3/12/87

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

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MAR 12 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Naled - Review of Studies Submitted under Accession No.'s 263583 and 263584. Caswell 586

TB Project 2106

EPA ID # 239-1633

TO: William Miller/Gary Otakie, PM 16
Registration Division (TS-767c)

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

THRU: Judy W. Hauswirth, Ph.D., Acting Head
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769c)

Judith W. Hauswirth 3/9/87

Registrant: Chevron Chemical

Action Requested (660): Review and evaluate the following studies submitted under cover letter of June 30, 1986 in response to the Naled Registration Standard (issued June 30, 1983):

Study 1: A Twenty-Eight Day Dermal Study with Naled Technical in Rats. Conducted by Bio/dynamics Inc, Project # 85-2981, dated May 21, 1986. Chevron Study #S-2542 (EPA ACCESSION NO. 263583)

Study 2: One-Year Chronic Oral Toxicity Study in Dogs with Naled Technical. Conducted by IRDC, Project # 415-044, dated June 10, 1986. Chevron Study #S-1920 (EPA ACCESSION NO. 263584)



1 of 45

TB CONCLUSIONS:

Study 1 (28-day rat dermal): CORE MINIMUM*
NOEL = 1 mg/kg/day (LDT)
LOEL = 20 mg/kg/day (severe dermal irritation and necrosis; inhibition of plasma, RBC and brain ChE)

Study 2: (One-year dog oral): CORE MINIMUM
NOEL = 0.2 mg/kg/day
LOEL = 2.0 mg/kg/day (inhibition of plasma and RBC ChE; decreased hemoglobin and hematocrit)

* Although the rabbit is preferred for studies of this type according to FIFRA Testing Guidelines, the rat is an acceptable substitute, providing justification for the substitution is submitted. In its cover letter of June 30, 1986, the registrant submits that, in contrast to rabbits, the rat is a more appropriate model for evaluating cholinesterase inhibitors (such as naled), since methods for detecting such inhibition are well-established for the rat, and are supported by a more sufficient database. TB accepts this justification for selecting the rat.

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EPA: 68-02-4225
DYNAMAC No. 247A
February 12, 1987

# DATA EVALUATION RECORD

## NALED (DIBROM)

## Chronic Toxicity Oral Gavage Study in Dogs

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 2-12-87

005774
EPA: 68-02-4225
DYNAMAC No. 247A
February 12, 1987

DATA EVALUATION RECORD

NALED (DIBROM)

Chronic Toxicity Oral Gavage Study in Dogs

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 2-12-87

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: _____
Date: _____

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-12-87

Irving Mauer, Ph.D.
EPA Reviewer, Section VI
TS 769C

Signature: [signature]
Date: 2-24-87

Judith Hauswirth, Ph.D.
Acting EPA Section Head

Signature: Judith W. Hauswirth
Date: 2/3/87

DATA EVALUATION REPORT

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TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: One-year chronic toxicity oral gavage study in dogs.

ACCESSION NUMBER: 263584.

TEST MATERIAL: Naled, technical; 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate.

SYNONYMS: Dibrom.

STUDY NUMBER(S): S-1920; IRDC 415-044.

SPONSOR: Chevron Chemical Co.

TESTING FACILITY: International Research & Development Corp., Mattawan, MI.

TITLE OF REPORT: One-year chronic oral toxicity study in dogs with naled technical.

AUTHOR(S): Johnson, D. E., Lochner, D. H., Geil, R. G., and Goldenthal, E. I.

REPORT ISSUED: June 10, 1986.

CONCLUSIONS:

An increase in the incidence of emesis and diarrhea was found in male and female dogs receiving 2.0 or 20.0 mg/kg/day naled. Decreased erythrocyte counts and hemoglobin and hematocrit values were seen at these same doses in males and females at several study intervals. Platelet counts were increased in high-dose males and females at several study intervals. Plasma protein and albumin were decreased in high-dose males and females, and calcium levels were slightly decreased. Plasma cholinesterase activity was decreased in males and females receiving 2.0 and 20.0 mg/kg/day and there was a dose-related decrease in erythrocyte cholinesterase activity at the same dose levels. Brain cholinesterase activity was depressed at 12 months in mid- and high-dose females and in high-dose males. There were increases in the mean weights and organ-to-body weight ratios of liver and kidney in both males and females receiving 20 mg/kg/day when compared to controls. There were no compound-related histopathologic lesions with the possible exception of an increased incidence of testicular degeneration (trace) in males receiving 2.0 and 20 mg/kg/day. The LOEL is 2.0 mg/kg/day and the NOEL is 0.2 mg/kg/day based on inhibition of plasma and erythrocyte cholinesterase activities and decreased hemoglobin and hematocrit values in both sexes.

Core Classification: The study is Core Minimum since there was one mortality.

A. MATERIALS:

1. Test Compound: Naled, technical, lot No. SX-1397; purity: 91.4 percent.

2. Test Animals: Species: dog; strain: Beagle; mean age: approximately 6 months; weight: approximately 10.5 kg for males and 8.3 kg for females at initiation; source: Ridglan Farms Inc., Mount Horeb, WI. The dogs had been vaccinated by the supplier for distemper, hepatitis, parainfluenza, Bordetella, leptospirosis, parvovirus, and rabies and were revaccinated by the testing laboratory for rabies and treated for coccidiosis.

B. STUDY DESIGN:

1. Animal Assignment: Animals were weighed and assigned to the following groups using a computerized random selection in a block design based on body weight:

| Test group | Dosage (mg/kg/day) | Number of Dogs Males | Females |
|---|---|---|---|
| 1 Control | 0 | 6 | 6 |
| 2 Low (LDT) | 0.2 | 6 | 6 |
| 3 Mid (MDT) | 2.0 | 6 | 6 |
| 4 High (HDT) | 20.0 | 6 | 6 |

Dogs were individually housed in metabolism cages in an environmentally controlled room with a 12-hour light/dark cycle.

2. Dose Preparation: Dosing suspensions of the test material were prepared daily. A 1 percent suspension of naled in 0.5 percent aqueous solution of carboxymethylcellulose was prepared using a Waring blender and this stock solution was diluted with the vehicle so that the appropriate dosage was administered in a volume of 2.5 mL/kg body weight. Samples of the dosing suspensions were provided to the sponsor for homogeneity analysis prior to study initiation and for analysis of test material concentration on the first day of weeks 1, 2, 3, and 4 and every 4 weeks thereafter. On all other study days, 10 g samples of each dosing suspension and vehicle control were collected and retained frozen. Samples of the test material were provided to the sponsor at initiation, day 180, and at study termination to analyze for stability.

   Results: The test material contained 92.0 percent naled when analyzed 2 months prior to study initiation, 89.6 percent at initiation, and 87.6 and 87.7 percent at 6 and 12 months, respectively. Data on analysis of dosing formulations are summarized in Table 1. The formulations were below nominal levels (82.1 to 94.4 percent). The coefficients of variation were 4.0 and 7.7 percent at the 0.8 and 8.0 mg/kg dose levels, respectively.

3. Dogs were dosed orally by gavage, 7 days a week, at a volume of 2.5 mL/kg; the volume administered to each dog was adjusted weekly based on body weight. Animals were conditioned to the dosing regimen by gavage of 2.5 mL water/kg for 7 days prior to initiation. Dogs were offered water and diet (Ralston-Purina Certified Canine Diet No. 6007) ad libitum. Animals were fasted for 16 to 19 hours prior to blood collection.

4. Statistics: Body weights, food consumption, clinical pathology parameters, and organ weights were analyzed using analysis of variance and Bartlett's test. Treatment groups were compared to

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TABLE 1. Formulation Analysis Data for Naled

| Dose Level (mg/kg)[a] | No. of Samples | Mean Concentration (mg/kg)[b] | Percent of Theoretical | Coefficient Variation (%) | Range (%) |
|---|---|---|---|---|---|
| 0.08 | 16 | 0.0586 | 82.1 | 15 | (50.3-98.6) |
| 0.8 | 16 | 0.664 | 93.0 | 4.0 | (84.9-97.6) |
| 8.0 | 16 | 6.74 | 94.4 | 7.7 | (82.5-105) |

[a]Expressed in terms of naled technical; not corrected for purity.

[b]Expressed in terms of active ingredient.

the control group, by sex, using the appropriate t-statistic (equal or unequal variances). Nonparametric procedures (i.e., rank transformation[1]) were used to analyze total bilirubin, uric acid, gamma glutamyltranspeptidase, lactic acid dehydrogenase, erythrocyte cholinesterase activity, and urine specific gravity and volume. Unless otherwise noted, the statistical notations in this DER are those of the report authors.

5. A quality assurance statement was signed and dated June 10, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected at least twice daily for mortality, moribundity and signs of overt toxicity; detailed observations were conducted at least once weekly. Detailed physical examinations were conducted pretest and at 3, 6, 9, and 12 months; heart and lung sounds were included in these examinations.

   Results: A high-dose male dog (No. 5387) was sacrificed moribund during week 50. The moribundity was not considered related to dosing. This dog showed loss of appetite, weight loss, decreased activity, dehydration, and weakness prior to sacrifice.

   There was an increased incidence of soft stool and/or diarrhea in mid- and high-dose males from week 14 to 52 and in high-dose females throughout the study. Emesis was frequent in all dosed groups; this occurred at an average of 40 minutes after the gavage dosing. The emesis did not noticeably affect the intake of test compound. The frequency of soft stool/diarrhea and emesis is summarized in Table 2. All other observations, with the possible exception of salivation in high-dose males and females, were considered incidental and not related to dosing.

2. Body Weight: Dogs were weighed weekly.

   Results: There was no effect of dosing on mean body weights. Representative weight data are summarized in Table 3. Dog No. 5387, a high-dose male that was sacrificed moribund at week 50, showed no marked change in weight gain until week 47. There was a weight loss of 3 kg in the 3 weeks prior to sacrifice.

3. Food consumption: Food consumption was calculated weekly.

   Results: Food consumption was similar in all groups of males and females. There were fluctuations in mean values from week to week and between some groups, but there were no apparent dose-related trends. Table 4 presents representative data. Food efficiency was not calculated.

