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

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Office of Prevention, Pesticides
and
Toxic Substances

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

013522

MEMORANDUM

SUBJECTIVE: Phosmet: Review of a 90-neurotoxicity study in rats

DP Barcode: D 255515 &256411 **PC code.** 059201
Submission No. S561064 & S562751 **MRID No.** 44811801

TO: Linda Werrell/Kathy Monk, PM Team 52
SRRD (7508W)

FROM: Whang Phang, Ph.D. 6/23/99
Branch Senior Scientist
Reregistration Branch I/HED (7509C)

THROUGH: Mike Metzger, Chief
Reregistration Branch I/HED (7509C)

The registrant, Gowan, submitted a 90-neurotoxicity study in rats (MRID No. 44811801). This study has been reviewed by Dynamac, and this reviewer has performed the secondary review on the report and the DER. Currently, the registrant has also submitted an amended version of this study which you have sent a copy to HED without a MRID number (DP barcode 256411; Submission No. S562751). This reviewer has evaluated the relevant information in the amended version and have incorporated it into the DER. The DER for this study is attached. The citation and conclusion of this study are presented below:

Cappon, G.D., (1999) A Dietary Subchronic (90-Day) Neurotoxicity Study of Phosmet in Rats. WIL Research Laboratories, Inc.; Study Number WIL-331002, April 21, 1999. MRID 44811801. Unpublished.

In this subchronic oral neurotoxicity study (MRID 44811801), Phosmet (97.3% a.i.) was administered for 90 days to 32 Crl:CD(SD)IGS BR rats/sex/dose at nominal dietary concentrations of 0, 25, 50, or 150 ppm. **Actual mean dietary concentrations of**

phosmet based on analysis of weekly batches of diet were 0, 21.6, 39.7, and 136 ppm (equivalent to achieved doses of 0/0, 1.5/1.6, 2.7/3.1, or 9.4/11.0 mg/kg/day [M/F]). Cholinesterase activity levels were determined using modified Ellman method in the blood and brains of 2 rats/sex/dose during the pretest and 6 rats/sex/dose during study weeks 3, 7, and 13. The remaining 12 rats/sex/dose were subjected to the functional observational battery (FOB) and motor activity measurements during the pretest period and study weeks 3, 7, and 12, and were then perfused *in situ* at study termination. Five animals/sex from the control and high-dose groups were used for neurohistological examination.

No animals died during the study. No changes in appearance or behavior, body weights or body weight gains, or food consumption were observed in any of the treated groups when compared to concurrent controls. Results of the FOB indicated no treatment-related findings during the home cage, handling, open field, sensory, neuromuscular, physiological, and locomotor activity observations. There were no adverse effects on mean ambulatory, total motor activity, or gross neurological findings. No treatment-related effects were observed on brain weight in non-perfused and perfused animals. An increase in the incidence of neuropathological changes (characterized by digestion chambers in the sciatic & peroneal nerves) was observed in the high-dose perfused males.

In general, whole blood and red blood cell (RBC) cholinesterase activity levels were decreased in all dose groups of both sexes. Plasma and regional brain choliesterase activities were decreased in all dose groups of the treated females. The details are summarized below:

> **In the 22 ppm group**, mean RBC cholinesterase activity was significantly decreased ($p<0.01$) in the males at week 13 (↓19%) and females at week 7 (↓42%). Significant decreases ($p<0.05$ or 0.01) were also observed at week 13 in mean plasma cholinesterase activity in the females (↓29%), mean whole blood cholinesterase in both sexes (↓16-19%), and cholinesterase activity of the olfactory bulb (↓36%) and brain stem (↓21%) regions in the females.
>
> **In the 40 ppm group**, the following significantly decreased ($p<0.05$ or 0.01) mean cholinesterase activity levels were observed: (i) plasma cholinesterase in the males at week 3 (↓21%) and females at weeks 3 and 13 (↓27-46%); (ii) RBC cholinesterase in the males at weeks 3, 7, and 13 (↓26-39%) and females at week 7 (↓38%); (iii) whole blood cholinesterase at weeks 3, 7, and 13 in both sexes (↓24-36%); and (iv) whole brain cholinesterase at week 7 in the females (↓20%) and at weeks 3 and 7 in the males (↓11-17%).
>
> **In the 136 ppm group**, the following decreased ($p<0.05$ or 0.01) mean cholinesterase activity levels were observed: (i) plasma cholinesterase at weeks 3, 7, and 13 in both sexes (↓23-71%); (ii) RBC cholinesterase at weeks 3, 7, and 13 in males (↓65-70%) and at weeks 3 and 7 in females (↓66-89%); (iii) whole blood cholinesterase in both sexes (↓59-74%) at weeks 3, 7, and 13; (iv) whole brain

cholinesterase in both sexes (↓43-68%) at weeks 3 and 7; and (v) in all six brain regions in the females (↓36-67%) and in all brain regions (except for olfactory region) in the males (↓27-52%) at week 13.

