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

DEC 14 1998

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Mr. John J. Wrubel
Product Registration Manager
U.S. Plant Regulatory Affairs
American Cyanamid Company
Agricultural Products Research Division
P.O. Box 400
Princeton, NJ 08543-0400

Dear Mr. Wrubel:

Enclosed is a copy of the Hazard Identification Assessment Review Committee's (HIARC's) report concerning the evaluation of the rat acute oral neurotoxicity study for terbufos, which was submitted by you during the 60-day comment period for the chemical. In addition, enclosed is a copy of EPA's review of the terbufos rat acute oral neurotoxicity study. Both these documents are being placed in the terbufos open docket.

HIARC recommendations include that American Cyanamid generate an acute oral toxicity study in dogs and that a protocol be submitted to EPA for review prior to study commencement. Please submit a response that indicates (1) the company's decision on whether this study will be conducted; (2) if the study will be conducted, the date by which a protocol will be submitted to the Agency for review; and (3) an estimate (in months) of the length of time to conduct and submit the study (from study commencement to Agency submission).

We are interested in including this information as part of the Agency's risk management proposal for terbufos. Consequently, your response needs to be submitted by no later than 10 working days following the receipt of this letter. Please contact Pam Noyes at (703) 308-8179 with any questions.

Sincerely,

Robert McNally, Chief
Special Review Branch
Special Review and
Reregistration Division

Enclosure

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

Bill Hoyle

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Date: November 23, 1998

MEMORANDUM

SUBJECT: TERBUFOS - Report of the Hazard Identification Assessment Review Committee

TO: William J. Hazel, Ph.D.
Chemist
Reregistration Branch I/HED (7509C)

FROM: Elizabeth Méndez, Ph.D. *[Signature]* 12/1/98
Toxicologist
Reregistration Branch I/HED (7509C)

and

Brenda Tarplee *[Signature]*
Executive Secretary
Hazard Identification Assessment Review Committee

THROUGH: Michael Ioannou, Branch Chief - Toxicology Branch I *[Signature]* 12/1/98
Co-Chairperson
Hazard Identification Assessment Review Committee

and

Pauline Wagner, Branch Chief - Reregistration Branch II *[Signature]* 12/1/98
Co-Chairperson
Hazard Identification Assessment Review Committee

On November 19, 1998, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the acute oral neurotoxicity study in rats submitted by the registrant, American Cyanamid Company. After extensive deliberation, the committee has decided to maintain the current acute reference dose (RD) based on the 28-day oral toxicity study in dogs. Given the significant differences in the NOAELs obtained in the acute neurotoxicity study in rats and the 28-day feeding studies in dogs, it is the committee's view that the dog may be a more sensitive species to assess the inhibitory effect on cholinesterase activity of terbufos. Consequently, HIARC recommends that an acute oral toxicity study be conducted in dogs. This study should include cholinesterase activity measurements at the time of peak effect, observation for clinical signs, and histopathology. The committee also recommends that a proposed protocol be submitted to the Agency prior to the initiation of the study.

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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Date: November 5, 1998

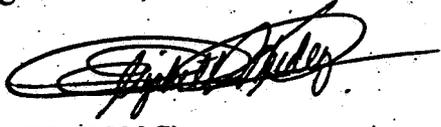
MEMORANDUM

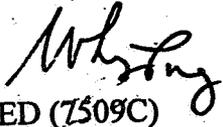
SUBJECT: Terbufos: Review of an Acute Oral Neurotoxicity Study in Rat.

DP BARCODE:D250353
P.C. CODE:105001

SUBMISSION CODE:S550251
MRID 44672003

TO: P. Noyes/ R. McNally PM60
Special Review and Reregistration Division (7508W)

FROM: Elizabeth Méndez, Ph.D. 
Toxicologist
Reregistration Branch I/HED (7509C)

THROUGH: Whang Phang, Ph.D. 
Branch Senior Scientist
Reregistration Branch I/HED (7509C)

The registrant, American Cyanimid Company, submitted an acute oral neurotoxicity study in rats. This study has been reviewed and a DER for this study is attached. The citation of this study and the conclusion of the review are presented below:

Mandella, R.C. (1998). An Acute Neurotoxicity Study with AC 92100 in the Rat via Oral Gavage Administration. Huntingdon Life Sciences. Study No. 98-4525 and 97-4517. October 7, 1998. MRID No. 44672003. Unpublished.

