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DATA EVALUATION RECORD

FOSTHIAZATE

Study Type: Non-guideline Comparative Cholinesterase Activity Study in Rats

Work Assignment No. 3-1-106 B (MRID 46744902)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; Comparative Cholinesterase Activity Study in Rats (gavage)

PC CODE: 129022 **DP BARCODE:** D326791
TXR#: 0054097

TEST MATERIAL (PURITY): Fosthiazate technical (94.7% a.i.)

SYNONYMS: *O*-ethyl *S*-(1-methylpropyl) (2-oxo-3-thiazolidinyl)phosphonothioate; IKI-1145

CITATION: Beck, M. J. (2006) An oral (gavage) age-sensitivity study of technical fosthiazate in rats. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Study No.: WIL-282005, January 25, 2006. MRID 46744902. Unpublished.

SPONSOR: Ishihara Sangyo Kaisha, Ltd, 1-chome, Edobori, Nishi-ku, Osaka, Japan

EXECUTIVE SUMMARY - A non-guideline comparative cholinesterase activity study (MRID 46744902) was conducted in Sprague-Dawley rats to determine the potential of fosthiazate technical (94.7% a.i.; Lot No. 21007013) to induce effects on maternal, fetal, neonatal, and young adult cholinesterase activity in the blood components and brain after maternal gestational exposure, or after acute or short-term repeated exposure to rats of various ages. These effects were evaluated in a series of 4 phases, where doses of 0, 0.1, 0.7 or 5 mg/kg/day were administered to rats by gavage in a volume of 5 mL/kg, using deionized water as a vehicle. Blood and brain were collected from animals at sacrifice. Samples from viable fetuses were pooled by litter, and samples from pups at post-natal day (PND 11) were pooled 2/sex/litter. Plasma, red blood cell (RBC), and whole brain cholinesterase assays were performed. In Phase I, maternal gestational exposure was evaluated by dosing 12 bred females/dose group once daily from gestation day (GD) 6 through 20. Dams were sacrificed on GD 20. In Phase II, time of peak effect was determined by administering a single dose to 60 pups/sex/dose on PND 11 and 30 pups/sex/dose on PND 21. Pups were sacrificed (10 [PND 11] or 5 [PND 21] pups/sex/dose) at 10 minutes, 30 minutes, and 1, 2, 4, and 24 hours post-treatment. In Phase III, acute exposure was evaluated by administering a single dose to 20 pups/sex/dose on PND 11, 10 pups/sex/dose on PND 21, and 10 young adult rats/sex/dose on approximately PND 42. Animals were euthanized at the time of peak inhibition of cholinesterase. In Phase IV, the effect of repeated exposure was evaluated by administering a single daily dose to pups at PND 11-21 (10 pups/sex/dose) or for 11 consecutive days to young adults (approximately 5 weeks of age) beginning on approximately PND 42. Animals were sacrificed at the time of peak inhibition of cholinesterase following the final dose.

No treatment-related effect was observed on mortality, brain weights, or gross pathology.

In the Phase I study, systemic toxicity was observed in the 5 mg/kg/day dams as follows: (i) decreased mean gravid body weight on PND 20; (ii) decreased body weight gains during GD 18-20 and 6-20; and (iii) decreased absolute and relative food consumption on GD 18-20. Additionally, the following CNS findings were noted in the treated group but not in the controls: repetitive jaw movement, tremors, piloerection, and prostrate posture. Other clinical signs observed in the treated group but not the control included: wet yellow material in the urogenital area; unkempt appearance; dried red material on right forelimb, around the nose, on the left ear, and around both eyes; and decreased defecation and urination. Decreased ($p<=0.01$) cholinesterase was observed in the plasma, red blood cells, and brain of the 0.7 and 5 mg/kg/day dams (decr 44-99%), except in the 0.7 mg/kg/day brains (decr 5%; $p<=0.01$). Decreased ($p<=0.05$) cholinesterase was also observed in the plasma, packed RBCs, and brain of the 5 mg/kg/day fetuses (decr 22-33%). Thus, the fetuses were less sensitive than the dams.

In Phase II in the 5 mg/kg groups at PND 11 and 21, the maximum observed decreases in cholinesterase levels (hours post-dose) and the decrease at 24 hours post-dose were as follows: plasma (decr 58-64% at 4 hours; decr 40-42% at 24 hours), RBC (33-55% at 2 hours; decr 9-40% at 24 hours), and brain (decr 14-28% at 4 hours; decr 14% to incr 2% at 24 hours). The greatest magnitude of inhibition occurred in the plasma, and maximum inhibition was 4 hours post-dose in both plasma and brain. Consequently, the time of peak effect in pups can be regarded as 4 hours.

In Phase III, there was no clear effect of age on the inhibition of plasma or RBC cholinesterase. In the 5 mg/kg group, cholinesterase levels were decreased at all ages tested by 42-67% in plasma, 18-47% in red blood cells, and 14-16% in brain (no decrease in young adults). These decreases were significant ($p<=0.05$) in plasma in both sexes, in the brain in both sexes (except males at PND 21), and in female RBC (except at PND 21).

In Phase IV, repeated dosing resulted qualitatively in effects similar to a single dose. Decreased ($p<=0.05$) plasma, red blood cell, and brain cholinesterase levels were observed in the 5 mg/kg/day group (decr 22-99%) at PND 21 and in young adults. However, inhibition ($p<=0.05$) of cholinesterase in red blood cells was particularly severe following repeated exposure (decr 87-99% repeated vs decr 23-47% single), and brain cholinesterase levels were also decreased more following repeated exposure (decr 22-65% repeated vs decr 0-16% single).

This study is classified **acceptable/non-guideline**.