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[1] Conover, W. J. and Iman, R. L. (1981) Rank transformation as a bridge between parametric and nonparametric statistics. American Statistician 35:124-133.

TABLE 2. Selected Clinical Observations of Dogs Dosed Orally with Naled for 1-Year

| Observation | Study Interval (weeks) | Dosage Group (mg/kg/day) 0 | 0.2 | 2.0 | 20.0 |
|---|---|---|---|---|---|
| | | **Males** | | | |
| Soft stool/ diarrhea | 1-13 | 6[a](100)[b] | 5 (83) | 6(100) | 5 (83) |
| | 14-26 | 4 (67) | 4 (67) | 5 (83) | 6 (100) |
| | 27-39 | 2 (33) | 1 (17) | 3 (50) | 6 (100) |
| | 40-52 | 2 (33) | 1 (17) | 4 (67) | 6 (100) |
| Emesis | 1-13 | | 6 (100) | 6(100) | 6 (100) |
| | 14-26 | 2 (33) | 2 (33) | 6(100) | 6 (100) |
| | 27-39 | 1 (17) | 2 (33) | 6(100) | 6 (100) |
| | 40-52 | 4 (67) | 5 (83) | 5(83) | 6 (100) |
| | | **Females** | | | |
| Soft stool/ diarrhea | 1-13 | 4[a](67) | 3 (50) | 3 (50) | 6 (100) |
| | 14-26 | - | 3 (50) | 1 (17) | 6 (100) |
| | 27-39 | - | 1 (17) | 1 (17) | 6 (100) |
| | 40-52 | - | 2 (33) | - | 6 (100) |
| Emesis | 1-13 | 2 (33) | 3 (50) | 5 (83) | 6 (100) |
| | 14-26 | 2 (33) | 3 (50) | 4 (67) | 6 (100) |
| | 27-39 | 1 (17) | 2 (33) | 6 (100) | 6 (100) |
| | 40-52 | 2 (33) | 4 (67) | 4 (67) | 6 (100) |

[a]Number of animals affected during interval.

[b]Percent affected, based on number of animals surviving at start of study interval.

TABLE 3. Representative Body Weights of Dogs Dosed Orally with Naled for 1 Year

| Dosage Group[a] (mg/kg/day) | Mean Body Weight (kg) ± SD at Week | | | |
|---|---|---|---|---|
| | 0 | 13 | 26 | 52 |
| | | | Males | |
| 0 | 10.4±2.26 | 12.3±2.69 | 12.9±2.54 | 13.3±2.75 |
| 0.2 | 10.4±2.26 | 11.7±2.19 | 12.6±1.96 | 13.0±1.93 |
| 2.0 | 10.5±2.41 | 12.6±2.46 | 13.8±2.93 | 14.7±3.05 |
| 20.0 | 10.6±2.15 | 11.3±2.12 | 12.1±2.00 | 13.7±1.68[b] |
| | | | Females | |
| 0 | 8.1±1.01 | 9.3±1.57 | 10.1±1.90 | 10.6±2.10 |
| 0.2 | 8.2±1.14 | 9.3±1.20 | 10.0±1.28 | 10.5±1.55 |
| 2.0 | 9.4±1.72 | 9.5±2.52 | 10.3±2.78 | 10.9±3.06 |
| 20.0 | 8.2±0.96 | 9.4±1.40 | 10.5±1.00 | 11.2±0.79 |

[a]Group = six dogs/group.

[b]Five dogs/group; one animal died in extremis at week 50.

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TABLE 4. Representative Food Consumption Values of Dogs Dosed Orally with Naled for 1 Year

| Dosage Group[a] (mg/kg/day) | Mean Food Consumption (g/kg/day ± SD) | | | |
|---|---|---|---|---|
| | 0 | 13 | 26 | 52 |
| | | | Males | |
| 0 | 31.8± 2.04 | 30.9±6.22 | 30.3± 7.99 | 28.6± 6.00 |
| 0.2 | 34.7±13.33 | 30.8±7.18 | 32.1±10.51 | 34.9±10.01 |
| 2.0 | 31.4± 5.42 | 27.9±4.96 | 25.1± 4.31 | 26.2± 4.42 |
| 20.0 | 26.6± 5.55 | 31.7±9.19 | 29.4± 9.17 | 31.6± 5.08[b] |
| | | | Females | |
| 0 | 30.4±3.14 | 32.2±15.85 | 34.1±10.4 | 30.5±10.37 |
| 0.2 | 31.3±7.42 | 26.8± 5.98 | 29.2± 7.71 | 32.1±10.16 |
| 2.0 | 30.9±3.12 | 32.0± 8.25 | 35.0±10.94 | 36.6±15.26 |
| 20.0 | 30.8±4.29 | 36.7±12.98 | 29.8± 9.73 | 32.9± 7.07 |

[a]Group = six dogs/group.

[b]Five dogs/group; one animal died in extremis at week 50.

4. Ophthalmological examinations were performed pretest and at weeks 26 and 52 on all animals.

   Results: No compound-related abnormalities were noted.

5. Blood was collected before study initiation and at 1, 3, 6, 9, and 12 months for hematology and clinical analysis on all dogs. The CHECKED (X) parameters were examined.

   a. Hematology:

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†
X Reticulocyte count
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Reticulocyte count
X Prothrombin time
X Activated partial prothrombin time

   Results: Decreased erythrocyte counts (RBC), hemoglobin concentration (HGB), and hematocrit (HCT) were seen in both males and females receiving 2.0 or 20.0 mg/kg/day. These values differed significantly from controls at several intervals (Table 5). Platelet counts were increased when compared to controls in males and females receiving 20 mg/kg/day; there were no effects on platelets at 0.2 or 2.0 mg/kg/day (Table 6).

   b. Clinical Chemistry

Electrolytes
X Calcium†
X Chloride†
  Magnesium†
X Phosphorus†
X Potassium†
X Sodium†

Enzymes
X Alkaline phosphatase (ALP)
X Cholinesterase
X Creatinine phosphokinase†
X Lactic acid dehydrogenase
X Serum alanine aminotransferase (also SGPT)†
X Serum aspartate aminotransferase (also SGOT)†
X Gamma glutamyl transaminase

Other
X Albumin†
X Blood creatinine†
X Blood urea nitrogen† (BUN)
X Cholesterol†
X Globulins
X Glucose†
X Total bilirubin†
X Total protein†
X Triglycerides

†Recommended by Subdivision F (October 1982) guidelines for chronic studies.

TABLE 5. Mean Hematologic Values (±SD) in Dogs Dosed Orally with Naled for 1 Year

| Parameter/ Month | Males/Dosage (mg/kg/day) 0 | 0.2 | 2.0 | 20.0 | Females/Dosage (mg/kg/day) 0 | 0.2 | 2.0 | 20.0 |
|---|---|---|---|---|---|---|---|---|
| Erythrocytes ($10^3/mm^3$) | | | | | | | | |
| Pretest | 6.09±0.435 | 5.98±0.457 | 5.97±0.499 | 6.32±0.556 | 5.32± 0.599 | 6.10±0.386 | 6.17±0.230 | 6.17±0.417 |
| 1 | 5.96±0.366 | 6.10±0.281 | 5.79±0.329 | 5.88±0.599 | 6.28±0.312 | 6.13±0.431 | 6.22±0.367 | 5.74±0.105* |
| 3 | 6.12±0.525 | 5.73±0.587 | 5.08±0.575* | 4.62±0.845** | 6.55±0.312 | 6.00±0.556 | 5.58±0.424** | 5.27±0.352** |
| 6 | 5.58±0.801 | 6.59±0.523 | 5.62±0.508* | 5.56±0.470* | 6.39±0.387 | 6.68±0.455 | 5.78±0.303* | 5.65±0.406** |
| 9 | 6.43±0.536 | 6.23±0.511 | 5.37±0.486** | 5.58±0.333* | 6.48±0.278 | 6.19±0.254 | 5.90±0.267* | 5.36±0.434* |
| 12 | 6.59±0.452 | 6.30±0.334 | 5.63±0.39** | 5.34±0.473** | 6.23±0.465 | 6.28±0.479 | 6.04±0.325 | 5.70±0.773 |
| Hemoglobin (g/dL) | | | | | | | | |
| Pretest | 16.2±0.77 | 15.3±1.09 | 16.1±1.28 | 16.8±1.12 | 15.3±1.63 | 16.3±1.13 | 16.3±0.45 | 16.5±1.19 |
| 1 | 15.4±0.83 | 15.4±0.55 | 15.1±1.14 | 14.7±1.29 | 16.1±0.44 | 15.7±1.32 | 15.9±0.56 | 14.6±1.01* |
| 3 | 16.2±1.04 | 15.3±1.43 | 14.0±1.50 | 12.5±2.23** | 17.4±0.91 | 16.1±1.58 | 15.1±0.96** | 14.5±0.98** |
| 6 | 17.6±1.29 | 17.9±1.26 | 15.8±1.56 | 15.1±1.23** | 17.7±0.89 | 18.2±1.43 | 16.1±1.16 | 15.8±1.15* |
| 9 | 17.8±1.20 | 17.3±1.19 | 15.4±1.14 | 14.9±2.09** | 18.1±1.01 | 17.4±0.83 | 16.7±0.44* | 15.3±1.30** |
| 12 | 17.8±0.82 | 17.2±0.69 | 15.8±0.85** | 14.2±2.60* | 17.1±1.19 | 17.2±1.27 | 16.9±0.064 | 15.5±1.59 |
| Hematocrit (%) | | | | | | | | |
| Pretest | 45.7±2.35 | 43.3±3.08 | 45.4±3.78 | 47.7±2.67 | 43.8±3.97 | 46.4±3.20 | 46.6±1.18 | 46.8±3.29 |
| 1 | 44.6±2.26 | 46.0±1.89 | 44.4±3.83 | 44.4±4.15 | 47.3±1.66 | 46.4±3.45 | 47.2±2.11 | 43.7±1.95 |
| 3 | 46.1±2.90 | 43.4±4.40 | 39.5±4.49 | 35.3±6.16** | 49.9±2.47 | 45.6±4.83 | 43.1±2.76** | 40.8±2.64** |
| 6 | 49.0±4.10 | 49.7±3 52 | 43.4±4.11* | 42.1±3.36* | 48.6±3.06 | 50.2±3.95 | 44.2±2.47 | 43.3±3.29* |
| 9 | 47.0±3.23 | 45.8±3.46 | 40.3±3.3/* | 39.9±5.46* | 48.1±2.26 | 46.7±1.78 | 44.5±0.93* | 40.4±3.15** |
| 12 | 48.1±2.45 | 46.2±2.08 | 42.4±2.45* | 38.7±5.81** | 46.3±3.07 | 46.0±3.50 | 45.5±1.76 | 42.8±4.95 |

