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DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity Screening Battery - Rats [OPPTS 870.6200, OPP §81-8]

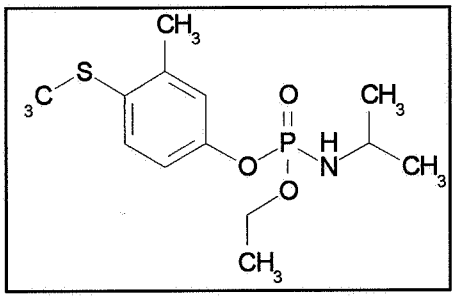
DP BARCODE: D227925
P.C. CODE: 100601

SUBMISSION NO.: S508216
TOX CHEM NO.: 453A

TEST MATERIAL: SRA 3886, Technical

SYNONYMS: Fenamiphos, Technical

STRUCTURE:



CITATION: Dreist, M. (16 October 1995), SRA 3886 (Common Name: Fenamiphos) Acute Oral Neurotoxicity Screening Study in Wistar Rats, Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No.: 24408, Study No.: T 6058166, MRID No.: 440415-01, Unpublished

SPONSOR: Bayer AG, Department of Toxicology, Wuppertal, Germany

EXECUTIVE SUMMARY: Fasted (overnight) male and female Wistar rats (18/sex/dose) were orally gavaged once with Fenamiphos at 0 (vehicle), 0.4, 1.6, or 2.4 mg/kg (analytically confirmed doses: 0, 0.37, 1.52, and 2.31 mg/kg). The main study animals (12 rats/sex/dose, except the high-dose male group which contained 15 rats) were used for a routine neurotoxicity screening battery with behavioral testing at the peak time of effect (25 min postdosing) and at Days 7 and 14 postdosing; neuropathological examination was carried out at terminal sacrifice (Day 14). Plasma, RBC and brain cholinesterase activities were measured in 6 rats/sex/dose) at approximately 50 min postdosing.

No treatment-related changes were noted in mean body weights, absolute and relative brain weights and the incidences of gross and neurohistopathological

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lesions.
At the high-dose, Fenamiphos toxicity was observed within 21 to 31 min postdosing (lethality 7/15 males, 1/12 females), with clinical signs of cholinesterase inhibition persisting to approximately 2 hr 45 min postdosing. At 4 to 8 hr postdosing, all treatment-related clinical signs were absent. Although plasma and RBC ChE activities were markedly and rapidly (50 min postdosing) inhibited, brain ChE was unaffected. The following treatment-related effects were observed:

At 2.31 mg/kg
- Lethality in 7/15 males and 1/12 females
- Muscle fasciculations, gait incoordination, nasal and oral staining, constricted pupils, salivation, lacrimation (females only), and piloerection.
- Statistically significant decreases in plasma (-61% males, -85% females) and RBC (-76% males, -80% females) ChE activities
- Decreased motor (-32%) and locomotor (-41%) activities in males.

At 1.52 mg/kg
- Muscle fasciculations in males
- Statistically significant decreases in plasma (-64% males, -77% females) and RBC (-70% males, -51% females) ChE activities.

At 0.37 mg/kg
- Statistically significant decreases in plasma ChE in females (-55%) and RBC ChE in males (-24%) with a non-significant decrease in plasma ChE in males (-23%).

Based on the results of this study (inhibition of plasma and RBC), the LOEL was established at 0.37 mg/kg; the NOEL was not established.

This study is classified as **Acceptable** and satisfies guideline requirements (§81-8) for an acute neurotoxicity screening battery in the rat.

Compliance: Quality assurance was documented by signed and dated GLP and quality assurance statements; the sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria; and a statement of "no confidentiality claims" was provided.

