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

OFFICE OF PREVENTION PESTICIDES AND TOXIC SUBSTANCES

Date: August 16, 2005
TXR: 0052747

MEMORANDUM

SUBJECT: **EPTC: Data Evaluation Record of a Developmental Neurotoxicity Study**

PC Code: 041401 Reregistration Case #: 0064
DP Barcode: D305793

FROM: Jess Rowland.
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THRU: Brenda May, Branch Senior Scientist
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I. Conclusions

Attached is the Data Evaluation Record for Developmental Neurotoxicity (DNT) Study with EPTC (MRID No. 46319101). This study is classified Acceptable and may be used for regulatory purposes. It, however, does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control data. This classification scheme is applicable only to the Developmental Neurotoxicity studies as determined by DNT Work Group.

II. Action Requested

Review/prepare a Data Evaluation Record for Developmental Neurotoxicity Study with EPTC. (MRID No. 46319101.

DATA EVALUATION RECORD

EPTC

Study Type (§83-6): Developmental Neurotoxicity Study in the Rat

Work Assignment No. 2-01-44; formerly 1-01-44 (MRID 46319101)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 041401
TXR#: 0052747

DP BARCODE: D305793
SUBMISSION NO.: None

TEST MATERIAL (PURITY): EPTC (98.1% a.i.; Batch #: FL021317)

SYNONYMS: S-Ethyl-N,N-dipropylthiocarbamate

CITATION: Lees, D (2004) EPTC: Developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID: CTL Study Number: RR0926, July 2, 2004. MRID 46319101. Unpublished.

SPONSOR: Gowan Company, PO Box 5569, Yuma, AZ 85366

EXECUTIVE SUMMARY - In a developmental neurotoxicity study (MRID 46319101) EPTC (98.1% a.i.; Batch #: FL021317) was administered in the diet to pregnant Wistar rats (30/dose) from gestation day (GD) 7 to lactation day (LD) 23 at nominal doses of 0, 100, 300, or 1000 ppm (equivalent to 0/0, 7.6/16.4, 21.9/47.9, and 67.2/157.3 mg/kg/day [gestation/lactation]). Dams were allowed to deliver naturally and were killed on LD 29. On postnatal day (PND) 5, litters were standardized to 8 pups/litter; the remaining offspring and dams were sacrificed and discarded without further examinations. Subsequently, 1 pup/litter/group (at least 10 pups/sex/dose when available) were allocated to subsets for FOB, motor activity, acoustic startle response, learning and memory evaluation, and neuropathological examination. Positive control data were not submitted with this study; however, summaries of positive control data previously submitted to the Agency were obtained and reviewed.

The maternal LOAEL is 1000 ppm (67.2 mg/kg/day) based on clinical signs (piloerection, hunched posture, sides pinched in); decreased body weight, body weight gain, and food consumption; and increased incidence of whole litter losses. The maternal NOAEL is 300 ppm (21.9 mg/kg/day).

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In the offspring, no treatment-related effects were noted in developmental landmarks, FOB (maternal or F_1), motor activity, auditory startle reflex habituation, learning and memory (watermaze), neuropathology, or brain morphology at any dose level. At the high dose (1000 ppm), the number of whole litter losses was significantly increased (6/28 treated vs 1/30 controls; Table 6a). When whole litter losses were included, the following differences were noted at 1000 ppm: (i) live birth index was slightly decreased (96.3 treated vs 99.7% controls); (ii) mean litter size (PND 5) was decreased ($p \leq 0.05$) by 19%; and (iii) survival (PND 1-5) was decreased ($p \leq 0.01$; 74.0% treated vs 91.9% controls). However, when whole litter losses were excluded, live birth index, mean litter size, and survival (PND 1-5) were comparable to controls. Survival (PND 1-5; excluding whole litter losses) was decreased at 300 ppm (89.4% treated vs 95.1% control); however, this finding was not dose-dependent. On PND 1, increased incidences of pups considered to be cold (all treatment groups) and pups displaying hypothermia (1000 ppm). On PND 1, pup body weights were decreases ($p < 0.01$) by 8-9% at 100 ppm group. Absolute brain weights of female pups were decreased (5%) on both PND 12 and 63 at the high dose only.

The offspring LOAEL is 100 ppm (to 7.6 mg/kg/day; LDT) based on dose-depended decreases in absolute brain weights in male pups on PND 63 at all dose levels. An offspring NOAEL was not established.

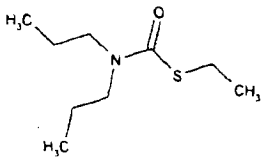
This study is classified **Acceptable** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: EPTC

Description:	Amber liquid
Batch #:	FL021317
Purity:	98.1% a.i.
Compound stability:	The test material was shown to be stable in the diet for up to 12 days at room temperature or for 42 days at -20°C.
CAS # of TGAI:	759-94-4
Structure:	

2. Vehicle - Diet

3. Test animals (P)

Species:	Rat	
Strain:	Wistar (Alpk:AP$_f$SD)	
Age at study initiation:	10-12 weeks	
Weight on arrival:	220-300 g (females)	
Source:	Rodent Breeding Unit, Alderley Park, Macclesfield, Cheshire, UK	
Housing:	Dams were kept individually in solid plastic cages. The F1 animals were kept with their parent dam until PND 29 when the litters were separated by sex and housed separately (4/sex/cage) in wire mesh cages. Environmental enrichment items were supplied.	
Diet:	CT1 diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum*, except during behavioral testing	
Water:	Tap water, *ad libitum*, except during behavioral testing	
Environmental conditions:	**Temperature:**	22±3 °C
	Humidity:	30-70%
	Air changes:	≥15/hr
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	6 days	

B. PROCEDURES AND STUDY DESIGN

1. In-life dates - Start: 03/04/2003 End: 10/03/2003

2. Study schedule - The maternal animals were mated and assigned to study. The P females were administered the test substance continuously in the diet from gestation day (GD) 7 until lactation day (LD) 23. On postnatal day (PND) 5, the litters containing >8 pups were randomly standardized to 8 pups/litter (with equal sexes where possible) to reduce the variability. Litters containing 7-8 pups with at least 3 males and 3 females were used for selection of the F_1 generation. All other litters and all P females without a litter were sacrificed, and were discarded without further examinations. F_1 pups remained on study for up to PND 63 (study termination).

3. **Mating procedure** - The animals were mated by the breeder, and the day of successful mating (verified by the presence of sperm in a vaginal smear) was designated as gestation day (GD) 1. Twenty pregnant females were supplied on each of 6 days for a total of 120 females on study.

4. **Animal assignment** - Time-mated females were randomly assigned to the test groups shown in Table 1.

Table 1. Study design [a]

Experimental Parameter	Dose (ppm)			
	0	100	300	1000
Dams				
# of maternal animals	30	30	30	30
FOB (GDs 10, 17 & LDs 2, 9)	30	30	30	30
Offspring				
FOB (PND 5, 12, 22, 36, 46, 61)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter
Motor activity (PND 14, 18, 22, 60)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter
Auditory startle habituation test (PND 23, 61)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter
Watermaze (PND 21, 24 and 59, 62)	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter
Brain weight and neuropathology [b] (PND 12) (PND 63)	1pup/litter 1pup/litter	1pup/litter 1pup/litter	1pup/litter 1pup/litter	1pup/litter 1pup/litter
Perfusion fixation, brain weight, and neuropathology (including morphometry)[b] (PND 63)	1pup/litter	1pup/litter	1pup/litter	1pup/litter

a Data were obtained from pages 18-19 and 21-25 of the Study Report.
b At each sacrifice time 1 pup/litter was taken to give at least 10 pups/sex/dose.

