

US EPA ARCHIVE DOCUMENT

3/20/86



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Naled - Review of Data Submitted in Response to Data Call-In, Accession Nos. 257455, 257458, 257459, 257460, 257461, 257462, and 257463

Caswell No. 586

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Handwritten initials and scribbles

TO: William Miller, PM 16
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

and

Gary F. Otakie, PMT 16
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

THRU: Jane E. Harris, Ph.D., Head
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

JEM 3/20/86
Def. 4/15
3/22/86

Registrant: Chevron

Action Requested:

Review and evaluate the following studies submitted in response to the Naled Data Call-In Notice, identified as test data requirements in the Naled Registration Standard:

1. The Acute Dermal Toxicity of Chevron Naled Technical (SX-1397) in Adult Male and Female Rabbits. SCCAL 2293. February 11, 1985, S-2501. Accession No. 257458.

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2. The Acute Inhalation Toxicity of Naled Technical (SX-1554) in Rats. SOCAL 2266. February 19, 1985, S-2453. Accession No. 257453.
3. Pilot Teratology Study in Rabbits with Chevron Naled Technical (SX-1397). SOCAL 2194. January 24, 1985, S-2192. Accession No. 257453.
4. Teratology Study in Rabbits with Chevron Naled Technical (SX-1397). SOCAL 2206. February 28, 1985, S-2193. Accession No. 257453.
5. Mouse Bone Marrow Micronucleus Assay with Chevron Naled Technical (92.0% Purity, SX-1397) SOCAL 2213. November 21, 1984, S-2213. Accession No. 257452.
6. A Pilot Rat Reproduction Study with DIBROM. Final Report. Bio/dynamics, Inc. Project No. 82-2611. March 7, 1983, S-2018. Accession No. 257464.
7. Addendum to Pilot Reproduction Study in Rats with Chevron Naled Technical (SX-1380). Bio/dynamics Project No. 82-2611. Chevron No. S-2018. Dosage Formulation Analyses. September 10, 1982. Accession No. 257456.
8. Two-Generation Reproduction Study in Rats with DIBROM. Project No. 82-2612. Volume I thru V. March 22, 1985, S-2019. Accession No. 257459 to 257463.
9. Addendum to Two-Generation Reproduction Study in Rats with Naled Technical (SX-1397). Chevron No. S-2019. Dosage Formulation Analyses. June 1, 1984. Accession No. 257455.

Toxicology Branch Conclusions:

Toxicology Branch (TB) Data Reviews for studies 1, 2, and 5 (cited above) have already been transmitted to Registration Division (attachments to memorandum: Mauer to Miller, dated December 9, 1985, TB. DOC. No. 004838).

Attached to this memorandum are Data Reviews for studies 3/4 (rabbit teratology) and 6-9 (rat reproduction). In summary, TB's conclusions on these two studies are:

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<u>(3/4) Rabbit Teratology</u>	<u>Core Grade</u>
(Chevron #S-2192/ S-2193)	<u>Supplementary:</u> The highest dose tested did not elicit any maternal or fetal toxicity; methods for fetal visceral/skeletal examination were not cited by reference nor adequately described.
(6-9) <u>Rat Reproduction</u> (Chevron #S-2018/ S-2019)	<u>Minimum.</u>

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

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EPA: 68-02-4225
DYNAMAC NO. 1-39-A3b
January 14, 1986

DATA EVALUATION RECORD

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Teratogenicity Study in Rabbits

STUDY IDENTIFICATION: Fitzgerald, L., Hardy, L., Parker, J., and Richter, W. Teratology study in rabbits with Chevron naled technical (SX-1397) (Unpublished study No. SOCAL 2206 by Chevron Environmental Health Center, Inc., Richmond, CA, for Chevron Chemical Company, Richmond, CA; dated February 28, 1985.) Accession No. 257458.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 1-14-86

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1. CHEMICAL: Naled; 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate; Dibrom.
2. TEST MATERIAL: Chevron naled technical, lot/batch No. SX-1397, was described as a clear, colorless liquid with a specific gravity of 1.97 and a purity of 92.5 percent.
3. STUDY/ACTION TYPE: Teratogenicity study in rabbits.
4. STUDY IDENTIFICATION: Fitzgerald, L., Hardy, L., Parker, J., and Richter, W. Teratology study in rabbits with Chevron naled technical (SX-1397). (Unpublished study No. SOCAL 2206 by Chevron Environmental Health Center, Inc., Richmond, CA, for Chevron Chemical Company, Richmond, CA; dated February 28, 1985.) Accession No. 257458.

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Robin B. Phipps, B.S.
Principal Reviewer
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Date: 01-14-86

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EPA Section Head

Signature: Jane Harris
Date: 1/31/86

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7. CONCLUSIONS:

- A. No significant maternal effects were noted at any dose level used in this study; therefore, 8 mg/kg/day, the highest dose tested, was assessed as the tentative NOEL for maternal toxicity in rabbits. The LOEL for maternal effects was not demonstrated in this study. A definitive assessment of fetal effects was precluded by the inadequate description of methods used for fetal examination.
- B. Core Classification: Due to the absence of maternal effects, even at the highest dosage tested, and to deficiencies in the description of methods used for fetal examinations, this study did not provide sufficient information for our assessment of the teratogenic potential of naled when administered by gavage to pregnant rabbits. Therefore, this study is classified Core Supplementary.

