



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

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586 JAN 6 1995

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: NALED---Tox Data Submitted Under MRID 432239-01, -02,
and -03
ID # 034401

(PCCODE)
Chemical: 034401 (586)
RD Record: S469741
HED Project: D205390

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I
Health Effects Division (7509C) *Irving Mauer*
12/12/94

TO: L. Schnaubelt/B. Lowery, PM #72
Reregistration Branch
Special Review and Reregistration Division (7508W)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I
Health Effects Division (7509C) *Karl P. Baetcke*
12/13/94

Registrant: Valent USA, Walnut Creek, CA

Request: Review and evaluate the following subchronic neurotoxicity studies.

- (1) (82-7) "A Subchronic (13-Week) Neurotoxicity Study of Naled Technical in Rats", performed by WIL Research Laboratories, Ashland, OH, WIL Laboratory Project No. 194008/VP-10104 (5 volumes), Final Report dated April 28, 1994 (MRID 43223901).
- (2) (82-6) "A 28-Day Subchronic Delayed Neurotoxicity Study in Laying Hens (Gallus gallus domesticus)" (MRID 43223902), together with: "A Range-Finding Study for a Subchronic Delay[ed] Neurotoxicity Study in Laying Hens (Gallus gallus domesticus)" (MRID 43223903), both performed by Wildlife International, Easton MD, Laboratory Project No. 263-132/VP-10103, and 263-129/VP-10103, both Final Reports dated April 29, 1994.

TB CONCLUSIONS: These studies have been adjudged as follows
(full detailed reviews are attached to this memo):

①



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Study (MRID)	Reported Results	TB EVALUATION
(82-7) Subchronic (90-day) oral neurotoxicity-rat (43223901)	<u>Doses tested:</u> 0, 0.4, 2.0, 10.0 mg/kg/day for 90 days by oral gavage. NOAEL = 2.0 mg/kg/day (females only) LOAEL = 10.0 mg/kg/day (transient tremors in females only) NOAEL > 10 mg/kg (males)	<u>ACCEPTABLE</u>
(82-6) 28-Day delayed neurotoxicity-hen (43223903, 43223902)	<u>Doses tested:</u> 0, 0.4, 2.0, 4.0 mg/kg/day for 28 days by oral intubation. Clinical NOEL = 2.0 mg/kg/day LOEL = 4.0 mg/kg/day (transient BW depression; AChE reduction) Delayed neurotoxic NOEL > 4.0 mg/kg/day (HDT)	<u>ACCEPTABLE</u>

Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I, HED (7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I, HED (7509C)

Irving Mauer
11/21/94
Karl P. Baetcke
12/2/94

DATA EVALUATION REPORT

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MRID No.: 432239-01
PC No.: 034401
RD Record No.: S469741
EPA ID No.: 034401
Tox Chem No.: 586
Project No.: D205390

I. SUMMARY

Study Type: (82-7) Subchronic (90-Day) Neurotoxicity Screening
Battery - Rat

Chemical: Naled [1,2-dibromo-2,2-dichloroethyl
dimethylphosphate]

Synonyms: Dibrom

Sponsor: Valent USA
Walnut Creek, CA

Testing Facility: WIL Research Labs., Inc. (WIL)
Ashland, OH

Title of Report: A Subchronic (13-Week) Neurotoxicity Study of
Naled Technical in Rats

Author: Ian C. Lamb

Study Number: WIL-194008/VP-10104

Report Issued: April 28, 1994

Executive Summary: Test article (Naled technical, 94.35% a.i.) was administered by gavage to Sprague-Dawley rats for 90 days at doses of 0, 0.4, 2.0 and 10.0 mg/kg/day, and neurological parameters measured by both the Functional Observation Battery (FOB) and Locomotor Activity (LA) procedures. Minimal neurotoxic effects (tremors) were registered in 3/10 high-dose females, but no other clinical effects were observed at any dose. Hence the NOEL and LOEL for females were considered as 2 and 10 mg/kg/day, respectively, and the NOEL for males as 10 mg/kg/day.

