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WASHINGTON, D.C. 20460

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MEMORANDUM #586

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: NALED---Tox Data Submitted Under MRID 43189601 and 42861301
ID # 034401

Chemical: PC Code 034401 (586)
RD Record: S445518
HED Project: D193724

FROM: Irving Mauer, Ph.D., Geneticist
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9-14-94

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THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I
Health Effects Division (7509C)
9/19/94

Registrant: Valent USA, Walnut Creek, CA

Request: Review and evaluate the following acute neurotoxicity studies (GDLN 81-8-SS)

(1) An Acute Neurotoxicity Study of Naled Technical in Rats, performed by WIL Research, Ashland, OH, Laboratory Project No. 194007, Final Report dated July 12, 1993 (MRID 428613-01).

(2) A Range-Finding Acute Study of Valent Naled Technical in Rats, performed by WIL Research, Laboratory Project No. 194006, Final Report dated February 9, 1994 (MRID 431896-01).

TB CONCLUSIONS: This study is judged ACCEPTABLE. Although a no-effect level was not established for females (the LDT, 25 mg/kg, the NOEL for males, was considered to cause mild tremors, exophthalmos and reduced hindlimb resistance in a few animals), an estimate of this parameter can reasonably be set for females at 5 mg/kg, based upon minimal neurological compromise at 25 mg/kg in the main study, coupled with no toxicity at 5 or 25 mg/kg in the preliminary range-finding study.

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ATTACHMENT: DER

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Karl P. Baetcke 9/19/94

DATA EVALUATION RECORD

MRID No.: 428613-01
PC No.: 034401
RD Record No.: S445518
EPA ID No.: 034401
Tox Chem. No.: 586
Project No.: D193724

I. SUMMARY

STUDY TYPE: (81-8-SS) Acute neurotoxicity-rat

CHEMICAL: Naled Technical (1, 2-dibromo-2, 2-dichloro ethyl dimethyl-phosphate)

SYNONYMS: DIBROM

SPONSOR: Valent USA, Walnut Creek, PA

TESTING FACILITY: WIL Research, Ashland, OH

TITLE OF REPORT: An Acute Neurotoxicity Study of Naled Technical in Rats

AUTHOR: Ian C. Lamb

STUDY NUMBER: WIL-194007

DATE ISSUED: July 12, 1993

EXECUTIVE SUMMARY: Groups of rats were administered test article at single doses of 0, 25, 100 and 400 mg/kg, and examined 30 minutes after dosing and weekly thereafter for neurotoxic changes by appropriate test procedures for the Functional Observation Battery (FOB) and motor activity.

Transient functional neurotoxicity consistent with inhibition of cholinesterase was evident in both sexes at doses of 100 and 400 mg/kg, but not in males given 25 mg/kg (the NOAEL); a few females manifested minimal neurological effects at this LDT. However, since these effects in this main study were marginal, and no toxicity was observed in the preliminary range-finding study at 25 mg/kg or the next lower dose, 5 mg/kg, the latter may be considered a reasonable and conservative estimate of the NOAEL for females.

TB-I EVALUATION: The study is ACCEPTABLE.

II. DETAILED REVIEW

A. TEST MATERIAL: Naled Technical (Amvac Chemical, Los Angeles, CA)

Description:	Clear viscous liquid
Batches (Lots):	204026
Purity (%):	92.7 (100% assumed for dosage calculations)
Solvent/carrier/diluent:	0.5% Aqueous Carboxymethylcellulose (CMC)

B. TEST ORGANISM: Rodent

Species:	Rat
Strain:	Crl: CD® BR (Sprague-Dawley)
Age:	43 days
Weights - males:	189-249 g
females:	141-185 g
Source:	Charles River, Portage, MI