5. **Dose selection rationale** - The doses presented in Table 1 were selected based on the results of a developmental neurotoxicity range-finding study (CTL study # RR0931/F0). In this study, pregnant rats received EPTC in the diet at doses of 250, 500, or 1000 ppm from GD 7 through LD 23. It was stated that in the 1000 ppm dams reduced body weight was observed throughout gestation and lactation, and reduced food consumption was noted during lactation. Additionally at 1000 ppm, total litter weights were decreased as a result of reduced pup body weights. No treatment-related effects on dams or pups were observed at ≤500 ppm.

6. Dosage preparation, administration, and analysis - Test diets were prepared (20 kg batches) by mixing the appropriate amount of the test material with 1000 g of milled diet to form a premix. The premix was further diluted with diet (up tp 19 kg) to achieve the appropriate doses. The dams were supplied dietary admixtures beginning on GD 7 and continuing through LD 23. F_1 animals were not directly supplied with the test diets. Homogeneity (top, middle, bottom) was determined from samples of the 100 and 1000 ppm diets at the beginning of the study. Stability in the diet was determined for up to 12 days at room temperature or up to 42 days at -20°C using the same samples evaluated for homogeneity. Concentration was determined for each dietary formulation using samples collected prior to the start of the study and once during the study.

Results - Homogeneity (range as % nominal): 96.0-105.3%

Stability (range as % of initial):
After 12 days at room temperature: 88.9-90.0%
After 42 days at -20°C: 96.2-97.4%

Concentration (range as % of nominal):

Dose (ppm)	% of Nominal
100	100.5-102.6
300	100.7-107.3
1000	98.8-105.2

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

C. OBSERVATIONS

1. In-life observations

a. Maternal animals - The main study dams were checked twice daily (cage-side) for mortality and clinical signs of toxicity. Detailed physical examinations were recorded at the same time that body weights were recorded. Body weights were measured on arrival at CTL, on GDs 7 (prior to administration of test material), 15, and 22, on LDs 1, 5, 8, 12, 15, and 22, and at termination (LD 29). Food consumption was recorded at intervals throughout gestation (GD 1-22) and lactation (LD 1-23), and calculated as g/rat/day.

The dams were subjected to a modified functional observation battery (FOB) outside of the home cage on GDs 10 and 17, and on LDs 2 and 9. The functional observations included, but were not limited to, the following:

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Lacrimation and salivation 2) Piloerection 3) Urination and defecation 4) Ptosis 5) Exophthalmos 6) Pupillary function
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions, and general signs of toxicity (thin, altered muscle tone, dehydrated, or altered fur appearance).

b. Offspring

1) Litter observations - Each litter was examined as soon as possible after completion of parturition (Day 1), and always within 24 hours. On PND 1 and 5, the sex, weight, clinical condition, and number of pups were determined. On PND 5, the litters containing >8 pups were randomly standardized to 8 pups/litter (with equal sexes where possible) to reduce the variability. Litters with 7-8 pups and at least 3 males and 3 females were used for F_1 evaluations; excess pups were sacrificed and discarded. The F_1 pups were evaluated for mortality and morbidity daily. Clinical observations were recorded daily throughout the study. From PND 5, detailed clinical observations were recorded at the same time the rats were weighed. Body weights were recorded on PNDs 1, 5 (precull and post-cull), 12, 18, and 22, and then weekly thereafter until sacrifice (PND 63). On PND 22 all pups were weaned. Post-weaning food consumption was not reported.

2) Developmental landmarks - Beginning on PND 41, male offspring were examined daily for preputial separation. Beginning on PND 29, female offspring were examined daily for vaginal patency. Additionally, body weight on the exact days of preputial separation or vaginal patency was recorded.

3) Neurobehavioral evaluations

i) Functional observational battery (FOB) - The evaluation criteria for the FOB were not provided. On PNDs 5, 12, 22, 36, 46, and 61, selected animals (1 pup/litter/dose) were subjected to a modified FOB in the open-field, as appropriate for the developmental stage being observed. The same parameters assessed in the maternal FOB were examined in the offspring. The technicians were 'blind' as to the dose group of the animals.

ii) Motor activity testing - Motor activity measurements were performed on selected animals (1 pup/litter/dose) on PNDs 14, 18, 22, and 60 using an automated activity recording apparatus (no further details provided) in a separate testing room. Data were collected in five-minute intervals over the course of 50 minutes. Total number of movements (counts) were evaluated.

iii) Auditory startle reflex habituation - Auditory startle response and habituation of responses with repeated presentation of stimuli were evaluated for selected animals (1 pup/litter/dose) on PNDs 23 and 61. The rats were tested using an automated recording apparatus (no further details provided). No details as to the duration (msec). level (dBA), or intervals of the stimulus were provided. It was not reported if any "blank" (baseline) trials were performed. The mean response amplitude and time to maximum amplitude were analyzed in 5 blocks of 10 trials each.

iv) Learning and memory testing - Learning and memory testing was performed on two sets of selected animals (1 pup/sex/litter/dose at each time point). Watermaze testing was performed with the first set of animals on PNDs 21 and 24, and a second set of animals at PNDs 59 and 62.

The watermaze test consisted of 2 parts (learning ability on the first day, and memory ability 3 days later). The learning ability phase consisted of 6 trials (intervals not reported) for each rat. On each test trial, the rat was placed into the starting position (base of a Y-maze stem farthest from the two arms) and required to find the escape ladder. The scoring criteria and details of each trial were not provided. After 3 days, the memory phase was performed (6 trials for each animal) using the same animals and the same escape route. Additionally, each animal was placed in a straight channel (to measure swimming speed) after concluding the 6th trial on each day.

2. Postmortem observations

a. Maternal animals - Dams that did not deliver were sacrificed, and their uteri were examined to confirm pregnancy status (no tissues were collected). Dams with total litter loss or with litters not required for F_1 selection on Day 5 were sacrificed and discarded without further examination. All other dams were sacrificed on LD 29 and discarded without further examination.

b. Offspring - All pups found dead or culled on PND 5 were discarded without further examination. The animals selected for sacrifice on PND 12 (at least 10/sex/dose) were sacrificed via CO_2 asphyxiation, and the brain was immediately exposed and immersion fixed in 10% neutral buffered formalin. The brains were weighed after 24 hours fixation. The brains of the control and 1000 ppm animals were embedded in paraffin, and routinely processed for microscopic evaluation. On PND 63, selected animals (at least 10 pups/sex/dose) were sacrificed via CO_2 asphyxiation, and the brains were weighed prior to fixation in formalin. Additionally, 1 pup/litter/dose was anaesthetized with sodium pentobarbitone (i.p.), and sacrificed via perfusion fixation with formalin fixative. The brains were removed and weighed. The CHECKED (X) tissues listed below were removed from all animals and preserved in an appropriate fixative.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Olfactory bulbs	X	Sciatic nerve (proximal)
X	Frontal lobe		
X	Parietal lobe		**OTHER**
X	Midbrain with occipital and temporal lobe		Sural nerve
X	Pons	X	Tibial nerve (proximal and distal)
X	Medulla oblongata		Peroneal nerve
X	Cerebellum	X	Lumbar dorsal root ganglion
	SPINAL CORD	X	Lumbar dorsal root fibers
X	Cervical swelling	X	Lumbar ventral root fibers
X	Lumbar swelling	X	Cervical dorsal root ganglion
	OTHER	X	Cervical dorsal root fibers
	Gasserian ganglia with nerve	X	Cervical ventral root fibers
	Pituitary gland		
X	Eyes (with retina and optic nerve)		
X	Skeletal muscle (gastrocnemius)		

The central nervous system tissues, the eye (with retina and optic nerve), and gastrocnemius muscle were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The peripheral nerves (proximal sciatic, proximal tibial, distal tibial) were embedded in resin, sectioned, and stained with toluidine blue. All tissues from the control and 1000 ppm groups were examined microscopically. Morphometric evaluation was performed on the cortex, hippocampus, corpus callosum, thalamus, and cerebellum.

