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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

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

MEMORANDUM

Date: 9/9/09

SUBJECT: Phorate. Review of Special Comparative Cholinesterase Inhibition Studies (Companion Study to Developmental Neurotoxicity Study)

PC Code: 057201
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0052690
MRID No.: 46241401, 46241402, and 46248101

DP Barcode: D304304, D304005
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: 298-02-2
40 CFR: NA

FROM: Paul Chin, Ph.D. Paul Chin 9/9/09
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THROUGH: Kit Farwell, D.V.M. Kit Farwell 9/9/09
Michael S. Metzger, Chief Michael S. Metzger 9/16/09
Risk Assessment Branch VII
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TO: Jennifer Howenstine/Tom Moriarty
Special Review and Reregistration Division (7508P)
Office of Pesticide Programs

I. CONCLUSIONS

The registrant, BASF Corporation, submitted Special Comparative Cholinesterase Inhibition Studies (Companion Study to Developmental Neurotoxicity Study). These studies (MRIDs 46241401, 46241402, and 46248101) were reviewed by the contractor, Oak Ridge National

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Laboratory, and went through the secondary review process in HED. Two studies (MRIDs 46248101 and 46241402) are classified **Acceptable/Nonguideline** for the determination of serum, RBC, and brain cholinesterase activities following acute or repeated treatment with phorate in adult and juvenile rats. The other study (MRID 46241401) is classified **Unacceptable/Nonguideline** for the determination of a time to peak effect for all compartments. In this study, a time to peak effect could not be determined because the dose levels employed were not high enough to cause enzyme inhibition. The DERs for these studies are attached to this memorandum and the citations and the conclusions of these studies are presented below.

II. & III. ACTION REQUESTED and BACKGROUND

The registrant, BASF Corporation, submitted these studies. SRRD requested RAB VII, HED to review and prepare DER for these studies.

IV. REVIEW SUMMARY

CITATION: Schneider, S., Deckardt, K., and van Ravenswaay, B. (2004) BAS 225 I (phorate) - Study of the effects on cholinesterase activities in juvenile and adult Wistar rats after single administration ("time-to peak" study) oral administration (gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Laboratory ID 06R0091/02033, Doc. No. 2004/1010809. March 22, 2004. MRID 46241401. Unpublished.

Schneider, S., Deckardt, K., and van Ravenswaay, B. (2004) BAS 225 I (phorate) - range-finding developmental neurotoxicity study in Wistar rats oral administration to the dams and pups (gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Laboratory ID 29R0091/02030, Doc. No. 2004/1010806. March 19, 2004. MRID 46241402. Unpublished.

Schneider, S., Deckardt, K., and van Ravenswaay, B. (2004) BAS 225 I (phorate) - Study of the effects on cholinesterase activities in juvenile and young adult Wistar rats (age sensitivity) oral administration (gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Laboratory ID 06R0091/02060, Doc. No. 2004/1011014. April 2, 2004. MRID 46248101. Unpublished.

EXECUTIVE SUMMARY: In a series of special comparative cholinesterase inhibition (ChEI) studies, Phorate (BAS 225 I; 91.8% a.i., lot #AC 9429-41) was administered by gavage to groups of Wistar rats. For time-course evaluation (MRID 46241401) 5 animals/sex/group were given a single oral dose of 0 or 0.2 mg/kg on PND 21 or at approximately 12 weeks and sacrificed 0.5, 1, 2, 4, or 8 hours later. In two acute studies (MRIDs 46241402, 46248101) groups of 8-10 rats/sex were given a single oral dose of 0, 0.03, 0.1, or 0.2 mg/kg on PNDs 11, 21, or 60 and sacrificed 2-4 hours post-dosing. The effects of maternal treatment (MRID 46241402) were studied in groups of 8 pregnant rats given 0, 0.03, 0.1, or 0.2 mg/kg/day on either gestation days (GDs) 6-

20 or on GD 6 - lactation day 10; ChE activity was measured in GD 20 dams and fetuses and in PND 4 pups. Finally, repeated administration was studied by giving eleven daily doses of 0, 0.03, 0.1, or 0.2 mg/kg/day to groups of 8-10 rats/sex beginning on either PND 11 or 60; animals were sacrificed 2-3 hours or 4 hours, respectively, after the last dose (MRID 46248101). Serum, RBC, and brain ChE activities were measured in all animals in each study. No rationale for dose selection was given in any study.

All adult and juvenile animals survived to scheduled sacrifice. Pup survival during lactation was not affected by treatment of dams or pups. The only treatment-related clinical sign of toxicity was salivation in pregnant animals after dosing with 0.2 mg/kg/day. No other clinical signs were observed in adult or juvenile animals in any study. Survival, body weight, food consumption, and reproductive performance were not affected by treatment with the test article.

Acute exposure (adult and juvenile animals)
In adult animals, no treatment-related inhibition was observed in any compartment following a single dose of the test article to male and female adult. In pre-weaning rats, RBC and brain enzyme activities were not inhibited in PND 11 or 21 males and females at any dose. In serum, limited inhibition was observed at the highest dose, however, biological significance was marginal.

Repeated Exposure (adult and juvenile animals)
Inhibition of ChE activity was apparent in all compartments of pre-weaning rats and in RBC and plasma of adult female rats after repeated dosing. No effects were observed on enzyme activity in any compartment of adult males. At a dose of 0.2 mg/kg/day in PND 21 males and females, serum activity was inhibited 46% and 50%, respectively, RBC activity was inhibited 41% and 33%, respectively, and brain activity was inhibited 39% and 54%, respectively. Serum and RBC enzyme activities were inhibited 41% and 15%, respectively, in adult females at a dose of 0.2 mg/kg/day.