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the acute neurotoxicity potential of the test article when administered once orally to rats, which were evaluated using a neurotoxicity screening battery, according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Based upon a preliminary range-finding acute study[1], groups of rats (12/sex/dose group) were gavaged once at doses of 0 (CMC vehicle), 25, 100, or 400 mg/kg (16 animals/sex at the HDT), and observed twice daily for 14 days. A battery of prescribed functional neurotoxicity and

[1]WIL-194006: A Range-Finding Acute Study of Naled Technical in Rats (MRID # 431896-01), in which dose-related signs of OP-toxicity were evident at doses from 35 to 150 mg/kg, (but not at 25 mg/kg and below), and mortality occurred at 300 mg/kg and above.

motor activity tests (FOB)[2] were performed during the pre-test period, 30-minutes post-dosing (presumed time of peak effect), and again on days Study Days 7 and 14. Animals were weighed before dosing, as well as on Days 0, 7 and 14; those found dead-on-study (DOS), as well as all survivors, were necropsied, the latter the day after the final FOB evaluation (Study Day 15).

After completion of each phase of the FOB, locomotor activity was evaluated at the same times (pretest, 30 minute post-dose, Study Days 7 and 14) using the Digiscan "Micro" Animal Activity System (Omnitech Electronics, Columbus, OH), and 40 minutes (as four ten-minute sessions) of data collection per animal. Locomotor activity was divided in two categories: "Total, and ambulatory, activity". The first measured fine motor skills (grooming, interruption of one or two photobeams), the latter the ability to interrupt several photobeams consecutively.

Necropsy examinations of animals DOS included all external surfaces, orifices, and body cavities (cranial, thoracic, abdominal-pelvic), and associated viscera. Carcasses were discarded. Survivors to 14 days were killed by CO_2, perfused <u>in situ</u>, and central

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a. Home Cage Observations
- Posture
- Convulsions/tremors
- Feces consistency
- Biting
- Palpebral (eyelid) closure

b. Handling Observations
- Ease of removal from cage
- Lacrimation/Chromodacryorrhea
- Piloerection
- Palpebral closure
- Red/Crusty deposits
- Eye prominence
- Ease of handling animal in hand
- Salivation
- Fur appearance
- Respiratory rate/character
- Mucous Membranes/eye color/skin color
- Muscle tone

c. Open Field Observations (evaluated over a 2 minute observation period)
- Mobility
- Rearing
- Convulsions/tremors
- Grooming
- Bizarre/Sterotypic behavior
- Time to first step (seconds)
- Gait
- Arousal
- Urination/defecation
- Gait score
- Backing

d. Sensory Observations
- Approach response
- Startle response
- Pupil response
- Forelimb extension
- Air righting reflex
- Touch response
- Tail pinch
- Eyeblink response
- Hindlimb extension
- Olfactory orientation

e. Neuromuscular Observations
- Hindlimb extensor strength
- Hindlimb foot splay
- Grip strength-hind and forelimb
- Rotarod performance

f. Physiological Observations
- Catalepsy
- Body Temperature
- Body weight

and peripheral neural tissues[3] from five control and 5 high-dose animals/sex preserved for histopathological examination. Brain dimensions (weight, length/width) and any gross changes were recorded.

Data generated were analyzed by conventional statistical tests, including ANOVA, followed by Dunnett's Test. The data from the FOB and Locomotor Activity sequences were analyzed by SAS/STAT software, including CATMOD procedures (Creason, 1989), fed into a Digital Micro VAX 3400 computer with appropriate programming.

E. RESULTS: (A selective summary of relevant findings is tabulated on the pages following:)

Dosing suspensions of the test material were analyzed by HPLC using UV detection at 210 nm (Report APPENDIX D). A linear response with concentration was observed in the range 33.76 to683.4 ug/ml, and homogenicity assured within ± 15% of nominal dose concentrations for six hours at room temperature, up to 23 days when frozen.