The LOAEL is 22 ppm (equivalent to 1.5/1.6 mg/kg/day [Male/Female])(LDT) based on dose-related decreases in plasma, RBC, whole blood, and brain cholinesterase activity levels. The NOAEL was not established.

This 90 day subchronic oral neurotoxicity study is classified **acceptable/guideline** and satisfies the guideline requirements for a subchronic neurotoxicity study in rats (§82-7).

013522

DATA EVALUATION RECORD

013522

PHOSMET

Study Type: §82-7, 90-Day Dietary Neurotoxicity Study in Rats

Work Assignment No. 1-01-31 (MRID 44811801)

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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Registration Branch I/HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Dietary Subchronic Neurotoxicity Study - rat (§82-7) (870.6200)

DP BARCODE: D255515
P.C. CODE: 059201
SUBMISSION CODE: S561064
TO. CHEM. NO.: 543

TEST MATERIAL (PURITY): Phosmet (97.3% a.i.)

SYNONYMS: *N*-(mercaptomethyl)phthalimide *S*-(O,O-dimethyl phosphorodithioate); Imidan®

CITATION: Cappon, G.D., (1999) A Dietary Subchronic (90-Day) Neurotoxicity Study of Phosmet in Rats. WIL Research Laboratories, Inc.; Study Number WIL-331002, April 21, 1999. MRID 44811801. Unpublished.

SPONSOR: Gowan Company, P.O. Box 5569, Yuma, AZ

EXECUTIVE SUMMARY: In this subchronic oral neurotoxicity study (MRID 44811801), Phosmet (97.3% a.i.) was administered for 90 days to 32 Crl:CD(SD)IGS BR rats/sex/dose at nominal dietary concentrations of 0, 25, 50, or 150 ppm. **Actual mean dietary concentrations of phosmet based on analysis of weekly batches of diet were 0, 21.6, 39.7, and 136 ppm (equivalent to achieved doses of 0/0, 1.5/1.6, 2.7/3.1, or 9.4/11.0 mg/kg/day [M/F]).** Cholinesterase activity levels were determined using modified Ellman method in the blood and brains of 2 rats/sex/dose during the pretest and 6 rats/sex/dose during study weeks 3, 7, and 13. The remaining 12 rats/sex/dose were subjected to the functional observational battery (FOB) and motor activity measurements during the pretest period and study weeks 3, 7, and 12, and were then perfused *in situ* at study termination. Five animals/sex from the control and high-dose groups were used for neurohistological examination.

No animals died during the study. No changes in appearance or behavior, body weights or body weight gains, or food consumption were observed in any of the treated groups when compared to concurrent controls. Results of the FOB indicated no treatment-related findings during the home

cage, handling, open field, sensory, neuromuscular, physiological, and locomotor activity observations. There were no adverse effects on mean ambulatory, total motor activity, or gross neurological findings. No treatment-related effects were observed on brain weight in non-perfused and perfused animals. An increase in the incidence of neuropathological changes (characterized by digestion chambers in the sciatic & peroneal nerves) was observed in the high-dose perfused males.

In general, whole blood and red blood cell (RBC) cholinesterase activity levels were decreased in all dose groups of both sexes. Plasma and regional brain choliesterase activities were decreased in all dose groups of the treated females. The details are summarized below:

In the 22 ppm group, mean RBC cholinesterase activity was significantly decreased ($p<0.01$) in the males at week 13 (↓19%) and females at week 7 (↓42%). Significant decreases ($p<0.05$ or 0.01) were also observed at week 13 in mean plasma cholinesterase activity in the females (↓29%), mean whole blood cholinesterase in both sexes (↓16-19%), and cholinesterase activity of the olfactory bulb (↓36%) and brain stem (↓21%) regions in the females.

In the 40 ppm group, the following significantly decreased ($p<0.05$ or 0.01) mean cholinesterase activity levels were observed: (i) plasma cholinesterase in the males at week 3 (↓21%) and females at weeks 3 and 13 (↓27-46%); (ii) RBC cholinesterase in the males at weeks 3, 7, and 13 (↓26-39%) and females at week 7 (↓38%); (iii) whole blood cholinesterase at weeks 3, 7, and 13 in both sexes (↓24-36%); and (iv) whole brain cholinesterase at week 7 in the females (↓20%) and at weeks 3 and 7 in the males (↓11-17%).

In the 136 ppm group, the following decreased ($p<0.05$ or 0.01) mean cholinesterase activity levels were observed: (i) plasma cholinesterase at weeks 3, 7, and 13 in both sexes (↓23-71%); (ii) RBC cholinesterase at weeks 3, 7, and 13 in males (↓65-70%) and at weeks 3 and 7 in females (↓66-89%); (iii) whole blood cholinesterase in both sexes (↓59-74%) at weeks 3, 7, and 13; (iv) whole brain cholinesterase in both sexes (↓43-68%) at weeks 3 and 7; and (v) in all six brain regions in the females (↓36-67%) and in all brain regions (except for olfactory region) in the males (↓27-52%) at week 13.

