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12/12/84

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

004128

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

December 12, 1984

Caswell # 586

SUBJECT: NALED RS - Company Response August 1, 1984 to DCI, under Accession No.'s 254215 through 254228 (14 Volumes). EPA 239-1633.

TO: William Miller, PM -16
Registration Divison (TS-767c)

FROM: Irving Mauer
Toxicology Branch/Hazard Evaluation Division (TS-769c)

THRU: Jane E. Harris, Section Head (VI)
Toxicology Branch/HED (TS-769c)

REGISTRANT: Chevron Chemical Co., Richmond CA

ACTION REQUESTED: (660) Review and evaluate the following studies identified as DATA GAPS in the NALED REGISTRATION STANDARD (issued June, 1983):

1. DIBROM Chronic Oral Toxicity/Carcinogenicity Study in Rats. Bio-Research Labs. Ltd Project 9394, June 7, 1984. S-1802. Volumes I through VII.

2. Lifetime Oral Carcinogenicity Study in Mice. IRDC. March 19, 1984. S-1664. Volumes I through 4.

3. Pilot Evaluation of CHEVRON Naled Technical in the Mouse Somatic Cell Mutation Assay. Amended Report. Litton Bionetics, Project NO. 20994, June, 1984. S-2139.

4. Evaluation of CHEVRON Naled Technical/DIBROM in the Mouse Somatic Cell Mutation Assay. Litton Bionetics. Project No. 20994, June, 1984. S-2038.

TB EVALUATION (see attached DATA REVIEWS):

Study	CORE GRADE	DEFICIENCIES
1	Chronic, SUPPLEMENTARY Oncogenicity, SUPPLEMENTARY	1. No rational given for gavage administration. 2. No evidence the HDT approached an MTD. 3. No individual clinical data. 4. Insufficient gross pathology. 5. Unexplained increased mortality in controls.
2	SUPPLEMENTARY	1. No rational given for gavage administration. 2. No evidence the HDT approached the MTD. 3. No explanation for excessive mortality in controls. 4. Study was not lifetime, since terminated at 89 weeks.

1837

STUDY	CORE GRADE	DEFICIENCIES
3/4	ACCEPTABLE	(None)

12-18-84

TOXICOLOGY BRANCH: DATA REVIEW

CHEMICAL: Naled

Caswell: 586 004128
EPA Chem.: #034401

STUDY TYPE: Mutagenicity: Mouse Spot Test (Russell)

CITATION: Evaluation of Chevron Naled Technical/Dibrom® In the Mouse Somatic Cell Mutation Assay.

ACCESSION NO./MRID No.: 254216/NA

SPONSOR/TESTING LAB.: Chevron Chemical Company, Agricultural Chemicals Division, 940 Hensley Street, Richmond, California 94804/Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20895.

STUDY No./DATE: LBI 20994/June, 1984

TEST MATERIAL: Naled tech (SX-1397), 92.5% a.i., suspended in 0.5% sodium carboxymethyl cellulose (CMC) for oral administration.

PROCEDURES: Acceptable as given in the attached M&M from the Final Report (Procedures, pp. 7-11). Briefly, groups of plugged (presumably pregnant) C57Bl/6 females previously mated to T-strain males were orally gavaged on 4 gestation days (D 8-1/2to D 12-1/2) with test substance at 0 (CMC control), 3, 20 or 150 mg/kg/day, or injected once i.p. with ethylnitrosourea (ENU, 50 mg/kg on D 10-1/2), and litters scored for coat color mutations ("spots") according to referenced criteria on post-partum days 12 and 28.

Results and Conclusions: In contrast to positive results for ENU-treated litters, the data presented for naled treatment showed no significant increase over CMC controls in the number of pups from naled-treated dams with recessive coat spots (RCS), the only presumed indicator of mutation sampled, even at doses producing maternal lethality (15% at the HDT) and/or toxicity (decreased BW at 12/2 da. in plugged females that did not litter), as well as decreased pup survival during lactation at the HDT (see tabulation). A significant increase in pups with midventral spots (WMVS) was observed in the high-dose group (150 mg/kg/day naled) scored on day 12, but not in survivors on day 28. WMVS, however, record melanocyte toxicity, and are not necessarily indicative of mutation potential; further, these spots vary spontaneously with female age, time of year and diet.

This reviewer concurs with the author's conclusions that, under the conditions of this study, naled exhibited no potential to induce coat-color spots presumably indicative of mutational events consisting of intragenic base-pair changes, deletions and somatic crossing-over.

TB EVALUATION: ACCEPTABLE.

12-11-84

Effect of Naled in the Mouse Spot Test*

Study Group (mg/kg)	No. of Females	Maternal Deaths	Number Litters	Number pups: Born	Number pups: Dead	Number survived: Day 12	Number survived: Day 28	No. of pups with Spots*** on: Day 12 RCS	Day 12 WMVS	Day 28 RCS	Day 28 WMVS
Control (CMC)	120	0	38	252	20	168	160	3	0	5	2
Naled: 3	120	1	36	225	20	133	125	1	5	3	7
20	146	1	35	223	21	128	122	2	1	4	1
150	181	27**	37	252	34	113**	111**	3	5**	7	5
ENU: 50	124	1	34	242	24	132**	125	39**	9**	39**	11**
TOTALS	691	30	180	1194	119	674	643	48	20	58	26

*Summarized from Final Report, Tables 1 to 15.

**Significantly different from CMC-control by Fisher's Exact Test ($p \leq 0.05$).

***RCS, recessive coat color spots; WMVS, white mid-ventral spots.

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SAFETY NO. 7909A

00412

FINAL REPORT

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EVALUATION
OF
CHEVRON NALED TECHNICAL/DIBROM®
IN THE MOUSE SOMATIC
CELL MUTATION ASSAY

721.11 Dibrom S-2033 EC-59A

SUBMITTED TO

CHEVRON CHEMICAL COMPANY
AGRICULTURAL CHEMICALS DIVISION
940 HENSLEY STREET
RICHMOND, CALIFORNIA 94804

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20895

PROJECT NO. 20994

5

JUNE 1984

Bionetics
14911-A/B

Naled toxicology review

Page _____ is not included in this copy.

Pages 6 through 10 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

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EPA: 68-01-6561
TASK: 82
December 6, 1984

DATA EVALUATION RECORD

NALED (DIBROM)

Chronic Toxicity/Oncogenicity Study in Rats

CITATION: Batham, P., Osborne, B.E., Bier, C., et al. Dibrom chronic oral toxicity/carcinogenicity study in rats. (Unpublished report No. 9394, prepared by Bio-Research Laboratories, LTD. Quebec, Canada, for Chevron Chemical (Canada) Limited, Burlington, Ontario, dated June 7, 1984.)

REVIEWED BY:

Nicolas P. Hajjar, Ph.D.
Senior Scientist
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: December 6, 1984

William McLellan, Ph.D.
Senior Scientist
Dynamac Corporation

Signature: William L. McLellan
Date: 6 December 1984

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____
Date: _____

APPROVED BY:

Irving Mauer, Ph.D.
EPA Scientist

Signature: [illegible]
Date: 12-12-84

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DATA EVALUATION RECORD

STUDY TYPE: Chronic toxicity/oncogenicity study in rats.