D. DATA ANALYSIS

1. **Statistical analyses** - All statistical tests were 2-sided, and significance was denoted at $p \leq 0.05$. Data were subjected to the following statistical procedures:

Parameter	Statistical test
LD 1 maternal body weight, maternal food consumption, gestation length, litter size, PND 1 mean pup body weight, total litter weight, PND 5 litter based mean body weights for selected F1 animals, motor activity measurements, maximum amplitude and time to maximum amplitude startle response, litter based time to preputial separation or vaginal patency, mean litter body weight at the time of developmental landmark, brain weights for selected F1 animals, brain morphometry data, and swimming times in the straight channel and individual trial times in the Y-maze	Analysis of variance
Maternal gestation and lactation body weights, mean pup body weights after PND 1, litter based mean pup body weights after PND 5, brain weights for selected F1 animals, and brain morphometry data	Analysis of covariance
Proportions of: whole litter losses, litters with gestation length of 22 days and of >22 days, pups born live, pups surviving, litters with all pups surviving, and male pups	Fisher's Exact Test
Percentages of live born pups, pre- and post-cull pup survival, pup sex, and successful trials in the Y-maze	Double arcsine transformation of Freeman and Tukey followed by analysis of variance

Analyses of body weights, brain weights, motor activity, brain morphometry data, swimming times in the straight channel, and individual trial times and percentage of successful trials in the Y-maze were performed separately for male and female pups. Analyses of *post partum* body weights and food consumption, litter size, and pup survival were presented excluding whole litter losses.

2. **Indices** - The reviewers calculated the following indices using the formulas below and included the data in the summary tables.

Fertility index (%) = $\frac{\text{\# of pregnant}}{\text{\# of females mated}} \times 100$

Live birth index (%) = $\frac{\text{\# of liveborn pups}}{\text{Total \# of pups born}} \times 100$

Gestation index (%) = $\frac{\text{\# of females with live pups on day of birth}}{\text{\# of females pregnant}} \times 100$

3. **Positive control data** - Positive control data previously submitted to the Agency are under review.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality, clinical signs, and functional observations - Two dams (one each at 300 and 1000 ppm) were sacrificed prior to scheduled termination as they failed to litter. Examination of these animals uteri revealed that they were not pregnant. Seven dams (one control and six 1000 ppm) were sacrificed because of whole litter losses. One 1000 ppm dam was sacrificed due to parturition difficulties. Additionally, 2-4 dams/dose were sacrificed due to insufficient (7 pups with at least 3 males and 3 females) pups. All other dams with sufficient pups for F_1 selection survived to scheduled sacrifice. Increased incidence of piloerection, hunched posture, and sides pinched in were noted in the 1000 ppm dams near the time of parturition (GD 22-26; Table 2). No treatment-related clinical signs were observed in the ≤300 ppm groups. No adverse FOB effects were noted at any dose throughout the study.

Table 2. Incidence [# affected/30 (# of times observed)] of clinical signs in P females administered EPTC in the diet from GD 7 to LD 23. [a]

Observation	Dose (ppm)			
	0	100	300	1000
Piloerection	2 (4)	3 (4)	2 (2)	8 (15)
Hunched Posture	0 (0)	0 (0)	1 (1)	5 (5)
Sides Pinched-In	0 (0)	0 (0)	0 (0)	3 (3)

a Data were obtained from the Study Report Table 4, pages 50 and 51; findings were observed during GD 22-26.

2. Body weight and food consumption - At 1000 ppm, body weight was decreased ($p \leq 0.01$) by 2-3% on GDs 15 and 22 (Table 3a) and overall (GDs 1-22) body weight gain (calculated by reviewers) was decreased by 13%. No treatment-related effects on body weight or body weight gain were noted at ≤300 ppm during gestation or at any dose during lactation.

At 1000 ppm, food consumption (g/animal/day) was decreased ($p \leq 0.01$) throughout the gestation treatment period (GDs 7-22, ↓11-16%) and during most of the lactation period (↓7-19%; Table 3b). Additionally, food consumption was decreased ($p \leq 0.05$) by 6% at 300 ppm on GDs 7-15. The decreases noted at 1000 ppm during GDs 7-22 corresponded to the decreased body weights and overall body weight gain noted during gestation. The decrease in food consumption noted at 300 ppm was considered unrelated to treatment because there was no corresponding effect on body weight at GD 7 or 15.

Table 3a. Selected mean (± SD) body weights (g) for P females administered EPTC in the diet from GD 7 to LD 23. [a]

Interval (Days)	Dose (ppm)			
	0	100	300	1000
Gestation (n=29-30)				
1	259.8±18.1	262.1±16.4	267.5±15.5	263.7±13.1
15	329.1	328.9	323.4	321.4** (-2)
22	389.0	390.0	394.7	375.5** (-3)
Overall body weight gain (GD 1-22)[b]	129.2	127.9	127.2	111.8 (-13)
Lactation (n=18-30)[c]				
1	296.4±29.5	300.2±28.2	299.7±23.9	288.2±21.3
22	352.2	359.2	360.5	342.2
29 [d]	336.3	344.4	344.3	339.9
Overall body weight gain (LD 1-23)[b]	55.8	59.0	60.8	54.0

a Data were obtained from the Study Report Tables 5 and 6, pages 72-73. Percent difference from control (calculated by reviewers) is presented parenthetically.
b Values were calculated by the reviewers using the unadjusted means obtained from pages 72-73.
c Excluding whole litter losses
d Post-weaning
** Significantly different from controls at $p \leq 0.01$

Table 3b. Selected mean (± SD) absolute (g/animal/day) food consumption for P females administered EPTC in the diet from GD 7 to LD 23. [a]

Interval (Days)	Dose (ppm)			
	0	100	300	1000
Gestation (n=28-30)				
1-7	18.5±2.6	18.7±4.4	18.4±2.8	17.5±2.5
7-15	24.9±3.7	24.1±2.7	23.4±1.9* (-6)	20.9±2.7** (-16)
15-22	25.8±3.2	26.5±3.5	26.1±2.2	22.9±3.2** (-11)
Lactation (n=18-30)				
1-5	30.1±8.0	30.3±7.7	28.2±7.3	24.4±6.0** (-19)
12-15	62.1±6.6	61.2±6.4	60.5±5.0	57.6±5.7** (-7)
21-23	75.1±7.0	76.2±8.6	74.6±4.3	69.1±4.1** (-8)

a Data were extracted from the Study Report Tables 7 and 8, pages 74-75. Percent difference from control (calculated by reviewers) is presented parenthetically.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

3. **Test substance intake** - Mean compound intake (mg/kg bw/day) during the gestation and lactation periods was calculated based on maternal food consumption, nominal dose, and body weight (Table 4).

Table 4. Mean (±SD) test substance intake (mg/kg/day) for P females administered EPTC in the diet from GD 7 to LD 23. [a]

Interval	Nominal Dose (ppm)	Actual Dose (mg/kg/day)
GD 7-22	100	7.6
	300	21.9
	1000	67.2
LD 1-22	100	16.4
	300	47.9
	1000	157.3

a Data were obtained from the Study Report Appendix H, pages 211-212.

4. **Reproductive performance** - Gestation index was reduced at 1000 ppm (75.9% treated vs 96.7% controls). One 1000 ppm female was sacrificed due to difficult parturition (dystocia). All other indices (fertility index and gestation length) were comparable between treated and control animals (Table 5).

