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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

013167

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Phosmet: Acute Neurotoxicity study

EPA ID NOs: MRID No.: 44673301
Pesticide Chemical Code: 059201
Toxicology Chemical Code: 543
DP Barcode: D250525
Submission No.: S550557
CAS Reg. No.: 00732-11-6

FROM: Kathleen Raffaele, Ph.D. Kathleen C. Raffaele
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THRU: Stephen C. Dapson, Ph.D. Stephen C. Dapson 2/3/99
Branch Senior Scientist
Toxicology Branch II
Health Effects Division (7509C)

REGISTRANT: Gowan Company, Yuma, AZ

CHEMICAL: Phosmet

ACTION REQUESTED: Review acute neurotoxicity study

The acute neurotoxicity study [Cappon, G.D. (1998). An acute neurotoxicity study of phosmet in rats. WIL Research Labs, Ashland OH, Laboratory Study Number WIL-331004, October 8, 1998, MRID#44673301.] has been reviewed. The DER is attached. The study is classified as ACCEPTABLE pending submission of the range-finding study and additional documentation of the sensitivity of the motor activity procedure (as discussed in the DER) and satisfies guideline requirements (Guideline 870.6200, formerly Section 81-8) for an acute neurotoxicity study in rats.

013167

Reviewed by: Kathleen C. Raffaele, Ph.D. Kathleen C. Raffaele 1/26/99
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1/27/99

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity Study in Rats

EPA ID NOs: MRID No.: 44673301
Pesticide Chemical Code: 059201
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CAS Reg. No.: 00732-11-6

TEST MATERIAL: Phosmet

CITATION: Cappon, G.D. (1998). An acute neurotoxicity study of phosmet in rats. WIL Research Labs, Ashland OH, Laboratory Study Number WIL-331004, October 8, 1998, MRID#44673301.

SPONSOR: Gowan Company, Yuma, AZ

EXECUTIVE SUMMARY:

Phosmet (94.4% purity) was administered to Sprague Dawley rats (30/sex/group) at 0, 3.0, 4.5, or 22.5 mg/kg as one dose, orally, by gavage. Body weights were recorded weekly, and clinical observations were recorded daily. Cholinesterase activity was measured in plasma, red blood cells, and brain at time of peak effect (approximately 3 hours post-dosing) on day 0, and on days 7 and 14 post-dosing, in 6 animals/sex/group. Neurobehavioral assessment (functional observation battery and motor activity testing) was performed in 12 animals/sex/group prior to the start of testing, at the time of peak effect, and on days 7 and 14 post-dosing. Brain weights were determined on days 0, 7, and 14 (unperfused animals, 6/sex/group). At study termination, 12 animals/sex/group were perfused in situ, and brain weight, length, and width were measured. Of the perfused animals, 5/sex from the control and high dose groups were subjected to histopathological evaluation of brain and peripheral nervous system.

No effects of treatment were seen in the 3.0 or 4.5 mg/kg groups. At 22.5 mg/kg, there were decreases in plasma cholinesterase in both sexes, at the time of peak effect only (decreased 46% in females, 57% in males). Red blood cell cholinesterase was also decreased at the time of peak effect in both sexes (88% in females, 75% in males); inhibition persisted on days 7 (25%) and 14 (40%) for females only. Brain cholinesterase was inhibited in both sexes at all three time points (for males and females, respectively; 61 and 70% on day 0, 15 and 20% on day 7, and 9 and 17% on day 14). The only other effect of treatment was a decrease in motor activity seen in both sexes at the time of peak effect on day 0. No treatment-related changes were seen in FOB parameters or in neuropathological findings.

Based on the effects seen in this study, the LOAEL was 22.5 mg/kg (based on cholinesterase inhibition [plasma, red blood cell, and brain] and decreased motor activity in both sexes). The NOAEL is 4.5 mg/kg.

The study is classified acceptable for acute neurotoxicity in rats [Guideline 870.6200, formerly 81-8], pending submission of the range-finding study and additional documentation of the sensitivity of the motor activity procedure (as discussed below). The classification will be reevaluated upon receipt of the requested information.

COMPLIANCES: Statement of No Data Confidentiality Claims, p. 2, dated 10/9/98; GLP Compliance Statement, p. 3, dated 10/9/98; Flagging Statement, p. 4, dated 10/9/98; Quality Assurance Unit Statement, p. 37, dated 10/8/98.