Three (of 16) males and 8 (of 16) females given 400 mg/kg (the HDT) died, most within 45 minutes of dose administration (all 3 males, and 4 of the 8 females), or within 24 hours of compound gavage (the remaining 4 females). All other high-dose animals, and those given lower doses survived until study termination (Day 15). (Table 1, Appendix A.) Most of the surviving high-dose animals manifested consistent orange-yellow staining of body fur, as well as reddish oral/nasal discharges, one

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Central Nervous System tissues[a]

Brain-forebrain, center of cerebrum, midbrain, cerebellum and pons, and the medulla oblongata
Spinal cord - at cervical swellings C3-C8 and at lumbar swellings T13-L4
Gasserian Ganglion/Trigeminal nerves
Lumbar dorsal root ganglion at T13-L4
Lumbar dorsal root fibers at T13-L4
Lumbar ventral root fibers at T13-L4
Cervical dorsal root ganglion at C3-C8
Cervical dorsal root fibers at C3-C8
Cervical Ventral root fibers at C3-C8
Optic Nerves
Eyes

[a] - embedded in paraffin

Peripheral Nervous System tissues[b]

Sciatic nerves (mid-thigh region and at sciatic notch)
Sural nerves
Tibial nerves
Peroneal nerves
Forelimbs[c]
Tail[c]
[b] - embedded ubokastic
[c] - preserved for possible future neuropathological evaluation

The effects of Acute Administration of Naled Technical on Neurotoxicity Parameters in Sprague-Dawley Rats[1]

Observation/Response		Dose (mg/kg) 0 M[2] (12)	0 F (12)	25 M (12)	25 F (12)	100 M (12)	100 F (12)	400 M (13)	400 F (8)
Daily Clinical Observations:									
DOS[3]		0	0	0	0	0	0	3	8
Body fur staining		0	0	0	0	0	0	7	8
Rales		0	0	0	0	1	0	1	1
Oral/nasal discharge		1	0	0	0	1	0	3	2
Mean body weight gain (g):									
Day 0-7		53	27	50	22	51	20	43	27
Day 0-15		108	41	102	41	105	42	95	47
FOB (Day 0-1): [A][4]	Extended limbs	0	0	0	0	4	8	11	7
	Convulsions[5]	0	0	0	1	5	3	2	0
	Tremors[6]	0	0	0	0	4	9	10	8
[B]	Lacrimation	0	1	0	0	4	4	1	3
	Salvation	0	0	0	0	2	6	7	8
	Exophthalmos	0	1	0	2	1	4	2	5
[C]	First step (sec)	0.6	0.6	0.7	0.6	0.6	4.3	3.1	0.9
	Impair. mobil.	0	0	0	0	2	3	7	7
	Ataxia	1	2	1	2	11	10	9	6
	Rearing (sec)	8.4	9.8	7.5	9.5	3.2	2.8	1.7	0.5
[D]	Absent approach response	0	0	0	0	2	3	5	6
	Absent touch response	0	0	0	0	0	2	4	5
	Absent tail pinch response	0	0	0	0	1	4	6	5
	Absent pupil response	0	0	0	0	10	9	5	7
	Altered air-righting reflex	0	0	0	0	4	6	5	5
	Limb extension	0	0	0	0	0	0	1	0
[E]	Abs. hind limb strength	0	0	0	1	6	4	5	5
	Rotarod perf. (sec.)	85.9	78.8	97.3	73.1	38.5	41.7	25.0	39.0
[F]	Catalepsy (sec.)	0.7	0.7	0.7	0.6	1.2	0.9	1.1	1.9
	Body T (°C)	39.1	39.5	39.1	39.2	37.3	36.4	36.3	35.4
[G]	Total motor activity (counts)	1741	1763	1640	1488	742	772	573	640

[1]Selected, statistically significant clinical effects and neurotoxic responses abstracted from Report Tables 1 through 51, and Appendices A and K

[2](Number in parentheses=group size at scheduled sacrifice.)

