.92	3.0
19	22.8	0.95	2.7
20	23.3	0.98	2.0
21	23.8	1.00	100

Lot No. I62976

System 1:

Instrument: Tracor MT 220
Detector: Flame ionization
Inlet temperature: 225°C
Detector temperature: 310°C
Column: 10% Carbowax 20 M-TPA on 80/100 Chromosorb W AW, 1.8 m x 4 mm I.D., glass
Oven temperature program: 5 min at 75°C, then 75 to 200°C at 10°C/min
Results: Major peak and 32 impurities. One of these has an area of 0.89% of the major peak, one 0.20, one 0.19, and one 0.18% of the major peak. The others individually constitute < 0.1% of the major peak and total 0.4% of the major peak.

Peak	Retention Time (min)	Retention Time (Relative to Bis(2-chloro-1-methylethyl) ether)	Relative Areas (Compared to Major Peak)
1	0.51	0.046	0.0001
2	0.56	0.052	0.0005
3	0.86	0.078	0.003
4	1.0	0.096	0.003
5	1.2	0.11	0.0006
6	1.8	0.16	0.0007
7	2.1	0.19	0.009
8	2.3	0.21	0.005
9	4.8	0.44	0.19

Peak	Retention Time (min)	Retention Time (Relative to Bis(2-chloro-1-methylethyl) ether)	Relative Areas (Compared to Major Peak)
(continued)			
10	6.6	0.60	0.0005
11	7.2	0.66	0.008
12	7.5	0.68	shoulder, 0.006-0.02
13	7.7	0.70	0.18
14	8.0	0.74	shoulder, 0.0006-0.004
15	8.2	0.76	0.0005
16	8.6	0.78	0.0005
17	8.8	0.81	0.05
18	9.0	0.82	shoulder, 0.0002-0.006
19	9.5	0.88	0.001
20	9.8	0.90	0.0008
21	10.9	1.00	100
22	11.5	1.06	0.89
23	12.3	1.12	0.02
24	12.4	1.14	0.03
25	12.6	1.16	0.002
26	13.0	1.20	0.05
27	13.5	1.24	0.01
28	13.9	1.28	0.005
29	14.3	1.32	0.20
30	14.6	1.34	shoulder, 0.002-0.009
31	14.8	1.36	0.03
32	15.2	1.39	0.02
33	15.6	1.44	0.06

System 2:

Instrument: Tracor MT 220
Detector: Flame ionization
Inlet temperature: 225°C
Detector temperature: 310°C
Column: 20% SP 2100/0.1% Carbowax 1500 on 100/120 Supelcaport, 1.8 m x 4 mm I.D., glass
Oven temperature program: 5 min at 75°C, then 75 to 200°C at 10°C/min
Results: Major peak and 32 impurities. One of these has an area 0.94% of the major peak, one 0.18, one 0.16 and one 0.15% of the major peak. The others individually constitute < 0.1% of the major peak and total < 0.5% of the major peak.

Peak	Retention Time (min)	Retention Time (Relative to Bis(2-chloro-1-methylethyl) ether)	Relative Areas (Compared to Major Peak)
1	0.66	0.05	0.0002
2	0.91	0.06	0.0004
3	1.6	0.12	0.002
4	2.2	0.16	0.003
5	2.7	0.20	0.001
6	3.4	0.25	0.004
7	4.1	0.30	0.18
8	4.8	0.36	0.15
9	5.8	0.42	0.03
10	7.6	0.55	0.001
11	8.0	0.58	0.001
12	8.6	0.62	0.02
13	9.2	0.68	0.0002
14	9.4	0.70	0.004
15	10.2	0.75	0.08
16	10.6	0.78	0.16
17	11.0	0.80	0.03
18	11.2	0.82	shoulder 0.002-0.001
19	11.4	0.83	0.009
20	11.8	0.86	0.008
21	12.1	0.88	0.94
22	12.4	0.90	0.02
23	12.8	0.94	0.04
24	13.7	1.00	100
25	14.2	1.04	0.03
26	14.6	1.06	0.04
27	14.8	1.08	shoulder 0.002-0.004
28	15.0	1.10	0.008
29	15.2	1.11	0.02
30	15.4	1.12	0.008
31	15.8	1.16	0.01
32	16.1	1.18	0.001
33	16.5	1.20	0.05

