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03/26/96

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity Screening Battery - Rats [OPPTS 870.6200, OPP §82-7]

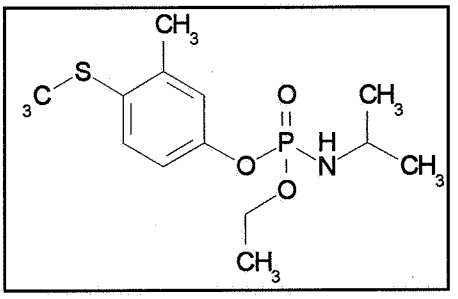
DP BARCODE: D227925
P.C. CODE: 100601

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

TEST MATERIAL: SRA 3886, Technical

SYNONYMS: Fenamiphos, Technical

STRUCTURE:



CITATION: Dreist, M. and Popp, A. (26 March 1996), SRA 3886 (Common Name: Fenamiphos) Subchronic Neurotoxicity Screening Study in Wistar Rats (Thirteen-Week Administration in the Diet, Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No.: 24948, Study No.: T 9058277, MRID No.: 440514-01, Unpublished

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

EXECUTIVE SUMMARY: In this subchronic neurotoxicity screening battery, male and female Wistar rats (12/sex/dose) were fed diets containing Fenamiphos at 0 (basal diet), 1, 10, or 50 ppm (equivalent to 0, 0.06, 0.61, or 3.13 mg/kg/day, males; 0, 0.08, 0.8, 3.98 mg/kg/day, females) for at least 13 weeks. Routine neurotoxicity screening battery consisting of Functional Observational Battery and motor activity measurements were performed at prestudy and after 4, 8 and 13 weeks of treatment. Gross pathology (all animals) and neuropathological (6/sex/dose) examinations were carried out at terminal sacrifice. Plasma and RBC cholinesterase activities were measured in 6/sex/dose at Week 4; plasma, RBC and brain cholinesterase activities were measured on animals not selected for neuropathological examination at Week 15.

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No treatment-related changes were noted in mean body weights or absolute and relative brain weights. The incidences of gross and neuropathological finding of treated animals were comparable to controls. Dose-related increases in motor and locomotor activity were observed in females at Week 13. This effect was judged to be equivocal since a similar "dose-related" increase was observed during the pre-study evaluations. Additionally, none of the motor or locomotor activities achieved statistical significance.

No treatment-related effects were observed in animals dosed at 1 ppm. Treatment-related effects are summarized as follows:

At 10 ppm, decreases in plasma ChE activity at Week 4 (-30%, males; -71%, females) and Week 15 (-39%, males; -77%, females) and RBC ChE activity at Week 4 (-5%, males; -25%, females) and Week 15 (-25%, males; -20%, females).

At 50 ppm, an increased incidence of muscle fasciculations in all (12/12) females during weeks 1 to 3. Statistically significant decreases in plasma ChE activity at Week 4 (-68%, males; -88%, females) and Week 15 (-69%, males; -91%, females) and RBC ChE activity at Week 4 (-88%, males; -86%, females) and Week 15 (-93%, males; -96%, females). Brain ChE was slightly (but statistically significant) decreased (-12%) at Week 15 in females.

Based on the results (inhibition of plasma and RBC ChE) of this study, the LOEL was established at 10 ppm (0.61 mg/kg/day, males; 0.8 mg/kg/day, females); the NOEL was established at 1 ppm (0.06 mg/kg/day, males; 0.08 mg/kg/day, females).

This study is classified as **Acceptable** and satisfies guideline requirements (§82-7) for an subchronic 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 - 95.7%
Storage: Room temperature
CAS Registry No.: 22224-92-6

B. Test Animals
Species (Strain): SPF-bred Wistar Rat (Hsd Cpb:WU)
Age at Initiation (Weeks): Approximately 7 weeks
Weight at Initiation: 129 - 179 g (males), 104 - 139 g (females)
Source: Harlan Winkelmann GmbH, Borchen
Housing: Individually in polycarbonate cages with low-dust wood granule bedding
Feed: Altromin 1321 Diet for Rats and Mice (Altromin GmbH and Com. KG, Lage) mixed with 1% peanut oil and tap water, *ad libitum*,
Water: Tap water, *ad libitum*, supplied in 700 ml bottles
Environment: Temperature: 22 ± 2°C; Humidity: 55±5% Light/Dark cycle: 12 hr/12 hr; Air changes: 15-20/ hour
Acclimation Period: Six days

