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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

November 17, 2004

MEMORANDUM

SUBJECT: Secondary Review of the Developmental Neurotoxicity Studies.

FROM: Jess Rowland
Chief, Science Information Management Branch
Health Effects Division (7509C)

Attached for your review is a package on Profenofos prepared by Ayaad Assaad.

A meeting to review these chemicals is scheduled for Wednesday, November 24, 2004 at 9:00 am in Room 813 CM2.

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DATA EVALUATION RECORD

PROFENOFOS

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

Work Assignment No. 1-01-19 (MRIDs 46025401 and 46025402)

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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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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Registration Action Branch 3, Health Effects Division (7509C) **Date**

Template version 11/01

DATA EVALUATION RECORD

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

PC CODE: 111401 **DP BARCODE:** D293052
TXR#: 0052102 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Profenofos (91.8% a.i.)

SYNONYMS: O-4-bromo-2-chlorphenyl O-ethyl S-propyl phosphorothioate, CGA-15324

CITATION: Milburn, G.M. (2003) Profenofos: Developmental neurotoxicity study in rats. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Project ID: RR0928, June 10, 2003. MRID 46025401. Unpublished.

Milburn, G.M. (2002) Profenofos (CGA 15324): Preliminary developmental neurotoxicity study in rats. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Project ID: RR0927, September 24, 2002. MRID 46025402. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Rd. PO Box # 18300, Greensboro, NC

EXECUTIVE SUMMARY - In a developmental neurotoxicity study (MRIDs 46025401 and 46025402) Profenofos (91.8% a.i.; Batch/Lot #: 66719888) was administered in the diet to pregnant Wistar rats (30/dose) from gestation day (GD) 7 to lactation day (LD) 22 at nominal doses of 0, 3, 60, or 600 ppm (equivalent to 0/0, 0.3/10.5, 5.1/10.7, and 50.6/103.4 mg/kg/day [gestation/lactation]). Additionally, satellite groups of 10 pregnant rats/dose were similarly treated and the dams and F_1 pups were evaluated for cholinesterase activity. 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, and are included as an Appendix to this DER.

A. MATERNAL ANIMALS

Three dams were sacrificed prior to scheduled termination as they failed to litter, one in the control group and two at 600 ppm. All other dams survived to scheduled sacrifice. No treatment-related clinical signs or FOB effects were noted at any dose throughout the study.

At 600 ppm, body weights were generally decreased from GD 15 through LD 22 (↓2-5%). Overall body weight gains were decreased by 11% during GDs 1-22 and by 17% during LDs 1-22. These decreases corresponded to the reductions noted in absolute food consumption (↓4-11%) during the gestation and lactation periods. No effects on body weight or body weight gain were noted at ≤60 ppm.

Food consumption was reduced in the 600 ppm dams during GDs 7-22 (↓4-6%), and during LDs 12-23 (↓7-11%). Additionally, food consumption was decreased by 5% in the 60 ppm dams on LDs 18-21; however, as there were no corresponding significant decreases in body weight observed in the 60 ppm animals, this finding is not considered to be treatment-related. The increased food consumption noted in the 60 ppm dams during LDs 8-12 was considered incidental.

All indices of reproductive performance (fertility index, gestation length, gestation index, and incidence of dystocia) were comparable between treated and control animals.

No macroscopic or microscopic examination was performed, other than examining the uteri of the females that did not litter, no macroscopic evaluations of the dams were performed.

Cholinesterase activity was decreased at ≥60 ppm in the erythrocytes (↓50-59%, GD 22 and ↓55-57%, LD 22) and plasma (↓60-84%, GD 22 and ↓59-78%, LD 22). Plasma cholinesterase activity was also decreased by 13% at 3 ppm on LD 22. Additionally at 600 ppm, brain cholinesterase activity was decreased by 44% on GD 22, and by 26% (not statistically significant) on LD 22.

(5.1 mg/kg/day) JR

The maternal LOAEL is 60 ppm based on decreased body weight, body weight gains, and food consumption. The maternal NOAEL was 3 ppm. (0.3 mg/kg/day) JR

The cholinesterase LOAEL was 60 ppm for both the brain and red blood cells. (5.1 mg/kg/day)
The cholinesterase NOAEL was 3 ppm for both compartments. (0.3 mg/kg/day)

The plasma cholinesterase LOAEL was 3 ppm based on LD 22.
The plasma cholinesterase NOAEL ~~< 3 ppm based on LD 22.~~
not established JR

B. OFFSPRING

No significant differences in live litter size or post-natal survival were observed in any treated group through PND 5. On PND 5, all litters contained 7 or 8 animals (assumed by the reviewers); however, the mean number of pups/litter was not reported from PND 5 (post-cull) to

PND 22. The sex ratio (% of male pups) was increased slightly at ≥60 ppm (55.6-59.4% treated vs 49.5% controls); however, this finding was not considered to be treatment-related. Clinical signs were limited to hypothermia on PND 1; however, this finding was generally limited to animals that accounted for whole litter losses.

Throughout pre-weaning (Days 5-22), body weights were decreased in both sexes at 600 ppm (↓6-12%). Also, overall (Days 5-22) pre-weaning body weight gain was decreased in both sexes at 600 ppm (↓11-12%). Post-weaning body weights were decreased in the 600 ppm males (↓5%, PND 29) and females (↓3-5%, PND 29-36); however, body weights were similar between treated and control groups from PND 43-63. Overall (PND 22-63) body weight gains were similar between treated and control groups. Food consumption was not reported for the F_1 animals.

No treatment-related effect on mean time to preputial separation or mean time to vaginal patency was observed.

No treatment-related behavioral effects were observed at any dose in either sex.

No significant differences in motor activity from controls were noted in overall session activity counts in either sex at any dose. Overall activity counts were increased on PND 18-60 compared to PND 14. Several isolated significant differences were noted at various intervals in the females on PNDs 14, 18, and 60. No significant findings were observed in the males during any sub-session at any time point. Habituation was unaffected by treatment.

No treatment-related differences in auditory startle reflex habituation from control were noted in startle response maximum amplitude at any dose in either sex on PNDs 23 or 60. On PND 60, latency was increased in the 60 ppm females during Block 1 (↑20%, Table 14). No significant differences in latency were observed in the males on PNDs 23 or 60, or in the females on PND 23.

No treatment-related differences in learning or memory testing were noted in any treated group relative to concurrent controls in the water maze test. 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 from control were noted in the 3 and 600 ppm males and in the ≥3 ppm females. These findings are not considered to be toxicologically significant, because they were transient and there was no clear dose-response. The increased straight channel swimming times noted in the 3 and 60 ppm males and in the 600 ppm females were considered unrelated to treatment because there was no clear dose-response.

On PND 12, absolute brain weight was decreased in the 600 ppm males (↓4%). However, because there was no difference in relative (to body) brain weight, this finding was considered to be related to the decreased terminal body weight (↓7%, not statistically significant), and not an effect of treatment. Absolute brain weights were similar between treated and control females throughout the study. Relative (to body) brain weights were similar between treated and control groups in both sexes throughout the study.

No treatment-related gross or microscopic pathological findings were noted in any treated group. No adverse neuropathological effects were noted in the 600 ppm animals on PND 63. The demyelination noted in the peripheral nerves occurred with equal frequency in the control and 600 ppm animals, and is commonly noted in rats. Minor differences in various morphometric measurements were noted at 600 ppm in both sexes on PND 12 and 63. However, there was no consistency in the areas affected either between sexes or at different ages, other measurements for the same structures at other levels showed no differences, and the values were within the historical control ranges provided; therefore, these findings were not considered to be treatment-related.

No effects on cholinesterase activity (brain, erythrocyte, or plasma) were noted in the fetuses at any dose in either sex examined at GD 22. However, postnatally in the 600 ppm pups, the following decreases in cholinesterase activity were noted: (i) erythrocyte, ↓22-40% in the females on PNDs 12 and 22; (ii) plasma, ↓25-50% in the males and ↓24-54% in the females on PNDs 12 and 22; and brain, ↓11% in females at PND 5. Additionally, plasma cholinesterase activity was decreased by 23% in the 60 ppm females on PND 22.

50.6 m/kD

The offspring LOAEL is 600 ppm, based on decreased body weights and body weight gains. The offspring NOAEL is 60 ppm. (5.1 m/kD)

No evidence of developmental neurotoxicity was observed at any dose.

The cholinesterase LOAEL was 60 ppm. The cholinesterase NOAEL was 3 ppm.

5.1 m/kD 0.3

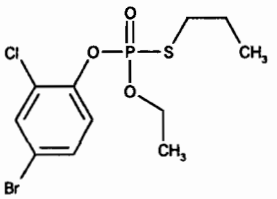
This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Profenofos

Description:	Yellow/brown liquid
Batch/Lot #:	66719888
Purity:	91.8% a.i.
Compound Stability:	The test material was shown to be stable in the diet for up to 27 days at room temperature or at -20°C.
CAS # of TGAI:	41198-08-7
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:	208-260 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.	
Diet:	Powdered 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: 06/25/2002 End: 03/06/2003

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 postnatal day (PND) 22. On PND 5, the litters containing >8 pups were randomly standardized to 8 pups/litter (with equal sexes where possible) to reduce the variability. 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 successful mating was verified by the presence of sperm in a vaginal smear. The animals were supplied on the same day that successful breeding was determined (GD 1).