Table 5. Delivery observations in P females administered EPTC from GD 7 to LD 22. [a]

Observation	Dose (ppm)			
	0	100	300	1000
# of females mated	30	30	30	30
# of females pregnant Fertility index (%)	30 100.0	30 100.0	29 96.7	29 96.7
Mean (±SD) gestation length (days)	22.0±0.2	22.0±0.2	22.1±0.3	22.1±0.4
# of females with liveborn Gestation index (%)	29 96.7	30 100.0	29 100.0	22 75.9
# of litters with less than 8 pups	2	1	1	1
Incidence of dystocia	0	0	0	1

a Data were obtained from Study Report Tables 9-10, pages 76-78.

5. **Maternal postmortem results**

a. **Macroscopic examination** - The uteri of the two females (one each at 300 and 1000 ppm) that did not litter lacked implantation sites. No other macroscopic evaluations of the dams were performed.

b. **Microscopic examination** - No microscopic examinations were conducted on the dams.

B. OFFSPRING

1. **Viability and clinical signs** - The number of whole litter losses was significantly increased at 1000 ppm (6/28 treated vs 1/30 controls; Table 6a). When whole litter losses were included, the following differences were noted at 1000 ppm: (i) live birth index (calculated by reviewers) was

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slightly decreased (96.3 treated vs 99.7% controls); (ii) mean litter size (PND 5) was decreased ($p \leq 0.05$) by 19%; and (iii) survival (PND 1-5) was decreased ($p \leq 0.01$; 74.0% treated vs 91.9% controls). However, when whole litter losses were excluded, live birth index, mean litter size, and survival (PND 1-5) were comparable to controls. Survival (PND 1-5; excluding whole litter losses) was decreased at 300 ppm (89.4% treated vs 95.1% control); however, this finding was not dose-dependent. There was no treatment-related effect on sex ratio at any dose. The mean number of pups/litter was not reported from PND 5 (post-cull) to PND 22.

Table 6a. F_1 live litter size and viability. [a]

Observation	Dose (ppm)			
	0	100	300	1000
Number of litters	30	30	29	28
Whole litter losses	1	0	0	6*
Total # of pups delivered [b]	357	343	358	350
# of liveborn	356	342	355	337
# of stillborn	1	1	3	13
Live birth index (%)[c]	99.7	99.7	99.1	96.3
Total # of pups delivered [d]	345	343	358	265
# of liveborn	344	342	355	260
# of stillborn	1	1	3	5
Live birth index (%)[c]	99.7	99.7	99.1	98.1
Sex ratio (% male)				
PND 1	46.3	52.0	48.6	49.7
PND 5e	46.7	51.5	50.1	49.3
% Survival (PNDs 1-5[be])	91.9	95.1	89.4	74.0**
% Survival (PNDs 1-5[de])	95.1	95.1	89.4	94.2
Mean litter size [b]				
PND 1	11.9±2.4	11.4±2.3	12.3±2.8	12.1±2.7
PND 5[e]	10.9±3.2	10.8±2.4	10.8±3.1	8.8±5.3* (119)
Mean litter size [d]				
PND 1	11.9±2.4	11.4±2.3	12.3±2.8	11.9±2.8
PND 5[e]	11.2±2.4	10.8±2.4	10.8±3.1	11.2±2.7

a Data were obtained from Study Report Tables 10-14, pages 77-84. Percent difference from control (calculated by reviewers) is presented parenthetically.
b Including whole litter losses
c Live birth index = # pups born live/total # pups born x 100
d Excluding whole litter losses
e Before culling
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

On PND 1, increased incidences of pups considered to be cold (all treatment groups) and pups displaying hypothermia (1000 ppm) were noted (Table 6b). Additionally, the numbers of pups found dead and missing/presumed dead were increased at 1000 ppm between PND 1-5 (pre-cull).

Table 6b. Incidence [# of pups (number of litters)] of clinical signs in F_1 pups. [a]

Observation	Dose (ppm)			
	0	100	300	1000
Cold (PND 1)	4 (2)	14 (1)	22 (2)	26 (3)
Hypothermia (PND 1)	0	1 (1)	0	15 (2)
Found Dead (PND 1-5)	14 (3)	1 (1)	9 (4)	44 (11)
Missing, Presumed Dead (PND 2-5)	18 (11)	17 (7)	36 (14)	59 (15)

a Data were obtained from Study Report Table 17, page 88.

2. **Body weight and food consumption** - On PND 1, body weights were decreased ($p \leq 0.01$) by 8-9% in the 1000 ppm animals (Table 7a). Additionally during pre-weaning, body weight was decreased ($p \leq 0.05$) by 5% on PND 22 in the 1000 ppm females. Overall (PND 5-22, calculated by reviewers) body weight gains were similar to controls at all doses in both sexes.

Table 7a. Selected mean (± SD) F_1 pup pre-weaning body weights and body weight gains (g). [a]

Post-natal Day	Dose (ppm)			
	0	100	300	1000
Males				
1	5.9±0.6	5.9±0.7	5.7±0.6	5.4±0.4** (18)
5[b]	9.3	9.4	8.8	8.4
5[c]	9.1±1.1	9.4±1.4	9.0±1.2	8.7±1.2
22	50.6	49.7	49.9	39.5
Overall (Days 5-22) Gain [d]	41.3	40.6	40.9	48.3
Females				
1	5.6±0.5	5.6±0.6	5.4±0.6	5.1±0.5** (19)
5[b]	8.9	8.8	8.5	8.0
5[c]	8.8±1.1	8.9±1.3	8.8±1.2	8.3±1.1
22	49.3	47.9	49.1	46.7* (15)
Overall (Days 5-22) Gain [d]	40.4	39.1	40.6	38.7

a Data were obtained from Study Report Tables 15 and 19, 85-86 and 119-122. Percent difference from controls (calculated by reviewers) is presented parenthetically. During pre-weaning, n=21-30 litters (pre-culling) or n=19-28 litters (post-culling).
b Pre-culling
c Post-culling
d Calculated by reviewers using unadjusted mean data from Days 5 (post-cull) to 22.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

Post-weaning body weight was decreased ($p \leq 0.05$) by 4% in the 1000 ppm males on PND 50 and 57 (Table 7b); however, body weights were similar between treated and control groups at all other time points during post-weaning. Post-weaning body weights were similar to controls in all treated females. Overall (PND 22-63) body weight gains were similar between treated and control groups. Food consumption was not reported for the F_1 animals.

Table 7b. Selected mean F_1 pup post-weaning body weights and body weight gains (g). [a]

Post-natal Day	Dose (ppm)			
	0	100	300	1000
Males				
29	89.4	89.1	88.4	85.9
57	308.7	307.9	304.2	297.6* (-4)
63	339.2	340.6	338.1	330.2
Overall (Days 22-63) gain [b]	288.6	290.9	288.2	281.9
Females				
29	85.2	83.6	84.7	81.7
63	211.2	210.3	213.5	208.4
Overall (Days 22-63) gain [b]	161.9	162.4	164.4	161.7

a Data were obtained from Study Report Table 19, pages 119-122; n=19-28 litters. Percent difference from controls (calculated by reviewers) is presented parenthetically.
b Calculated by the reviewers using unadjusted means from Study Report Table 19.
* Significantly different from controls at $p \leq 0.05$

3. Developmental landmarks

a. Sexual maturation - No treatment-related effect on mean time to preputial separation or mean time to vaginal patency was observed (Table 8). Time to vaginal patency was increased ($p \leq 0.05$) by less than a day at 1000 ppm (34.5±1.1 treated vs 33.9±0.9 controls); however, as this value was within the historical control range (34±2 to 36.9±1.7), this finding was considered to be unrelated to treatment. Body weights at the time of sexual maturity were similar to controls in all treated males and females.