[3]DOS, found dead-on-study, before scheduled sacrifice.

[4]Bracketed letters correspond to categories of the FOB as given on p3 of this DER.

[5]Clonic tremors of limbs

[6]Whole body tremors

to two days post-dosage, which the authors attributed to naled treatment; comparable staining among low (25 mg/kg) and mid-dose (100 mg/kg) animals was discounted because the incidence was low and similar to controls. Additionally, rales were observed in one high-dose male (No. 93061) and one high-dose female (No. 93114), as well as in one mid-dose male (No. 93046); gait disturbances (rocking,lurching, swaying), tremors and hypoactivity were visible in a further 400 mg/kg group male (No. 96063) (Table 1, APP. K).

High-dose male group mean body weight gain was transiently but significantly lower (36%, $p<0.01$) than control for Days 0 to 7, which resulted in lower (12%) overall mean cumulative body weight gains for the periods 0 to 14 days and 0 to 15 days, as well as lower mean body weights for 7, 14 and 15 (2% and 4%); neither of the latter, however, reached statistical significance. No such treatment-related effect on body weights or gains were observed among high-dose females, nor in any of the lower dose groups (Tables 2, 3; APP. A).

Elements of the FOB (Functional Observational Battery) were altered in a statistically significant and dose-related fashion in mid-dose (100 mg/kg) rats and high-dose (400 mg/kg) survivors, and thus considered treatment related by the investigators (Tables 4 through 11; Figure 1; APP. A, compared also to historical control data provided in APP. G). For example, altered posture was evident in 5/12 mid-dose males and 8/12 mid-dose females, but in 11/13 males and 7/8 females in the high-dose group. Likewise, clonic convulsions as well as tremors were observed in the majority of both mid-dose and high-dose animals. One low-dose (25 mg/kg) female (No. 93178)---but no males---exhibited mild tremors on the day of dosing, recovering the next day. Since this sign was not observed in this laboratory's historical control data base (APP. G), it was considered a treatment-related effect. All animals observed to be so affected immediately after dosing recovered by Study Day 7.

Enhanced physiological responses following handling (lacrimation, salivation, eye prominence, respiration) were significantly altered in mid-dose and high-dose groups (Table 12-19; Fig. 2; APP. A; compared to historical control data, APP. G). Changes in eye prominence (specifically exophthalmus) were observed in all dose groups, exceeding either concurrent controls, during pre-treatment, or the WIL FOB historical background: High-dose, 2/13 males and 5/8 females; mid-dose, 1/12

males and 4/12 females; low-dose, 2/12 females (but no males); and control, 1/12 females. Hence, the author considered the exophthalmos in these 25 mg/kg females to represent a potential treatment effect. Changes in respiration (principally, rales and/or retching) occurred at the high-dose in 3/13 males and 3/8 females, at 100 mg/kg in 2/12 males and 1/12 females, but in no low-dose animals (or controls).

Open field observations (impaired mobility, gait alterations, convulsions, altered arousal, bizarre behavior) were affected by Naled treatment at the mid- and high- dose levels (Tables 20 to 27; (Fig. 3; APP. A; compared to historical control data, APP. G). For example, gait alterations --- as manifested by ataxia, tip-toeing, and limb-splaying---were observed in almost every mid-dose and high-dose animal, while convulsions and/or tremors were noted in about half these dose groups, with lesser incidences for other elements of these responses (impaired mobility, rearing scores, altered arousal, etc.). These effects were no longer apparent at Study Days 7 or 14 in affected mid-dose or high-dose animals, nor at any time among low-dose (25 mg/kg) animals.

At 100 and 400 mg/kg Naled, dose-related signs of toxicity were evident for a number of sensory parameters (Tables 28 to 35; Fig. 4; APP. A; compared to historical control data as provided in APP. G), as summarized below. As with other FOB effects, these also disappeared by Study Day 7.