In an acute oral neurotoxicity study (MRID No. 44672003) 5 groups of 7 week old Sprague-Dawley CD® rats (20/sex/group) were given a single oral dose (by gavage) of Terbufos, 89.7% a.i., in corn oil at doses of 0, 0.15, 0.30, and 0.90 mg/kg of body weight. Before the initiation of the main study, an additional 10 females were exposed to 0.90 mg/kg of Terbufos to determine survivability (satellite study).

Clinical signs such as ano-genital stains, oral stains, lethargy, reduced defecation and food

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consumption were observed in females treated with 0.90 mg/kg of the test substance (main study and satellite study). A red exudate from the eye was reported in one male treated at the 0.30 mg/kg dose level. These symptoms did not persist beyond day 5 of the study. One female treated at the 0.90 mg/kg dose level died 5.5 hours after treatment with the test article. No other mortalities were reported during the test period.

The first indication of a compound-related effect (miosis) detected by the FOB was reported at the 0.3 mg/kg and 0.90 mg/kg doses approximately 6 hours after exposure to the test substance in both males and females. In addition to miosis, females at the 0.90 mg/kg dose level showed evidence of excessive salivation, lacrimation, ataxia, stupor, soiled coat, decreased forelimb strength, slightly impaired locomotion, and tremors. These symptoms resolved within the first week after treatment with the test article and were no longer evident at the Day 7 observation period.

Ten animals/sex/dose were tested for plasma, erythrocyte, and brain cholinesterase activity (PChE, RChE, and BChE, respectively). Significant cholinesterase inhibition was reported in both males and females at the 0.30 mg/kg and 0.90 mg/kg doses.

Gross necropsy examination and histopathology testing of animals that were euthanized at the end of the study revealed no compound-related abnormalities.

Under the conditions of this study, the NOAEL for acute neurotoxicity and plasma cholinesterase inhibition is 0.15 mg/kg/day. Given the findings of the FOB testing and cholinesterase inhibition, the LOAEL is established at 0.30 mg/kg/day.

This study is acceptable and satisfies the guideline requirements for an acute oral neurotoxicity study (81-8) in the rat.

Terbufos

Acute Oral Neurotoxicity Study (81-8)

EPA Reviewer: Elizabeth Méndez, Ph.D.
RRB1/HED (7509C)

EPA Secondary Reviewer: Whang Phang, Ph.D.
RRB1/HED (7509C)

Elizabeth Méndez 11/10/98

Whang Phang 11/10/98

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DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Neurotoxicity - RAT
OPPTS 870.6200 [§81-8]

DP BARCODE: D250353
PC CODE: 105001

SUBMISSION CODE: S550251
MRID NO: 44672003

TEST MATERIAL (PURITY): Terbufos (89.7% a.i.)

SYNONYMS: AC 92100

CITATION: Mandella, R.C. (1998). An Acute Neurotoxicity Study with AC 92100 in the Rat via Oral Gavage Administration. Huntingdon Life Sciences. Study No. 98-4525 and 97-4517. October 7, 1998. MRID No. 44672003. Unpublished.

SPONSOR: American Cyanimid Company

EXECUTIVE SUMMARY: In an acute oral neurotoxicity study (MRID No. 44672003) 5 groups of 7 week old Sprague-Dawley CD® rats (20/sex/group) were given a single oral dose (by gavage) of Terbufos, 89.7% a.i., in corn oil at doses of 0, 0.15, 0.30, and 0.90 mg/kg of body weight. Before the initiation of the main study, an additional 10 females were exposed to 0.90 mg/kg of Terbufos to determine survivability (satellite study).

Clinical signs such as ano-genital stains, oral stains, lethargy, reduced defecation and food consumption were observed in females treated with 0.90 mg/kg of the test substance (main study and satellite study). A red exudate from the eye was reported in one male treated at the 0.30 mg/kg dose level. These symptoms did not persist beyond day 5 of the study. One female treated at the 0.90 mg/kg dose level died 5.5 hours after treatment with the test article. No other mortalities were reported during the test period.