C. Diet Preparation: Test diets were prepared on a weekly basis by blending sufficient amount of Fenamiphos to achieve the desired dose. Peanut oil (1%) and tap water were mixed with the feed to reduce the amount of dust formation during preparation. During the study one-half of the test diet was presented to the animals the day after preparation; the other half was stored frozen and after thawing, presented to the animals four days later. Stability, homogeneity and achieved concentration of Fenamiphos were determined at pre-study. These analyses were performed on diets prepared at nominal concentrations of 0.5 and 100 ppm, that bracketed the range of concentrations used in the study.

II. STUDY DESIGN

A. In-life Study Dates: 23 Jan to 9 May 1995

B. Study Design: Animals were randomly assigned to test groups and fed test diets at the doses indicated for 13 to 14 weeks (Table 1). Neurobehavioral evaluations (Functional Observational Battery and motor activity) were performed on all animals at pre-study and after 4, 8 and 13 weeks of treatment. Neuropathological evaluations were carried out on randomly selected animals (6/sex/dose) at terminal sacrifice (Week 13).

Plasma and RBC cholinesterase (ChE) activities were measured at Weeks 4 and 15; brain ChE activity was measured at Week 15 only. ChE activities were measured on 6 animals/sex/dose at Week 4 and on animals not selected for neuropathological evaluation at Week 15.

TABLE 1: Animal Assignment

Dose Group	Dose (ppm)	Males	Females
Control	0	12	12
Low	1	12	12
Mid	10	12	12
High	50	12	12

C. Dose Selection: The doses selected for the present study were based on a subchronic toxicity study (Bayer AG Report No.: 745, 29 March 1968) and a chronic toxicity study (Bayer AG Report No.:721, 28 February 1986). In the subchronic toxicity study, rats were fed diets containing 0, 4, 8, 16, or 32 ppm Fenamiphos for 13 weeks. Inhibition of plasma (-28% females) and RBC (-27% males, -39% females) ChE activities in animals dosed at 8 ppm (LOEL); the NOEL was established at 4 ppm. In the chronic toxicity study, rats were fed diets containing 0, 0.36, 0.6, 1, 2, 10, or 50 ppm Fenamiphos for 2 years. The LOEL for this study was establish at 2 ppm, based on inhibition of plasma ChE activity (-42% females); the NOEL for this study was established at 1 ppm. Based on the results of these studies doses selected for the present study were 0, 1, 10, and 50 ppm.

D. Statistical Evaluations: Group mean and standard deviations (S.D.) were calculated for terminal body weights, brain 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.

III. METHODS

A. Clinical Observations: Cage-side observations were performed twice daily (once on holidays and weekends) for clinical signs, mortality and moribundity. Detailed physical examinations were carried out daily for the

first three weeks of treatment and weekly, thereafter.

B. Body Weight: Body weights were measured at prestudy, the first day of treatment and at weekly intervals, thereafter.

C. Feed and Water Consumption: Feed and water consumption were measured on a weekly basis. The achieved intake was calculated from the analytical concentration of test compound in the feed and the feed consumption.

D. Ophthalmology: Ophthalmic examinations were performed on all animals at pre-study and at Week 13.

E. Neurobehavioral Tests

1. Functional Observational Battery (FOB): FOB was performed at pre-study and after 4, 8 and 13 weeks of treatment. 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 containing 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.

3. Neurobehavioral: FOB evaluations, motor activity, and neuropathology were evaluated in a positive control study (MRID No.: 440415-02) in male and female rats. This study verified the ability of the performing laboratory to adequately conduct the neurobehavioral testing.

F. Pathology

1. Gross pathology and organ weights: All animals were examined grossly at the time of death or terminal sacrifice. Brain weights were determined after removal from the skull and before being placed in preservative.

2. Neurohistopathology: The 6 animals/sex/group selected for neuropathological examinations were anesthetized (pentobarbital), perfused *in situ* with phosphate-buffered sodium nitrite followed by universal fixative (2% glutaraldehyde and 2% formaldehyde). All the tissues listed below were collected; those of the control and high-dose groups were examined microscopically.

