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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Dichlorvos (DDVP) - Submission of a 12-Month Chronic Oral Study in Dogs (EPA ID No. 5481-96)

TOX Chem No.: 328
Project No.: 0-1891
Submission No.: S381707

FROM: William B. Greear, M.P.H. William B. Greear 11/29/90
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Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: Jane M. Talerico/Lois Rossi, PM Team 74
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THRU: Marion P. Copley, D.V.M., Section Head
Review Section II
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Health Effects Division (H7509C)
KB 12/1/90

I. CONCLUSIONS

The 12-month chronic oral study in dogs (HLA Study No. 2534-102, August 6, 1990) satisfies the requirement for a Guideline Series 83-1 Chronic Toxicity Study in Dogs.

II. ACTION REQUESTED

In a letter dated August 14, 1990, Diane Allemang of Jellinek, Schwartz, Connolly & Freshman, Inc. submitted the report on the chronic toxicity of DDVP in dogs (HLA Study No. 2534-102, August 6, 1990) on behalf of AMVAC Corporation to further support AMVAC's DDVP reregistration.

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II. COMMENTS

In the letter dated August 14, 1990 from Diane Allemang, it is stated that the data requirement for a chronic toxicity study in dogs is listed as being satisfied according to the DDVP Registration Standard (September 1987). This new 12-month dog study was conducted in accordance with the data requirements of California's Birth Defects Prevention Act (SB 950).

The previously submitted and reviewed chronic dog feeding study has been recently invalidated (see memorandum of Henry Spencer dated November 5, 1990).

This new study satisfies the requirements for a chronic oral dog study.

12-Month Chronic Oral Study - Dog (EPA Registration No. 2534-102; August 6, 1990)

NOEL = 0.05 mg/kg/day
LEL = 1.0 mg/kg/day (plasma and RBC ChE inhibition in males and females as early as the first time point measure (2-weeks) and brain ChE inhibition in males)

2-Week Range-Finding Study - Dog (EPA Registration No. 2534-101; No Date)

Plasma and RBC ChE were inhibited at the 1.0 mg/kg/day dose level and at higher dose levels. Brain ChE was decreased in the 0.1 mg/kg/day dose level in the animals in the 5.0, 10.0, and 30.0 mg/kg/day dose levels.

Reviewed By: William B. Greear, M.P.H. William B. Greear 11/27/90
Review Section II, Toxicology Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. Marion P. Copley 11/28/90
Review Section II, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT

Study Type: Guideline Series 83-1
1-Year Oral - Dog

TOX Chem No.: 328

Test Material: DDVP

MRID No.: 415931-01

Synonyms: 2,2-Dichlorovinyl dimethyl phosphate, Dimethyl 2,2-dichlorovinyl phosphate, Dichlorvos, Herkol, Dedevap, Oko, Vapona

Study Number: HLA Study No. 2534-102

Sponsor: AMVAC Chemical Corporation
Los Angeles, CA 90023

Testing Facility: Hazleton Laboratories America, Inc.
Vienna, VA 22182

Title of Report: A 52-Week Chronic Toxicity Study on DDVP in Dogs.

Author: Victoria R. Markiewicz

Report Issued: August 6, 1990

Conclusions:

NOEL = 0.05 mg/kg/day
LEL = 1.0 mg/kg/day (plasma and RBC ChE inhibition in males and females as early as the first time point measure (2 weeks) and brain ChE inhibition in males)

[The sponsor should submit Flow Chart No. 1 for examination.]

Classification:

Core-Guideline. The study satisfies the requirement for a Guideline Series 83-1 Chronic Oral Study in Dogs.

A. Materials:

1. Test Compound - DDVP, Description: A clear, oily liquid; Lot No. 802097; Purity: Not reported; Contaminants: Not reported.