D. Spectral Data

1. Infrared: All lots gave infrared absorption spectra (figures 3 to 5) which were consistent with spectra given in the literature (Sadtler Standard Spectra)

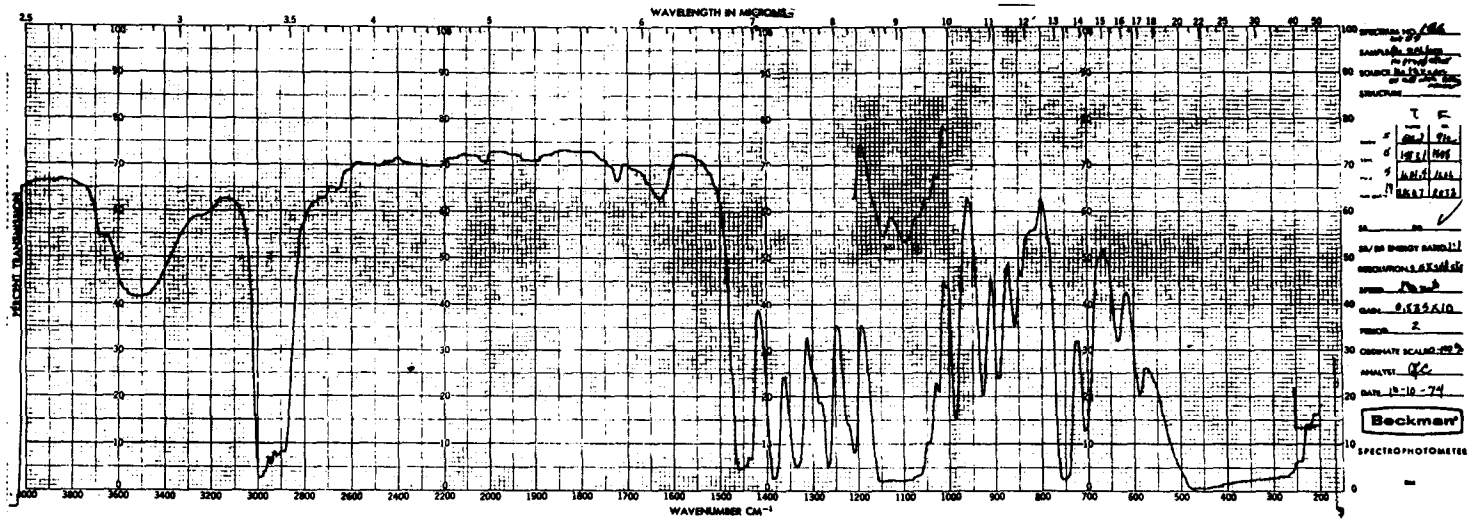

Figure 3. Infrared Absorption Spectrum of Bis(2-chloro-1-methylethyl) ether, Lot No. 7