8. RECOMMENDATIONS:

In the event that further work is conducted, the following steps are recommended:

- A. The highest dose level selected for a teratogenicity study should elicit some systemic maternal toxicity.
- B. Methods for fetal visceral and ^{or fetal} skeletal examinations should be cited or described in detail.
- C. Nonparametric methods should be used for statistical analysis of data that does not follow a normal distribution, such as the incidence of fetal abnormalities. Furthermore, the study authors should indicate whether statistics were performed on a per fetus and/or per litter basis.

9. BACKGROUND:

A pilot study of naled (No. SOCAL 2194) was conducted with groups of eight artificially inseminated New Zealand white rabbits. The rabbits were dosed by gavage at dosages of 0.2, 2, 10, or 40 mg/kg/day on days 7 through 19 of gestation. The high-dose level produced death and marked cholinergic signs. At 10 mg/kg/day, marked cholinergic signs, decreased body weight gain, and decreased fetal weights were reported. Results for the 2- and 0.2-mg/kg/day dosages were not reported. Based on these data, 8 mg/kg/day was chosen as the high dose for the definitive study.

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Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOL): (See Appendix A for details.)

- A. Test Material: The test material, technical grade Chevron naled, was described as a clear, colorless liquid with a purity of 92.5 percent.

Dosing suspensions were prepared daily on a weight for weight basis by mixing the test material with the vehicle, aqueous 0.5 percent sodium carboxymethyl cellulose. The high-dose suspension was prepared first and then serially diluted to prepare the mid- and low-dose levels. Final concentrations of the dosing suspensions were 0.04, 0.40, and 1.60 mg/g. Dosing suspensions were analyzed for homogeneity prior to initiation of dosing and weekly during the dosing period.

- B. Test Animals and Test System: Sexually mature male and female New Zealand white rabbits (specific pathogen free) were supplied by Hazleton-Dutchland, Inc., Denver, PA. The animals were acclimated to laboratory conditions for approximately 4 weeks prior to study initiation. Twenty healthy females, approximately 6 months old, were assigned to each of four groups by a computer program for weight randomization. The females were artificially inseminated 4-6 hours after intravenous administration of human chorionic gonadotropin. The day of insemination was designated as day 0 of gestation. Females were dosed by gastric intubation on days 7 through 19 of gestation at dosages of 0 (vehicle only), 0.2, 2.0, or 8.0 mg/kg/day. The dose volume was 5 mL/kg and was adjusted following each body weight measurement. On day 29 of gestation, all females were sacrificed and necropsied, and the fetuses were removed by cesarean section.

- C. Parameters Evaluated: Animals were observed at least once daily during gestation for clinical signs. Individual body weights were recorded on days 0, 7-19, 24, and 29 of gestation. Food consumption was measured daily. At necropsy, maternal gross observations were recorded and abnormal tissues were preserved for possible histological examination. The ovaries and uteri were removed, corpora lutea were counted, and gravid uterine weights were recorded. The number and placement of implantations, resorptions, and live and dead fetuses were recorded. Fetuses were individually identified, weighed, and examined externally. Live fetuses were sacrificed by subcutaneous injection of sodium pentobarbital and dissected for visceral examination, then eviscerated, skinned, and processed for skeletal evaluation.

¹Only items appropriate to this DER have been included.

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12. REPORTED RESULTS:

- A. Test Material: No data on the stability of the test material or the dose suspensions were reported. In general, results of analyses for homogeneity were within ± 10 percent of the target levels.
- B. Maternal Effects: The study authors reported no adverse compound-related effects on maternal clinical signs, body weight (Table 1), food consumption, or gross necropsy findings. Three control females aborted their litters on day 20 or 21 of gestation, and a low-dose female aborted on day 23. Comparable pregnancy rates and mean numbers of corpora lutea, implantations, and resorptions were reported for all groups (Table 2). The authors stated that preimplantation loss was elevated in the dosage groups compared to controls; however, the study authors did not attribute this to dosing because implantation (theoretically) occurs prior to initiation of treatment.
- C. Litter Effects: There were no dead fetuses in any group. Mean litter size and fetal weight were comparable among all groups (Table 2). The fetal incidence of incomplete ossification of the sternbrae was significantly increased in all dosage groups compared to control. The percentages of fetuses affected were 46, 61, 60, and 63 for the control, low-, mid-, and high-dose groups, respectively. However, the study authors stated that "this finding is commonly seen in rabbit fetuses and is not considered indicative of a teratogenic response." External and/or visceral malformations were noted in all groups and included hydrocephaly, cleft palate, space between brain and skull, and ectopic kidney (Table 3). Skeletal malformations were noted in the low-dose (4 fetuses from 3 litters) and mid-dose (2 fetuses from 2 litters) groups only and included nonossified rib, vertebral arches or centra, and fused rib or sternbrae.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

- A. The study authors stated that "no significant maternal or embryotoxic effects were demonstrated at any dose level. Thus, Chevron naled technical was not considered teratogenic in the rabbit."
- B. A signed quality assurance statement, dated March 4, 1985, was present in the final report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Maternal Effects: No compound-related effects were apparent for maternal body weight, body weight gain, clinical observations, or food consumption. Gross lesions that were examined microscopically were limited to pale or friable livers, characterized as vacuolation of hepatocytes, in one control, one mid-dose, and one

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TABLE 1. Mean Maternal Body Weight and Weight Gain during Gestation in Rabbits Dosed with Naled

Gestation Day(s)	Dosage (mg/kg/day)			
	0	0.2	2.0	8.0
	<u>Body Weight (g±SD)</u>			
0	3531±242	3518±167	3599±244	3525±254
7	3691±264	3655±164	3722±257	3672±267
19	3736±242	3695±167	3748±243	3681±277
29	3875±313	3904±206	3984±283	3951±334
	<u>Body Weight Gain (g±SD)</u>			
0- 7 ^a	168± 45	137± 81	156± 63	147± 71
7-19 ^a	47± 74	40± 96	26±142	9±104
19-29 ^a	148±143	204±144	236±153	271±116
0-29 ^a	370±165	378±177	401±149	426±173

^aAnalyzed by reviewers, using analysis of variance ($\alpha = 0.05$).