2. Test Animals - Species: Dog; Strain: Beagle; Age: 6 to 7 months; Weight: Males 8.7 to 11.7 kg, Females 5.2 to 8.9 kg; Source: Hazleton Research Products, Inc., Cumberland, VA.

B. Study Design:

1. Animal Assignment - Animals were assigned via a weight randomization program to the following test groups:

Test Group	Dose by Capsule (mg/kg/day)	52 Weeks Male	52 Weeks Female
Control	0	4	4
Low	0.1[a]	4	4
Mid	1.0	4	4
High	3.0	4	4

[Dosages were selected based on the results of a 2-week range-finding study (HLA No. 2534-101).]
[a]The dose was lowered to 0.05 mg/kg/day on day 22 due to the inhibition of plasma ChE noted after 12 days dosing at 0.1 mg/kg/day.

2. Animal Dosing - Animals received the test material by capsule. Capsules were prepared weekly based on the most recently recorded body weight, and stored refrigerated and protected from light. Prior to preparing the capsules, the test material was stored refrigerated.

Results - The purity of the test material as percent relative purity compared to an analytical standard was 97.3 percent when determined at the beginning of a 2-week range-finding study (HLA No. 2534-101). The stability of the test material from September 21, 1988 to January 8, 1990 was greater than 98 percent when compared to an analytical standard.

3. Animals received food (Purina Certified Canine Diet Meal® No. 5007) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data, body weights, body weight changes, total food consumption, clinical pathology data

(except for plasma and RBC, ChE, cell morphology and urinalyses), and organ weight data of the treated groups were compared to the control data using Levene's Test of Homogeneity of Variances. Homogenous data and heterogenous data (after transformation) were subjected to ANOVA. Dunnett's control vs. treatment comparisons were made for equal variances and unequal variances if heterogenous. Group comparisons were analyzed at the 5 percent, 2-tail probability level. RBC and plasma ChE values were analyzed by factorial repeated measures analysis of variance (ANOVA) techniques. These techniques allow each individual animal to serve as its own control. Brain ChE data were analyzed for each individual sex by one-way ANOVA techniques as indicated in "Flow Chart No. 1." ["Flow Chart No. 1" should be submitted for examination.] Statistical analyses were conducted at the $p \leq 0.05$ level. Group comparisons (2-tailed) were performed at the same level.

5. A Quality Assurance Statement was provided and signed by Janet Milazzo on August 6, 1990.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for mortality and moribundity. Cage-side observations for signs of toxicity were made at least once daily for approximately 1 hour postdosing. Physical examinations were made once weekly.

   Results - No deaths occurred. Ataxia, salivation, and dyspnea were observed in one male in the 3.0 mg/kg/day group on 1 day during Week 33. The investigator believes these signs were due to a slight overdose with DDVP. Formulation and dosing records could not confirm that an overdose occurred. Emesis containing the test material or capsule was noted in one male and three females in the 3.0 mg/kg/day group, one female in the 1.0 mg/kg/day group, and in one male and one female in the 0.05 mg/kg/day group.

2. Body Weight - They were weighed weekly for Weeks 1 to 16 and once every 4 weeks thereafter.

   Results - Mean cumulative body weight gain was decreased in males in the 3.0 mg/kg/day group from Week 1 to Week 7. However, after Week 7, cumulative body weight gain of males in the 3.0 mg/kg/day group was comparable to the other groups (see Table 1 below).

Table 1. Mean Cumulative Body Weight Gain (kg) in Males

Group (mg/kg/day)	Week 2	4	6	8	12	24	52
0	.2	.3	.3	.3	.7	1.5	.9
0.05	0	.4	.5	.8	1.2	2.4	2.0
1.0	0	.4	.6	.7	1.3	2.4	1.9
3.0	-.5	-.3	-.1	.1	.8	2.0	1.3

3. Food Consumption and Compound Intake - Consumption was determined weekly for Weeks 1 to 16 and then once every 4 weeks thereafter.

Results - Unremarkable.