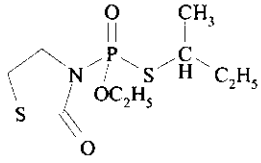
COMPLIANCE - Signed and dated GLP Compliance, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Fosthiazate technical

Description: Translucent pale yellow liquid
Lot No.: 21007013
Purity: 94.7% a.i.
Stability of compound: Stable in deionized water for up to 10 days while refrigerated
CAS #: 98886-44-3
Structure:



2. Vehicle – Deionized Water

3. Test animals

Species: Rat
Strain: Crl:CD®(SD)
Age and group mean weight at initiation of treatment:
Phase I: Young adults were 42-46 days of age and 258-260 g adult females
Phase II: PND 11 (24.1-25.7 g) or PND 21 (49.0-52.1 g)
Phase III: PND 11 (24.4-26.2 g) or PND 21 (49.4-55.0 g) or PND 42 (197-198 g males and 160-165 g females)
Phase IV: PND 11 (24.3-26.0 g) or PND 42 (202-205 g males and 160-163 g females)
Source: Charles River Laboratories, Inc., Raleigh, NC
Housing: Individually in suspended, stainless steel wire mesh cages in general; breeder females were individually housed in plastic maternity cages with nesting material
Diet: Certified Rodent LabDiet® 5002 (PMI Nutrition International, LLC, St. Louis, MO) , *ad libitum*
Water: Reverse osmosis-purified water, *ad libitum*
Environmental conditions
Temperature: 21-22°C
Humidity: 38-45%
Air changes: Approximately 10/h
Photoperiod: 12 h light/12 h dark
Acclimation period: ≥10 days

B. STUDY DESIGN

1. **In life dates** - Start: April 25, 2005 End: July 14, 2005

2. **Animal assignment** - The rats were randomly assigned, stratified by weight (on GD 0 for Phase I, on PND 7 or 17 for pups in Phases II-IV, and on weight at the end of acclimation for young adults in Phases III-IV), to the test groups shown in Table 1.

Table 1. Study design [a]

Phase I: Gestational Exposure		
Dose to animal (mg/kg/day)	**Number of dams and litters** [b, c]	**Time dosed**
0	12	Once daily during gestation day (GD) 6-20
0.1		
0.7		
5		

Phase II: Time of Peak Effect			
Dose to animal (mg/kg/day)	**Number of rats/sex** [d]		**Time dosed**
	PND 11	**PND 21**	
0	60	30	A single dose on postnatal day (PND) 11 or PND 21
0.1			
0.7			
5			

Phase III: Acute Exposure				
Dose to animal (mg/kg/day)	**Number of rats/sex**			**Time dosed**
	PND 11 [e, f]	**PND 21** [e, f]	**Young adult**	
0	20	10	10	A single dose on PND 11, 21, or 44±2
0.1				
0.7				
5				

Phase IV: Repeated Exposure			
Dose to animal (mg/kg/day)	**Number of rats/sex**		**Time dosed**
	PND 21 [e, g]	**Young adult**	
0	10	10	Once daily during PND 11-21 or for 11 consecutive days beginning on PND 43-46
0.1			
0.7			
5			

a Data were obtained from pages 33-34 of MIRD 46744902. All doses were administered by gavage in a volume of 5 mL/kg, using deionized water as a vehicle.
b The litter was considered the experimental unit.
c 12 Dams/group were treated. All females/group that met criteria for pregnancy and live litter size were used for assessment.
d Animals were obtained from 105 litters.
e Age of animal at euthanasia
f Animals were obtained from 41 litters.
g Animals were obtained from 14 litters.

3. **Breeding procedures** – Females of approximately 12 weeks of age were paired with mature males (same strain and source) until positive evidence of mating was confirmed by the

presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage. Each mating pair was examined daily. The day on which evidence of mating was identified was termed GD 0.

4. **Study design and purpose** – Box diagrams detailing the study design for each phase are included as an Appendix to this DER. The overall objective of this study was to determine the potential of fosthiazate to induce effects on maternal, fetal, neonatal, and young adult cholinesterase activity in the blood components and brain after maternal gestational exposure or after acute or short-term repeated exposure to rats of various ages. These effects were evaluated in a series of 4 phases, where doses of 0, 0.1, 0.7 or 5 mg/kg/day were administered by gavage in a volume of 5 mL/kg. Blood and brains were collected from animals at sacrifice. Samples from viable fetuses were pooled by litter, and samples from pups at PND 11 were pooled 2/sex/litter. Plasma, red blood cell (RBC), and whole brain cholinesterase assays were performed. Briefly, the study design for each phase was as follows. In Phase I, maternal gestational exposure was evaluated by dosing 12 bred females/dose group once daily from GD 6 through 20. Dams were sacrificed on GD 20. In Phase II, time of peak effect was determined by administering a single dose to 60 pups/sex/dose on PND 11 and 30 pups/sex/dose on PND 21. Pups were sacrificed (10 PND 11 or 5 PND 21 pups/sex/dose) at 10 minutes, 30 minutes, and 1, 2, 4, and 24 hours post-treatment. In Phase III, acute exposure was evaluated by administering a single dose to 20 pups/sex/dose on PND 11, 10 pups/sex/dose on PND 21, and 10 young adult rats/sex/dose on approximately PND 42. Animals were euthanized at the time of peak inhibition of cholinesterase. In Phase 4, the effect of repeated exposure was evaluated by administering a single daily dose to pups during PND 11-21 (10 pups/sex/dose) or to young adults (approximately 5 weeks of age) for 11 consecutive days beginning on approximately PND 42. Animals were sacrificed at the time of peak inhibition of cholinesterase following the final dose.

5. **Dose-selection rationale** – The Sponsor stated that doses were selected based on the results of previous 28-day feeding and 90-day neurotoxicity studies. Further information was not provided.

6. **Treatment preparation and analysis** – Dose formulations were prepared approximately weekly, divided into aliquots for daily dispensation, and stored refrigerated. A stock suspension was prepared first by suspending the test article in deionized water. Aliquots of the stock suspension were diluted with deionized water to achieve the desired concentrations in each dose formulation. The stock suspension and formulations were stirred continuously throughout the preparation, sampling, and dose administration procedures.

   Prior to the initiation of treatment, duplicate samples were collected from the top, middle, and bottom strata of each formulation to confirm homogeneity. Additional samples were stored refrigerated for 5 or 10 days to test stability. Duplicate samples of each dose formulation were analyzed for concentration.