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TABLE 2. Selected Reproduction and Litter Data for Rabbits Dosed with Naled

	Dosage (mg/kg/day)			
	0	0.2	2.0	8.0
males inseminated	20	20	20	20
pregnant	18 (90%)	16 (80%)	16 (80%)	15 (75%)
abortions	3	1	0	0
per litter:				
corpora lutea	9.4± 2.3	8.3± 1.8	9.4± 1.7	9.5± 1.9
implantations	8.8± 3.0	7.3± 3.0	8.0± 2.6	8.3± 2.0
resorptions	0.9± 1.2	1.0± 1.8	0.5± 0.7	0.9± 0.8
live fetuses	7.9± 3.0	6.3± 3.0	7.5± 2.4	7.5± 1.8
implantation loss (%) ^a	14.3±17.0	23.7±27.1	19.3±21.5	10.9±16.7
implantation loss (%) ^a	10.6±12.6	12.0±18.7	5.3± 7.6	9.9± 9.4
ear weight (g)	42.2± 6.4	45.5± 6.8	44.0± 6.6	44.3± 6.3

^a Data obtained by reviewers from individual litter data presented in the study report and analyzed by analysis of variance ($\alpha = 0.05$).

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TABLE 3. Effect of Naled on Incidence of Malformations in Fetal Rabbits

	Dosage (mg/kg/day)			
	0	0.2	2.0	8.0
Fetuses evaluated	118	94	120	112
Litters evaluated	15	15	16	15
<u>External Malformations (litter incidence)</u> ^a				
Domed head	2 (13.3)	0	0	1 (6.7)
Cleft palate	0	0	0	1 (6.7)
<u>Visceral Malformations (litter incidence)</u>				
Cleft palate	1 (6.7)	0	0	0
Hydrocephaly	2(13.3)	0	1 (6.3)	0
Space between brain and skull	0	1 (6.7)	0	0
Ectopic kidney	0	1 (6.7)	0	0
<u>Skeletal Malformations (litter incidence)</u>				
Thoracic vertebral centra nonossified	0	1 (6.7)	1 (6.3)	0
Thoracic vertebral arches nonossified	0	0	1 (6.3)	0
Sternebrae fused	0	2(13.3)	1 (6.3)	0
Rib fused	0	0	1 (6.3)	0
Rib nonossified	0	1 (6.7)	0	0

^a Values in parentheses represent the percent incidence of each malformation.

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high-dose animal, and a firm nodule, characterized as a hemangioma, in the abdominal cavity of a low-dose female. These findings, as well as other gross findings, were considered incidental and unrelated to compound administration. Reproduction data including pregnancy and abortion rates, mean numbers of corpora lutea and implantations, and litter size appeared comparable between control and dosage groups. Thus, in the absence of maternal effects, we concluded that the dose levels selected for this study were unacceptably low.

Although no treatment-related fetal effects were apparent, the fetotoxic and/or teratogenic potential of naled could not be assessed for these reasons:

1. Maternal toxicity was not demonstrated.
2. Because the methods for fetal examinations were not adequately described in the study report, we could not assess their sensitivity or acceptability; therefore, the resulting data is of limited value in assessing possible compound-related effects.

- B. Differences Between Study Authors' and Reviewers' Assessments:
We do not agree with the authors' statement that preimplantation loss was elevated in the dosage groups compared to controls. The mean incidence of preimplantation loss was increased at the low and mid doses, but lower at the high dose when compared to control.

We assess that the authors' conclusion that Chevron naled technical is not teratogenic in the rabbit is not substantiated by the data. Deficiencies in the study design, conduct of the study, and the absence of maternal toxicity at any test dosage precluded our assessment of the teratogenic potential of naled in rabbits.

- C. Deficiencies: The following deficiencies in the study design and final report were noted:

1. The methods used for fetal visceral and skeletal examinations were not cited or adequately described in the final report. Appendix XVI, the study protocol, indicated that fetuses were to be evaluated according to "CEHC SOP TER.8.8.2." However, this SOP was not included in the final report, nor does the methods section clearly indicate whether the SOP was followed.
2. The highest dosage tested in this study, 8 mg/kg/day, did not elicit maternal toxicity.

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APPENDIX A
Materials and Methods

Naled toxicology review

Page _____ is not included in this copy.

Pages 14 through 20 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
 - FIFRA registration data
 - The document is a duplicate of page(s) _____
 - The document is not responsive to the request
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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.