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

3. Neuropathological Positive Controls: Neuropathology positive controls were evaluated in a previously submitted study (MRID No.: 440415-02).

G. Cholinesterase Determination: Plasma and RBC ChE activities were determined at Week 4 on 6 animals/sex/dose. Plasma, RBC and brain ChE activities were determined at Week 15 on animals not selected for neuropathological examination. Solubilized brain tissue (J. Neurochemistry 16: 1505-1513) and plasma ChE activities were determined using the modified method of Ellman (Biochem. Pharmacol. 7: 88-95, 1961), while RBC ChE activity was determined by the method of Okabe et al. (Clin. Chim. Acta 80: 87-94, 1977).

IV. RESULTS

A. Analytical Chemistry: The stability and homogeneity analyses were determined on two separate batches of test diets prepared at nominal concentrations of 0.5 and 100 ppm. These concentrations were selected to

bracket the dose levels to be used in the study. The concentration of Fenamiphos, measured in samples taken from different locations within the sample container, was found to be homogeneously distributed within the 0.5 ppm diet (coefficients of variation 6.6 to 7.3%) and the 100 ppm diet (coefficient of variation 1.7 to 6.5%). Test diets were stable at room temperature for at least four days; assayed levels of Fenamiphos were within 90.6 and 90.3% of the initial target concentrations of 0.5 and 100 ppm, respectively. During the study, the concentration of Fenamiphos in the 1, 10, and 50 ppm test diets were within 86.0 to 240.6%, 85.1 to 101.4%, and 89.2 to 102.3%, respectively. of the target concentrations. On one occasion (Week 14), the low-dose test diet was approximately 240% of the target value. For this reason, animals not selected for neuropathological examination were treated for an additional week. The concentration of Fenamiphos in the low-dose diet for Week 15 was 90.3% of the target concentration. This deviation did not appear to affect the results of this study.

B. Clinical Observations: All animals survived to terminal sacrifice. Muscle fasciculations were observed in all (12/12) high-dose females during the first three weeks; no adverse clinical signs were observed in low- and mid-dose females and all treated males.

C. Body Weight: No treatment-related differences in body weights were noted during the study. High-dose males showed a slight decrease (-3%) in mean bodyweight throughout the study, the effect was not considered to be toxicologically significant.

D. Feed and Water Consumption and Achieved Test Compound Intake: No treatment-related differences were note in the mean feed and water consumption during the study. The achieved intake of test compound is summarized in Table 2.

Table 2: Overall (Weeks 1 to 13) Achieved Dosage

SEX	Achieved Dosage (mg/kg/day)		
	1 ppm	10 ppm	50 ppm
Male	0.06	0.61	3.13
Female	0.08	0.8	3.98

D. Ophthalmology: Ophthalmic examinations did not reveal any treatment-related effects.

E. Neurobehavioral Findings

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1. FOB Findings: FOB evaluations at Weeks 4, 8 and 13 did not reveal any treatment-related effects.

2. Motor and Locomotor Activity: No statistically significant differences were noted in either motor or locomotor activity data between control and treated animals (Table 3). Motor and locomotor activities of treated males were comparable to control values at each of the evaluation times. There appeared to be, however, a dose-related increase in motor and locomotor activities in females at Week 13. The significance of this effect is not clear, however, since measurements at pretreatment, revealed a similar "dose-related" effect. At pretreatment, motor and locomotor activities were both 33% higher than control values, and were both greater than the 25% range of normal variability for motor and locomotor activities. At Week 13, low-, mid- and high-dose females had motor activity 26, 47, and 64%, respectively, greater than control value and locomotor activity 39, 57, and 82%, respectively, greater than controls values. There does not appear to be a time-related tend in activities, since intermediate determinations at Weeks 4 and 8 were generally lower than either the pretreatment or Week 13 values. Based on these results, the observed motor and locomotor activities effects were considered to be equivocal by the reviewer.