Maternal exposure on GDs 6-20 or GD 6-PND 10 (ChE activity in GD20 dams, GD 20 fetuses, and PND 4 pups)
Pregnant rats treated with 0.1 or 0.2 mg/kg/day had dose-related inhibition of serum (19% and 40%, respectively) and RBC (14% and 23%, respectively) activities. Brain activity was inhibited 41% at the highest dose. No treatment-related enzyme inhibition was observed in any compartment of GD 20 fetuses or PND 4 pups following maternal treatment.

Time-course of inhibition
During time-course investigations in both juvenile and adult rats, no statistically or biologically significant inhibition of enzyme activity occurred in any compartment at any time. Therefore, a time to peak effect could not be determined.

For acute oral exposure to phorate, the overall adult LOAEL for cholinesterase inhibition in rats is not identified and the adult NOAEL is 0.2 mg/kg, the highest dose tested.

For acute oral exposure to phorate, the overall offspring LOAEL for cholinesterase inhibition in rats is not identified and the offspring NOAEL is 0.2 mg/kg, the highest dose tested.

For repeated oral exposure to phorate, the overall adult LOAEL for cholinesterase inhibition in rats is 0.2 mg/kg/day based on enzyme inhibition in red blood cells from females; the adult NOAEL is 0.1 mg/kg/day.

For repeated oral exposure to phorate, the overall offspring LOAEL for cholinesterase inhibition in rats is 0.2 mg/kg/day based on enzyme inhibition in red blood cells and brain; the offspring NOAEL is 0.1 mg/kg/day.

The cholinesterase activity measurements following acute and repeated oral dosing of phorate demonstrate that juvenile rats are more susceptible than adults and that adult females were more susceptible than adult males. In pups and adult females the serum ChE activity appeared to be more sensitive than RBC or brain enzyme activity. This susceptibility was observed in terms of the dose level at which an effect was observed (i.e., the LOAEL for cholinesterase inhibition was lower for juveniles than for adults and lower for serum than for RBC or brain). Above the juvenile LOAEL, similar doses to young and adult rats produced a greater magnitude of effect in juveniles compared with the adults. Pregnant rats were more sensitive than non-pregnant females and showed effects similar to juveniles.

Two studies (MRIDs 46248101 and 46241402) are classified **Acceptable/Nonguideline** for the determination of serum, RBC, and brain cholinesterase activities following acute or repeated treatment with phorate in adult and juvenile rats.

The other study (MRID 46241401) is classified **Unacceptable/Nonguideline** for the determination of a time to peak effect for all compartments. In this study, a time to peak effect could not be determined because the dose levels employed were not high enough to cause enzyme inhibition.

DATA EVALUATION RECORD

PHORATE (BAS 225 I)

Study Type: SPECIAL STUDIES, CHOLINESTERASE INHIBITION [NON-GUIDELINE]
MRID 46241401 (time-course); 46241402 (range-finding, dams and pups)
46248101 (acute - preweaning; repeated - young adults)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 67-2004

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Date: OCT 27 2004

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Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: OCT 27 2004

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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Risk Assessment Branch III, Health Effects Division (7509P)

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Date 9/16/09
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Date 09/16/2009

TXR#: 0052690

DATA EVALUATION RECORD

STUDY TYPE Special Study, Effects on Cholinesterase in Adult and Juvenile Rats, Companion Study to Developmental Neurotoxicity Study 870.6300

PC CODE: 057201 **DP BARCODE:** D304005, D304304

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

SYNONYMS: *O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate; BAS 225 I

CITATION: Schneider, S., Deckardt, K., and van Ravenswaay, B. (2004) BAS 225 I (phorate) - Study of the effects on cholinesterase activities in juvenile and adult Wistar rats after single administration ("time-to peak" study) oral administration (gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Laboratory ID 06R0091/02033, Doc. No. 2004/1010809. March 22, 2004. MRID 46241401. Unpublished.

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SPONSOR: BASF Corporation, Agricultural Products, RTP, NC

EXECUTIVE SUMMARY: In a series of special comparative cholinesterase inhibition (ChEI) studies, Phorate (BAS 225 I; 91.8% a.i., lot #AC 9429-41) was administered by gavage to groups of Wistar rats. For time-course evaluation (MRID 46241401) 5 animals/sex/group were given a single oral dose of 0 or 0.2 mg/kg on PND 21 or at approximately 12 weeks and sacrificed 0.5, 1, 2, 4, or 8 hours later. In two acute studies (MRIDs 46241402, 46248101) groups of 8-10 rats/sex were given a single oral dose of 0, 0.03, 0.1, or 0.2 mg/kg on PNDs 11, 21, or 60 and sacrificed 2-4 hours post-dosing. The effects of

maternal treatment (MRID 46241402) were studied in groups of 8 pregnant rats given 0, 0.03, 0.1, or 0.2 mg/kg/day on either gestation days (GDs) 6-20 or on GD 6 - lactation day 10; ChE activity was measured in GD 20 dams and fetuses and in PND 4 pups. Finally, repeated administration was studied by giving eleven daily doses of 0, 0.03, 0.1, or 0.2 mg/kg/day to groups of 8-10 rats/sex beginning on either PND 11 or 60; animals were sacrificed 2-3 hours or 4 hours, respectively, after the last dose (MRID 46248101). Serum, RBC, and brain ChE activities were measured in all animals in each study. No rationale for dose selection was given in any study.