The LOAEL is 22 ppm (equivalent to 1.5/1.6 mg/kg/day [Male/Female])(LDT) based on dose-related decreases in plasma, RBC, whole blood, and brain cholinesterase activity levels. The NOAEL was not established.

This 90 day subchronic oral neurotoxicity study is classified **acceptable/guideline** and satisfies the guideline requirements for a subchronic neurotoxicity study in rats (§82-7).

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

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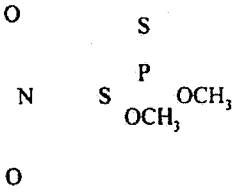
I. MATERIALS AND METHODS

A. Materials:

1. Test material: Phosmet
   Description: Pink powder
   Lot/Batch #: CGH2604
   Purity: 97.3% a.i. (determined by HPLC); the report also stated that 94.4% was use by the testing laboratory for dose calculations based on a correction factor of 1.059.
   Stability: Stable in the diet at room temperature for up to 10 days. (Chemical was stable at room temperature)
   CAS #: 732-11-6
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Rat
   Strain: Crl:CD®(SD)IGS BR
   Age and weight at the start of dosing: 44 days old; males, 106-156 g; females, 99-145 g
   Source: Charles River Laboratories, Raleigh, NC
   Housing: Individually in stainless steel wire-mesh cages. Motor activity measurements were conducted in polycarbonate cages with wire covers.
   Diet: PMI Nutrition International, Inc., Certified Rodent Lab Diet® 5002 (Purina Mills, Richmond, Indiana), ad libitum
   Water: reverse-osmosis purified water, ad libitum
   Environmental conditions:
   Temperature: 22-23°C
   Humidity: 33-55%
   Air changes: Not reported
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: 14 days

B. Study design:

1. In life dates - start: 4/21/98 end: 8/31/98

2. Animal assignment - The rats were randomly assigned to the test groups shown in Table 1 (stratified by weight) using a computerized random selection protocol.

Table 1. Study design. [a]

Test Group	Concentration in diet (ppm)		Mean Dose (mg/kg/day)[b] [M/F]		Animals Assigned	
	Nominal	Achieved[c]	Nominal	Achieved[c]	Males	Females
Control	0	0	0/0	0/0	32	32
Low	25	21.6	1.7/1.9	1.5/1.6	32	32
Mid	50	39.7	3.4/3.9	2.7/3.1	32	32
High	150	136	10.4/12.1	9.4/11.0	32	32

a It was stated that dose selection was based on previous toxicology studies (not provided) conducted by the Sponsor.

b Mean daily test substance intake values, based on actual food consumption, are reported on page 32 of the study report.

c Achieved mean dose (mg/kg/day) and achieved phosmet concentration in the diet (ppm) are based on diet analyses conducted by WIL Research Laboratories, Appendix C, page 1112.

3. Treatment preparation and dosing - The test substance was weighed, mixed with a small amount of feed, and additional feed was added to obtain the desired concentrations. Fresh batches (18 kg) of test diet were prepared weekly. To assess homogeneity and stability of the test substance in the diet, samples (top, middle, and bottom strata) of the 0 week batch were sampled. To assess stability, samples were stored at room temperature for 10 days, then frozen prior to analysis. To assess concentration of the test substance, samples were collected from the middle of each test batch. Samples (5 and 25 gm) from each strata of the 0 week batch and mid-level samples from weeks 1, 2, 3, 7, and 11 were shipped on dry ice to PTRL West, Inc. for analysis, and duplicate mid-level samples from each test diet were retained in frozen storage by WIL Research Laboratories.

Analytical Results from PTRL West, Inc:

Mean concentration (weeks 0, 1, 2, 3, 7 and 11):

25 ppm: 38.8-156% of nominal
50 ppm: 49.4-69.4% of nominal
150 ppm: 57.4-78.3% of nominal

Homogeneity (% of nominal):

Nominal Concentration	Top	Middle	Bottom	Mean ± RSD
25 ppm	47.6	37.6	77.0	54.1 ± 37.9
50 ppm	49.8	68.3	49.8	56.0 ± 19.1
150 ppm	72.1	52.4	64.4	63.0 ± 15.7

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Stability analysis (% of 0 day analysis):
25 ppm: 139%
50 ppm: 90.4%
150 ppm: 100.5%

Due to the high degree of variability and low levels of phosmet determined in the initial analyses of the test diet by PTRL West, a new batch of test diet was formulated and analyzed for homogeneity by WIL Research Laboratories to examine the effect of analytical sample size on determination of phosmet concentration. These homogeneity tests suggested that increasing the sample aliquot to 100 g (5 and 25 g samples were used by PTRL) would improve the consistency of the analyses and give a better estimate of the phosmet actually consumed. Consequently, additional concentration analyses of the test diet were conducted at WIL Research Laboratories using the stored mid-level samples from each batch of diet. Results from the analysis of 100 g aliquots of test diet from weeks 0 through 13 by WIL Research Laboratories are presented below:

Mean Concentration Range (% nominal)		Group Mean ± %RSD (%nominal)
25 ppm:	52.4-117	86.5 ± 21
50 ppm:	64.4-107	79.4 ± 16
150 ppm:	63.7-118	90.8 ± 17

The achieved dietary concentrations and actual consumption of phosmet based on the analyses of the diet conducted by WIL Research Laboratories are presented in Table 1. Given the 9-20% difference in the nominal and actual dietary concentrations of phosmet, references to the various dose groups in this report use the actual dietary levels determined by WIL Laboratories (0, 22, 40, and 136 ppm) rather than the nominal concentrations.