CITATION: Batham, P., Osborne, B.E., Bier, C., et al. Dibrom chronic oral toxicity/carcinogenicity study in rats. (Unpublished report No. 9394, prepared by Bio-Research Laboratories, LTD. Quebec, Canada, for Chevron Chemical (Canada) Limited, Burlington, Ontario, dated June 7, 1984.)

ACCESSION NUMBER: 254217 - 254224.

LABORATORY: Bio-Research Laboratories Ltd., Senneville, Quebec, Canada.

QUALITY ASSURANCE STATEMENT: Present, signed, and dated June 8, 1984.

TEST MATERIAL: The test material was identified as dibrom technical (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) an organophosphate insecticide, with a purity of 91%. Dibrom was a yellow viscous liquid from Lot # SX-1278.

PROCEDURES:

1. Male and female Sprague-Dawley CD rats were obtained from Charles River Breeding Laboratories, Portage, Michigan. The animals were 28 days old and were allowed a 13-day acclimation period prior to treatment. Male and female rats were housed individually in wire-mesh bottom cages, in rooms maintained at 22.6 ± 3.3° C, and 61 ± 19.8% relative humidity with a 12 hour light/dark cycle.

2. All animals were weighed prior to dosing and randomly assigned to 4 groups with initial mean body weights approximately equalized. The group size of 65 animals/sex was used initially to assure sufficient animals on study in the event of early deaths. The number of animals was reduced to 55 animals/sex/group after 8 weeks of treatment. An additional group of 30 males and 30 females was assigned to a pre-treatment health screen group. Rats were permanently identified by an individual ear notch and tail tattoo.

3. The test material was administered by gavage; the decision to use gavage rather than mixing the test material in the diet was not explained. The dosing solutions for each group were prepared daily. A 1% suspension of the test material in 0.5% aqueous sodium carboxymethylcellulose (vehicle) was mixed in a blender at high speed

for a minimum of 5 minutes. The suspension was then serially diluted with carboxymethylcellulose to concentrations of 0.1, 0.02, and 0.002% and then mixed in a blender for 5 minutes. The appropriate concentration was administered by gavage at a constant dose volume of 10 ml/kg body weight. The control group received the vehicle at 10 ml/kg body weight. The dosages received by the animals were 0, 0.2, 2, or 10 mg/kg/day and were based on data obtained from a 4-week preliminary study. However, the data for the 4-week study were not presented.

4. The homogeneity of the test material in 0.5% aqueous sodium carboxymethylcellulose was determined by gas-liquid chromatography weekly for the first 4 weeks of study and monthly thereafter. For the first 8 months, the analyses were conducted at the testing facility, whereas subsequent determinations were performed by the sponsor.

   Clinical observation for individual animals were performed daily while detailed examinations which included palpation for masses were performed weekly. Ophthalmologic examinations were performed prior to study initiation and during weeks 52 and 102 of the study.

   Individual animal body weights were determined twice prior to study initiation and weekly thereafter. The individual food consumption for animals was recorded weekly.

   Prior to initiation of the study, 10 male and 10 female rats from the pretreatment health screen group were used for hematology and cholinesterase determinations, clinical chemistry evaluation, and urinalysis.

   Hematology and cholinesterase determinations were performed during weeks 25, 51, 77, 102 (females), and 104 (males) on blood samples from 10 males and 10 females randomly selected at each interval from each group. The following hematology analyses were performed: red blood cell count, hematocrit, hemoglobin, reticulocyte count, Wintrobe's constants (MCH, MCV, MCHC), total and differential white blood cells count, and platelet count. The cholinesterase determinations were performed on plasma and red blood cells. Brain cholinesterase was performed only at final sacrifice on 10 animals/sex/group. The entire left hemisphere of the brain was utilized for analysis.

   Clinical chemistry and urinalysis were performed during weeks 25, 51, 77, and 102/104 on blood samples and urine from an additional number of 10 malesand 10 females randomly selected at each interval from each group. Urine samples were collected from individual animals placed in metabolism cages for 16-18hours overnight. The following clinical chemistry analyses were performed: blood urea nitrogen, total protein, globulin, lactic dehydrogenase, alkaline phosphatase, SGPT, SGOT, total and direct bilirubin, inorganic phosphorus, glucose, albumin, chloride, sodium, potassium, creatinine, cholesterol, calcium, and A/G ratio (calculated). The following parameters for urinalysis were performed: volume, color/character, specific gravity, protein, pH, ketones, urobilinogen, glucose, bilirubin, occult blood, and microscopy on urinary sediment.

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All animals that died during the study or were killed at the end of the replacement period (8 wks), and those killed at termination (females at week 102 and males at week 104) were subjected to gross examination including examination of the following: external surface, all orifices, cranial cavity, carcass, external and sectioned surfaces of the brain and spinal cord, thoracic, abdominal and pelvic cavities and their viscera, cervical tissues, and organs. Animals that were killed during study or at termination were sacrificed by carbon dioxide asphyxiation and exsanguination. The following organs from final sacrifice animal were weighed: brain with entire brainstem, heart, liver, kidneys, testes, and ovaries. Organ-to-body weight ratios were also calculated.

All animals that died during the study or sacrificed at termination had the following tissues examined microscopically: all gross lesions (including normal contiguous tissue), liver, kidneys, heart, lungs with mainstem bronchi, pituitary, thyroid with parathyroids (unless grossly abnormal parathyroids were reported or if found in the routine section), adrenals, and gonads. All rats in the control and 10 mg/kg/day groups that died during study or sacrificed at termination, and 15 male and 15 female rats from the low- and mid-dose groups that were sacrificed at termination had the following tissues examined:

bone (sternum with marrow)	lymph nodes	spleen
brain (fore, mid and hind)	mammary gland (inguinal)	stomach
cecum	optic nerves	thymus (if present)
cervix	pancreas	trachea
colon	prostate	urinary bladder
duodenum	salivary gland (mandibular)	uterus
epididymides	sciatic nerve	
esophagus	skeletal muscle	
eyes	skin	
ileum	spinal cord (cervical and lumbar)	
jejunum		

Partial histopathologic examinations (of the liver, kidneys, heart, lungs, pituitary, thyroid, adrenals, gonads and all gross lesions) were performed on the remaining animals of the low- and mid-dose groups.

5. Statistical Methods: The group mean values were statistically analyzed using Dunnett's "t" test. Microscopic findings were analyzed by the proposed method of Gart et al.[1] Life table adjusted analysis was performed according to Tarone.[2]

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[1]Gart, J.J., et al. 1979. J. Nat'l. Cancer Inst. 62:957-974.
[2]Tarone, R.E. 1975. Biometrika 62:679-682.

RESULTS:

Clinical Signs: Four females from the high-dose group showed slight tremors on single occasions during weeks 36, 37/38 and 43/45. These episodes lasted for approximately one minute, except for one rat which showed slight lethargy for 5 minutes. No further signs were observed after the tremors had ceased. No tremors were noted for the male groups or the low- and mid-dose female groups.