All adult and juvenile animals survived to scheduled sacrifice. Pup survival during lactation was not affected by treatment of dams or pups. The only treatment-related clinical sign of toxicity was salivation in pregnant animals after dosing with 0.2 mg/kg/day. No other clinical signs were observed in adult or juvenile animals in any study. Survival, body weight, food consumption, and reproductive performance were not affected by treatment with the test article.

Acute exposure (adult and juvenile animals)
In adult animals, no treatment-related inhibition was observed in any compartment following a single dose of the test article to male and female adult. In pre-weaning rats, RBC and brain enzyme activities were not inhibited in PND 11 or 21 males and females at any dose. In serum, limited inhibition was observed at the highest dose, however, biological significance was marginal.

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Inhibition of ChE activity was apparent in all compartments of pre-weaning rats and in RBC and plasma of adult female rats after repeated dosing. No effects were observed on enzyme activity in any compartment of adult males. At a dose of 0.2 mg/kg/day in PND 21 males and females, serum activity was inhibited 46% and 50%, respectively, RBC activity was inhibited 41% and 33%, respectively, and brain activity was inhibited 39% and 54%, respectively. Serum and RBC enzyme activities were inhibited 41% and 15%, respectively, in adult females at a dose of 0.2 mg/kg/day.

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For repeated oral exposure to phorate, the overall adult LOAEL for cholinesterase inhibition in rats is 0.2 mg/kg/day based on enzyme inhibition in red blood cells from females; the adult NOAEL is 0.1 mg/kg/day.

For repeated oral exposure to phorate, the overall offspring LOAEL for cholinesterase inhibition in rats is 0.2 mg/kg/day based on enzyme inhibition in red blood cells and brain; the offspring NOAEL is 0.1 mg/kg/day.

The cholinesterase activity measurements following acute and repeated oral dosing of phorate demonstrate that juvenile rats are more susceptible than adults and that adult females were more susceptible than adult males. In pups and adult females the serum ChE activity appeared to be more sensitive than RBC or brain enzyme activity. This susceptibility was observed in terms of the dose level at which an effect was observed (i.e., the LOAEL for cholinesterase inhibition was lower for juveniles than for adults and lower for serum than for RBC or brain). Above the juvenile LOAEL, similar doses to young and adult rats produced a greater magnitude of effect in juveniles compared with the adults. Pregnant rats were more sensitive than non-pregnant females and showed effects similar to juveniles.

Two studies (MRIDs 46248101 and 46241402) are classified **Acceptable/Nonguideline** for the determination of serum, RBC, and brain cholinesterase activities following acute or repeated treatment with phorate in adult and juvenile rats.

The other study (MRID 46241401) is classified **Unacceptable/Nonguideline** for the determination of a time to peak effect for all compartments. In this study, a time to peak effect could not be determined because the dose levels employed were not high enough to cause enzyme inhibition.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Phorate

Description:	clear to slightly turbid liquid
Lot/Batch #:	AC 9429-41
Purity:	91.8 % a.i.
Compound Stability:	proven by reanalysis
CAS # of TGAI:	298-02-2

2. Vehicle and/or positive control: Corn oil was used as the vehicle in all studies. No positive control was used.

3. Test animals (P):

Species:	rat
Strain:	Wistar (CrlGl x BrlHan:WI)
Age and wt. at study initiation:	Adults: Male and females, 10-12 weeks, males 280.7-329.1 g, females 192.8-229.5 g Mated females approx. 70-84 days old delivered on GD 0, 134.8-189.9 g Juvenile animals: males 30.9-52.2 g, females 31.4-50.0 g
Source:	Charles River Laboratories, Germany

Housing:	adults in type DK III stainless steel wire mesh cages; dams from GD 18 - PND 21 with litters in Makrolon type M III cages with bedding material	
Diet:	Kliba maintenance diet mouse/rat meal or pellets, Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*	
Water:	tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	20-24 °C (nominal)
	Humidity:	30-70% (nominal)
	Air changes:	not stated
	Photoperiod:	12 hrs light/dark
Acclimation period:	about 6 days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates** - MRID 46241401: Start: November 4, 2002 End: November 6, 2002
   MRID 46241402: Start: October 29, 2002 End: December 12, 2002
   MRID 46248101: Start: June 12, 2003 End: September 12, 2003

2. **Study design**: Table 1 shows the treatment groups allocated for the study.

TABLE 1. Study design for cholinesterase inhibition studies

MRID	Dose(s) (mg/kg/day)	Sex; No. of animals/ dose	Treatment and termination
46241401 (acute)	0, 0.2	M&F; 25	Single oral dose on PND 22 or approx. 12 weeks of age; five animals/group terminated 0.5, 1, 2, 4, or 8 hours post-dosing
46241402 (gestational)	0, 0.03, 0.1, 0.2	F; 20	Dams: daily oral dose on GD 6-20 or GD 6 - PND 10; Pups: daily oral dose on PND 11-21; eight animals/group terminated 2-3 hours (dams) or 2 hours (pups) after the last dose
46248101 (repeated)	0, 0.03, 0.1, 0.2	M&F; 10 or 20	Single oral dose on PND 11 and 21; Eleven daily doses beginning on PND 60-70; terminated 4 hour after the last dose

3. **Mating procedure**: In those studies in which preweaning or juvenile animals were treated, dams with litters were delivered to the testing facility on day 0 post coitum. The day of parturition was designated as day 0 *post partum*.