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

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TABLE 6. Platelet Counts in Dogs Dosed Orally with Naled for 1 Year

| | Males/Dosage (mg/kg/day) | | Females/Dosage (mg/kg/day) | |
|---|---|---|---|---|
| Parameter/Month | 0 | 20.0 | 0 | 20.0 |
| Platelets ($10^3$/mm$^3$) | | | | |
| Pretest | 349±66.9 | 350±53.4 | 375±92.6 | 424±47.0 |
| 1 | 322±82.1 | 338±61.8 | 344±78.4 | 382±71.4 |
| 3 | 379±84.4 | 394±97.7 | 387±46.4 | 474±54.3* |
| 6 | 344±113.6 | 441±67.7 | 368±70.9 | 496±54.1** |
| 9 | 276±78.8 | 419±118.7* | 297±50.6 | 417±32.0** |
| 12 | 301±61.7 | 428±81.8* | 344±47.7 | 482±77.7** |

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

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

Plasma and erythrocyte cholinesterase activities were determined twice pretest (a week apart), at 7 days, and at months 1, 3, 6, 9, and 12. Brain cholinesterase activity was determined at study termination.

Results: Decreased albumin and total protein levels were found in both males and females receiving 20 mg/kg/day throughout the study; decreased albumin levels were also noted in males receiving 2.0 mg/kg/day at months 1, 9, and 12 (Table 7). There were slight decreases in calcium levels in males and females receiving 20 mg/kg/day. An increase in chloride level, particularly in the high-dose animals, was stated to be of doubtful toxicologic significance since it did not correlate with other findings. Chloride and calcium levels are presented in Table 7.

Data on cholinesterase activities are summarized in Table 8. Plasma cholinesterase activity was markedly depressed in both males and females in the 2.0- and 20-mg/kg/day groups. The activity in the 2.0-mg/kg/day group of males was significantly lower ($p \leq 0.01$) than in controls from 30 days onward, whereas in the corresponding female group it was significantly lower as early as day 7. There were significantly lower ($p \leq 0.05$) activities in the 0.2-mg/kg/day males and females beginning at 91 and 30 days, respectively. At 1 year, the activity in low-dose males was 96.5 percent of the pretest activity and in low-dose females it was 80.9 percent of the pretest activity. The authors assessed that the depressed activity in the 0.2-mg/kg/kg groups was not of biological significance.

Erythrocyte cholinesterase activity was depressed in males and females receiving 2.0 and 20.0 mg/kg/day, but there was no effect in the 0.2-mg/kg/day groups. The depression of activity was dose related; the activity at 12 months was 48.1 and 26.7 percent of the pretest value in males receiving 2.0 and 20.0 mg/kg/day and was 53.1 and 25.5 percent of the pretest value in females receiving the same doses.

Brain cholinesterase activity was significantly depressed in high-dose males ($p < 0.01$) and in mid- ($p \leq 0.05$) and high-dose females ($p \leq 0.01$).

6. Urinalyses: Urine was collected from fasted animals at 6 and 12 months. The CHECKED (X) parameters were examined.

| | | | |
|---|---|---|---|
| X | Appearance (and color)† | X | Glucose† |
| X | Volume† | X | Ketones† |
| X | Specific gravity† | X | Bilirubin† |
| X | pH† | X | Blood† |
| X | Sediment (microscopic)† | X | Nitrite |
| X | Protein† | X | Urobilinogen |

†Recommended by Subdivision F (October 1982) guidelines for chronic studies.

TABLE 7. Selected Clinical Chemistry Data for Dogs Dosed Orally with Naled for 1 Year

| Parameter/ Month | Males/Dose (mg/kg/day) 0 | 0.2 | 2.0 | 20.0 | Females/Dose (mg/kg/day) 0 | 0.2 | 2.0 | 20.0 |
|---|---|---|---|---|---|---|---|---|
| **Chloride (mEq/L)** | | | | | | | | |
| Pretest | 113±1.1 | 114±1.4 | 113±0.8 | 113±2.8 | 114±1.0 | 114±2.3 | 115±1.8 | 114±1.0 |
| 1 | 110±3.3 | 114±0.9 | 115±1.4 | 126±9.8** | 112±1.0 | 112±2.1 | 114±1.5 | 130±10** |
| 3 | 110±4.6 | 114±1.2 | 116±1.2* | 148±36.3 | 111±1.4 | 112±0.8 | 116±1.6** | 155±27** |
| 6 | 115±3.3 | 120±4.2 | 123±1.9** | 170±23.1** | 116±1.2 | 117±2.3 | 121±3.1** | 192±35.3** |
| 9 | 117±2.6 | 118±2.1 | 118±2.4 | 127±5.1** | 116±1.9 | 116±1.8 | 119±2.6* | 135±10.3** |
| 12 | 115±2.3 | 120±2.8* | 120±2.3* | 131±3.8* | 116±1.4 | 119±1.0** | 119±2.1* | 126± 9.6** |
| **Calcium (mg/dL)** | | | | | | | | |
| Pretest | 11.4±0.26 | 11.0±0.37 | 11.4±0.42 | 11.1±0.51 | 11.2±0.65 | 11.4±0.20 | 11.1±0.49 | 11.2±0.41 |
| 3 | 10.9±0.21 | 10.6±0.30 | 10.6±0.32 | 10.2±0.39** | 10.7±0.36 | 10.8±0.17 | 10.7±0.28 | 10.2±0.48 |
| 6 | 10.4±0.52 | 10.5±0.31 | 10.6±0.37 | 9.7±0.31* | 10.5±0.43 | 10.5±0.49 | 10.5±0.38 | 9.7±0.38** |
| 9 | 11.2±0.43 | 11.1±0.43 | 11.3±0.37 | 10.5±0.12** | 11.0±0.32 | 11.2±0.29 | 11.0±0.31 | 10.3±0.39** |
| 12 | 10.9±0.39 | 10.8±0.24 | 10.9±0.24 | 9.9±0.23** | 10.9±0.38 | 10.8±0.35 | 10.9±0.26 | 10.3±[illegible] |
| **Total Protein (g/dL)** | | | | | | | | |
| Pretest | 5.0±0.13 | 4.9±0.24 | 5.2±0.24 | 5.0±0.19 | 4.9±0.05 | 4.9±0.21 | 4.8±0.43 | 4.9±0.35 |
| 1 | 5.1±0.12 | 5.3±0.14* | 5.1±0.09 | 4.5±0.37** | 5.1±0.09 | 5.2±0.25 | 5.0±0.33 | 4.5±0.26** |
| 3 | 5.1±0.10 | 5.0±0.20 | 5.1±0.17 | 4.2±0.29** | 5.1±0.17 | 5.1±0.20 | 4.9±0.33 | 4.4±0.50** |
| 6 | 5.8±0.24 | 5.7±0.37 | 5.8±0.10 | 4.7±0.12** | 5.8±0.18 | 5.5±0.29 | 5.4±0.28 | 4.7±0.48** |
| 9 | 5.9±0.19 | 5.9±0.23 | 6.1±0.32 | 5.0±0.34** | 5.9±0.17 | 5.7±0.24 | 5.6±0.31 | 4.9±0.29** |
| 12 | 5.7±0.24 | 5.8±0.29 | 6.1±0.40 | 4.5±0.25** | 5.7±0.31 | 5.6±0.29 | 5.8±0.35 | 4.9±0.[illegible]** |
| **Albumin (g/dL)** | | | | | | | | |
| Pretest | 3.2±0.08 | 3.1±0.75 | 3.2±0.24 | 3.0±0.13 | 3.0±0.20 | 3.0±0.10 | 3.0±0.20 | 3.0±0.24 |
| 1 | 3.3±0.21 | 3.2±0.10 | 3.1±0.18* | 2.8±0.26 | 3.3±0.19 | 3.3±0.17 | 3.2±0.12 | 2.8±0.29** |
| 3 | 3.4±0.15 | 3.2±0.10 | 3.2±0.10 | 2.7±0.23** | 2.4±0.15 | 3.4±0.13 | 3.2±0.15 | 2.8±0.36** |
| 6 | 3.5±0.20 | 3.4±0.15 | 3.3±0.13 | 2.7±0.20** | 3.5±0.23 | 3.5±0.05 | 3.3±0.23 | 2.7±0.27** |
| 9 | 3.6±0.16 | 3.5±0.10 | 3.3±0.14* | 2.9±0.29** | 27.7±0.15 | 3.6±0.10 | 3.4±0.16* | 2.8±0.21** |
| 12 | 3.5±0.21 | 3.4±0.10 | 3.3±0.15* | 2.6±0.08** | 3.4±0.18 | 3.5±0.15 | 3.4±0.18 | 2.8±0.36** |