Clinical observations noted in rats included: patchy alopecia, ocular or nasal discharge, staining of the fur, swollen pinnae, salivation, and varying degrees of respiratory distress. Based on the frequency, duration, severity, and time of onset none of these observations were considered by the authors as compound related. However, individual data were not presented. Approximately 20-25% of all male rats in all groups exhibited recurrent swelling in the perigenital region associated with ulceration and scab formation. These were considered to be preputial gland abscesses.

Ophthalmologic examination at week 52 showed no eye abnormalities for any dose group that were compound related (CBI Report, page 24 and Appendix 7). However, at the preterminal examination (week 102), the incidence of corneal opacity was slightly, but signficantly higher in the high-dose males (18%) when compared to controls (12%) (CBI Report, page 24 and Appendix 7). The majority of rats at weeks 7 and 8 and again during weeks 73 and 74 showed signs of sialodacryoadenitis virus. These rats recovered without further residual effects.

Mortalities: There were no differences in survival among compound treated and control animals throughout the study (Table 1). However, because of the abnormally high incidence of mortality for this strain in the female control group towards the end of the study, all of the females were sacrificed after 102 weeks of dosing.

TABLE 1. Percent Survival of Rats Dosed with Naled

Group/Dose (mg/kg/day) Week:	Percent Survival 52	76	102[a]/104
	Males		
Control	93 (51)[b]	82 (45)	38 (21)
0.2	93 (51)	87 (48)	45 (25)
2.0	96 (53)	84 (46)	44 (24)
10.0	100 (55)	89 (49)	49 (27)
	Females		
Control	98 (54)	82 (45)	22 (12)
0.2	96 (53)	85 (47)	47 (26)
2.0	98 (54)	87 (48)	51 (28)
10.0	96 (53)	80 (44)	35 (19)

[a] Female rats were sacrificed at week 102.
[b] Number of surviving rats.

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Body Weight: The mean body weights of females receiving 10 mg/kg/day (the reported HDT) were significantly higher than the control group starting with week 34 of the study (Table 2). The mean body weights of the mid-dose females were also higher than the controls during weeks 34-92 but the differences were not statistically significant. There were no other differences noted in the body weights of treated animals when compared to controls.

Food Consumption: The mean food consumption of females receiving 10 mg/kg/day were significantly higher than the control group during weeks 51-85. The mean food consumption of mid-dose females was also slightly higher than the control group during the same period. There were no other differences noted in food consumption between dosed and control groups (CBI Report, Table 6). Concomitant with the sialodacryoadenitis virus infection during weeks 73-74, food consumption in all groups decreased.

Hematology: There was a dose- and time-related decrease in erythrocyte count, hemoglobin, and hematocrit values in dosed males and females at weeks 26, 52, and 78 of the study. Decreased erythrocyte counts were observed in all dosed males at week 26 (Table 3). In addition, decreased hemoglobin hematocrit and platelet counts were observed in the mid and high dose males. In females, decreased erythrocyte counts were observed in the mid- and high-dose groups and decreased hemoglobin and hematocrit values in high-dose animals at week 26. These changes were also accompanied by corresponding increases in MCV, MCH, or MCHC. These differences were less pronounced during weeks 52 and 78 of the study, and by termination all values were similar among dosed and control animals (Table 3).

Clinical Chemistry: There was a significant increase in glucose levels in high-dose males on months 18 and 24 when compared to control values (Table 4). In females, glucose levels were significantly higher than control values in the high-dose group at month 18 and the mid- and high-dose groups at month 24. There was also a time- and dose-related effect on lactate dehydrogenase activities in male and female animals at months 12, 18, and 24 of the study, when compared to control (Table 4). Other isolated differences were noted in dosed rats, but none was dose- or time-related.

Urinalysis: Individual urinalysis data for months 6, 12, 18, and 24 of the study did not reveal a compound-related effect in dosed animals (CBI Report, Appendix 11).

Cholinesterase Activity: Plasma and red blood cell cholinesterase inhibition was observed in the mid- and high-dose male and female rats at months 6, 12, 18, and 24 of the study (Table, 5 and 6). However, at month 24, statistically significant differences were only noted for plasma of high-dose males and red blood cells of high-dose females. Brain cholinesterase inhibition was also observed in the mid- and high-dose male and female rats at final sacrifice (Table 6).

Gross Observations: Summary data for gross findings were not presented. Examination of individual animal data by our reviewer did not reveal any differences in findings among dosed and control rats. However, only those

TABLE 2. Mean Body Weights of Rats Dosed with Naled

Group/Dose (mg/kg/day)	Body Weight (g)[a] Week: 0	26	52	78	102/104[b]
Males					
Control	183 ± 9.6	624 ± 65.9	726 ± 88.4	810 ± 113.2	781 ± 100.3
0.2	181 ± 9.8	616 ± 70.7	732 ± 97.7	834 ± 113.6	799 ± 139.4
2.0	181 ± 9.1	616 ± 55.0	722 ± 77.8	811 ± 106.3	764 ± 192.3
10.0	184 ± 6.8	616 ± 66.4	720 ± 93.9	806 ± 119.6	768 ± 129.5
Females					
Control	141 ± 8.4	331 ± 35.9	407 ± 59.0	467 ± 84.2	527 ± 145.5
0.2	143 ± 8.7	337 ± 39.0	410 ± 63.1	466 ± 82.3	497 ± 99.6
2.0	142 ± 8.5	342 ± 40.3	415 ± 65.0	493 ± 95.0	503 ± 125.4
10.0	145 ± 8.8	349 ± 45.6	444 ± 72.6*	553 ± 113.0**	563 ± 81.8

[a] Mean value and standard deviation.
[b] Female rats were sacrificed at week 102.
* Significantly different from control value $p < 0.05$.
** Significantly different from control value $p < 0.01$.

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TABLE 3. Mean Hematology Values for Rats Dosed with Naled[a]