4. Ophthalmological Examinations were performed prior to and at termination using an indirect ophthalmoscope and a 1% Mydriacyl solution.

Results - Unremarkable.

5. Blood was collected before treatment (Week -2) and during Weeks 26 and 52 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)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)
X	Absolute reticulocyte count[1]	X	Activated partial thromboplastin time
X	Cell morphology		
X	Corrected leukocyte count	X	Prothrombin time
X	Reticulocyte count[1]		

[1]Determined only during Week -2 due to technician error.

Results - During Week 26, one male (G26238) in the 3.0 mg/kg/day group exhibited a decrease in the HCT (31.1%) and HGB (10.7 g/dl). This appeared to be a transient event in that the HCT and HGB recovered by Week 52. Because the decreases were not observed throughout the study, the decreases were slight, and significant decreases were not observed in other animals, this indication of slight anemia observed in male #G26238 at Week 26 was probably not treatment related.

b. Clinical Chemistry

X	Electrolytes
X	Calcium
X	Chloride
	Magnesium
X	Phosphorous
X	Potassium
X	Sodium
	Enzymes
X	Alkaline phosphatase
X	Cholinesterase[1]
X	Creatinine phosphokinase
	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (also SGPT)
X	Serum aspartate aminotransferase (also SGOT)

X	Other
X	Albumin
X	Blood creatinine
X	Blood urea nitrogen
X	Cholesterol
X	Globulins
X	Glucose
X	Total bilirubin
X	Total protein
	Triglycerides
X	A/G ratio

[1]Determined on Days -7, -4, -2, and during Weeks 2, 6, 13, 26, 39, and 52 approximately 3 hours postdosing on nonfasted animals. Brain ChE determined at termination (see Attachment 1 for methods).

Results - One female (G26225) in the 0.05 mg/kg/day group had increased SGPT (500+ U/L), SGOT (85 U/L) and alkaline phosphatase (120+ U/L) at 26 and/or 52 weeks. This appeared to be an incidental finding that was not observed in other treated animals. Plasma ChE was decreased (21.1 to 66.6%)* in males and females in the 0.1, 1.0, and 3.0 mg/kg/day groups during Week 2 (see Table 2). Because of the degree of plasma ChE inhibition in the 0.1 mg/kg/day group, the dosage was decreased to 0.05 mg/kg/day on Day 1 of Week 4. From Week 6 on, plasma ChE was decreased by less than 12 percent in the 0.05 mg/kg/day group; less than 60 percent in the 1.0 mg/kg/day group; and less than 75 percent in the 3.0 mg/kg/day group. RBC ChE was only minimally decreased (less than 2%) in males and females in the 0.1 mg/kg/day group at Week 2. On Week 2, animals in the 1.0 and 3.0 mg/kg/day group exhibited decreases in RBC ChE of approximately 33 and 72 percent, respectively (see Table 3). On Week 6, males and females in the 0.05 mg/kg/day group exhibited 23.6 and 50.1 percent RBC ChE inhibition, respectively. From Week 13 on, RBC ChE was decreased by less than 13 percent in the 0.05 mg/kg/day group;

*The degree of mean group ChE inhibition is being compared to pretreatment mean values for each group.

less than 54 percent in the 1.0 mg/kg/day group; and less than 87 percent in the 3.0 mg/kg/day group.

Statistical analysis of the data prior to Week 13 was not conducted because 1) the 0.1 mg/kg/day group's dosage was lowered to 0.05 mg/kg/day on Day 1 of Week 4; 2) the decrease in plasma ChE in the 0.1 mg/kg/day group at Week 2; and 3) what the authors believe to be the residual effect on RBC ChE in the 0.05 mg/kg/day group at Week 6. The statistical analysis revealed a significant depression in plasma and RBC ChE from Week 13 in males and females in the 1.0 and 3.0 mg/kg/day groups. In addition, brain ChE was significantly inhibited in males and females in the 3.0 mg/kg/day group and in males in the 1.0 mg/kg/day group at termination. Brain ChE inhibition was approximately 22 percent in males in the 1.0 mg/kg/day group and 63 and 31 percent, respectively, in males and females in the 3.0 mg/kg/day group (see Table 4).