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EPA: 68-02-4225
DYNAMAC No. 1-0398-2a
March 13, 1986

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION [EO 12065]

DATA EVALUATION RECORD

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Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Schroeder, R. E. and Daly, I. W. Two-generation reproduction study in rats with Dibrom. (Unpublished study No. 82-2612 prepared by Bio/dynamics, Inc., East Millstone, NJ, for Chevron Chemical Company, Agricultural Chemicals Division, Richmond, CA; dated March 22, 1985.) Accession Nos. 257455, 257459, 257460, 257461, 257462, and 257463.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 3-13-86

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1. CHEMICAL: Naled; Dibrom; 1,2-dibromo-2,2-dichloroethyldimethylphosphate.
2. TEST MATERIAL: Dibrom technical, lot No. SX-1397, containing 91.0% active ingredient, was described as a clear, colorless liquid.
3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.
4. STUDY IDENTIFICATION: Schroeder, R. E. and Daly, I. W. Two-generation reproduction study in rats with Dibrom. (Unpublished study No. 82-2612 prepared by Bio/dynamics, Inc., East Millstone, NJ, for Chevron Chemical Company, Agricultural Chemicals Division, Richmond, CA; dated March 22, 1985.) Accession Nos. 257455, 257459, 257460, 257461, 257462, and 257463.

5. REVIEWED BY:

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Principal Reviewer
Dynamac Corporation

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Date: 13 MARCH 86

Patricia A. Turck, M.S.
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EPA Reviewer

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Jane Harris, Ph.D.
EPA Section Head

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Date: 3/14/86

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7. CONCLUSIONS:

- A. From the results of oral administration of Dibrom at levels of 0, 2, 6, and 18 mg/kg by gavage to two generations of rats, we assess the NOEL and LOEL for parental effects to be 6 and 18 mg/kg of Dibrom, respectively, based on decreases in male body weight gain during both generations.

The NOEL and LOEL for progeny are 6 and 18 mg/kg of Dibrom, respectively, based on decreases in survival of offspring in the F₁ and F_{2b} litters, decreases in the number of pups born in the F_{2b} litters, and consistent decreases in pup body weights at 18 mg/kg or higher.

- B. This study is classified Core Minimum.

Item 8--see footnote 1.

9. BACKGROUND:

The study reviewed for this document was preceded by a range-finding study in rats (Bio/dynamics, No. 82-2611, entitled, "A Rat Pilot Reproduction Study with Dibrom"). The study was conducted using Dibrom technical, analyzed as 91% active ingredient. The range-finding study was conducted using a 1% suspension of Dibrom technical. The dosages, administered by gavage, were 0, 2, 10, and 20 mg/kg/day, through one generation using Sprague-Dawley CD rats. Each group consisted of four breeding pairs (one male and two females). The results of this range-finding study suggested a NOEL of 2 mg/kg and a LOEL of 10 mg/kg based on decreased body weight gain in the males and a decreased body weight in the females during lactation.

Based on the results of this study the doses for the definitive two-generation study were set at 2, 6, and 18 mg/kg Dibrom.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

- A. Materials and Methods: (See Appendix A.)

1. Test Material: The test material, Dibrom, was described as a clear, colorless liquid produced by Chevron Chemical Company,

¹ Only items appropriate to this DER have been included.

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Richmond, CA. The lot used for this study (No. SX-1397) contained 91% active ingredient.

Dosing suspensions were prepared daily by diluting a 1% stock solution of Dibrom technical with carboxymethyl cellulose to obtain concentrations of 2, 6, and 18 mg/kg. Dose volumes were adjusted to 10 mL/kg. Adult animals received a single daily dose via gavage throughout the study, beginning with the F₀ generation for 102 days before cohabitation. Rats selected for the F₁ adult generation began receiving daily oral doses after weaning of the last litter, and dosing continued for a 120-day period prior to mating; subsequently, the dosing continued through mating, gestation, lactation, and for a 36-day postweaning period.

Dosing suspensions were sampled daily throughout the study. Representative samples of each dose levels were frozen and sent to the sponsor for analysis. Homogeneity and stability studies were conducted prior to the initiation of this study.

2. Animals and Test System: The animals used for the study were male and female Sprague-Dawley-derived Charles River CD rats from Charles River Breeding Laboratories, Inc., Portage, MI. The animals were received at the testing laboratory at 26 days of age and were placed on test at 42 days of age (initiation of F₀ dosing). Animals considered suitable for study were randomized by computer according to body weight. Fifteen male and 30 female rats were assigned to each of four groups. Mating was initiated when F₀ and F₁ animals were 143 to 148 days of age. The females were paired with males on a two to one basis for 14 days, after which unmated females were remated with proven males. Vaginal smears were evaluated daily for evidence of sperm. The day on which evidence of mating (copulatory plug or sperm) was found was designated day 0 of gestation.

Following a 3-week postmating period, the F₀ parental males were sacrificed and subjected to gross necropsy; reproductive organs were saved for histopathological evaluation. After weaning of the F₁ litters, parental F₁ females and weanlings not selected for the F₁ parental generation were sacrificed and examined grossly. Selected tissues from five randomly chosen male and female weanlings were saved. Tissue inventories are provided in Appendices A and B (attached).

Fifteen male and 30 female weanlings were randomly selected as F₁ parental animals and began receiving daily oral doses at approximately 28 days of age. The F₁ parental animals were mated twice to produce F_{2a} and F_{2b} litters and approximately 36 days after weaning of F_{2b} litters, parental animals were sacrificed and necropsied. Selected tissues were fixed for histopathological examination. At weaning (21

days of age), litters were sacrificed and pups were subjected to gross necropsies. Tissues from five randomly selected male and female weanlings from each group were examined microscopically. Pups dying prior to lactational day 4 were weighed, eviscerated, and fixed in 70% ethanol; those dying after lactational day 4 were weighed and given a gross necropsy and discarded. Only abnormal tissues were saved. Tissues inventories are provided in Appendices A and B (Attached).