I. MATERIALS

A. Test Compound: Phosmet; Description: pink powder; Batch No: CGH2604; Purity: 94.4% (from study report, p. 18; note that purity stated on Certificate of Analysis, Appendix A was 97.3%); Contaminants: not described.

B. Test Animals:

Species: Rat

Strain: Crl:CD(SD)IGS BR

Age: at least 45 days at start of dosing

Acclimation: 14 days prior to initiation of dosing

Weight at initiation (g): 185-261 (M); 143-194 (F)

Source: Charles River Laboratories, Raleigh, NC

Housing: Animals were housed in stainless steel suspended wire mesh cages. On receipt, animals were housed in groups of 3 (segregated by sex) for three days; thereafter, animals were housed individually.

Feed: PMI Nutrition International, Inc. Certified Rodent LabDiet 5002, ad libitum.

Water: Municipal water supply, ad libitum

Environmental: Temperature range during the study was 71.9-72.7°F, humidity range was 49-68%. Light cycle was 12/12 hours light/dark.

In-life dates: 4/22/98-5/5/98.

II. **METHODS**

A. Study Design:

Animals were randomly assigned to study groups (stratified by body weight) as indicated in Table 1 (below). One acute dose of test substance was administered orally, by gavage, in a dosage volume of 5 ml/kg (test substance was suspended in corn oil). Animals were not fasted prior to test substance administration.

Table 1. Experimental Design.

Group	Treatment	Dose (mg/kg)	Dosage Concentration (mg a.i./ml)*	Total # animals /sex	ChE evaluation	Behavior/ pathology
1	Corn Oil	0	0	30	18	12
2	Phosmet	3.0	0.6	30	18	12
3	Phosmet	4.5	0.9	30	18	12
4	Phosmet	22.5	4.5	30	18	12

*As noted above, an adjustment factor of 1.059 was applied to correct for purity of test substance.

In each treatment group, 18 animals/sex were assigned for evaluation of cholinesterase activity (6/sex/time point) in plasma, blood, and brain. Cholinesterase inhibition was evaluated at time of peak effect and on days 7 and 14 after treatment. Twelve animals/sex were assigned for behavioral testing (Functional Observation Battery [FOB] and motor activity). Behavioral testing was performed prior to test substance administration (pre-test), at the time of peak effect (3 hours post-dosing), and on days 7 and 14 post-dosing. Of the animals assigned for behavioral testing, 5/group were assigned for neuropathological evaluation at study termination.

Dosing was performed as four replicates, balanced by sex and treatment group. Replicates were dosed on four consecutive days.

Doses were selected based on a range-finding study (WIL-331003), described on p. 20 of the report (Vol. 1), but not submitted. Briefly, doses of 1.5, 3, 6, 9, and 36 mg/kg phosmet were administered. The report states that the only clinical signs seen in the range-finding study were whole body tremors, gait alterations, and salivation at 36 mg/kg. Plasma cholinesterase levels were said to be reduced at 9 and 36 mg/kg. Time of peak effect was stated to be 2-4 hours post-dosing. This study should be submitted, for verification of time of peak effect. The results as described do not support the doses chosen for use in the current study.

B. Test substance administration: To prepare dosing solutions, an appropriate amount of test substance was weighed (adjusted for compound purity, adjustment factor of 1.059) and mixed with corn oil [Mazola Corn Oil, CPC International, Englewood Cliffs, NJ]. Formulations were homogenized to obtain a uniform mixture, and dosing solutions were stirred throughout dose administration using a magnetic stirrer. Formulations were

prepared daily prior to dosing. All prepared formulations were analyzed for concentration, and the preparations from the first dosing day were analyzed for homogeneity. Homogeneity samples were collected prior to dosing; time of collection was not specified for concentration samples. No stability analyses were conducted.

C. Observations

1. **Mortality and clinical observations**: Animals were observed twice daily for mortality and moribundity. Clinical signs were evaluated once daily, except that animals receiving FOB/motor activity testing were not evaluated for clinical signs on the days that testing was performed.

2. **Body weights**: Body weights were recorded pre-study (day -6), and on study days 0 (day of dosing), 7, and 14, and at study termination (day 15).