[It is noted that male #26220 exhibited 22.8 percent inhibition of plasma ChE at Week 52. However, from Week 13 on, significant inhibition (greater than 20%) of plasma and/or RBC ChE failed to be exhibited in male #26220 and all other animals in the group at all other time intervals. In addition, plasma ChE was somewhat variable, varying from 4.3 to 22.8 percent in male #26220 depending on the week of measurement. This apparent increase in plasma ChE inhibition of male #26220 appears to be a spurious event.]

Table 2. Mean Percent Plasma ChE Inhibition Compared to Pretreatment Mean Values

Group	Week					
Males	**2**	**6**	**13**	**26**	**39**	**52**
0	7.0	11.7	-4.3	-0.5	7.2	2.0
0.05*	21.1	11.0	-2.4	-1.9	6.0	11.7
1.0	59.0	59.2	48.1	39.1	47.5	52.9
3.0	66.6	74.3	68.1	71.2	65.1	71.5
Females	**2**	**6**	**13**	**26**	**39**	**52**
0	-0.7	1.9	-14.3	-1.9	-8.1	-0.9
0.05*	25.7	10.3	-4.8	-8.6	-10.7	-9.3
1.0	59.3	56.7	41.0	51.4	43.7	51.8
3.0	65.2	73.9	61.1	74.2	67.6	64.6

Table 3. Mean RBC ChE Inhibition Compared to Pretreatment Mean Values

Group	Week					
Males	**2**	**6**	**13**	**26**	**39**	**52**
0	-0.8	-2.4	-5.7	-8.6	2.7	-8.7
0.05*	1.6	23.6	7.0	3.1	8.0	-2.6
1.0	33.6	65.2	53.9	43.0	44.0	53.4
3.0	75.3	94.0	86.9	84.7	81.2	85.1
Females						
0	-2.1	0.0	0.7	-5.8	7.8	-1.1
0.05*	-1.4	50.1	6.5	3.4	12.8	2.3
1.0	33.1	63.2	51.9	38.0	39.7	45.2
3.0	67.5	90.2	82.4	82.5	79.2	81.1

*Initial dosage was 0.1 mg/kg/day until Day 1 of Week 4 when it was decreased to 0.05 mg/kg/day.

Table 4. Mean Brain ChE Inhibition (umol/g) at Termination and Percent Inhibition When Compared to Controls

	Group (mg/kg/day)			
	0	0.05	1.0	3.0
Males	9.0	8.0 (-12.5)	7.0* (-22.2)	4.8* (-46.7)
Females	8.5	9.0 (+5.9)	7.9 (-7.1)	6.0* (-29.4)

*Statistically significant at $p \leq 0.05$.

6. Urinalysis - Urine was collected from fasted animals prior to initiation of dosing and during Weeks 26 and 52. The CHECKED (X) parameters were examined.

X	
X	Appearance
X	Volume
X	Specific gravity
X	pH
X	Sediment (microscopic)
X	Protein

X	
X	Glucose
X	Ketones
X	Bilirubin
X	Blood
	Nitrate
X	Urobilinogen
X	Reducing substances

Results - Unremarkable.

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, in addition, were weighed.