3. Parameters Measured: The animals were observed twice daily for signs of toxicity and mortality; detailed physical examinations were conducted weekly. Body weights of parental males were measured weekly. Parental females were weighed weekly during the growth phases and on gestational days (GD) 0, 7, 14, 17, and 20, on lactational days 0, 8, 12, and 21, and weekly thereafter until sacrifice. Food consumption of parental animals was measured during the growth phases.

The day of parturition was designated as lactational day 0. The number, sex, and individual body weight measurements of offspring were recorded on lactational days 0, 4, 8, 12, and 21 for each litter.

4. Statistical Methods: The statistical methods used for normally distributed parametric data included ANOVA, Dunnett's, Kruskal-Wallis, Dunn's rank sum, and Jonckheere's tests. The tests used for incidence data included Chi-square, Fisher's exact, and Armitage test for linearity. Statistical significance was reported at $p < 0.05$ and $p < 0.01$ levels of confidence.

B. Protocol: See Appendix B.

2. REPORTED RESULTS:

- A. Test Material Analysis: Results of the test material analysis revealed that actual dose concentrations ranged from 72.8-158% of nominal concentrations. However, the low and high values were obtained from analysis of the low-dose samples and occurred only once. The majority of samples were within 10% of target concentrations. Dosing suspensions were found to be stable throughout the dosing period.

B. Parental Effects:

1. Mortality and Clinical Observations: Several animals died during the F_0 generation: two males from the mid-dose (6 mg/kg) group, two males from the high-dose (18 mg/kg) group, and one female from the mid-dose group. None of these deaths were attributable to the test material; the authors

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stated that postmortem findings in the two high-dose males suggested dose-related injury as the cause of death, while the single mid-dose female was killed in a moribund condition during the mating period (unstated causes).

During the F₁ generation, the following mortalities occurred: one and three males in the low- and high-dose groups, respectively, and one and four females in the control and high-dose groups, respectively. The authors reported that although there were increased mortalities noted in the high-dose group (not statistically significant), the results did not clearly indicate a compound-related effect; for one of the high-dose males and the single control female, death was attributable to dose-related injury.

During the F₀ pre mating period, incidences of ocular lesions and lacrimation indicative of a sialodacryoadenitis (SDA) viral infection were noted (enumerated in Appendix M of the Final Report). However, all groups were equally affected and the authors did not consider the observations to be compound related. No toxicological signs were noted in either generation that could be attributed to test material administration.

2. Body Weight: In the F₀ generation, body weights of the males from the low- and mid-dose groups were comparable to controls prior to mating, whereas weight gain in the high-dose group was 9.1% less than controls during this same period (not statistically significant). A statistically insignificant lower mean body weight in high-dose males (7.1% decrease when compared to controls) was also recorded at week 13 during this period. Mean body weight and body weight gain of the females from all test groups were comparable to controls throughout the pre mating period during the F₀ generation.

In the F₁ generation, there was a statistically significant dose-related decrease in body weights and body weight gain for males in all dose groups when compared to controls during the pre mating period. Body weights for females in all dose groups were comparable to controls throughout the pre mating period. Table 1 summarizes the effect of Dibrom on the body weights of F₀ and F₁ parental animals during the pre mating period.

No toxicologically significant changes in maternal body weight were noted during gestation or lactation (Table 2) for any litter interval (F₁, F_{2a}, or F_{2b}).

3. Food Consumption: Mean weekly food consumption by F₀ males from the mid- and high-dose groups was generally higher than controls (Table 3). Mean weekly food consumption of F₀

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TABLE 1. Effects of Dibrom on Mean Body Weights for the Premating Periods in Two Generations of Rats¹

Generation	Dose Level (mg/kg)	Mean Body Weight (g) at Premating Week					Body Weight Change (g)
		0	6	11	14	17	
F ₀ Males	0	185.5	373.2	443.1	456.2	--	270.7
	2	184.3	369.3	428.1	439.7	--	255.3
	6	182.5	362.4	424.3	439.9	--	257.3
	18	184.9	357.9	413.1	431.1	--	246.1
F ₀ Females	0	137.6	227.2	250.9	258.8	--	121.1
	2	137.2	226.9	255.3	261.9	--	124.7
	6	136.6	217.9	242.7	249.5	--	112.9
	18	137.1	227.8	253.3	264.9	--	127.9
F ₁ Males	0	147.4	398.1	488.5	518.1	547.5	400.2
	2	147.9	357.6*	434.1**	467.8**	484.9**	337.1**
	6	169.7	371.9	446.0*	475.1*	498.9*	329.1**
	18	169.7	366.1	425.5**	449.6**	473.9**	304.3**
F ₁ Females	0	114.9	228.5	257.8	269.3	278.5	153.6
	2	121.0	223.6	257.8	269.2	273.3	152.3
	6	128.3	220.0	253.9	264.8	275.4	147.1
	18	128.6	224.2	254.8	266.0	283.8	155.5

*Significantly different from control value (p <0.05).

**Significantly different from control value (p <0.01).

¹ Extracted by the reviewers from Tables 1 and 2 and Appendices F and G of the Final Report.