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

Table 1. Study design [a]

Experimental Parameter	Dose (ppm)			
	0	3	60	600
Dams				
# of maternal animals (Main study)	30	30	30	30
FOB (GDs 10, 17 & LDs 2, 9)	30	30	30	30
# of maternal animals (Satellite study)[b]	10	10	10	10
Cholinesterase determinations (GD 22) (LD 22)	 5 5	 5 5	 5 5	 5 5
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 test (PND 23, 61)	1 pup/litter	1 pup/litter	1 pup/litter	1 pup/litter
Learning and Memory (PND 21, 24) (PND 59, 62)	 1 pup/sex/litter 1 pup/sex/litter	 1 pup/sex/litter 1 pup/sex/litter	 1 pup/sex/litter 1 pup/sex/litter	 1 pup/sex/litter 1 pup/sex/litter
Cholinesterase determinations (GD 22)[c] (PND 5, 12, 22)[bd]	 5 litters 5 pups/sex	 5 litters 5 pups/sex	 5 litters 5 pups/sex	 4 litters 5 pups/sex
Brain weight and neuropathology [e] (PND 12) (PND 63)	 10 pups/sex 10 pups/sex	 10 pups/sex 10 pups/sex	 10 pups/sex 10 pups/sex	 10 pups/sex 10 pups/sex
Perfusion fixation , brain weight, and neuropathology (including morphometry) (PND 63)	 10 pups/sex	 10 pups/sex	 10 pups/sex	 10 pups/sex

a Data were obtained from pages 21, 24 -28 of the study report.
b Satellite animals assigned for cholinesterase determinations only.
c The fetuses from the 4-5 maternal animals in the satellite group sacrificed on GD 22 were pooled by sex and evaluated for cholinesterase activity.
d One pup/litter was taken where possible.
e 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 (MRID 46025402). This study is summarized and included as Appendix I of this DER.

6. Dosage preparation, administration, and analysis - Test diets were prepared by mixing the appropriate amount of the test material with a small amount of diet to form a premix. The premix was further diluted with diet to achieve the appropriate doses. The dams were supplied dietary admixtures beginning on GD 7 and continuing through PND 22 (inclusive). F_1 animals were not directly supplied with the test diets. Homogeneity (top, middle, bottom) was determined from samples of the 3 and 600 ppm diets at the beginning of the study. Stability in the diet was determined using samples from the 3 and 600 ppm dietary formulations at room temperature and -20°C for up to 27 days. Concentration was determined for each dietary formulation using samples collected on Days 1 (excluding 3 ppm), 9 (3 ppm only), 16, 29, and 55.

Results - Stability (range as % of initial):
After 27 days at room temperature: 88.7-98.2%
After 27 days at -20°C: 98.3-113.7%

Homogeneity (range as % of nominal): 102-119%

Concentration (range as % of nominal):

Dose (ppm)	% of Nominal
3	91.7-105.0%
60	95.8-104.5%
600	93.7-103.7%

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 for mortality and clinical signs of toxicity. Detailed physical examinations were performed at the times of body weight measurement. Body weights were measured on GDs 7 (immediately prior to administration of test material), 15, and 22, on LDs 1, 5, 8, 12, 15, and 22, and at termination. Food consumption was recorded on GDs 1, 7, 15, and 22, and on LDs 1, 5, 8, 12, 15, 18, 21, and 23.

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. It was assumed by reviewers that the technicians were blind as to the dose group, because it was stated as such during the FOB of the F_1 generation. 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).

In the satellite group, body weight, food consumption, clinical observations, and detailed physical examinations were measured/performed on the same days as the main study animals. These animals were not subjected to a FOB.

b. Offspring

1) Litter observations - On PND 1 and 5, the status (sex, weight, and clinical condition) and number of all delivered pups were determined. Pups were evaluated for mortality and morbidity daily. Clinical observations were recorded daily throughout the study. Body weights were recorded on PNDs 5 (precull and post-cull), 12, 18, and 22, and then weekly thereafter until sacrifice. Post-weaning food consumption was not reported. The following additional litter observations (X) were made (Table 2):

Table 2. Litter observations. [a]

Observation	1	5[c]	5[d]	12	18	22
Number of live pups [b]	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
Number of dead pups [b]	X	X	X	X	X	X
Sex of each pup	X	X	X			

a Data were obtained from pages 22-24 of the study report.
b Observed daily
c Preculling
d Post-culling

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 with at least 3 males and 3 females were used for F_1 evaluations; excess pups were sacrificed and discarded.

2) Developmental landmarks - Beginning on PND 41, selected male offspring were examined daily for preputial separation. Beginning on PND 29, selected female offspring were examined

daily for vaginal patency. The exact days of preputial separation or vaginal patency were recorded.

3) Postweaning observations - After weaning on PND 22, offspring were examined for mortality and morbidity daily. Detailed physical observations and body weights were recorded weekly until sacrifice.

4) Neurobehavioral evaluations

i) Functional observational battery (FOB) - The evaluation criteria for the modified FOB were not provided. On PNDs 5, 12, 22, 36, 46, and 61, selected animals (10 pups/sex/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.

ii) Motor activity testing - Motor activity measurements were performed on selected animals (1 pup/litter) 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) 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 latency to the peak of the response 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). 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 6^{th} trial on each day.

5) Cholinesterase determinations - The modified Ellman method (Ellman *et al*, 1961) was used for cholinesterase activity determinations. Erythrocytes were lysed using saponin, thiol groups were released from acetyl thiocholine iodide with DTNB (5,5' dithiobis-2-nitrobenzoic acid) in phosphate buffer (pH 8.0) at 37°C. Absorption was measured at 405 nm using a Konelab 60i automated analyzer.

In the satellite groups, five dams/dose were sacrificed on GD 22, blood was taken for plasma and erythrocyte cholinesterase activities, and the brain was removed for cholinesterase determinations. The uterus was removed by caesarean section and samples from the fetuses (pooled by sex) were taken for plasma, erythrocyte, and brain cholinesterase activities. On LD 22, the remaining five dams/dose were sacrificed, blood was taken for plasma and erythrocyte cholinesterase activities, and the brain was removed for cholinesterase determinations. In the F_1 satellite group, 5 pups/sex/dose (1 pup/litter when possible) were sacrificed on PNDs 5, 12, and 22. The brains were removed for cholinesterase determinations, and blood was taken for plasma and erythrocyte cholinesterase activities. The carcasses were discarded without further examinations.

2. Postmortem observations

a. Maternal animals - Dams that did not deliver a litter 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 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 and culled on PND 5 were discarded without further examination. Also, those animals used for neurobehavioral evaluations were sacrificed and discarded without further examination after conclusion of their respective investigations.

In the main study, 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 600 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. An additional 10 rats/sex/dose were anaesthetized with sodium pentobarbitone (i.p.), and sacrificed via perfusion fixation with neutral buffered formalin. The brains were removed, weighed, and measured. 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 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. Tissues from the control and 600 mg/kg groups were examined microscopically. Morphometric evaluations were 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, 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, 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, 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. The statistical analyses were considered appropriate; however, it was not reported if homogeneity of variances or normality of the data were verified. These assumptions should be verified prior to performing parametric analyses.

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 were not submitted with this study; however, summaries of positive control data previously submitted to the Agency were obtained and reviewed, and are included as Appendix II of this DER.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality, clinical signs, and functional observations - Three dams were sacrificed prior to scheduled termination as they failed to litter, one in the control group and two at 600 ppm. All other dams survived to scheduled sacrifice. No treatment-related clinical signs or FOB effects were noted at any dose throughout the study.

2. Body weight and food consumption - Body weights and body weight gains for the P females are presented in Table 3. At 600 ppm, body weights were generally decreased ($p \leq 0.05$) from GD 15 through LD 22 (↓2-5%). Overall body weight gains (calculated by the reviewers) were decreased by 11% during GDs 1-22 and by 17% during LDs 1-22. These decreases corresponded to the reductions ($p \leq 0.05$) noted in absolute food consumption (↓4-11%) during the gestation and lactation periods (Table 4). No effects on body weight or body weight gain were noted at ≤60 ppm.

Table 3. Selected mean (± SD) body weights (g) for P females administered Profenofos in the diet from GD 7 to LD 22. [a]

Interval (Days)	Dose (ppm)			
	0	3	60	600
Gestation (n=37-40)				
1	244.4±14.5	243.5±15.9	242.5±15.6	242.2±14.9
7	279.4±16.1	277.5±16.5	276.5±18.6	278.4±16.9
15[b]	323.3	325.8	324.1	308.2** (↓5)
22[b]	390.4	395.3	394.6	373.9** (↓4)
Overall body weight gain (GD 1-22)[c]	147.6	151.3	150.4	132.0 (↓11)
Lactation (n=23-34)				
1	301.4±24.3	301.2±21.7	295.1±20.0	287.6±19.1** (↓5)
5[b]	315.1	314.0	314.4	308.2* (↓2)
12[b]	335.2	335.0	334.3	330.4
15[b]	352.4	351.8	348.1	337.6** (↓4)
22[b]	361.4	360.6	356.2	347.7** (↓4)
29[d]	338.7	342.0	339.9	338.8
Overall body weight gain (LD 1-22)[c]	64.7	61.1	60.7	54.0 (↓17)

a Data were obtained from pages 74-75 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.
b Adjusted means
c Values were calculated by the reviewers using the unadjusted means obtained from pages 74-75.
d Post-weaning
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

Food consumption (g/animal/day) was reduced ($p \leq 0.05$) in the 600 ppm dams during GDs 7-22 (↓4-6%), and during LDs 12-23 (↓7-11%, Table 4). Additionally, food consumption was decreased ($p \leq 0.05$) by 5% in the 60 ppm dams on LDs 18-21; however, as there were no corresponding significant decreases in body weight observed in the 60 ppm animals, this finding is not considered to be treatment-related. The increased ($p \leq 0.05$) food consumption noted in the 60 ppm dams during LDs 8-12 was considered incidental.

Table 4. Selected mean (± SD) absolute (g/animal/day) food consumption for P females administered Profenofos in the diet from GD 7 to LD 22. [a]

Interval (Days)	Dose (ppm)			
	0	3	60	600
Gestation (n=36-40)				
1-7	22.0±2.2	21.8±2.6	22.0±2.8	22.1±2.3
7-15	27.0±2.5	27.2±3.1	27.4±3.1	25.3±4.9* (↓6)
15-22	29.4±3.4	29.6±3.3	28.8±2.8	28.2±3.4* (↓4)
Lactation (n=21-34)				
1-5	36.6±7.7	36.2±7.2	39.6±4.8	34.9±8.7
8-12	54.4±7.6	56.4±7.5	58.8±8.4* (↑8)	55.1±6.6
12-15	61.4±4.8	62.8±6.5	61.1±4.7	57.3±7.7* (↓7)
15-18	69.0±6.4	70.9±8.1	68.7±6.8	61.3±6.8** (↓11)
18-21	77.0±5.4	78.1±9.0	73.2±8.6* (↓5)	70.4±8.3** (↓9)
21-23	80.1±6.7	79.8±7.5	78.3±8.2	73.1±9.3** (↓9)

a Data were extracted from pages 76-77 of the study report. 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 determined based on maternal food consumption and body weight (Table 5).