4. **Animal assignment**: MRID 46248101 stated that the test article was administered to randomly selected animals. No other information was given in any report about the method of animal assignment.

5. **Dose selection rationale**: No rationale for dose selection was given in any study.

6. **Dosage administration**: All single or multiple doses were administered to the adult males and females and selected offspring in the groups shown in Table 1 by daily oral gavage at a volume of 5 mL/kg calculated from the most recent individual body weight.

7. **Dosage preparation and analysis**: Dosing solutions were prepared on the day of use (MRID 46241401, 46241402) or at intervals which accounted for the analytical results of stability analyses. For preparation, an appropriate amount of test article was weighed into a graduated flask, topped with corn oil, and mixed by shaking. Solutions for the dose groups were prepared by dilution.

Concentration and stability of the dosing solutions were determined in all studies. Homogeneity was not measured since the preparations were stated as being true solutions. The results for each study are given below.

Results -
MRID 46241401: Absence of test article was confirmed in the vehicle. Concentration of the dosing solution was 96.9% of nominal. After 12 days of storage at room temperature, the concentration was 102.8% of the initial measured concentration.

MRID 46241402: The mean concentrations were 97.7-105.5%, 95.1-97.7%, and 97.0-97.5% of nominal for the 0.66, 2.20, and 4.40 mg/100 mL dose preparations, respectively. Stability was measured in MRID 43241401.

MRID 46248101: The mean concentrations were 91.7-99.1%, 94.7-101.8%, and 98.5-103.9% of nominal for the 0.66, 2.20, and 4.40 mg/100 m dose preparations, respectively. Stability was measured in MRID 43241401.

The analytical data indicated that the difference between nominal and actual dosage to the study animals was acceptable for all studies.

C. OBSERVATIONS:

1. In-life observations:

a) **Clinical evaluation:** All animals were checked twice daily for mortality and once daily for clinical signs of toxicity. No information was given on whether the timing of the observations correlated with the expected time to peak effect. Body weight was recorded for each animal immediately prior to dosing. Dams were weighed on GDs 0, 6/7, 13/14, and 20/21 and on lactation days 1, 7, 11, 14, and 21. Food consumption for dams was determined at approximately weekly intervals. Pups were weighed on PNDs 1, 11, and 21 (MRID 46248101) or PNDs 1, 4, 7, and 11-21 (MRID 46241402).

b) **Reproductive performance:** Nesting, littering, and lactation behavior of the dams was checked at least once daily. As soon as possible after birth, pups were examined for sex, numbers of live and stillborn, and any gross malformations. On PND 4 litters were culled to 4 pups/sex, where possible.

2. **Termination schedule and sample collection**: Adult and pre-weaning animals were terminated according to the schedule shown in Table 1. No rationale was provided regarding the selection of time of sacrifice in relation to time of dosing. All animals were killed by either cervical dislocation or decapitation under isofluoran anesthesia.

   Blood samples were collected from adult animals from the retroorbital venus plexus and from pups from the vena cava cranialis after decapitation. The brain was collected immediately after blood collection. In all studies, ChE activity was determined in RBCs, plasma, and brain tissue.

3. **Cholinesterase determination**: Cholinesterase assays were performed on all blood and brain samples using a modified Ellman method adapted to a Cobra Fara analyzer. RBC samples were measured using DTNA as chromogen. For specific ChE activity in RBC and brain, hematocrit and total protein content, respectively, were determined.

D. **DATA ANALYSIS**: Cholinesterase activity data were considered by non-parametric one-way analysis using the Kruskal-Wallis test; if significance was found, each dose group was compared with the control group using the Wilcoxon test. Body weight and food consumption data, duration of gestation, and number of pups/litter were analyzed by comparison of all dose groups with the control group using the Dunnett test. Reproduction indices, females with live born and stillborn, and pups stillborn, died, cannibalized, and sacrificed moribund were analyzed by pairwise comparison of each dose group with the control group using Fisher=s Exact test. All statistical tests were two-sided and p0.05 and p0.01 were designated by * and **, respectively.

RESULTS:

A. **Mortality and clinical observations**: All adult and juvenile animals survived to scheduled sacrifice. Pup survival during lactation was not affected by treatment of dams or pups. In MRID 46241402, salivation was observed after treatment with 0.2 mg/kg/day in five dams during gestation and in one dam during lactation. No adverse clinical signs of toxicity were observed in any animal of any other study. Reproductive performance was similar between treated and control groups.

B. **Body weight:** Body weight and food consumption were not adversely affected by treatment of adults or pups in any study.

C. **Brain weight:** Brain weight data were not obtained in any study

D. **Cholinesterase activity:** The serum, RBC, and brain cholinesterase activity data for treated adult male and female rats and offspring are shown in Tables 2, 3, 4, and 5 for acute exposure, repeated exposure, maternal, and time-course studies, respectively.