4. Statistics - The mean with standard deviation was presented for each parameter. Data on body weight, changes in body weight, food consumption, cholinesterase levels, brain weight and dimensions, and the continuous data from the FOB were analyzed using a one-way ANOVA. If significant differences were indicated by the ANOVA, then a Dunnett's test was used to compare control and treated groups. Noncontinuous data from the FOB were analyzed using Fisher's Exact test to determine significant differences between the treated and control groups. Locomotor activity data were analyzed using a two-way repeated measures ANOVA. If significant treatment or treatment-time interactions occurred, then a one-way ANOVA was conducted at each time point. If significant treatment effects were observed at a time point, then Dunnett's multiple T-test was conducted to determine significant ($p<0.05$) differences between control and treated groups.

C. Methods

1. Observations

a. Clinical signs - All animals were observed twice daily for mortality and moribundity, and detailed clinical observations were recorded daily on all test animals.

b. Functional observational battery (FOB) and motor activity - Animals (12/sex/group) were subjected to the FOB and motor activity assessments during the pretest period and during study weeks 3, 7 and 12. The FOB assessment included the following parameters:

Home Cage Observations
Posture
Biting
Convulsions/Tremors
Palpebral closure
Feces consistency

Handling Observations
Ease of removal from cage
Ease of handling animal in hand
Lacrimation\Chromodacryorrhea
Salivation
Piloerection
Fur appearance
Palpebral closure
Respiratory rate/character
Red/Crusty deposits
Mucous membranes/eye/skin color
Eye prominence
Muscle tone

Open Field Observations
Mobility
Gait
Rearing
Arousal
Convulsion\Tremors
Urination\Defecation
Grooming
Gait score
Bizarre/Stereotypic behavior
Backing
Time to first step (seconds)

Neuromuscular Observations
Hindlimb extensor strength
Grip strength-hind and forelimb
Hindlimb foot splay
Rotarod performance

Sensory Observations
Approach response
Touch response
Startle response
Tail pinch response
Pupil response
Eyeblink response
Forelimb extension
Hindlimb extension
Air Righting reflex
Olfactory orientation

Physiological Observations
Catalepsy
Body weight
Body temperature

Following each administration of the FOB, motor activity was assessed using a Digiscan 'Micro' Activity System (AccuScan Instrument, Inc., Columbus, OH) to measure ambulatory and total activity (counts). Ambulatory activity was defined as activity in which ≥3 consecutive photobeams are interrupted, and total activity was defined as the combination of ambulatory activity and fine motor skill activity (e.g. grooming, in which one or two adjacent photobeams are interrupted). Motor activity was evaluated over a 40 minute period and the number of movements was tabulated and reported for four 10-minute intervals and for the total 40-minute period.

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c. Positive controls - Summaries were provided of four neurotoxicity studies conducted by the performing laboratory on rats to generate positive control data and validate the procedures and observers used to perform motor activity measurements and assess FOB tests, neurotoxicity, and behavioral effects. The chemicals used in these studies included: D-amphetamine sulfate (0, 0.5, 1, or 2 mg/kg, administered as a single i.p. injection); chlorpromazine hydrochloride (0, 2.5, 5, or 10 mg/kg, administered as a single i.p. injection); carbaryl (0, 2, 10 or 50 mg/kg, administered as a single i.p. injection); acrylamide (0, 5, 10, or 20 mg/kg/day, administered orally for 28 days); trimethyltin chloride (0 or 7.5 mg/kg, administered as a single i.p. injection); and 3,3'-iminodipropionitrile (IDPN, 0 or 2 g/kg, administered as a single oral dose).

Increased activity (FOB and motor activity) was observed in animals dosed with D-amphetamine sulfate while decreased activity (FOB and motor activity) was observed in animals dosed with chlorpromazine hydrochloride. During performance of the FOB, altered posture and an increase in the number of rearing episodes were observed in some of the animals dosed with D-amphetamine sulfate. The chloropromazine hydrochloride treated animals demonstrated numerous altered responses during home cage, handling, open-field, sensory, neuromuscular, and physiological observations of the FOB. Clinical signs of neuropathy such as convulsions, tremors, salivation, and decreased motor activity were observed after administration of carbaryl; these signs were transient in nature. Following repeated dosing with acrylamide, clinical signs of peripheral neuropathy such as alterations in muscle tone, hindlimb extensor strength, and grip strength were observed; histopathological evidence of peripheral nervous system damage (i.e. axonal degeneration and demyelination) was also noted. Histopathological changes to the central nervous system were observed following dosing with trimethyltin. Following dosing with IDPN, multiple observers noted changes in open field observations (mobility, gait, gait score, and head flick), sensory observations (startle response and air righting reflex) and neuromuscular observations (fore- and hindlimb strength).