Table 8. Sexual maturation (mean days ± SD) in F_1 generation rats. [a]

Parameter	Dose (ppm)				Historical Control Range [b]
	0	100	300	1000	
N (M/F)	83/87	100/89	86/87	66/65	NA
Preputial separation	44.1±0.9	44.2±0.9	44.2±0.9	44.5±1.1	NA
Vaginal patency	33.9±0.9	34.4±1.2	34.3±0.8	34.5±1.1*	34±2 to 36.9±1.7

a Data were obtained from Study Report Table 20, pages 123-124 of the study report.
b Data were obtained from Study Report Appendix 1, page 213; n=70-93.
* Significantly different from controls at $p \leq 0.05$
NA Not applicable

4. Behavioral assessments

a. Functional observational battery - No treatment-related behavioral effects were observed at any dose in either sex.

b. Motor activity - Overall session activity counts were similar to controls at all time points (Table 9a). Several significant findings were noted at various intervals in both sexes throughout the study (Tables 9b and 9c); however, as these findings were isolated and/or not dose-dependent they were considered to be unrelated to treatment. Habituation was unaffected by treatment.

Table 9a. Mean (±SD) session motor activity data (counts) in F_1 pups. [a]

Post-natal Day	Dose (ppm)			
	0	100	300	1000
Males				
14	118.2±53.4	205.9±150.1	143.1±122.6	205.0±139.3
18	184.6±105.3	131.4±82.4	149.2±106.4	186.9±168.6
22	373.8±114.0	250.0±179.0	340.8±142.1	342.7±181.9
60	597.4±124.0	589.1±122.9	527.8±110.9	618.0±90.1
Females				
14	139.4±85.1	157.9±109.2	176.3±188.0	179.6±152.2
18	143.2±75.3	179.8±137.6	122.6±86.6	195.4±120.9
22	383.5±118.2	293.8±149.1	342.8±153.2	441.6±137.1
60	606.2±91.6	606.5±98.2	679.6±65.5	624.7±120.1

a Data were obtained from Study Report Table 21, pages 125-132; n=9-14.

Table 9b. Mean (±SD) sub-session motor activity (counts) in male F_1 pups. [a]

Interval	Dose (ppm)			
	0	100	300	1000
PND 14				
1-5	10.9±9.4	35.4±34.0*(1225)	19.9±21.4	27.5±25.4
6-10	14.3±18.7	30.8±22.6	20.0±20.3	15.6±22.1
11-15	33.9±19.1	14.4±15.5*(158)	16.8±21.2*(150)	17.3±17.7*(149)
16-20	11.6±15.9	21.1±20.3	8.4±11.8	26.8±23.8
21-25	10.3±14.0	29.0±31.4*(1182)	8.8±14.5	20.7±25.4
26-30	13.3±15.3	23.0±26.9	19.2±25.5	17.9±25.8
31-35	12.4±13.5	8.0±13.2	14.4±23.1	22.6±19.9
36-40	6.8±8.4	19.7±24.0	9.3±11.7	26.1±26.4*(1284)
41-45	2.1±3.0	14.6±21.0	10.5±17.5	16.5±21.5
46-50	2.5±4.0	9.9±14.2	15.9±23.5	14.0±22.4
PND 18				
1-5	17.8±14.5	16.6±16.9	21.3±22.9	28.9±27.4
6-10	16.9±15.5	14.0±16.8	15.5±21.6	17.2±27.9

(table continues next page)

Interval	Dose (ppm)			
	0	100	300	1000
11-15	22.2±22.8	15.6±16.5	10.7±12.8	22.6±29.6
16-20	21.6±20.3	6.6±8.6*(169)	8.2±19.1	21.0±21.1
21-25	11.0±13.2	4.9±10.1	12.9±17.7	12.8±17.0
26-30	18.7±26.9	8.1±13.1	10.9±19.5	19.5±24.0
31-35	16.8±21.2	11.1±13.1	11.8±19.7	18.6±21.0
36-40	30.8±22.8	21.7±22.2	15.4±25.6	21.3±24.9
41-45	16.1±16.7	17.4±22.7	20.5±27.1	11.9±14.9
46-50	12.9±22.5	15.4±17.9	22.0±23.9	13.1±17.8
PND 22				
1-5	45.0±15.8	41.4±22.3	49.8±23.6	50.9±24.3
6-10	29.2±16.9	29.4±23.3	38.1±24.7	44.4±31.2
11-15	34.3±20.4	20.1±24.3	34.2±26.2	32.7±28.7
16-20	32.9±26.2	19.1±19.8	25.7±20.9	38.5±29.5
21-25	29.3±22.6	18.1±21.6	31.1±27.2	24.5±23.6
26-30	29.8±26.2	23.7±24.4	30.9±25.2	24.8±28.3
31-35	40.5±32.6	27.8±28.3	30.7±27.0	29.2±20.2
36-40	46.9±26.2	20.8±27.0*(156)	33.1±25.5	32.4±31.4
41-45	49.5±15.1	24.2±29.3*(151)	34.7±32.2	36.4±30.7
46-50	36.4±29.4	25.4±26.7	32.6±26.7	28.9±24.3
PND 60				
1-5	70.5±7.1	70.8±13.1	72.0±6.1	67.1±10.2
6-10	72.6±12.4	70.2±13.4	69.1±11.2	75.1±14.2
11-15	72.2±10.0	71.1±10.6	66.0±11.0	68.1±12.9
16-20	72.3±14.9	70.2±10.0	64.2±12.3	67.3±10.6
21-25	63.5±13.4	58.1±15.2	55.6±17.9	62.4±21.9
26-30	64.6±20.0	58.5±25.4	53.6±36.2	59.3±23.0
31-35	48.1±26.2	51.2±29.2	43.5±27.5	60.7±18.0
36-40	44.7±29.5	45.5±28.8	28.5±27.6	50.2±14.0
41-45	39.2±29.7	46.1±32.1	29.2±26.0	46.7±26.8
46-50	49.8±24.1	47.4±27.3	46.1±27.5	61.1±15.2

a Data were obtained from Study Report Table 21, pages 125-132; n=10-14. Percent difference from control (calculated by reviewers) is presented parenthetically.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

Table 9c. Mean (±SD) sub-session motor activity (counts) in female F_1 pups. [a]

Interval	Dose (ppm)			
	0	100	300	1000
PND 14				
1-5	26.1±18.8	27.2±22.9	27.0±27.1	13.6±15.0
6-10	24.7±18.2	22.2±25.8	21.7±26.4	19.9±25.2
11-15	13.6±12.1	23.2±23.2	15.6±21.9	15.8±17.2
16-20	11.7±21.5	15.7±21.7	23.2±27.6	13.9±15.0
21-25	14.0±15.5	22.6±29.7	18.5±29.9	17.1±18.6
26-30	9.6±13.7	12.5±20.7	19.3±23.2	24.7±29.4
31-35	12.1±21.0	6.6±11.5	18.6±26.3	22.3±27.9
36-40	11.6±15.5	11.7±18.9	12.8±17.8	28.8±22.9*(↑148)
41-45	6.3±13.2	7.7±11.1	10.3±15.1	13.8±18.2
46-50	9.7±20.8	8.7±11.4	9.4±15.8	9.8±19.6
PND 18				
1-5	15.7±13.6	14.0±14.4	18.8±22.8	26.1±20.2
6-10	12.2±13.3	14.5±20.1	13.8±13.8	28.3±26.3
11-15	9.6±12.4	15.0±11.0	19.9±24.0	24.2±19.7
16-20	13.8±17.7	21.0±22.6	14.1±19.0	21.8±28.4
21-25	13.8±22.0	16.8±18.9	12.1±19.2	12.8±13.4
26-30	17.8±19.9	25.4±21.3	7.3±15.1	18.1±21.7
31-35	28.7±23.1	18.1±25.2	8.6±15.7*(↓70)	23.4±19.2
36-40	12.8±17.9	15.8±20.0	7.5±13.9	18.2±27.5
41-45	10.9±19.9	16.8±18.6	5.1±7.5	17.3±18.1
46-50	7.8±14.1	22.4±26.1	15.5±24.5	5.1±13.5
PND 22				
1-5	39.8±14.6	39.6±20.0	44.7±21.8	50.8±9.2
6-10	42.2±21.6	29.6±23.2	29.1±21.7	37.0±17.1
11-15	38.0±20.2	20.7±20.8*(↓46)	32.3±27.7	37.7±15.1
16-20	32.9±22.3	25.1±21.1	30.6±22.8	38.6±20.5
21-25	38.8±17.1	25.9±25.1	25.1±21.7	40.9±24.2
26-30	38.3±23.4	25.5±21.9	35.0±29.9	46.3±20.6
31-35	30.5±29.3	34.5±25.6	28.4±26.8	55.3±24.9*(↑81)
36-40	38.4±21.5	38.2±19.8	42.0±34.9	46.9±29.0
41-45	43.6±25.0	22.6±25.4*(↓48)	39.8±26.2	48.6±22.6
46-50	40.8±26.4	32.2±27.4	35.8±28.7	39.6±22.7
PND 60				

