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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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DATE: **May 29, 2001**

MEMORANDUM

SUBJECT: *NALED:* - Report of the Hazard Identification Assessment Review Committee.

FROM: Abdallah Khasawinah, Ph.D., Toxicologist A. Khasawinah
Reregistration Branch 4
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair 5/30/01
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee E.A. Doyle
Health Effects Division (7509C)

TO: Susan Hummel, Branch Senior Scientist
Reregistration Branch 4
Health Effects Division (7509C)

PC Code: 034401

On May 1, 2001, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) **reevaluated** the toxicology data base of naled in view of the results of a newly submitted 28-day dermal toxicity study in rats and selected the toxicology endpoints for use as appropriate in occupational/residential exposure risk assessments. HIARC also reaffirmed acute and reference doses (RfDs) selected previously (RfD/Peer review committee: HED Doc No. 011199, August 31, 1994) and (TES Document, September 26, 1996). The potential increased susceptibility of infants and children from exposure to naled as required by the Food Quality Protection Act (FQPA) of 1996 had been evaluated previously by HIARC (HED Doc No.012316, 012329: September 18, 1997 & HED Doc No. 012479: February 12, 1998). The Committee's conclusions are presented in this report.

THIS REPORT SUPERSEDES THE PREVIOUS RFD, TES & HIARC REPORTS.

Committee Members in Attendance

Members present were: William Burnam, Pamela Hurley, Elizabeth Mendez, David Nixon, Ayaad Assaad, Yung Yang, Jess Rowland, Paula Deschamp, Jonathan Chen, Brenda Tarplee (Executive Secretary)

Members in absentia were: None

Data evaluation prepared by: Abdallah Khasawinah, Ph.D., RRB4

Also in attendance were: Ray Kent, Sanjivani Diwan, Tim Leighton, Tom Myers, Susan Hummel,

Data Evaluation / Report Presentation:

Abdallah Khasawinah
Toxicologist

1. INTRODUCTION

On May 1, 2001, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) **reevaluated** the toxicology data base of naled in view of the results of a newly submitted 28-day dermal toxicity study in rats and selected the toxicology endpoints for use as appropriate in occupational/residential exposure risk assessments. HIARC also reaffirmed acute and reference doses (RfDs) selected previously (RfD/Peer review committee: HED Doc No. 011199, August 31, 1994) and (TES Document, September 26, 1996). The potential increased susceptibility of infants and children from exposure to naled as required by the Food Quality Protection Act (FQPA) of 1996 had been evaluated previously by HIARC (HED Doc No.012316, 012329: September 18, 1997 & HED Doc No. 012479: February 12, 1998). The Committee's conclusions are presented in this report. This report includes the decisions made by the RfD, TES, HIARC and the FQPA Safety Factor Committees.

THIS REPORT SUPERSEDES ANY PREVIOUS RFD, TES & HIARC REPORTS.

2. HAZARD IDENTIFICATION

2.1 Acute Reference Dose (RfD)

Study Selected : 28- Day Oral Feeding Study - Rats Guideline #: 82-1(a)

MRID No.: 00088871, 00246496

Executive Summary: In a 28-day oral study, rats (10/sex/dose level) received 0, 0.25, 1, 10, or 100 mg/kg/day of naled by gavage. The 100 mg/kg/day dose level produced mortality and marked cholinergic signs. The 10 mg/kg/day dose produced mild cholinergic signs (after the 6th dose), 50% reduction in plasma (at end of 3-4 weeks of treatment) and brain cholinesterase (at end of 4 weeks of treatment). The 1 mg/kg/day dose level produced 15% plasma cholinesterase inhibition without clinical signs. The NOAEL was 1 mg/kg/day, and the LOAEL was 10 mg/kg/day based on cholinergic effects.

Dose and Endpoint for Establishing Acute RfD: 1 mg/kg/day based on cholinergic signs and plasma and brain cholinesterase inhibition at 10 mg/kg/day

Uncertainty Factor: 100

Comments about Study/Endpoint/Uncertainty Factor(s): The cholinergic signs and cholinesterase inhibition can occur after a single exposure to naled and therefore, this endpoint is appropriate for this acute dietary risk assessment. The NOAEL and LOAEL of 1 and 10 mg/kg/day, respectively, for acute effects are supported by the following additional data: (1). 1-year dog study - 43% RBC cholinesterase inhibition in dogs treated with 20 mg/kg/day **for 7 days** and 27% plasma cholinesterase inhibition at 2 mg/kg/day **for 7 days**. No effect on cholinesterase at 0.2 mg/kg/day after 7 days (MRID 00160751). (2). Acute neurotoxicity study - neurotoxic signs in the functional observational battery were seen in rats given a single oral dose of 25 mg/kg (the lowest dose tested) and higher (MRID 42861301). However, cholinesterase measurements were not done in this study.