3. **Food consumption**: Not recorded

4. **Ophthalmology**: Not done

D. Cholinesterase Determination: Cholinesterase levels in brain, plasma, and red blood cells were evaluated for 6 animals/sex/group at the time of peak effect on the day of dosing (day 0, approximately 3 hours post-dosing), and on days 7 and 14 post-dosing. Blood was collected from the inferior vena cava following euthanization. Red blood cell cholinesterase activity (RBC ChE) was derived from measured values for whole blood cholinesterase activity, plasma cholinesterase activity, and hematocrit (formula provided in the study report, Vol. 1, p. 24). For determination of brain cholinesterase, the brain was removed from the skull, weighed, homogenized in 1% Triton X-100 solution, and centrifuged; the supernatant was evaluated for cholinesterase activity levels. Cholinesterase activity was evaluated using a modification of the Ellman method (references provided, Study report Vol. 4, p. 1007).

E. Neurobehavioral Assessment:

Neurobehavioral assessments (Functional Observation Battery [FOB] and motor activity) were conducted for 12 animals/sex/dose at pre-test (not otherwise specified), day 0 at the time of peak effect (3 hours after test substance administration), and on days 7 and 14 post-dosing. Motor activity testing was performed after completion of the FOB.

1. Functional Observational Battery:

FOB was performed in a sound-proofed room with a background noise generator set at 70 db, except that home cage observations were performed in the animal room. Testing was performed `blind' to treatment status of animals, by the same technician whenever possible. The following parameters were evaluated:

Home cage observations: posture, convulsions/tremors, feces consistency, biting, palpebral closure;
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 (during a two-minute observation period): mobility, gait, rearing, arousal, convulsions/tremors, urination/defecation, grooming, gait score, bizarre/stereotypic behavior, backing, time to first step;
Sensory observations: approach response, touch response, startle response, tail pinch response, pupil response, eyeblink response, forelimb extension, hindlimb extension, air righting reflex, olfactory orientation;
Neuromuscular observations: hindlimb extensor strength, hind- and forelimb grip strength, hindlimb foot splay, rotarod performance;
Physiological observations: catalepsy, body temperature, body weight.

2. Locomotor activity:

Motor activity testing was performed using a `Digiscan Micro Animal Activity System' (AccuScan Instruments, Inc., Columbus, OH, 43228; a clear plastic rectangular cage, with activity measured by interruption of infrared photobeams). Motor activity was recorded as total and ambulatory activity, during a 41-minute session (data for the first minute were discarded). Motor activity data were presented for total session activity (40 minutes) and for 4 ten-minute subsessions.

F. Sacrifice and Pathology:

Gross Pathology: None performed

Neuropathology: On the day of scheduled sacrifice, the 12 animals from the neurobehavioral assessment group were euthanized by CO_2 inhalation, and perfused in situ. Peripheral nervous system tissues were dissected and preserved. Brains were removed, and

brain weight and dimensions (length and width) were recorded. Gross lesions were also recorded at this time. For five animals per sex for control and high dose groups, the following tissues were evaluated histopathologically (central nervous system tissues were embedded in paraffin, peripheral nervous system tissues were embedded in plastic; staining was with hematoxylin and eosin):

Central Nervous System: Brain (forebrain, center of cerebrum, midbrain, cerebellum and pons, medulla oblangata), spinal cord (at cervical and lumbar swellings), lumbar dorsal root ganglia, lumbar dorsal root fibers, lumbar ventral root fibers, cervical dorsal root ganglia, cervical dorsal root fibers, cervical ventral root fibers, optic nerves, eyes;
Peripheral Nervous System: Sciatic nerves (mid-thigh region and sciatic notch), sural nerves, tibial nerves; forelimbs and tail were preserved but not evaluated.

G. Positive Controls: The following positive control study summaries were submitted: 1) WIL-99026, A Validation Study of the Digiscan `Micro' Animal Motor Activity System (Study Report Vol. 4, p. 976, performance date 11/5/90-11/30/90, test substances D-Amphetamine sulfate and chlorpromazine hydrochloride were evaluated for effects on FOB and motor activity); 2) WIL-99032, An Acute Neurotoxicity Study of 1-Naphthyl-N-Methylcarbamate (Carbaryl) in Rats (Study Report Vol. 4, p. 992, performance date 12/3/90-12/21/90, Carbaryl was evaluated for effects on FOB and motor activity); 3)WIL-99034, A Repeated Dose Neurotoxicity Study of Acrylamide in Rats and An Acute Neurotoxicity Study of Trimethyltin Chloride in Rats (Study Report Vol. 4, p. 996, performance dates 1/14/91-2/15/91, Acrylamide was evaluated for effects on FOB, motor activity, and peripheral neuropathology, Trimethyltin was evaluated for effects on central nervous system pathology); 4) WIL-99035, An Inter-Observer Reliability Study of Technicians Assigned to Perform F.O.B. Observations for Neurotoxicity Studies (Study Report Vol. 4, p. 1004, performance dates 8/1/91-8/9/91, Inter-rater reliability was evaluated for FOB scores of rats treated acutely with IDPN).