TB-I Evaluation: ACCEPTABLE, in consideration of previous repeat dose and chronic studies, which record 10 mg/kg/day as an effect level (= LOEL).

II. DETAILED REVIEW:

A. Test Material: Naled Technical (from AMVAC, Los Angeles, CA)

Description: Clear viscous liquid
Batches (Lots): 204026
Purity (%): 94.35%
Solvent/carrier/diluent: 0.25% Aqueous
Carboxymethylcellulose (CMC)

B. Test Organism: Rodent

Species: Rat
Strain: Sprague-Dawley; Crl:CD®BR
Age: 44 days
Weights: males: 176-209 g
females: 129-166 g
Source: Charles River, Portage, MI

C. Study Design (Protocol): This study was designed to assess the neurotoxicity potential of the test article when administered orally to rats for 13 weeks, and determining functional and locomotor parameters, according to established (published) procedures and FIFRA/OECD 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: Groups of rats (10/sex/dose level) were administered test article by oral gavage in a volume of 5 ml/kg at daily dose levels of 0 (CMC vehicle only), 0.4, 2.0 and 10.0 mg/kg/day¹ for

¹ Doses selected on the basis of the following previous repeat dose toxicity studies with Naled:

DIBROM® Four-Week Subchronic Oral Toxicity study in Rats. Bio-Research Laboratories, Ltd., Project No. 9393, October 1, 1981, MRID No. 00088871, which resulted in minimal clinical toxicity at 10 mg/kg/day but lethality at 100 mg/kg/day; and

DIBROM® Chronic Oral Toxicity/Carcinogenicity Study in Rats. Bio-Research Laboratories, Ltd., Project No. 9394, June 7, 1984, MRID No. 00141784, which reported tremors in females and reduced plasma, RBC and brain cholinesterase in both

90 days. Animals were observed twice daily; weighed, and food consumption recorded, weekly; and measurements from a Functional Observation Battery (FOB)² as well as motor and locomotor activity (using the Digiscan "Micro" Animal

sexes treated at the HDT, 10 mg/kg/day.

² a. Home Cage Observations

Posture	Biting
Convulsions/Tremors	Palpebral (eyelid) closure
Feces consistency	

b. Handling Observations

Ease of removal from cage	Ease of handling animal in hand
Lacrimation/Chromodacryorrhea	Salivation
Piloerection	Fur appearance
Palpebral closure	Respiratory rate/character
Red/Crusty deposits	Mucous membranes/Eye/Skin color
Eye prominence	Muscle tone

c. Open Field Observations (evaluated over a 2 minute observation period)

Mobility	Gait
Rearing	Arousal
Convulsions/Tremors	Urination/Defecation
Grooming	Gait Score
Bizarre/Stereotypic behavior	Backing
Time to first step (seconds)	

d. Sensory Observations

Approach response	Touch response
Startle response	Tail pinch response
Pupil response	Eyeblink response
Forelimb extension	Hindlimb extension
Air righting reflex	Olfactory orientation

e. Neuromuscular Observations

Hindlimb extensor strength	Grip strength-hind and forelimb
Hindlimb foot splay	Rotarod performance

f. Physiological Observations

Catalepsy	Body weight
Body temperature	

Activity System provided by Omnitech Electronics)³ recorded during Study Weeks -1 (pretest), 1, 3, 7 and 12.

At study termination, survivors were euthanized (CO₂ inhalation), perfused in situ, and central and peripheral neural tissue⁴ collected for neurohistopathological examination from 5 animals/sex from control and high-dose groups. Brain weight and dimensions (length, width) were recorded for all animals; any gross changes or lesions were also recorded.

Data other than from the FOB and Locomotor Activity (LA) runs, were analyzed statistically by ANOVA using a Digital MicroVAX 3400 computer with appropriate program for body and brain weight/dimension, food consumption, and histopathological findings; significance (at both 5% and 1% levels) was determined by either Dunnett's Test (continuous data), or the one-tailed Kolmogorov-Somirnov Test (discontinuous data).