Interval/ Parameter	Group/ Dose: Sex:	Control M	Control F	0.2 M	0.2 F	2 M	2 F	10 M	10 F
Week 26									
RBC		7.99 ± 0.4	6.77 ± 0.2	7.45 ± 0.5**	6.60 ± 0.2	6.97 ± 0.2**	6.26 ± 0.3**	7.07 ± 0.3**	6.16 ± 0.3**
HGB		14.9 ± 0.4	14.4 ± 0.7	14.9 ± 0.6	14.3 ± 0.6	14.3 ± 0.5**	14.1 ± 0.6	14.3 ± 0.5**	13.6 ± 0.5*
HCT		39.5 ± 1.8	36.0 ± 1.2	37.9 ± 2.2	35.4 ± 1.5	35.4 ± 1.2**	35.0 ± 0.9	36.9 ± 1.6**	34.0 ± 0.8**
PLT		478 ± 114	679 ± 237	525 ± 125	714 ± 203	723 ± 265**	763 ± 266	869 ± 128**	933 ± 144
Week 51/52									
RBC		8.05 ± 0.42	6.87 ± 0.39	7.81 ± 0.37	6.87 ± 0.34	7.07 ± 0.45**	6.39 ± 0.16**	7.06 ± 0.30**	6.05 ± 0.43**
HGB		14.9 ± 0.55	14.0 ± 0.85	14.9 ± 0.58	14.3 ± 0.52	14.1 ± 0.50*	14.1 ± 0.44	14.1 ± 0.50*	13.6 ± 0.80
HCT		41.7 ± 1.89	38.8 ± 2.15	40.8 ± 1.87	38.5 ± 1.78	36.6 ± 1.96**	36.1 ± 0.99**	37.0 ± 1.89**	34.6 ± 1.90**
PLT		587 ± 227.1	793 ± 232.0	509 ± 293.5	892 ± 243.9	656 ± 206.8	920 ± 242.2	827 ± 300*	1036 ± 310.3
Week 77/78									
RBC		7.41 ± 0.59	6.72 ± 0.33	7.13 ± 0.85	6.58 ± 0.56	6.70 ± 0.58*	6.45 ± 0.30	6.55 ± 0.40*	6.09 ± 0.33**
HGB		14.1 ± 1.11	14.1 ± 0.54	14.3 ± 1.96	14.1 ± 1.16	14.1 ± 1.14	14.2 ± 0.48	13.6 ± 1.04	13.7 ± 0.62
HCT		38.3 ± 2.91	37.1 ± 2.02	37.9 ± 4.89	36.5 ± 3.10	36.2 ± 2.74	36.4 ± 1.08	35.4 ± 2.41	35.2 ± 2.04
Week 101/104									
RBC		5.96 ± 1.36	6.04 ± 0.80	6.57 ± 0.73	6.66 ± 0.67	6.16 ± 0.55	6.64 ± 0.12	5.76 ± 1.05	5.96 ± 0.43
HGB		11.8 ± 2.08	12.8 ± 1.21	13.5 ± 1.47	14.2 ± 1.23*	12.9 ± 1.61	14.3 ± 0.65**	11.7 ± 2.29	13.5 ± 0.70
HCT		32.7 ± 5.96	35.2 ± 3.22	35.4 ± 3.78	37.6 ± 2.96	34.1 ± 3.11	37.5 ± 1.58	31.8 ± 4.87	36.1 ± 1.20

[a] Mean value from 10 animals/group and standard deviations.
* Significantly different from control value $p < 0.05$.
** Significantly different from control value $p < 0.01$.

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TABLE 4. Mean Blood Chemistry Values for Rats Dosed with Naled[a]

Interval/ Parameter	Group/ Dose: Sex:	Control M	Control F	0.2 M	0.2 F	2 M	2 F	10 M	10 F
Week 52									
Glucose		118 ± 13.33	120 ± 11.33	122 ± 8.82	114 ± 9.47	120 ± 11.90	114 ± 10.59	130 ± 10.11	118 ± 13.56
LDH		1467 ± 586.72	1247 ± 606.26	995 ± 449.62	925 ± 317.85	1136 ± 424.74	1170 ± 587.52	522 ± 502.24*	1325 ± 859.28
Week 77/78									
Glucose		110 ± 16.02	101 ± 16.32	130 ± 14.51**	100 ± 12.54	119 ± 11.59	115 ± 16.30	135 ± 14.35**	116 ± 9.38*
LDH		1833 ± 595	1449 ± 521.93	936 ± 421.22**	1277 ± 603.93	876 ± 585.29**	919 ± 578.26	541 ± 406.32**	611 ± 259.51**
Week 101/104									
Glucose		75 ± 18.10	83 ± 11.52	93 ± 31.96	94 ± 6.61	89 ± 32.13	103 ± 20.37*	111 ± 15.40*	105 ± 22.17*
LDH		2431 ± 1024.6	1467 ± 532.87	1772 ± 540.16	1196 ± 442.95	1318 ± 761.12**	1266 ± 410.07	892 ± 507.49**	726 ± 576.36[b]

[a]Mean value from 10 animals/group and standard deviations.
[b]Statistical analyses conducted by these reviewers using the ANOVA with Dunnett's test for multiple comparison.
*Significantly different from control value $p < .05$.
**Significantly different from control value $p < .01$.

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TABLE 5. Mean Plasma Cholinesterase Activities (μmoles/min/ml) in Rats Dosed With Naled

Group/Dose (mg/kg/day) Week:	Plasma 25/26	51/52	77/78	102/104
Males				
Control	0.6561	1.0417	1.2241	1.0708
0.2	0.6718 (102)[a]	0.9001 (86)	0.9711* (79)	1.2206 (114)
2.0	0.4606** (70)	0.5833** (56)	0.7124** (58)	0.7628 (71)
10.0	0.2994** (46)	0.4082** (39)	0.4269** (35)	0.4780* (45)
Females				
Control	3.0995	2.7435	2.7074	1.9825
0.2	3.4793 (112)	3.2368 (118)	2.7404 (101)	2.7019* (136)
2.0	2.3606* (76)	2.1611* (79)	1.8704** (69)	1.5117 (76)
10.0	1.3523** (44)	1.2044** (44)	1.0847** (40)	0.9998 (50)

[a] Percent of control value.

* Significantly different from control value $p < 0.05$.
** Significantly different from control value $p < 0.01$.

TABLE 6. Mean Red Blood Cell (μmoles/min/ml) and Brain (μmoles/min/g) Cholinesterase Activites in Rats Dosed With Naled

Group/Dose (mg/kg/day) Week:	Red Blood Cells 25/26	51/52	77/78	102/104	Brain 102/104
Males					
Control	3.0259	2.7900	2.2007	2.0237	7.178
0.2	2.9801 (98)[a]	2.6940 (97)	2.2[illegible]0* (104)	2.2399 (111)	6.957 (97)
2.0	2.0172** (67)	2.4232* (87)	2.0499 (93)	1.9516 (96)	5.489** (76)
10.0	1.7617** (58)	2.1942** (79)	1.5915** (72)	1.5061 (74)	2.899** (40)
Females					
Control	2.1351	2.6460	2.3645	2.1743	7.197
2.0	2.0499 (96)	2.6459 (100)	2.0761 (88)	2.1044 (97)	7.126 (99)
2.0	1.8470* (87)	2.2660** (86)	1.9582** (83)	1.5980 (73)	5.436** (76)
10.0	1.9647 (92)	1.9648** (74)	1.9845** (84)	1.4472** (67)	2.960** (41)

[a] Percent of control value.

* Significantly different from control value $p < 0.05$.
** Significantly different from control value $p < 0.01$.

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tissues and organs showing abnormalities were presented. In addition, individual tissue masses observed during necropsy were in agreement with histopathology findings.

Organ Weights: There were no changes noted in the mean organ weights and organ-to-body weight ratios of dosed animals when compared to controls, except for an increase in mean kidney weights of male rats receiving the low-dose (CBI Report, Tables 14 and 15).

Histopathologic Examinations: Complete histopathologic examinations were performed on all control and high-dose animals and for 15 male and 15 female rats from the low- and mid-dose groups; major tissues and gross lesions were examined from the remaining animals in the low- and mid-dose groups. The neoplasms observed most frequently at final sacrifice are summarized in Table 7. The incidence of all neoplastic lesions observed in dosed animals was comparable to those observed in controls. There was a slight increase in the incidence of mammary gland fibroadenomas in high-dose females, but the increase was not statistically significant.