Acute RfD = $\frac{1 \text{ mg/kg (NOAEL)}}{100 \text{ (UF)}}$ = **0.01 mg/kg**

2.2 Chronic Reference Dose (RfD)

Study Selected: Chronic/Oncogenicity 2- year Feeding Study Guideline #: 83-2a

MRID No.: 00128701, 00088871, 00141784 & 40418901

Executive Summary: In a two-year chronic toxicity/carcinogenicity study naled was administered by gavage to male and female Sprague-Dawley CD rats at doses of 0, 0.2, 2, or 10 mg/kg/day. Plasma, RBC and brain cholinesterase activities were decreased at dose levels of 2 and 10 mg/kg/day. No other treatment-related findings were observed. The NOAEL for cholinesterase inhibition was 0.2 mg/kg/day and the LOAEL was 2 mg/kg/day. The NOAEL for systemic toxicity was the highest dose tested, 10 mg/kg/day (MRID 00141784, 00088871).

Dose and Endpoint for Establishing RfD: 0.2 mg/kg/day based on inhibition of brain cholinesterase activity at 2.0 mg/kg/day

Uncertainty Factor: 100

Comments about Study/Endpoint/Uncertainty Factor(s): The route and duration of exposure is appropriate for assessing chronic dietary risk.

Chronic RfD = $\frac{0.2 \text{ mg/kg/day (NOAEL)}}{100 \text{ (UF)}}$ = **0.002 mg/kg/day**

2.3 Occupational/Residential Exposure

2.3.1 Short-Term (1 - 7 days) Incidental Oral Exposure :

Hazard identification (dose and endpoint) was not previously made for this exposure scenario.

Study Selected : 28- Day Oral Feeding Study - Rats Guideline #: 82-1(a)

MRID No.: 00088871, 00246496

Executive Summary: **See 2.1 above.**

Dose and Endpoint for Establishing Risk Assessment: 1 mg/kg/day based on cholinergic signs and plasma and brain cholinesterase inhibition at 10 mg/kg/day.

Comments about Study/Endpoint: In the 1 - year dog study, plasma cholinesterase inhibition was seen at 2 mg/kg/day for 7 days, RBC ChE inhibition was seen at 20 mg/kg/day for 7 days. The endpoint is appropriate for the population (infants and children) and duration of concern.

2.3.2 Intermediate-Term (1-Week to Several Months) Incidental Oral Exposure

Study Selected: 1-Year Oral Gavage Study - Dogs § 83-1

MRID No: 00160751

Executive Summary: Beagle dogs (6/sex/group) were given 0, 0.2, 2 or 20 mg/kg/day of naled technical by gavage in carboxymethyl cellulose for 1 year. An increase in the incidence of emesis and diarrhea was found in male and female dogs receiving 2 or 20 mg/kg/day naled. Red blood cell count, hemoglobin and hematocrit were significantly reduced, by as much as 25% throughout most of the study in males and females of the 2 and 20 mg/kg/day groups. Platelet counts were increased by as much as 40% in males and females of the 20 mg/kg/day group throughout most of the study. Serum protein and albumin levels were significantly decreased in both sexes of the high dose group throughout the study. Serum levels were also slightly reduced in these groups. Plasma and red blood cell ChE levels were significantly reduced in both sexes (2 and 20 mg/kg/day groups) throughout most of the study (first determination after treatment was at 7 days). There was 43% RBC ChE inhibition (20 mg/kg/day group females) and 27% plasma ChE inhibition (2 mg/kg/day group females) **after 7 days of treatment**. Statistically significant reductions in plasma ChE activity were reported in the 0.2 mg/kg/day group males and females, but were not considered biologically significant due to their relatively small magnitude in comparison with the other higher dose groups. At terminal sacrifice, brain ChE levels were significantly reduced by 18% in the 20 mg/kg/day males, 17 % in the 2 mg/kg/day females and 29 % in the 20 mg/kg/day females. Absolute and relative liver and kidney weights were significantly increased in males and females of the high dose group. There were no compound-related histopathologic lesions with the possible exception of an increased incidence of testicular degeneration (trace) in males receiving 2 and 20 mg/kg/day. The **LOAEL** is 2 mg/kg/day and the **NOAEL** is 0.2 mg/kg/day based on inhibition of plasma and RBC ChE activities and decreased hemoglobin and hematocrit values in both sexes. The study is classified as **Acceptable** and satisfies the guideline requirement for a chronic study (83-1)