Results:
Homogeneity (range as % CV): 0.5-1.6%

Stability (% of time 0): 98-105%

Concentration (range as % of nominal): 84.9-103%

Concentration (mg/mL)	Range (% of nominal)
0.02	86.0-103
0.14	84.9-96.0
1	94.2-99.3

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

7. **Statistics** – Analyses were conducted using two-tailed tests, $p \leq 0.05$ and 0.01. Where applicable, the litter was used as the experimental unit. Mean maternal body weights, body weight changes and food consumption (Phase I), pup and young adult body weights (Phases II-IV) and body weight changes (Phase IV), and plasma, RBC and whole brain cholinesterase values (Phases I-IV, at each age and for each sex separately, as appropriate) were subjected to a parametric one-way analysis of variance (ANOVA) followed by Dunnett's test if ANOVA was significant. Provided that the assumptions of homogeneity of variance and normal distribution of the data were verified before proceeding with parametric analysis, the reviewers considered these analyses appropriate.

C. METHODS

1. **Observations** – In the Phase I study, all maternal rats were observed twice daily for moribundity and mortality. Detailed clinical examinations were performed twice weekly for each maternal female from GD 0 until necropsy. All maternal animals were also observed for signs of toxicity approximately 3 hours following dose administration.

   Litters from Phases II-IV were examined daily for moribundity and mortality. Detailed clinical examinations were performed on PND 7, 11, 13, 15, 17, 19, and/or 21 as appropriate for each study phase. Pups were also observed for signs of toxicity approximately 30 minutes and 1, 2, 4, and 24 hours post-dose (Phase II) and approximately 4 hours post-dose (Phases III and IV). Pups were sexed on PND 7, 11, and/or 21 as appropriate.

   All young adult rats (Phases III and IV) were observed twice daily for moribundity and mortality. Detailed clinical examinations were performed twice weekly from animal receipt until necropsy. Each rat was also observed for signs of toxicity approximately 3 hours post-dose.

2. **Breeding pups for Phases II-IV** – For Phases II-IV, breeder females were allowed to deliver naturally and rear their young to the scheduled day of euthanasia (PND 11 or 21). During the

period of expected parturition, the breeder females were observed twice daily for initiation and completion of parturition and for signs of dystocia. Beginning on PND 0, pups were sexed and examined for gross malformations, and the numbers of stillborn and live pups were recorded. The litters were randomly culled on PND 4 to 8 pups/litter with 4 rats/sex when possible. Culled pups were weighed, euthanized, and discarded. Breeder females were euthanized and discarded after all data from their litters had been collected. Pretest data for breeder females, offspring, and young adults were recorded, but were not reported.

3. **Body weight** – All rats were weighed prior to group assignment.

   In the Phase I study, individual maternal body weights were measured on GD 0, 6, 9, 12, 15, 18, and 20. Group mean body weight changes were reported for each corresponding interval and also for GD 6-20.

   Pups from Phases II-III were weighed individually on PND 7 and 11 (pups dosed on PND 11) or PND 17 and 21 (pups dosed on PND 21). Phase IV pups were weighed individually on PND 7, 11, 13, 15, 17, 19, and 21; and group mean body weight changes were reported for each corresponding interval.

   Young adult rats were weighed twice weekly, beginning on the first day of dose administration and continuing until necropsy. Corresponding body weight changes were also calculated for each body weight interval in the Phase IV study.

4. **Food consumption** – Food consumption was reported as g/animal/day and g/kg/day for corresponding body weight change intervals in the Phase I study. Individual maternal food consumption was recorded on GD 0, 6, 9, 12, 15, 18, and 20.

5. **Cholinesterase assays** – Samples were generally kept at approximately 4°C from the time collected, through processing, and until analysis. Plasma, RBC, and brain cholinesterase activities were determined using an assay based on a modification of the Ellman reaction. Whole brains were weighed, diluted 1:10 with 1% Triton X-100 buffer, and homogenized. The homogenate was centrifuged, and the supernatant was analyzed. Blood samples were centrifuged to obtain plasma, and the red blood cells were diluted 1:20 with 1% Triton X-100 buffer. The diluted red blood cells were mixed and analyzed. Cholinesterase determinations were made on all animals from each phase when possible.

6. **Sacrifice and pathology** – It was stated that other maternal tissues/organ of the Phase I dams were preserved in 10% neutral buffered formalin but were examined only as deemed necessary by gross findings. Except where specified otherwise, blood was collected from each animal, and the brain was excised and weighed. In Phase I, maternal females were euthanized on GD 20 by carbon dioxide inhalation. Uteri with no macroscopic evidence of implantation were opened and placed in 10% ammonium sulfide solution for determination of pregnancy status. All pups in Phase II-IV were killed by decapitation at PND 11 or by carbon dioxide inhalation at PND 21 followed by exsanguination. The carcass of each pup was discarded without further examination. All Phase III and IV young adults were killed by

carbon dioxide inhalation followed by exsanguination, and the carcasses were discarded without further examination.

II. RESULTS

A. OBSERVATIONS

1. **Mortality** - No treatment-related fatalities occurred during the study. One 0.1 mg/kg/day female was nongravid in the Phase I study. One young adult female control from the Phase IV study was found dead and findings, such as distended bladder with multiple calculi, indicated disease.

2. **Clinical signs of toxicity** – No treatment related clinical findings were reported, except in the 5 mg/kg/day females of Phase I on GD 19 and 20. CNS findings (vs 0/12 controls) included repetitive jaw movement, tremors, and prostrate posture observed in 2-4/12 rats on 2-5 occasions; piloerection was observed once in one female. The following findings were also observed in 2-4/12 rats on 2-4 occasions (vs 0 controls): (i) wet yellow material in the urogenital area; (ii) unkempt appearance; (iii) dried red material on right forelimb, around the nose, on the left ear, and around both eyes; and (iv) decreased defecation and urination.

