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Chemical:	**Isofenphos**
PC Code:	**109401**
HED File Code	**13000 Tox Reviews**
Memo Date:	**08/26/1997**
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG 26 1997

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ISOFENPHOS: Review of Subchronic Neurotoxicity Toxicity Study

EPA Identification Nos.: Tox Chem Code: 109401
MRID No: 44236601
Caswell No.: 447AB
DP Barcode: D235981
Submission No.: S523830

FROM Robert F. Fricke, Ph.D. Robert F. Fricke 21 Aug 97
Reregistratioin Branch II
Health Effects Division (HED) (7509C)

THRU: Alan Nielsen Al Nie
Branch Senior Scientist
Reregistration Branch II, HED

TO: Ruby Whiters
Chemical Review Manager
Special Review and Reregistration Division (7508C)

To support the reregistration of Isofenphos by Bayer Corporation, a subchronic neurotoxicity Screening Battery in the rat was reviewed. The results of this study is summarized as follows:

CITATION: Dreist, M. and Popp, A. (3 Dec 1996), SRA 12869 (Common Name: Isofenphos) Subchronic Neurotoxicity Screening Study in Wistar Rats (Thirteen-Week Administration in the Diet), Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No.: 25722, Study No.: T 2059080, MRID No.: 44236601, Unpublished

EXECUTIVE SUMMARY: Male and female Wistar rats (12/sex/dose) were fed diets containing Isofenphos (91.6%) at 0 (basal diet), 1, 25, or 125 ppm (mg/kg/day equivalents: 0, 0.06, 1.62, or 8.45, males; 0, 0.09, 2.07, or 11.54, females) for at least 13 weeks. Neurobehavioral evaluations, 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. Six animals/sex/dose were selected for determination of plasma and RBC cholinesterase (ChE) activities at week 4 and plasma, RBC and brain ChE activities at week 14.

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Treatment-related, cholinergic signs were observed during the clinical evaluations of high-dose males and females. During the first two to four weeks of treatment, males and females showed piloerection and tremors; high incidences of palmus and non-specific behavioral disturbances (females only) were observed during the entire study. No treatment-related clinical signs were observed in the low and mid-dose groups. All animals survived to terminal sacrifice.

Mean body weights of high-dose males and females were statistically significantly lower than control values during the first six to seven weeks of the study. These decreases appear to be a result of decreased body weight gains of 51% in males and 100% (no weight gain) in females during the first week of the study. The decreased body weight gains appear to be a result of decreased food consumption (g/animals/day) of 19% in males and 35% in females. Excluding the body weight data for the first week of the study, the body weight gains for weeks 1 to 13 were the same as the control value in males and 11% greater than control value in females.

Plasma, RBC and brain ChE activities of mid- and high-dose animals were all significantly decreased. The evaluations at week 4 for mid-dose animals showed significant decreases in plasma (54%, males; 84%, females) and RBC (64%, males; 81%, females) ChE activities. At week 14, mid-dose animals had decreases in plasma, RBC and brain ChE activities of 54%, 63% and 32% in males, respectively and 88%, 66% and 60% in females, respectively. At week 4, high-dose animals had decreases in plasma and RBC ChE activities of 85% and 98%, in males, respectively and 97% and 100% in females, respectively. At week 14, plasma, RBC and brain ChE activities of high-dose animals were decreased 84%, 96%, and 75% in males, respectively and 97%, 97%, and 89% in females, respectively.

Neurobehavioral evaluations revealed treatment-related effects in high-dose males and females, with females being more affected than males. Treatment-related FOB effects consisted in part, of muscle fasciculations in both sexes and abnormal gait and decreased grip strength in females. Motor and locomotor activities were significantly decreased in high-dose females.

Ophthalmological examination at week 13 revealed a slow pupillary reflex in five high-dose females, this is regarded as a treatment-related effect.

The incidences of gross and neuropathological finding of treated animals were comparable to controls.

Based on the results of this study (inhibition of plasma, RBC E and brain ChE), the LOEL was established at 25 ppm (1.62 mg/kg/day, males; 2.07 mg/kg/day, females); the NOEL was established at 1 ppm (0.06 mg/kg/day, males; 0.09 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.