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TABLE 2. Effects of Dibrom on Mean Maternal Body Weights (g) During Gestation and Lactation of Two Generations of Rats¹

Parental/ Litter Generation	Dose Level (mg/kg)	Gestational Day				Body Wt. Change (g)	Lactational Day			Body Wt. Change (g)
		0	7	14	20		0	8	21	
F ₀ /F ₁	0	259.8	283.6	307.3	366.6	106.8	294.2	312.1	309.5	14.3
	2	265.7	287.4	311.6	374.2	108.5	291.9	309.2	306.3	13.1
	6	252.6	269.6	295.5	354.0	101.4	280.7	294.3	293.4	13.0
	18	262.8	285.2	310.6	367.0	104.2	299.5	306.1	302.3	3.1
F ₁ /F _{2a}	0	274.6	296.1	319.4	381.3	106.7	305.3	313.4	307.0	-0.1
	2	269.3	295.4	317.7	376.0	106.6	300.2	303.8	299.3	-0.5
	6	270.6	297.4	313.7	365.2	94.6	292.1	301.1	292.3	1.1
	18	268.8	287.4	309.5	364.8	96.0	297.4	303.5	291.1	-9.1
F ₁ /F _{2b}	0	285.6	307.1	331.1	390.7	105.1	323.8	328.3	313.9	-8.8
	2	292.6	313.2	339.3	401.5	108.9	325.3	339.3	314.3	-11.0
	6	274.2	303.9	322.9	365.2	91.0	312.4	322.8	312.1	-0.3
	18	281.9	309.5	332.9	380.1	98.2	319.6	324.1	310.0	-17.2

¹Extracted from Table 8 and Appendix C of the Final Report.

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TABLE 3. Effects of Dibrom on Mean Food Consumption in Rats During the Premating Period¹

Generation	Dose Level (mg/kg)	Mean Food Consumption (g/kg/day) at Premating Week				
		1	6	11	14	17
F ₀ Males	0	110.3	69.4	63.6	56.6	--
	2	107.9	68.8	61.3	53.9	--
	6	108.6	72.1	65.6	58.8	--
	18	110.9	74.2**	66.7	63.9**	--

F ₀ Females	0	112.4	82.5	80.0	75.0	--
	2	114.3	86.1*	83.0	76.5	--
	6	112.7	86.3*	83.9*	75.0	--
	18	115.0	89.7**	85.4**	79.3*	--

F ₁ Males	0	127.9	67.8	55.8	53.4	52.0
	2	130.2	69.7	59.6	56.9	53.1
	6	116.9	67.8	57.3	54.3	51.4
	18	119.1	67.8	57.4	55.3	54.0

F ₁ Females	0	132.0	76.5	70.0	66.3	64.0
	2	127.6	78.2	70.0	64.7	65.0
	6	124.2	82.7*	71.3	68.0	66.9
	18	127.7	79.9	70.5	67.8	61.3

*Significantly different from control value (p <0.05).

**Significantly different from control value (p <0.01).

¹Extracted from Tables 1 and 2 and Appendices F and G of the Final Report.

females was also increased sporadically throughout the pre-mating period. The authors stated that the reasons for increases in food consumption were "unclear." No toxicologically significant changes in food consumption were noted during the F₁ generation.

4. Reproductive Parameters: A summary of effects of Dibrom on parental reproductive parameters is presented in Table 4. Slight decreases in the mating indices were noted for F₀ females in the low- and high-dose groups but the study authors did not consider these to be toxicologically significant.

Mating indices for F₀ males in the dose groups were considered comparable to the controls. Pregnancy rates and fertility indices were not affected by treatment of the males. The study authors concluded that no compound-related differences were evident in mating, pregnancy, or fertility indices for the F₀ generation. In addition, the mean gestational length was comparable between control (22.0 days) and dose groups (21.8-22.2 days) for the first generation (data in Final Report Table 9, not shown here). The mean number of live and total pups for control and mid- and high-dose groups, however, traversed the low range of historical control data (10.6-14.6 and 10.8-15.1, recorded for 12 studies conducted during the period 1978-1983), attributable in part to several females with very small litters (3 pups or less).

The authors reported decreases (not statistically significant) in both male and female mating indices for the high-dose group during the F_{2a} litter interval. No differences were noted in the other dose groups when compared to controls, and the fertility indices, gestational length, parturition, and litter size data were comparable between control and dose groups. The mean number of live pups per litter was slightly lower in the dose versus the control groups during the F_{2a} litter interval, but these differences were not statistically significant, and no dose-response relationship was evident.

Female mating indices were comparable for all groups during the production of F_{2b} litters. For the low-dose group, the mating index was lower than controls (73.3% vs. 93.3%) but this was not statistically significant. Since the mid- and high-dose groups were not affected, this decrease was not considered biologically significant. Pregnancy rates were comparable across groups, and no statistically significant differences were noted. Male fertility indices were comparable between the control and high-dose groups but lower than controls in the low- and mid-dose groups (not statistically significant). Mean gestation lengths for the F_{2b} litter interval were comparable across groups. During the F_{2b} interval, the mean numbers of live and total pups were

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TABLE 4. Summary of Reproductive Parameters in Two Generations of Rats Given Dibrom¹