I. MATERIALS

A. Test Material: Fenamiphos, Technical
Description: Colorless to brownish crystalline solid
Batch No: 809334134
Purity: 95.2%
Storage: Room temperature
CAS Registry No.: 22224-92-6

B. Test Animals
Species (Strain): SPF-bred Wistar Rat (Hsd Win:WU)
Age at Initiation (Weeks): 8 to 9 (males), 9 - 10 (females)
Weight at Initiation (mean ± SD): 181 ± 7 g (males), 150 ± 7 g (females)
Source: Harlan Winkelmann GmbH, Borchen
Housing: Individually in polycarbonate cages with low-dust wood granule bedding
Feed: Altromin 1324 Diet for Rats and Mice, *ad libitum*, Altromin GmbH and Com. KG, Lage
Water: Tap water, *ad libitum*
Environment: Temperature: 22 ± 2°C; Humidity: 30-70% Light/dark cycle: 12 hr/12 hr; Air changes: 12 - 15/ hour
Acclimation Period: 11 - 15 days

C. Vehicle: 2% (v/v) Cremophor EL in deionized water

II. STUDY DESIGN

A. In-life Study Dates: 22 Aug to 16 Sept 1994

B. Animal Assignment: Animals were randomly assigned to test groups as shown in Table 1. Due to the complexity of the study, main study animals were subdivided into four subgroups and stagger-started over a four day period. Animals assigned to the cholinesterase (ChE) study were also randomized and stagger-started over a two day period. All animals were fasted overnight prior to dosing.

TABLE 1: STUDY DESIGN

Group	Dose (mg/kg)	Main Study		Cholinesterase Study	
		Males	Females	Males	Females
Control	0	12	12	6	6
Low	0.4	12	12	6	6
Mid	1.6	12	12	6	6
High	2.4	15[a]	12	6	6

[a] Four high-dose males, which died within the first 32 min post-dosing, were replaced with three others of approximately the same body weight.

C. Dose Selection and Determination of the Peak Time of Effect: Based an acute oral LD_{50} values of approximately 6.0 mg/kg in male and female rats, doses of 0, 0.4, 1.2, 2.4, and 3.0 mg/kg were selected for a range finding study (Study No.: T 0058052). Signs of neurotoxicity increased in a dose-dependent manner from 1.2 mg/kg to 3.0 mg/kg, with deaths occurring at 3.0 mg/.kg in males (1/5) and females (1/5). From this study, the NOEL was established at 0.4 mg/kg and a LOEL at 1.2 mg/kg. Doses of 0.4, 1.2, and 2.4 mg/kg were selected for the acute neurotoxicity study. signs of neurotoxicity were present at approximately 25 min (minimum) post-dosing. The FOB portion of the study was conducted from 25 min to 1 hr 5 min post dosing, and motor activity, from 1 hr 35 min to 2 hr 45 min.

E. Statistical Evaluations: Group mean and standard deviations (SD) were calculated for terminal body and organ weights and ChE activities. Data for treated groups were compared with control data using the Mann -Whitney and Wilcoxon tests with significance levels of $p \leq 0.05$ and $p \leq 0.01$. Motor activity and FOB results were analyzed using Repeated-Measures Analysis of Variance (ANOVA) followed by an one-way ANOVA if significant interactions were detected. Categorical data were analyzed using General Linear Modeling or Categorical Modeling Procedures followed by Dunnett's test or Analysis of Contrasts, respectively, for post-hoc data analysis. Fischer's Exact test was used to evaluate pupil response.

D. Neurobehavioral and Neuropathological Positive Controls: FOB evaluations, motor activity, and neuropathology were evaluated in a positive control study (MRID No.: 440415-02) in male and female rats. This study verifies the ability of the performing laboratory to adequately conduct the acute neurotoxicity screening battery.

III. METHODS

A. Clinical Observations: Animals were observed twice daily (once on holidays and weekends) for clinical signs, mortality and moribundity. Detailed physical examinations were carried out daily.