The first indication of a compound-related effect (miosis) detected by the FOB was reported at the 0.3 mg/kg and 0.90 mg/kg doses approximately 6 hours after exposure to the test substance in both males and females. In addition to miosis, females at the 0.90 mg/kg dose level showed evidence of excessive salivation, lacrimation, ataxia, stupor, soiled coat, decreased forelimb strength, slightly impaired locomotion, and tremors. These symptoms resolved within the first week after treatment with the test article and were no longer evident at the Day 7 observation period.

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Ten animals/sex/dose were tested for plasma, erythrocyte, and brain cholinesterase activity (PChE, RChE, and BChE, respectively). Significant cholinesterase inhibition was reported in both males and females at the 0.30 mg/kg and 0.90 mg/kg doses.

Gross necropsy examination and histopathology testing of animals that were euthanized at the end of the study revealed no compound-related abnormalities.

Under the conditions of this study, the NOAEL for acute neurotoxicity and plasma cholinesterase inhibition is 0.15 mg/kg/day. Given the findings of the FOB testing and cholinesterase inhibition, the LOAEL is established at 0.30 mg/kg/day.

This study is acceptable and satisfies the guideline requirements for an acute oral neurotoxicity study (81-8) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: AC 92100

Description: Colorless to pale yellow liquid with a mercaptan-like odor

Lot #: AC 9429-26

Purity: 89.7% a.i.

Preparation and Stability of Test Substance in Vehicle: The dosing suspensions were prepared once and used in staggered dosing over a 4-day period. The stability of the substance in the vehicle was tested in the pilot acute neurotoxicity study (Huntingdon Life Sciences Study No. 97-4517; American cyanamid Protocol No. 971-97-131). Dosing suspensions were stored under refrigeration. Analysis to confirm homogeneity of the dosing suspensions were performed at dose levels (0.15 mg/kg and 1.2 mg/kg) that ranged the dose levels used in this study.

2. Vehicle: Corn Oil

3. Test animals: Species: Rat

Strain: Albino rats (Outbred) VAF/Plus®

CD® (Sprague-Dawley derived) [CrI:CD® (SD) IGS BR]

Age at dosing: 7 weeks

Weight at dosing: Males: 184 - 251 g

Females: 145 - 199 g

Source: Charles River Laboratories

Acclimation Period: approximately 2 weeks

Diet: Certified Rodent Diet # 5002 *ad libitum* except during fast prior to dosing and euthanasia.

Water: Tap water *ad libitum*.

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Environmental Conditions: Temperature: 20 - 23°C
 Humidity: 50 - 79%
 Photoperiod: 12 hour light/dark cycle

B. STUDY DESIGN AND METHODS

1. In life dates - start: June 2,3,4, or 5, 1998 end: June 16,17,18, or 19, 1998

2. Animal assignment and treatment - Animals were assigned to the test groups noted in Table 1. Rats were given a single dose of the test substance by gavage at a rate of 5 mL/kg and at doses of 0.15, 0.30, and 0.90 mg/kg. These dosages were selected based on the results of a pilot study (Study No. 97-4517) with AC 92100 conducted at the Testing Facility. Terbufos was administered via gavage at the dose levels of 0.025, 0.05, 0.15, 0.40, 1.2, and 1.6 mg/kg for males, and 0.025, 0.05, 0.15, 0.30, 0.90, and 1.2 mg/kg for females. This one-day study (6 hour duration) was conducted in February 1998 and showed that a dose of 0.9 mg/kg would exhibit evidence of neurotoxicity while a low dose of 0.15 mg/kg would produce no effect. The mid-dose was chosen to produce intermediate effects. The report also noted that prior to the initiation of the study a group of 10 females were treated with 0.90 mg/kg to ensure that no excessive mortality would result from treatment with the test article at this dose level.