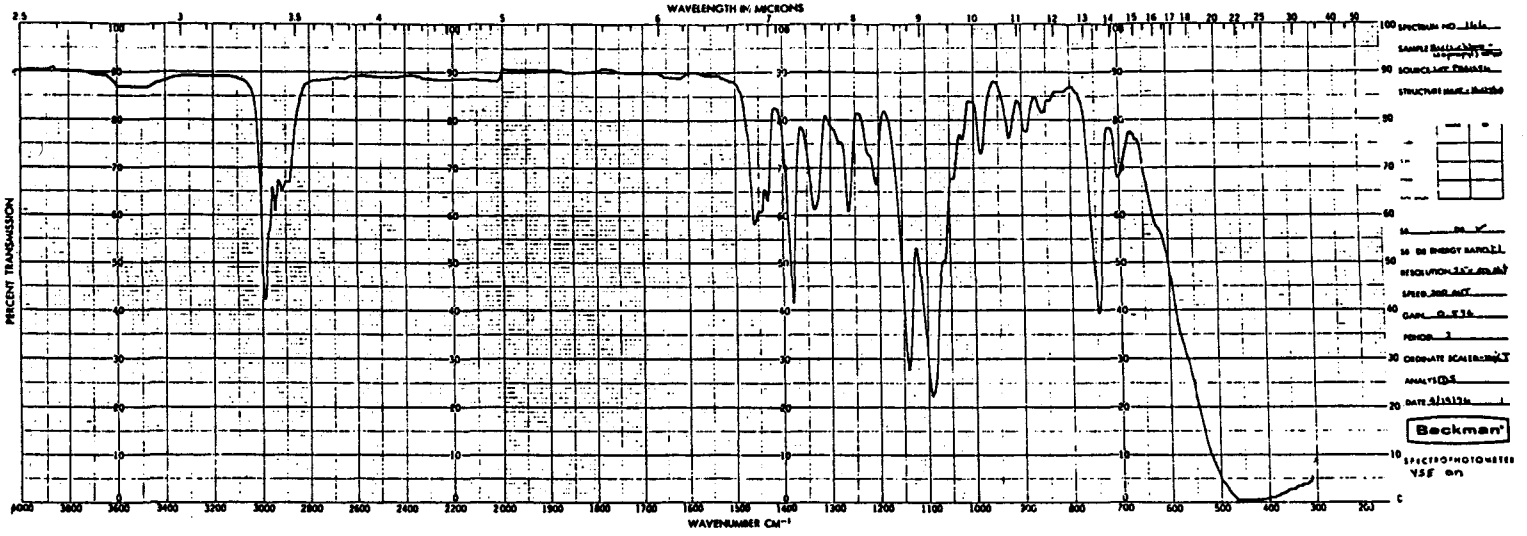

Figure 4. Infrared Absorption Spectrum of Bis(2-chloro-1-methylethyl) ether, Lot No. PB 41576

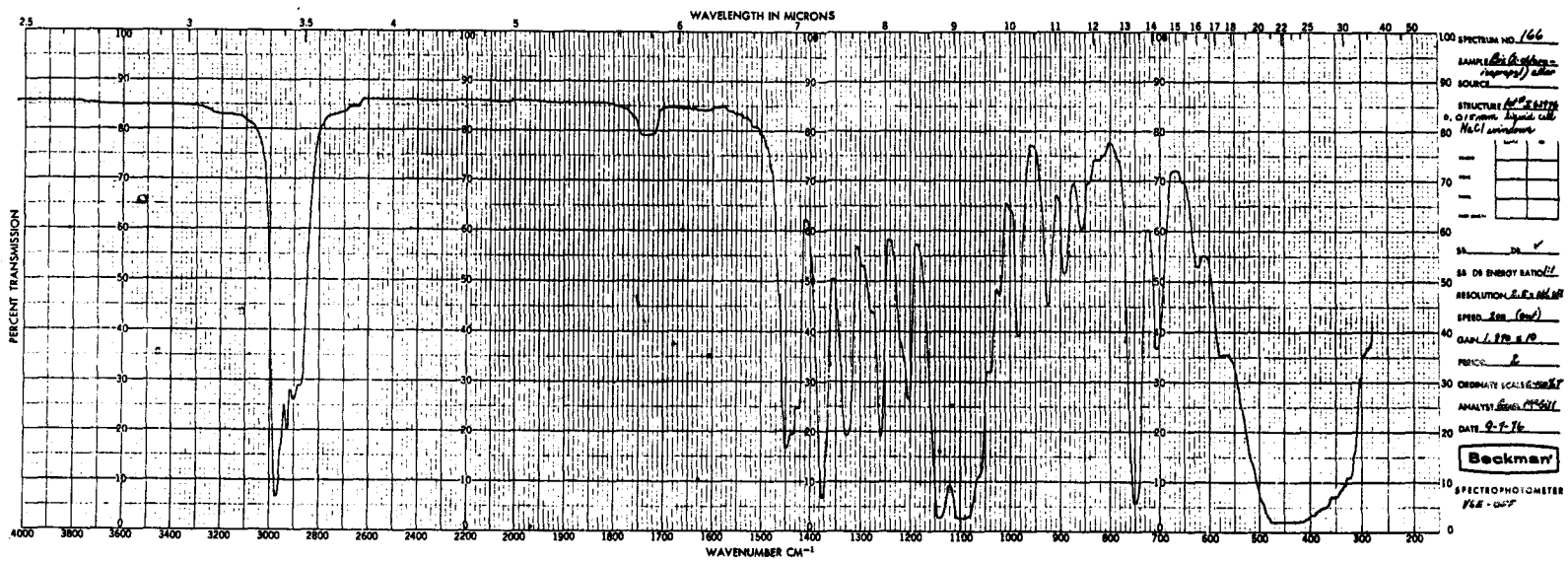

Figure 5. Infrared Absorption Spectrum of Bis(2-chloro-1-methylethyl) ether, Lot No. I62976