B. BODY WEIGHT AND WEIGHT GAIN – During gestation of the Phase I dams, decreased ($p \leq 0.05$) mean gravid body weight (Table 2) was noted at 5 mg/kg/day on PND 20 (↓7%). Decreased ($p \leq 0.01$) body weight gains were observed at 5 mg/kg/day during GD 18-20 (↓68%) and 6-20 (↓24%).

In Phase IV, there was a 17% decrease ($p \leq 0.05$) in body weight gain in the 5 mg/kg/day males during PND 11-21; however, only an 8% decrease (not statistically significant [NS]) was observed in their body weights at PND 21. Furthermore, the decrease in body weight gains in these rats from PND 7-21 (calculated by reviewers) was only 10%. Other body weight and body weight gains in Phases II-IV treated groups were similar to controls.

Table 2. Mean (∀SD) gravid body weights and cumulative body weight gains (g) at selected intervals in Phase I rats treated with fosthiazate by gavage. [a]

Day(s)	Dose (mg/kg/day)			
	0	0.1	0.7	5
0	259±17	260±11	260±11	258±14
6	293±18	295±12	297±12	292±14
18	377±17	379±16	382±18	371±20
20	411±18	413±23	416±20	381±38* (↓7)
18-20	34±5	34±9	34±6	11±32** (↓68)
6-20	118±11	118±14	119±11	90±33** (↓24)

a Data (n=11-12) were obtained from pages 101-104 of MRID 46744902. Percent difference from controls, calculated by reviewers, is included in parentheses.

* Significantly different from controls; p#0.05

** Significantly different from controls; p#0.01

C. **FOOD CONSUMPTION** – Decreases ($p \leq 0.05$) in absolute and relative food consumption were noted in the 5 mg/kg/day females from Phase I on GD 18-20 (↓24% each). Other differences ($p \leq 0.05$) were unrelated to dose.

E. CHOLINESTERASE ASSAYS

1. **Phase I** – Decreased ($p \leq 0.01$) cholinesterase was observed in the plasma, red blood cells, and brain of the 0.7 and 5 mg/kg/day dams (↓44-99%), except in the 0.7 mg/kg/day brains (↓5%; $p \leq 0.01$; Table 3). Decreased ($p \leq 0.05$) cholinesterase was also observed in the plasma, packed RBCs, and brain of the 5 mg/kg/day fetuses (↓22-33%). Other values were similar to controls.

Table 3. Mean (∀SD) cholinesterase (U/L) in rats treated with fosthiazate by gavage. [a]

Parameter		Dose (mg/kg/day)			
		0	0.1	0.7	5
Plasma	Dams	3147±410	3008±467	852±139** (↓73)	142±25** (↓95)
	Fetuses	491±41	506±39	495±51	329±59** (↓33)
Red blood cells	Dams	3931±1474	3831±757	2193±712** (↓44)	20 [b] ±0** (↓99)
	Fetuses	2644±644	3283±992	2893±738	1851±593* (↓30)
Brain	Dams	49446±2190	48974±1364	47135±1510** (↓5)	5152±1719** (↓90)
	Fetuses	6612±680	6328±476	6251±650	5182±684** (↓22)

a Data (n=11-12) were obtained from pages 111-112 of MRID 46744902. Percent difference from controls, calculated by reviewers, is included in parentheses.

b For statistical analyses, the lower limit for quantitation (20 U/L) was used for values that were under instrument range.

* Significantly different from controls; p#0.05

** Significantly different from controls; p#0.01

2. **Phase II** – In the 5 mg/kg groups at PND 11 and 21, the plasma cholinesterase levels fell over the course of 4 hours post-dose (↓58-64% at 4 hours) and slowly recovered thereafter (↓40-42% at 24 hours post-dose; Table 4a). The RBC cholinesterase levels fell over the course of 2 hours post-dose (↓33-55% at 2 hours) and slowly recovered thereafter (↓9-40% at 24 hours post-dose; Table 4b). The brain cholinesterase levels fell over the course of 4 hours post-dose (↓14-28%) and slowly recovered thereafter (↓14-↑2 at 24 hours post-dose; Table 4c), except that levels remained decreased by 14% in the PND 11 males.

   Values in the 0.1 and 0.7 mg/kg/day groups were generally similar to controls and no clear trends were observed in cholinesterase levels over time. The following decreases ($p \leq 0.05$) in cholinesterase levels were considered incidental and/or transient: red blood cell cholinesterase at 10 minutes post-dose in the 0.1 mg/kg/day females (↓38%) at PND 11; and plasma cholinesterase at 4 hours post-dose in the 0.1 and 0.7 mg/kg/day females (↓16-18%) at PND 21.

Table 4a. Mean (±SD) plasma cholinesterase (U/L) in rats treated with fosthiazate by gavage [a]				
Time point	**0 mg/kg males**	**5 mg/kg males**	**0 mg/kg females**	**5 mg/kg females**
PND 11				
10 minutes	1546±93	1414±87 (↓9)	1532±73	1396±169 (↓9)
4 hours	1589±80	603±177** (↓62)	1565±159	650±111** (↓58)
24 hours	1490±61	861±114** (↓42)	1502±142	875±77** (↓42)
PND 21				
10 minutes	1058±70	1052±89	1182±174	1052±177 (↓11)
4 hours	1148±229	451±80** (↓61)	1154±38	418±82** (↓64)
24 hours	1061±117	613±56** (↓42)	1054±67	634±113** (↓40)

a Data (n=4-5) were obtained from pages 130-145 of MRID 46744902. Percent difference from controls, calculated by reviewers, is included in parentheses.