Table 5. Mean (±SD) test substance intake (mg/kg/day) for P females administered Profenofos from GD 7 to LD 22. [a]

Interval	Nominal Dose (ppm)	Actual Dose (mg/kg/day)
GD 7-22	3	0.3
	60	5.1
	600	50.6
LD 1-23	3	10.5
	60	10.7
	600	103.4

a Data were obtained from pages 216-217 of the study report.

4. Reproductive performance - All indices (fertility index, gestation length, gestation index, and incidence of dystocia) were comparable between treated and control animals (Table 6).

Table 6. Delivery observations in P females administered Profenofos from GD 7 to LD 22. [a]

Observation	Dose (ppm)			
	0	3	60	600
# of females mated [b]	40	40	40	40
# of females pregnant Fertility index (%)	39 98	40 100	40 100	39 98
Mean (±SD) gestation length (days)	22±0.0	22±0.0	22±0.2	22±0.0
# of females with liveborn Gestation index (%)	39 100	40 100	40 100	38 97
Incidence of dystocia	0	0	0	1

a Data were obtained from pages 78-84, 785-786, and 817-818 of the study report.
b Includes main study (30/dose) and satellite (10/dose) animals.

5. Maternal postmortem results

a. Macroscopic examination - Other than examining the uteri of the females that did not litter, no macroscopic evaluations of the dams were performed. One 600 ppm female (animal # 137) that did not litter had 2 dead fetuses lodged in the right uterine horn at the junction of the uterine horns. The control and other 600 ppm females that did not litter lacked implantation sites.

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

c. Cholinesterase determinations - Cholinesterase activity was decreased ($p \leq 0.01$) at ≥60 ppm in the erythrocytes (↓50-59%, GD 22 and ↓55-57%, LD 22) and plasma (↓60-84%, GD 22 and ↓59-78%, LD 22; Table 7). Plasma cholinesterase activity was also decreased ($p \leq 0.01$) by 13% at 3 ppm on LD 22. Additionally at 600 ppm, brain cholinesterase activity was decreased by 44% ($p \leq 0.01$) on GD 22, and by 26% (not statistically significant) on LD 22.

Table 7. Mean (±SD) cholinesterase activity in P females administered Profenofos in the diet from GD 7 to LD 22. [a]

Compartment	Dose (ppm)			
	0	3	60	600
Gestation Day 22 (n=5)				
Brain (IU/g)	6.20±1.97	5.62±0.97	5.38±1.26	3.48±0.54** (↓44)
Erythrocyte (U/L)	2295±140	2184±172	1151±83** (↓50)	951±111** (↓59)
Plasma (U/L)	955±127	1010±158	386±30** (↓60)	150±9** (↓84)
Lactation Day 22 (n=5)				
Brain (IU/g)	5.07±0.80	6.28±0.98	7.02±3.14	3.77±1.12 (↓26)
Erythrocyte (U/L)	2329±99	2169±340	1058±75** (↓55)	999±131** (↓57)
Plasma (U/L)	611±56	529±29** (↓13)	251±35** (↓59)	134±24** (↓78)

a Data were obtained from page 166 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.
** Significantly different from controls at $p \leq 0.01$

B. OFFSPRING

1. **Viability and clinical signs** - No significant differences in live litter size or post-natal survival were observed in any treated group through PND 5 (Table 8). On PND 5, all litters contained 7 or 8 animals (assumed by the reviewers); however, the mean number of pups/litter was not reported from PND 5 (post-cull) to PND 22. The sex ratio (% of male pups) was increased ($p \leq 0.05$) slightly at ≥60 ppm (55.6-59.4% treated vs 49.5% controls); however, this finding was not considered to be treatment-related. Clinical signs were limited to hypothermia on PND 1; however, this finding was generally limited to animals that accounted for whole litter losses.

Table 8. F_1 live litter size and viability. [a]

Observation	Dose (ppm)			
	0	3	60	600
Number of litters	34	35	35	33
Whole litter losses	0	1	3	3
Total # of pups delivered	399	423	461*	399
# of liveborn	398	419	453	393
# of stillborn	1	4	8	6
Sex ratio (% male)				
PND 1	49.5±11.7	52.5±13.0	55.6±12.7*	59.4±16.2**
PND 5[b]	49.2±13.3	52.3±13.1	55.2±12.6*	59.8±15.2**
# of deaths (PNDs 1-5[b])	12	17	12	10
Mean litter size[d]				
PND 1	11.7±2.6	11.9±2.4	12.8±2.2	11.8±2.6
PND 5[b]	11.4±2.8	11.4±2.5	12.4±2.1	11.4±2.4
PND 5[c]	NR	NR	NR	NR
PND 12	NR	NR	NR	NR
PND 18	NR	NR	NR	NR
PND 22	NR	NR	NR	NR
Live birth index (%)	99.8	99.0	98.6	98.7

a Data were obtained from pages 79-84 of the study report.
b Before culling
c After culling
d Excluding whole litter losses
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$
NR Not reported

2. **Body weight and food consumption** - Throughout pre-weaning (Days 5-22), body weights were decreased ($p \leq 0.01$) in both sexes at 600 ppm (↓6-12%, Table 9a). Also, overall (Days 5-22) pre-weaning body weight gain was decreased in both sexes at 600 ppm (↓11-12%). Post-weaning body weights were decreased ($p \leq 0.05$) in the 600 ppm males (↓5%, PND 29) and females (↓3-5%, PND 29-36; Table 9b); however, body weights were similar between treated

and control groups from PND 43-63. 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 9a. Selected mean (± SD) F_1 pup pre-weaning body weights and body weight gains (g). [a]

Post-natal Day	Dose (ppm)			
	0	3	60	600
Males				
1	6.0±0.5	6.0±0.5	5.8±0.5	5.8±0.4
5[bd]	9.4	9.3	9.3	8.8** (↓6)
5[c]	9.8±1.2	9.7±1.2	9.2±0.9	8.9±1.1** (↓9)
22[d]	51.9	53.1	51.5	47.4** (↓9)
Overall (Days 5-22) Gain [e]	42.7	43.9	42.0	37.5 (↓12)
Females				
1	5.6±0.5	5.6±0.5	5.5±0.5	5.5±0.4
5[bd]	8.9	8.8	8.9	8.3** (↓7)
5[c]	9.4±1.2	9.2±1.2	8.9±1.0	8.3±0.9** (↓12)
22[d]	49.9	50.8	49.9	46.0** (↓8)
Overall (Days 5-22) Gain [e]	41.2	42.0	40.9	36.5 (↓11)

a Data were obtained from pages 85, 86, 120, and 122 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically. During pre-weaning, n=31-34 litters (pre-culling) or n=23-27 litters (post-culling).
b Pre-culling
c Post-culling
d Adjusted means
e Calculated by reviewers using unadjusted mean data from Days 5 (post-cull) to 22.
** Significantly different from controls at $p \leq 0.01$

Table 9b. Selected adjusted mean F_1 pup post-weaning body weights and body weight gains (g).[a]

Post-natal Day	Dose (ppm)			
	0	3	60	600
Males				
29	93.8	95.2	93.5	89.0** (↓5)
50	259.6	259.5	258.8	252.7
63	348.9	349.2	349.6	339.6
Overall (Days 22-63) gain [b]	297.0	296.1	298.1	292.2
Females				
29	87.2	88.6	87.5	82.9** (↓5)
36	126.9	127.5	126.4	123.4* (↓3)
63	211.7	211.1	211.2	213.1
Overall (Days 22-63) gain [b]	161.8	160.3	161.3	167.1

a Data were obtained from pages 120-123 of the study report. Percent difference from controls (calculated by reviewers) is presented parenthetically.
b Calculated by reviewers using adjusted mean data.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

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 10).

Table 10. Sexual maturation (mean days ± SD) in F_1 generation rats.[a]

Parameter	Dose (ppm)			
	0	3	60	600
N (M/F)	90/93	90/91	91/85	79/77
Preputial separation (Males)	43.9±1.1	44.3±1.1	43.9±1.0	44.5±1.7
Vaginal patency (Females)	34.6±1.3	35.1±1.5	35.0±1.5	34.9±1.0

a Data were obtained from pages 124-125 of the study report.

4. Behavioral assessments

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

b. Motor activity - No significant differences from controls were noted in overall session activity counts in either sex at any dose (Table 11). Overall activity counts were increased on PND 18-60 compared to PND 14. Several isolated significant differences were noted at various intervals in the females on PNDs 14, 18, and 60 (Table 12). No significant findings were observed in the males during any sub-session at any time point. Habituation was unaffected by treatment.

Table 11. Mean (±SD) motor activity data (counts) in F_1 pups. [a]

Post-natal Day	Dose (ppm)			
	0	3	60	600
Males				
14	166.8±137.9	151.6±148.6	130.2±150.8	171.6±131.2
18	247.6±121.3	212.2±168.8	254.0±184.2	180.2±136.5
22	419.5±176.6	411.1±147.6	400.8±159.8	501.4±175.2
60	555.8±136.2	553.9±142.4	572.6±80.0	548.7±107.5
Females				
14	230.2±153.2	151.3±102.4	170.5±112.4	140.9±107.1
18	159.6±104.0	243.8±127.6	182.9±118.2	192.7±132.4
22	446.4±143.5	446.5±153.2	497.7±141.8	433.1±135.0
60	641.4±94.0	628.0±50.6	565.1±160.3	555.5±104.3

a Data were obtained from pages 126-133 of the study report; n=11-14.