TABLE 1. Doses and Testing, Animals Treated with Terbufos

Dose (mg/kg)	Number of Animals							
	Animals Treated		FOB & Motor Activity Evaluation		Cholinesterase Activity Evaluation		Neuropathology	
	♂	♀	♂	♀	♂	♀	♂	♀
Vehicle Control (0)	20	20	10	10	10	10	5	5
0.15	20	20	10	10	10	10	5	5
0.30	20	20	10	10	10	10	5	5
0.90	20	20	10	10	10	10	5	5

♂ = Males; ♀ = Females

3. FOB and Motor Activity Testing - Functional Observational Battery (FOB) testing was performed before dosing, approximately 6 hours after dosing (time of peak effect), and subsequently at days 8 and 15 of the study. The FOB consisted of the following parts:

- Home cage observations - posture, vocalization and palpebral closure.
- Handling evaluations - reactivity to general stimuli; autonomic compound-related effects (lacrimation, salivation, crusty deposit around the eyes, etc.).
- Open Field Evaluations - gait, convulsions, tremors, defecation and urination count, arousal levels, piloerection, exophthalmia, etc.

- d. Reflex/Physiologic Observations - reflex assessments, grip strength, landing foot play, air righting ability, and body weight.

Motor activity was determined by testing animals individually for 60 minutes using an automated Photobeam Activity System. Each 60 minute test session was divided into 12 consecutive 5 minutes intervals according to Schulze's method.

4. Clinical Pathology: Cholinesterase Activity Measurements - Ten animals/sex/dose (not subjected to behavioral observations) were used for cholinesterase activity testing 6 hours after treatment with the test article (time of peak effect). They were tested for plasma and erythrocyte cholinesterase activity levels immediately followed by collection of brain tissue for determination of brain cholinesterase activity (BChE)¹. Blood (0.5 mL) was collected via venipuncture of the orbital sinus after animals were anesthetized with CO₂/O₂. Samples for PChE and RChE were also collected from animals subjected to neurobehavioral evaluations (10/sex/dose) on Day 8 and Day 15 (5/sex/dose)². Blood collection on day 15 of the study was followed by terminal sacrifice and collection of brain tissue for BChE determination. Cholinesterase activity was measured according to a modified Ellman reference method.

5. Sacrifice and Pathology - Ten animals/sex/dose (including 5 animals/sex/dose allocated for perfusion fixation and tissue collection) were sacrificed after the last test session (day 15) A gross pathology examination was performed on all animals except those sacrificed on Day 1 for BChE determinations. The necropsies involved an examination of all orifices and external surfaces, external surfaces of the brain and spinal cord, and body cavities. Animals that were not designated for tissue collection (15 animals/sex/dose) were sacrificed by an overdose of inhaled carbon monoxide. Tissue collected for further examination was obtained from rats anesthetized with sodium pentobarbital and euthanized by transcardial perfusion with phosphate-buffered saline followed by 1% glutaraldehyde and 4% paraformaldehyde in the same buffer.

The brain, eyes (including optical nerve and retina), spinal cord, sciatic nerve, tibial nerve, sural nerve, trigeminal ganglia, dorsal root ganglia (from C₃ to C₆ and L₁ to L₄), dorsal root fibers (from C₃ to C₆ and L₁ to L₄), and ventral root fibers (from C₃ to C₆ and L₁ to L₄), were subjected to a histopathology examination. Peripheral nerves, trigeminal ganglia, dorsal root ganglia, and dorsal and ventral root fibers were prepared for microscopic examination by embedding in glycol methacrylate and sectioning at approximately 2 µm. These tissues were stained with toluidine blue. All other tissues, including the brain and spinal cord, were embedded in paraffin and sectioned at approximately 5-6 µm. Sections were then stained with hematoxylin and eosin, Luxol Fast Blue and Sevier-Munger stains.

6. Statistical Analysis -

- a. Multiple Group Analysis - Statistical evaluations of equality of means were performed using the one way ANOVA method, followed by a multiple comparison

¹ PChE = Plasma cholinesterase activity; RChE = Erythrocyte cholinesterase activity; BChE = Brain cholinesterase activity.

² Blood collections on days 8 and 15 were done after behavioral observations were completed.

procedure if needed. Initially, Bartlett's test³ was used to determine if groups had equal variance. If they did, a parametric procedure was used (one way ANOVA using the F distribution) to assess significance. If significant differences among the means were determined, the Dunnett's test was used to ascertain which means were significantly different from the control. If the variances were not equal, a non-parametric procedure for testing equality of means was used (Kruskal-Wallis test). If differences were determined, the Dunn's summed rank test was used to determine which treatments were different from the control.