B. Body Weight: Body weights were measured at prestudy, just prior to dosing on Day 0, and on Days 7 and 14.

C. Neurobehavioral Tests

1. Functional Observational Battery (FOB): FOB was performed on Days -7, 0 (at time of peak effect), 7 and 14. Animals were presented in a blind manner to a trained observer, who evaluated the following parameters:

HOME CAGE
- Posture
- Tremors/Convulsions
- Gait abnormalities
- Piloerection
- Vocalizations
- Level of activity
- Other abnormal signs

HAND-HELD
- Ease of removal from cage
- Reaction to handling
- Muscle tone
- Palpebral closure
- Pupil size
- Lacrimation
- Salivation
- Stains
- Other abnormal signs

OPEN FIELD
- Piloerection
- Respiratory abnormalities
- Posture
- Tremors/Convulsions
- Stereotypic/Bizarre Behavior
- Gait Abnormalities
- Vocalizations
- Activity
- Arousal level
- No. of rearings in 2 min
- Urination
- Defecation
- Other abnormal signs

REFLEXES/RESPONSES
- Approach response
- Touch response
- Auditory response
- Tail pinch response
- Righting reflex

QUANTITATIVE
- Hindlimb grip strength
- Forelimb grip strength
- Landing foot splay
- Body weight
- Body temperature

2. Motor Activity: Following FOB evaluation, animals were evaluated for motor activity using figure-eight shaped automated activity chambers which contained eight infrared emitter/detector pairs. Animals were evaluated individually over a 70 min period consisting of seven, 10-minute intervals. Motor activity was defined as the total number of beam breaks, while locomotor activity was calculated by elimination of consecutive counts for a single beam. To minimize acoustical variations during the study, white background noise of 70 dB was used.

D. Cholinesterase Determination: Plasma, RBC and brain cholinesterase activities were determined at the peak time of effect (approximately 50 min post-dosing) on 6/sex/dose. Brain tissue (solubilized, J. Neurochemistry 16: 1505-1513) and plasma cholinesterase activities were determined using the modified method of Ellman (Biochem. Pharmacol. 7: 88-95, 1961), while RBC cholinesterase activity was determined by the method of Okabe et al. (Clin. Chim. Acta 80: 87-94, 1977).

E. Pathology

1. Gross pathology and organ weights: All animals, except those assigned to the ChE study, were examined grossly at the time of death or terminal sacrifice. Brains were weighed prior to placement in fixative.

2. Neurohistopathology: The 6 animals/sex/group selected for neuropathological examinations were anesthetized with pentobarbital, perfused *in situ* with phosphate-buffered sodium nitrite followed by universal fixative (2% glutaraldehyde and 2% formaldehyde). The tissues listed below were examined.

Brain: Olfactory lobe, forebrain, midbrain, cerebellum, medulla oblongata
Spinal Cord:: Cervical, thoracic & lumbar
Spinal Nerve Root Fiber and Ganglia: Dorsal and ventral, cervical;
dorsal and ventral, lumbar
Eyes and optic nerves
Gasserian ganglia
Gastrocnemius muscle
Peripheral Nerves: Sciatic, tibial, & sural

IV. RESULTS

A. Analytical Chemistry: The stability and homogeneity of the dosing solutions were determined prior to the start of the study using nominal concentrations of 0.001 and 0.2% that bracketed the range of the test concentrations (0.004 and 0.024%) used in the study. Test solutions were stable to at least four days with values of 103% and 100.5% of the target concentration of 0.001 and 0.2%, respectively. Samples taken from the top, middle and bottom were homogeneous with coefficients of variance of 1.57 and 1.23% for the 0.001 and 0.2% concentrations, respectively. The achieved doses for the 0.4, 1.6 and 2.4 mg/kg doses were 0.37, 1.52 and 2.31 mg/kg, respectively.

B. Clinical Observations: During the first 21 to 31 min post-dosing (prior to FOB evaluation), four males and one female were found dead (Table 2). Three replacement males dosed at 2.4 mg/kg also died and were not replaced. At the first scheduled (Day 0 at approximately four to eight postdosing) and subsequent clinical evaluations, no treatment-related clinical signs were observed in any of the surviving animals.

TABLE 2: LETHALITIES OCCURRING at 21 to 31 MINUTES POST-DOSING

OBSERVATION	SEX	DOSE (mg/kg)			
		0	0.4	1.6	2.4
Found dead	Male	0/12	0/12	0/12	7/15[a]
	Female	0/12	0/12	0/12	1/12

a Includes three replacement animals which also died.