2. Nuclear Magnetic Resonance: The nuclear magnetic resonance spectra of all three lots (figures 6 to 8) contained 2 methyl resonances (at 1.19 and 1.46 ppm). One resonance (1.19 ppm) agrees with the structure, and the second (1.46 ppm) agrees with the resonance for a methyl group next to a carbon bonded to one proton and one chlorine atom. The integration ratios indicated that these were present in a ratio of 80:20. It was postulated at the time of analysis that if the isomers were randomly distributed among the possible combinations, the samples contained 65% bis(2-chloro-1-methylethyl) ether, 4% bis(2-chloro-n-propyl) ether, and 31% mixed ethers. No reference spectrum was found in the literature.

E. Special Analysis

Additional analysis on Lot No. I62976 by vapor-phase chromatography and gas chromatography/mass spectrometry analysis after completion of the bioassay indicated that this lot contained 69.4% bis(2-chloro-1-methylethyl) ether, 2.1% bis(2-chloro-n-propyl) ether, and 28.5% mixed ether.

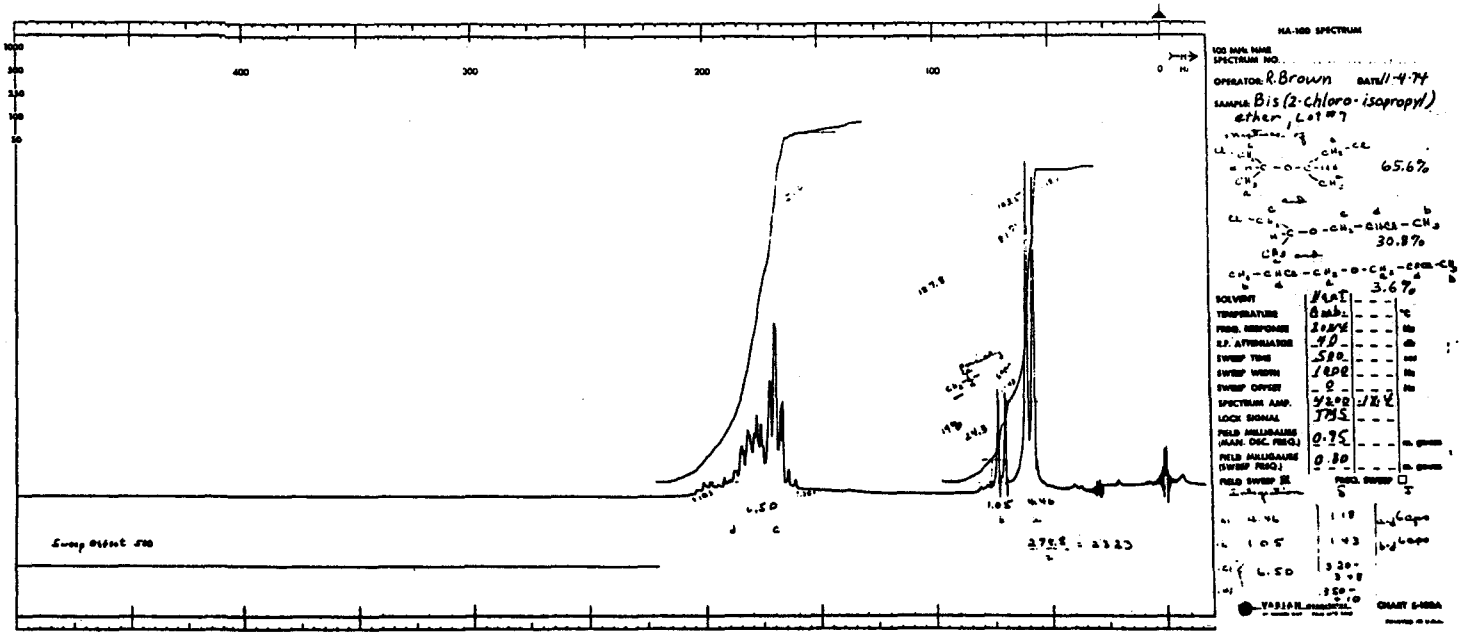

Figure 6. Nuclear Magnetic Resonance Spectrum of Bis(2-chloro-1-methylethyl) ether, Lot No. 7