Neuromuscular parameters (e.g., hind/forelimb extensor strength and splay; rotarod performance) were affected in about 50% of the animals receiving 100 and 400 mg/kg Naled Technical (Report Table 36-43; Fig. 5; APP. A; as compared to historical control data, APP. G). In addition, one low-dose female displayed reduced hindlimb resistance; since this effect was not seen in any control (concurrent or historical) or pre-treatment animal, the authors consider this also to be a possible effect of Naled treatment.

Physiological disturbances (catalepsy, altered body temperature) were evident among mid-dose and high-dose animals immediately following dosing (Report Table 44-47; Fig. 6; APP. A; compared to historical control data, App. G.); these effects were no longer apparent in affected animals at Study Days 7 or 14, nor in low-dose animals at any time. As noted above, body weight among high-dose (400 mg/kg) animals was slightly (insignificantly) reduced compared to concurrent

controls (5% and 2%).

Locomotor activity was also temporarily compromised in about half the animals receiving 100 or 400 mg/kg, especially during the earlier subsessions (Report Tables 48-51; Fig. 7, 8; APP. A; compared to historical control data, as supplied in APP. G). These effects disappeared in the affected animals by Study Day 7, and were never evident among low-dose animals at any time (see summary tabulation herein).

Gross pathological examination of the high-dose animals DOS (3 males, 8 females) revealed reddened renal cortico-medullary junctions in four females, dark red mucosa in the glandular portion of the stomach in one male and two other females, and white foamy contents in lungs and trachea of a seventh female, but no apparent anomalies in the remaining animals that died before scheduled termination, nor in scheduled sacrifices (Table 52). Finally there were no differences between any treated group and controls in brain weights or dimensions (Table 53), and no microscopic lesions in CNS or PNS among high-dose scheduled sacrifices (Table 54).

From these observations and testing results, the investigators concluded that the single oral administration of Naled Technical resulted in (as stated on pp 37, 38 of the Final Report):

"A. No remarkable differences in brain weight or dimensions for any treated animal compared to control group animals.

B. No treatment-related neuropathological lesions upon microscopic evaluation of 5 animals/sex in the 400 mg/kg group.

C. Three males (19% mortality) and eight females (50% mortality) died following dose administration with 400 mg/kg Naled Technical. The three males and six of the females died on day 0; 78% (three males, four females) of these deaths occurred within 45-minutes of dose administration. The remaining two females in the 400 mg/kg/group died on the day following test article administration (day 1).

D. A transient lower body weight gain (for study days 0 to 7) was observed in the 400 mg/kg group males that resulted in lower cumulative body weight gains (12%) for study days 0 to 14 and 0 to 15.

E. Treatment-related clinical signs of orange and/or yellow material on various body surfaces and red material around the mouth, nose and/or eyes were observed for males and females in the 400 mg/kg group, generally on the day following administration. Gait alterations, tremors and hypoactivity were observed for a single 400 mg/kg group male on day 1. Rales were generally limited occurrences in the 100 and/or 400 mg/kg groups.

F. Remarkable differences were observed between animals that received 100 and 400 mg/kg Naled Technical and control group animals when the functional observational battery was performed on Study Day 0: In general the responses occurred approximately 30 minutes following treatment, and were dose-related; however, they were transient in nature (a very limited number of signs were apparent on Study Day 7, and none were apparent on Study Day 14). Specific test results included:

1. Altered posture, convulsions (clonic) and tremors during the home cage observations;

2. Salivation, changes in eye prominence and respiratory character, and lacrimation during the handling observations;

3. Impaired mobility, gait alterations, convulsions (clonic), tremors and decreased rearing behavior during the open field observations. Time to first step was increased for the 400 mg/kg male and female groups and a single 100 mg/kg group female. In addition, one 400 mg/kg group male displayed an alteration of arousal and another male in this group displayed bizarre/stereotypic behavior (head flick);