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

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

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TABLE 8. Cholinesterase Activity in Dogs Administered Naled for 1 Year

| Day of Study | Males (mg/kg/day) | | | | Females (mg/kg/day) | | | |
|---|---|---|---|---|---|---|---|---|
| | Control (µmol/mL/min) | 0.2 (Percent of Control) | 2.0 (Percent of Control) | 20.0 (Percent of Control) | Control (µmol/mL/min) | 0.2 (Percent of Control) | 2.0 (Percent of Control) | 20.0 (Percent of Control) |
| **Plasma Cholinesterase** | | | | | | | | |
| Pretest 1 | 7.2±0.87 | 85 | 103 | 93 | 7.5±1.10 | 89 | 95 | 83 |
| Pretest 2 | 7.0±1.58 | 76 | 103 | 87 | 7.3±0.90 | 99 | 94 | 86 |
| 7 | 6.0±1.16 | 80 | 85 | 67** | 6.2±0.94 | 82 | 73** | 68** |
| 30 | 6.3±0.97 | 83 | 76* | 59** | 6.7±0.95 | 79* | 60** | 60** |
| 91 | 3.9±0.89 | 78* | 68** | 61** | 6.2±0.90 | 79* | 63** | 63** |
| 180 | 6.6±0.96 | 79* | 68** | 61** | 7.5±1.00 | 72** | 56** | 52** |
| 270 | 6.0±0.91 | 78* | 68** | 63** | 6.8±1.01 | 78* | 56** | 50** |
| 360 | 6.6±0.96 | 83 | 65** | 56** | 7.9±0.86 | 70** | 52** | 53** |
| **Erythrocyte Cholinesterase** | | | | | | | | |
| Pretest 1 | 2.8±0.69 | 107 | 100 | 82 | 2.8±0.76 | 111 | 86 | 93 |
| Pretest 2 | 2.7±0.60 | 107 | 96 | 101 | 3.1±0.78 | 106 | 81 | 94 |
| 7 | 2.2±0.54 | 105 | 91 | 59 | 2.3±0.42 | 104 | 74 | 57*[a] |
| 30 | 2.3±0.69 | 96 | 57 | 30** | 2.5±0.72 | 96 | 48** | 24** |
| 91 | 2.3±0.51 | 109 | 57** | 30** | 2.6±0.46 | 96 | 46** | 23** |
| 180 | 2.7±0.68 | 93 | 48* | 22** | 3.0±0.59 | 92 | 37** | 20** |
| 270 | 3.2±0.60 | 103 | 47** | 28** | 3.3±0.76 | 94 | 36** | 15** |
| 360 | 3.1±0.67 | 84 | 42** | 19* | 3.1±0.71 | 94 | 42** | 23** |
| **Brain Cholinesterase** | | | | | | | | |
| 360 | 2.2±0.27 | 100 | 95 | 82** | 2.4±0.12 | 92 | 83* | 71** |

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

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

[a]Statistical analysis of erythrocyte cholinesterase was by nonparametric methods (i.e., rank transformation); analysis by report authors.

Results: There were no compound-related effects for any urinary parameters. All values in dosed groups were similar to controls.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

| | | | | | |
|---|---|---|---|---|---|
| | Digestive system | | Cardiovasc./Hemat. | | Neurologic |
| | Tongue | X | Aorta† | XX | Brain (fore, mid, and hind)† |
| X | Salivary glands† | XX | Heart† | X | Peripheral nerves† |
| X | Esophagus† | X | Bone marrow† | X | Spinal cord (3 level) |
| X | Stomach† | X | Lymph nodes† | XX | Pituitary† |
| X | Duodenum† | XX | Spleen† | X | Eyes (optic nerve)† |
| X | Jejunum† | X | Thymus† | | Glandular |
| X | Ileum† | | Urogenital | XX | Adrenals† |
| X | Cecum† | XX | Kidneys† | | Lacrimal gland |
| X | Colon† | X | Urinary bladder† | X | Mammary gland† |
| X | Rectum† | XX | Testes† | XX | Parathyroids† |
| XX | Liver† | X | Epididymides | XX | Thyroids† |
| X | Gallbladder† | X | Prostate | | Other |
| X | Pancreas† | | Seminal vesicle | X | Bone (femur)† |
| | Respiratory | XX | Ovaries | X | Skeletal muscle† |
| X | Trachea† | X | Uterus | X | Skin |
| X | Lung† | | | X | All gross lesions and masses |

Results:

a. Organ Weights: Mean weights and organ-to-body weight ratios of liver, kidney, and adrenal glands are presented in Table 9. There was a significant increase in the mean weight of liver and the liver-to-body weight ratio in both high-dose males and females. There was a significant increase in the weight of each kidney as well as an increase in the kidney-to-body weight ratios in high-dose females. There was a slight but nonsignificant ($p > 0.05$) increase in absolute and relative kidney weights in high-dose males when compared to controls. Both adrenals had increased mean weights ($p \leq 0.05$) in high-dose females; adrenal weights in high-dose males were increased but did not differ significantly from control. The report authors considered the increases in liver and kidney weight as related to dosing.

b. Gross Pathology: No compound-related macroscopic changes were seen. Edema of the intestinal mucosa was seen in three males and one female that received 20 mg/kg/day; however, this was not confirmed on histologic examination.

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†Recommended by Subdivision F (October 1982) guidelines for chronic studies.

TABLE 9. Selected Mean Body Weights, Organs, and Organ-to-Body Weight Ratios of Dogs Dosed Orally with Naled for 1-Year

| Dose Group (mg/kg/day) | Body Weight (g) | Left Kidney | | Right Kidney | | Liver | | Left Adrenal | | Right Adrenal | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Absolute (g) | Relative (%x10) | Absolute (g) | Relative (%x10) | Absolute (g) | Relative (%) | Absolute (g) | Relative (%x10) | Absolute (g) | Relative (%x10) |
| **Males** | | | | | | | | | | | |
| 0 | 13.0±3.01 | 24.66±5.8 | 1.93±0.38 | 25.33±5.5 | 1.96±0.23 | 293.95±60.14 | 2.29±0.38 | 0.64±0.13 | 4.96±0.64 | 0.64±0.12 | 4.82±0.60 |
| 0.2 | 12.7±2.00 | 27.94±8.3 | 2.16±0.37 | 27.35±9.1 | 2.12±0.46 | 280.52±54.56 | 2.20±0.23 | 0.61±0.07 | 4.87±1.10 | 0.65±0.11 | 5.23±1.15 |
| 2.0 | 14.4±3.05 | 27.22±3.4 | 1.94±0.26 | 26.89±3.2 | 1.92±0.27 | 334.92±51.78 | 2.38±0.30 | 0.64±0.07 | 4.65±1.01 | 0.64±0.10 | 4.64±1.28 |
| 20.0 | 13.2±1.67 | 30.79±4.2 | 2.36±0.36 | 29.22±3.0 | 2.25±0.38 | 395.66±35.78* | 3.02±0.263** | 0.74±0.11 | 5.68±0.87 | 0.78±0.06 | 5.97±0.86 |
| **Females** | | | | | | | | | | | |
| 0 | 10.3±2.08 | 18.87±2.4 | 1.85±0.19 | 18.49±2.3 | 1.82±0.20 | 232.33±44.77 | 2.26±0.26 | 0.59±0.10 | 5.77±0.81 | 0.57±0.13 | 5.68±1.69 |
| 0.2 | 10.1±1.46 | 21.06±3.6 | 2.08±0.23 | 20.77±3.8 | 2.05±0.22 | 224.21±28.86 | 2.22±0.13 | 0.63±0.08 | 6.37±1.48 | 0.63±0.06 | 6.41±1.41 |
| 2.0 | 10.6±3.03 | 23.79±8.3 | 2.24±0.36* | 22.14±4.3 | 2.15±0.38 | 259.75±51.05 | 2.51±0.36 | 0.61±0.11 | 6.23±2.39 | 0.60±0.10 | 6.01±1.92 |
| 20.0 | 10.7±0.80 | 26.58±2.8** | 2.49±0.23** | 25.12±3.3* | 2.35±0.26** | 335.61±27.74** | 3.14±0.23** | 0.76±0.11* | 7.09±0.74 | 0.75±0.12* | 7.01±0.82 |

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

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

c. Microscopic Pathology (Nonneoplastic): The incidences of lesions that were increased in dosed groups when compared to controls are summarized in Table 10. None of these lesions were considered "toxicologically significant." In males, the spontaneous lesions were esophageal inflammation, foci of mineralization of the spinal cord, splenic siderosis, and testicular degeneration. In females, the lesions were foci of mineralization in the spinal cord and splenic siderosis. Trace mineralization of the kidneys was a common finding in both sexes. The high-dose male that was sacrificed in extremis during week 50 had diffuse gastric mucosal fibrosis. This lesion correlated with a reduced gastric function and a maldigestive syndrome that was evidenced by anorexia and weight loss observed.

Neoplastic: There were no neoplastic nor preneoplastic lesions.

STUDY AUTHORS CONCLUSIONS:

In dogs dosed with naled technical at 2.0 and 20.0 mg/kg/day (mid- and high-dose), there were several compound-related effects noted throughout the 1-year study. There was an increased incidence of soft stool and/or diarrhea and emesis; however, this did not affect weight gain. Erythrocyte counts and hemoglobin and hematocrit values were decreased at various intervals in both males and females in the mid- and high-dose groups, and platelets were increased in high-dose males when compared to controls. Decreased total plasma protein and globulin levels and slight decreases in calcium levels were seen in both sexes at the high dose. Plasma cholinesterase activities were depressed in the mid- and high-dose males and females and there was a dose-related significant decrease in erythrocyte cholinesterase activity in the same groups. At 12 months, brain cholinesterase activity was depressed for mid- and high-dose females and for high-dose males. There was a slight depression of plasma cholinesterase activity at 0.2 mg/kg/day but it was not considered of toxicologic importance. There were increases in mean absolute weights of liver and kidney and in the organ-to-body weight ratios of these organs in both sexes receiving 20.0 mg/kg/day; however, these weight changes were not accompanied by macroscopic or microscopic findings. There were no compound-related nonneoplastic lesions and no neoplasms were found. The LOEL was determined by the study authors to be 2.0 mg/kg/day and the NOEL to be 0.2 mg/kg/day.

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TABLE 10. Selected Nonneoplastic Findings in Dogs Dosed Orally with Naled for 1 Year

| Organ/Finding | Dosage (mg/kg/day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | | | Females | | | |
| | 0 | 0.2 | 2.0 | 20 | 0 | 0.2 | 2.0 | 20.0 |
| Esophagus | (6)[a] | (6) | (6) | (6) | (6) | (6) | (6) | (6) |
| Inflammation | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 0 |
| Spleen | (6) | (6) | (6) | (6) | (6) | (6) | (6) | (6) |
| Siderosis | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 1 |
| Spinal cord | (6) | (6) | (6) | (6) | (6) | (6) | (6) | (6) |
| Mineralization | | | | | | | | |
| Cervical | 0 | 1 | 1 | 3 | 1 | 1 | 0 | 1 |
| Lumbar | 1 | 3 | 5 | 4 | 0 | 1 | 2 | 4 |
| Thoracic | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 1 |
| Testis | (6) | (6) | (6) | (6) | | | | |
| Degeneration, trace | 0 | 0 | 2 | 3[b] | | | | |
| Lung | (6) | (6) | (6) | (6) | (6) | (6) | (6) | (6) |
| Perivasculitis | 2 | 0 | 4 | 1 | 1 | 0 | 0 | 1 |
| Kidney | (6) | (6) | (6) | (6) | (6) | (6) | (6) | (6) |
| Trace mineralization | 1 | 6 | 4 | 3 | 4 | 6 | 4 | 5 |
| Liver | (6) | (6) | (6) | (6) | (6) | (6) | (6) | (6) |
| Capsular fibrosis | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Inflammation | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |

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

[b]Moderate in one animal.

REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were adequate and adhered to Guideline requirements. The emesis noted in the clinical observations may have been due, in part, to the gavage procedure or the vehicle used since it also occurred in control dogs. This did not adversely affect the weight gain in the dogs. There was one moribundity in a high-dose male that was not related to dosing but was probably due to a preexisting gastric malfunction, which was not of sufficient severity to be identified in the pretest examination. This lesion, identified histologically as ulcerative gastritis with fibrosis of the gastric mucosa, was not found in other high-dose dogs so is of doubtful toxicologic importance. The anemia seen in the dogs receiving 2.0 and 20.0 mg/kg/day and the increased platelet counts in high-dose males and females did not seriously affect the general health of these dogs or result in histopathologic changes. These effects appear to be compound-related, however, as were the decreases in plasma protein and globulin observed in high-dose dogs.

We assess that the study authors correctly interpreted that the statistically significant decreases in plasma cholinesterase activities observed in both males and females at the low dose (0.2 mg/kg/day) were not of biological significance. The activities were generally about 80 percent of the control activity. It was noted, however, that the pretest activities in the groups receiving 0.2 mg/kg/day were lower than in the control group. The pretest values were 6.1 and 5.3 µmol/mL/min in males receiving 0.2 mg/kg/day when compared to 7.2 and 7.0 µmol/mL/min in pretest controls, and 6.7 and 6.9 µmol/mL/min in low-dose females when compared to pretest values of 7.5 and 7.3 µmol/mL/min in controls. The activities of plasma cholinesterase at 12 months, expressed as percent of pretest activity, were 96.5 and 80.9 percent in low-dose males and females, respectively.

The significant increases in mean kidney and liver weights in high-dose males and females at 12 months were not accompanied by any histologic changes or clinical chemistry correlations. The toxicologic significance of these finding is not known but cannot be dismissed. There were no histologic changes clearly related to dosing. Trace testicular degeneration was found in two males each receiving 2.0 and 20 mg/kg/day and moderate degeneration in one male receiving 20 mg/kg/day. This is a common finding of aging and its toxicologic importance is questionable.

We assess that based on depression of brain and erythrocyte cholinesterase activities and anemic changes in both sexes the LOEL for the study should be 2.0 mg/kg/day and that the NOEL is 0.2 mg/kg/day.

EPA: 68-02-4225
DYNAMAC No. 2678
March 5, 1987

# DATA EVALUATION RECORD

## NALED

## Twenty-Eight Day Dermal Study in Rats

APPROVED BY:

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

Signature: William L. McLellan for

Date: 3-5-87

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EPA: 68-02-4225
DYNAMAC No. 247B
March 5, 1987

DATA EVALUATION RECORD

NALED

Twenty-Eight Day Dermal Study in Rats

REVIEWED BY:

Margaret E. Brower, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Margaret E Brower
Date: 3/5/87

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 3-5-87

APPROVED BY:

I. Cecil Felkner, Ph.D.
Subchronic and Chronic Studies
Department Manager
Technical Quality Control
Dynamac Corporation

Signature: William L. McLellan for
Date: 3-5-87

Irving Mauer, Ph.D.
EPA Reviewer, Section VI
(TS-769C)

Signature:
Date: 03-09-87

Judith Hauswirth, Ph.D.
Acting EPA Section Head, Section VI
(TS-769C)

Signature: Judith W. Hauswirth
Date: 3/9/87

DATA EVALUATION REPORT

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Twenty-eight day dermal study in rats.

ACCESSION NUMBER: 263583.

TEST MATERIAL: Naled, technical; 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate.

SYNONYMS: Dibrom.

STUDY NUMBER(S): 85-2981.

SPONSOR: Chevron Environmental Health Center, Inc., Richmond, CA.

TESTING FACILITY: Bio/dynamics Inc., East Millstone, NJ.

TITLE OF REPORT: A Twenty-Eight Day Dermal Study with Naled Technical in Rats.

AUTHOR(S): Rausina, G. A., and Zimmerman, R. A.

REPORT ISSUED: May 2, 1986.

CONCLUSIONS:

Under the conditions of the study, severe dermal irritation and necrosis were found to occur after repeated dermal exposure to naled at dose levels of 20 and 80 mg/kg/day for 28 days. There were no dermal effects at 1 mg/kg/day. Plasma, erythrocyte, and brain cholinesterase activities were significantly inhibited in males and females receiving 20 and 80 mg/kg/day. Mean body weights of mid- and high-dose males were significantly decreased from day 7 to study termination. Decreased erythrocyte counts and hemoglobin and hematocrit values and increased reticulocyte counts were seen in high-dose males and females. Plasma protein and albumin levels were decreased and blood urea nitrogen (BUN) levels and BUN/creatinine ratios were increased in these same groups. Absolute and relative adrenal weights were found to be increased in high-dose males and females; high-dose females also displayed increased absolute and relative liver weights. The LOEL is 20 mg/kg/day and the NOEL is 1 mg/kg/day based on dermal response and plasma, erythrocyte, and brain cholinesterase activity inhibition.

Classification: Core Minimum.

A. MATERIALS:

1. Test Compound: Naled, technical; description: straw-colored liquid, lot No. SX-1655; purity: 90 percent.

2. Test Animals: Species: rats; strain: CD/Sprague Dawley; age: 7 weeks at initiation; mean weights: males--197-225 g, females--144-169 g; source: Charles River Breeding Laboratories, Inc., Wilmington, MA.

B. STUDY DESIGN:

1. Animal Assignment: After 9 days of acclimation, animals were assigned to the following test groups using a computer-generated randomization scheme:

| Test Group | Dosage Level (mg/kg/day) | Dosage Volume (mL/kg/day) | Main Study (28 Days) Males | Females |
|---|---|---|---|---|
| 1 Control | 0 | 1.5 | 12 | 12 |
| 2 Low (LDT) | 1 | 1.5 | 12 | 12 |
| 3 Mid (MDT) | 20 | 1.5 | 12 | 12 |
| 4 High (HDT) | 80 | 1.5 | 12 | 12 |

Animals were housed in an environmentally controlled room with a 12-hour light/dark cycle.

Rationale for Dose Selection: The dose levels selected for this study were based on a range-finding study in which rats were dosed via dermal application at levels of 0, 50, 120, or 300 mg/kg/day for 4 consecutive days. Erythema and atonia of the skin were found in all (4/4) males and females receiving 300 mg/kg/day, 2 of 4 males receiving 120 mg/kg/day, and 2 of 4 and 3 of 4 females receiving 50 and 120 mg/kg/day, respectively.

2. Test Material Preparations: Due to the rapid decomposition rate of the test material in aqueous media, dosing suspensions were prepared daily and used within 3 hours of preparation. A suspension of naled in a 0.5 percent aqueous solution of carboxymethylcellulose was prepared using a Waring blender; this stock solution was diluted with the vehicle so that the appropriate dosage was administered in a volume of 1.5 mL/kg body weight. Samples of the high- and low-dosing suspensions were provided to the sponsor for homogeneity analysis on each day of dosing; all dosing suspensions were provided to the sponsor for analysis of test material concentrations and stability. Samples were frozen and maintained in the frozen state during transit. The test material was found to be stable if refrigerated at 4°C; direct sunlight and contact with metallic surfaces were avoided. Results of the analyses performed by the sponsor were not reported.

3. Preparation of Animal Skin: Prior to dosing, the hair was removed from the scapular region of the back of each animal by clipping. Animals were reclipped when necessary throughout the study period. The test material, in a volume of 1.5 mL/kg, was applied to the clipped area and uniformly spread over the application site once per day, 5 days/week, for a total of 20-21 applications in 29-30 days. Individual doses were adjusted by most recent body weight. The area was covered and wrapped with an occlusive bandage for 6 hours, after which time the test site was wiped with moistened gauze. Application sites were alternated between the shoulder and an area caudal to the shoulder on a daily basis. Control animals were treated with the vehicle control formulation (without active ingredient) in volumes equivalent to those applied on test animals.

4. Food and Water: Animals received food (Purina Certified Rodent Chow No. 5002) and water ad libitum.

5. Statistics: Body weights, food consumption, clinical pathology parameters, and organ weights were analyzed using Bartlett's test to determine equality of variances. The analysis of variance was used if parametric procedures were indicated; Dunnett's test was used if significant differences were found among these parameters. The Kruskal-Wallis test was used if nonparametric procedures were indicated; the ranked sum test was used if significant differences were found. Standard regression techniques were used for parametric trends, Jonckheere's test was used for nonparametric trends.

6. Quality Assurance: A quality assurance statement was signed and dated April 29, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for mortality and signs of overt toxicity. Detailed physical examinations and dermal evaluations were conducted pretest and on days 2, 5, 9, 12, 17, 19, 22, 26, and 28 or 29.

Results: There were no mortalities during the study. Clinical signs found in dosed animals were of low incidence and were transient. Soft stool was found in one low-dose and two high-dose males on day 9; anogenital staining was found in two mid-dose females on this same day. Coarse or fine tremors were observed in one low-dose, one mid-dose, and two high-dose females on days 2, 5, or 9 for a total of four observations. The authors reported these signs to be similar to those found in the pilot study and to be possibly related to dosing; however, they were considered of little toxicological significance because of their low incidence and transient nature.