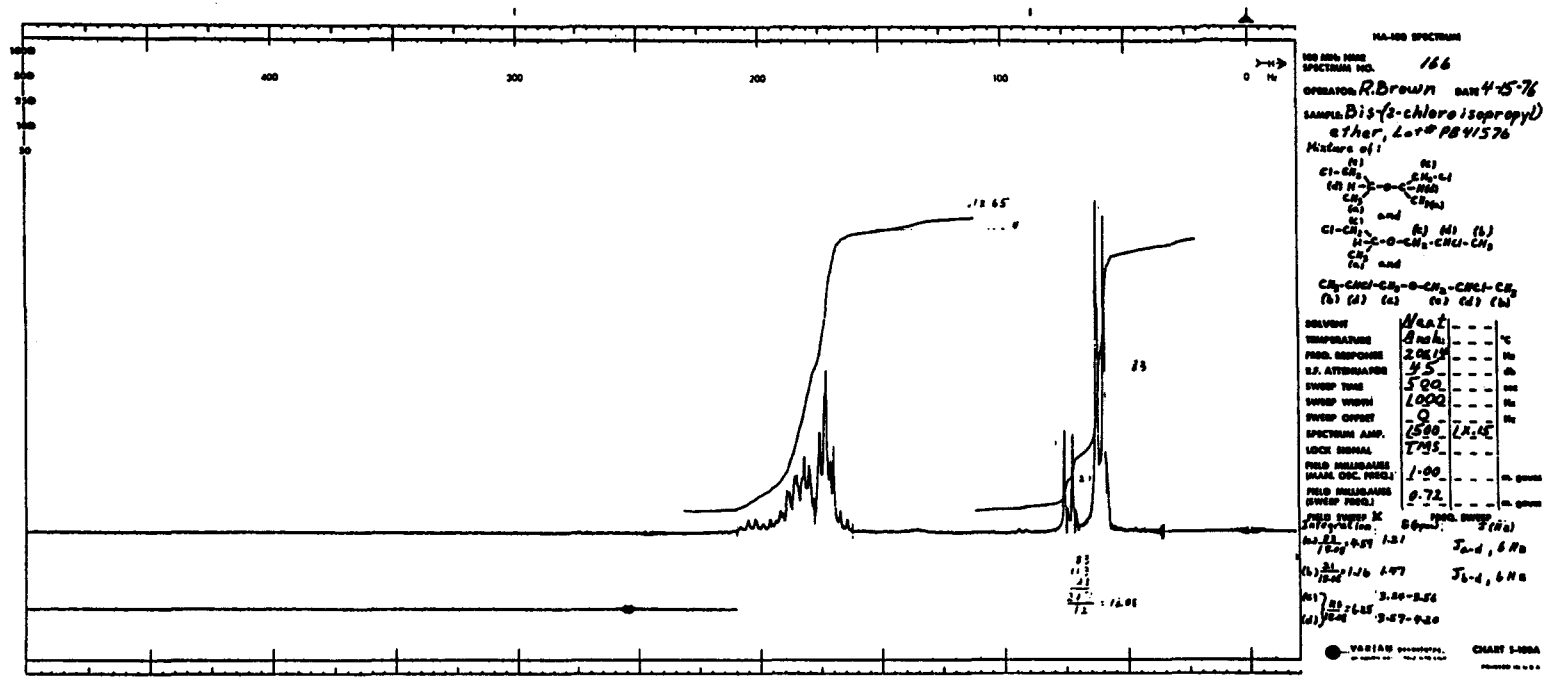

Figure 7. Nuclear Magnetic Resonance Spectrum of Bis(2-chloro-1-methylethyl) ether, Lot No. PB41576