Dose and Endpoint for Risk Assessment: 0.2 mg/kg/day based on inhibition of plasma and RBC ChE activities and decreased hemoglobin and hematocrit values in male and female dogs at 2 mg/kg/day.

Comments about Study/Endpoint/Uncertainty Factor(s): The route and duration of exposure is appropriate for assessing intermediate-term exposures of 7 days to several months. The ChE inhibition occurred within 7 - 30 days of exposure at the LOAEL of 2 mg/kg/day and persisted for the three month duration of the naled administration.

2.3.3 Dermal Absorption: (Revised Assessment) Since the last HIARC meeting (HED Doc. 013939: Organophosphates: Evaluation of the Dermal Absorption Factor, February 24, 1999), a new naled dermal absorption study was made available by the registrant.

Study Selected: Dermal Penetration - Rat Guideline #: 85-3

MRID No.: 45099301

Executive Summary: In a dermal absorption study (MRID 45099301), young adult male rats (4/dose/exposure duration) received single applications of ^{14}C- naled EC formulation on a 10 cm^2 shaven dorso-lumbar area at 0.045, 0.19, 0.52 or 4.2 mg/rat for durations of 0.5, 1, 2, 4, 10 or 24 hours. Rats were housed individually in metabolism cages where urine and feces were collected separately, and exhaled volatile metabolites collected over 10 and 24 hours. At the end of the exposure periods charcoal filters were removed and the skin was washed to remove the unabsorbed dose. Rats were killed by cardiac puncture and blood samples (volume not reported) were collected. Samples analyzed for radioactivity were skin wash, filters, O-rings, skin of application site and stratum corneum. These represented unabsorbed radioactivity. Blood, carcass, urine, feces, volatile traps, and cage wash represented absorbed radioactivity.

Blood concentration of ^{14}C-naled radioactivity increased with increasing dose, but the time of the peak radioactivity was independent of the dose. The blood concentration peaked at 0.5, 2, 10 and 24 hours at doses of 4.5, 19, 52 and 420 $\mu g/cm^2$. The saturation of absorption occurred at 420 $\mu g/cm^2$. Blood plasma concentration of radioactivity was 1.5 times that of the whole blood.

The mean percentage of absorbed radioactivity at the three lower doses was 2-3 times higher than that absorbed at the high dose during the first 10 hours of exposure (7.7-21.45% vs 3.25-10.20%) indicating a saturation of absorption at this dose. At 420 $\mu g/cm^2$, the absorbed radioactivity peaked at 20.69% after 24 hours of exposure. For the lower doses absorbed radioactivity fluctuated at various intervals. The absorbed radioactivity at 52, 19 and 4.5 $\mu g/cm^2$ peaked at 24, 2 and 24 hours, respectively. No explanation was offered for these irregularities in this absorption pattern.

Total recovery of radioactivity at various exposure intervals ranged from 88-98%, 89-95%, 90-95%, and 87-92% at 420, 52, 19 and 4.5 $\mu g/cm^2$, respectively. The majority of the unabsorbed radioactivity remained on the skin surface and was removed by skin washing. It gradually decreased (84.74% at 0.5 hours to 38.29% at 24 hours) in the 420 $\mu g/cm^2$ dosed group and at all lower dose levels with increasing exposure duration.

In conclusion, the test material, naled is moderately absorbed through the skin at peak levels of 21 - 23% after 10- 24 hours of exposure.

The study is classified **Acceptable** and satisfies the guideline requirement 870.7600 (85-3) for a dermal absorption study.

Dermal Absorption Factor: 21%

Comments about Dermal Absorption: Dermal absorption at 10 hour exposure period was selected to simulate a normal working day scenario.