Table 12. Mean (±SD) sub-session motor activity (counts) in F_1 female pups. [a]

Sub-session (Minutes)		Dose (ppm)			
		0	3	60	600
PND 14	1-5	57.3±29.0	38.5±30.2	48.1±30.3	38.0±28.0
	6-10	39.6±22.6	23.3±15.9	28.5±23.1	24.1±26.1
	11-15	30.4±25.8	14.7±13.5* (↓52)	24.8±18.7	11.8±14.9* (↓61)
	16-20	22.6±21.9	14.5±15.0	17.9±14.4	11.9±13.9
	21-25	15.1±16.9	14.0±16.3	14.2±19.8	14.2±15.4
	26-30	15.4±14.8	9.3±11.2	13.1±17.0	11.4±11.5
	31-35	11.1±22.1	9.8±15.5	5.8±10.8	13.8±17.7
	36-40	9.8±19.8	11.1±17.8	9.2±17.2	9.3±12.5
	41-45	17.6±26.2	9.8±16.4	5.5±8.8	4.4±7.8
	46-50	11.3±20.9	6.4±13.1	3.4±5.8	2.1±2.7
PND 18	1-5	17.9±12.1	18.2±12.1	33.5±29.0*(↑89)	28.1±20.7
	6-10	15.4±15.4	27.2±21.8	18.8±18.3	20.3±16.8
	11-15	17.6±19.7	16.3±13.7	13.9±18.4	12.6±17.7
	16-20	13.0±14.6	21.6±22.1	17.8±17.8	21.8±17.6
	21-25	20.3±19.3	18.5±17.7	22.3±28.7	10.3±8.7
	26-30	18.1±19.5	30.2±23.9	17.3±22.2	16.6±21.5
	31-35	15.6±13.9	29.3±26.8	17.2±22.3	20.3±24.1
	36-40	13.4±19.2	29.5±23.8*(↑120)	7.2±10.8	16.9±23.1
	41-45	17.0±24.4	26.4±22.3	12.9±18.4	18.8±24.2
	46-50	11.3±19.6	26.8±20.9	21.9±25.4	27.1±24.6

(table continues next page)

Sub-session (Minutes)		Dose (ppm)			
		0	3	60	600
PND 22	1-5	53.3±21.2	52.1±23.1	56.5±20.8	46.5±19.5
	6-10	44.6±15.9	45.8±20.4	48.9±22.8	43.2±14.6
	11-15	45.6±19.8	52.1±24.7	60.2±18.9	36.8±22.6
	16-20	49.3±23.8	50.6±19.2	45.2±22.9	44.5±25.6
	21-25	42.9±26.6	51.5±13.5	52.2±22.4	47.3±20.6
	26-30	47.3±21.5	42.8±22.4	50.2±16.4	44.4±23.5
	31-35	39.6±20.1	39.8±21.5	54.8±24.2	47.2±18.1
	36-40	42.6±18.5	35.8±26.7	48.2±21.6	43.8±22.0
	41-45	40.8±27.2	38.7±30.0	37.1±22.1	42.7±25.0
	46-50	40.3±22.0	37.3±30.8	44.4±30.1	36.9±21.7
PND 60	1-5	70.1±7.8	65.7±10.0	61.5±20.5	60.8±8.5
	6-10	67.9±11.8	67.7±9.7	61.8±19.7	59.7±10.3
	11-15	64.4±10.7	67.6±12.4	66.8±20.1	59.9±16.8
	16-20	65.1±15.1	60.3±9.6	62.7±18.0	58.8±16.6
	21-25	60.2±15.9	62.5±15.0	54.7±18.6	52.4±19.7
	26-30	63.1±17.3	60.8±7.1	48.2±24.3* (↓24)	47.5±17.4* (↓25)
	31-35	64.3±14.0	65.5±9.5	47.2±20.5** (↓27)	58.5±16.8
	36-40	62.3±16.1	58.3±14.4	48.1±25.5* (↓23)	52.3±13.8
	41-45	62.7±16.1	59.7±10.8	56.2±25.1	54.0±15.9
	46-50	61.4±10.7	59.8±12.5	57.7±27.1	51.5±20.3

a Data were obtained from pages 127, 129, 131, and 133 of the study report; n=12-14. Percent difference from control (calculated by reviewers) is presented parenthetically.

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

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

c. <u>Auditory startle reflex habituation</u> - No treatment-related differences from control were noted in startle response maximum amplitude at any dose in either sex on PNDs 23 or 60 (Table 13). On PND 60, latency was increased ($p \leq 0.05$) in the 60 ppm females during Block 1 (↑20%, Table 14). No significant differences in latency were observed in the males on PNDs 23 or 60, or in the females on PND 23.

Table 13. Mean (±SD) auditory startle reflex maximum amplitude (g) in F_1 rats. [a]

Observation [b]		Dose (ppm)			
		0	3	60	600
Males					
PND 23	Block 1	273.5±118.5	336.5±181.3	282.6±74.4	254.3±88.9
	Block 2	214.2±83.7	285.0±162.8	221.9±57.1	201.8±68.4
	Block 3	202.8±55.5	215.1±86.0	192.4±47.4	202.5±60.4
	Block 4	179.7±58.2	199.7±82.0	169.4±51.7	171.4±64.4
	Block 5	191.4±53.9	181.5±74.6	177.0±48.7	173.6±50.7
PND 61	Block 1	923.6±338.2	861±7±301.5	953.8±211.7	732.5±241.4
	Block 2	745.4±261.0	686.2±188.4	770.7±229.8	721.3±282.8
	Block 3	625.7±274.2	647.5±231.1	656.7±111.7	584.1±242.5
	Block 4	570.1±227.6	586.6±233.7	537.9±219.2	520.8±307.4
	Block 5	566.8±249.4	513.6±197.2	508.3±172.1	455.9±251.2
Females					
PND 23	Block 1	248.5±65.6	262.9±72.4	242.9±64.6	197.4±77.6
	Block 2	236.4±80.2	232.9±55.3	209.7±62.2	198.6±69.8
	Block 3	204.7±73.5	190.7±48.6	191.6±66.3	174.2±60.3
	Block 4	187.2±53.8	176.4±35.6	180.2±59.7	153.1±60.2
	Block 5	172.6±44.8	160.8±39.5	164.1±50.7	150.9±64.1
PND 61	Block 1	595.8±172.6	612.9±263.0	500.5±229.4	645.1±277.7
	Block 2	637.7±263.1	492.9±149.9	546.6±361.1	594.6±283.4
	Block 3	421.4±221.0	432.0±106.9	441.5±195.9	515.8±297.4
	Block 4	436.7±201.1	376.8±122.1	389.8±224.7	439.4±234.4
	Block 5	424.2±202.6	370.5±122.0	373.7±160.8	424.4±169.2

a Data were obtained from pages 134-137; n=11-14.
b Block=10 consecutive trials

Table 14. Mean (±SD) auditory startle reflex latency (msec) in F_1 rats. [a]

Observation [b]		Dose (ppm)			
		0	3	60	600
Males					
PND 23	Block 1	28.3±8.7	30.5±11.0	25.0±4.6	24.6±6.2
	Block 2	25.0±6.5	24.1±9.6	20.8±2.5	22.3±6.1
	Block 3	23.9±9.3	26.2±11.2	22.0±2.8	22.9±6.5
	Block 4	27.3±11.1	26.0±9.4	22.1±4.1	23.5±6.2
	Block 5	24.8±8.1	27.1±10.0	21.6±3.0	22.1±4.6
PND 61	Block 1	26.2±9.0	25.7±6.7	22.6±2.1	24.3±3.0
	Block 2	22.0±3.3	22.6±2.8	21.5±1.8	22.3±3.2
	Block 3	24.5±2.9	24.3±3.6	22.4±3.4	24.7±4.4
	Block 4	25.8±2.8	24.1±3.2	24.0±2.7	25.8±5.2
	Block 5	24.4±2.6	25.1±4.0	24.4±3.2	26.5±4.0
Females					
PND 23	Block 1	26.4±8.1	26.2±6.3	25.7±4.4	28.1±5.2
	Block 2	22.5±6.4	23.0±4.4	23.2±6.9	24.3±4.5
	Block 3	23.6±8.0	23.1±6.6	23.3±5.6	25.1±6.5
	Block 4	23.0±5.0	23.4±3.1	26.0±11.3	23.5±4.1
	Block 5	23.8±5.0	23.6±4.5	24.2±5.1	24.1±5.4
PND 61	Block 1	22.7±2.6	25.0±4.0	27.3±6.4* (↑20)	24.8±3.9
	Block 2	22.8±2.8	23.0±3.8	26.2±6.0	24.0±4.1
	Block 3	24.7±3.9	24.3±3.4	25.2±4.3	26.9±6.1
	Block 4	24.7±4.1	25.0±2.6	24.8±5.8	28.0±6.3
	Block 5	24.2±4.5	25.4±3.3	26.0±5.2	27.0±8.6

a Data were obtained from pages 138-141; n=11-14. Percent difference from control (calculated by reviewers) is presented parenthetically.

b Block=10 consecutive trials

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

d. <u>Learning and memory testing</u> - No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the water maze test (Table 15a). 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 were noted in the 3 and 600 ppm males and in the ≥3 ppm females. These findings are not considered to be toxicologically significant, because they were transient and there was no clear dose-response. The increased ($p \leq 0.05$) straight channel swimming times noted in the 3 and 60 ppm males and in the 600 ppm females were considered unrelated to treatment because there was no clear dose-response (Table 15b).