EPA Reviewer: Robert F. Fricke, Ph.D.
Reregistration Branch II, HED (7509C)

Robert F. Fricke 21 Aug 97 01231

EPA Secondary Reviewer: David Anderson, Ph.D.
Reregistration Branch II, HED (7509C)

D. Anderson 8/21/97

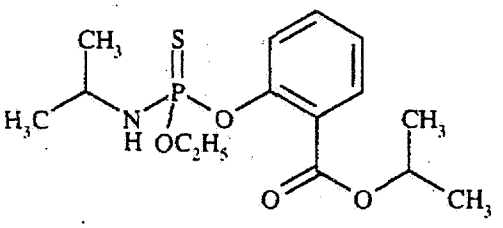
DATA EVALUATION RECORD

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

DP BARCODE: D235981
SUBMISSION NO.: S235981
P.C. CODE: 109401
TOX CHEM NO.: 447AB

TEST MATERIAL: SRA 12869, Technical

SYNONYMS: Isofenphos, Technical



CITATION: Dreist, M. and Popp, A. (3 Dec 1996), SRA 12869 (Common Name: Isofenphos) Subchronic Neurotoxicity Screening Study in Wistar Rats (Thirteen-Week Administration in the Diet), Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No.: 25722, Study No.: T 2059080, MRID No.: 44236601, Unpublished

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

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Treatment-related, cholinergic signs were observed during the clinical evaluations of high-dose males and females. During the first two to four weeks of treatment, males and females showed piloerection and tremors; high incidences of palmus and non-specific behavioral disturbances (females only) were observed during the entire study. No treatment-related clinical signs were observed in the low and mid-dose groups. All animals survived to terminal sacrifice.

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Neurobehavioral evaluations revealed treatment-related effects in high-dose males and females, with females being more affected than males. Treatment-related FOB effects consisted in part, of muscle fasciculations in both sexes and abnormal gait and decreased grip strength in females. Motor and locomotor activities were significantly decreased in high-dose females.

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The incidences of gross and neuropathological finding of treated animals were comparable to controls.

Based on the results of this study (inhibition of plasma, RBC E and brain ChE), the LOEL was established at 25 ppm (1.62 mg/kg/day, males; 2.07 mg/kg/day, females); the NOEL was established at 1 ppm (0.06 mg/kg/day, males; 0.09 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.

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I. MATERIALS

A. Test Material: Isofenphos, Technical
Description: Yellow-brown fluid
Batch No: 809416817
Purity: 91.6%
Storage: Room temperature
CAS Registry No.: 25311-71-1

B. Test Animals
Species (Strain): SPF-bred Wistar Rat (Hsd Cpb:WU)
Age at Initiation (Weeks): Approximately 7 weeks
Weight at Initiation: 138 - 186 g (males), 106 - 140 g (females)
Source: Harlan Winkelmann GmbH, Borchen
Housing: Individually in polycarbonate cages with low-dust wood granule bedding during the study
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 (corrected for % purity of a.i.) of Isofenphos to achieve the desired dose. Peanut oil (1%) and tap water were mixed with the feed (including control diet) to reduce the amount of dust formation during preparation. Room temperature stability and homogeneity of test diets prepared at 0.5 and 150 ppm were determined prior to initiation of the study. These doses bracketed to the range used in the actual study. The achieved concentration of Isofenphos in the test diets were determined on random samples taken during weeks 1, 4 and 14 of the study.

II. STUDY DESIGN

A. In-life Study Dates: 6 June to 14 September 1995

B. Study Design: Animals were randomly assigned to test groups and fed test diets at the doses indicated for at least 13 weeks (Table 1). Neurobehavioral evaluations (Functional Observational Battery and motor activity) were performed on all animals at pre-study (designated as Day 0, week 0) 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 on 6 animals/sex/dose

at week 4. At week 14, plasma, RBC and brain ChE activities were measured on animals not selected for neuropathological evaluation.

TABLE 1: Animal Assignment

Dose Group	Dose (ppm)	Males	Females
Control	0	12	12
Low	1	12	12
Mid	25	12	12
High	125	12	12

C. Dose Selection: The doses selected for the present study were based on three previously conducted studies in rats: a subchronic (13-week) feeding study (Bayer AG Report No.: 4094, 23 March 1977), a chronic (2-year) feeding study (Bayer AG Report No.: 6979, 2 September 1977), and a 4-week range finding study.