1. Acute exposure (Table 2):
Two studies evaluated serum, RBC, and brain ChE activity following a single dose of the test article to male and female adult or pre-weaning rats. Clear dose-related inhibition of enzyme activity was not observed in any compartment of any group. In PND 11 males and females treated with 0.2 mg/kg, serum enzyme activity was significantly inhibited in both studies. Serum ChE activity was also inhibited in PND 21 females administered 0.1 mg/kg but the magnitude was not biologically significant. RBC enzyme activity was not inhibited in PND 11 or 21 males and females at any dose. In adult animals, no treatment-related inhibition was observed in any compartment. Brain enzyme activity from adult females was significantly inhibited at all doses in MRID 44248101; but the inhibition of brain activity in all treated groups of adult females was not dose-related and is probably statistically significant due to a high value for the control group. In addition, no effects on brain activity were observed in any other study (MRID 46241402).

2. Repeated Exposure (Table 3): Dose-related inhibition of ChE activities was apparent in all compartments of pre-weaning rats and in RBC and plasma of adult female rats after repeated dosing. Significant inhibition of serum, RBC, and brain enzyme activity occurred at a dose of 0.2 mg/kg/day in both sexes of PND 21 animals. Serum enzyme activity was also slightly inhibited in PND 21 animals at 0.1 mg/kg/day but the magnitude was not biologically significant. Serum and RBC enzyme activities were statistically inhibited in adult females at a dose of 0.2 mg/kg/day. No effects were observed on enzyme activity in adult males.

3. Maternal exposure (Table 4): Pregnant rats treated with 0.1 or 0.2 mg/kg/day had dose-related inhibition of serum and RBC activity and brain activity was inhibited at the highest dose. No treatment-related enzyme inhibition was observed in any compartment of GD 20 fetuses or PND 4 pups following maternal treatment.

4. Time-course of inhibition (Table 5): For both juvenile and adult rats, no statistically or biologically significant inhibition of enzyme activity occurred in any compartment at any time. Therefore, a time to peak effect could not be determined and dosing was considered inadequate.

TABLE 2. RBC, plasma, and brain ChE activity in adult and pre-weaning rats treated with phorate: Acute exposure with termination 2-4 hours post-dosing

Cholinesterase	Dose (mg/kg/day)			
	0	0.03	0.1	0.2
PND 11 Males (MRID 46248101)				
Serum (μkat/L)	19.25 ± 1.37	18.68 ± 1.82	18.08 ± 1.27	15.00** ± 1.64 (22)[a]
RBC (μkat/L)	23.20 ± 5.32	19.77 ± 3.47	20.99 ± 4.14	18.67 ± 3.50 (20)
Brain (μkat/g)	1.70 ± 0.30	1.65 ± 0.33	1.77 ± 0.24	1.40 ± 0.31 (18)
PND 11 Females (MRID 42648101)				
Serum (μkat/L)	18.07 ± 1.43	18.15 ± 2.01	17.44 ± 0.83	14.99** ± 1.31 (17)
RBC (μkat/L)	23.25 ± 3.66	23.07 ± 3.21	24.62 ± 5.54	20.87 ± 3.44 (10)
Brain (μkat/g)	1.44 ± 0.36	1.51 ± 0.26	1.68 ± 0.39	1.42 ± 0.28
PND 11 Males (MRID 46241402)				
Serum (μkat/L)	18.32 ± 1.11	17.77 ± 2.15	17.37 ± 1.48	15.18* ± 2.01 (17)
RBC (μkat/L)	27.47 ± 6.9	25.67 ± 6.84	28.98 ± 6.69	27.92 ± 9.55
Brain (μkat/g)	1.28 ± 0.32	1.28 ± 0.56	1.12 ± 0.15	0.92 ± 0.22 (28)
PND 11 Females (MRID 46241402)				
Serum (μkat/L)	17.77 ± 1.20	17.61 ± 1.43	16.38 ± 1.36	15.07** ± 1.39 (15)
RBC (μkat/L)	26.25 ± 6.81	29.23 ± 11.18	23.82 ± 3.54	24.89 ± 4.53 (5)
Brain (μkat/g)	1.17 ± 0.39	1.10 ± 0.22	0.98 ± 0.16	0.87 ± 0.15 (26)
PND 21 Males (MRID 46248101)				
Serum (μkat/L)	14.31 ± 2.07	14.56 ± 1.19	13.64 ± 1.62	13.43 ± 1.79 (6)
RBC (μkat/L)	39.28 ± 4.58	40.86 ± 5.02	41.24 ± 5.36	36.87 ± 5.50 (16)
Brain (μkat/g)	2.27 ± 0.54	2.11 ± 0.28	2.48 ± 0.56	1.90 ± 0.46 (6)
PND 21 Females (MRID 46248101)				
Serum (μkat/L)	15.06 ± 1.09	14.04 ± 1.80	13.67* ± 1.22 (9)	12.95** ± 1.48 (14)
RBC (μkat/L)	37.41 ± 5.91	41.24 ± 4.81	39.45 ± 3.25	37.40 ± 3.72
Brain (μkat/g)	2.36 ± 0.49	2.17 ± 0.49	2.05 ± 0.43	2.15 ± 0.54 (9)
Adult (PND 60) Males (MRID 46248101)				
Serum (μkat/L)	10.51 ± 1.55	9.95 ± 0.92	10.16 ± 1.46	10.66 ± 2.16
RBC (μkat/L)	30.44 ± 3.49	31.32 ± 2.18	31.28 ± 3.41	31.26 ± 3.77
Brain (μkat/g)	2.38 ± 1.00	2.01 ± 0.88	2.43 ± 1.09	1.97 ± 0.68 (17)
Adult (PND 60) Females (MRID 46248101)				
Serum (μkat/L)	39.03 ± 6.96	36.35 ± 11.79	34.79 ± 6.67	33.00 ± 7.56 (15)
RBC (μkat/L)	27.35 ± 2.88	29.60 ± 2.68	27.94 ± 3.03	27.54 ± 2.47
Brain (μkat/g)	2.75 ± 0.99	2.01* ± 0.78 (27)	2.03* ± 0.94 (26)	1.81** ± 0.52 (34)