C. Body Weight: No treatment-related differences in body weights were noted during the study.

D. Neurobehavioral Findings

1. FOB Findings: FOB findings are summarized in Table 3. At Day 0 (time of peak effect) increased incidences of home cage, hand-held and open field observations were noted in treated males and females. Increased incidences of piloerection, gait incoordination, muscle

fasciculations, constricted pupils, nasal and oral staining, lacrimation (females only), and, salivation were noted in high-dose animals. Mid-dose males also showed gait incoordination, and oral staining. Muscle fasciculations were observed in mid-dose males and females.

No treatment-related FOB observations were noted at the Day 7 and 14 evaluations.

TABLE 3: INCIDENCE of FOB EFFECTS OBSERVED on DAY 0 at PEAK TIME of EFFECT[a]

OBSERVATION		SEX	DOSE (mg/kg)			
			0	0.4	1.6	2.4
HOME CAGE and HAND-HELD OBSERVATIONS						
Piloerection		Males Females	0/12 0/12	0/12 0/12	0/12 0/12	7/8* 5/11*
Gait Incoordination	(slight to moderate) (slight)	Males Females	0/12 0/12	0/12 0/12	1/12 0/12	5/8* 4/11*
Muscle Fasciculations	(slight to moderate)	Males Females	0/12 0/12	0/12 0/12	9/12* 1/12	7/8* 6/11*
Constricted Pupils		Males Females	0/12 0/12	0/12 0/12	1/12 0/12	3/8* 5/11*
Red Stains	Nasal (slight) Oral (slight)	Males	0/12 0/12	0/12 0/12	0/12 1/12	2/8* 4/8*
	Nasal (slight to moderate) Oral (slight to moderate)	Females	0/12 0/12	0/12 0/12	0/12 0/12	2/11 4/11*
Excretions	Salivation (moderate)	Males	0/12	0/12	0/12	1/8
	Lacrimation (slight) Salivation (moderate)	Females	0/12 0/12	0/12 0/12	0/12 0/12	1/11 1/11
OPEN FIELD OBSERVATIONS						
Piloerection		Males Females	0/12 0/12	0/12 0/12	0/12 0/12	7/8* 5/11*

Muscle Fasciculations (slight to moderate)	Males	0/12	0/12	10/12*	8/8*
	Females	0/12	0/12	3/12	7/11*
Gait Incoordination (slight to moderate)	Males	0/12	0/12	4/12	6/8*
	Females	0/12	0/12	0/12	5/11*

[a] Data summarized from Table 1 (pp. 69 to 72, pp. 85 to 88)

2. Motor Activity: Motor and locomotor activity on Day 0 at the peak time of effect revealed decreases (non-statistically significant) in high-dose males (Table 4). No treatment-related effects were noted in low- and mid-dose males and low-, mid- and high-dose females. Even though these decreases noted in males were not statistically significant, the differences were greater than the 20% variation for historical controls observed by the performing laboratory.

At Days 7 and 14, mean motor and locomotor activity of treated animals were comparable to control values.

TABLE 4: MOTOR and LOCOMOTOR ACTIVITIES on DAY 0 at PEAK TIME of EFFECT[a]

OBSERVATION	SEX	DOSE (mg/kg)			
		0	0.4	1.6	2.4
Motor Activity	Males	192	226	183	131(-32)[b]
	Females	198	241	248	222
Locomotor Activity	Males	78	101	85	46 (-41)
	Females	83	98	94	94

[a] Data summarized from Table 1 (pp 234 to 237)
[b] Values in parentheses are the % of control

E. Pathology and Terminal Body and Brain Weights

1. Gross Examination: Gross examination did not reveal any treatment-related effects.

2. Brain weights and terminal body weights: Terminal body weights and absolute and relative brain weights of treated animals were comparable to controls.

3. Neuropathology: Neuropathological findings of treated animals were comparable to control animals.

F. ChE Activity: Plasma, RBC and brain ChE results are summarized in Table 5. At the peak time of effect (25 to 50 min post-dosing) on Day 0, animals dosed at 1.6 mg/kg and higher showed statistically significant, treatment-related inhibition of plasma and RBC ChE activities. At 0.4 mg/kg, statistically significant inhibition of ChE was observed in males (RBC only) and females (plasma only). Brain ChE activity was not affected by treatment.