A statistical test for trend in the dose levels was also undertaken. In the cases of equal variance, standard regression methods with a test for trend and lack of fit was used. If variances were not equal, the Jonckheere's test for monotonic trend was performed.⁴

b. Motor Activity Counts - Since the motor activity counts were performed in 12 consecutive 5 minute intervals, the set of counts gave a "profile" of an animal's motor activity. These intervals were then compared within each study period using a multivariate model to determine the profiles from different groups had the same shape (parallel hypothesis). If that was the case, active-treatment groups were compared with control in terms of total motor activity (level hypothesis). The parallel hypothesis was tested by the Wilks' Lambda method. The level hypothesis, on the other hand, was tested by a two-way ANOVA. This was at times followed by the Dunnett's t test to find groups that were different from controls. The Blom inverse normal transformation was applied to achieve normality of the residuals from the multivariate model when $p < 0.01$ for the Shapiro-Wilk test.⁵

II. RESULTS

A. PHYSICAL OBSERVATIONS AND MORTALITY

No compound-related effects were seen in males at any dose level. The red exudate from the eye observed in 1 male at the 0.30 mg/kg dose is considered incidental due to the limited number of animals exhibiting the condition. Clinical signs were observed in females treated with 0.90 mg/kg (main study and satellite study). Symptoms included ano-genital staining, oral staining, lacrimation, excessive salivation, lethargy, tremors of paws, fasciculations, decreased defecation, and decreased food intake as shown in Table 2. The decrease in fecal output may be correlated to a decrease in food intake. One female treated with the high dose (0.90 mg/kg) was found dead 5.5 hours after treatment with the test article.

³ Conducted at the 1%, two-sided risk level.

⁴ With the exception of Bartlett's test, all statistical tests were conducted at the 5% and 1%, two-sided risk level.

⁵ All other statistical analysis was considered significant at the $p = 0.05$ level.

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TABLE 2. Clinical Signs and Mortality

Clinical Signs	Dose (mg/kg)								
	Vehicle (0) ^{a,b}		0.15 ^{a,b}		0.30 ^{a,b}		0.90 ^{a,b}		0.90 (Satellite Study) ^{b,c}
	♂	♀	♂	♀	♂	♀	♂	♀	♀
Dead	0	0	0	0	0	0	0	1 (1)	0
Red exudate from the eye	0	0	0	0	1 (8)	0	0	0	0
Ano-genital staining	0	0	0	0	0	0	0	1 (1-4)	3 (1-3)
Oral staining	0	0	0	0	0	0	0	1 (2-3)	0
Lacrimation	0	0	0	0	0	0	0	0	1 (1)
Excessive salivation	0	0	0	0	0	0	0	0	1 (1)
Lethargy	0	0	0	0	0	0	0	1 (1)	0
Tremors (forepaws and hindpaws)	0	0	0	0	0	0	0	0	6 (1)
Fasciculations	0	0	0	0	0	0	0	0	2 (1)
Decreased Defecations	0	0	0	0	0	0	0	1 (2-4)	3 (2)
Decreased food consumption	0	0	0	0	0	0	0	1 (3-5)	0

^a 20 rats/sex/dose were observed on day 1 of the study; 10 rats/sex/dose were observed for the remainder of the study.

^b incidence (days observed)

^c 10 female rats/dose were assigned to this study group.

[Excerpted from p. 55-57 MRID 44672003]

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B. BODY WEIGHT - The animals were weighed twice before initiation of the study, on the day before exposure to the test article, weekly during the study and prior to euthanasia (after fasting). The body weights of treated male rats were comparable to those of the controls, and those of 0.15 and 0.90 mg/kg females were slightly decreased (\approx 7%). The mean body weights of the mid-dose (0.30 mg/kg) females were comparable to controls. Although the mean body weight gains of females treated at the 0.30 and 0.90 mg/kg dose were decreased (21% and 26%, respectively on day 7; 16% and 15%, respectively on day 14), a clear dose response could not be established. Consequently, the decreases in mean body weight and body weight gains observed in females at the 0.90 mg/kg dose were deemed equivocal.