The results of these reports were presented as short summaries of procedures and conclusions, with no supporting raw data.

H. Statistical Evaluations: Statistical evaluations, other than for locomotor activity, were performed using a Digital MicroVAX 3400 computer with `appropriate programming.' Continuous data were evaluated using one-way analysis of variance (ANOVA), significant differences were further evaluated using Dunnet's test for paired comparisons. Scalar or descriptive data from the FOB were evaluated using Fisher's Exact Test. Data from Locomotor activity testing were evaluated using two-way repeated measures (ANOVA) with SAS/STAT software. Significant findings were further evaluated using Dunnett's multiple T-test.

IV. RESULTS

A. Analytical Chemistry: As noted above, the purity stated on the Certificate of Analysis was greater than that stated in the procedural section of the study report. The results from concentration analyses of the prepared formulations of test substance are presented in Table 2.

Table 2. Analytical concentration of prepared test solutions.

Dose Group	Nominal Concentration (mg/ml)	Actual concentration (mg/ml)
Control	0	0
Phosmet 3.0 mg/kg	0.6	0.49-0.67
Phosmet 4.5 mg/kg	0.9	0.82-0.89
Phosmet 22.5 mg/kg	4.5	3.74-4.3

Data were extracted from study report, Vol. 4, pp. 943-945. Actual concentration ranges were calculated by the reviewer.

Actual concentrations were all within an acceptable range of nominal concentration. Homogeneity analyses demonstrated adequate homogeneity for all formulations.

B. Clinical signs and mortality: All animals survived until scheduled termination. There were no treatment-related clinical observations. Observations were limited to scabbing, hair loss, dried material around eyes or nose, or decreased defecation, and were similarly distributed across treatment groups.

C. Body weight and body weight gain: Body weights were similar across treatment groups for all time periods.

D. Food consumption and achieved compound intake: Food consumption was not measured. Compound was administered by gavage, as noted above.

E. Ophthalmic examinations: Not done.

F. Cholinesterase activities: RBC and plasma cholinesterase activities were significantly decreased in high dose males and females, on day 0. For both sexes, plasma cholinesterase values were similar for all groups on days 7 and 14 (the statistically significant increase in plasma cholinesterase activity of low dose males on day 7 is considered spurious). For high dose males, RBC cholinesterase had returned to control values by day 7. For high dose females, RBC cholinesterase was still decreased on days 7 and 14 (statistically significant on day 14 only). Although the RBC

cholinesterase activity level of high dose females on day 7 was not statistically different from control values, the decrease is of sufficient magnitude (25%) that it is considered treatment-related (especially given the high variability of these values). Brain cholinesterase activity was also significantly decreased at the high dose for both sexes, at all three time points. Data are presented in Table 3, below.

Table 3. Cholinesterase activity.

Observation	Dose (mg/kg)			
	0	3.0	4.5	22.5
Plasma ChE				
Males				
Day 0	621±40	587±87.5 [5]	549±124.7 [12]	266±37.4** [57]
Day 7	488±40.8	602±79.7*	487±91.3	474±52.9 [3]
Day 14	533±87.6	603±127.6	527±73.5	502±65.2 [6]
Females				
Day 0	1125±271.6	1290±268.1	1037±370.6 [8]	610±206.3* [46]
Day 7	1314±243.1	1413±392.9	1415±362.2	1561±541.0
Day 14	1906±481.3	1761±698.7	1675±477.9	2239±774.6
RBC ChE				
Males				
Day 0	3696±417.7	3779±208.1	3162±553.4 [14]	928±239.4** [75]
Day 7	3560±745.9	3418±361.1	3791±482.2	3405±435.7 [4]
Day 14	3110±591.7	3114±746.4	3185±278.0	3056±648.6 [2]
Females				
Day 0	3848±431.6	3259±563.2 [15]	3339±294.3 [13]	443±318.7** [88]
Day 7	3101±544.4	3276±1279.9	2673±845.2 [14]	2332±720.6 [25]