2. Body weight - Animals were weighed weekly beginning one week prior to treatment, when FOB and motor activity assessments were conducted, and at scheduled termination. Weekly body weights and body weight gains were reported.

3. Food consumption and compound intake - Food consumption was measured at weekly intervals beginning one week prior to dosing until study termination and was reported as g/animal/day. Mean compound intake (mg/kg/day) was calculated using the mean food consumption (g/kg/day) and the concentration of phosmet (ppm) in the diet.

4. Cholinesterase and brain weight determinations - Levels of cholinesterase activity in whole blood, plasma, and brain were determined for 2 rats/sex/dose prior to initial dosing and for 6 rats/sex/dose at weeks 3, 7, and 13. Following euthanization by CO_2 inhalation, blood

samples were collected from the inferior vena cava of each animal and the brain was excised from the skull and weighed. Whole brain cholinesterase levels were determined for pretest and weeks 3 and 7. For week 13, the brain was dissected into the olfactory bulb, cerebral cortex, cerebellum, brain stem, hippocampus, and midbrain, and cholinesterase levels for each brain region was determined. Absolute and relative (to body) brain weights of non-perfused animals were reported prior to dosing (2/sex/dose) and at 3, 7, and 13 weeks (6/sex/dose); brain regional weights (absolute, relative to brain weights, and relative to body weights; 6/sex/dose) were also reported at week 13.

The method for quantitating the plasma, RBC, and whole brain cholinesterase activity was report as follows:

- From a single blood sample obtained from the test animal, a samall sub-sample was removed for evaluation of hematocrit.

- A 0.1 ml was then used from determination of whole blood cholinesterase activity. Prior to the assay, this sample was diluted 1:10 with distilled water.

- Plasma was separated from the orignal whole blood sample and assay for cholinesterase activity.

- The method for cholinesterase determinations was based on the modified Ellman procedure. Two different concentrations of substrate were used depending on the sample to be assayed. In tissues with high hemoglobin content (brain and whole blood), the substrate was based on the modification of the Boehringer Mannheim Cholinesterase kit described by Hunter et. al. *Toxicology Methods* 7:43-53. For plasma samples, the substrate concentration of 24 mM final working concentration was employed as recommended by Boehringer Mannheim.

5. Sacrifice and pathology - At study termination, the 12 animals/sex/dose utilized for FOB and motor evaluations were sacrificed by CO_2 inhalation and perfused *in situ*. Central and peripheral nervous system tissues were dissected and preserved. Absolute brain (including olfactory bulb) weights and dimensions were recorded after removal, and any abnormalities (gross changes, discoloration, or lesions) in the brain or spinal cord were noted. Central and peripheral nervous system tissues were embedded in paraffin and plastic, respectively. For neuropathological examination, the following tissues from 5 rats/sex from the control and high-dose groups were sectioned, stained with hematoxylin and eosin, and examined qualitatively:

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Central Nervous System		
Brain		
Forebrain	Center of Cerebrum	Midbrain
Cerebellum and pons	Medulla oblongata	
Spinal cord		
Cervical swellings C_3-C_7	Lumbar swellings T_{13}-L_4	
Other		
Gasserian ganglion/ Trigeminal nerves	Lumbar dorsal root ganglion at T_{13}-L_4	Lumbar dorsal root fibers at T_{13}-L_4
Lumbar ventral root fibers at T_{13}-L_4	Cervical dorsal root ganglion at C_3-C_7	Cervical dorsal root fibers at C_3-C_7
Cervical ventral root fibers at C_3-C_7	Optic Nerves	Eyes
Peripheral Nervous System		
Sciatic nerve (mid-thigh and at sciatic notch)	Sural nerve	Tibial nerve
Peroneal nerve		

II. RESULTS

A. Observations

1. Mortality - With the exception of one female sacrificed *in extermis* one day prior to initial dosing, no animals died during the study.

2. Clinical signs - No treatment-related changes in appearance or behavior were noted in any of the treated groups during the study. The report indicated that 1 to 2 females of the high-dose group had "dried red material around the eye". This finding might suggest that these animals had chromadacryrrhea which was consistent with choliesterase inhibition.