Severe dermal irritation and tissue destruction were found in all males and females receiving 20 and 80 mg/kg/day naled technical (Tables 1A and 1B). Mid- and high-dose males and females exhibited atonia and moderate to severe erythema and edema within 9 days after test initiation. All of the animals of these test groups also developed necrosis and eschar formation, accompanied in many animals by fissuring and exfoliation. Onset of dermal irritation was reported at day 2 in high-dose males as compared to day 9 in mid-dose males; otherwise, incidence and severity were similar between these groups. Onset of dermal irritation began to appear at day 2 for mid- and high-dose females, although the number of females exhibiting irritation at this time was not as pronounced as in the group of high-dose males. Incidence and severity of irritation were similar between mid- and high-dose females. No dermal irritation was found in control or low-dose males or females.

2. Body Weight: Rats were weighed 5 days prior to dosing, on days 1, 5, 9, 12, 15, 19, 22, 25, and 28, and at termination.

Results: Mean body weights of mid- (4-7% lower than control weights) and high-dose (14-18% lower than controls) males were found to be significantly ($p < 0.05$) decreased when compared to controls from day 7 to study termination (Table 2). The body weights of low-dose males and all females were comparable to control weights.

3. Food Consumption and Compound Intake: Food consumption was determined weekly beginning 1 week prior to dosing.

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TABLE 1A. Summary of Dermal Observations[a] in Male Rats Administered Naled for 28 Days

| Dosage Group (mg/kg/day) | Observation | Test Day 2 | 5 | 9 | 12 | 17 | 19 | 22 | 26 | 28/29 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Erythema | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Edema | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Atonia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Fissuring | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Eschar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Exfoliation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Necrosis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | Erythema | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Edema | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Atonia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Fissuring | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Eschar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Exfoliation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Necrosis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | Erythema | 0 | 0 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Edema | 0 | 0 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Atonia | 0 | 0 | 11 | 11 | 11 | 11 | 11 | 12 | 12 |
| | Fissuring | 0 | 0 | 3 | 3 | 3 | 1 | 0 | 10 | 8 |
| | Eschar | 0 | 0 | 8 | 8 | 8 | 10 | 10 | 12 | 12 |
| | Exfoliation | 0 | 0 | 2 | 1 | 3 | 2 | 2 | 8 | 6 |
| | Necrosis | 0 | 0 | 11 | 11 | 11 | 11 | 11 | 12 | 12 |
| 80 | Erythema | 9 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Edema | 11 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Atonia | 0 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Fissuring | 0 | 3 | 5 | 4 | 4 | 6 | 6 | 2 | 3 |
| | Eschar | 0 | 0 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Exfoliation | 0 | 0 | 2 | 0 | 3 | 3 | 3 | 11 | 11 |
| | Necrosis | 0 | 2 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |

[a]Number presented represents the number of rats in each group (based on a total of 12 rats/group) exhibiting a given observation.

TABLE 1B. Summary of Dermal Observations[a] in Female Rats Administered Naled for 28 Days

| Dosage Group (mg/kg/day) | Observation | Test Day 2 | 5 | 9 | 12 | 17 | 19 | 22 | 26 | 28/29 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Erythema | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Edema | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Atonia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Fissuring | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Eschar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Exfoliation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Necrosis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | Erythema | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Edema | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Atonia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Fissuring | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Eschar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | Exfoliation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Necrosis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | Erythema | 2 | 11 | 12 | 12 | 12 | 11 | 12 | 12 | 11 |
| | Edema | 0 | 10 | 12 | 12 | 12 | 11 | 12 | 12 | 12 |
| | Atonia | 0 | 10 | 12 | 12 | 12 | 11 | 12 | 11 | 11 |
| | Fissuring | 0 | 0 | 3 | 11 | 11 | 4 | 7 | 2 | 2 |
| | Eschar | 0 | 0 | 10 | 12 | 12 | 11 | 12 | 11 | 10 |
| | Exfoliation | 0 | 0 | 3 | 12 | 10 | 7 | 12 | 6 | 4 |
| | Necrosis | 0 | 1 | 12 | 12 | 12 | 11 | 12 | 11 | 10 |
| 80 | Erythema | 4 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Edema | 4 | 8 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Atonia | 0 | 8 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Fissuring | 0 | 3 | 3 | 10 | 9 | 9 | 10 | 9 | 7 |
| | Eschar | 0 | 0 | 11 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Exfoliation | 0 | 0 | 2 | 12 | 12 | 12 | 12 | 12 | 12 |
| | Necrosis | 0 | 1 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |

[a]Number presented represents the number of rats in each group (based on a total of 12 rats/group) exhibiting a given observation.

TABLE 2. Representative Results of Mean Body Weights (±SD) of Rats Treated with Naled for 28 Days[a] 005774

| Dosage Group (mg/kg/day) | Mean Body Weights (g) on Day | | | |
|---|---|---|---|---|
| | 1 | 7 | 14 | 28 |
| | | | Males | |
| 0 | 211.0±8.1 | 271.0±12.1 | 302.5±13.3 | 338.7±16.2 |
| 1 | 211.4±8.1 | 268.9± 9.0 | 302.3±12.3 | 333.3±15.5 |
| 20 | 211.1±8.6 | 259.6±12.2* | 287.0±15.7* | 316.3±20.0* |
| 80 | 211.4±8.8 | 231.7±10.3** | 256.7± 9.4** | 276.9±19.8** |
| | | | Females | |
| 0 | 155.0±6.6 | 180.8± 7.6 | 192.2±12.0 | 205.7±11.0 |
| 1 | 155.3±7.0 | 176.9± 7.1 | 193.5± 9.5 | 210.0±10.3 |
| 20 | 155.1±6.8 | 173.6± 9.8 | 191.8± 9.9 | 205.9±10.9 |
| 80 | 154.8±6.8 | 175.5± 8.6 | 196.0±10.1 | 205.2±10.0 |

[a]Based on 12 rats/sex/group.

* Significantly different from control value ($p < 0.05$) as evaluated by the study authors using Dunnett's test.

** Significantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunnett's test.

Results: Mean food consumption values were found to be significantly ($p < 0.01$) increased for males and females receiving 80 mg/kg/day from days 21 through study termination when compared to controls (Table 3). The study authors reported that this increase in high-dose males may be a result of the decreased body weight in that group; however, this body weight decrease was not found in females. The food consumption of males receiving 20 mg/kg/day was also found to be significantly ($p < 0.05$) increased on days 7 and 28 and that of males receiving 1 mg/kg/day on day 28. These latter changes were not found to be consistent over time.

4. Ophthalmological examinations (pupillary response) were performed 6 hours after test material application on days 1, 9, 18, 22, and 28 or 29.

   Results: One female in the mid-dose group had a negative pupil response on days 22 and 28, and one low-dose female had a unilateral corneal opacity from day 5 through study termination. These effects were not considered to be of toxicological significance.

5. Blood was collected via venipuncture of the orbital sinus at study termination for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

   a. Hematology

| | | | |
|---|---|---|---|
| X | Hematocrit (HCT)† | | Total plasma protein (TP) |
| X | Hemoglobin (HGB)† | X | Leukocyte differential count |
| X | Leukocyte count (WBC)† | | Mean corpuscular HGB (MCH) |
| X | Erythrocyte count (RBC)† | | Mean corpuscular HGB concentration (MCHC) |
| X | Platelet count† | | Mean corpuscular volume (MCV) |
| X | Reticulocyte count | X | Erythrocyte morphology |

†Recommended by Subdivision F (October 1982) Guidelines.

Results: Erythrocyte counts (RBC), hemoglobin concentration (HGB), and hematocrit (HCT) were found to be slightly but nonsignificantly ($p < 0.05$) decreased in females receiving 80 mg/kg/day when compared to controls; HGB and RBC counts were slightly but nonsignificantly ($p < 0.05$) decreased in males at this dose level. Reticulocyte counts were found to be significantly ($p < 0.05$) increased in high-dose males and females when compared to controls (Table 4). The study authors considered these observations to be suggestive of increased erythropoiesis in high-dose males and females. Platelet counts were slightly increased in high-dose males and significantly ($p < 0.01$) increased in high-dose females when compared to controls; there were no effects on platelets at 1 or 20 mg/kg/day (Table 4). The total leukocyte count (WBC) was found to be significantly ($p < 0.05$) decreased in low- and mid-dose females; however, this was not dose related and no change in WBC was found in males. This effect was not considered to be compound related.

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TABLE 3. Representative Mean Food Consumption (±SD) Values of Rats Dosed with Naled for 28 Days[a]

| Dosage Group (mg/kg/day) | Mean Food Consumption (g/kg/day) on Day 1 | 7 | 14 | 28 |
|---|---|---|---|---|
| | | Males | | |
| 0 | 102.2± 5.8 | 91.9±3.0 | 93.6± 5.9 | 79.3±2.1 |
| 1.0 | 105.1± 4.5 | 94.6±3.1 | 92.7± 4.4 | 85.7±5.5** |
| 20.0 | 100.3± 5.1 | 98.0±3.2** | 92.8± 4.2 | 85.3±3.8* |
| 80.0 | 101.5± 4.8 | 93.2±7.8 | 95.0± 5.4 | 94.0±7.1** |
| | | Females | | |
| 0 | 110.8± 8.0 | 102.2±8.1 | 100.6± 7.0 | 97.3±8.4 |
| 1.0 | 104.2±10.3 | 101.3±6.8 | 108.7±14.2 | 97.2±7.0 |
| 20.0 | 109.6± 9.1 | 107.8±8.2 | 100.8± 8.3 | 96.2±5.0 |
| 80.0 | 107.8± 8.5 | 95.6±7.9 | 109.8± 8.1 | 108.9±9.7**[b] |

[a]Based on 12 rats/sex/group.

* Significantly different from control value ($p < 0.05$) as evaluated by the study authors using Dunn's Rank Sum Test.

** Significantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunn's Rank Sum Test.

**[b] Significantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunnett's Test.

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TABLE 4. Representative Mean Hematology Values (± SD) at Selected Intervals in Rats Dosed with Naled for 28 Days[a]

| | Males/Dosage (mg/kg/day) | | Females/Dosage (mg/kg/day) | |
|---|---|---|---|---|
| Parameter/Day | 0 | 80 | 0 | 80 |
| Platelets ($\times 10^5/\mu L$) | 10.09±1.45 | 11.56±2.18 | 10.74±1.25 | 12.86±1.62** |
| Reticulocytes (% RBC) | 0.2±0.1 | 0.5±0.3* | 0.2±0.1 | 0.5±0.3** |

[a]Based on 12 animals/sex/group with the exception of the reticulocyte count in high-dose females in which 10 animals were evaluated.