In the subchronic feeding study, rats were fed diets containing 0, 0.5, 1, 5, 25, or 125 ppm Isofenphos for 13 weeks. At 125 ppm, cholinergic signs (muscle fasciulations, tremors and salivation) were observed during the first two weeks of the study. Decreases in body weight gain were observed after 4-weeks (males,-18%; females, -13%) and 13 weeks (males,-12%; females, -5%) of treatment. Biologically significant inhibition of RBC ChE was observed at 5 ppm and higher in females (-39% to -90%) and 25 ppm and higher in males (-68% to -87%). Brain ChE activity at week 13 was decreased at 25 ppm and higher in males (-48% to -78%) and females (-63 to -84%).

In the chronic feeding study, animals were fed diets containing 0, 1, 10, or 100 ppm Isofenphos. At 100 ppm, decreases in body weight gain were observed after 4-weeks (males,-26%; females, -13%) and 13 weeks (males,-13%; females, -8%) of treatment. Biologically significant inhibition of RBC ChE was observed at 10 ppm and higher in males (-49% to -88%) and females (-43% to -84%).

In the range finding study, animals were dosed at 0, 1, 25, and 125 ppm Isofenphos for four weeks. RBC ChE activities were reduced at 25 ppm (males, -64%; -82%, females) and 125 ppm (males, -96%;females, -98%). Brain ChE were also decreased at 25 ppm (males, -37%; -58%, females) and 125 ppm (-78%, males; -88%, females). Cholinergic signs were observed in high-dose animals, but were less prominent in males.

Based on the results of these studies doses of 0, 1, 25, and 125 ppm were selected for the present study.

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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 (immediately before treatment) and at weekly intervals, thereafter.

C. Food Consumption: Food consumption were measured on a weekly basis. The achieved test compound 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. Animals showing abnormalities were excluded from the study.

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 observers, 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, the motor activity was measured on individual animals using figure-eight shaped automated activity chamber containing eight infrared emitter/detector pairs. Animals were evaluated 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 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 verified the ability of the performing laboratory to adequately conduct the neurobehavioral testing. Further, inter-observer reliability and consistency for the FOB evaluations were established prior to initiation of the study (Bayer AG Report Nos.: 23337 (12 September 1994) and 24294, (7 September 1996)).

F. Cholinesterase (ChE) Determinations: Animals (6/sex/dose, non-fasted) not selected for neuropathological evaluation were bled via the retroorbital sinus for determination of plasma and RBC ChE activities. At weeks 4 and 14, plasma and RBC ChE activities were measured and at week 14, brain ChE activity. RBC ChE activity was determined using the method of Okabe *et al.* (Clin. Chim. Acta 80: 87-94, 1977). 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).

G. 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 but 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 in phosphate buffer). All the tissues listed below were collected; those of the control and high-dose groups were examined microscopically. The low- and mid-dose groups were examined if lesions were observed in the high-dose group. Tissues from non-perfused animals with gross lesions were also examined microscopically.

Brain: 10 sections total from olfactory lobe, forebrain, midbrain, pons, cerebellum (anterior and posterior), medulla oblongata
Spinal Cord: Cervical, thoracic & lumbar
Spinal Nerve Root Fiber and Ganglia: Dorsal and ventral, cervical (bilateral) and dorsal and ventral, lumbar (bilateral)
Eyes and optic nerves
Gasserian ganglia
Gastrocnemius muscle
Peripheral Nerves: Sciatic, tibial, & sural (all bilateral)

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

H. 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 followed by an one-way analysis of variance 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.

IV. RESULTS

A. Analytical Chemistry (Summarized from Analytical Chemistry Report, Appendix VI, pp 54 to 68): The stability and homogeneity analyses were determined on two separate batches of test diets prepared at nominal concentrations of 0.5 and 150 ppm. These concentrations were selected to bracket the dose levels to be used in the study. The concentration of Isofenphos, 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 6.7 to 9.3%) and the 150 ppm diet (coefficient of variation 7.2 to 7.8%). Test diets were stable at room temperature for at least 11 days; compared to the initial day 0 values, assayed levels of Isofenphos were 91.8 to 118.8% for the 0.5 ppm test diet and 108.5 to 118.5% for the 150 ppm test diets. During the study, the concentration ranges of Isofenphos were 0.98 to 1.17 ppm for the 1 ppm test diet, 26.94 to 31.04 ppm for the 25 ppm test diet, and 116.1 to 131.05 ppm for the 125 ppm test diet.