3. Functional observational battery - Results of the FOB indicated no treatment-related findings during the home cage, handling, open field, sensory, neuromuscular, physiological, and locomotor activity observations. When compared to concurrent controls, an increase ($p<0.05$) in mean hindlimb grip strength in the low-dose females was observed on week 3 (↑36%); this increase was an isolated incidence, not dose related, and not of toxicological concern. At weeks 7 & 12, there was an ever so slight decrease in the mean hindlimb grip strength in the high-dose males. However, the individual animal data showed that the decrease was contributed mainly by 1 or 2 animals. No other differences from controls were noted in FOB parameters in any of the treated groups.

4. Motor activity - There were no treatment-related effects on mean ambulatory and total motor activity. In general, values were similar among the treated groups and their concurrent controls.

B. Body weight and body weight gain - No treatment-related effects on body weights or body weight gains were observed in either sex of any treated groups. During the first week of dosing, mean body weight gains were decreased ($p<0.05$ or 0.01) compared to controls in the 22, 40, and 136 ppm males (↓7-9%). This could be due to an initial palatability problem as data indicated that the body weight gains in the males were similar to controls for the remainder of the study. Body weight gains of all female groups were similar to the controls throughout the study.

C. Food and compound intake/water consumption

1. Food consumption - Food consumption in all treated groups was unaffected by treatment.

2. Compound consumption - The nominal mean dosages based on nominal dietary concentrations and mean food consumption are shown in Table 1. For the achieved mean dosages, the nominal mean dosages were adjusted based on the diet analyses conducted by WIL Research Laboratories.

D. Cholinesterase and brain weight determinations - Mean cholinesterase values are presented in Tables 2a and 2b.

Mean plasma cholinesterase activity (Table 2a) was decreased in all dose groups of **males** at various examination periods, but statistically significant difference ($p<0.05$ or 0.01) from the controls was seen at week 3 in 40 ppm males and at all examination periods in high-dose males. **In females**, the plasma cholinesterase activity was decreased in all dose groups at various times. However, statistically significant decrease was seen at weeks 13 in the 22 ppm group, at weeks 3 and 13 in 40 ppm group, and at all examination periods in the high dose group.

Mean RBC cholinesterase activity (Table 2a) was significantly decreased ($p<0.01$) in the **22 ppm** males at week 13 (↓19%) and females at week 7 (↓42%), in the **40 ppm** males at weeks 3, 7, and 13 (↓26-39%) and females at week 7 (↓42%), and at weeks 3, 7, and 13 in the **136 ppm** males (↓65-70%) and females at weeks 3 and 7 (↓66-89%).

Mean whole blood cholinesterase activity (Table 2a) was significantly decreased ($p<0.05$ or 0.01) in the **22 ppm** males (↓19%) and females (↓16%) at week13; in the **40 ppm** males (↓24-36%) and females (↓28-33%) at weeks 3, 7, and 13; and in the **136 ppm** males (↓59-64%) and females (↓64-71%) at weeks 3, 7, &13.

Table 2a. Mean blood cholinesterase activity levels (U/L) [a]

Dose (ppm)	Week -1	Week 3	Week 7	Week 13
Males				
Plasma Cholinesterase (U/L)				
0	754	555	586	589
22	576	505 (19)	488 (117)	514 (113)
40	541	437* (121)	479 (118)	527 (111)
136	624	327**(141)	450*(123)	373**(137)
Erythrocyte Cholinesterase (U/L)				
0	4018	3619	3321	3582
22	4961	3145(113)	3240	2887**(119)
40	3902	2690**(126)	2043**(139)	2601**(127)
136	4692	1258**(165)	1130**(166)	1091**(170)
Whole Blood Cholinesterase (U/L)				
0	211	194	184	196
22	232	170	170	158**(119)
40	180	146 **(125)	117**(136)	149**(124)
136	223	74**(162)	76**(159)	70**(164)
Females				
Plasma Cholinesterase (U/L)				
0	698	2609	2657	3250
22	661	2065 (121)	2790	2312*(129)
40	761	1398*(146)	1936	2363*(127)
136	860	756**(171)	1066**(160)	1698**(148)
Erythrocyte Cholinesterase (U/L)				
0	4377	2065	2312	1295
22	4153	2196	1336**(142)	1626[b]
40	3959	2035	1431**(138)	594 (154)
136	4999	710**(166)[b]	252**(189)[d]	176[c]

Dose (ppm)	Week -1	Week 3	Week 7	Week 13
Whole Blood Cholinesterase (U/L)				
0	220	234	250	239
22	208	212	214	201*(↓16)
40	204	168**(↓28)	171**(↓32)	159**(↓33)
136	249	68 **(↓71)	66**(↓74)	86**(↓64)

a: These data were extracted from study report Table 55, pages 245 through 248; n=2 at -1 week, and n=6 at weeks 3, 7, and 13, except where noted.
b: n=5; c: n=1; d: n=4 ( ): % of controls
* or **: Significantly different from controls at $p<0.05$ or 0.01, respectively.

Mean whole brain cholinesterase activity (Table 2b) was significantly decreased ($p<0.05$ or 0.01) in the **40 ppm females** (↓20%) at 7 weeks, in the **40 ppm males** (↓11-17%) at 3 and 7 weeks, and in the **136 ppm males (↓43-49%) and females** (↓61-68%) at 3 and 7 weeks.