*Significantly different from control value ($p < 0.05$) as evaluated by the study authors using Dunnett's test.

**Significantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunnett's test.

b. Clinical Chemistry

| Electrolytes | | Other | |
|---|---|---|---|
| X | Calcium† | X | Albumin† |
| X | Chloride† | X | Blood creatinine† |
| | Magnesium† | X | Blood urea nitrogen† (BUN) |
| X | Phosphorus† | X | BUN/creatinine ratio |
| X | Potassium† | X | Cholesterol† |
| X | Sodium† | X | Globulins (calculated) |
| | | X | Glucose† |
| Enzymes | | X | Total bilirubin† |
| X | Alkaline phosphatase (ALP) | X | Direct bilirubin |
| X | Cholinesterase (brain, RBC, plasma)* | X | Total protein† |
| | | X | Triglycerides |
| X | Creatine phosphokinase† | X | Uric acid |
| X | Lactic acid dehydrogenase† | | |
| X | Serum alanine aminotransferase (also SGPT) | | |
| X | Serum aspartate aminotransferase (also SGOT) | | |

†Recommended by Subdivision F (October 1982) Guidelines.
*At the sponsor's request, plasma cholinesterase determinations were performed using two different methods.

Results: Representative clinical chemistry values are presented in Tables 5A and 5B. Significantly increased ($p < 0.01$) blood-urea-nitrogen (BUN) values and BUN/creatinine ratios were found in males and females receiving 80 mg/kg/day/day; creatinine was decreased in both groups and was significant ($p < 0.01$) in females. The study authors considered this trend to be suggestive of an alteration in renal function at the high dose. Total protein and albumin were also found to be significantly ($p < 0.01$) decreased in high-dose males and females; albumin was slightly but significantly ($p < 0.01$) decreased in males receiving 20 mg/kg/day. Globulin values in dosed animals were comparable to controls. A decrease in glucose in mid- and high-dose males, a decrease in calcium in high-dose males, and an increase in cholesterol and chloride values in this same group were stated to be of doubtful toxicologic significance, possibly a result of the decrease in body weight seen in these animals. These changes were not found in females, although an increase in phosphorus was found in high-dose females when compared to controls.

Cholinesterase activity data are summarized in Tables 5A and 5B. Plasma cholinesterase (PCHE) activities were found to be markedly ($p < 0.01$) depressed in both males and females in the 20- and 80 mg/kg/day groups at study termination. The depression of activity was dose related. PCHE activities in mid- and high-dose males were 54 and 36 percent of control values, respectively; PCHE activities in mid- and high-dose females were 47 and 17

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TABLE 5A. Representative Mean Clinical Chemistry Values (±SD) of Male Rats Dosed with Naled for 28 Days[a]

| Parameter | Dosage Group (mg/kg/day) 0 | 1 | 20 | 80 |
|---|---|---|---|---|
| BUN[b] (mg/dL) | 14.1±2.7 | 13.9±2.8 | 15.7±2.2 | 19.8±4.3** |
| Creatinine (mg/dL) | 0.5±0.1 | 0.5±0.1 | 0.5±0.1 | 0.4±0.1 |
| BUN/Creatinine | 32.2±10.2 | 33.9±13.7 | 33.3±9.9 | 48.2±18.4* |
| Glucose (mg/dL) | 72±8 | 69±11 | 57±6** | 52±7** |
| Total Protein (g/dL) | 5.9±0.3 | 5.7±0.2 | 5.8±0.3 | 5.3±0.3** |
| Albumin (g/dL) | 3.6±0.1 | 3.5±0.1 | 3.4±0.2** | 2.9±0.1** |
| Cholesterol (mg/dL) | 48±12 | 46±11 | 50±10 | 60±13* |
| Chloride (mEq/L) | 100±1.0 | 101±1.0 | 101±2.0 | 104±2** |
| Calcium (mg/dL) | 10.3±0.2 | 10.1±0.3 | 10.0±0.3 | 9.7±0.2** |
| RCHE (IU/mL) | 5.8±1.0 | 5.8±1.0(100)[c] | 4.6±1.0*(79) | 4.8±1.2(83) |
| PCHE (IU/mL) | 0.314±0.031 | 0.302±0.054(89) | 0.191±0.45**(54) | 0.136±0.025**(36) |
| BCHE (IU/mL) | 9.7±0.6 | 9.7±0.5(100) | 3.9±0.2**[d](40) | 2.9±0.2**[d](30) |

[a]Based on 12 animals/group.

[b]Abbrevations: BUN = blood urea nitrogen; RCHE = red cell cholinesterase; PCHE = plasma cholinesterase; BCHE = brain cholinesterase.

[c]Numbers in parentheses represent percentage of control.

*Significantly different from control value ($p < 0.05$) as evaluated by the study authors using Dunnett's test.

**Significantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunnett's test.

**[d]Significantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunn's Rank Sum Test.

TABLE 5B. Representative Mean Clinical Chemistry Values (±SD) of Female Rats Dosed with Naled for 28 Days[a]

| Parameter | Dosage Group (mg/kg/day) 0 | 1 | 20 | 80 |
|---|---|---|---|---|
| BUN[b] (mg/dL) | 16.5±2.6 | 15.4±1.9 | 16.6±2.0 | 19.8±3.6* |
| Creatinine (mg/dL) | 0.5±0.1 | 0.5±0.1 | 0.4±0.1 | 0.4±0.1** |
| BUN/Creatinine | 32.9±6.2 | 32.8±5.5 | 41.3±4.9 | 60.0±20.7**e |
| Total Protein (g/dL) | 6.1±0.3 | 6.2±0.3 | 6.0±0.3 | 5.5±0.3** |
| Albumin (g/dL) | 3.5±0.1 | 3.6±0.2* | 3.5±0.2 | 3.0±0.1** |
| Phosphorus (mg/dL) | 7.3±0.6 | 7.1±1.0 | 7.5±0.6 | 8.8±1.3** |
| RCHE (IU/mL) | 5.2±0.8 | 4.8±0.5(92)[c] | 3.9±0.8**(75) | 3.7±0.9**(71) |
| PCHE (IU/mL) | 0.925±0.282 | 1.263±0.482(136) | 0.462±0.164*d(47) | 0.207±0.039**e(17) |
| BCHE (IU/mL) | 10.0±0.6 | 9.8±0.6(98) | 4.0±0.4**(40) | 3.1±0.2**(31) |

[a]Based on 12 animals/group.

[b]Abbreviations: BUN = blood urea nitrogen; RCHE = red cell cholinesterase; PCHE = plasma cholinesterase; BCHE = brain cholinesterase.

[c]Numbers in parentheses represent percentage of control.

*Significantly different from control value ($p < 0.05$) as evaluated by the study authors using Dunnett's test.

**Significantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunnett's test.

*dSignificantly different from control value ($p < 0.05$) as evaluated by the study authors using Dunn's Rank Sum Test.

**eSignificantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunn's Rank Sum Test.

percent of control values. Pretest activity was not measured. There was no effect in the PCHE activities of low-dose males and females.

Red cell cholinesterase (RCHE) activities were depressed in males and females in the 20- and 80-mg/kg/day groups. This depression was significant in mid-dose males ($p < 0.05$) and mid- and high-dose females ($p < 0.01$). The depression of activity was dose related in females where the activity was 92, 75, and 71 percent of control values in 1-, 20-, and 80-mg/kg/day groups.

Brain cholinesterase (BCHE) activities were significantly ($p < 0.01$) depressed in mid- and high-dose males and females. The BCHE activities in mid- and high-dose males were 40 and 30 percent of control values, respectively; BCHE activities in mid- and high-dose females were 40 and 31 percent of control values, respectively. There was no effect in the BCHE activities of low-dose males and females.

6. Urinalyses: Urinalyses were not performed.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

| | Digestive system | | Cardiovasc./Hemat. | | Neurologic |
|---|---|---|---|---|---|
| | Tongue | | Aorta† | XX | Brain† |
| | Salivary glands† | | Heart† | | Peripheral nerves† |
| | Esophagus† | | Bone marrow† | | Spinal cord (3 level) |
| | Stomach† | | Lymph nodes† | | Pituitary† |
| | Duodenum† | X | Spleen† | | Eyes (optic nerve)† |
| | Jejunum† | | Thymus† | | Glandular |
| | Ileum† | | Urogenital | XX | Adrenals† |
| | Cecum† | XX | Kidneys† | | Lacrimal gland |
| | Colon† | | Urinary bladder† | | Mammary gland† |
| | Rectum† | XX | Testes† | | Parathyroids† |
| XX | Liver† | | Epididymides | | Thyroids† |
| | Gall bladder† | | Prostate | | Other |
| | Pancreas† | | Seminal vesicle | | Bone (femur)† |
| | Respiratory | XX | Ovaries | | Skeletal muscle† |
| | Trachea† | | Uterus† | X | Skin (treated and untreated) |
| X | Lung† | | Vagina | X | All gross lesions and masses |

†Recommended by Subdivision F Guidelines (October 1982).

Histopathology was performed on selected tissues of control and high-dose animals, with the exception of the skin, which was examined histologically for all animals. Due to technical problems, only the brain weight was recorded for one high-dose female (animal No. 4515).

Results:

a. Organ Weights: Mean weights of brain, adrenal, liver, and kidney and organ-to-body and organ-to-brain weight ratios are presented in Tables 6A and 6B. Mean liver weights and liver-to-body and liver-to-brain weight ratios for high-dose females were found to be significantly increased ($p < 0.01$) when compared to controls. These differences were reported to be compound related by the study authors. Kidney weights and kidney-to-body and kidney-to-brain weight ratios of high-dose females were slightly increased; these increases were nonsignificant. Adrenal weights and adrenal-to-body and adrenal-to-brain weight ratios were found to be increased in high-dose males and females; most of these changes were found to be significant ($p < 0.05$). Liver and kidney weights of males receiving 20 mg/kg/day and liver, kidney, and testicular weights of males receiving 80 mg/kg/day were decreased when compared to controls; these differences were significant ($p < 0.05$) for liver and kidney weights. These differences were considered by the study authors to be consistent with the reduced body weights in these groups and were not considered to be compound related.

b. Gross Pathology: Dermal observations at study termination included erythema, eschar, atonia, necrosis, exfoliation, and fissures of both mid- and high-dose males and females (Table 7). There were no dermal changes found in controls or low-dose males and females. Other sporadic gross pathological changes were not considered to be compound related.

c. Microscopic Pathology: Compound-related dermal changes (rated moderate to moderately severe) were found in males and females receiving 20 and 80 mg/kg/day (Table 8). Minimal to mild inflammatory response of the surface skin was found in 3 out of 12 females receiving 1 mg/kg/day naled. There were no dermal histologic changes found in controls or low-dose males. Other sporadic histologic changes were not considered to be compound related.