2.3.4 Short-term Dermal (1 - 7 days) Exposure:

Study Selected: 28-Day Repeated Dose Dermal Study- Rat, Guideline # 82-2
10 - Day Range Finding Study, Guideline # 82-2

MRID No.: 45222001

Executive Summary: In the range finding portion of the study, Crl:CD(SD)BR rats (2/sex/group) received dermal applications of naled at 0, 5, 10, 20, 40 or 80 mg/kg/day for 10 days. The 80 mg/kg/day group was terminated after 5 days due to dermal irritation and systemic toxicity. Rats in the 20 and 40 mg/kg groups showed signs of skin irritation. Decreased brain, plasma, and erythrocyte ChE activities were observed in the range finding study in both sexes at ≥ 20 mg/kg/day. Activities at 20 mg/kg/day were 101%, **69%, and 71%** of control for brain, erythrocyte, and plasma, respectively, for males and **64%, 51%, and 68%** of control for brain, erythrocyte, and plasma, respectively, for females.

In the main dermal toxicity study, groups of 10 male and 10 female Crl:CD(SD)BR rats received 21 daily dermal applications of 0, 5, 10, or 40 mg/kg/day Naled (95.84%, Batch No. JF110299) in corn oil over a 28-day period (dermal occlusion for 6 hours/day). Additional groups of 5 male and 5 female rats were designated satellite animals for determination of brain, erythrocyte, and plasma cholinesterase (ChE) activity.

There were no treatment-related deaths or signs of systemic toxicity and no treatment-related effects on body weight, food consumption, functional observational battery, locomotor activity or opthalmology. Treatment-related dermal effects included slight to moderate desquamation, erythema, and/or edema in all dose-groups in both males and females. Additionally, skin discoloration and erythema were noted on the treatment area of 6/20 mid-dose and 3/20 high-dose animals. Hyperkeratosis was noted in treated skin of 5/20, 6/20, 15/20, and 17/20 rats receiving 0, 5, 10, and 40 mg/kg/day, respectively. Acanthosis was noted in treated skin of 1/20, 0/20, 1/20, and 6/20 rats receiving 0, 5, 10,and 40 mg/kg/day, respectively. The acanthosis and hyperkeratosis were classified as "minimal" in all females and in control and low-dose males and were described as "slight" in mid- and high-dose males.

Decreased brain, plasma, and erythrocyte ChE activities were observed at 40 mg/kg/day in males and females compared to controls. Activities were **54%, 72%, and 79%** of control for brain, erythrocyte, and plasma, respectively, for males and

53%, 65%, and 55% of control for brain, erythrocyte, and plasma, respectively, for females.

There was an increase ($p<0.05$, 7.7% increase compared to controls) in relative kidney weight of high-dose males. This effect, when considered with the observed unilateral hydronephrosis in 4/10 high-dose males, suggests a minimal treatment-related effect.

The systemic NOAEL is 10 mg/kg/day and the **systemic LOAEL** is 40 mg/kg/day based on minimal kidney effects (increased relative weight and hydronephrosis) observed in males. Based on the **combined findings of main and range finding studies, the ChE activity NOAEL** is 10 mg/kg/day and the **ChE activity LOAEL** is 20 mg/kg/day based on decreased brain ChE activity in females and decreased erythrocyte, and plasma ChE activity in males and females. **The dermal LOAEL** is 5 mg/kg/day based on slight erythema in both sexes and slight edema in females and a **dermal NOAEL** was not identified.

This study is classified as **Acceptable/Guideline** and does satisfy the guideline requirements for a repeated-dose dermal study [OPPTS 870.3200 (§82-2)] in rats.

Dose and Endpoint for Risk Assessment: 10 mg/kg/day based on decreased brain ChE activity in females and decreased erythrocyte and plasma ChE activities in males and females occurring within 10 days of exposure at 20 mg/kg/day.

Comments about Study/Endpoint: The 10 mg/kg/day dose in the range finding study selected at this HIARC meeting is higher than the previously selected NOAEL of 1 mg/kg/day (TES Document, 9/26/1996). The 1 mg/kg/day dose was based also on a 28-day dermal study where naled was applied to intact skin at dose levels of 0, 1, 20 or 80 mg/kg/day for 6 hours, 5 days/week (MRID No. 00160750). The NOAEL from this study was 1 mg/kg/day based on dermal irritation, reduced body weight gain and cholinesterase inhibition at 20 mg/kg/day. There were no intermediate doses between the 1 and 20 mg/kg/day doses used in the original study. Therefore, the NOAEL of 1 mg/kg/day was an artifact of dose selection. In the current study (MRID No. 45222001), additional doses were used including 5 and 10 mg/kg/day where no adverse effects were observed at these dose levels, ChE activities were depressed at 20 mg/kg /day exposure to naled for **10 days** in the range finding study. The route and duration of exposure in this study are appropriate for the dermal risk assessment.