TABLE 3: MOTOR and LOCOMOTOR ACTIVITIES (MEAN ± S.D.) for FEMALE RATS[a]

OBSERVATION	Week	Beam Interruptions (% of Control)			
		0 ppm	1 ppm	10 ppm	50 ppm
Motor Activity	-1	493 ± 137	593 ± 180 (+20)	631 ± 233 (+28)	657 ± 215 (+33)
	4	627 ± 298	587 ± 133 (-6)	560 ± 194 (-11)	788 ± 222 (+26)
	8	507 ± 236	492 ± 143 (-3)	717 ± 255 (+41)	650 ± 178 (+28)
	13	350 ± 105	441 ± 146 (+26)	513 ± 193 (+47)	574 ± 182 (+64)
Locomotor Activity	-1	248 ±69	293 ± 83 (+18)	311 ± 120 (+25)	329 ± 118 (+33)
	4	301 ± 86	325 ± 83 (+8)	306 ± 122 (+2)	426 ± 116 (+42)
	8	276 ± 92	282 ± 84 (+2)	352 ± 100 (+28)	376 ± 95 (+36)
	13	176 ± 73	244 ± 79 (+39)	276 ± 116 (+57)	321 ± 119 (+82)

[a] Data summarized from Table 1 (pp 279 and 281)

F. 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.

G. ChE Activity: Plasma, RBC and brain ChE activities are presented in Table 4. At Week 4, high-dose males and females had statistically significant decreases in plasma (-68%, males; -88%, females) and RBC (-88%, males; -86%, females) ChE activities, plasma ChE activity of mid-dose females was significantly decreased by 71%. At Week 15, plasma and RBC ChE activities of high-dose females were inhibited by greater than 90%; these animals also had significant decreases (-12%) in brain ChE activities. At 10 ppm, statistically significant decreases in RBC ChE activity were observed in males (-25%) and plasma ChE, in females (-77%); decreases (not statistically significant) were observed in plasma ChE activity in males (-39%) and RBC ChE activity, in females (-20%). , Although the study states that the sample size for statistical analysis of ChE activities in high-dose males was too small, plasma and RBC ChE activities were markedly inhibited 69% and 93%, respectively.

TABLE 4: PLASMA, RBC and BRAIN ChE ACTIVITIES (Mean ± S.D.) at WEEKS 4 and 15[a]

SEX	DOSE (ppm)	ChE ACTIVITY (% of Control)		
		PLASMA (U/L)	RBC (U/L)	BRAIN (U/g)
WEEK 4				
Male	0	440 ± 102	740 ± 197	---
	1	390 ± 52 (-11)	830 ± 160 (+12)	---
	10	310 ± 60 (-30)	700 ± 123 (-5)	---
	50	140 ± 37** (-68)	90 ± 71** (-88)	---
Female	0	1290 ± 392	810 ± 186	---
	1	920 ± 259 (-29)	850 ± 207 (+5)	---
	10	380 ± 101** (-71)	610 ± 135 (-25)	---
	50	150 ± 56** (-88)	110 ± 82** (-86)	---
WEEK 15				
Male	0	520 ± 156	710 ±136	12.54 ± 0.763
	1	440 ± 111 (-15)	670 ± 101 (-6)	11.87 ± 0.557 (-5)
	10	320 ± 51 (-39)	530 ± 50* (-25)	11.26 ± 2.275 (-10)
	50	160 ± 49#(-69)	50 ± 5#(-93)	11.37 ± 0.494# (-9)

Female	0	1850 ± 566	690 ± 106	11.94 ± 0.400
	1	1260 ± 445 (-32)	780 ± 157 (+13)	12.59 ± 0.333* (+5)
	10	430 ± 90** (-77)	550 ± 218 (-20)	11.74 ± 0.525 (-2)
	50	170 ± 50** (-91)	30 ± 14** (-96)	10.48 ± 0.421** (-12)

[a] Data summarized from Text Table (pg 33) and Appendix XVI (pp 403 to 409).
--- Not determined
* $p \leq 0.05$, ** $p \leq 0.01$
# Sample too small for statistical analyses

V. DISCUSSION and CONCLUSIONS: Male and female Wistar rats (12/sex/dose) were fed diets containing Fenamiphos at 0 (basal diet), 1, 10, or 50 ppm (equivalent to 0, 0.06, 0.61, or 3.13 mg/kg/day, males; 0, 0.08, 0.8, 3.98 mg/kg/day, females) for at least 13 weeks. Routine neurotoxicity screening battery consisting of Functional Observational Battery and motor activity measurements were performed at prestudy and after 4, 8 and 13 weeks of treatment. Gross pathology (all animals) and neuropathological (6/sex/dose) examinations were carried out at terminal sacrifice. Plasma and RBC cholinesterase activities were measured in 6/sex/dose at week 4, plasma; RBC and brain cholinesterase activities were measured on animals not selected for neuropathological examination at Week 15.

