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

OFFICE OF PREVENTION,
PESTICIDES AND
TOXIC SUBSTANCES

Date: 9/20/2005

TXR No. 0052102

MEMORANDUM

SUBJECT: **PROFENOFOS: Data Evaluation Record of a Developmental Neurotoxicity Toxicity Study.**

PC Code: 111401
CAS # 41198-08-7
DP Barcode D293052

FROM: Ayaad Assaad, D.V.M., Ph.D.
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THRU: Alberto Protzel, Ph.D.,
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Alberto Protzel 9/20/05

I. Conclusions

Attached is the Data Evaluation Record for Developmental Neurotoxicity (DNT) Study with Profenofos (MRID No. 46025401 & 46025402). 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 Profenofos (MRID No. 46025401 & 46025402).

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

Health Effects Division
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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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 Unpublished.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, 0.3, 5.1, and 50.6 mg/kg/day [gestation]). 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.

Maternal toxicity manifested as sacrifice of three dams prior to scheduled termination as they failed to litter, one in the control group and two at the high dose (600 ppm). No treatment-related clinical signs, FOB, or reproductive performances were observed at dose level.

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. 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.

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 systemic LOAEL was 600 ppm (50.6 mg/kg/day) based on decreases in body weight, body weight gain and food consumption. The maternal systemic NOAEL was 60 ppm (5.1 mg/kg/day).

The maternal cholinesterase LOAEL was 60 ppm (5.1 mg/kg/day) based on significant inhibition of erythrocyte and brain cholinesterase activity. The maternal cholinesterase NOAEL was 3 ppm (0.3 mg/kg/day).

Treatment had no adverse effects on offspring survival, clinical signs, FOB, developmental landmarks, motor activity, auditory startle reflex, learning and memory, or neuropathology.

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. .

On PND 12, absolute brain weight was decreased (↓4%) in the high dose males only. No treatment-related gross or microscopic pathological findings were noted in any treated group. The demyelination noted in the peripheral nerves occurred with equal frequency in the control and high dos groups animals, and is commonly seen in rats of this age. Significant differences in various morphometric measurements were seen in both sexes of the high dose animals on PND 12 and PND 63.

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 systemic LOAEL was 600 ppm (50.6 mg/kg/day) based on decreased body weights, body weight gains, decreased brain weights in males on PND 12 and changes in the brain morphometric parameters. The offspring systemic NOAEL was 60 ppm (5.1 mg/kg/day).

The offspring cholinesterase LOAEL was 600 ppm (50.6 mg/kg/day) based on inhibition of plasma, erythrocyte and brain cholinesterase activity. The cholinesterase NOAEL was 60 ppm (5.1 mg/kg/day).

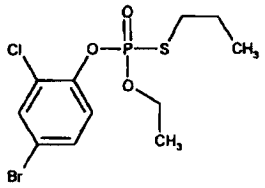
This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive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

COMPLIANCE - Signed and dated 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 (MRID Nos. 44064701, 44064702, 44064703, 44064704 and 44064705).

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≤0.05
** Significantly different from controls at p≤0.01

Food consumption (g/animal/day) was reduced (p≤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≤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≤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 (0/39, 1/40, 3/40, 3/40 at 0, 3, 60, 600 ppm, respectively).

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.

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. **Auditory startle reflex habituation** - 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* (120)	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. These findings are not considered to be toxicologically significant, because there was no clear dose-response. The significant decrease in swim times in PND 62 females at all dose levels is not considered treatment-related because of the abnormally high control values seen during those time periods (Trials 5 and 6) 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 not considered to be toxicologically significant 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≤0.05
** Statistically different from controls at p≤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≤0.05
** Statistically different from controls at p≤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).

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 treatment-related gross or microscopic pathological findings were noted in any treated group. The demyelination noted in the peripheral nerves occurred with equal frequency in the control and high dos groups animals, and is commonly seen in rats of this age. Significant ($p \leq 0.05$) differences in various morphometric measurements were seen at the high dose in both sexes on PND 12 and 63 and are considered to be treatment-related (Table 17). Because effects were seen at the high dose, in accordance with the guideline specifications, morphometric analyses of all brain regions for both sexes at the low and mid dose groups are required for a complete analyses.

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 -

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.

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.

rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control data.