2.3.5 Intermediate-term Dermal (1-Week to Several Months) Exposure:

Study Selected: 28-Day Repeated Dose Dermal Study- Rat Guideline #: 82-2

MRID No.: 45222001

Executive Summary: **See 2.3.4. above**

Executive Summary: **See 2.3.4. above**

Dose and Endpoint for Risk Assessment: 10 mg/kg/day based on decreased brain ChE activity in females and decreased erythrocyte and plasma ChE activities in males and females occurring within 10 days of exposure at 20 mg/kg/day.

Comments about Study/Endpoint: **See 2.3.4 above.**

2.3.6 Long-term Dermal (Several Months to Lifetime)

Study Selected: Chronic/Oncogenicity 2- year Feeding Study Guideline #: 83-2a

MRID No.: 00128701, 00141784 & 40418901

Executive Summary: A two-year chronic toxicity/carcinogenicity study administered naled to male and female Sprague-Dawley CD rats at doses of 0, 0.2, 2, or 10 mg/kg/day by gavage. Plasma, RBC and brain cholinesterase activities were depressed at dose levels of 2 and 10 mg/kg/day. No other treatment-related findings were observed. The NOAEL for cholinesterase inhibition was 0.2 mg/kg/day and the LOAEL was 2 mg/kg/day. The NOAEL for systemic toxicity was the highest dose tested, 10 mg/kg/day (MRID 00141784, 00088871).

Dose and Endpoint for Risk Assessment: 0.2 mg/kg/day based on inhibition of brain cholinesterase activity at 2.0 mg/kg/day.

Comments about Study/Endpoint: the dose and endpoint are relevant because the chronic dosing regimen simulates the exposure period of concern (several months to life-time). **Since an oral study was used a dermal absorption factor of 21% should be applied.**

2.3.7 Inhalation Exposure (All Durations)

Study Selected: Inhalation- 3 month Guideline #: 82-4

MRID No.: 00164224, 00265678, 265680

Executive Summary: In a 13-week inhalation study, male and female Fischer-344 rats were exposed (whole body) to filtered air (control group) or aerosols containing 0.2, 1, or 6 µg/L of naled for 6 hours/day, 5 days/week (actually analyzed as 0, 0.23, 1.29 or 5.8 µg/L equivalent to 0.0, 0.053, 0.298 and 1.336 mg/kg/day, respectively). Additional control and high dose groups were allowed to recover for 6 weeks. Exposure to the highest concentration of 5.8 µg/l resulted in clinical signs of toxicity manifest as tremors, salivation, nasal discharge, abnormal respiration, and anogenital staining. The clinical signs were consistent with cholinergic effects and the observed inhibition of cholinesterase activity. Brain cholinesterase was inhibited at 5.8 µg/l. Plasma and RBC cholinesterases were inhibited at 1.29 and 5.8 µg/l. Only plasma cholinesterase continued to be inhibited six weeks after exposure to the high concentration. No other treatment-related effects were observed.

The NOAEL for cholinesterase inhibition was 0.23 µg/l (0.053 mg/kg/day), and the LOAEL was 1.29 µg/l (0.298 mg/kg/day) based on depression of plasma and RBC cholinesterase activities. The NOAEL for systemic toxicity was 1.29 µg/l, and the LOAEL was 5.8 µg/l based on clinical signs of toxicity.

Dose and Endpoint Selected for Risk Assessment: 0.053 mg/kg/day, based on inhibition of plasma and RBC cholinesterase activities.

Comments about Study/Endpoint: Plasma and RBC cholinesterase were inhibited as early as day 15 of the study. The results of the 90-day study are supported by a 21-day inhalation study (MRID 400872-01). In this study, plasma and RBC cholinesterase inhibition was reported by day 5 of treatment at a dose level of 3.4 µg/L. In addition, there were decreases in food consumption and body weight when measured on day 7. Nasal epithelial lesions were also observed at this dose level. In order to convert the NOAEL's for cholinesterase (0.23 µg/L/day) into mg/kg/day, the following calculations are made:

1. 0.23 µg/L = 0.00023 mg/L
2. $$\frac{\text{mg/L} \times RV_A \times AM \times \text{study hours/day}}{BW_A} = \text{mg/kg/day}$$

where:
RV_A = respiratory volume - liters of air inspired during each animal exposure. For F344 rats the values are 15.4 L/hour for a 0.4 kg male rat and 10.8 L/hour for a 0.25 kg female rat.
AM = absorbed material - the ratio of material inhaled by an animal which is absorbed into systemic circulation (expressed in a decimal). In this case, due to lack of data, we will assume 100% absorption, which is a very conservative value.
BW_A = Mean body weight of the animals in kg.