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Interval	Dose (ppm)			
	0	100	300	1000
1-5	66.5±7.5	69.7±8.6	66.2±7.8	73.4±12.9
6-10	66.6±8.6	70.5±11.1	70.3±9.5	70.2±10.8
11-15	70.3±12.1	66.9±11.9	67.4±9.4	68.2±14.7
16-20	62.1±15.6	69.9±11.9	70.3±10.5	67.4±11.5
21-25	56.8±16.6	62.0±12.4	71.5±13.7*(↑26)	59.2±16.1
26-30	57.2±20.0	59.7±23.3	68.8±11.0	59.6±22.7
31-35	55.5±20.8	49.9±26.7	64.4±19.0	61.3±21.1
36-40	48.0±22.0	43.1±26.5	68.0±12.5*(↑42)	50.7±20.6
41-45	55.9±21.1	54.0±20.3	64.8±11.7	52.3±25.6
46-50	67.4±12.8	60.8±20.0	67.8±8.9	62.2±11.3

a Data were obtained from Study Report Table 21, pages 125-132; n=9-13. Percent difference from control (calculated by reviewers) is presented parenthetically.

* Significantly different from controls at $p \leq 0.05$

c. <u>Auditory startle reflex habituation</u> - No treatment-related effects on auditory startle reflex maximum amplitude or time to maximum amplitude were observed at any dose in either sex on PND 23 or 61. Startle response maximum amplitude was decreased ($p \leq 0.05$) in the 1000 ppm females (↓35%) during Block 5 on PND 23 (Table 10a). Several significant differences in latency were observed in both sexes on PNDs 23 or 61 (Table 10b). As these significant findings in latency were within the historical control range means (provided in Study Report Appendix I, pages 214-219), they were considered to be unrelated to treatment.

Table 10a. Mean (±SD) auditory startle reflex maximum amplitude (g) in F_1 rats. [a]

Observation [b]		Dose (ppm)			
		0	100	300	1000
Males					
PND 23	Block 1	303.4±124.4	257.0±151.8	290.1±127.3	210.3±50.8
	Block 2	238.0±107.5	198.3±65.2	220.5±43.2	179.4±58.8
	Block 3	190.8±70.0	172.5±65.5	170.1±38.3	154.6±57.4
	Block 4	171.6±74.2	150.6±52.8	156.1±67.2	154.7±41.1
	Block 5	171.9±84.1	148.6±55.2	163.2±40.7	148.6±40.1
PND 61	Block 1	1040.6±618.1	848.2±298.1	987.4±396.4	832.0±289.3
	Block 2	834.5±411.7	766.5±308.0	688.3±318.4	787.6±317.2
	Block 3	700.7±398.3	636.8±234.8	606.9±197.5	692.9±330.8
	Block 4	743.0±400.8	552.1±177.4	636.9±187.2	613.6±252.3
	Block 5	582.0±305.3	515.7±143.2	577.4±207.7	533.2±191.7
Females					
PND 23	Block 1	246.4±74.4	289.2±227.0	215.2±80.5	174.5±59.9
	Block 2	194.9±54.5	215.7±128.2	185.7±72.3	161.3±32.9
	Block 3	180.9±43.7	177.2±78.5	166.7±83.3	149.0±64.5
	Block 4	159.8±42.6	168.6±83.8	155.6±62.6	149.7±28.3
	Block 5	181.4±45.9	170.9±76.8	153.7±60.7	118.0±50.5* (135)
PND 61	Block 1	599.3±169.6	543.1±280.0	600.3±225.6	430.3±137.7
	Block 2	592.0±245.5	497.6±273.9	600.2±180.5	409.6±222.5
	Block 3	486.2±205.3	471.2±234.2	529.9±314.0	424.7±242.2
	Block 4	450.3±246.4	464.9±231.6	476.8±164.4	420.9±255.0
	Block 5	419.9±175.3	411.7±179.9	463.8±82.2	325.4±122.3

a Data were obtained from Study Report Table 22, pages 133-136; n=8-14. Percent difference from control (calculated by reviewers) is presented parenthetically.

b Block=10 consecutive trials

* Significantly different from controls at $p \leq 0.05$

Table 10b. Mean (±SD) time to maximum amplitude (msec) in F_1 rats. [a]

Observation [b]		Dose (ppm)			
		0	100	300	1000
Males					
PND 23	Block 1	27.2±7.2	25.1±3.8	26.2±6.7	25.1±2.0
	Block 2	20.8±2.4	21.9±3.1	21.0±2.4	22.4±4.5
	Block 3	22.8±3.1	21.0±2.3	21.1±1.8	22.5±4.5
	Block 4	21.0±1.2	22.7±4.3	22.5±3.6	21.3±1.6
	Block 5	21.9±4.5	20.9±1.8	21.0±2.0	22.1±2.2
PND 61	Block 1	28.8±5.6	25.8±3.5	24.7±3.3*(↓14)	25.9±5.2
	Block 2	24.6±3.6	24.1±2.5	24.5±2.9	21.8±2.1*(↓11)
	Block 3	24.3±3.2	25.1±2.1	24.7±2.8	23.0±2.6
	Block 4	27.4±4.1	26.9±3.2	24.7±2.2*(↓10)	23.2±2.5**(↓15)
	Block 5	29.4±3.5	25.5±2.7**(↓13)	25.3±3.1**(↓14)	25.9±3.8*(↓12)
Females					
PND 23	Block 1	21.0±2.6	25.5±8.8	22.6±4.2	23.3±4.2
	Block 2	20.0±2.9	22.2±4.7	22.2±3.3	23.8±3.8*(↑19)
	Block 3	20.4±2.5	22.3±2.5	23.2±4.3*(↑14)	21.8±2.9
	Block 4	20.7±2.4	23.4±7.7	21.6±3.6	20.9±2.8
	Block 5	21.0±2.6	21.7±2.5	21.3±3.4	21.4±2.4
PND 61	Block 1	25.7±4.8	27.1±5.0	25.1±5.1	28.8±3.3
	Block 2	24.6±4.4	24.5±2.0	24.8±3.7	25.8±4.3
	Block 3	25.6±5.1	25.6±6.0	25.5±3.0	27.3±3.3
	Block 4	24.7±3.4	27.1±4.8	23.5±2.7	25.8±2.4
	Block 5	27.1±4.4	26.7±4.7	24.3±3.1	28.4±3.5

a Data were obtained from Study Report Table 23, pages 137-140; n=8-14. Percent difference from control (calculated by reviewers) is presented parenthetically.
b Block=10 consecutive trials
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

d. Learning and memory testing - No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the water maze test (Table 11). All groups showed evidence of learning (the time to complete Trial 1 was greater than the times to complete subsequent trials) and memory (the time to complete Trial 1 of the memory phase was less than the time to complete Trial 1 of the learning phase). Several differences ($p \leq 0.05$) from control in swimming times were noted in the 1000 ppm males (↓50-52% during Trials 2 and 6) and females (↑44-71% during Trials 1 and 5) on PND 21 (learning phase). These findings are not considered to be toxicologically significant, because they were transient (not observed on PND 24) and there was no clear pattern of effect. Additionally, several decreases ($p \leq 0.05$) in swimming times were noted in the females at all doses on PND 59 (learning phase); however, it

was stated that there were several longer swimming times in the controls which resulted in a high control mean value, outside the historical control range (provided in Study Report Appendix I, page 220). All other noted differences from controls were minor and/or not dose-dependent. The straight channel swimming times were comparable to controls throughout the study, with the exception of a minor decrease (↓23%; $p \leq 0.05$) in the 100 ppm females on PND 62.