** Significantly different from controls; p#0.01

Table 4b. Mean (±SD) red blood cell cholinesterase (U/L) in rats treated with fosthiazate by gavage [a]				
Time point	**0 mg/kg/day males**	**5 mg/kg/day males**	**0 mg/kg/day females**	**5 mg/kg/day females**
PND 11				
10 minutes	6030±1842	5993±494.2	9128±3364	6126±818* (↓33)
2 hours	6066±1190	3730±265** (↓39)	7194±1715	4219±1363 (↓41)
4 hours	5752±1320	3557±912* (↓38)	5832±2070	3935±1061 (↓33)
24 hours	5910±376	3955±1596 (↓33)	6476±2265	4077±1919 (↓37)
PND 21				
10 minutes	5748±1924	6499±2322 (↑13)	5171±1456	5188±847
2 hours	5204±1930	3485±969 (↓33)	7530±3026	3418±856** (↓55)
4 hours	6682±4036	4522±2008 (↓32)	5467±694	4732±1144 (↓13)
24 hours	4818±410	4366±1209 (↓9)	6082±1878	3639±1018 (↓40)

a Data (n=4-5) were obtained from pages 130-145 of MRID 46744902. Percent difference from controls, calculated by reviewers, is included in parentheses.

* Significantly different from controls; p#0.05

** Significantly different from controls; p#0.01

Table 4c. Mean (±SD) brain cholinesterase (U/L) in rats treated with fosthiazate by gavage[a]				
Time point	**0 mg/kg/day males**	**5 mg/kg/day males**	**0 mg/kg/day females**	**5 mg/kg/day females**
PND 11				
10 minutes	24317±1568	23705±435	24524±1647	24258±1294
4 hours	27513±9300	23669±9733 (↓14)	27383±8142	19624±1774 (↓28)
24 hours	26000±380	22425±2432* (↓14)	25985±794	26420±9359
PND 21				
10 minutes	38224±886	36429±4702 (↓5)	38943±1005	39650±4090
4 hours	38816±1311	31778±2018** (↓18)	39670±1055	31688±942** (↓20)
24 hours	39477±2589	34024±1341** (↓14)	41133±1494	33609±2025** (↓18)

a Data (n=4-5) were obtained from pages 130-145 of MRID 46744902. Percent difference from controls, calculated by reviewers, is included in parentheses.

* Significantly different from controls; p#0.05

** Significantly different from controls; p#0.01

3. **Phase III** – There was no clear effect of age on the inhibition of plasma or RBC cholinesterase (Table 5). In the 5 mg/kg group, cholinesterase levels were decreased at all ages tested by 42-67% in plasma, 18-47% in red blood cells, and 14-16% in brain (no decrease in young adults). These decreases were significant ($p \leq 0.05$) in plasma in both sexes, in the brain of both sexes (except males at PND 21), and in female red blood cells (except at PND 21). Cholinesterase levels in the 0.1 and 0.7 mg/kg groups were generally similar to the controls. Increased ($p \leq 0.05$) plasma cholinesterase was noted in the 0.1 mg/kg females at PND 11; however, the value was within the historical control range.

Table 5. Mean (±SD) cholinesterase (U/L) in rats treated with fosthiazate by gavage

Age[b]	0 mg/kg males	5 mg/kg males	0 mg/kg females	5 mg/kg females
Plasma				
PND 11	1536±151	633±62** (↓59)	1482±64	641±60** (↓57)
PND 21	1094±149	432±46** (↓61)	1123±157	422±65** (↓62)
Young adult	888±63	519±99** (↓42)	1423±399	472±96** (↓67)
Red blood cell				
PND 11	4701±1008	3866±3075 (↓18)	5558±2940	3110±832* (↓44)
PND 21	4929±2165	3085±1264 (↓37)	4058±1576	2777±879 (↓32)
Young adult	3351±636	2582±478 (↓23)	4469±2965	2387±475* (↓47)
Brain				
PND 11	24360±1177	20730±1386** (↓15)	24800±933	21286±1277** (↓14)
PND 21	36661±1971	31019±1088 (↓15)	36797±2081	30784±1843** (↓16)
Young adult	47611±2804	47783±2016	48377±2815	47798±3075

a Data (n=10-11) were obtained from pages 170-175 of MRID 46744902. Percent difference from controls, calculated by reviewers, is included in parentheses.

b Analyses were conducted 4 hours post dose in rats at PND 11 and PND 21, and at 3 hours post-dose in young adult rats.

* Significantly different from controls; p#0.05

** Significantly different from controls; p#0.01

4. **Phase IV** – Repeated dosing resulted qualitatively in effects similar to a single dose. Decreased ($p \leq 0.05$) plasma, red blood cell, and brain cholinesterase levels were observed in the 5 mg/kg/day group (↓22-99%) at PND 21 and in young adults (Tables 6 a-b). However, inhibition ($p \leq 0.05$) of cholinesterase in red blood cells was particularly severe following repeated exposure (↓87-99% repeated vs 23-47% single), and brain cholinesterase levels were also decreased more following repeated exposure (↓22-65% repeated vs ↓0-16% single). Values in the other dose groups were within the historical control range.

Table 6a. Mean (∀SD) cholinesterase (U/L) in rats at PND 21 treated with fosthiazate by gavage. [a]					
Parameter	**0 mg/kg/day**	**0.1 mg/kg/day**	**0.7 mg/kg/day**	**5 mg/kg/day**	**Historical control range [b]**
Males					
Plasma	1085±110	1055±100	849±137** (↓22)	439±180** (↓60)	729-1791
Red blood cells	8725±3387	7522±2623 (↓14)	9528±10383	1107±1942* (↓87)	3282-13376
Brain	39177±1109	40093±2031	37563±825	19692±11644** (↓50)	37616-47209
Females					
Plasma	1019±254	1061±151	832±193 (↓18)	346±104** (↓66)	747-1623
Red blood cells	7595±3821	7661±4412	6523±1382 (↓14)	508±510** (↓93)	3292-10142
Brain	37284±8313	40936±1082 (↑10)	34444±6957 (↓8)	13125±1810** (↓65)	39240-53198

a Data (n=10) were obtained from pages 67 and 201-204 of MRID 46744902. Percent difference from controls, calculated by reviewers, is included in parentheses.
b Details, such as when these tests were performed, were not provided.
** Significantly different from controls; p#0.01