B. Clinical Observations and Mortality: All animals survived to terminal sacrifice. Treatment-related clinical signs were observed only in high-dose animals; no treatment-related clinical signs were observed in animals in the low- and mid-dose groups. The weekly incidences of treatment-related clinical signs of high-dose animals are summarized in Table 2. Piloerection and tremors were generally observed during weeks 1 - 2 in males and weeks 1 - 4 in females; a higher number of females were affected than males. A non-specific behavioral disturbance (no further description was given in the study) was observed in during the last three weeks of the study. For weeks 1 -13, a high incidence of palmus was observed in both males and females, while spastic gait was only observed in females.

C. Body Weights and Body Weight Gains: Treatment-related changes in mean body weights were limited to high-dose males and females (Table 3).

From weeks 1 to 4, mean body weights were statistically significantly decreased by 10 to 14% in males and 10 to 15% in females. At week 6, decreases of 9% were noted in both males and females, and at week 7, females had a decrease of 8%.

Mean body weight gains are summarized in Table 4. During the first week of the study body weight gains by high-dose males and females were 51% and -100% lower, respectively, than the concurrent control values. From the second week of the study and on, body weight gain by both males and females were generally comparable to control values. The overall body weight gains for weeks 0-13 were lower than control values in both males (-9%) and females (-12%), however, after excluding the data for week 1, the resulting body weight gains for weeks 1-13 were the same as or greater than the control values in males (0%) and females (+11%).

Table 2: Time-Related Incidences of Clinical Observations in High-Dose Animals for Weeks 1 to 13[a]

Observation	Week												
	1	2	3	4	5	6	7	8	9	10	11	12	13
High-Dose (125 ppm) Males													
Alive Animals	12	12	12	12	12	12	12	12	12	12	12	12	12
Piloerection	1	1	0	0	0	0	0	0	0	0	0	0	0
Tremors	1	1	0	0	0	0	0	0	0	0	0	0	0
Palmus	10	12	9	7	5	11	10	5	7	11	11	9	6
High-Dose (125 ppm) Females													
Alive Animals	12	12	12	12	12	12	12	12	12	12	12	12	12
Piloerection	6	7	2	1	0	0	0	0	0	1	1	0	0
Tremors	7	10	1	0	0	0	0	0	0	1	0	0	0
Non-specific Behavioral Disturbance[b]	0	0	0	0	0	0	0	0	0	0	8	10	11
Spastic Gait	3	4	10	8	7	9	9	8	10	11	11	11	11
Palmus	12	12	12	12	12	12	12	12	12	12	12	12	12

[a] Summarized from Appendix VIII pages 75 to 88.
[b] No further description of "non-specific behavioral disturbances" was given in the study.

Table 3: Mean Body Weights (g)[a]

Week	Dose Level (ppm)			
	0	1	25	125
Male				
0	174	170	170	166
1	219	215	211	188** (-14)[b]
2	257	256	247	227** (-12)
3	289	289	277	257* (-11)
4	305	307	296	274* (-10)
6	352	351	340	319* (-9)
13	431	435	421	400
Female				
0	122	120	122	123
1	145	141	143	123** (-15)
2	160	157	160	138** (-14)
3	172	169	172	152** (-12)
4	181	175	180	162** (-10)
6	198	194	199	181* (-9)
7	205	202	208	189* (-8)
13	227	222	230	215

[a] Data summarized from Appendix IX (pp. 93-95)
[b] Values in parentheses are the percent change from the concurrent control value.
* $p \leq 0.05$, ** $p \leq 0.01$ compared to concurrent control value.

Table 4: Mean Body Weight Gains[a]

Weeks	Dose Level (ppm)			
	0	1	25	125
Male				
0-1	45	45	41	22 (-51)[b]
1-2	38	41	36	39 (+3)
1-13	212	220	210	212 (0)
0-13	257	265	251	234 (-9)
Female				
0-1	23	21	21	0 (-100)
1-2	15	16	17	15 (0)
1-13	82	81	87	92 (+11)
0-13	105	102	108	92 (-12)

[a] Calculated by reviewer from mean body weight data in Appendix IX (pp. 93-95)
[b] Values in parentheses are the percent change from the concurrent control value.