Table 11. Mean (±SD) water maze performance (swimming times [sec]) in F_1 rats. [a]

Parameter	Trial	Dose (ppm)			
		0	100	300	1000
Males (n=16-28)					
Learning (PND 21)	SC	4.23±3.38	3.70±2.61	4.44±3.02	3.25±1.87
	1	16.89±9.22	17.52±9.45	14.34±8.01	14.44±7.94
	2	13.01±8.22	13.75±9.23	11.71±7.69	6.45±3.64**(↓50)
	3	9.50±5.68	10.68±6.92	9.75±7.16	8.01±7.72
	4	7.80±4.06	8.12±5.85	9.22±7.33	7.72±6.48
	5	7.71±6.40	9.56±7.62	8.96±7.37	6.67±6.24
	6	8.87±6.29	6.73±5.88	6.65±4.09	4.22±1.66**(↓52)
Memory (PND 24)	SC	3.75±3.52	3.39±1.99	3.83±2.34	2.52±1.08
	1	7.08±3.14	9.01±4.97	6.59±3.80	5.90±3.47
	2	4.00±2.74	4.30±2.34	4.29±2.76	5.77±4.88
	3	6.24±5.77	3.99±2.00*(↓36)	4.92±3.77	4.43±2.63
	4	4.37±2.84	3.78±2.17	4.57±3.22	3.11±0.89
	5	4.15±2.72	3.62±1.87	3.72±1.73	4.65±2.47
	6	3.81±2.31	3.30±1.22	4.91±3.10	4.55±3.45
Learning (PND 59)	SC	2.85±0.62	2.63±0.90	3.15±1.36	3.12±1.08
	1	10.27±4.34	10.00±4.13	12.10±5.32	10.29±2.74
	2	5.12±2.91	5.74±3.27	5.17±3.22	5.63±2.73
	3	5.02±2.67	5.21±2.65	4.23±1.93	5.06±2.72
	4	4.30±2.36	4.47±3.10	4.95±3.10	4.59±1.93
	5	4.01±1.36	4.12±1.95	4.11±2.34	3.84±1.22
	6	4.94±2.28	4.79±2.31	5.28±3.66	5.04±3.00
Memory (PND 62)	SC	2.65±1.21	2.64±0.84	2.44±1.24	2.29±0.34
	1	4.40±2.65	5.34±2.64	5.43±2.34	5.89±4.70
	2	4.49±2.82	6.02±6.65	4.86±5.02	5.04±6.99
	3	5.29±4.64	3.81±2.51	9.33±8.90*(↑76)	5.48±7.04
	4	7.54±6.94	5.64±5.69	6.85±6.54	7.47±7.02
	5	7.30±4.18	7.45±6.45	8.94±8.14	8.48±7.53
	6	5.76±5.86	6.83±4.79	7.74±7.65	5.84±4.36

(table continues next page)

Parameter	Trial	Dose (ppm)			
		0	100	300	1000
Females (n=19-28)					
Learning (PND 21)	SC	4.17±3.46	3.84±3.06	3.22±2.35	2.89±1.51
	1	12.28±7.95	14.23±8.31	12.41±6.75	17.72±8.30*(144)
	2	6.77±5.55	10.85±7.43*(160)	11.14±6.15*(165)	8.64±6.08
	3	6.88±4.33	8.27±7.49	7.60±4.69	8.43±6.93
	4	6.16±4.07	8.10±4.84	6.45±5.68	8.16±4.40
	5	5.39±3.35	6.00±3.27	5.42±3.25	9.19±5.64**(171)
	6	6.81±6.12	5.60±4.21	7.61±6.57	5.36±3.89
Memory (PND 24)	SC	4.32±3.06	3.17±1.88	3.66±1.94	3.18±1.64
	1	6.82±5.24	6.87±4.27	7.51±5.32	6.48±4.50
	2	3.91±1.87	4.19±4.15	4.05±2.55	3.44±2.09
	3	4.81±3.43	3.30±1.63*(131)	4.29±2.22	4.33±3.47
	4	4.62±2.43	3.28±1.57*(129)	3.89±1.42	3.73±3.03
	5	4.17±2.37	3.63±1.72	3.80±2.29	3.27±1.39
	6	4.10±3.05	3.92±3.08	4.73±4.80	4.62±3.40
Learning (PND 59)	SC	3.21±1.46	2.92±1.20	2.80±0.90	2.82±0.90
	1	12.23±4.06	13.72±5.65	12.82±5.33	10.75±3.12
	2	6.63±4.36	6.31±2.94	5.94±2.29	7.62±6.72
	3	5.59±2.56	5.02±2.05	4.20±1.80*(125)	4.88±1.52
	4	5.48±2.98	4.87±2.23	3.91±1.63*(129)	4.26±2.64
	5	6.05±4.84	3.81±1.53*(139)	4.15±2.61*(131)	3.81±1.55*(139)
	6	4.92±3.71	4.11±1.79	4.55±1.95	4.57±3.16
Memory (PND 62)	SC	2.66±1.13	2.06±0.69*(123)	2.64±1.27	2.21±0.72
	1	5.06±3.49	5.18±3.45	6.46±5.68	3.82±2.70
	2	4.39±3.45	6.05±6.64	5.74±6.25	3.51±2.52
	3	5.94±6.92	6.42±7.64	7.54±8.78	5.78±7.07
	4	6.04±6.61	6.12±7.62	9.17±8.03	7.60±6.14
	5	8.48±8.14	7.27±7.67	12.61±9.33	6.96±6.04
	6	8.67±9.41	8.79±9.26	10.32±9.02	6.73±7.28

a Data were obtained from Study Report Table 24, pages 141-148. Percent difference from control (calculated by reviewers) is presented parenthetically.

SC Straight channel trial

* Statistically different from controls at $p \leq 0.05$

** Statistically different from controls at $p \leq 0.01$

5. Postmortem results

a. Brain weights - No treatment-related changes in brain weights were seen in male offspring on PND 12. (Table 12a). On PND 12, absolute brain weights were decreased ($p \leq 0.05$) by 5% in the 1000 ppm females (Table 12b). On PND 63 (non-perfused), absolute brain weights were decreased ($p \leq 0.05$) by 4-6% in the ≥100 ppm males and by 5% in the 1000 ppm females (Tables 12a and 12b).