Continuous FOB and LA data were subjected to a computer program employing SAS/STAT software, analyzed by ANOVA, followed by Dunnett's Multiple t-Test for significance ($p \leq 0.05$). FOB parameters yielding ordinal (scalar) or descriptive data were analyzed the SAS/CATMOD procedure, with significance determined by Fisher's Exact Test (or Dunnett's).

-
- ³ Four 10-minute sessions, measuring: (i) total motor activity (grooming, interruption of singular photobeams); as well as (ii) ambulatory activity (interruption of three or more consecutive photobeams)

⁴Central 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 nerve
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

Peripheral Nervous System tissues^b

Sciatic nerve (mid-thigh region and at sciatic notch)
Sural nerve
Tibial nerve
Peroneal nerve
Forelimbs^c
Tail^c

^a = embedded in paraffin

^b = embedded in plastic

^c - Preserved but not examined

[To provide evidence that this lab could conduct reliable assays for behavioral effects and neuropathological lesions that meet the criteria specified in ADDENDUM 10 - NEUROTOXICITY of the Agency's PAG-Subdivision F, WIL tested (during the period 11/5/90 to 8/9/91) a variety of chemicals as positive controls capable of producing neurotoxic effects, namely: D-amphetamine sulfate, chlorpromazine HCl, carbaryl, acrylamide, trimethyltin chloride, and 3,3-iminodipropionitrile (Report APPENDIX G). The results of these studies validated both the sensitivity of the systems employed and the competence of the lab staff to perform these operations.]

- E. Results: [A selective, summary of significant data from the Final Report is presented on the following page.] Dosing suspensions were analyzed by HPLC with UV photodiode array detection, and corrected concentrations were found to range within acceptable limits of nominal (Report Appendix D).

All animals survived to scheduled termination (Report Table 1; Appendix A). The singular adverse clinical effects recorded were sporadic occurrences of tremors (of the forelimb, or hindlimb, and/or whole body) in three high-dose (10 mg/kg/day) females at the 30-minute post dose observation period, on Study Day 19 for one animal, and again on six occasions during Days 73-87, but later (Study Day 65 and 81) for the other two. Other clinical signs noted in Naled-treated animals were either a common finding in lab rats (e.g., hair loss), or were observed with incidences comparable to concurrent controls (e.g. oronasal/perineal staining of fur), hence were considered to be not attributable to the test article (Report Tables 1, 2; Appendix B). No consistent effect of treatment was apparent at any dose on either mean body weight or gain (Report Tables 3, 4, 4A; Appendix A) or food consumption (Report Tables 5, 6; Appendix A).

No significant differences were recorded in any group for any element of the FOB or MA/LA as evaluated during the pre-test period vs Study Weeks 3, 7 or 12 (Report Tables 7 through 54; Appendices A, B, as compared to historical control data, provided in Report Appendices I and J).

Gross pathological examination revealed no adverse effects in any test group on mean brain weight or dimensions (Report Table 55; Appendix A). Microscopic inspection (Report Table 56; Appendix A) uncovered sporadic incidences of neurohistopathological lesions among a minority of treated as well control animals (digestion chambers in trigeminal nerves of one high-dose group female, the sciatic nerve of one-high dose male and

two high dose females, as well as one control male, and the tibial nerves of another on 10 mg/kg/day male; unilateral retinal degeneration in another 10 mg/kg/day female), but were discounted as treatment related because similar incidences of these lesions have been noted in control animals from other neurotoxicity studies conducted at this laboratory (Report Appendix K).