No treatment-related changes were noted in mean body weights or absolute and relative brain weights. The incidences of gross and neuropathological finding of treated animals were comparable to controls. Equivocal increases in motor and locomotor activity were observed in females at Week 13. This effect was judged to be equivocal since similar "dose-related" increases were also observed during the pre-study evaluations. Additionally, none of the motor or locomotor activities achieved statistical significance.

No treatment-related effects were observed in animals dosed at 1 ppm. Treatment-related effects are summarized as follows:

At 10 ppm:
- Decreases in plasma ChE activity at Week 4 (-30%, males; -71%, females) and Week 15 (-39%, males; -77%, females). RBC ChE activity was decreased at Week 4 (-25%, females) and Week 15 (-25%, males; -20%, females).

At 50 ppm:
- Increased incidence of muscle fasciculations in all (12/12) females during weeks 1 to 3.
- Statistically significant decreases in plasma ChE activity at Week 4 (-68%, males; -88%, females) and Week 15 (-69%, males; -91%, females) and RBC ChE activity at Week 4 (-88%, males; -86%, females) and Week 15 (-93%, males; -96%, females). Brain ChE was slightly (but statistically significant) decreased (-12%) at Week 15 in females.

Based on the results (inhibition of plasma and RBC ChE) of this study, the LOEL

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was established at 10 ppm (0.61 mg/kg/day, males; 0.8 mg/kg/day, females); the NOEL was established at 1 ppm 0.06 mg/kg/day, males; 0.08 mg/kg/day, females).

This study is classified as **Acceptable** and satisfies guideline requirements (§82-7) for an subchronic neurotoxicity screening battery in the rat.

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STUDY TYPE: OPP 82-7, OPPTS: 870.6200
Subchronic Neurotoxicity Screening Battery
Species: Rat

TEST MATERIAL: 100601 - Fenamiphos, Technical (SRA 3886, technical)
Batch No. 809335134
Purity: 95.2 to 95.7%

EPA MRID NOs.: 440514-01
440415-02

TESTING FACILITY: Bayer AG

STUDY NOs.: T 9058277
T 3060610

REPORT ISSUED: 26 March 1996

EXECUTIVE SUMMARY: Male and female Wistar rats (12/sex/dose) were fed diets containing 0 (basal diet), 1, 10 or 50 ppm (equivalent to 0, 0.06, 0.61, or 3.13 mg/kg/day, males; 0, 0.08, 0.8, or 3.98 mg/kg/day, females) for 13 weeks. FOB and motor activity were evaluated at prestudy and weeks 4, 8, and 13. Cholinesterase activities were measured at Weeks 4 (plasma and RBC only) and 15 (plasma, RBC and brain).

No treatment-related effects were observed in animals dosed at 1 ppm. Treatment-related effects are summarized as follows:

At 10 ppm: Decreases in plasma ChE activity at Week 4 (-30%, males; -71%, females) and Week 15 (-39%, males; -77%, females). RBC ChE activity was decreased at Week 4 (-25%, females) and Week 15 (-25%, males; -20%, females).

At 50 ppm: Increased incidence of muscle fasciculations in all (12/12) females during weeks 1 to 3. Statistically significant decreases in plasma ChE activity at Week 4 (-68%, males; -88%, females) and Week 15 (-69%, males; -91%, females) and RBC ChE activity at Week 4 (-88%, males; -86%, females) and Week 15 (-93%, males; -96%, females). Brain ChE was slightly (but statistically significant) decreased (-12%) at Week 15 in females.

Based on the results of this study the LOEL is established at 10 ppm (0.61 mg/kg/day, males; 0.80 mg/kg/day, females) and the NOEL at 1 ppm (0.06 mg/kg/day, males; 0.08 mg/kg/day, females).