Table 6b. Mean (∀SD) cholinesterase (U/L) in young adult rats treated with fosthiazate by gavage. [a]					
Parameter	**0 mg/kg/day**	**0.1 mg/kg/day**	**0.7 mg/kg/day**	**5 mg/kg/day**	**Historical control range [b]**
Males					
Plasma	773±156	828±77 (↑7)	756±152	325±38** (↓58)	674-1261
Red blood cells	2941±793	2915±950	2821±644	392±306** (↓87)	1902-5588
Brain	49176±1372	51198±1784	49595±907	38237±4347** (↓22)	44255-56124
Females					
Plasma	1991±433	2006±754	1152±170** (↓42)	253±27** (↓87)	852-2289
Red blood cells	2548±847	3178±1249 (↑25)	2196±298 (↓14)	20±0** [c] (↓99)	1998-10410
Brain	50227±2570	50726±1031	48882±1781	21476±4545** (↓57)	45616-62316

a Data (n=9-10) were obtained from pages 68 and 201-204 of MRID 46744902. Percent difference from controls, calculated by reviewers, is included in parentheses.
b Details, such as when these tests were performed, were not provided.
c For statistical analyses, the lower limit for quantitation (20 U/L) was used for values that were under instrument range.
** Significantly different from controls; p#0.01

F. SACRIFICE AND PATHOLOGY

1. **Brain weight** - No treatment-related effects were observed on brain weights.

2. **Gross pathology** - No treatment-related effects were noted at necropsy. One 0.1 mg/kg/day female was nongravid in the Phase I study. The mean numbers of viable fetuses were similar in all groups.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS= CONCLUSIONS – Directly exposed maternal females were more sensitive to cholinesterase inhibition than fetuses exposed *in utero*. RBC cholinesterase inhibition was severe following repeated exposure. Inhibition in the 5 mg/kg/day maternal animals corresponded to functional deficits, including tremor and repetitive jaw movement. Plasma cholinesterase was determined to be the most sensitive compartment. The NOAEL was 0.1 mg/kg/day, based on cholinesterase inhibition in the: (i) plasma of dams; (ii) plasma of juveniles and young adults following repeated exposure; and (iii) red blood cells of dams. The NOAEL was 0.7 mg/kg/day for fetuses (plasma, RBC, and brain), juveniles and young adults (single or repeated exposure; plasma, RBC, and brain, excluding plasma for repeated exposure).

B. REVIEWER COMMENTS - No treatment-related effect was observed on mortality, brain weights, or gross pathology.

In the 5 mg/kg/day females of Phase I, CNS findings (vs 0/12 controls) included repetitive jaw movement, tremors, and prostrate posture observed in 2-4/12 rats on 2-5 occasions; piloerection was observed once in one female. The following findings were also observed in 2-4/12 rats on 2-4 occasions (vs 0 controls): (i) wet yellow material in the urogenital area; (ii) unkempt appearance; (iii) dried red material on right forelimb, around the nose, on the left ear, and around both eyes; and (iv) decreased defecation and urination.

During gestation of the Phase I dams, systemic toxicity was noted. Decreased ($p \leq 0.05$) mean gravid body weight was noted at 5 mg/kg/day on PND 20 (↓7%). Decreased ($p \leq 0.01$) body weight gains were observed at 5 mg/kg/day during GD 18-20 (↓68%) and 6-20 (↓24%). Decreased ($p \leq 0.05$) absolute and relative food consumption was noted in the 5 mg/kg/day females from Phase I on GD 18-20 (↓24% each).

In Phase I, decreased ($p \leq 0.01$) cholinesterase was observed in the plasma, red blood cells, and brain of the 0.7 and 5 mg/kg/day dams (↓44-99%), except in the 0.7 mg/kg/day brains (↓5%; $p \leq 0.01$). Decreased ($p \leq 0.05$) cholinesterase was also observed in the plasma, packed RBCs, and brain of the 5 mg/kg/day fetuses (↓22-33%). Thus, the fetuses were less sensitive than the dams.

In Phase II in the 5 mg/kg groups at PND 11 and 21, the plasma cholinesterase levels fell over the course of 4 hours post-dose (↓58-64% at 4 hours) and slowly recovered thereafter (↓40-42% at 24 hours post-dose). The RBC cholinesterase levels fell over the course of 2 hours post-dose (↓33-55% at 2 hours) and slowly recovered thereafter (↓9-40% at 24 hours post-dose). The brain cholinesterase levels fell over the course of 4 hours post-dose (↓14-28%) and slowly recovered thereafter (↓14-↑2 at 24 hours post-dose), except that levels remained decreased by 14% in the PND 11 males. The greatest magnitude of inhibition occurred in the plasma (↓58-64% at 4 hours post-dose) compared to the RBCs (↓33-55% at 2 hours post-dose) and brain (↓14-28% at 4 hours post-dose). Consequently, the time of peak effect in pups can be regarded as 4 hours through experimental observation. Mathematical procedures were not used to determine a more precise time of peak effect.

In Phase III, there was no clear effect of age on the inhibition of plasma or RBC cholinesterase. In the 5 mg/kg group, cholinesterase levels were decreased at all ages tested by 42-67% in plasma, 18-47% in red blood cells, and 14-16% in brain (no decrease in young adults). These decreases were significant ($p \leq 0.05$) in plasma in both sexes, in the brain in both sexes (except males at PND 21), and in female red blood cells (except at PND 21).

In Phase IV, repeated dosing resulted qualitatively in effects similar to a single dose. Decreased ($p \leq 0.05$) plasma, red blood cell, and brain cholinesterase levels were observed in the 5 mg/kg/day group (↓22-99%) at PND 21 and in young adults. However, inhibition ($p \leq 0.05$) of cholinesterase in red blood cells was particularly severe following repeated exposure (↓87-99% repeated vs ↓23-47% single), and brain cholinesterase levels were also decreased more following repeated exposure (↓22-65% repeated vs ↓0-16% single).