Observation	Dose (mg/kg)			
	0	3.0	4.5	22.5
Day 14	2128±406.7	2146±528.0	2197±883.8	1274±234.4* [40]
Brain ChE				
Males				
Day 0	22.83±0.892	22.57±0.499	22.59±1.084	8.88±0.870** [61]
Day 7	22.88±0.535	22.82±0.372	22.65±0.439	19.54±0.828** [15]
Day 14	19.61±1.173	20.21±0.842	19.79±0.825	17.87±0.651** [9]
Females				
Day 0	23.27±0.734	22.85±0.858	22.39±0.680	6.89±1.311** [70]
Day 7	23.80±0.827	23.30±0.588	22.91±1.086	19.11±0.736** [20]
Day 14	21.15±1.130	20.29±1.317	20.08±1.086	17.59±0.892** [17]

Values for cholinesterase activity are international units/liter, mean±s.d.; n=6 except where otherwise noted. Values in brackets represent percent inhibition. *=significantly different from control values, p<.05; ** significantly different from control values, p<.01. Data were extracted from the study report, volume 1, pp. 216-219.

Note that the variances for blood cholinesterase measurements (plasma cholinesterase in females and red blood cell cholinesterase in both sexes), are very high. This raises questions about the reliability of the analytical method used in this study, with respect to the ability to detect changes in these parameters (the smallest statistically significant change detected in blood measures was 40%).

G. Neurobehavioral results

1. **FOB Findings**: There were no treatment-related findings on the parameters evaluated in the FOB. One statistically significant effect, an increase in the number of high-dose females with alert posture on day 0 (number of females with this finding was 0, 1, 2, 5 for 0, 3.0, 4.5, and 22.5 mg/kg groups,

respectively), was not considered treatment-related because a similar number of control females had this finding on day 7 (number of females with this finding was 6, 5, 8, and 4 for 0, 3.0, 4.5, and 22.5 mg/kg groups, respectively).

2. **Motor activity**: There was a statistically significant decrease in motor activity, for specific subsessions only (1 and/or 2), in high dose males and females at the time of peak effect. At later subsessions (3 and 4), there was an increase in motor activity at the high dose, for both males and females (greater for males than females, with increases approaching 300% above control values). An decrease in activity early in the session, accompanied by increased activity late in the session, could be interpreted as a lack of habituation in high dose animals. Due to the high variance in this measure (see comments below), the increases in activity for subsessions 3 and 4 were not statistically significant, except for ambulatory activity in males for subsession 4. The results for day 0 are detailed in Table 4, below. Motor activity was similar across treatment groups for all other time points.

Table 4. Motor activity at day 0, time of peak effect.

Group	Subsession				Total
	1	2	3	4	
Total Activity					
Males					
0	468±118.2	226±99.5	50±56.3	35±25.8	779±187.9
3.0 mg/kg	419±164.6	213±87.6	125±109.3	127±118.8	884±225.2
4.5 mg/kg	463±183.1	193±113.4 [-15]	130±118.9	80±141.1	865±391.1
22.5 mg/kg	288±123.2* [-38]	110±92.1* [-51]	147±90.3 [+194]	136±122.0 [+289]	681±280.7 [-13]
Female					
0	648±223.2	211±173.4	132±148.5	133±178.2	1124±510.8
3.0 mg/kg	525±76.4	203±90.8	125±128.8	82±88.1	936±282.7
4.5 mg/kg	625±171.6	246±131.1	221±106.3	175±125.3	1266±344.0
22.5 mg/kg	440±176.9* [-32]	204±121.6 [-3]	178±114.2 [+34]	183±166.7 [+38]	1005±443.5 [-11]

Group	Subsession 1	2	3	4	Total
Ambulatory Activity					
Males					
0	276±80.3	105±53.1	27±29.8	22±19.7	431±121.1
3.0 mg/kg	243±85.1	113±41.7	52±54.1	55±49.9	462±137.2
4.5 mg/kg	278±109.8	92±65.0 [-12]	61±66.8	16±15.8	447±171.7
22.5 mg/kg	174±77.5* [-37]	65±55.4 [-38]	70±46.7 [+159]	68±65.7* [+209]	377±173.8 [-13]
Females					
0	391±138.9	132±116.6	82±102.1	87±107.3	692±328.8
3.0 mg/kg	315±63.3	109±58.0	67±90.0	36±52.2	527±183.6
4.5 mg/kg	352±95.9	144±77.5	125±72.3	103±84.6	724±180.6
22.5 mg/kg	257±80.6* [-34]	122±81.1 [-8]	87±56.3 [+6]	101±90.9 [+16]	566±232.4 [-18]

Values presented are activity counts; n=12 except where otherwise noted. Values represent mean±s.d. Values in brackets represent percent change from control. *=significantly different from control values, p<.05; ** significantly different from control values, p<.01. Data were extracted from the study report, volume 1, pp. 204-207.