Generation	Dose Level (mg/kg)	Total No. Paired		Total No. Mating		Mating ^a Index (%)		No. Females Pregnant	Fertility ^b Index (%)		Total No. Litters Born	Mean No. Pups/Litter	Viability ^c Index
		M	F	M	F	M	F		M	F			
1	0	15	30	12	30	80.0	100.0	25	91.7	83.3	25	10.7	94.8
	2	15	30	12	26	80.0	86.7	23	100.0	88.5	23	12.1	92.8
	6	14	29	14	28	100.0	96.6	24	92.9	85.7	24	10.8	96.9
	18	15	30	12	26	80.0 ^d	86.7	26	100.0	100.0	26	10.8	97.9
2a	0	15	30	15	29	100.0	96.7	21	86.7	72.4	21	12.2	98.0
	2	15	30	15	29	100.0	96.7	25	100.0	86.2	25	10.7	94.4
	6	15	30	15	28	100.0	93.3	18	93.3	64.3	18	9.4	96.4
	18	15	29	11	22	73.3	75.9	15	81.8	68.2	15	10.9	97.6
2b	0	15	30	14	28	93.3	93.3	19	92.9	67.9	19	11.4	92.6
	2	14	30	13	22	92.9	73.3	12	69.2	54.5	12	11.8	97.2
	6	15	30	14	27	93.3	90.0	15	71.4	55.6	15	8.1*	95.0
	18	13	28	12	26	92.3	92.9	20	91.7	76.9	18 ^e	8.5*	95.3

No. paired/total No. mating.

No. achieving a pregnancy/total No. mating.

No. live pups per litter/total No. pups born per litter.

(12/14) according to reviewers' calculations.

uses data from two females dying during lactation.

ificantly different from control value ($p \leq 0.05$).

ated from Tables 7 and 9 and Appendices D and N of the Final Report.

comparable between the control and low-dose groups. In the mid- and high-dose groups, however, there were significantly lower numbers of live and total pups when compared to concurrent controls and outside this laboratory's range of historical control data (see above).

Male to female sex ratios were comparable between control and dose groups for both generations (as recorded in Appendix D of the Final Report).

5. Macroscopic and Microscopic Observations: No compound-related effects were reported at necropsy or after histopathologic examination for the F_0 or F_1 parental animals (as recorded in Appendix L, "Pathology Report," of the Final Report).

C. Progeny Effects:

1. Body Weight: F_1 pup weights from lactational days 0-4 were comparable between control and dose groups (Table 5). However, from day 8 through weaning, mean pup weights were consistently, but not dose relatedly lower than controls for all dose groups. These differences reached statistical significance ($p < 0.05$) when compared to control for the mid- and high-dose groups (-17.9 and -15.7%, respectively) at day 8 and at all doses on day 12. At day 21 of lactation, mean pup weights were -10.8, -10.2, and -13.9% of control values; however, these differences were not statistically significant.

In F_{2a} and F_{2b} litters, mean pup weights of the high-dose group (only) were consistently lower than controls at days 12 and 21. Although not statistically significant, the authors considered this indicative of a compound-related effect.

2. Survival: For the F_1 litter interval, pup survival indices during lactational days 0-4 were comparable between the control and the low-dose groups and were significantly lower than control at the mid- and high-dose levels (but not dose-related, see Table 5). During the interval from lactational days 4-21, the mid- and high-dose groups were comparable to, and the low-dose group was lower than, controls. The overall pup survival index for lactational days 0-21 was significantly lower than control for all dose groups (but again, no dose relationship was evident). During this interval, several females experienced complete litter mortality: five control litters (total of 39 pups), two low-dose litters (total of 17 pups), four mid-dose litters (total of 44 pups), and four high-dose litters (total of 39 pups).

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TABLE 5. Summary of Effects of Dibrom on Group Pup Survival and Body Weights During Lactation in Two Generations of Rats¹

Generation	Dose Level (mg/kg)	Pup Survival (%) ^a During Lactational Days			Mean Pup Body Weight (g) at Lactational Days				
		0-4	4-21	0-21	0	4	8	12	21
F ₁	0	83.1	94.8	78.7	6.0	8.3	14.0	21.0	36.1
	2	87.7	80.8**	69.0*	5.7	7.7	11.9	17.5*	32.2
	6	65.6**	89.0	58.4**	5.7	7.3	11.5*	17.7*	32.4
	18	74.9*	93.7	70.2*	5.9	7.7	11.8*	17.7*	31.1
F _{2a}	0	88.8	97.5	85.6	5.7	8.6	14.1	21.3	38.1
	2	96.4**	100.0	96.4**	6.0	9.2	14.9	21.7	40.0
	6	90.2	98.6	89.0	6.1	8.9	14.7	21.0	38.1
	18	86.3	96.4	83.1	5.9	8.4	12.9	19.0	34.0
F _{2b}	0	95.0	99.5	94.3	5.9	9.3	15.2	23.0	40.7
	2	99.3	98.5	97.8	6.0	9.6	16.1	23.1	41.2
	6	93.9	98.1	92.2	6.4	9.6	16.1	23.9	44.6
	18	87.1*	85.3**	73.3**	6.3	8.7	14.2	20.6	37.1

^a Expressed as the ratio of $\frac{\text{Total No. pups alive at the end of specified lactation interval}}{\text{Total No. live pups on lactation day 0}}$

* Significantly different from control value ($p \leq 0.05$).

** Significantly different from control value ($p \leq 0.01$).

¹ Extracted from Tables 9 and 10 and Appendix D of the Final Report.