4. Alterations in the approach, touch, startle, tail pinch, pupil, and eyeblink responses and in air righting reflex during the sensory observations. Altered forelimb and hindlimb extension observed for a single 400 mg/kg group male may have been related to treatment:

5. Reduced hindlimb resistance, rotarod performance, forelimb grip strength and hindlimb grip strength during the neuromuscular observations;

6. Increased catalepsy times and decreased body temperature means during the physiological observations.

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7. Conduct of the day 0 functional observational battery on animals dosed at 25 mg/kg revealed a few treatment-related signs in a limited number (a total of 4) of females. Specific test results include:

a. Slight tremors of the limbs in one 25 mg/kg group female during the home cage observations.

b. A change in eye prominence (exophthalmus) in two females during the handling observations was considered a potential effects of treatment;

c. Reduced hindlimb resistance for one 25 mg/kg group female during the neuromuscular observations was considered a potential effects of treatment.

8. Dose-related reductions in mean ambulatory and total motor activity were apparent on day 0 following Naled Technical administration in males and females dosed at levels of 100 and 400 mg/kg.

9. There were no apparent treatment related signs of neurotoxicity persisting to Day 7. A single 400 mg/kg group male had altered respiratory character (rales) during the handling observations on day 7. This was consistent with the rales observed for this animal at the daily clinical examinations on study days 1 to 2, 4 to 6 and 8 to 10. This finding may represent a potential primary, secondary or non-specific effect of dosage administration."

Thus, all six of the functional domains for the FOB and motor activity evaluations described by Moser (1991) were affected by 100 mg/kg and 400 mg/kg Naled, albeit transiently, with no apparent pathological consequences. Based upon this study's results, the authors consider 25 mg/kg to be the NOAEL for acute neurotoxicity in males, but the LOAEL (minimal effect) in females.

F. <u>TB CONCLUSIONS</u>: TB tends to agree with the categorization of altered neurological responses in naled-treated <u>male</u> groups, providing the following toxicology parameters:

NOAEL = 25 mg/kg (the LDT)
LOAEL = 100 mg/kg

Although this study did not establish a NOAEL for females, the neurological effects seen at 25 mg/kg may be considered marginal, so that a firm no-effect level may be estimated at a lower level, if for example, we include the results from the preliminary range-finding assay, in which no toxicity was evident at either 5 or 25 mg/kg. Thus a conservative (reasonable) estimate of the NOAEL for females may be set at 5 mg/kg.

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APPENDIX

Validation studies to assure the lab's capability to perform and adherence to the Agency's criteria to detect endpoints indicative of neurotoxicity (as specified in PAG Addendum 10-Neurotoxicity, Series 81-8, 82-7 and 83-1), were performed by WIL using appropriate reference neurotoxins, and the results reported as Studies 099026, 099032, 099034 and 099035 (WIL-194007, REPORT APPENDIX J):

Study No.	Test Procedure (Equipment)	Ref. Compound	Expected Response	Results*
099026	Activity Counts (Digiscan "Micro")	Amphetamine Sulphate	Increased counts	Males: +136-246% Females: +53-84%
		Chlorpromazine HCl	Decreased counts	Males: -25 to 59% Females: -36 to 74%
099032	F. O. B.	Carbaryl	Potent Inhibitor AChE activity	Tremors; salivation; altered gait, arousal, and tail pinch, etc.
099034	F. O. B.	Acrylamide	Neuromuscular deficit	Increased alterations in musle tone, hindlimb extension, rotarod performance.
		Trimethyltin Cl	CNS pathology	Loss of dentate gyrus (2/5). Chromatolysis of gasserian ganglion neurons (1/5)
099035	{Reliability between Observers}	3'-3'Iminodipropr-ionitride (IDPN)	Altered behavior	"Consistent" scores for gait, startle response, and other behavioral IDPN-toxicity.

* $P \leq 0.05$

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