TABLE 5: PLASMA, RBC and BRAIN ChE ACTIVITIES at the PEAK TIME of EFFECT on DAY 0 and HISTORICAL CONTROL VALUES[a]

SEX	DOSE (mg/kg)	ChE ACTIVITY, Mean ± SD (% of Control)		
		PLASMA (U/L)	RBC (U/L)	BRAIN (U/g)
Male	0	390 ± 76	930 ± 141	11.80 ± 0.551
	0.4	300 ± 49 (-23)	710 ± 131* (-24)	12.56 ± 0.559* (+6)
	1.6	140 ± 37** (-64)	280 ± 144** (-70)	11.84 ± 0.530 (0)
	2.4	150 ± 72** (-61)	220 ± 158** (-76)	11.58 ± 0.721 (-2)
Female	0	1030 ± 254	780 ± 311	11.89 ± 0.429
	0.4	460 ± 103** (-55)	750 ± 112 (-4)	12.26 ± 0.660 (+3)
	1.6	240 ± 104** (-77)	380 ± 219* (-51)	12.17 ± 0.632 (+2)
	2.4	150 ± 58** (-85)	160 ± 74** (-80)	11.72 ± 0.615 (-1)
Historical Controls				
Male		470 ± 89	800 ± 179	12.66 ± 1.507
Female		1470 ± 438	920 ± 234	13.46 ± 1.430

[a] Data summarized from Text Table (pg 30), Study Table 1 (pp 338 & 339), and Appendix II (pg 41).
* $p \leq 0.05$, ** $p \leq 0.01$

V. DISCUSSION and CONCLUSIONS: Male and female Wistar rats (18/sex/dose) were fasted overnight and then orally gavaged once with Fenamiphos at 0 (vehicle), 0.4, 1.6, or 2.4 mg/kg (analytically confirmed doses: 0, 0.37, 1.52 or 2.31 mg/kg, respectively). The main study consisted of 12 rats/sex/dose (except the high-dose male group which was increased to 15 animals due to lethality), with a satellite group of 6 rats/sex/dose. Main study animals were used for a routine neurotoxicity screening battery with behavioral testing at the peak time of effect (25 min post-dosing) and at Days 7 and 14; neuropathological examination was carried out at terminal sacrifice (Day 14). The satellite group was used for

determination of plasma, RBC and brain ChE activities at approximately 50 min postdosing.

No treatment-related changes were noted in mean body weights, absolute and relative brain weights and the incidences of gross and neurohistopathological lesions.

The toxic effects of Fenamiphos were observed rapidly (within 21 to 31 min postdosing) and persisted to approximately 2 hr 45 min. At 4 to 8 hr postdosing, all treatment-related clinical signs were absent. Although plasma and RBC ChE activities were markedly and rapidly (50 min postdosing), brain ChE was unaffected. The following treatment-related effects were observed:

At 2.31 mg/kg
- Lethality in 7/15 males and 1/12 females
- Muscle fasciculations, gait incoordination, nasal and oral staining, constricted pupils, salivation, lacrimation (females only), and piloerection
- Statistically significant decreases in plasma (-61% males, -85% females) and RBC (-76% males, -80% females) ChE activities
- Decreased motor (-32%) and locomotor (-41%) activities in males.

At 1.52 mg/kg
- Muscle fasciculations in males
- Statistically significant decreases in plasma (-64% males, -77% females) and RBC (-70% males, -51% females) ChE activities.

At 0.37 mg/kg
- Statistically significant decreases in plasma ChE in females (-55%) and RBC ChE in males (-24%) with non-significant decreases in plasma ChE (-23%) in males.

Based on the results of this study (inhibition of plasma and RBC), the LOEL was established at 0.37 mg/kg; the NOEL was not established.

This study is classified as **Acceptable** and satisfies guideline requirements (§81-8) for an acute neurotoxicity screening battery in the rat.

Sign-off date: 08/16/96
DP Barcode: D227925
HED DOC Number: "NONE"
Toxicology Branch: TB2

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