The decreases in motor activity seen at the high dose, in both males and females, are considered to be treatment-related effects. No effects were seen at the mid or low dose for either sex. Note, however, that because of the extremely high variability seen for both subsession and total activity measures, a very large change in activity is needed before a change would be detected in this measure (this problem becomes even more obvious when the individual data are examined). These problems raise questions about the sensitivity of the procedure used in this study. The positive control data submitted with this study (cited above, consisting of summary data describing differences in total session activity) were not adequate to document the sensitivity of this procedure. Additional documentation of the sensitivity of this procedure, and its ability to satisfy the guideline requirements, should be submitted.

H. Sacrifice and pathology:

1. **Gross pathology**: Not evaluated.

2. **Neuropathology**: No differences were detected in brain weight (for cholinesterase animals or for perfused animals), or in brain length and width for perfused animals. Values in treated animals were similar to those for controls for all treatment groups.

There were no histopathological lesions related to treatment. Scattered incidences of neutrophil infiltrate in the perineurium (trigenimal nerve), or axonal degeneration in sciatic nerve (all graded minimal) were similarly distributed among control and high dose animals.

V. **DISCUSSION and CONCLUSIONS:**

Phosmet (94.4% purity) was administered to rats (30/sex/group) at 0, 3.0, 4.5, and 22.5 mg/kg, orally, by gavage. Rats were evaluated for behavioral effects (using the FOB and motor activity levels) as well as cholinesterase inhibition at 3 time points (time of peak effect (approximately 3 h post-dosing), days 7 and 14 post-dosing). The data were well reported, and historical control data, and positive control data summaries were provided.

Effects of exposure to phosmet were seen only at the highest dose (22.5 mg/kg). At this dose, cholinesterase inhibition was observed in plasma (peak effect only), red blood cells (peak effect only for males, all time points for females), and brain (all time points for both sexes). No effects were seen on the FOB at any dose, but there were statistically significant decreases in motor activity (subsessions 1 and/or 2 only) in both sexes at the time of peak effect. There were no neuropathological findings related to treatment.

The study report failed to provide an acceptable rationale for the doses selected for use in this study. Although no deaths or severe clinical signs were seen at doses up to 36 mg/kg in the range-finding study, the highest dose selected for use was 22.5. Also, the range-finding study apparently demonstrated cholinesterase inhibition at doses of 9 mg/kg or above (no inhibition was seen at 6 mg/kg). The two lower doses selected for use in this study, 3.0 and 4.5, were well below doses at which cholinesterase inhibition was demonstrated. Thus, although effects in the current study at the high dose were sufficient to demonstrate toxicity at that dose, no information is available regarding the dose response curves for cholinesterase inhibition or behavioral effects. This is especially relevant since similar levels of inhibition (60-75%) were seen in brain and red blood cell cholinesterase at the high dose, with brain inhibition persisting throughout the study.

No information documenting the choice of peak effect time was submitted. Registrants should be asked to submit the range-finding study so that the appropriateness of the time used for the current study, approximately 3 hours post-dosing, can be verified.

Extremely high variability was noted in the data from the motor activity testing, raising questions about the sensitivity of the procedures used in this study. For example, an increase in subsession activity approaching 300% above control levels was not found to be statistically significantly different from controls. Thus, it is possible that differences in activity level may be caused by the compound, but be obscured by the large variability and insensitivity of the measurement method. Additional data, documenting the sensitivity of the motor activity procedure used in this study, including data documenting habituation (i.e. activity during subsessions as well as total session activity) and ability to reliably detect increases and decreases in motor activity, should be submitted.

There was also large variability in some of the blood cholinesterase measurements (especially for red blood cells), such that decreases of 25% were not statistically significant. Again, it is possible that true differences caused by exposure to phosmet might be obscured by the high variability of the measure.

Based on the effects seen in this study, the LOAEL was 22.5 mg/kg (based on cholinesterase inhibition [plasma, red blood cell, and brain] and decreases in motor activity in both sexes). The NOAEL is 4.5 mg/kg.

The study is classified **acceptable** for acute neurotoxicity in rats, pending submission of the range-finding study and additional documentation of the sensitivity of the motor activity procedure (as discussed above). The classification will be reevaluated upon receipt of the requested information.