X	Digestive System
	Tongue
X	Salivary glands
X	Esophagus
X	Stomach
X	Duodenum
X	Jejunum
X	Ileum
X	Cecum
X	Colon
X	Rectum
XX	Liver
X	Gall bladder
X	Pancreas
	Respiratory
X	Trachea

X	Cardiovasc./Hemat.
X	Aorta
XX	Heart
X	Bone marrow
X	Lymph nodes
X	Spleen
X	Thymus
	Urogenital
XX	Kidneys
X	Urinary bladder
XX	Testes
X	Epididymides
X	Prostate
	Seminal vesicle
XX	Ovaries
X	Uterus

X	Neurologic
XX	Brain
X	Periph. nerve
X	Spinal cord (3 levels)
X	Pituitary
X	Eyes (optic n.)
	Glandular
XX	Adrenal gland
	Lacrimal gland
X	Mammary gland
	Parathyroids
X	Thyroids
	Other
	Bone
X	Skeletal muscle
X	Skin

X		X		X	
X	Lung Nosa Pharynx Larynx	X X	Cervix Vagina	X	All gross lesions and masses

Results

a. Organ Weight - There was an increase in the absolute and relative weight of the ovaries in the control group. This was attributed to female #26215 that had several cysts on both ovaries.

b. Gross Pathology - Unremarkable.

c. Microscopic Pathology

1) Non-neoplastic - Unremarkable.

2) Neoplastic - Unremarkable.

D. Discussion:

No deaths occurred. Ataxia, salivation, and dyspnea were observed in one male in the 3.0 mg/kg/day group on 1 day in Week 33. (This may be attributable to an accidental overdose.) Mean cumulative body weight gain was decreased in males in the 3.0 mg/kg/day group during Week 1 to Week 7 At termination, mean cumulative body weight gain was comparable among groups. Plasma ChE was decreased in males (21.1%) and females (25.7%) at Week 2 in the 0.1 mg/kg/day group. In order to achieve a NOEL, the dosage was dropped to 0.05 mg/kg/day at Day 1 of Week 4. After Week 2, plasma ChE was only significantly decreased in males (39.1 to 59.2%) and females (41.0 to 56.7%) in the 1.0 mg/kg/day group and in males (65.1 to 74.3%) and females (61.1 to 74.2%) in the 3.0 mg/kg/day group at all other later time intervals. RBC ChE was decreased in males (23.6%) and females (50.1%) at Week 6 in the 0.05 mg/kg/day group. This is believed to be the residual effect on RBC ChE of the initial higher dose of 0.1 mg/kg/day. (Much lower levels were observed in this group after Week 6). After Week 6, RBC ChE was only significantly decreased in males (43.0 to 53.9%) and females (38.0 to 51.9%) in the 1.0 mg/kg/day group and in males (81.2 to 86.9%) and females (79.2 to 82.5%) in the 3.0 mg/kg/day group at all other later time intervals. The percent inhibition did not appear to increase with time. Brain ChE was significantly decreased in males (approximately 22%) in the 1.0 mg/kg/day group and in males (approximately 47%) and females (approximately 29%) in the 3.0 mg/kg/group.

NOEL = 0.05 mg/kg/day
LEL = 1.0 mg/kg/day (plasma and RBC ChE inhibition and brain ChE inhibition in males)

Attachments

Attachment 1

Methods Used for ChE Determinations

Plasma, Erythrocyte, and Brain Cholinesterase - AutoAnalyzer®

General operating instruction manual (1966) Section C-4. Technicon Instruments Corporation, Chaucey, NY.

Levine, J.B.; Scheidt, R.A.; Nelson, V.A. (1966) An automated micro determination of serum cholinesterase. Technicon Symposium 1965 Automation in Analytical Chemistry. Mediad, Inc., New York, NY, pages 582-585.

Mersmann, H.J.; Sanguinetti, M.C. (1974) Automated determination of plasma and erythrocyte cholinesterase in various species. Am. J. Vet. Res. 35, page 579.

Tietz, N. (1976) Fundamentals of Clinical Chemistry, 2d ed. W.B. Saunders Company, Philadelphia, PA, page 47.

Ward, F.P.; Hess, T.H. (May 1969) An automated method with a microadaptation for cholinesterase assays in the dog. Department of the Army, Edgewood Arsenal, EATR 4279.