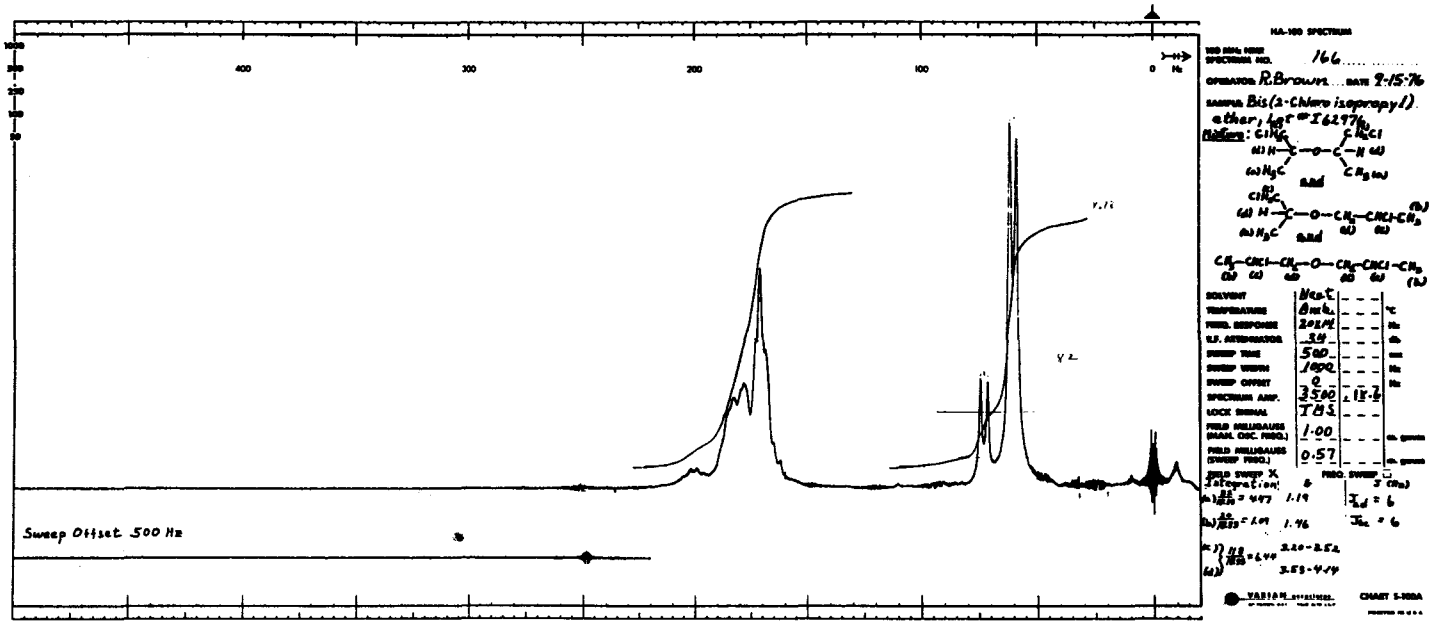

Figure 8. Nuclear Magnetic Resonance Spectrum of Bis(2-chloro-1-methylethyl) ether, Lot No. I62976

Review of the Bioassay of Bis(2-chloro-1-methylethyl)ether* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

May 1, 1979

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute of the Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Bis(2-chloro-1-methylethyl)ether.

The primary reviewer for the report on the bioassay of Bis(2-chloro-1-methylethyl)ether said that the compound was not carcinogenic in treated Fischer 344 rats, under the conditions of test. After briefly commenting on the experimental design, he noted a significant increased incidence of esophageal hyperkeratosis among high dose treated animals of both sexes and an increased incidence of esophageal acanthosis among high dose treated female rats. The maximum tolerated dose may have been exceeded, as indicated by the poor survival rate in the high dose treatment groups of both sexes. Although he agreed with the conclusions given in the report, the primary reviewer noted that the assessment of the carcinogenicity of Bis(2-chloro-1-methylethyl)ether was limited in that it had been tested in only a single species.

The secondary reviewer pointed out that only 69 per cent of the tested material was Bis(2-chloro-1-methylethyl)ether; the remainder being comprised of different isomers. He recommended that this fact be reflected in the title of the report. He also commented on the fact that the test material was obtained from three different sources and that the tumor incidence was lower in treated animals than controls. The latter may be attributed to inadequate sampling of histopathological materials or weight loss among treated animals.

It was moved that the report on the bioassay of Bis(2-chloro-1-methylethyl)ether be accepted with the limitations noted in the reviewers' critiques. The motion was seconded and approved unanimously.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
David B. Clayson, University of Nebraska Medical Center
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Sheldon Samuels, AFL-CIO
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
Kenneth Wilcox, Michigan State Health Department

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or for other reasons. Thus, certain comments and criticisms reflected in the review may no longer be applicable.

☆U.S. GOVERNMENT PRINTING OFFICE: 1979—281-217:3217

NIH Publication No. 79-1747