Table 12a. Mean (±SD) absolute (g) and relative (to body, %) brain weights in F_1 male rats. [a]

Parameter	Dose (ppm)			
	0	100	300	1000
PND 12 (n=9-13)				
Terminal Body Weight (g)	21.1±2.8	22.0±2.6	21.5±1.4	21.3±2.8
Absolute Brain Weight (g)	1.10±0.05	1.13±0.10	1.11±0.04	1.07±0.07
Relative (to body) Weight (%)	5.27±0.56	5.19±0.46	5.17±0.32	5.08±0.40
Adjusted for Body Weight (g)	1.11	1.12	1.11	1.08
PND 63 (n=10-15)				
Terminal Body Weight (g)	338.7±16.6	334.5±20.5	331.7±24.0	326.9±22.3
Absolute Brain Weight (g)	2.03±0.06	1.94±0.05** (-4)	1.94±0.09** (-4)	1.90±0.07** (-6)
Relative (to body) Weight (%)	0.60±0.04	0.58±0.03	0.59±0.03	0.58±0.02
Adjusted for Body Weight (g)	2.02	1.94** (-4)	1.94** (-4)	1.91** (-5)
PND 63 (post-perfusion, n=10-13)				
Terminal Body Weight (g)	338.0±22.4	344.5±24.5	335.8±19.5	328.0±28.2
Absolute Brain Weight (g)	1.94±0.10	1.93±0.09	1.94±0.08	1.88±0.08
Relative (to body) Weight (%)	0.58±0.03	0.56±0.04	0.58±0.04	0.58±0.05
Adjusted for Body Weight (g)	1.94	1.92	1.95	1.89

a Data were obtained from Study Report Tables 26 and 27, pages 165-167. Percent difference from control (calculated by reviewers) is presented parenthetically.

** Statistically different from controls at $p \leq 0.01$

Table 12b. Mean (±SD) absolute (g) and relative (to body, %) brain weights in F_1 female rats. [a]

Parameter	Dose (ppm)			
	0	100	300	1000
PND 12 (n=10-15)				
Terminal Body Weight (g)	20.9±2.4	20.5±3.2	21.7±2.0	20.5±3.2
Absolute Brain Weight (g)	1.10±0.05	1.07±0.06	1.08±0.06	1.04±0.05* (↓5)
Relative (to body) Weight (%)	5.29±0.43	5.30±0.66	4.98±0.30	5.16±0.61
Adjusted for Body Weight (g)	1.10	1.07	1.07* (↓3)	1.05** (↓5)
PND 63 (n=10-13)				
Terminal Body Weight (g)	210.5±14.4	211.5±13.4	212.3±11.3	202.4±12.7
Absolute Brain Weight (g)	1.82±0.05	1.83±0.07	1.82±0.04	1.76±0.08* (↓5)
Relative (to body) Weight (%)	0.87±0.05	0.87±0.04	0.86±0.05	0.87±0.06
Adjusted for Body Weight (g)	1.82	1.82	1.82	1.78
PND 63 (post-perfusion, n=10-15)				
Terminal Body Weight (g)	213.4±11.3	208.9±18.2	209.5±14.2	219.5±22.3
Absolute Brain Weight (g)	1.79±0.08	1.79±0.08	1.80±0.08	1.75±0.09
Relative (to body) Weight (%)	0.84±0.05	0.86±0.08	0.86±0.07	0.80±0.10
Adjusted for Body Weight (g)	1.79	1.79	1.80	1.74

a Data were obtained from Study Report Tables 26 and 27, pages 165-167. Percent difference from control (calculated by reviewers) is presented parenthetically.
* Statistically different from controls at $p \leq 0.05$
** Statistically different from controls at $p \leq 0.01$

b. Neuropathology

1) Macroscopic examination - Macroscopic examinations were not performed.

2) Microscopic examination - No compound-related neuropathological effects were noted in either sex on PNDs 12 or 63. On PND 12, the following increases ($p \leq 0.05$) in morphometric measurements were noted in the 1000 ppm females (Table 13): (i) thickness of the corpus callosum (↑31%); (ii) width of the thalamus (↑5%); (iii) overall width of the thalamus/cortex (↑4%); and (iv) length from midline of the hippocampus (↑15%). No significant differences from controls were observed in any morphometric parameter in the 1000 ppm males on PND 12. On PND 63, the following differences ($p \leq 0.05$) in morphometric measurements were noted at 1000 ppm: (i) decreased thickness of the molecular layer of the cerebellum at the preculminate fissure in the males (↓10%); (ii) decreased overall width of the thalamus/cortex in females (↓5%); and (iii) increased thickness of the inner granular layer of the cerebellum at the preculminate fissure in the females (↑14%). Brain morphometric changes in the corpus callosum in the control animals are at the low end of the historical control range, while the high dose values are at the high end of the control range. However, these findings were considered unrelated to treatment, because the values were all within the range of historical control means and there were no corroborative neuropathological or neurobehavioral effects.

Table 13. Mean (±SD) morphometric measurements in F_1 rats. [a]

Parameter		Dose (ppm)		Range of Historical Control Means [b]
		0	1000	
PND 12				
Females				
Corpus callosum	Thickness	0.49±0.11	0.64±0.06** (131)	0.494-0.641
Thalamus	Width	7.55±0.43	7.92±0.34* (105)	6.85-8.28
Thalamus/Cortex	Overall width	11.88±0.52	12.37±0.52* (104)	11.6-13.9
Hippocampus	Length from midline	3.63±0.49	4.18±0.24** (115)	3.37-4.51
PND 63				
Males				
Cerebellum	Thickness of the molecular layer of the preculminate fissure	221.9±20.4	198.7±23.2* (110)	193-223
Females				
Thalamus/Cortex	Overall width	14.60±0.19	13.83±0.80* (105)	13.0-14.6
Cerebellum	Thickness of the inner granular layer of the preculminate fissure	159±17	181±16** (114)	126-189

a Data were obtained from Study Report Tables 27 and 28, pages 170-185; n=8-10. Percent difference from control (calculated by reviewers) is presented parenthetically.
b Data obtained from Study Report Appendix I, pages 223-229.
* Statistically different from controls at $p \leq 0.05$
** Statistically different from controls at $p \leq 0.01$

III. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u> - The investigators concluded that treatment with EPTC at 1000 ppm in the diet caused the following signs of general toxicity in dams: (i) clinical signs (piloerection, hunched posture, and sides pinched in); (ii) decreased body weight at GD 15 and 22; (iii) reduced food consumption throughout the dosing period (GD 7-23) and lactation; and (iv) increased incidence of whole litter losses. Additionally, effects in F_1 pups were limited to decreased body weights at 1000 ppm on PND 1. No evidence of developmental neurotoxicity was observed at up to 1000 ppm.

B. <u>REVIEWER'S COMMENTS</u>

Repeated oral (dietary) administration of EPTC to pregnant rats during gestation and lactation resulted in maternal toxicity characterized as clinical signs (piloerection, hunched posture, sides pinched in); decreased body weight, body weight gain, and food consumption; and increased incidence of whole litter losses.

Following *in utero* and post natal exposure, no treatment-related effects were noted in FOB parameters, motor activity, auditory startle response, learning and memory in the offspring. No treatment-related neuropathology or alterations in the brain morphology were seen. EPTC at 1000 ppm caused decreases in pup body weights and decreases in absolute brain weights in the female PND 12 and 63 pups and in male PND 63 pups.

The maternal LOAEL is 1000 ppm (67.2 mg/kg/day) based on clinical signs (piloerection, hunched posture, sides pinched in); decreased body weight, body weight gain, and food consumption; and increased incidence of whole litter losses. The maternal NOAEL is 300 ppm (21.9 mg/kg/day).

The offspring LOAEL is 100 ppm (to 7.6 mg/kg/day; LDT) based on dose-depended decreases in absolute brain weights in male pups on PND 63 at all dose levels. An offspring NOAEL was not established.

This study is classified **Acceptable** and may be used for regulatory purposes. however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control data.

C. **STUDY DEFICIENCIES** - The following deficiencies were noted, but do not change the conclusions of this DER:

- The evaluation criteria for the functional observational battery were not provided.
- The scoring criteria and details for the auditory startle response test and watermaze test were not provided.