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Pup survival indices during lactational days 0-4, 4-21, and 0-21 of the F_{2a} litter interval were comparable between the control and mid- and high-dose groups. In the low-dose group, pup survival indices for lactational days 0-4 and 0-21 were significantly higher than controls.

Pup survival indices during lactational days 0-4, 4-21, and 0-21 for the F_{2b} litter interval were comparable between the control and low- and mid-dose groups. In the high-dose group, however, pup survival indices were significantly and consistently lower than controls during all lactational day intervals.

3. Macroscopic and Microscopic Observations: No malformations or other gross lesions were noted in pups born dead or dying during lactation or at termination for any litter interval (as recorded in Appendix L of the Final Report).

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

- A. The study authors concluded that no mortalities or clinical observations noted in parental animals from either generation were compound related. No differences in body weights occurred in F₀ or F₁ parental females or F₀ parental males. However, the authors concluded that significant body weight decreases for the F₁ parental males at all doses when compared to controls were compound related. A dose-related trend was evident. Increases in food consumption did not clearly indicate a compound-related effect. No significant differences were noted between control and dose groups for the mating, pregnancy, and fertility indices for either generation. Reproductive performance of the F₀ and F₁ generation animals was not considered to be adversely affected by treatment. During the F_{2a} litter interval, mating indices for the high-dose males and females were reduced when compared to controls; however, these differences were not statistically significant, and a similar effect on mating performance was not observed during the F_{2b} litter interval. Some variability was encountered in both pregnancy rates and male fertility indices between the control and dose groups during all litter intervals; however, none of these differences were significantly different from controls and no trend indicative of a compound-related effect was noted.

The authors stated that no adverse effects of treatment were evident for any group in gestation length, pup body weights, numbers of live and dead pups, or litter size at parturition. In the mid- and high-dose groups from the second generation, a statistically significant reduction in the mean total number of pups at birth was evident only in the F_{2b} litter interval. Although there were significant decreases in pup body weights at the low- and mid-dose levels during F₁ litter interval, no

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other differences were noted at these levels for any other litter interval; therefore, the authors did not consider these decreases to be toxicologically significant. However, the authors stated that the consistent decreases in pup body weights at the high-dose level were compound-related effects.

The changes occurring in pup survival at the low-dose level were not considered to be attributable to test material administration. However, compound-related decreases in survival were noted in the mid-dose group during the F₁ litter interval and in the high-dose group during the F₁ and F_{2b} litter intervals.

B. A quality assurance statement was signed and dated March 20, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. We agree with the authors that no clinical signs of toxicity were noted during the study. Decreases in body weight occurred in males at all doses from both generations, although the only significant decrease occurred in the F₁ parental males. Increases in food consumption occurred in second generation males and females dosed at the 6- and 18-mg/kg levels. Since the active ingredient of Dibrom is a cholinesterase-inhibiting organophosphate, the decreases in body weight and increases in food consumption suggest a possible effect on gut motility, which in turn would affect nutrient absorption. This interpretation may be supported by the fact that increased acetylcholine activity at the neuromuscular junction would markedly increase energy utilization, resulting in increased food consumption without increased body mass.

There were increased mortalities in the high-dose group during the second generation: three males (20%) and four females (16%). No toxicologically significant findings were observed at necropsy. However, evidence that these deaths were not compound-related is inconclusive.

No differences in mating performance or fertility occurred in the F₁ or F_{2b} litter intervals. Mating and fertility indices in males and females in the high-dose group were decreased (not significantly). However, in subsequent mating (F_{2b} litter interval), the majority of animals mated successfully. Therefore, we did not consider these decreases to be compound related.

There were significant decreases in the mean number of total pups born in the mid- and high-dose groups during the F_{2b}, but not in the F₁ or F_{2a} litter intervals, when compared to controls; however, the viability indices were comparable between the control and dose groups, suggesting that a prenatal effect occurred.

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However, we were unable to assess whether sperm viability, ovulation, fertilization, and/or implantation were affected. No other differences occurred in any dose group when compared to controls.

Although significant decreases in survival of offspring occurred somewhat randomly in the low- and mid-dose groups during the F₁ litter interval, we do not consider these to be toxicologically significant since similar decreases were not noted at these dose levels in the F_{2a} and F_{2b} litter intervals; in fact survival for the low dose during the F_{2a} litter interval was significantly greater than the control value. However, we consider the significant decreases at the high-dose level occurring during the F₁ and the F_{2a} litter intervals to be compound related.

- B. Minor discrepancies as well as some typographical and arithmetic errors were noted. For example, the daily dose levels are stated in the prefaced Abstract as "2,8 and 16 mg/kg" and throughout the text and tabulation of the Report as 2, 6, and 18 mg/kg. However, no errors that would change the interpretation of the results were found by the reviewers.

Item 15--see footnote 1.

16. CBI APPENDICES: Appendix A, Materials and Methods (Final Report, pages 3-16); Appendix B (Final Report, Protocol, Appendix S, pp. 2-16).

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APPENDIX A
Materials and Methods

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- 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
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APPENDIX B

Protocol

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Standard Oil Company of California

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PROTOCOL NO. 11.103

TWO GENERATION (ONE LITTER) REPRODUCTION
STUDY IN RATS WITH DIBROM

SEPTEMBER 1, 1981

Designed to meet proposed EPA-FIFRA Requirements
(Federal Register 43 (163), August 22, 1978)

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