Table 15a. Mean (±SD) water maze performance (swimming times [sec]) in F_1 rats. [a]

Parameter	Trial	Dose (ppm)			
		0	3	60	600
Males					
Learning (PND 21)	1	17.31±8.69	18.46±8.44	16.46±9.18	14.55±8.31
	2	10.83±8.56	11.08±7.20	12.94±7.16	12.10±7.77
	3	10.09±6.62	12.52±7.94	10.04±6.27	9.42±8.56
	4	9.62±7.86	10.97±7.87	7.86±5.63	9.27±6.67
	5	9.69±7.23	8.45±5.82	8.79±6.47	7.01±4.69
	6	7.38±5.17	7.85±4.25	8.07±5.99	8.11±6.05
Memory (PND 24)	1	8.84±4.34	5.62±1.99** (↓36)	7.38±4.35	9.50±5.90
	2	4.50±2.19	4.39±2.33	3.63±1.98	5.01±3.10
	3	4.69±3.46	5.29±3.98	3.99±2.99	3.16±0.91
	4	4.30±2.52	4.33±2.74	4.56±3.51	4.38±2.67
	5	5.14±4.14	4.23±3.29	4.95±4.01	4.56±3.69
	6	5.26±4.25	4.45±2.45	4.91±3.05	4.21±2.20
Learning (PND 59)	1	10.98±2.85	10.56±4.43	9.97±4.17	9.45±4.91
	2	6.09±4.37	5.09±2.49	7.11±3.54	5.44±2.44
	3	4.91±2.24	4.77±2.56	4.80±1.93	5.38±2.33
	4	4.48±2.61	5.51±3.49	5.60±2.23	5.66±3.63
	5	4.79±2.60	7.05±6.45	5.60±4.56	4.97±3.19
	6	5.11±2.64	5.53±2.69	5.90±3.99	4.70±2.62
Memory (PND 62)	1	4.49±3.79	5.57±3.27	5.23±2.83	6.23±5.74
	2	5.00±5.13	7.83±7.32	8.04±7.53	9.75±8.78* (↑95)
	3	6.52±5.96	7.69±6.02	5.02±3.43	7.76±7.72
	4	5.18±4.29	7.66±8.89	7.42±6.95	6.76±5.40
	5	6.19±5.56	7.21±7.14	7.64±7.94	7.53±7.39
	6	6.13±6.26	8.74±9.15	7.62±6.93	6.29±3.71
Females					
Learning (PND 21)	1	15.72±7.46	14.49±6.69	14.43±6.05	13.62±6.77
	2	9.59±6.03	11.55±8.44	10.18±7.49	9.95±6.57
	3	7.13±4.59	11.93±7.13* (↑67)	13.24±8.40** (↑86)	11.51±7.83* (↑61)
	4	7.64±4.86	9.47±5.58	8.53±6.40	8.53±6.84
	5	6.87±6.03	7.46±4.25	6.69±5.47	7.31±6.35
	6	9.54±7.28	8.23±6.56	7.53±5.81	7.54±5.05

(table continues next page)

Parameter	Trial	Dose (ppm)			
		0	3	60	600
Memory (PND 24)	1	7.48±4.20	7.58±4.42	7.08±3.77	7.89±5.72
	2	4.81±3.21	4.48±2.84	4.71±2.81	3.69±1.87
	3	3.72±2.35	3.17±1.29	5.25±4.32	4.80±3.00
	4	5.27±4.57	4.02±2.63	5.24±4.07	4.11±3.77
	5	4.85±3.24	4.90±2.93	5.66±5.12	4.16±2.05
	6	4.86±3.99	3.99±1.57	4.01±2.17	4.34±2.44
Learning (PND 59)	1	11.80±5.53	10.65±3.61	11.29±3.22	11.63±4.26
	2	6.22±3.51	7.60±4.86	7.96±4.46	7.83±5.08
	3	5.11±3.08	5.49±3.80	7.17±3.52* (↑40)	6.42±3.70
	4	4.97±2.78	4.53±2.55	7.97±5.20** (↑60)	5.47±2.53
	5	5.16±3.46	5.28±3.53	5.54±2.59	8.51±5.71** (↑65)
	6	6.97±6.80	5.05±2.91	6.58±5.86	7.14±6.41
Memory (PND 62)	1	5.82±3.97	4.84±2.52	5.56±3.28	6.45±6.45
	2	7.12±6.67	5.91±5.67	6.14±5.86	10.57±9.74
	3	7.70±8.34	7.99±8.22	7.75±6.45	10.23±9.57
	4	7.81±6.98	6.87±4.54	7.36±6.91	9.92±7.83
	5	10.09±9.23	5.58±5.47* (↓45)	8.56±8.05	7.05±8.28
	6	10.00±9.48	5.77±5.21* (↓42)	5.28±4.22* (↓47)	5.69±6.97* (↓43)

a Data were obtained from pages 142-149 of the study report; n=23-27. Percent difference from control (calculated by reviewers) is presented parenthetically.
* Statistically different from controls at $p \leq 0.05$
** Statistically different from controls at $p \leq 0.01$

Table 15b. Mean (±SD) straight channel swimming times (sec) in F_1 rats. [a]

Observation	Dose (ppm)			
	0	3	60	600
Males				
Learning (PND 21)	3.23±0.71	5.11±4.44* (↑58)	4.14±1.99	4.93±3.86
Memory (PND 24)	2.86±0.75	2.80±0.97	3.81±2.18** (↑33)	2.74±0.60
Learning (PND 59)	3.20±1.51	3.27±0.99	3.21±1.23	3.27±1.10
Memory (PND 62)	2.80±1.83	2.69±1.03	2.72±0.69	2.96±1.48
Females				
Learning (PND 21)	3.28±1.67	3.77±2.17	3.52±1.56	6.26±6.99** (↑91)
Memory (PND 24)	3.14±1.65	3.74±2.24	3.24±1.68	3.10±1.58
Learning (PND 59)	3.30±1.51	2.75±0.72	3.89±2.51	2.54±0.66
Memory (PND 62)	2.85±0.71	2.64±1.14	2.77±0.94	2.75±1.05

a Data were obtained from pages 142-149 of the study report; n=23-27. Percent difference from control (calculated by reviewers) is presented parenthetically.
* Statistically different from controls at $p \leq 0.05$
** Statistically different from controls at $p \leq 0.01$

5. Postmortem results

a. Brain weights - On PND 12, absolute brain weight was decreased ($p \leq 0.05$) in the 600 ppm males (↓4%, Table 16). However, because there was no difference in relative (to body) brain weight, this finding was considered to be related to the decreased terminal body weight (↓7%, not statistically significant), and not an effect of treatment. Absolute brain weights were similar between treated and control females throughout the study. Relative (to body) brain weights were similar between treated and control groups in both sexes throughout the study.

Table 16. Mean (±SD) absolute (g) and relative (to body, %) brain weights in F_1 rats. [a]

Parameter	Dose (ppm)			
	0	3	60	600
Males				
PND 12 (n=12-14)				
Terminal Body Weight (g)	22.1±2.3	23.0±3.5	21.8±3.0	20.5±2.3 (↓7)
Absolute Brain Weight (g)	1.13±0.05	1.14±0.05	1.14±0.06	1.09±0.06* (↓4)
Relative (to body) Weight (%)	5.17±0.46	5.05±0.66	5.30±0.69	5.33±0.41
PND 63 (n=12-14)				
Terminal Body Weight (g)	353.9±28.4	355.1±26.9	346.3±19.5	337.5±19.3
Absolute Brain Weight (g)	2.04±0.06	2.05±0.09	2.03±0.05	1.99±0.07
Relative (to body) Weight (%)	0.58±0.04	0.58±0.03	0.59±0.03	0.59±0.04
PND 63 (post-perfusion, n=11-13)				
Terminal Body Weight (g)	357.4±32.2	352.3±27.5	352.1±22.5	340.0±22.3
Absolute Brain Weight (g)	2.00±0.09	2.05±0.09	2.04±0.07	1.97±0.06
Relative (to body) Weight (%)	0.56±0.05	0.59±0.04	0.58±0.05	0.58±0.05
Females				
PND 12 (n=12-14)				
Terminal Body Weight (g)	21.8±2.4	21.2±2.7	21.0±2.7	19.7±2.8
Absolute Brain Weight (g)	1.11±0.06	1.09±0.05	1.10±0.04	1.08±0.08
Relative (to body) Weight (%)	5.11±0.50	5.18±0.59	5.34±0.79	5.55±0.64
PND 63 (n=12-14)				
Terminal Body Weight (g)	212.3±17.1	211.0±18.0	218.4±10.5	215.0±17.8
Absolute Brain Weight (g)	1.89±0.06	1.86±0.08	1.87±0.04	1.86±0.08
Relative (to body) Weight (%)	0.89±0.07	0.89±0.07	0.86±0.05	0.87±0.06
PND 63 (post-perfusion, n=11-13)				
Terminal Body Weight (g)	218.8±12.6	220.2±15.3	212.8±16.9	206.9±13.3
Absolute Brain Weight (g)	1.86±0.08	1.86±0.07	1.85±0.09	1.86±0.07
Relative (to body) Weight (%)	0.85±0.06	0.85±0.05	0.87±0.08	0.90±0.05

a Data were obtained from pages 171-173 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.

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

b) Neuropathology

1) Macroscopic examination - No treatment-related gross pathological findings were noted in any treated group.

2) Microscopic examination - No adverse histopathological findings were noted in any group. No adverse neuropathological effects were noted in the 600 ppm animals on PND 63. The demyelination noted in the peripheral nerves occurred with equal frequency in the control and 600 ppm animals, and is commonly noted in rats. Minor differences ($p \leq 0.05$) in various morphometric measurements were noted at 600 ppm in both sexes on PND 12 and 63 (Table 17). However, there was no consistency in the areas affected either between sexes or at different ages, other measurements for the same structures at other levels showed no differences, and the values were within the historical control ranges provided; therefore, these findings were not considered to be treatment-related.

Table 17. Mean (±SD) morphometric measurements in F_1 rats. [a]

Parameter		Dose (ppm)		Historical Control Range [b]
		0	600	
PND 12				
Males				
Hippocampus	Width of dentate gyrus	0.48±0.03	0.45±0.03* (↓6)	0.27-0.565
Cerebellum	Height	3.83±0.16	3.54±0.25** (↓8)	2.86-4.57
	Thickness of inner granular layer of the pre-culminate fissure	146±19	131±15* (↓10)	95-187
	Thickness of molecular layer of the pre-pyramidal fissure	66.9±14.5	56.4±8.0* (↓16)	24.8-78.5
Females				
Hippocampus	Length from midline	2.81±0.17	2.60±0.21* (↓7)	1.79-3.22
Corpus callosum	Thickness	0.52±0.08	0.61±0.06** (↑17)	0.28-0.82
Piriform cortex	Thickness	1.01±0.05	1.06±0.05* (↑5)	0.63-1.28
PND 63				
Males				
Piriform cortex	Thickness	1.38±0.09	1.29±0.07* (↓7)	1.08-1.87
Females				
Thalamus	Width	7.72±0.36	7.34±0.40* (↓5)	6.6-8.29

a Data were obtained from pages 178-193 of the study report; n=6-14. Percent difference from control (calculated by reviewers) is presented parenthetically.

b Data obtained from Appendix I on pages 222-223 of the study report; n=6-12 studies.