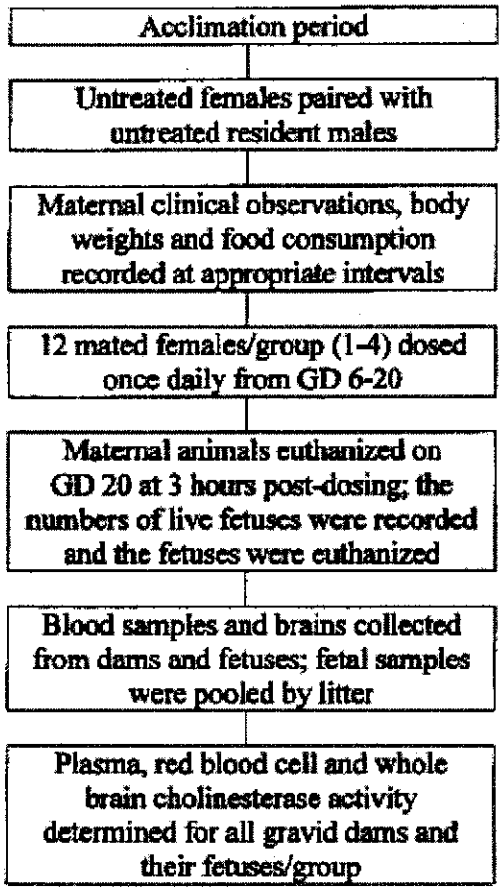
This study is classified **acceptable/non-guideline**.

APPENDIX

The following pages are from the study report pages 28-31.

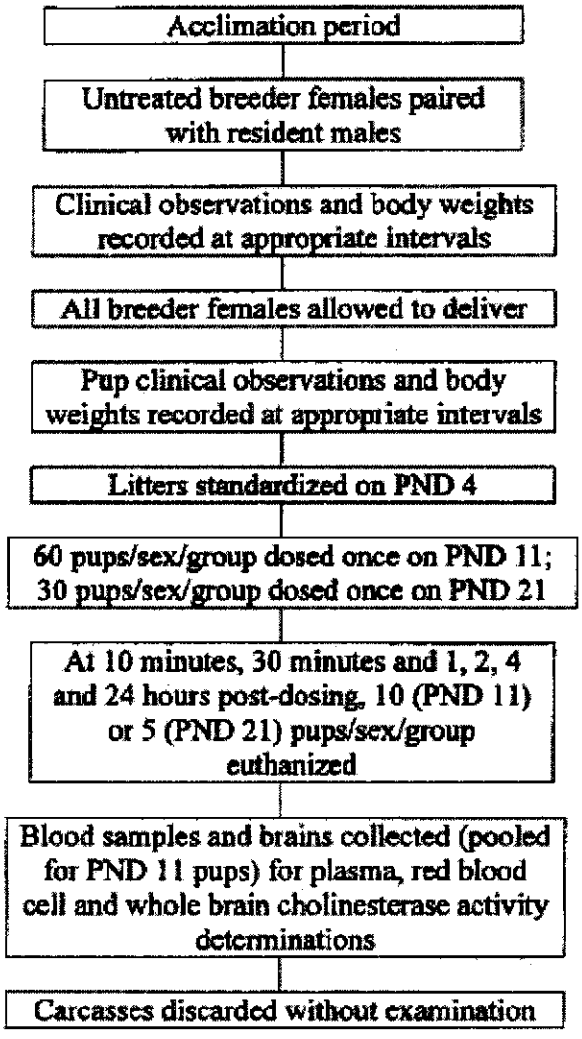
3. STUDY DESIGN

3.1. PHASE I (GESTATIONAL EXPOSURE)



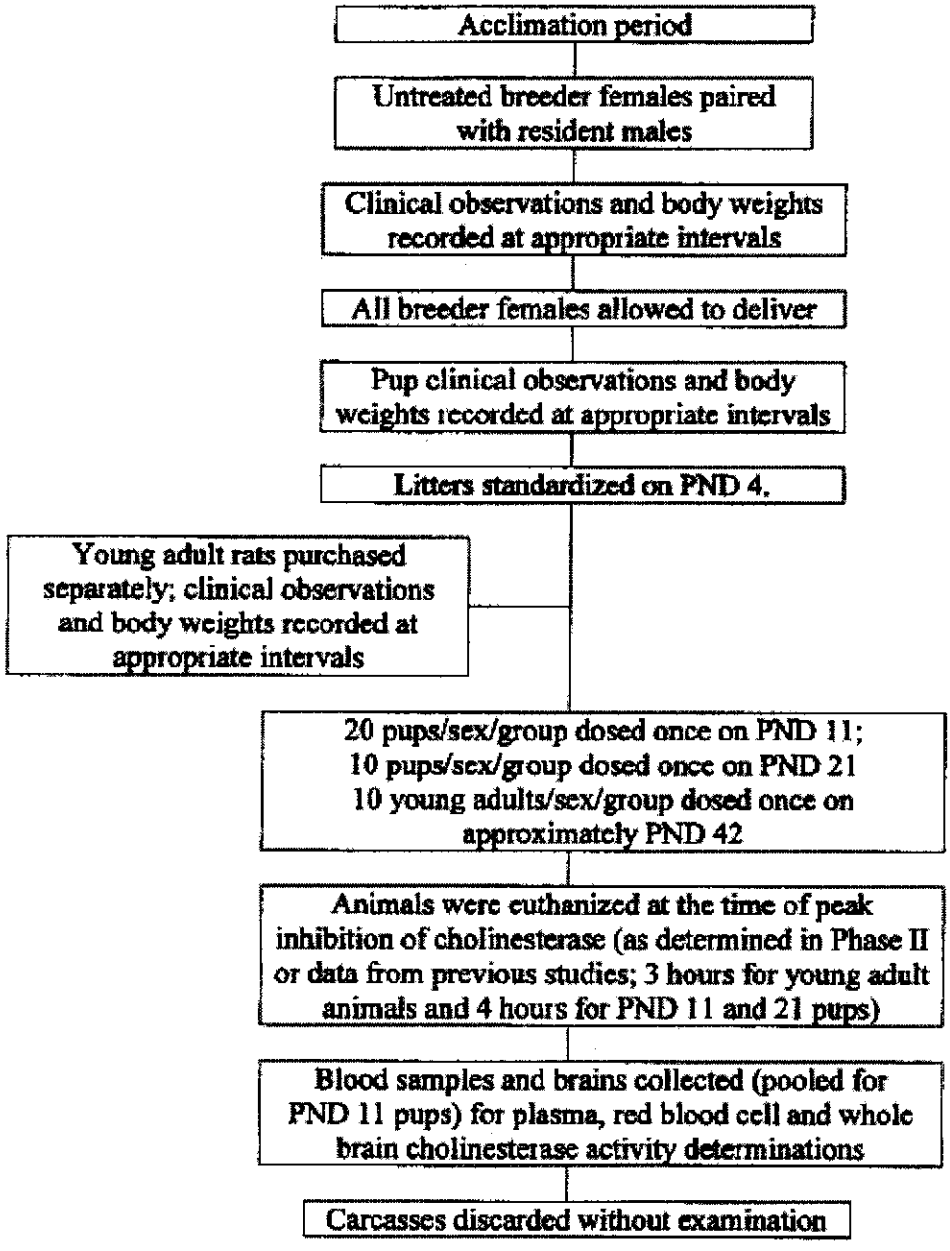
STUDY DESIGN (CONTINUED)