At the final sacrifice (week 13), there were no statistically significant decreases in **regional (6 regions) brain cholinesterase activity** in the 22 or 40 ppm males (Table 2b). However, decreased ($p<0.05$ or 0.01) cholinesterase activity was observed in the olfactory (↓36%) and brain stem (↓21%) regions of 22 ppm females and in the olfactory (↓27%) region of 40 ppm females. Decreased ($p<0.01$) cholinesterase activity was observed for all six brain regions in the 136 ppm females (↓36-67%) and in all brain regions (except for olfactory region) in the 136 ppm males (↓27-52%); cholinesterase activity in the olfactory region in the high-dose males was decreased compared to controls (↓41%), but the decrease was not statistically significant.

Table 2b. Mean cholinesterase activity levels (U/G) for whole brain and 6 brain regions[a]

Cholinesterase Activity (U/G)				
Interval	0 ppm	22 ppm	40 ppm	136 ppm
Males				
Whole Brain				
Week -1	22.28	22.02	23.22	22.05
Week 3	22.93	22.58	20.45* (↓11)	11.61**(↓49)
Week 7	20.59	20.17	17.09**(↓17)	11.72**(↓43)

Table 2b. Continued.

Cholinesterase Activity (U/G)				
Interval	0 ppm	22 ppm	40 ppm	136 ppm
Hippocampus				
Week 13	15.16	15.73	14.27	8.22**(146)
Olfactory				
Week 13	29.80	29.61	34.38	17.56[b]
Midbrain				
Week 13	23.70	22.18	22.55	12.42**(148)
Brainstem				
Week 13	20.25	19.41	20.09	12.77**(137)
Cerebellum				
Week 13	6.90	7.16	7.15	5.01**(127)
Cortex				
Week 13	24.86	23.58	23.06	11.87**(152)
Females				
Whole Brain				
Week -1	21.95	22.53	22.87	23.90
Week 3	23.42	21.88	20.89	9.06**(161)
Week 7	21.44	19.30 (114)	17.26**(120)	6.90**(168)
Hippocampus				
Week 13	20.79	14.19	13.77	7.04**(166)
Olfactory				
Week 13	35.82	22.92**(136)	26.10*(127)	12.00**(167)
Midbrain				
Week 13	23.49	24.08	18.60	12.72**(146)
Brainstem				
Week 13	22.03	17.44**(121)	18.57 (116)	12.13**(145)
Cerebellum				
Week 13	7.55	7.00	6.62	4.83**(136)
Cortex				
Week 13	24.09	22.38	20.16	9.12**(162)

a These data were extracted from study report Table 55, pages 249 through 252; n=2 at -1 week, and n=6 at weeks 3, 7, and 13, except where noted; values listed parenthetically are percent difference from controls.

b n=4

*, ** Significantly different from controls at $p<0.05$ or 0.01.

E. Sacrifice and pathology of perfused animals

1. Gross pathology and brain weights - No gross neurological findings were reported for any of the test animals. No treatment-related effects were observed on the brain weights and brain region weights (absolute, relative to body, and relative to whole brain) in either sex of the non-perfused animals. In the perfused animal, there was a slight increase in mean brain weight in the 136 ppm females, and the increase showed a statistical significance (↑7%, $p<0.01$). The significanceof this finding is not cleared because the increase was so slight and no histological changes in the brain were seen in any female dose group.

Table 3. Brain weight (gm) at 13 weeks.

Dose Groups (ppm)	Perfused (gm)		Non-perfused (gm)	
	Males	Females	Males	Females
0	2.10± 0.06	1.94± 0.08	2.16± 0.06	1.95± 0.11
22	2.12± 0.07	1.98± 0.07	2.18± 0.11	1.97± 0.01
40	2.14± 0.09	2.01± 0.09	2.12± 0.08	1.95± 0.09
136	2.19± 0.12	2.07± 0.07** (↑7%)	2.15± 0.092	1.77± 0.03

Data excerpted from Table 65 (pp. 277 & 278) of the report (MRID 44811801).
**: Statistical significance difference from the control ($p<0.01$). ( ): % increase relative to the controls.

2. Microscopic pathology - When compared to concurrent controls, high-dose male rats (perfused) exhibited digestion chambers (minimal) of the sciatic nerve [3/5 (60%) treated vs 0/5 controls] and peroneal nerve (1/5 treated vs 0/5 controls) (Table 4). The submitted historical control ranges for digestion chambers are 4.9-17.0% for the sciatic nerve and 0.8-1.1% for the peroneal nerve. The registrant cited a published study[1] which reported that mild nerve fiber degeneration characterized by digestion chambers was a common finding in rats used in subchronic neurotoxicity studies. However, the published study was conducted in Fischer 344 rats which are different (strain) from the Crl:CD(SD)IGS BR rats used in the current study. In addition, the incidence of digestion chambers of the sciatic nerve (3/5) was substantially greater than that seen in the concurrent controls (0/5). Therefore, it could not be ruled out that this was a treatment-related effect.