C. FUNCTIONAL OBSERVATIONAL BATTERY - The first evidence of compound-related effects were reported approximately 6 hours after treatment with the test article at the 0.30mg/kg dose and higher in both males and females. While only miosis was observed in males (3 and 6 at the 0.30 and 0.90 mg/kg dose levels, respectively), females' symptoms encompassed a wide array of neuromuscular, autonomic, and CNS effects suggestive of acute cholinergic toxicity as shown in Table 3. Animals in all test groups had recovered completely by the next test session (day 8 of the study).

TABLE 3. Functional Observational Battery (FOB) - Day of Treatment (Females)

SYMPTOMS		DOSE (mg/kg)			
		0*	0.15*	0.30*	0.90*
Home Cage Observations	Abnormal Posture	0	0	0	0
	Abnormal Vocalization	0	0	0	0
	Abnormal Palpebral Closure	0	0	0	0
Handling Evaluations	Abnormal Cage Removal	0	0	0	0
	Abnormal Handling	0	0	0	0
	Chromodacryorrhea	0	0	0	0
	Excessive Lacrimation (moderate to severe)	0	0	0	2
	Soiled Coat (moderate to severe)	0	0	0	2
	Excessive Salivation (slight)	0	0	0	1

SYMPTOMS		DOSE (mg/kg)				
		0*	0.15*	0.30*	0.90*	
Open Field Evaluations	Abnormal Gait (Ataxia)	0	0	0	2	
	Impaired Locomotion (slight)	0	0	0	2	
	Abnormal Arousal (stupor)	0	0	0	1	
	Piloerection	0	0	0	0	
	Exophthalmia	0	0	0	0	
	Defecation (partially formed)	1	0	0	0	
Reflex/Physiologic	Approach Response (no reaction)	0	0	0	1	
	Tail Pinch Response (walks towards or away from stimulus)	3	2	7	4	
	Abnormal Finger Snap Response	0	0	0	0	
	Pupil Response (Miosis)	0	0	1	10	
	Abnormal Air Righting Reflexes	0	0	0	0	
	Forelimb Grip Strength (Mean, kg)	Trial 1	.92	.93	.96	.70
		Trial 2	.98	.97	.91	.68
	Hindlimb Grip Strength (Mean, kg)	Trial 1	.68	.65	.65	.56
		Trial 2	.63	.68	.65	.52
	Landing Foot Splay (Mean, cm)	Trial 1	5.8	4.1*	4.2	5.1
Trial 2		5.4	4.5	5.1	5.3	
Abnormal Movements	Convulsions	0	0	0	0	
	Tremors (slight)	0	0	0	4	

Excerpted from p. 107-111 MRID 44672003

* incidence based on 10 animals

* p ≤ 0.05

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D. MOTOR AND LOCOMOTOR ACTIVITY

No statistically significant differences in mean motor activity were observed in any dose group when compared to the control group on days 1, 8, and 15.

E. CHOLINESTERASE ACTIVITY

The extent of cholinesterase inhibition 6 hours after treatment with the test substance was substantial in the mid- and high-dose groups (0.30 and 0.90 mg/kg, respectively) for both males and females as detailed in Table 4.

TABLE 4. Mean Cholinesterase Activity

Dose (mg/kg)	PChE (IU/mL)		RChE (IU/mL)		BChE (IU/mL)	
	Males	Females	Males	Females	Males	Females
0	0.58 ±0.12	0.99 ±0.11	1.44 ±0.39	1.39 ±0.39	19.70 ±0.64	19.56 ±1.3
0.15	0.51 ±0.12 (11.1%)	1.05 ±0.27 (-6.3%)*	1.25 ±0.17 (13.2%)	1.51 ±0.45 (-8.5%)*	18.99 ±0.64 (3.7%)	19.35 ±0.55 (1.1%)
0.30	0.40* ±0.08 (30.8%)	0.79** ±0.18 (20.2%)	1.15 ±0.22 (20.1%)	1.27 ±0.28 (9.2%)	18.64 ±0.71 (5.4%)	19.05 ±1.02 (2.6%)
0.90	0.18* ±0.09 (68.5%)	0.10* ±0.09 (89.7%)	0.47* ±0.35 (66.9%)	0.14* ±0.15 (90.1%)	15.53* ±2.25 (21.2%)	9.54* ±4.60 (51.2%)

PChE = Plasma Cholinesterase; RChE = Erythrocyte cholinesterase; BChE = Brain cholinesterase
 ± = Standard Deviation; () = Percent Inhibition; * = $p \leq 0.01$; ** = $p \leq 0.05$
 * Apparent cholinesterase activity enhancement.