Therefore, the calculations are:

$$\frac{0.00023 \text{ mg/L} \times 15.4 \text{ L/hr (♂ F344)} \times 1 \times 6 \text{ hours/day}}{0.4 \text{ kg}} =$$

0.053 mg/kg/day = NOAEL for cholinesterase inhibition for males
0.059 mg/kg/day = NOAEL for cholinesterase inhibition for females

2.3.8 **Margins of Exposure for Occupational/Residential Assessments**: A MOE of 100 is adequate for occupational and residential exposures via the dermal and inhalation routes.

2.4 **Recommendations for Aggregate Risk Assessments**: For acute, short, intermediate and long term aggregate exposure risk assessments, the oral, dermal, and inhalation exposures can be combined because of the common toxicity endpoint (ChE Inhibition) via these routes.

3. CLASSIFICATION OF CARCINOGENIC POTENTIAL:

3.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats:

MRID No.: 00141784, 00088871, 40418901

Executive Summary: In a two-year chronic toxicity/carcinogenicity study naled was administered by gavage to male and female Sprague-Dawley CD rats at doses of 0, 0.2, 2, or 10 mg/kg/day. Except for depressed plasma, RBC and brain ChE activities at 2 and 10 mg/kg/day dose levels, there were no treatment related effects.

Discussion of Tumor Data: No neoplastic lesions were related to treatment.

Adequacy of the Dose Levels Tested: The high dose of 10 mg/kg/day was considered adequate to assess the carcinogenic potential of naled in rats based on the results of a 28-day pilot study demonstrating mortality at 100 mg/kg/day and mild cholinergic signs (lethargy and muscle weakness) accompanying 50% reductions in plasma and brain cholinesterase activities at 10 mg/kg/day.

3.2 Carcinogenicity Study in Mice.

MRID No.: MRID 00141785, 00148569

Executive Eummary: In an 89-week carcinogenicity study naled was administered by gavage to male and female CD-1 mice at doses of 0, 3, 15, or 75 mg/kg/day. The high dose of 75 mg/kg/day was reduced to 50 mg/kg/day after 26 weeks due to high mortality. Mortality was 10 and 13% for high dose males and females, respectively, compared to 2% for control after 26 weeks. Tremors were observed in 3 of 8 high dose females that died during the first 26 weeks. The only other treatment-related finding was a slight reduction (3-5%) in weight gain by males showing a dose related trend at the middle and high dose levels. Cholinesterase activity was not determined.

Discussion of Tumor Data: No neoplastic findings were related to treatment.

Adequacy of the Dose Levels Tested: The doses tested were considered adequate to assess the carcinogenic potential of naled in mice. The dose selection was supported by the results of a pilot study, which indicated the use of a high dose between 50 and 100 mg/kg/day in the carcinogenicity study to avoid excessive toxicity and mortality. In the pilot study, a dose level of 300 mg/kg/day for two weeks produced mortality (60 to 80%), 150 mg/kg/day for two weeks produced cholinergic signs and 50 mg/kg/day for four weeks produced a slight decrease in body weight gain and a significant reduction in food consumption. The mortality rate associated with the 75 mg/kg/day dose level after 26 weeks justified reduction of the high dose to 50 mg/kg/day.

3.3 Classification of Carcinogenic Potential

In accordance with the Guidelines for Cancer Risk Assessment (April 10, 1986), naled was classified as a Category E Chemicaln; no evidence of carcinogenicity in humans. This classification is based on the lack of evidence for carcinogenicity in rats or mice.

4. MUTAGENICITY

An *in vivo* gene mutation study (mouse spot test) was conducted with pregnant C57BL/6 mice given 0, 3, 20, or 150 mg/kg/day of naled by gavage for four days of gestation (days 8-12). Litters were scored for coat color mutations ("spots") on post-partum days 12 and 28. The test was presumably indicative of mutation events consisting of intragenic base-pair changes, deletions and somatic crossing-over. The high dose of naled was very toxic producing maternal mortality, decreased maternal body weight and decreased pup survival. Naled exhibited no potential to induce coat color spots (MRID 00141571).