PND 60-70 data extracted from Table IB, pp. 87-88, MRID 46248101.
Pre-weaning data extracted from Table IB, pp. 83-86, MRID 46248101 and Table IB, pp. 96-97, MRID 46241402.
N = 8-10/sex/group; MRID 46248101 sacrificed 4 hours post-dosing; MRID 46241402 sacrificed 2-3 hours post-dosing.
[a] Numbers in parenthesis are percent inhibition relative to control; calculated by reviewer.

TABLE 3. Blood and brain ChE activity in adult and pre-weaning rats treated with phorate: Repeated exposure to eleven daily doses with termination 2 or 4 hours after last dose

	Dose (mg/kg/day)			
Cholinesterase	**0**	**0.03**	**0.1**	**0.2**
PND 21 Males (MRID 46241402)				
Serum (μkat/L)	14.74 ± 2.11	14.20 ± 1.55	12.52* ± 1.67 (15)[a]	8.01** ± 1.87(46)
RBC (μkat/L)	38.94 ± 6.28	38.42 ± 11.22	38.28 ± 5.98	22.95** ± 4.59 (41)
Brain (μkat/g)	1.61 ± 0.22	1.69 ± 0.38	1.48 ± 0.22 (8)	0.98** ± 0.31 (39)
PND 21 Females (MRID 46241402)				
Serum (μkat/L)	15.11 ± 1.89	14.03 ± 1.42	13.12* ± 1.12 (13)	7.56** ± 1.50 (50)
RBC (μkat/L)	34.94 ± 7.76	41.39 ± 3.89	36.36 ± 4.20	23.43** ± 4.33 (33)
Brain (μkat/g)	1.76 ± 0.29	1.62 ± 0.37	1.81 ± 0.55	0.81** ± 0.16 (54)
Adult (PND 70) Males (MRID 46248101)				
Serum (μkat/L)	10.31 ± 1.30	10.58 ± 1.37	9.82 ± 1.40	9.86 ± 0.90
RBC (μkat/L)	27.64 ± 3.78	28.23 ± 2.83	26.83 ± 2.89	27.28 ± 2.83
Brain (μkat/g)	1.63 ± 0.53	2.06 ± 0.74	1.90 ± 0.77	1.77 ± 0.65
Adult (PND 70) Females (MRID 46248101)				
Serum (μkat/L)	37.31 ± 5.97	37.77 ± 4.53	32.72 ± 7.82	22.03** ± 5.91 (41)
RBC (μkat/L)	26.22 ± 4.56	32.16** ± 2.98	27.78 ± 4.29	22.20* ± 3.19 (15)
Brain (μkat/g)	2.89 ± 1.26	2.40 ± 1.19	2.55 ± 1.08	3.02 ± 1.24

Data from Table IB, pp. 89-90, MRID 46248101 and Table IB, pp. 98-99, MRID 46241402.
N = 8-10/sex/group; MRID 46248101 sacrificed 4 hours post-dosing; MRID 46241402 sacrificed 2-3 hours post-dosing.
[a] Numbers in parenthesis are percent inhibition relative to control; calculated by reviewer.
Significantly different from control: *p≤ 0.05, **p ≤ 0.01

TABLE 4. Blood and brain ChE activity in dams and offspring: Maternal treatment on GDs 6-20 or GD 6 - lactation day 10 with termination 2-3 hours after dosing

Cholinesterase	Dose (mg/kg/day)			
	0	0.03	0.1	0.2
GD 20 Dams				
Serum (µkat/L)	60.74 ± 10.74	61.08 ± 6.23	49.46* ± 7.28 (19)[a]	36.60** ± 4.94 (40)
RBC (µkat/L)	35.98 ± 1.12	33.92 ± 3.76	30.99* ± 4.82 (14)	27.64** ± 5.16 (23)
Brain (µkat/g)	2.95 ± 0.54	2.88 ± 0.74	2.94 ± 0.70	1.73** ± 0.67 (41)
GD 20 Male fetuses				
Serum (µkat/L)	5..68 ± 0.53	5.88 ± 0.42	5.71 ± 0.33	5.76 ± 0.43
RBC (µkat/L)	7.05 ± 0.83	5.72** ± 0.51 (19)	5.69** ± 0.66 (19)	6.42 ± 0.56 (9)
Brain (µkat/g)	0.57 ± 0.01	0.58 ± 0.04	0.56 ± 0.03	0.60* ± 0.03
GD 20 Female fetuses				
Serum (µkat/L)	5.79 ± 0.37	6.17 ± 0.30	6.08 ± 0.49	5.86 ± 0.33
RBC (µkat/L)	6.80 ± 0.99	5.81 ± 0.91 (15)	5.48 ± 0.89 (19)	6.28 ± 0.78 (8)
Brain (µkat/g)	0.59 ± 0.04	0.57 ± 0.04	0.58 ± 0.02	0.59 ± 0.02
PND 4 Males				
Serum (µkat/L)	13.05 ± 1.17	12.70 ± 1.54	13.26 ± 1.55	11.94 ± 1.30 (9)
RBC (µkat/L)	13.74 ± 1.67	13.59 ± 1.06	14.44 ± 3.33	14.75 ± 2.97
Brain (µkat/g)	1.09 ± 0.10	1.12 ± 0.13	1.15 ± 0.13	1.15 ± 0.09
PND 4 Females				
Serum (µkat/L)	12.88 ± 1.36	13.60 ± 1.69	13.44 ± 1.25	12.27 ± 0.61 (5)
RBC (µkat/L)	15.11 ± 1.63	14.28 ± 2.08 (5)	14.10 ± 2.40 (7)	14.62 ± 1.57 (3)
Brain (µkat/g)	1.11 ± 0.13	1.14 ± 0.07	1.16 ± 0.06	1.09 ± 0.07 (2)