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

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

c) Cholinesterase determinations - No effects on cholinesterase activity (brain, erythrocyte, or plasma) were noted in the fetuses at any dose in either sex examined at GD 22 (Table 18). However, postnatally in the 600 ppm pups, the following decreases ($p \leq 0.05$) in cholinesterase activity were noted: (i) erythrocyte, ↓22-40% in the females on PNDs 12 and 22; (ii) plasma, ↓25-50% in the males and ↓24-54% in the females on PNDs 12 and 22; and brain, ↓11% in females at PND 5. Additionally, plasma cholinesterase activity was decreased by 23% in the 60 ppm females on PND 22.

Table 18. Mean (±SD) cholinesterase activities in the satellite group F_1 fetuses and pups. [a]

Compartment	Dose (ppm)							
	0	3	60	600	0	3	60	600
	Males				Females			
Gestation Day 22 (n=4-5)								
Brain (IU/g)	1.34±0.05	1.35±0.12	1.43±0.11	1.36±0.15	1.46±0.16	1.41±0.13	1.39±0.15	1.36±0.13
Erythrocyte (U/L)	2012±356	2503±389	2224±264	1956±303	2068±258	2102±573	2639±416	2202±514
Plasma (U/L)	336±35	345±19	371±21	330±28	364±19	342±28	355±33	334±17
PND 5 (n=3-5)								
Brain (IU/g)	2.44±0.14	2.49±0.15	2.44±0.18	2.79±1.05	2.30±0.13	2.42±0.09	2.37±0.14	2.05±0.05*(↓11)
Erythrocyte (U/L)	2328±334	2365±422	2259±370	2224±529	2517±266	2453±291	2552±477	2297±383
Plasma (U/L)	651±43	600±26	611±46	588±103	635±44	587±51	584±41	555±118
PND 12 (n=2-5)								
Brain (IU/g)	4.52±0.21	4.23±0.19	4.42±0.25	4.05±0.77	4.20±0.51	4.32±0.34	4.33±0.33	4.29±0.47
Erythrocyte (U/L)	3397±133	3203±420	3266±421	3027±45	3240±335	3213±262	3075±434	2529±223*(↓22)
Plasma (U/L)	842±16	768±71	817±74	635±85*(↓25)	823±86	784±61	785±72	623±164*(↓24)
PND 22 (n=2-5)								
Brain (IU/g)	4.41±0.28	4.82±0.43	4.61±0.27	4.58±1.13	4.36±0.27	4.32±0.19	4.60±0.33	4.38±0.56
Erythrocyte (U/L)	2949±574	2816±227	2539±347	2144±571	3109±644	2780±402	2600±45	1880±334*(↓40)
Plasma (U/L)	729±49	664±34	678±274	366±130*(↓50)	704±44	608±54	543±86**(↓23)	323±60**(↓54)

a Data were obtained from pages 167-170 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.

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

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

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigators concluded that treatment with Profenofos at 600 ppm caused decreased body weights and food consumption in the parent animals. Additionally, cholinesterase activity was decreased in dams receiving ≥60 ppm. No adverse effects on reproductive performance were observed at any dose. In the offspring, general toxicity was characterized by decreased body weight and body weight gains at 600 ppm. Cholinesterase activity was decreased at 60 ppm (females) and 600 ppm (both sexes). No effects on motor activity or learning and memory were observed at any dose in either sex. No adverse neuropathological effects on various brain or peripheral nerve tissues were observed. The maternal NOAEL was 3 ppm. The offspring NOAEL was 60 ppm.

B. REVIEWER'S COMMENTS - The was well conducted based on the many tables that showed tight mean values and standard deviations. However, the contractor wrote an extermly poor executive summary, which the EPA reviewer had to re-write. The study has two main points to focus on, the maternal effects of the Profenofos, as well as the offspring effects. EPA reviewer disagreed with the contractor's review regarding plasma Cholinesterase activity in the plasma of the maternal animals based on LD 22. The contractor used brain and red blood cells (60 ppm for LOAEL and 3 ppm for NOAEL). EPA reviewer divided the three compartments into two, the brain and red blood cells with 60 ppm for LOAEL and 3 ppm for NOAEL, the plasma with a LOAEL of 3 ppm, and a NOAEL of < 3 ppm.

B.1. MATERNAL ANIMALS

Three dams were sacrificed prior to scheduled termination as they failed to litter, one in the control group and two at 600 ppm. All other dams survived to scheduled sacrifice. No treatment-related clinical signs or FOB effects were noted at any dose throughout the study.

At 600 ppm, body weights were generally decreased from GD 15 through LD 22 (↓2-5%). Overall body weight gains were decreased by 11% during GDs 1-22 and by 17% during LDs 1-22. These decreases corresponded to the reductions noted in absolute food consumption (↓4-11%) during the gestation and lactation periods. No effects on body weight or body weight gain were noted at ≤60 ppm.

Food consumption was reduced in the 600 ppm dams during GDs 7-22 (↓4-6%), and during LDs 12-23 (↓7-11%). Additionally, food consumption was decreased by 5% in the 60 ppm dams on LDs 18-21; however, as there were no corresponding significant decreases in body weight observed in the 60 ppm animals, this finding is not considered to be treatment-related. The increased food consumption noted in the 60 ppm dams during LDs 8-12 was considered incidental.

All indices of reproductive performance (fertility index, gestation length, gestation index, and incidence of dystocia) were comparable between treated and control animals.

No macroscopic or microscopic examination was performed, other than examining the uteri of the females that did not litter, no macroscopic evaluations of the dams were performed.

Cholinesterase activity was decreased at ≥60 ppm in the erythrocytes (↓50-59%, GD 22 and ↓55-57%, LD 22) and plasma (↓60-84%, GD 22 and ↓59-78%, LD 22). Plasma cholinesterase activity was also decreased by 13% at 3 ppm on LD 22. Additionally at 600 ppm, brain cholinesterase activity was decreased by 44% on GD 22, and by 26% (not statistically significant) on LD 22.

The maternal LOAEL is 60 ppm based on decreased body weight, body weight gains, and food consumption. The maternal NOAEL was 3 ppm.

The cholinesterase LOAEL was 60 ppm for both the brain and red blood cells.
The cholinesterase NOAEL was 3 ppm for both compartments.

The plasma cholinesterase LOAEL was 3 ppm based on LD 22.
The plasma cholinesterase NOAEL < 3 ppm based on LD 22.

B.2 OFFSPRING

No significant differences in live litter size or post-natal survival were observed in any treated group through PND 5. On PND 5, all litters contained 7 or 8 animals (assumed by the reviewers); however, the mean number of pups/litter was not reported from PND 5 (post-cull) to PND 22. The sex ratio (% of male pups) was increased slightly at ≥60 ppm (55.6-59.4% treated vs 49.5% controls); however, this finding was not considered to be treatment-related. Clinical signs were limited to hypothermia on PND 1; however, this finding was generally limited to animals that accounted for whole litter losses.

Throughout pre-weaning (Days 5-22), body weights were decreased in both sexes at 600 ppm (↓6-12%). Also, overall (Days 5-22) pre-weaning body weight gain was decreased in both sexes at 600 ppm (↓11-12%). Post-weaning body weights were decreased in the 600 ppm males (↓5%, PND 29) and females (↓3-5%, PND 29-36); however, body weights were similar between treated and control groups from PND 43-63. Overall (PND 22-63) body weight gains were similar between treated and control groups. Food consumption was not reported for the F_1 animals.

No treatment-related effect on mean time to preputial separation or mean time to vaginal patency was observed.

No treatment-related behavioral effects were observed at any dose in either sex.

No significant differences in motor activity from controls were noted in overall session activity counts in either sex at any dose. Overall activity counts were increased on PND 18-60 compared to PND 14. Several isolated significant differences were noted at various intervals in the females on PNDs 14, 18, and 60. No significant findings were observed in the males during any sub-session at any time point. Habituation was unaffected by treatment.

No treatment-related differences in auditory startle reflex habituation from control were noted in startle response maximum amplitude at any dose in either sex on PNDs 23 or 60. On PND 60, latency was increased in the 60 ppm females during Block 1 (↑20%, Table 14). No significant differences in latency were observed in the males on PNDs 23 or 60, or in the females on PND 23.

No treatment-related differences in learning or memory testing were noted in any treated group relative to concurrent controls in the water maze test. 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 from control were noted in the 3 and 600 ppm males and in the ≥3 ppm females. These findings are not considered to be toxicologically significant, because they were transient and there was no clear dose-response. The increased straight channel swimming times noted in the 3 and 60 ppm males and in the 600 ppm females were considered unrelated to treatment because there was no clear dose-response.

On PND 12, absolute brain weight was decreased in the 600 ppm males (↓4%). However, because there was no difference in relative (to body) brain weight, this finding was considered to be related to the decreased terminal body weight (↓7%, not statistically significant), and not an effect of treatment. Absolute brain weights were similar between treated and control females throughout the study. Relative (to body) brain weights were similar between treated and control groups in both sexes throughout the study.

No treatment-related gross or microscopic pathological findings were noted in any treated group. No adverse neuropathological effects were noted in the 600 ppm animals on PND 63. The demyelination noted in the peripheral nerves occurred with equal frequency in the control and 600 ppm animals, and is commonly noted in rats. Minor differences in various morphometric measurements were noted at 600 ppm in both sexes on PND 12 and 63. However, there was no consistency in the areas affected either between sexes or at different ages, other measurements for the same structures at other levels showed no differences, and the values were within the historical control ranges provided; therefore, these findings were not considered to be treatment-related.

No effects on cholinesterase activity (brain, erythrocyte, or plasma) were noted in the fetuses at any dose in either sex examined at GD 22. However, postnatally in the 600 ppm pups, the following decreases in cholinesterase activity were noted: (i) erythrocyte, ↓22-40% in the females on PNDs 12 and 22; (ii) plasma, ↓25-50% in the males and ↓24-54% in the females on PNDs 12 and 22; and brain, ↓11% in females at PND 5. Additionally, plasma cholinesterase activity was decreased by 23% in the 60 ppm females on PND 22.