F. GROSS PATHOLOGY

No compound-related gross pathology abnormalities were observed in animals at any dose group.

G. HISTOPATHOLOGY

Gliosis along with degeneration of the optic nerve fiber was reported in 2 females from the control group and 1 female from the high-dose group (0.90 mg/kg). These findings are attributed to trauma during blood collection (via venipuncture of the orbital sinus) and are considered handling-related. Minimal degeneration of a nerve fiber was also reported in 1 male in the 0.90 mg/kg dose group. Given the limited number of animals exhibiting this condition, this finding is deemed incidental.

I. POSITIVE CONTROLS

Studies were conducted with Chlorpromazine, D-amphetamine sulfate, and Physostigmine to establish the sensitivity, reliability, and validity of the test procedures to detect signs of neurotoxicity. Furthermore, animals were also observed for increased (D-amphetamine sulfate) or decreased (Chlorpromazine) motor activity. Under the conditions of this study, the expected effects were observed upon treatment with the reference substances confirming that the test procedures used in the main study are adequate to assess compound-related effects. The submitted positive control study was conducted between November and December of 1996.

III. DISCUSSION

Clinical signs such as ano-genital stains, oral stains, lethargy, reduced defecation and food consumption were observed in females treated with 0.90 mg/kg of the test substance (main study and satellite study).

Ten animals /sex/dose were tested using the Functional Observational Battery (FOB) and Motor Activity tests. The first evidence of compound-related effects were reported approximately 6 hours after treatment with the test article at the 0.30mg/kg dose and higher in both males and females. Females, however, appear to be more severely affected by treatment with the test article as can be inferred by the myriad of symptoms they exhibit. While only miosis was observed in males (3 and 6 at the 0.30 and 0.90 mg/kg dose levels, respectively), females' symptoms encompassed a wide array of neuromuscular, autonomic, and CNS effects suggestive of acute cholinergic toxicity. In addition to miosis (1/10 females in the 0.30 mg/kg and 10/10 females in the 0.90 mg/kg dose groups), symptoms reported in females in the high-dose group were excessive salivation (1/10), lacrimation (2/10), ataxia (2/10), stupor (1/10), soiled coat (2/10), decreased forelimb strength (1/10), slightly impaired locomotion (2/10), tremors (7/10), and fasciculations (4/10). These symptoms resolved within the first week after treatment with the test article and were no longer evident at the Day 7 observation period.

No statistically significant differences in mean motor activity were observed in any dose group when compared to the control group on days 1, 8, and 15.

Ten animals/sex/dose were tested for plasma, erythrocyte, and brain cholinesterase activity (PChE, RChE, and BChE, respectively) 6 hours after treatment with the test article. The extent of cholinesterase activity inhibition in the 0.30 mg/kg males averaged 30.8%, 20.1%, and 5.4% in PChE, RChE, and BChE, respectively. Females in this dose group showed an average cholinesterase inhibition of 20.2%, 9.2%, 2.6% in PChE, RChE, and BChE, respectively. These same parameters were significantly reduced in the high dose test group. PChE, RChE, and BChE inhibition averaged 68.5%, 66.9%, and 21.2% for males and 89.7%, 90.1%, and 51.2%. Females once again appear to be more sensitive to the test article as was previously noted during the FOB testing.

Gross necropsy examination and histopathology testing of animals that were euthanized at the end of the study revealed no compound-related abnormalities.

One female in the 0.90 dose group died 5.5 hours after treatment with the test group. Nonetheless, no statistically significant mortality was reported at any dose group.

No deficiencies that would compromise the interpretation of the results were evident in this study.