Ward, F.P.; Hess, T.H. (March 1971) Automated cholinesterase measurement: canine erythrocytes and plasma. Am. J. Vet. Res. 32, pages 499-503.

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Review Section II, Toxicology Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. Marion P. Copley 11/30/90
Review Section II, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT

Study Type: 2-Week Range-Finding - Dog TOX Chem No.: 328

Test Material: DDVP MRID No.: 415931-01

Synonyms: 2,2-Dichlorovinyl dimethyl phosphate, Dimethyl 2,2-dichlorovinyl phosphate, Dichlorvos, Herkol, Dedevap, Oko, Vapona

Study Number: HLA Study No. 2534-101

Sponsor: AMVAC Chemical Corporation
Los Angeles, CA 90023

Testing Facility: Hazleton Laboratories America, Inc.
Vienna, VA 22182

Title of Report: A 2-Week Range-Finding Study on DDVP in Dogs.

Author: Victoria R. Markiewicz

Report Issued: Not reported

Conclusions:

Plasma and RBC ChE were decreased at the 1.0 mg/kg/day dose level and at higher dose levels. Brain ChE was decreased in the 0.1 mg/kg/day female and in animals in the 5.0, 10.0, and 30.0 mg/kg/day groups.

Classification: Core-Supplementary

A. Materials:

1. Test Compound - DDVP, Description: A clear, oily liquid; Lot No. 802097; Purity: Not reported; Contaminants: Not reported.

2. Test Animals - Species: Dog; Strain: Beagle; Age: 5 to 6 1/2 months; Source: Hazleton Research Products, Inc., Cumberland, VA.

B. Study Design:

1. Animal Assignment - Animals were assigned via random card draw to the following test groups:

Group No.	Animal Numbers Male	Female	Dosage Levels mg/kg/day
1	26174	26175	0
2/6	26176[a]	26179[a]	0.1
	26177[b]	26178[b]	0.1 (10.0)
3	26180[c]	26181[c]	1.0 (5.0)
4	26182	26183[d]	30 (15.0)
5	26184	26185	60

[a]Dosed at 0.1 mg/kg throughout study.

[b]Dosed at 0.1 mg/kg Days 1 to 7; 10 mg/kg Days 8 to 24.

[c]Dosed at 1.0 mg/kg Days 1 to 14; 5.0 mg/kg Days 15 to 24.

[d]Dosed at 30 mg/kg Days 1 to 6; not dosed Days 7 and 8; dosed at 15 mg/kg Days 9 to 15.

( ) = Adjusted dose where appropriate.

2. Animal Dosing - Animals received the test material by capsule once daily for 2 weeks. Dosage was based on the most recently recorded individual body weight. The dosing order was from high-dose to low-dose to control. The purity of the test material relative to an analytical standard was determined at the beginning of the study.

Results - The purity of the test material was 97.3 percent.

3. Animals received food (Purina® Certified Canine Diet No. 5007) and water ad libitum.

4. A Quality Assurance Statement was provided and signed by Janet Milazzo on August 6, 1990.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for mortality and moribundity. Cage-side observations for signs of toxicity were made at 1 and 3 hours postdosing starting on day 1 and starting 1/2 hours postdosing on day 2. Physical examinations were conducted at each weighing interval.

Results - Doses of 30 and 60 mg/kg/day produced death during the final week of dosing following the 5th or 6th dose (see Table 1 below).