Non-neoplastic lesions observed most frequently are summarized in Table 8. There was an increased incidence of subcapsular and cortical vacuolated cells of the adrenals in high-dose males and blood-filled cysts in the adrenals of mid- and high-dose females. There was an increased incidence of hepatocyte vacuolation and focal eosinophilic cells of the liver in high-dose female as compared to controls. In males, the incidences of spongiosis hepatis, focal cell necrosis, and bile duct hyperplasia were observed more frequently in high-dose animals when compared to controls but were not statistically significant. A significant increase in the incidence of pituitary hyperplasia was also observed in the high-dose males. There were no other treatment related changes noted in dosed animals.

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DISCUSSION:

The authors stated that "administration of dibrom did not elicit apparent consistent changes in the various clinical pathology parameters evaluated during the conduct of this study." The only treatment-related changes observed were cholinesterase inhibition in mid- and high-dose animals. Indeed the various effects observed except cholinesterase inhibition were not severe and were probably not related to compound administration. There were no adverse effects on body weight, food consumption, organ weights, gross pathology, and neoplastic lesions. The histologic observations noted in high-dose males (adrenals and pituitary) and females (adrenals and liver) are not particularly severe and their toxicological significance in relation to compound administration is not clear. However, there are several questions with regards to the cholinesterase data obtained and whether the high-dose used approached an MTD. Slight tremors observed in only 4 high-dose females during weeks 36, 37/38 and 43/45 were mild and apparently not life threatening, suggesting that cholinesterase inhibition may not be of clinical consequence. In addition, cholinesterase inhibition

TABLE 7. Summary of Neoplastic Lesions Most Frequently Observed in Rats Dosed with Naled[a]

Lesion	Group/ Dose: Sex:	Control M	Control F	0.2 M	0.2 F	2 M	2 F	10 M	10 F
Adrenals	N[b]	55	55	55	55	55	55	55	55
adenoma		5	0	2	3	2	3	6	0
Brain	N	55	55	15	15	15	15	55	55
glioma		1	2	1	0	0	1	1	0
Liver	N	51	54	53	55	55	53	50	51
carcinoma		0	0	3	0	0	0	1	1
Lungs	N	38	44	31	40	36	43	38	38
tumor		1	0	0	1	2	0	1	1
Mammary Glands	N	45	54	20	41	17	35	49	55
adenoma		0	6	0	5	0	1	0	5
fibroadenoma		1	13	0	17	0	13	0	21
adenocarcinoma		0	3	0	2	1	2	2	1
Pancreas	N	55	55	16	17	19	17	55	55
islet cell adenoma		3	1	3	0	3	0	3	0
Pituitary	N	54	54	55	55	55	55	54	55
tumor		30	40	18	34	24	34	25	39
Skin	N	55	55	28	16	27	17	55	55
fibrosarcoma		1	0	3	0	1	1	3	0
fibroma		0	0	4	0	0	0	2	0
Spleen	N	55	55	20	16	21	15	55	55
histiocytic sarcoma		2	0	2	0	0	0	1	0
leukemia		0	0	1	0	2	0	0	0
Thyroid	N	55	55	53	54	55	54	55	55
parafollicular cell		1	1	1	1	3	0	0	0

[a]Compiled by the reviewers; isolated incidences of other neoplasms were also noted, but none were significantly higher in dosed animals when compared to controls.

[b]Number of tissues examined.

TABLE 8. Summary of Non-Neoplastic Lesions Most Frequently Observed in Rats Dosed with Kalec[a]

Lesion	Group/ Dose: Sex:	Control M	Control F	0.2 M	0.2 F	2 M	2 F	10 M	10 F
Adrenals	N[b]	55	55	55	55	55	55	55	55
focal vacuolation		15	15	23	5	19	8	25*c	13
hyperplasia		9	2	11	1	8	1	10	2
cysts (blood)		3	34	7	42	13	45	6	47*c
Epididymides	N	55		55		55		55	
no sperm		12		9		11		12	
Heart	N	55	55	55	55	55	55	55	55
fibrosis		21	7	34	11	26	15	24	7
Kidneys	N	55	55	55	55	55	55	55	55
glomerulonephritis		48	20	49	24	47	34	45	25
mineralization		0	3	0	9	1	6	0	3
Liver	N	51	54	53	55	55	53	50	51
vacuolation		11	8	10	14	15	14	8	20*c
spongiosis		10	0	7	1	11	0	16	1
focal clear cells		2	1	2	1	2	1	6	6*
necrosis		1	2	1	5	4	2	4	3
atrophy of cords		1	2	2	0	4	1	5	1
bile duct hyperplasia		13	8	19	8	16	7	19	12
Lungs	N	38	44	31	40	36	43	38	38
histiocytosis		6	4	3	4	8	3	6	4
Mammary glands	N	45	54	20	41	17	35	49	55
cystic ducts		10	17	7	19	7	15	3	16
Ovaries	N		55		52		53		55
cyst			7		4		7		6
Pituitary	N	54	54	55	55	55	55	54	55
hyperplasia		4	8	7	10	11	16	12*c	12
Prostate	N	55		18		18		55	
exudate		34		10		9		32	
Skin	N	55	55	28	16	27	17	55	55
dermatitis		15	2	7	1	14	0	16	2
Spleen	N	55	55	20	16	21	15	55	55
hematopoiesis		10	9	3	1	4	1	9	6
hemosiderosis		1	10	0	0	1	0	2	14
Testes	N	55		55		55		55	
degeneration (epithelial)	12		13		17		12		
periarteritis nodosa		8		11		9		6	
Thyroid	N	55	55	53	54	55	54	55	55
hyperplasia		5	1	3	2	4	2	3	2
Uterus	N		55		22		20		55
endometrial hyperplasia			14		9		11		17

[a]Compiled by the reviewers.

[b]Number of tissues examined.

[c]Statistical analysis conducted by reviewer using the Fisher's exact test (for direct intergroup comparisons).

*Significantly different from control value $p < 0.05$.

observed during weeks 26, 52, and 78 may in part be due to changes noted in the hematology of the dosed animals particularly the decreased RBC count. Inhibition was less pronounced at the end of the study, when the hematology values in dosed animals were similar to control. The lower hematology values in control animals at the end of the study are probably age-related. Finally, the high-dose (10 mg/kg/day) is only 2-4% of the LD_{50} value reported in the available literature* and only 1/5-1/7.5 that used in a chronic study with mice (IRDC #415-038).

In addition, the following deficiencies were also noted in the study: individual clinical observation data were not present; there were no interim (52 week) sacrifice animals; the thoroughness of gross pathologic examination could not be determined; brain cholinesterase activity was determined from one hemisphere and not the whole brain; it was not specified whether only one or both adrenals of each animal were examined, although both the cortex and medulla were apparently examined. There was no apparent explanation present for the increased mortality of female control animals at the end of the study.