Effects of Naled Administered Orally by Gavage to Sprague-Dawley Rats ¹								
	Dose Groups (mg/kg/day)							
<u>OBSERVATION</u> (10/sex/group)	0		0.4		2.0		10	
Daily Examination (# affected)	M	F	M	F	M	F	M	F
Mortality	0	0	0	0	0	0	0	0
Hair loss	0	1	1	2	0	2	4	4
Fur stains	4	1	5	0	3	2	5	4
Tremors	0	0	0	0	0	0	0	3
<u>BODY WEIGHT GAIN(S)</u>								
Weeks 0-13	322	123	313	130	330	131	320	113
<u>FOB</u>								
Week - 12 Mean Urination count (n)	2.2	0.1	1.7	0	2.3	0	0.7*	0
Week - 3 Tail Pinch response (n)	2	6	6	5	9	3	7*	4
¹ Selective (and/or statistically significant) effects extracted from Summary Tables 1 through 56.								
* Although significantly different from concurrent control (p < 0.05), not considered treatment-related.								

The author concluded that oral administration of Naled Technical at doses of 0.4, 2.0 and 10 mg/kg/day for three months resulted in minimal clinical effects in females only, limited to transient sporadic occurrences of tremors in 3/10 high-dose females, but no effects on body weight, food consumption, FOB or LA neurotoxic evaluations, or pathological consequences. The NOAEL for neurotoxicity was considered to be 2.0 mg/kg/day for females and 10 mg/kg/day for males.

- F. TB-I Evaluation: Minimally ACCEPTABLE as a comprehensive evaluation of the neurotoxicity potential of Naled technical in rats, based upon the following qualifications.

Dosing in previous oral studies upon which dose-selection in this assay was based ranged generally in multiples of 5 (0.2, 2.0 and 10 mg/kg/day for the chronic) or 10 (1, 10, 100 mg/kg/day in the 28-day study), resulting in minimal clinical and/or biochemical effects at 10 mg/kg/day, but severe toxicity (including death) at 100 mg/kg; there were no attempts at evaluating intermediate doses below the lethal dose (say 15, 20, 25 mg/kg/day, etc.), which we consider a fault of these studies.

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Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I, HED (7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I, HED (7509C)

Irving Mauer
12/12/94
Karl P. Baetcke
12/13/94

DATA EVALUATION RECORD

MRID No.: 432239-02
PC No.: 034401
RD Record No.: S469741
EPA ID No.: 034401
Tox Chem. No.: 586
Project No.: D205390

I. SUMMARY

STUDY TYPE: (82-6) 28-Day delayed neurotoxicity - hen.

CHEMICAL: Naled [1, 2-dibromo-2, 2-dichloroethyl dimethyl phosphate]

SYNONYMS: DIBROM®

SPONSOR: Valent USA, Walnut Creek, CA

TESTING FACILITY: Wildlife International Ltd., Easton, MD

TITLE OF REPORT: A 28-Day Subchronic Delayed Neurotoxicity Study in Laying Hens (*Gallus gallus domesticus*)

AUTHOR(S): Joann B. Beavers and James W. Foster

STUDY NUMBER: 263-132/VP-10103

DATE ISSUED: April 29, 1994

EXECUTIVE SUMMARY: Groups of laying hens (14 per dose group) received naled technical (91.7% a.i.) at oral dose levels of 0, 0.4, 2.0 and 4.0 mg/kg/day for 28 days. Four animals per group were sacrificed two days after the final dose for determinations of brain acetylcholinesterase (AChE) and neurotoxic esterase (NTE); the remainder were observed for evidence of delayed neurotoxicity for up to three weeks, following which survivors were necropsied.

Minimal and transient body weight depression was recorded only in high-dose (4 mg/kg/day) birds, (and only during week-1 of treatment), and significant decreases in brain AChE at both 2.0 and 4.0 mg/kg/day, but no clinical or delayed neuropathy in any naled-treated group at any dose.

TB-I EVALUATION: ACCEPTABLE.