Naled was tested for gene mutation in the *Salmonella typhimurium* reverse mutation assay (Ames assay) using tester strain TA 100 with and without metabolic activation (PCB-induced mouse liver S9 fraction). Naled was tested at concentrations of 0.5, 1 and 2 μM. The highest concentration was toxic in the absence of metabolic activation but was mutagenic with metabolic activation. The middle concentration of 1 μM was positive both with and without metabolic activation. The low concentration of 0.5 μM was marginally positive (less than two-fold DMSO control) (MRID 00142662).

Naled was tested for DNA damage in *Proteus mirabilis* strains PG273 (wild type) and PG713 (thr^-, rec^-, hcr^-). Naled was negative in both strains at inhibitory concentrations of 10 and 40 μM (MRID 00142662).

Naled was tested for cytogenetic effects *in vivo* in the mouse bone marrow micronucleus assay. Naled was administered to male and female Swiss mice as a single oral dose by gavage. Dose levels were 0, 55, 110, or 220 mg/kg for males and 0, 55, 110, or 290 mg/kg for females. Dose selection was based on preliminary studies indicating oral LD_{50} values of 257 mg/kg for males and 336 mg/kg for females. Bone marrow cells were harvested 24, 48 and 72 hours after treatment. The highest dose produced mortality (16-24%) and clinical signs of toxicity. Naled had no cytotoxic effect on bone marrow at these dose levels and produced no nuclear anomalies (MRID 00146497).

In another *in vivo* cytogenetics study, male and female Sprague Dawley rats were administered naled as a single oral dose by gavage. Dose levels were 0, 3.88, 12.93, or 38.80 mg/kg for males and 0, 6.17, 20.57, or 61.70 mg/kg for females. Dose selection was based on preliminary studies conducted at the same laboratory indicating oral LD_{50} values of 85.1 mg/kg for males and 81.2 mg/kg for females. Bone marrow cells were harvested 6, 24 and 48 hours after treatment. High dose females showed signs of toxicity including ataxia, dyspnea and oral exudate. Cytotoxicity in bone marrow was not evident at any dose level. Naled had no clastogenic effect. The highest dose was considered to be near a maximum tolerated dose based on the clinical signs observed in females and the results of preliminary studies indicating the high dose for males was approximately one-half the oral LD_{50} (MRID 00142665).

In conclusion, naled was not mutagenic in the above assays.

5. FQPA CONSIDERATIONS

The FQPA Safety Factor Committee has concluded that the 10X FQPA safety factor should be removed based on the following factors (Memorandum of Brenda Tarplee and Jess Rowland: FQPA Safety Factor Recommendations for the Organophosphates, August 6, 1998):

(a) In prenatal developmental toxicity studies following *in utero* exposure in rats and rabbits, there was no evidence of developmental effects being produced in fetuses at lower doses as compared to maternal animals nor was there evidence of an increase in severity of effects at or below maternally toxic doses.

(b) In the pre/post natal two-generation reproduction study in rats, there was no evidence of enhanced susceptibility in pup when compared to adults (i.e., effects noted in offspring occurred at maternally toxic doses or higher).

(c) There was no evidence of abnormalities in the development of the fetal nervous system in the pre/post natal studies.

(d) There is no concern for positive neurological effects from the available neurotoxicity studies or for histopathology in the central nervous system from the other toxicological studies (e.g., subchronic rat, chronic dog, chronic mouse and rat).

(e) The toxicology data base is complete and there are no data gaps according to the Subdivision F Guideline requirements.

(f) Adequate actual data, surrogate data, and/or modeling outputs are available to satisfactorily assess dietary and residential exposure and to provide a screening level drinking water exposure assessment.

6. HAZARD CHARACTERIZATION

Naled (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) is an organophosphate insecticide registered for use primarily to control adult mosquito and blackfly populations. The toxicological database for naled is complete. By the oral, dermal, and inhalation exposure routes, technical naled is classified in Toxicity Category II. For eye and dermal irritation, naled is classified in Toxicity Category I. Naled was weakly positive in a guinea pig dermal sensitization study. In the oral acute toxicity studies, females appear to be more sensitive than males. Naled, like other organophosphates, has anticholinesterase activity in all species tested (including dogs, rabbits, hens, rats, and mice). Clinical signs of cholinesterase (ChE) inhibition include tremors, salivation, nasal discharge, and abnormal respiration. Inhibition of plasma, erythrocyte and brain cholinesterase activity occurs by the oral, dermal, and inhalation routes of exposure, and following exposure for various durations (acute, short- intermediate-term, and chronic).