D. Food Consumption and Achieved Test Compound Intake: Statistically significant decreases in mean food consumption, expressed as g/animals/day, were observed in high-dose males and females during week 1 of the study (Table 5). When expressed as g/kg body weight/day, significant decreases were observed only in high-dose females. For the remainder of the study, feed consumption by treated animals was generally comparable to control values.

The overall (weeks 1 - 13) achieved compound intake for the study is summarized in Table 6.

Table 5: Mean Food Consumption for Week 1 of the Study [a]

Sex	Dose Level (ppm)			
	0	1	25	125
g/animal/day				
Male	21	20	19	17** (-19)[b]
Female	17	16	15	11** (-35)
g/kg body wt/day				
Female	120	117	106	93* (-23)

[a] Data summarized from tables on pages 101 to 104 of Appendix X
[b] Values in parentheses are the percent change from the concurrent control value.
* $p \leq 0.05$, ** $p \leq 0.01$ Compared to concurrent control value

Table 6: Overall (Weeks 1 to 13) Achieved Dosage (mg/kg/day)[a]

Sex	Dose Level (ppm)		
	1 ppm	25 ppm	125 ppm
Male	0.06	1.62	8.45
Female	0.09	2.07	11.54

[a] Data summarized from text table on page 28 of the study

E. Ophthalmology: At week 13, five high-dose females had a slow pupillary reflex, which was regarded as a treatment-related effect.

F. Cholinesterase Activities: Plasma, RBC and brain ChE activities of mid- and high-dose animals were all significantly decreased (Table 7). At 25 ppm, plasma ChE activity of males and females was decreased from 54% to 84%, respectively, at week 4, and 54% to 88%, respectively, at week 14. At 125 ppm, plasma ChE activity was further decreased (-85%, males; -97%, females), and again similar decreases were noted at week 14. At week 4, RBC ChE was also markedly decreased at 25 ppm (-64%, males; -81%, females) and 125 ppm (-98%, males; -100%, females). At week 14, RBC ChE activities were decreased

at 25 ppm (-32%, males; -66%, females) and 125 ppm (-96%, males; -97%, females). Brain ChE activity, determined at terminal sacrifice, was markedly inhibited in males (-32%) and females (-60%) dosed at 25 ppm; and males (-75%) and females (-89%) dosed at 125 ppm.

Table 7: Plasma, RBC and Brain Cholinesterase Activities at Weeks 4 and 14[a]

Sex	ChE[b]	Dose Level (ppm)			
		0	1	25	125
Week 4					
Male	Plasma	0.41 ±0.083	0.42 ± 0.102[c] (+2)	0.19 ± 0.021** (-54)	0.06 ± 0.012** (-85)
	RBC	0.99 ± 0.193	0.82 ± 0.201 (-17)	0.36 ± 0.208** (-64)	0.02 ± 0.019** (-98)
Female	Plasma	1.53 ± 0.419	1.15 ± 0.521 (-25)	0.24 ± 0.049** (-84)	0.05 ± 0.008** (-97)
	RBC	0.99 ± 0.086	0.94 ± 0.227 (-5)	0.19 ± 0.148** (-81)	0.00 ± 0.008** (-100)
Week 14					
Male	Plasma	0.43 ± 0.097	0.46 ± 0.127 (+7)	0.20 ± 0.024** (-54)	0.07 ± 0.005** (-84)
	RBC	1.16 ± 0.321	1.08 ± 0.238 (-7)	0.43 ± 0.134** (-63)	0.05 ± 0.041** (-96)
	Brain	11.87 ± 0.488	12.16 ± 0.623 (+2)	8.11 ± 0.779** (-32)	3.00 ± 0.184** (-75)
Female	Plasma	2.17 ± 0.878	1.92 ± 0.224 (-12)	0.27 ± 0.029** (-88)	0.06 ± 0.010** (-97)
	RBC	1.24 ± 0.283	1.36 ± 0.098 (+10)	0.42 ± 0.165** (-66)	0.04 ± 0.012** (-97)
	Brain	12.03 ± 0.502	11.76 ± 0.743 (-2)	4.87 ± 0.798** (-60)	1.37 ± 0.213** (-89)

[a] Data summarized from of Appendix XV (pp. 415 to 421) and text table on page 35.
[b] Units: Plasma and RBC ChE, k/l; brain ChE, U/g
[c] Mean ± S.D. for n = 6, values in parentheses are the percent change from the concurrent control value.
* $p \leq 0.05$, ** $p \leq 0.01$ compared to concurrent control value.