II. DETAILED REVIEW

A. TEST MATERIAL: Naled Technical

Description: Clear liquid
Batches (Lots): VS-6-17 (Amvac 204057)
Purity (%): 91.7
Solvent/carrier/diluent: 0.25% Aqueous
Carboxymethylcellulose
Sodium (CMC)

B. TEST ORGANISM: Domestic bird

Species: Hen (White Leghorn)
Strain: Gallus gallus domesticus
Age: 49 weeks
Weights - females only: 1461-1525 g
Source: Wenger Feed Mills, Rheems, PA

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the delayed neurotoxicity potential of the test article when administered orally for 28 days to domestic laying hens, according to established (published) procedures and FIFRA Test Guidelines (GDLN 82-6).

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: Following preliminary dose-selection testing,¹ groups of hens (14 /dose group) were orally intubated with test article at dose levels of 0 (CMC vehicle), 0.4, 2.0 and 4.0 mg/kg/day (in a constant volume of 6 ml/kg) for 28 days. Four of each group were sacrificed 48 hours after the final dose for determination of acetylcholinesterase (AChE; brain) and neurotoxic esterase (NTE, brain and spinal cord). The remainder (10/group) were evaluated twice

¹ A Range-Finding Study for a Subchronic Delayed Neurotoxicity Study in Laying Hens (*Gallus gallus domesticus*), Wildlife International Ltd., Study No. 263-129 (MRID 432239-03), performed at four dose levels (2, 4, 8 and 16 mg/kg/day, for seven days) and resulting in deaths at 8 mg/kg/day (2/4 birds) and the HDT (all 4 birds), with evidence of cholinesterase inhibition at 4 mg/kg/day and above

weekly for locomotor activity² for 21-days following the last dose. At the end of this post-dose period all survivors were perfused for gross necropsy but neural tissues (brain, spinal cord, sciatic and tibial nerves) from only six control and six high-dose birds were prepared for histopathologic evaluation.

Two additional groups of 14 birds each were administered the known neurotoxin, triorthocresyl phosphate in corn oil (TOCP: 35 and 45 mg/kg/day), to serve as positive controls. Due to mortalities and increased clinical signs of delayed neurotoxicity, however, the TOCP-treated groups had to be terminated earlier than the 49-day study period for naled-test birds (35 mg/kg TOCP, on Study Day 41; 45 mg/kg-birds on Day 35).

Birds were weighed and feed consumption determined at the beginning of the dosing period, and weekly thereafter. Those dying-on-study (DOS) and all survivors to study termination were perfusion-fixed (10% neutral buffered formalin), and subjected to necropsy under supervision of a qualified pathologist³, who supervised the collection of neural tissues (brain, spinal cord, sciatic and proximal tibial nerves), which were transported to EPL for processing and histologic examination (by H & E/Luxol Fast Blue staining, and PAS counterstain).

Body weight, feed consumption, enzyme activities (AChE, NTE) and ataxia scores were statistically analyzed by Dunnett's Multiple Comparison Procedure, using the TOXSTAT program.

²"Drop, walk and hop" each activity scored according to the following ataxia point system:

POINTS

ATAXIA ASSESSMENT

- | | |
|---|--|
| 0 | No Ataxia |
| 1 | Slight incoordination; occasional stumbling or wing drooping, especially after exertion. |
| 2 | Staggering gait, tail and leg reflexes may be affected; bird lands awkwardly. |
| 3 | Continuous staggering gait, bird rests often, tail and leg reflexes usually noticeable affected. |
| 4 | Bird stands for short periods only, normally moves by shuffling on hocks; tail and leg reflexes usually noticeably affected. |
| 5 | Bird unable to stand, weak limb movements; tail and leg reflexes virtually non-existent. |

Each of the four activities was scored on a scale of 0 to 5 with 0 - No Ataxia and 5 - Maximum Effect. The highest possible total score for each hen was 20.

³From EXPERIMENTAL PATHOLOGY LABORATORIES (EPL), Herndon, VA

- E. RESULTS: [A synopsis of select (significant) results is presented on the following page.]
Dosing suspensions were analyzed by HPLC with UV photodiode array detection, and corrected measured concentrations were found to range from 93% to 113% of nominal (Report APPENDIX III).