[1] Eisenbrandt, DL, et.al. (1990). Spontaneous lesions in subchronic neurotoxicity testing of rats. *Toxicologic. Pathology*. 18(2): 154-164.

Table 4 Selected incidences of microscopic changes [a]

Observation	Dose (ppm)				
	0	22	40	136	Historical Controls (%)
Males					
Sciatic Nerve Digestion chambers (minimal)	0/5	NA[b]	NA	3/5 (60%)	4.9-17.0
Peroneal Nerve Digestion chambers (minimal)	0/5	NA	NA	1/5 (20%)	0.8-1.1
Females					
Lumbar Dorsal Ganglia Peripheral chromatolysis (minimal)	1/5 (20%)	NA	NA	1/5 (20%)	0.5-1.0

a Data obtained from the study report, Table 66, pages 280 through 282 and Appendix K, pages 1333 through 1339; percent incidence is listed parenthetically.

b NA - not applicable.

III. DISCUSSION

A. **Investigator's conclusions** -No evidence of systemic toxicity was observed in any of the treated groups. No treatment-related effects were observed during the FOB and motor activity evaluations. Plasma and brain cholinesterase levels were decreased in the 22 ppm females, and the 40 and 136 ppm males and females. Red blood cell and whole blood cholinesterase levels were decreased in both sexes at all dose levels. The NOAEL for cholinesterase inhibition is <22 ppm.

B. **Reviewer's discussion** -In this subchronic oral neurotoxicity study, phosmet was administered for 90 days to 32 Crl:CD(SD)IGS BR rats/sex/dose at actual mean dietary concentrations of 0, 22, 40, or 136 ppm (equivalent to achieved doses of 0/0, 1.5/1.6, 2.7/3.1, or 9.4/11.0 mg/kg/day [M/F]).

No animals died during the study. No treatment-related changes in appearance or behavior, body weights or body weight gains, or food consumption were observed in any of the treated groups. Results of the FOB indicated no treatment-related findings during the home cage, handling, open field, sensory, neuromuscular, physiological, and locomotor activity observations. There were no adverse effects on mean ambulatory, total motor activity, or gross neurological findings. No treatment-related effects on brain weight in non-perfused and perfused animals were observed. An increase in the neuropathological changes (characterized by digestion chamber in the sciatic and peroneal nerves) was seen in high dose male rats (perfused).

In the 22 ppm group, mean RBC cholinesterase activity was decreased ($p<0.01$) in the males at week 13 (↓19%) and females at week 7 (↓42%). Decreases ($p<0.05$ or 0.01) were also observed at week 13 in mean plasma cholinesterase activity in the females (↓29%), mean whole blood cholinesterase in both sexes (↓16-19%), and cholinesterase activity of the olfactory (↓36%) and brain stem (↓21%) regions in the females.

At 40 ppm, the following decreased ($p<0.05$ or 0.01) mean cholinesterase activity levels were observed: (I) plasma cholinesterase in the males at week 3 (↓21%) and females at weeks 3 and 13 (↓27-46%); (ii) RBC cholinesterase in the males at weeks 3, 7, and 13 (↓26-39%) and females at week 7 (↓38%); (iii) whole blood cholinesterase at weeks 3, 7, and 13 in males (↓24-36%) and females (↓28-33%); and (iv) whole brain cholinesterase at week 7 in the females (↓20%) and at weeks 3 and 7 in the males (↓11-17%).

At 136 ppm, the following decreased ($p<0.05$ or 0.01) mean cholinesterase activity levels were observed: (I) plasma cholinesterase at weeks 3, 7, and 13 in males (↓23-41%) and females (↓48-71%); (ii) RBC cholinesterase at weeks 3, 7, and 13 in the males (↓65-70%) and in females at weeks 3 and 7 (↓66-89%); (iii) whole blood cholinesterase in the males (↓59-64%) and females (↓64-74%) at weeks 3, 7, and 13; (iv) whole brain cholinesterase in the males (↓43-49%) and females (↓61-68%) at weeks 3 and 7; and (v) in all six brain regions in the females (↓36-67%) and in all brain regions (except for olfactory region) in the males (↓27-52%) at week 13. Cholinesterase activity in the olfactory region in the high-dose males was decreased compared to controls (↓41%), but the decrease was not statistically significant.

The LOAEL is 22 ppm (equivalent to 1.5/1.6 mg/kg/day [M/F]) (LDT) based on dose-related decreases in plasma, RBC, whole blood, and brain cholinesterase activity levels. The NOAEL was not established.

This 90 day subchronic oral neurotoxicity study is classified **acceptable/guideline** and satisfies the guideline requirements for a subchronic neurotoxicity study in rats (§82-7).

IV. STUDY DEFICIENCIES

A study deficiency which would interfere with the interpretation of the results was not identified.