CONCLUSIONS:

Under the conditions of this 2-year study, dibrom was not oncogenic when administered by gavage to Sprague-Dawley rats at levels up to 10 mg/kg/day. Brain cholinesterase was inhibited approximately 24 and 60 percent in both male and female rats receiving dibrom at dose levels of 2 and 10 mg/kg/day, respectively. There was a slight inhibition of red cell cholinesterase and moderate inhibition of plasma cholinesterase at 10 mg/kg/day. There were no other effects noted for clinical signs, body weight, food consumption, organ weights and gross and histopathologic findings that could be clearly related to compound administration. However, due to some limitation in the cholinesterase data and the fact that the high dose used was only 2-4% percent of the reported LD_{50} values and only 1/5 - 1/7.5 of that used in a companion study with mice, it is not evident that the high-dose approached a MTD.

CORE CLASSIFICATION:

Chronic toxicity, supplementary:

1. It was not evident that the high-dose approached a MTD.
2. Plasma, RBC and brain cholinesterase activities were apparently depressed but no accompanying clinical consequences were observed.
3. There were no interim sacrifice animals for brain cholinesterase determinations.

Oncogenicity, supplementary:

1. It was not evident that the high-dose approached a MTD.

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*Toxicology Chapter of the Naled Registration Standard.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

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EPA: 68-01-6561
TASK: 82
November 20, 1984

(see p. 11)

DATA EVALUATION RECORD

DIBROM

Oncogenicity Study in Mice

CITATION: Brewer, L. Dibrom: Lifetime oral carcinogenicity study in mice. (Unpublished report No. 415-038 prepared by International Research and Development Corporation for Chevron Chemical Company, dated March 19, 1984.)

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Date: 12-12-84

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DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity study in mice.

CITATION: Brewer, L. Dibrom: Lifetime oral carcinogenicity study in mice. (Unpublished report No. 415-038 prepared by International Research and Development Corporation for Chevron Chemical Company, dated March 19, 1984.)

ACCESSION NUMBER: 254225 - 254228.

LABORATORY: International Research and Development Corporation, Mattawan, Michigan 49071.

QUALITY ASSURANCE STATEMENT: Present, signed, and dated March 16, 1984.

TEST MATERIAL: Dibrom Technical (Naled, SX1203), 1,2-Dibromo-2,2-dichloroethyl dimethyl phosphate, was the test material. Formulation CC 10262, described as a solid white waxy substance, 92.7% pure, was used in the first year of the study; a formulation dated 7/10/81, described as a clear, slightly viscous liquid when refrigerated, was used thereafter. The purity of the second formulation was not stated.

PROCEDURES:

1. Twenty-eight day old CD-1 mice (source: Charles River Breeding Laboratories, Portage, Michigan) acclimated to laboratory conditions for 14 days, were used in the study. At study initiation, the mean weights of males and females were 27 and 21 g, respectively. Dibrom was administered at levels of 3, 15, and 75 mg/kg/day by gavage to groups of 60 male and 60 female mice. The rationale for the gavage route was not given. The control group (60 mice/sex) received 10 ml/kg of the vehicle solution (0.5% carboxymethyl cellulose). At study week 27 the high dosage level was reduced to 50 mg/kg/day due to excessive mortality in the group. The mice were individually housed in wire mesh cages in a temperature and humidity controlled environment on a 12 hour light/dark cycle. Feed and water were provided *ad libitum*.

2. A one percent base suspension of Dibrom was prepared by mixing 1 g of the test material with a 0.5% aqueous suspension of sodium carboxymethyl cellulose and stirring at high speed for 15 minutes. The base suspension was then volumetrically diluted with additional carboxymethyl cellulose to the appropriate dose levels. Samples of each dose level were analyzed for homogeneity by the testing facility. Samples

were analyzed weekly for the first four weeks of the study, and monthly thereafter. Fresh dosing suspensions were prepared daily.

3. Clinical observations for toxicity were made daily and detailed observations were performed weekly. Body weights and food consumption were measured weekly for the first 14 weeks and once every two weeks thereafter.

   Hematology was conducted on 10 males and 10 females in each group at 52 and 89 weeks of the study. The hematologic parameters measured were: erythrocyte count, hemoglobin, hematocrit, red cell indices, total leukocyte count, differential leukocyte count, platelet count, and reticulocyte count (if signs of anemia were present).

4. Postmortem examinations were conducted on all animals that died or were sacrificed moribund, on 10 males and 10 females from each group at the week 52 interim sacrifice and on all animals that survived to study termination, at week 89. Fasted body and organ weights were determined at the interim sacrifice and at study termination. The following organs were weighed: brain (with stem), heart, liver and gallbladder, kidneys, testes and ovaries.

   Hematoxylin-eosin stained slides were prepared for all animals and the following tissues were examined histologically:

All gross lesions	Bone and marrow (sterum)
Eyes (with Harderia gland)	Skeletal muscle (thigh)
Brain (fore, mid, hind-brain)	Stomach
Pituitary	Pancreas
Major salivary gland (mandibular)	Spleen
Thyroids (with parathyroid)	Small intestine (duodenum, jejunum, ileum)
Trachea	Large intestine (colon, cecum)
Esophagus	Lymph nodes (mediastinal, mesenteric)
Thymus	Kidneys
Heart	Gonads (testes/ovaries)
Lung/bronchi	Prostate/corpus and cervix uteri
Spinal cord (thoracic, lumbar)	Skin
Sciatic nerve	Blood smear
Mammary gland	Skull[a]
Liver	
Urinary bladder	

[a]Examined for 10 randomly selected mice/sex/group at termination to include tongue, nasal cavity, nasopharynx and middle ear. All other skulls preserved in formalin.

5. Body weights, food consumption, hematological parameters, organ weight and relative organ weight data were analyzed by the investigator, using one-way analysis of variance. Homogeneity of variance was determined with Bartlett's test. Equal or unequal variance was analyzed according to the t-test, using Dunnett's multiple comparison table to determine significance of the differences. Monotonic trends for body weight data were analyzed using a t-statistic based on the contrast as described by Neter and Wasserman[1].

RESULTS:

Diet Analyses: Mean analyzed doses throughout the study were 97±22, 90±15, and 98±20 percent of target values at nominal dosage levels of 3, 15, and 50 mg/kg/day.

Observations and Mortality: It was reported that animals treated with Dibrom showed no change in appearance or behavior when compared to the control groups (CBI; Table 2 and Appendix C). During the first 26 weeks of the study, increased mortality was observed in the highest dosage (75 mg/kg/day) groups. At week 26, the percent mortality was 10 and 13% for the male and female mice receiving 75 mg/kg/day as compared to 1.7% for the male and female control mice (Table 1).

Mortality was similar in all groups from weeks 26-89 with the exception that control females had a lower percent survival than females receiving Dibrom. Eighteen month (78 week) survival ranged from 72 to 88 percent in males and 64-78 percent in females (Table 1).

Body Weight and Food Consumption: Table 2 presents the mean body weights for male mice at selected intervals during the study. There was a statistically significant decreasing trend in mean body weights of males in the mid and high dose groups when compared to controls. However, the body weights were only 3-5% lower than in controls and at study termination mean body weights were similar (Table 2).

In females, the mean body weights were slightly lower at 50 mg/kg/day than in controls at sporadic intervals throughout the study. (CBI: Tables 3 and 4).