3.2. PHASE II (DETERMINATION TIME OF PEAK EFFECT)



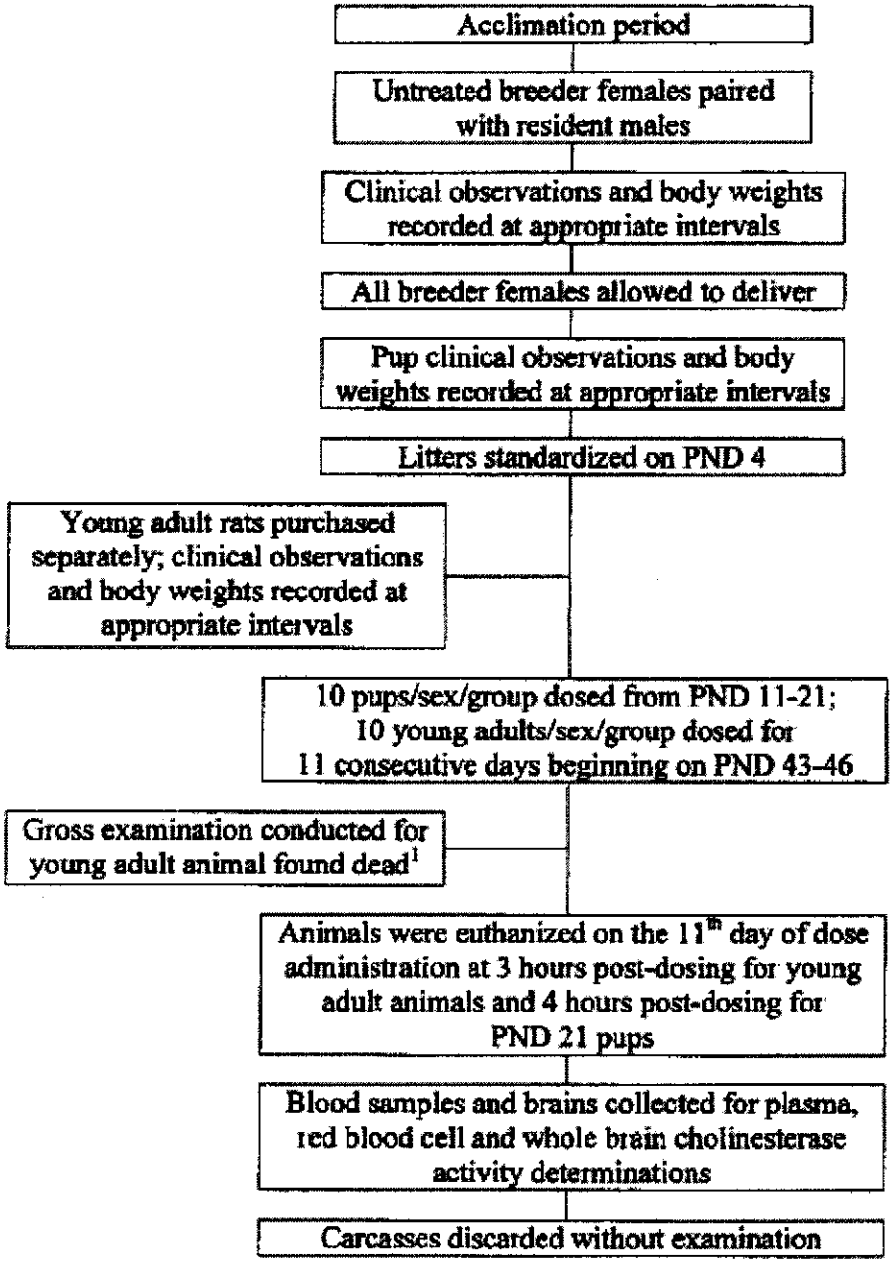
STUDY DESIGN (CONTINUED)

3.3. PHASE III (ACUTE EXPOSURE)



STUDY DESIGN (CONTINUED)

3.4. PHASE IV (REPEATED EXPOSURE)



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	2644	1851	
	49446	47135	
	49446	5152	
112	6612	5182	
130	1561	1197	
	1576	907	
	1544	645	
	1589	603	
131	1490	861	-42
	6066	3730	-39
132	5752	3557	-38
	24824	22184	-11
133	26000	22425	-14
134	1620	1098	-32
	1602	885	-45
	1468	682	-54
	1565	650	-58
135	1502	875	-42
	9128	5654	-38
	9128	6126	-33
137	24899	22810	-8
138	1019	672	-34
	1120	562	-50
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139	1061	613	-42
140	39048	35681	-9
141	41306	35806	-13
	38816	31778	-18
	39477	34024	-14
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173	1123	422	-62
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175	1423	472	-67
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	39177	19692	-50
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