Data from Table IB, pp. 89-95, MRID 46241402.
N = 8/sex/group
[a] Numbers in parenthesis are percent inhibition relative to control; calculated by reviewer.
Significantly different from control: *p≤ 0.05, **p ≤ 0.01

TABLE 5. Blood and brain ChE activity in juvenile and adult rats treated with a single dose of phorate: Time-course data

Cholinesterase	Dose (mg/kg)			
	0	0.2	0	0.2
	Males		Females	
PND 21 rats - Brain ChE (μkat/g)				
0.5 hr post-dosing	1.90 ± 0.36	1.66 ± 0.08 (13)	1.82 ± 0.20	1.77 ± 0.16
1 hr post-dosing	1.88 ± 0.44	2.10 ± 0.22	1.83 ± 0.29	1.82 ± 0.20
2 hr post-dosing	1.65 ± 0.25	1.80 ± 0.22	1.76 ± 0.20	1.79 ± 0.18
4 hr post-dosing	2.48 ± 0.54	2.45 ± 0.36	2.22 ± 0.45	2.12 ± 0.21 (5)
8 hr post-dosing	2.64 ± 0.90	2.11 ± 0.28 (20)	2.98 ± 0.73	2.40 ± 0.63 (19)
PND 21 rats - RBC ChE (μkat/L)				
0.5 hr post-dosing	42.07 ± 8.20	41.79 ± 7.64	50.18 ± 6.52	40.73 ± 8.10 (19)
1 hr post-dosing	47.46 ± 7.28	42.34 ± 1.76 (11)	44.70 ± 7.05	41.86 ± 3.13 (6)
2 hr post-dosing	42.24 ± 2.94	40.98 ± 3.91 (3)	44.01 ± 5.09	42.18 ± 5.99 (4)
4 hr post-dosing	38.56 ± 6.09	41.14 ± 6.73	42.04 ± 5.90	41.04 ± 8.49
8 hr post-dosing	43.53 ± 4.01	41.07 ± 3.32 (6)	41.32 ± 5.66	42.12 ± 4.30
PND 21 rats - Serum ChE (μkat/L)				
0.5 hr post-dosing	14.24 ± 2.03	13.68 ± 1.73 (4)	14.07 ± 1.13	13.37 ± 0.71 (5)
1 hr post-dosing	14.72 ± 0.73	14.02 ± 1.51 (5)	13.65 ± 1.70	13.11 ± 1.90 (4)
2 hr post-dosing	13.91 ± 1.02	12.42 ± 0.94 (10)	13.33 ± 0.92	14.37 ± 0.72
4 hr post-dosing	14.20 ± 1.08	12.85 ± 0.89 (10)	13.46 ± 1.69	13.26 ± 2.06
8 hr post-dosing	13.05 ± 1.40	13.53 ± 2.56	13.80 ± 0.86	13.23 ± 0.90
Adult rats - Brain ChE (μkat/g)				
0.5 hr post-dosing	1.80 ± 0.23	2.43 ± 0.68	2.69 ± 0.75	2.46 ± 1.32 (9)
1 hr post-dosing	2.61 ± 1.28	2.87 ± 0.64	3.16 ± 0.72	3.31 ± 0.64
2 hr post-dosing	3.15 ± 1.03	2.07 ± 0.70 (34)	1.77 ± 0.36	2.14 ± 0.28
4 hr post-dosing	1.97 ± 0.50	2.12 ± 0.78	2.69 ± 0.51	1.99 ± 0.35 (26)
8 hr post-dosing	2.61 ± 1.01	2.32 ± 0.50 (11)	2.71 ± 1.13	2.72 ± 0.73
Adult rats - RBC ChE (μkat/L)				
0.5 hr post-dosing	34.82 ± 3.42	28.98 ± 3.15 (17)	34.71 ± 2.36	36.64 ± 3.19
1 hr post-dosing	31.85 ± 2.81	32.03 ± 2.09	31.50 ± 1.20	32.59 ± 1.25
2 hr post-dosing	27.70 ± 2.94	32.08 ± 3.77	29.12 ± 4.33	32.26 ± 2.19
4 hr post-dosing	27.94 ± 4.11	28.33 ± 2.61	26.49 ± 4.76	30.60 ± 2.49
8 hr post-dosing	29.32 ± 2.66	26.05 ± 2.00 (11)	31.00 ± 5.85	27.80 ± 0.40 (10)
Adults rats - Serum ChE (μkat/L)				
0.5 hr post-dosing	13.38 ± 3.70	13.60 ± 2.17	69.39 ± 10.24	54.25 ± 8.17 (22)
1 hr post-dosing	12.68 ± 1.93	14.80 ± 3.72	57.72 ± 6.17	55.27 ± 11.83 (4)
2 hr post-dosing	14.79 ± 1.93	13.57 ± 1.98 (8)	54.49 ± 2.71	59.43 ± 10.83
4 hr post-dosing	11.07 ± 1.82	11.96 ± 1.41	52.52 ± 9.79	51.91 ± 13.79
8 hr post-dosing	10.45 ± 1.94	13.89 ± 2.17	51.47 ± 11.44	48.26 ± 8.22 (6)