The average food consumption for all groups of mice was similar throughout the study (CBI; p. 12 and Table 5).

Hematology: There were no compound-related effects on the hematology parameters at 52 or 89 weeks of study (CBI: Table 6).

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[1]Neter, J. and Wasserman, W. Applied Linear Statistical Models, Richard D. Irwin Inc., Homewood, IL, 1974.

TABLE 1. Cumulative Incidence of Mortality and Percent Survival in Mice Treated with Dibrom[a]

Dosage level (mg/kg/day)	Treatment Week 13	26[d]	52	78	89
Males					
0	0(100)[b]	1(98)	4(93)	6(88)	11(78)
3	3(95)	3(95)	6(90)	8(84)	12(76)
15	0(100)	0(100)	7(88)	9(82)	12(76)
75[c]	4(93)	6(90)	10(83)	14(72)	19(62)
Females					
0	1(98)	1(98)	8(87)	18(64)	24(52)
3	4(93)	4(93)	6(90)	8(84)	14(72)
15	0(100)	0(100)	5(92)	11(78)	16(68)
75[c]	7(88)	8(87)	11(82)	16(68)	18(64)

[a]Sixty animals/group/sex were initiated on the study and at week 52, 10 animals/group/sex were sacrificed.

[b]Percent survival is given in parenthesis.

[c]This dosage level was reduced to 50 mg/kg/day at week 27.

[d]There was no statistically significant trend in incidence of mortality in either males or females when analyzed by our reviewers using linear trend in porportions.

TABLE 2. Mean Body Weights (g) at Selected Intervals of Male Mice Treated with Dibrom

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Week	Dose group (mg/kg/day) 0	3	15	75[b]	Trend[a]
13	34 ± 3.0	33 ± 2.9	32 ± 2.9**	33 ± 2.2**	+
26	36 ± 3.5	35 ± 2.6	34 ± 3.4**	35 ± 3.2*	+
52	38 ± 3.8	37 ± 3.1	36 ± 3.5**	37 ± 3.4	+
80	38 ± 3.3	37 ± 3.1	36 ± 4.0**	36 ± 3.8*	+
89	38 ± 3.6	37 ± 3.4	37 ± 3.4	37 ± 3.8	-
No. of weeks significant at $p < 0.01$		—	18	14	

*Significantly different from control value ($p < 0.05$).
**Significantly different from control value ($p < 0.01$).
[a] Significant trend (+).
[b] This dosage level was reduced to 50 mg/kg/day at week 27.

Organ Weights: Organ weights were similar among all groups at the 12-month interim sacrifice. At the 21-month terminal sacrifice, the kidney-to-body weight ratio was slightly higher in males receiving 50 mg/kg/day as compared to controls but there was no significant effect at this dosage level on absolute kidney weights. In females, the mean absolute weight of livers was higher than controls at 3 and 15 mg/kg/day but not higher at 75 mg/kg/day; the relative liver-to-body weight ratio of females was significantly increased at 75 mg/kg/day but not at lower dosages when compared to controls (Table 3). There were no corresponding histologic lesions in liver and kidneys.

Gross Pathology: There were no dose-related macroscopic changes in treated groups at the 52 or 89 week sacrifices when compared to the corresponding control groups. Macroscopic findings that occurred were reported to be common to the strain and age of the mouse used on the study; these included a fairly high incidence of cystic lesions in the ovaries and uteri of all groups of females (CBI; Tables 7-10).

Histopathology: Table 4 summarizes neoplastic lesions in male and female mice. There did not appear to be any dose-related increase in tumors at any site. Table 5 summarizes the non-neoplastic lesions. There were occasional changes observed with higher incidence in dosed groups than in control groups but for most of the lesions there were no dose-related trends (adrenal degeneration, dacryoadenitis of the Harderian gland, acute

TABLE 3. Mean Weights in Grams of Kidneys and Livers at Terminal Sacrifice and Weights Relative to Body Weights[a] For Mice Treated with Dibrom

Organ		Dose Level (mg/kg/day) 0	3	15	75[b]
Kidney	Males	0.85(2.32)[a]	0.83(2.28)	0.83(2.33)	0.91(2.56*)
	Females	0.59(1.89)	0.64*(1.97)	0.65(1.95)	0.58(1.91)
Liver	Females	1.91(6.07)	2.07*(6.37)	2.14*(6.50)	2.04(6.66*)

[a]The values in parentheses are expressed as percent of body weight.
[b]This dose level was reduced to 50 mg/kg/day at week 27.
*Statistically different from control value at $p < 0.05$.

hepatitis, adenomatous hyperplasia of the liver, and mild axonal degeneration of the sciatic nerve). Renal amyloidosis was increased in all groups of dosed males as compared to controls but it was reported that the control incidence was below the "historical incidence of >50% in aging rats." There was an increase in galactoceles of the mammary gland in dosed females but no apparent dose-related trend. Bronchitis was more frequent in dosed males than in controls but the lesion was described as mild or trace, and was not life threatening.

There were no unusual lesions in animals that died in the first year of the study or were sacrificed at 12 months (CBI; Tables 13-16).

TABLE 4. Neoplastic Lesions in Mice Receiving Dibrom by Gavage

Organ/Finding	Dose Level (mg/kg/day) Males 0	Males 3	Males 15	Males 75[a]	Females 0	Females 3	Females 15	Females 75[a]
Adrenal	(46)[b]	(43)	(42)	(37)	(40)	(43)	(45)	(39)
Adenoma	1	0	0	0	3	0	0	0
Harderian gland	(46)	(44)	(42)	(40)	(42)	(43)	(45)	(39)
Adenoma	4	1	3	2	0	1	2	1
Liver	(46)	(44)	(43)	(40)	(42)	(44)	(45)	(39)
Hemangioma	0	1	1	0	1	1	0	1
Adenoma	4	4	4	4	0	0	1	0
Carcinoma	0	1	0	0	0	0	0	0
Lung[c]	(46)	(44)	(43)	(40)	(42)	(44)	(45)	(39)
Adenoma	14	11	14	8	3	7	11*	4
Carcinoma	1	0	0	0	0	0	0	0
Lymph node								
Hemangioma	0	0	0	0	0	0	1	1
Lymphoreticular	–	–	–	–	(81)	(3)	(6)	(2)
Lymphoma, histocyte	2	4	1	0	5	1	2	2
Lymphoma, lymphocytic	0	0	0	0	3	0	1	0
Lymphoma, mixed	0	1	2	1	0	5	4	0
Total lymphomas	2	5	3	1	8	6	7	2
Mammary gland					(36)	(44)	(42)	(36)
Adenoma					0	0	1	0
Carcinoma					0	0	1	0
Ovary								
Adenoma or cystadenoma					0	1	0	1
Parathyroid	(18)	(17)	(14)	(19)	(17)	(19)	(15)	(16)
Adenoma	1	0	0	0	1	0	0	0
Pituitary	(44)	(43)	(42)	(38)	(41)	(43)	(45)	(39)
Adenoma	0	0	0	0	0	1	1	0
Spleen	(46)	(44)	(43)	(40)				
Lymphoret	1	4	2	1				
Skin								
Carcinoma					(42)	(44)	(45)	(39)
Uterus					(42)	(44)	(45)	(39)
Hemangioma					0	1	2	1
Leiomyoma					5	1	3	1
Leiomyosarcoma					1	2	3	3
Adenocarcinoma					0	1	0	0
Polyp[a]					2	0	1	0

[a]This dosage level was reduced to 50 mg/kg/day at week 27.