The offspring LOAEL is 600 ppm, based on decreased body weights and body weight gains. The offspring NOAEL is 60 ppm.

No evidence of developmental neurotoxicity was observed at any dose.

The cholinesterase LOAEL was 60 ppm. The cholinesterase NOAEL was 3 ppm.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats.

C. **STUDY DEFICIENCIES** - The following deficiencies were noted:

- 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.

DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg	Doses tested mg/kg	NOAEL mg/kg	LOAEL mg/kg	Endpoint(s)	Comments
111401	46025401	dev neurotox	rats	GD 7-LD 22	oral	dietary	0.3-103.4	0/0, 0.3/10.5, 5.1/10.7, and 50.6/103.4 [gestation/lactation]	0.3	5.1	Decr BW, BWG, FC, CHeA (brain, plasma, RBC)	Maternal
111401	46025401	dev neurotox	rats	GD 7-LD 22	oral	dietary	0.3-103.4	0/0, 0.3/10.5, 5.1/10.7, and 50.6/103.4 [gestation/lactation]	0.3	5.1	Decr BW, BWG, CHeA (brain, plasma, RBC)	Offspring

APPENDIX I

Developmental neurotoxicity range-finding study in rats

Since this is a range-finding study, only a summary is provided to confirm the adequacy of the dose selection rationale used in subsequent studies.

In a developmental neurotoxicity range-finding study (MRID 46025402), Profenofos (91.8% a.i.; Batch/Lot #: 66719888) was administered in the diet to pregnant Wistar rats (15/dose) from gestation day (GD) 7 to lactation day (LD) 22 at nominal doses of 0, 4, 200, 400, or 600 ppm (equivalent to 0/0, 0.3/0.7, 15.5/33.9, 30.2/66.0, and 46.1/97.6 mg/kg/day [gestation/lactation]). On GD 22, five dams were sacrificed and cholinesterase evaluations (brain, plasma, and erythrocyte) were performed on the dams and fetuses. On PNDs 5, 12, and 22, 5 pups/sex/dose (1 pup/litter where possible) were sacrificed for cholinesterase determinations. On postnatal day (PND) 22, the remaining offspring and dams were sacrificed and discarded without further examinations.

In the dams, there were no treatment-related effects on gestation length, clinical signs, or gross pathology parameters. Decreases ($p \leq 0.05$) in body weight (↓2-6% at ≥200 ppm) and food consumption (↓10-15% at ≥400 ppm) were observed during gestation. No effect on body weight or food consumption was observed at any dose during lactation. The following decreases ($p \leq 0.01$) in cholinesterase activity were observed at ≥200 ppm: (i) brain, ↓21-52% on LD 22; (ii) erythrocyte, ↓42-51% on GD 22, and ↓49-53% on LD 22; and (iii) plasma, ↓81-86% on GD 22, and ↓76-83% on LD 22. Additionally, erythrocyte cholinesterase activity was decreased ($p \leq 0.01$) by 16% at 4 ppm on LD 22. Although cholinesterase activity was decreased during treatment, no adverse clinical signs of toxicity were observed at any dose.

The maternal LOAEL is 200 ppm based on decreased body weight and cholinesterase inhibition. The maternal NOAEL is 4 ppm.

In the pups, there were no treatment-related effects on % liveborn pups, litter size, sex distribution, or clinical signs of toxicity. Body weight was decreased ($p \leq 0.05$) by 10-11% at 600 ppm on PND 22. The following treatment-related decreases ($p \leq 0.01$) in cholinesterase activity were observed: (i) brain, ↓16% in the 600 ppm males on PND 22; (ii) erythrocyte, ↓43-46% in the ≥400 ppm males on PND 22; and (iii) plasma, ↓7-13% at 600 ppm (both sexes) on PND 5, ↓14-22% at ≥400 ppm (both sexes) on PND 12, and ↓36-65% at ≥200 ppm (both sexes) on PND 22. Although cholinesterase activity was decreased during treatment, no adverse clinical signs of toxicity were observed at any dose.

The offspring LOAEL is 200 ppm based on cholinesterase inhibition. The offspring NOAEL is 4 ppm.

This developmental neurotoxicity range-finding study is classified as **acceptable/guideline**.

COMPLIANCE - Signed and dated Data Confidentiality and GLP statements were provided. A Quality Assurance statement was not provided.

APPENDIX II

Zeneca

MAIN FORM

Laboratory	Zeneca Central Toxicology Laboratory, Cheshire, UK

Study No	MRID	TRX	Year	Citation
1	44064701	na	1993	1. S.L. Allen (1993) Molinate: Measurement of motor activity in rat pups. ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID Study Numbers XR4658. MRID 44064701. Unpublished. (QA included)
2	44064702	na	1993	2. S.L. Allen. (1994) Molinate: Assessment of learning and memory in rats. ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID Study Numbers XR 4712. MRID 44064702. Unpublished. (QA included)
3	44064703	na	1996	3. Allen, S.L. (1996) Trimethyltin chloride: Investigation of neurotoxicity in rat pups using morphometrics and startle response. ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID Study Number PR1054. MRID 44064703. Unpublished
4	44064704	na	1996	4. Horner, SA and SJ Duffell. (1996) Molinate: Morphometric evaluation of the developing rat brain. ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID Study Numbers PR1031. MRID 44064704. Unpublished.
5	44604705	na	1995	5. Allen, S.L. (1995) Molinate: developmental neurotoxicity study in rats using diet restriction. Zeneca Central Toxicology Laboratory, Macclesfield, Cheshire, England, laboratory study No.RR0638/F0 and RR0638/F1.CTL/P/4383. 960 p. MRID #: 44064705. Unpublished.

Positive Control Review Form

Testing Laboratory	Zeneca CTL	1. S.L. Allen (1993) Molinate: Measurement of motor activity in rat pups. ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID Study Numbers XR4658. MRID 44064701. Unpublished.	
Positive Control	amphetamine, chlorpromazine		
Date of Positive Control Data	1993		
Species/Strain	rat, Wistar (Alpk:APfSD)		
QA Review (yes/no)	Yes	**Date of Review**	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Ages (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB/CO									
Motor Activity	Coulbourn infrared	yes	yes	14, 18, 22	above	both	10/sex/group	yes	amphetamine
	Coulbourn infrared	yes	yes	14, 18, 22	above	both	10/sex/group	yes	chlorpromazine
Startle									
Learn/Memory									
Std Histopath									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Yes. Individual data were included, and methods were well described.
Methods/Results	Methods: Amphetamine sulphate (0.1 mg/kg) or chlorpromazine hydrochloride (10 mg/kg) were administered ip 1 h before dosing. Motor activity was measured in 50 minute sessions (10 5-min subsessions). Results: For controls the activity increased between day 14-18, overall level similar on 18 and 22, with a different pattern. Results for PC agents were inconsistent: Amphetamine: Day 14 males – no ss. increases, overall mean was higher but variance was large (>100%); d14 females - no effect (high variance (>100%); d 18 males, sig increase for most blocks, but not overall (variance around 100%); d 18 females - no effect, high variance; d22 males, overall activity was doubled, but not sig (large variance close to 100%) - sig at one block only; d 22 females, sig increase for two blocks, overall around 80%increase, but not sig (larve variance). Chlorpromazine: Day 14 males - decrease in first block, otherwise tendency to increase, overall mean increased w/ high variance; d14 females, tendency to decrease, none significant, generally variance was high; d 18 males, no sig changes, very slight tendency to decrease; d18 females, sig decrease overall and for several blocks, still high variance; d22 males, again slight tendency to decrease, especially 1st two blocks, but no ss; day 22 females no difference from controls.
Summary	Summary: Lack of effects on some days. Lack of adult data. Large variances. Failure to demonstrate sensitivity, since significant changes were sporadic and variance was large. Overall Conclusion: Proficiency = not demonstrated.

Positive Control Review Form

Testing Laboratory	Zeneca CTL	2. S.L. Allen. (1994) Molinate: Assessment of learning and memory in rats. ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID Study Numbers XR 4712. MRID 44064702. Unpublished. (QA included)	
Positive Control	scopolamine		
Date of Positive Control Data	1993		
Species/Strain	rat, Wistar (Alpk:APfSD)		
QA Review (yes/no)	Yes	**Date of Review**	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Ages (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB/CO									
Motor Activity									
Startle									
Learn/Memory	Y-shaped water maze	yes	adult only	52 days at receipt	0,10 mg/kg	both	20/sex	yes	
Std Histopath									
Morphometrics									

Is data report adequate (individual data, methods, etc)?	Yes. Individual data were included, and methods were well described.
Methods/Results	Methods: Groups of 20 male rats were given scopolamine ip prior to testing on Day 1 or Day 4. Three test groups, 1 = control, 2 = learning, 3 = retention. Each group received 10 trials in maze followed by 1 trial in straight channel on each test day (dosing given 30 min prior to testing in Group 2 prior to learning on Day 1 only, and to Group 3 prior to recall testing on Day 4 only). Ten 'learning' trials on Day 1 and ten 'recall' trials on Day 4. No testing on Day 2 or 3. Thirty sec maximum trial length. Straight channel swim test was done to determine if scopolamine affected swimming performance. The percentage of successful trials was used as the dependent variable. The criterion for success was varied from 3 to 10 secs, and all data were analyzed with a relatively sophisticated statistical analyses. Results: Scopolamine-treated male rats were slower on the first two trials of the learning phase (no difference for females), when treated prior to learning. They were also slightly slower (significant for females only) on the first few trials of the recall phase; rats treated during recall only were not different from controls. Even the significant differences were not large. In the straight channel, swim times were quicker for treated females during the learning phase. The statistical analysis was then re-done using a variety of cut-off times (was correct trial completed w/in X seconds, comparing % successful trials), and statistical significance was achieved for males for several times on day 1 and several different times on day 4; for females, statistical significance was seen for several times on day 4 only. These types of analyses were performed for the data in the molinate study, where only 6 trials per time point were used.
Summary	Summary: A variety of statistical manipulations (using different cut-off times to designate a successful trial, including only the first 6 trials in the analysis, etc.) were used to try to demonstrate sensitivity of test method. Also, the dose of scopolamine seems very high. Young animals were not tested. In general, this test method appears not to be very sensitive at adult ages; sensitivity at young ages is unknown. Overall Conclusion: Proficiency = very marginal for adults only.