Data from Table IB, pp. 49-68, MRID 46241401.
N = 5/group

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

Conclusions were made separately for each of the studies submitted. A peak effect was not determined in MRID 46241401. Administration of 0.2 mg/kg/day caused inhibition of serum, RBC, and brain enzyme activities in dam and pups while 0.1 mg/kg/day resulted in decreased serum and RBC activities in dams and slightly reduced serum enzyme activity in pups; no inhibition was found with 0.03 mg/kg/day (MRID 46241402). Age-related sensitivity was not found following either single or repeated dosing (MRID 46248101).

B. DISCUSSION AND REVIEWER COMMENTS:

A series of studies was conducted to determine ChE inhibition resulting from acute or repeated oral exposure of rats to phorate. These studies were submitted together for the assessment of comparative cholinesterase activity data as specified in the EPA Data-Call-In for adult and developmental neurotoxicity (DNT) studies on organophosphate pesticides. Most of the concerns raised in a protocol review (Raffaele, K., memo dated May 29, 2002; TXR #0050511) of the studies contained in this DER have been addressed in the current submissions. Deficiencies still include a lack of peak effect in the time-course study.

Acute exposure (adult and juvenile animals)
In adult animals, no treatment-related inhibition was observed in any compartment following a single dose of the test article to male and female adult. In pre-weaning rats, RBC and brain enzyme activities were not inhibited in PND 11 or 21 males and females at any dose. In serum, limited inhibition was observed at the highest dose, however, biological significance was marginal.

Repeated Exposure (adult and juvenile animals)
Inhibition of ChE activity was apparent in all compartments of pre-weaning rats and in RBC and plasma of adult female rats after repeated dosing. No effects were observed on enzyme activity in any compartment of adult males. At a dose of 0.2 mg/kg/day in PND 21 males and females, serum activity was inhibited 46% and 50%, respectively, RBC activity was inhibited 41% and 33%, respectively, and brain activity was inhibited 39% and 54%, respectively. Serum and RBC enzyme activities were inhibited 41% and 15%, respectively, in adult females at a dose of 0.2 mg/kg/day.

Maternal exposure on GDs 6-20 or GD 6-PND 10 (ChE activity in GD20 dams, GD 20 fetuses, and PND 4 pups)
Pregnant rats treated with 0.1 or 0.2 mg/kg/day had dose-related inhibition of serum (19% and 40%, respectively) and RBC (14% and 23%, respectively) activities. Brain activity was inhibited 41% at the highest dose. No treatment-related enzyme inhibition was observed in any compartment of GD 20 fetuses or PND 4 pups following maternal treatment.

Time-course of inhibition
During time-course investigations in both juvenile and adult rats, no statistically or biologically significant inhibition of enzyme activity occurred in any compartment at any time. Therefore, a time to peak effect could not be determined.

For acute oral exposure to phorate, the overall adult LOAEL for cholinesterase inhibition in rats is not identified and the adult NOAEL is 0.2 mg/kg, the highest dose tested.

For acute oral exposure to phorate, the overall offspring LOAEL for cholinesterase inhibition in rats is not identified and the offspring NOAEL is 0.2 mg/kg, the highest dose tested.

For repeated oral exposure to phorate, the overall adult LOAEL for cholinesterase inhibition in rats is 0.2 mg/kg/day based on enzyme inhibition in red blood cells from females; the adult NOAEL is 0.1 mg/kg/day.

For repeated oral exposure to phorate, the overall offspring LOAEL for cholinesterase inhibition in rats is 0.2 mg/kg/day based on enzyme inhibition in red blood cells and brain; the offspring NOAEL is 0.1 mg/kg/day.

The cholinesterase activity measurements following acute and repeated oral dosing of phorate demonstrate that juvenile rats are more susceptible than adults and that adult females were more susceptible than adult males. In pups and adult females the serum ChE activity appeared to be more sensitive than RBC or brain enzyme activity. This susceptibility was observed in terms of the dose level at which an effect was observed (i.e., the LOAEL for cholinesterase inhibition was lower for juveniles than for adults and lower for serum than for RBC or brain). Above the juvenile LOAEL, similar doses to young and adult rats produced a greater magnitude of effect in juveniles compared with the adults. Pregnant rats were more sensitive than non-pregnant females and showed effects similar to juveniles.

Two studies (MRIDs 46248101 and 46241402) are classified **Acceptable/Nonguideline** for the determination of serum, RBC, and brain cholinesterase activities following acute or repeated treatment with phorate in adult and juvenile rats.

The other study (MRID 46241401) is classified **Unacceptable/Nonguideline** for the determination of a time to peak effect for all compartments. In this study, a time to peak effect could not be determined because the dose levels employed were not high enough to cause enzyme inhibition.

C. STUDY DEFICIENCIES:

A major deficiency is lack of a definite time-to-peak effect. In this study, a time to peak effect could not be determined because the dose levels employed were not high enough to cause enzyme inhibition.

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