Table 1. Mortality

Group	Dose (mg/kg/day)	No. Deaths/No. Treated Males	Females
1	0	0/1	0/1
2	0.1	0/1	0/1
3	1.0 (5.0)	0/1	0/1
4	30 (15.0)	1/1	0/1
5	60	1/1	1/1
6	10	0/1	0/1

During the first week, severe clinical signs of toxicity observed in animals treated at 30 and 60 mg/kg/day included: salivation, ataxia, emesis, tremors, languid behavior, prostration, polypnea, dyspnea, miosis, cyanotic appearance, lacrimation, urine stains, soft feces (mucoid/red), and/or rough coat. During the first week, the female receiving 30 mg/kg/day exhibited soft feces, emesis, tremors, and ataxia. Once the dose had been lowered to 15.0 mg/kg after a 2-day recovery period, the female only exhibited emesis. The only effect observed at 1.0 mg/kg/day was soft mucoid feces. After the dose had been increased to 5.0 mg/kg/day during Days 15 to 24, the only clinical signs of toxicity observed were in the male and included emesis, tremors, alopecia, salivation, soft feces (mucoid/red), and few or no feces. The male in the 0.1 mg/kg/day group had emesis. When the dose was raised for one male and one female in the 0.1 mg/kg/day group to 10 mg/kg/day during Days 8 to 24, the animals exhibited soft feces, alopecia, emesis, and severely reduced food intake, thin appearance, few or no feces, languid/lethargic behavior, salivation, tremors, and/or ataxia (see Table 2 attached).

2. Body Weight - Individual body weights were recorded the week prior to treatment and at weekly intervals thereafter.

Results - The male in group 3 (1.0/5.0 mg/kg/day) lost approximately 19 percent of its weight at Week 2 after having received 5.0 mg/kg/day for 1 week. The group 6 male and female lost approximately 27 and 13 percent of their Week 1 weight, respectively, after receiving 10.0 mg/kg/day for 1 week. The group 4 female lost approximately 13 percent of its Week 1 weight after receiving 15.0 mg/kg/day beginning on Day 9 (see Table 3, attached).

3. Food Consumption - Individual food consumption was determined weekly.

Results - Control animals reduced their food consumption by up to 24 percent during Week 2 compared to Week 1. Food consumption was significantly decreased (> 50%) in the male in group 3 during Week 3 compared to Week 1 after receiving 5.0 mg/kg/day 2 weeks after receiving 1.0 mg/kg/day, and in the male and female in group 6 at Weeks 2 and 3 after receiving 10 mg/kg/day after previous dosing for 1 week at 0.1 mg/kg/day (see Table 4, attached).

4. Blood was collected once prior to treatment and on Day 13 and 22 for hematology and clinical analysis from all animals. In addition, blood was collected at three intervals during the last 1 to 6 days of the quarantine period and on Days 6, 9 (group 4 female #26183 only), 13, and 23 for ChE determinations. Blood (nonfasted) was collected for ChE determinations at approximately 3 and 6 hours postdose on Day 6 to determine the peak period of inhibition, prior to dosing on Day 9 to monitor recovery, and approximately 3 hours postdosing on Day 13 and 23 because maximal ChE inhibition occurred after 3 hours postdosing. 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)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)
X	Corrected leukocyte count	X	Prothrombin time
X	Cell morphology	X	Activated partial thromboplastin time

Results - Unremarkable.

b. Clinical Chemistry

X	Electrolytes
X	Calcium
X	Chloride
	Magnesium
X	Phosphorous
X	Potassium
X	Sodium
	Enzymes
X	Alkaline phosphatase
X	Cholinesterase
X	Creatinine phosphokinase
	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (also SGPT)
X	Serum aspartate aminotransferase (also SGOT)

X	Other
X	Albumin
X	Blood creatinine
X	Blood urea nitrogen
X	Cholesterol
X	Globulins
X	Glucose
X	Total bilirubin
X	Total protein
	Triglycerides
X	Albumin/Globulin ratio