In an acute delayed neurotoxicity study in hens, naled did not produce frank delayed neurotoxicity, but a degenerative neuronal effect was manifest in the spinal cord. In the hen subchronic neurotoxicity study, no delayed neuropathy was observed. No neurological effects were noted in the acute rat neurotoxicity study, however, in the subchronic rat neurotoxicity study minimal neurological effects were noted.

There is no evidence of increased susceptibility following *in utero* exposure to rats and rabbits and following pre/post natal exposure to rats. The Agency has determined that there is no evidence of carcinogenicity in humans for naled (i.e., Group E chemical). Naled is not mutagenic in *in vitro* and *in vivo* tests.

7. DATA GAPS

No data gaps have been identified.

The Agency waived the data requirement for a general rat metabolism study since the database is essentially complete and no cancer or developmental concerns are indicated at this time, and limited data already reviewed by the Agency allows basic characterization of metabolism of naled. No further data are required at this time.

8. ACUTE TOXICITY

Acute Toxicity of Naled

Guideline No.	Study Type	MRIDs #	Results	Toxicity Category
81-1	Acute Oral - Rat : vehicle: corn oil vehicle: carboxy methyl cellulose	00142660	LD_{50} = 325 mg/kg ♂ 230 mg/kg ♀; 191 mg/kg ♂, 92 mg/kg ♀	II
81-2	Acute Dermal-Rabbit	00146493	LD_{50} = 390 mg/kg ♂ 360 mg/kg ♀	II
81-3	Acute Inhalation - Rat 4 hr. exposure	00146494	LC_{50} = 0.20 mg/L ♂ 0.19 mg/L ♀	II
81-4	Primary Eye Irritation	00074826	severe irritation	I
81-5	Primary Skin Irritation	00074825	corrosive (escharotic)	I
81-6	Dermal Sensitization	00074657	weakly positive	N/A
81-7	Acute Neurotoxicity - Rats	42861301	No treatment-related pathological lesions of the central or peripheral nervous system.	N/A
81-8	Acute Neurotoxicity - Chicken	41630701	Oral LD_{50}: 42 mg/kg. No delayed neurotoxicity at LD_{50}, but increased histopathology and evidence of axonal degenrarion in the spinal cord and peripheral nerves, depressed brain ChE.	N/A

9. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION FOR NALED

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOAEL = 1.0 UF = 100	cholinergic signs and plasma and brain cholinesterase inhibition	28-Day Oral Toxicity - Rat
	Acute RfD = 0.01 mg/kg		
Chronic Dietary	NOAEL=0.2	Inhibition of brain cholinesterase activity	2-year Chronic toxicity - Rat
	Chronic RfD = 0.002 mg/kg/day		
Incidental Oral, Short - Term	NOAEL = 1.0	cholinergic signs and plasma and brain cholinesterase inhibition	28-Day Oral Toxicity - Rat
Incidental Oral, Intermediate-Term	NOAEL = 0.2	Plasma and RBC ChE inhibition and ↓hematocrit and hemoglobin values	1-Year Dog Study
Dermal, Short-Term	Dermal NOAEL = 10	Plasma, RBC and brain cholinesterase inhibition	28-Day Dermal - Rat
Dermal, Intermediate-Term	Dermal NOAEL = 10	Plasma, RBC and brain cholinesterase inhibition	28-Day Dermal - Rat
Dermal, Long-Term[a]	Oral NOAEL=0.2	Inhibition of brain cholinesterase activity	2-Year Chronic -Rat
Inhalation (Any time period)	NOAEL=0.05[b]	Plasma and RBC cholinesterase inhibition	90-Day Inhalation-Rat

a =Since an oral NOAEL is selected, appropriate route-to-route extrapolation should be done. The dermal exposure component (mg/kg/day), using a 21% dermal absorption factor, should be converted to an equivalent oral dose, and this dose should then be compared with the oral NOAEL.

b= The dose presented is a converted dose (i.e., the NOAEL in mg/L is converted to mg/kg/day). So the inhalation exposure should be compared to the NOAEL presented.

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