[b]The number in parentheses is the number of tissues examined. These did not include animals at interim sacrifice but combines those that died or were sacrificed in extremis between 12-21 months and those at terminal sacrifice (21 months).

[c]These values were derived from Table 16 of the report which listed tumor-bearing animals by number, disposition and date. They differed from the values given in the report's summary table of histologic lesions (12, 10, and 13 adenomas in males receiving 0, 3, and 15 mg/kg/day, respectively. Tumors that occurred before 12 months were censored in the author's summary table.

*Statistically significant at a p value of <0.05 using the Fisher exact test; analysis by our reviewers.

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TABLE 5. Non-Neoplastic Histologic Lesions in Mice Fed Dibromo[a]

Tissue/Finding	Dose Level (mg/kg/day) Males 0	Males 3	Males 15	Males 75[b]	Females 0	Females 3	Females 15	Females 75[b]
Adrenal	(46)[c]	(43)	(42)	(37)	(40)	(43)	(45)	(39)
degeneration	0	5	1	2	7	8	4	0
Harderian gland	(46)	(44)	(42)	(40)	(42)	(43)	(45)	(39)
dacryoadenitis	1	3	1	1	1	0	0	5
Heart	(46)	(44)	(43)	(40	(42)	(44)	(45)	(39)
arteritis	0	2	0	0	0	2	2	0
Kidneys	(46)	(44)	(43)	(40)	(42)	(44)	(45	(39)
amyloidosis	5	15	25	25	29	32	34	31
Liver	(46)	(44)	(43)	(40)	(42)	(44)	(45)	(39)
acute hepatitis	0	3	1	0	3	5	3	0
Lung	(46)	(44)	(43)	(40)	(42)	(44)	(45)	(39)
bronchitis	9	18	16	18	15	17	16	19
adenomatous hyperplasia	6	2	1	8	3	2	0	3
Sciatic nerve	(46)	(44)	(43)	(40)	(42	(44)	(45)	(39)
axonal degeneration, mild	3	0	4	4	4	13	1	7
Mammary gland					(36)	(44)	(42)	(36)
galactocele					9	27	35	19
Ovary					(39)	(44)	(42)	(36)
cyst					27	27	28	17

[a] Only frequently occurring lesions or lesions with an apparent increase in a dosed group compared to controls are included.

[b] This dosage level was reduced to 50/mg/kg/day at week 27.

[c] The number of tissues examined histologically is given in parentheses.

DISCUSSION:

This study was conducted and reported well. Individual and summary data were present and tabulated for all parameters.

No rationale for choice of dosage levels was presented in the report and on the basis of the available data, it is our assessment that the maximum dose to achieve optimum test sensitivity may not have been achieved in this study. The highest dosage tested was 75 mg/kg/day and this dosage was reduced to 50 mg/kg/day at week 27 since the investigators judged that there was excessive mortality at the higher dosage. However, after analysis of the data in Table 1 using linear trend in proportions, we did not observe a statistically significant dose-related trend in the mortality from weeks 1-26. There were very few accidental deaths.

Examination of data on clinical observations of animals receiving 75 mg/kg/day and dying during the first 26 weeks of the study revealed that none of the 6 males that died in this period had tremors but 3 of 8 females that died (at days 4, 60, and 91) had tremors. Table 6 summarizes incidence of observation of tremors. During the first 26 weeks, there was an apparent increase in the incidence of tremors in animals at the high dosage as compared to controls. There were no microscopic lesions in 5/6 males or 8/8 females receiving 75 mg/ kg/day that died between week 1 and 26 of the study. These cholinergic signs may not have been sufficient reason to reduce the dose level.

TABLE 6. Number of Animals Observed with Tremors

	Males/dosage level (mg/kg/day)				Females/dosage level (mg/kg/day)			
	0	3	15	50[a]	0	3	15	50[a]
Weeks 1-26	0	0	0	2	1	1	0	4
Weeks 27-89	3	3	6	5	12	6	11	3
Total	3	3	6	7	13	7	11	7

[a]The dosage level was 75 mg/kg/day for the first 26 weeks and 50 mg/kg/day thereafter.

The report showed that there was "a significant trend towards decreased body weights, primarily in males, with increasing dosage levels". The mean body weight gain during weeks 1 to 26 for males receiving 75 mg/kg/day Dibrom was 7 g compared to 8 g for controls. The mean body weights of males in the high-dose group were statistically significantly lower than controls at 21 of 52 weighing intervals; however, the mean weights were never more than 6% (2 g) lower than controls. At study termination mean

body weights in the high-dose group males were 2.6 percent lower than controls (not statistically significant). Hence, the toxicologic importance of the decreased body weight in high dose males is not apparent.

The toxicologic importance of the dose-related increase in liver-to-body weight ratios in female mice (Table 3) is difficult to assess since there were no cellular histologic changes noted. It is our assessment that the sensitivity of the study to detect an effect on liver would have been improved if the study had extended for 24 months. The study was terminated at 89 weeks when mortality approached 50% in control females.

A significant increase in lung adenomas only in females receiving 15 mg/kg/day when compared to controls was noted; the increase was not dose related (Table 4). These are common tumors in aging mice. The average time to tumor was 87 weeks for this group of females. Spontaneous historical incidence for adenomas of the lungs in CD-1 mice was not provided by the testing laboratory, and this may have been helpful in judging the toxicologic importance of the increased incidence.

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CONCLUSIONS:

Under the conditions of the study, Dibrom was not found to be carcinogenic in Charles River CD-1 mice. There were no dose-related changes in appearance, behavior, food consumption, hematology parameters, organ weights, and macroscopic or microscopic pathology findings. There was a dose-related increase in liver weights in female mice receiving Dibrom when compared to controls, but no corresponding effect in males.

CORE CLASSIFICATION: Supplementary. The deficiencies which made the study inadequate to assess oncogenicity are the following:

1. No rationale was given for the dosage choice.

2. The maximum tolerated dose may not have been approached.

   a. The high-dose was reduced from 75 mg/kg/day to 50 mg/kg/day at week 27 because of reported excessive mortality. However, we did not find a statistical dose-related trend in mortality in the first 26 weeks of the study.

   b. There was a statistically significant dose-related trend for body weight reduction. However, since the decrease compared to controls never exceeded 6 percent it may not have toxicologic meaning.

3. It is the reviewers' opinion that the sensitivity of the test to detect an oncogenic effect would have increased if the assay were terminated at 24 months (104 weeks, or equivalent to "lifetime") rather than at 21 months (89 weeks).

4. No explanation was given for the excessive mortality in control females, warranting termination of the study at 89 weeks.

5. Different batches of technical grade dibrom were used in the study and the purity of the material used in the second year of the study was not provided.