Results - The BUN was elevated in dogs in group 6 on Days 13 (M 22 mg/dl, F 43 mg/dl) and 22 (M 14 mg/dl, F 43 mg/dl) when compared to Day -7 (M 12 mg/dl, F 16 mg/dl). In addition, one male in group 3 had an increased BUN on Days 13 (23 mg/dl) and 22 (30 mg/dl) when compared to Day -7 (17 mg/dl). One female in group 6 had a very high creatine kinase level (6075 U/L) on Day 13. However, the level dropped to 101 U/L on Day 72. The reason for the high increase on Day 13 was not explained. Plasma and RBC ChE were decreased in dogs administered 1.0 mg/kg/day (see Tables 5 and 6 attached). The degree of ChE inhibition increased with dose. At 1.0 mg/kg/day, Day 6 values for plasma and RBC ChE were decreased by approximately 22 and 13 percent, respectively, when compared to Day -6, -4, and -1 values. Brain ChE was slightly decreased in females in the 0.1 mg/kg/day group on Day 15 by approximately 16 percent (See Table 7 attached) when compared to controls. Significant brain ChE inhibition (46%) was observed in the group 3 female in the 5.0 mg/kg/day dosing regimen on Day 24. Brain ChE levels were not determined at the 1.0 mg/kg/day level until after additional treatment at significantly higher dose levels for periods of time in excess of 1 week.

5. Urinalysis - Urine was collected by cage pan runoff from fasted animals at Days -7, 13, and 22. The CHECKED (X) parameters were examined.

X	
X	Appearance
X	Volume
X	Specific gravity
X	pH

X	
X	Glucose
X	Ketones
X	Bilirubin
X	Blood

X			X		
X	Sediment (microscopic)			Nitrate	
X	Protein		X	Urobilinogen	
			X	Reducing substances	

Results - Unremarkable.

6. 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, in addition, were weighed.

X			X			X	
	Digestive System			Cardiovasc./Hemat.			Neurologic
	Tongue			Aorta		XX	Brain
	Salivary glands		XX	Heart		X	Periph. nerve
X	Esophagus			Bone marrow			Spinal cord (3 levels)
X	Stomach			Lymph nodes			Pituitary
X	Duodenum		X	Spleen			Eyes (optic n.)
	Jejunum			Thymus			Glandular
	Ileum			Urogenital		XX	Adrenal gland
	Cecum		XX	Kidneys			Lacrimal gland
X	Colon		X	Urinary bladder			Mammary gland
	Rectum		XX	Testes		X	Parathyroids
XX	Liver		X	Epididymides		X	Thyroids
XX	Gallbladder			Prostate			Other
	Pancreas			Seminal vesicle			Bone
	Respiratory		XX	Ovaries			Skeletal muscle
	Trachea			Uterus			Skin
X	Lung					X	All gross lesions and masses

[Tissues were collected but not subjected to microscopic examination.]

a. Gross Pathology

Results - Unremarkable.

b. Organ Weight

Results - Unremarkable.

c. Microscopic Pathology - Not conducted.

D. Discussion:

Death occurred in the 30 and 60 mg/kg/day groups. Toxic signs observed at these dose levels included: salivation, ataxia, emesis, tremors, languid behavior, prostration, polypnea, dyspnea, miosis, cyanotic appearance, lacrimation, urine stains, soft feces (mucoid, red) and/or rough coat.

Soft, mucoid feces were noted at 1.0 mg/kg/day. At 5.0 mg/kg/day, emesis, tremors, alopecia, salivation, soft mucoid red feces, and/or few feces were observed. The male in the 0.1 mg/kg/day group exhibited emesis. Body weight loss and decreased food consumption were noted in the 5.0 and 10.0 mg/kg/day groups. The BUN was elevated in dogs in the 5.0 and 10.0 mg/kg/day groups. Plasma and RBC ChE was decreased in dogs in the 1.0 mg/kg/day group and at higher dose levels. Brain ChE was decreased in the female in the 0.1 mg/kg/day group by approximately 16 percent and in dogs at the higher dose levels. [Note: Brain ChE levels were not determined in the 1.0 mg/kg/day group until after the dose level had been changed for at least 1 week. Therefore, brain ChE levels could not be accurately determined at the 1.0 mg/kg/day level.]

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