Table 8: Cholinesterase Historical Control Data

Sex	ChE Activity (95% Confidence Interval)		
	Plasma (U/L)	RBC (U/L)	Brain (U/g)
Male	0.47 (0.29 - 0.65)	0.8 (0.45 - 1.16)	12.66 (9.65 - 15.67)
Female	1.47 (0.60 - 2.35)	0.92 (0.45 - 1.39)	13.46 10.60 - 16.32)

[a] Data summarized from Appendix II (pg 49)

G. Neurobehavioral Findings

1. FOB Findings: Treatment-related FOB findings were limited to animals in the high-dose group (Table 8). During the home-cage evaluations the incidence of muscle fasciculations were observed a large number of males and essentially all of the females at weeks 4, 8 and 13. Muscle fasciculations were also observed during the open field evaluations in females at weeks 4, 8 and 13. Stilted gait was observed in females during both the home-cage and open field evaluations at weeks 4, 8 and 13. At week 4, tremors and gait incoordination were observed in two females, while increased number of rearings was observed in males. At week 13, high-dose females showed more energetic approach, touch and tail pinch responses and increased difficulty in removal from the cage (increased rearing). Quantitative evaluations of high-dose animals showed significantly reduced hindlimb and forelimb grip strengths in females at weeks 4, 8, and 13; at week 4, males had a 10 % reduction in mean body weight.

2. Motor and Locomotor Activity: Treatment-related decreases in both motor and locomotor activity were limited to high-dose females (Table 9). At all of the evaluation times, motor activity were decreased by 40 to 49% and locomotor activity, by 37 to 52 % of the concurrent control values.

H. Pathology and Terminal Body and Brain Weights

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

2. Brain Weights: Brain weights at terminal sacrifice did not reveal any treatment-related differences.

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

V. DISCUSSION and CONCLUSIONS: Male and female Wistar rats (12/sex/dose) were fed diets containing Isofenphos at 0 (basal diet), 1, 25, or 125 ppm (mg/kg/day equivalents: 0, 0.06, 1.62, or 8.45, males; 0, 0.09, 2.07, or 11.54, females) for at least 13 weeks. Neurobehavioral evaluations, 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. Six animals/sex/dose were selected for determination of plasma and RBC cholinesterase (ChE) activities at weeks 4 and plasma, RBC and brain ChE activities at week 14.

Treatment-related, cholinergic signs were observed during the clinical evaluations of high-dose males and females. During the first two to four weeks of treatment, males

Table 8: Summary of FOB Findings[a]

Observation	Sex	Week	Dose Level (ppm)			
			0	1	25	125
HOME CAGE						
Muscle Fasciculations	Male	4	0/12	0/12	0/12	8/12*
		8	0/12	0/12	0/12	7/12*
		13	0/12	0/12	0/12	8/12*
	Female	4	0/12	0/12	0/12	12/12*
		8	0/12	0/12	0/12	11/12*
		13	0/12	0/12	0/12	12/12*
Abnormal Gait Stilted	Female	4	0/12	0/12	0/12	3/12*
		8	0/12	0/12	0/12	1/12
		13	0/12	0/12	0/12	3/12*
HAND-HELD						
Ease of Removal Rearing	Female	4	4/12	4/12	6/12	7/12
		13	0/12	0/12	0/12	11/12*
OPEN FIELD						
Gait Abnormalities Stilted	Female	4	0/12	0/12	0/12	7/12*
		8	0/12	0/12	0/12	8/12*
		13	0/12	0/12	0/12	9/12*
Muscle Fasciculations	Female	4	0/12	0/12	0/12	4/12*
		8	0/12	0/12	0/12	3/12*
		13	0/12	0/12	0/12	2/12
Incoordination	Female	4	0/12	0/12	0/12	2/12
Tremors	Female	4	0/12	0/12	0/12	2/12
No. of Rearings/2 min (mean)	Male	4	5.6	8.4	6.5	10.4*
RESPONSES/REFLEXES						
Approach - More energetic	Female	13	1/12	0/12	0/12	7/12*
Touch - More Energetic	Female	13	1/12	0/12	0/12	8/12*
Auditory - More Energetic	Female	13	0/12	0/12	0/12	2/12
Tail Pinch - More Energetic	Female	13	1/12	0/12	0/12	9/12*
QUANTITATIVE						
Body Weight (g)	Male	4	297	298	287	267*
Grip Strength Forelimb (kg)	Female	4	1.13	1.12	1.09	0.64*
		8	1.30	1.30	1.23	0.80*
		13	1.39	1.40	1.36	0.85*
Grip Strength Hindlimb (kg)	Female	4	0.61	0.64	0.67	0.46*
		8	0.79	0.72	0.79	0.56*
		13	0.84	0.81	0.85	0.59*