D. STUDY AUTHORS' CONCLUSIONS:

Dermal administration of naled technical at dosages of 20 and 80 mg/kg/day (mid and high doses) produced severe dermal irritation and significant ($p < 0.01$) dose-related cholinesterase inhibition (plasma, erythrocyte, and brain) in male and female CD/Sprague Dawley rats. Mean body weights of males receiving 20 and 80 mg/kg/day were decreased from day 7 to study termination when compared to controls.

TABLE 6A. Selected Organ Weights and Organ/Body, and Organ/Brain Weight Ratios in Rats Dosed with Naled for 28 Days[a]

| Dosage Group (mg/kg/day) | Brain | | Right Adrenal | | | Left Adrenal | | |
|---|---|---|---|---|---|---|---|---|
| | Absolute | Relative | Absolute | Relative | | Absolute | Relative | |
| | | | | Body | Brain | | Body | Brain |
| | (g) | (%) | (g) | (%) | (%) | (g) | (%) | (%) |
| **Males** | | | | | | | | |
| 0 | 1.92±0.098 | 6.13±0.34 | 0.027±0.004 | 8.73±1.24 | 1.43±0.21 | 0.033±0.007 | 1.05±0.25 | 1.71±0.39 |
| 1 | 1.92±0.114 | 6.21±0.47 | 0.027±0.004 | 8.72±1.47 | 1.41±0.26 | 0.028±0.003 | 0.91±0.10 | 1.48±0.19 |
| 20 | 1.90±0.077 | 6.43±0.43 | 0.027±0.004 | 8.99±1.03 | 1.40±0.19 | 0.032±0.007 | 1.08±0.31 | 1.68±0.40 |
| 80 | 1.84±0.086 | 7.08±0.62** | 0.033±0.005* | 12.53±1.70** | 1.78±0.25** | 0.034±0.004 | 1.32±0.16**[b] | 1.8/±0.21 |
| **Females** | | | | | | | | |
| 0 | 1.80±0.094 | 9.37±0.87 | 0.033±0.005 | 1.73±0.27 | 1.85±0.27 | 0.035±0.005 | 1.82±0.26 | 1.95±0.29 |
| 1 | 1.79±0.063 | 9.14±0.65 | 0.032±0.004 | 1.64±0.19 | 1.81±0.25 | 0.033±0.004 | 1.68±0.15 | 1.84±0.23 |
| 20 | 1.74±0.041 | 9.02±0.53 | 0.034±0.006 | 1.76±0.32 | 1.96±0.39 | 0.036±0.007 | 1.85±0.32 | 2.06±0.40 |
| 80 | 1.75±0.092 | 9.07±0.65 | 0.040±0.004* | 2.06±0.21* | 2.27±0.25** | 0.043±0.004** | 2.22±0.21** | 2.45±0.24** |

[a]Based on 12/animals/sex/dose with the exception of high-dose females, for which only brain weights were measured.

*Significantly different from control value ($p < 0.05$) as evaluated by the study authors using Dunnett's Test.

**Significantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunnett's Test.

**[b]Significantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunn's Rank Sum Test.

TABLE 68. Selected Organ Weights and Organ/Body and Organ/Brain Weight Ratios in Rats Dosed with Naled for 28 Days[a]

| Dosage Group (mg/kg/day) | Liver | | | Right Kidney | | | Left Kidney | | |
|---|---|---|---|---|---|---|---|---|---|
| | Absolute | Relative | | Absolute | Relative | | Absolute | Relative | |
| | | Body | Brain | | Body | Brain | | Body | Brain |
| | (g) | (%) | (%) | (g) | (%) | (%) | (g) | (%) | (%) |
| **Males** | | | | | | | | | |
| 0 | 9.49±0.50 | 3.03±0.14 | 4.95±0.28 | 1.34±0.12 | 4.26±0.32 | 6.97±0.71 | 1.32±0.12 | 4.22±0.38 | 6.91±0.81 |
| 1 | 9.17±0.51 | 2.95±0.13 | 4.77±0.40 | 1.34±0.12 | 4.29±0.34 | 6.96±0.83 | 1.28±0.13 | 4.12±0.37 | 6.68±0.84 |
| 20 | 8.37±0.73** | 2.82±0.18** | 4.41±0.43** | 1.26±0.09 | 4.27±0.27 | 6.65±0.42 | 1.25±0.14 | 4.21±0.38 | 6.57±0.68 |
| 80 | 7.87±0.78** | 3.02±0.16 | 4.30±0.49** | 1.14±0.15** | 4.35±0.36 | 6.20±0.82* | 1.12±0.14** | 4.28±0.37 | 6.10±0.79 |
| **Females** | | | | | | | | | |
| 0 | 6.20±0.47 | 3.21±0.18 | 3.45±0.37 | 0.81±0.03 | 4.19±0.19 | 4.49±0.30 | 0.79±0.49 | 4.11±0.23 | 4.41±0.32 |
| 1 | 6.32±0.47 | 3.23±0.19 | 3.55±0.32 | 0.81±0.07 | 4.15±0.28 | 4.57±0.47 | 0.80±0.07 | 4.09±0.33 | 4.50±0.49 |
| 20 | 6.31±0.51 | 3.26±0.24 | 3.62±0.28 | 0.83±0.06 | 4.28±0.36 | 4.75±0.36 | 0.81±0.05 | 4.20±0.34 | 4.66±0.32 |
| 80 | 6.86±0.45** | 3.57±0.20** | 3.94±0.31** | 0.84±0.04 | 4.39±0.34 | 4.84±0.21 | 0.83±0.04 | 4.35±0.30 | 4.79±0.29 |

[a]Based on 12/animals/sex/dose with the exception of high-dose females of which only brain weights were measured.

*Significantly different from control value ($p < 0.05$) as evaluated by the study authors using Dunnett's Test.

**Significantly different from control value ($p < 0.01$) as evaluated by the study authors using Dunnett's Test.

TABLE 7. Gross Necropsy Findings of Rats Dosed with Naled for 28 Days

| Findings (Treated Skin) | Males/ Dosage Group (mg/kg/day) | | | | Females/ Dosage Group (mg/kg/day) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 20 | 80 | 0 | 1 | 20 | 80 |
| | (12)[a] | (12) | (12) | (12) | (12) | (12) | (12) | (12) |
| Erythema | 0 | 0 | 9 | 9 | 0 | 0 | 7 | 11 |
| Eschar | 0 | 0 | 11 | 12 | 0 | 0 | 9 | 12 |
| Atonia | 0 | 0 | 6 | 12 | 0 | 0 | 7 | 10 |
| Exfoliation | 0 | 0 | 3 | 9 | 0 | 0 | 1 | 11 |
| Fissures | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 2 |
| Necrosis | 0 | 0 | 1 | 8 | 0 | 0 | 2 | 7 |
| Desquamation | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |

[a]Number of tissues examined.

TABLE 8. Histopathologic Findings of the Skin of Rats Dosed with Naled for 28 Days

| Findings (Treated Skin) | Males/ Dosage Group (mg/kg/day) | | | | Females/ Dosage Group (mg/kg/day) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 20 | 80 | 0 | 1 | 20 | 80 |
| | (12)[a] | (12) | (12) | (12) | (12) | (12) | (12) | (12) |
| Acute Inflammation | 0 | 0 | 4 | 1 | 0 | 2 | 8 | 0 |
| Acute Ulcerative Inflammation | 0 | 0 | 8 | 11 | 0 | 1 | 3 | 12 |
| Necrosis | 0 | 0 | 4 | 9 | 0 | 0 | 1 | 12 |
| Epidermal Hyperplasia | 0 | 0 | 8 | 11 | 0 | 0 | 6 | 11 |
| Hyperkeratosis/ Parakeratosis | 0 | 0 | 3 | 1 | 0 | 0 | 4 | 3 |
| Surface Inflammatory Debris | 0 | 0 | 12 | 12 | 0 | 3 | 9 | 11 |

[a]Number of tissues examined.

Erythrocyte counts and hemoglobin and hematocrit values were found to be slightly decreased in high-dose females, whereas reticulocyte and platelet counts were significantly increased in high-dose males and females. Increased blood-urea-nitrogen (BUN) values and BUN/creatinine ratios were found in high-dose males and females, while total protein and albumin were decreased in these groups. Absolute and relative adrenal weights were found to be increased in high-dose males and females, while only high-dose females displayed a compound-related absolute and relative liver weight increase. The LOEL was 20 mg/kg/day and the NOEL was 1 mg/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and complete and the conduct of the study and reporting of data were acceptable. However, the size of the application site was not reported. In addition, according to the dates reported, the test compound was administered 20-21 times in 29-30 days; the study is entitled a 28-day dermal study.

We agree with the study authors' conclusion that naled produced severe dermal irritation and significant dose-related inhibition of plasma, erythrocyte, and brain cholinesterase activities in males and females receiving 20 and 80 mg/kg/day. Decreased erythrocyte counts and hemoglobin and hematocrit values and increased reticulocyte counts in high-dose males and females suggested increased erythropoiesis. Increased BUN and BUN/creatinine ratios in high-dose males and females and slightly increased kidney weights in females may be suggestive of a compound-related renal effect; however, histological effects were not seen. We would not expect to see histological changes in a study conducted for only 28 days. Increased liver weights were also not accompanied by any histologic changes. These effects cannot be conclusively evaluated.

We assess that the LOEL for the study should be based on cholinesterase depression and dermal response at 20 mg/kg/day and that the NOEL is 1 mg/kg/day.