Positive Control Review Form

Testing Laboratory	Zeneca CTL	3. Allen, S.L. (1996) Trimethyltin chloride: Investigation of neurotoxicity in rat pups using morphometrics and startle response. ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID Study Number PR1054. MRID 44064703. Unpublished	
Positive Control	trimethyltin		
Date of Positive Control Data	1996		
Species/Strain	rat, Wistar (Alpk:APfSD)		
QA Review (yes/no)	Yes	Date of Review	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Ages (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB/CO	detailed clin obs	yes	yes	8,12,18, 23, 24	0, 8, 10 mg/kg	both	10/sex		
Motor Activity									
Startle	device not described	yes	yes	d. 23 only	0, 8 mg/kg	both	10/sex		
Learn/Memory									
Std Histopath	immersion fixation	yes	yes	12, 24	0, 8 mg/kg	both	6/sex		
Morphometrics	as above	yes	yes	12, 24	0, 8 mg/kg	both	6/sex		

Is data report adequate (individual data, methods, etc)?	Marginal; Methods were well described with some exceptions (type of startle device was not listed), individual data were included. Although appropriate measures were listed for clin obs (FOB), it is not clear whether these were done outside of the cage..
Methods/Results	Methods: Trimethyltin was administered ip 0, 8 or 10 mg/kg trimethyltin chloride. Clinical Observations - detailed clin obs on various days preweaning and postweaning. ; Startle habituation - device not described, 50 trials, 10 sec ITI, etc.; Pathology - immersion fixation on PND ??; morphometrics - on PND ?? ; many measures taken for each region evaluated. Results: Some animals at 10 mg/kg died so behavior and pathology examinations were done only on 0 and 8 mg/kg animals. Body weight loss at 8 and 10 mg/kg. ClinObs: Multiple clinical signs were seen at 0, a few at 8; it was unclear whether these were outside of cage observations. Startle: Decreases in amplitude was seen during first two blocks (not sig in females); apparent decrease in habituation was not mentioned in results; increase in time to peak amplitude, ss in both sexes, last two blocks Pathology: Decreased brain wgt., histopath lesions in hippocampus and/or piriform cortex and entorhinal cortex day 12, hippocampus only at day 24 Morphometrics: Decreases in cortical, thalamic, and hippocampal regions at both times, ss. varied but was present for some measures in both sexes at both time points.; no sig changes in cerebellum or corpus callosum
Summary	Summary: Study was well done and supports sensitivity for measures evaluated. Auditory startle was evaluated only on day 23 (no adult time point); histopath validation is also for early time point only, but includes both day 12 and day 24. In addition, auditory startle evaluated only decreased amplitude (increased time to peak amplitude), and not increased amplitude. No rationale for the use of TMT on PND8 when almost all published reports use PND5. Potential confound of body weight loss at 8 mg/kg was not mentioned even though this is a favorite way of dismissing effects by registrants. Overall Conclusion: Proficiency demonstrated for a developmental neurotoxicity study design for some endpoints. There were some endpoints not assessed at all or only at some time-points.

Positive Control Review Form

Testing Laboratory	Zeneca CTL	4. Horner, SA and SJ Duffell. (1996) Molinate: Morphometric evaluation of the developing rat brain. ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID Study Numbers PR1031. MRID 44064704. Unpublished.	
Positive Control	controls only		
Date of Positive Control Data	1996		
Species/Strain	rat, Wistar (Alpk:APfSD)		
QA Review (yes/no)	Yes	**Date of Review**	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Ages (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)									
FOB/CO									
Motor Activity									
Startle									
Learn/ Memory									
Std Histopath									
Morphometrics		yes	yes	7, 10, 12, 14, 16, 22, 29, 42, 63	controls only	both	6/sex		data good for historical controls only

's data report adequate ndividual data, methods, etc)?	Yes. Individual data were included, and methods were well described.
Methods/Results	Methods: No positive control agent used in this study. Animals at various ages were killed and processed for morphometric evaluations. No perfusion (immersion fixation at all ages); paraffin embedding, H&E staining, Measurements included brain length and width, morphometric measurements of cerebral cortex, piriform cortex, hippocampus, thalamus, corpus callosum, cerebellum Multiple measures were made in all regions. Results: Data tables were presented showing mean and sd for each measurement at each age, as well as figures charting measurements over time (without sd). In addition, figures showing location of various measures, on a brain schematic, were included. Individual data were also provided for all measures. There is no indication that statistical comparisons were made, thus it is unclear whether the differences noted were statistically significant. Changes were detected in all brain regions – the pattern of changes varied among regions evaluated. Study report referred to this data as historical control data, as well as providing reference data for brain development in the Alderley Park rat., and demonstrate competency in morphometric evaluations. Statistical evaluation to demonstrate detection of significant changes would have been a useful addition to the report.
Summary	Summary: Useful for historical controls. Overall Conclusion: Add some confidence of proficiency with control data, but not useful for showing sensitivity of methods. Lack of perfusion is not consistent with Guidelines at older ages.

Positive Control Review Form

Testing Laboratory	Zeneca CTL	5. Allen, S.L. (1995) Molinate: developmental neurotoxicity study in rats using diet restriction. Zeneca Central Toxicology Laboratory, Macclesfield, Cheshire, England, laboratory study No.RR0638/F0 and RR0638/F1.CTL/P/4383. 960 p. MRID #: 44064705	
Positive Control	None (diet restriction)		
Date of Positive Control Data	1995		
Species/Strain	rat,		
QA Review (yes/no)	Yes	**Date of Review**	November 2002

Methods	Method Codes	Data Present?	Age Relevant?	Ages (days)	Dose Levels	Sexes (m/f)	Group Size	Effects (y/n)	Comments
Dev Landmarks (PND X)	vaginal opening and preputial separation	yes	yes	29-51	none	both	30 litters	na	
FOB/CO	detailed clin obs	yes	yes	various	none	both			
Motor Activity	unknown, automated	yes	yes	14, 18, 22, 50	none	both	30	na	50 min total (10 blocks of 5 min)
Startle	unknown automated	yes	yes	23, 61	none	both	30	na	habituation testing
Learn/Memory	Y-maze	yes	yes	24, 62	none	both	30	na	6 trials on day 1 and day 4, same animals tested on PND24 and 62
Std Histopath		yes	yes	1263	none	both	8-10/sex /group	na	only groups 1 and 4 examined
Morphometrics		yes	yes	12, 63	none	both	37477	na	

Is data report adequate (individual data, methods, etc)?	Yes. Individual data were included, and methods were well described.
Methods/Results	Methods: Four groups of 30 time-mated rats: 1) control; 2) control diet - plus saline injections GD7 until PND11; 3) diet restriction from GD2 to PND11 - 6 hour/day food access; 4) diet restriction from GD2 to PND11 - 22 grams/day during gestation and 32 grams/day during lactation. The following were recorded in selected F1animals as per the DNT Guideline: body weights, clin obs, motor activity (14, 18, 22, 60), startle habituation, learning and memory (y-maze), sexual landmarks, histopathology on PND12 and 63. Note that animals were not weaned until PND29! Results: Despite body weight decreases (up to 17% lower than controls) there were no effects on any behavioral or morphological endpoints.
Summary	Summary: Not very useful as proficiency data. Overall Conclusion: Based on these data proficiency was not demonstrated. Data set useful for historical controls.

EPA Reviewer: Susan Makris **Signature:** _____
Toxicology Branch, Health Effects Division (7509C) **Date** _____
Work Assignment Manager: Ghazi Dannan, Ph.D. **Signature:** _____
Registration Action Branch 3, Health Effects Division (7509C) **Date** _____

Template version 11/01

DATA EVALUATION RECORD

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

PC CODE: 111401 **DP BARCODE:** D293052
TXR#: 0052102 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Profenofos (91.8% a.i.)

SYNONYMS: O-4-bromo-2-chlorphenyl O-ethyl S-propyl phosphorothioate, CGA-15324

CITATION: Milburn, G.M. (2003) Profenofos: Developmental neurotoxicity study in rats. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Project ID: RR0928, June 10, 2003. MRID 46025401. Unpublished.

Milburn, G.M. (2002) Profenofos (CGA 15324): Preliminary developmental neurotoxicity study in rats. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Project ID: RR0927, September 24, 2002. MRID 46025402. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Rd. PO Box # 18300, Greensboro, NC

ORIGINAL EXECUTIVE SUMMARY

In a developmental neurotoxicity study (MRIDs 46025401 and 46025402) Profenofos (91.8% a.i.; Batch/Lot #: 66719888) was administered in the diet to pregnant Wistar rats (30/dose) from gestation day (GD) 7 to lactation day (LD) 22 at nominal doses of 0, 3, 60, or 600 ppm (equivalent to 0/0, 0.3/10.5, 5.1/10.7, and 50.6/103.4 mg/kg/day [gestation/lactation]). Additionally, satellite groups of 10 pregnant rats/dose were similarly treated and the dams and F_1 pups were evaluated for cholinesterase activity. 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, and are included as an Appendix to this DER.

The maternal LOAEL is 60 ppm based on decreased body weight, body weight gains, and food consumption. The maternal NOAEL was 3 ppm.

The offspring LOAEL is 600 ppm, based on decreased body weights and body weight gains. The offspring NOAEL is 60 ppm.

No evidence of developmental neurotoxicity was observed at any dose.

The cholinesterase LOAEL was 60 ppm in both the parents and offspring. The cholinesterase NOAEL was 3 ppm in both the parents and offspring.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats.

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

13544

R161668

Chemical Name: **Profenofos**

PC Code: **111401**
HED File Code:
Memo Date: ***11/17/2004***
File ID: **00000000**
Accession #: **000-00-0126**

HED Records Reference Center
2/3/2009