[a] Data summarized from tables in Appendix XII (pp 120 - 160)

* $p \leq 0.05$ compared to concurrent control value

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TABLE 9: Motor and Locomotor Activities of Female Rats - Mean Number of Beam Interuptions[a]

Observation	Week	Dose Level (ppm)			
		0 ppm	1 ppm	25 ppm	125 ppm
Motor Activity	-1	588	766 (+30)[b]	629 (+7)	623 (+6)
	4	686	675 (-2)	595 (-13)	400* (-42)
	8	597	568 (-5)	526 (-12)	361* (-40)
	13	506	448 (-12)	400 (-21)	258* (-49)
Locomotor Activity	-1	303	362 (+20)	310 (+2)	326 (+8)
	4	383	370 (-3)	327 (-15)	183* (-52)
	8	302	287 (-5)	291 (-4)	189* (-37)
	13	245	246 (0)	209 (-15)	127* (-48)

[a] Data summarized from Appendix XIII, Tables 1 and 2 (pp 291 and 293) and text table on page 33.
[b] Values in parentheses are the percent change from the concurrent control value.
* $p \leq 0.05$ statistical significance compared to concurrent control value

and females showed piloerection and tremors; high incidences of palmus and non-specific behavioral disturbances (females only) were observed during the entire study. No treatment-related clinical signs were observed in the low and mid-dose groups. All animals survived to terminal sacrifice.

Mean body weights of high-dose males and females were statistically significantly lower than control values during the first six to seven weeks of the study. These decreases appear to be due to decreased body weight gains during the first week of the study in both males (51%) and females (100%). The decreased body weight gains appear to be a result of decreased food consumption (g/animals/day) of 19% in males and 35% in females. Excluding the body weight data for the first week of the study, the body weight gains for weeks 1 to 13 were the same as the control value in males and 11% greater than control values in females.

Plasma, RBC and brain ChE activities of mid- and high-dose animals were all significantly decreased. The evaluations at week 4 for mid-dose animals showed significant decreases in plasma (54%, males; 84%, females) and RBC (64%, males; 81%, females) ChE activities. At week 14, mid-dose animals had decreases in plasma, RBC and brain ChE activities of 54%, 63% and 32% in males, respectively and 88%, 66% and 60% in females, respectively. At week 4, high-dose animals had decreases in plasma and RBC ChE activities of 85% and 98%, in males, respectively and 97% and 100% in females, respectively. At week 14, plasma, RBC and brain ChE activities of high-dose animals were decreased 84%, 96%, and 75% in males, respectively and 97%, 97%, and 89% in females, respectively.

Neurobehavioral evaluations revealed treatment-related effects in high-dose males and females, with females being more affected than males. Treatment-related FOB effects consisted in part, of muscle fasciculations in both sexes and abnormal gait and decreased grip strength in females. Motor and locomotor activities were significantly decreased in high-dose females.

Ophthalmological examination at week 13 revealed a slow pupillary reflex in five high-dose females, this is regarded as a treatment-related effect.

The incidences of gross and neuropathological finding of treated animals were comparable to controls.

Based on the results of this study (inhibition of plasma, RBC E and brain ChE), the LOEL was established at 25 ppm (1.62 mg/kg/day, males; 2.07 mg/kg/day, females); the NOEL was established at 1 ppm (0.06 mg/kg/day, males; 0.09 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.