No control or Naled-treated birds died during the study period, whereas six TOCP-treated hens (2/14 at 35 mg/kg/day; 4/14 at 45 mg/kg/day) succumbed DOS before the scheduled study termination, following signs of severe toxicity, including: Loss of coordination, lower limb weakness, depression, wing droop, prostration, gasping/labored breathing (Report Table 1; APPENDIX IV). Transient lameness was manifest in six controls, as well as in five Naled-treated birds (2 low-dose; 2 mid-dose; 1 high-dose). However, no signs of ataxia typical of delayed neurotoxicity were observed in any test bird, in contrast to statistically significant numerical values ($p \leq 0.01$) different from controls for both TOCP groups (Report Table 2; APPENDIX V).

No apparent treatment-related gross or neuro-histopathological abnormalities or lesions attributable to Naled were recorded in any test group, in contrast to severe neuropathy (e.g. loss of muscle mass; myelin degeneration, etc.) found in TOCP-birds (APPENDICES IX, X).

A transient loss in mean body weight was recorded in high-dose Naled birds in Study Week 1 (7% less than pretreatment), which was followed by a compensatory body weight gain during the second week of treatment; no differences were evident during the remainder of the study period for either the 4.0 mg/kg/day test group, or at any time for low-dose or mid-dose, groups (Report Table 3; APPENDIX VI). Comparable effects on feed consumption were noted for the high-dose Naled group, specifically statistically significant ($p < 0.05$) reduction during Study Week 1 (71% of control value), but no other apparent effects on feed at any of the other measuring intervals (APPENDIX VII).

AChE, but not CNS-NTE, activity was significantly depressed ($p \leq 0.01$) in a dose-responsive manner by 2.0 (28.5%) and 4.0 (42.5%) mg/kg/day Naled (Report Table 5; APPENDIX VIII). In contrast, AChE responded only minimally to the higher dose of TOCP, but NTE activity plummeted in both TOCP groups (by 71% and 75%).

Based upon these results, the authors concluded that while 4.0 mg/kg/day Naled for 28 days produced

Neurotoxicity in Laying Hens Administered Naled Orally
for 28 Days¹

Observation (14/dose group)	Dose Group (mg/kg/day)			
	0	0.5	2.0	4.0
	F	F	F	F
Mortality	0	0	0	0
Ataxia	0	0	0	0
Body Wt. Change (g):				
(Study Wk-1)	-36	-61	-40	-98 ²
(Study wk-2)	16	15	10	65
(Study overall)	-51	-50	-27	+31
Neural Chemistry: ⁴				
Brain AChE uM/min.g	21.4	19.1	15.3 ³	12.3 ³
Brain NTE nM/min.g	1740	1630	1740	1730
Spinal NTE nM/min.g	525	619	646	674 ²

¹Extracted from Tables 1 through 5 and APPENDICES IV through X of the FINAL REPORT

²Statistically significant difference from control, $p < 0.05$ (an increase not considered biologically or toxicologically relevant)

³Statistically significant difference from control, $p < 0.01$

⁴From 4 birds/dose-group, and measured at 48 hours post-treatment.

transient and minimal clinical (body weight depression), and chemical (AChE depression) effects, it was considered negative for induction of delayed neurotoxicity at doses up to the HDT.

F. TB EVALUATION: ACCEPTABLE.

REVIEWER NOTE ADDED: Based upon the results of the preliminary 10-day dose range-finding study (No. 263-129, MRID 432239-03), the sponsor (and contract laboratory) might have been apprehensive with the obvious steep dose-response curve which would caution them against selecting a dose as the HDT for the main definitive study which had produced 50% mortality, namely, 8 mg/kg/day. Hence they probably reasoned that 4 mg/kg/day represented a sufficiently high level for the purpose of the assay. Therefore, selecting some intermediate dose level as the HDT (say 6 mg/kg/day) might have better served to satisfy the criteria for this type of assay.

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