UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Date: March 19, 2008
TX Number: 0052717

SUBJECT: Terbufos – Reviews of the Following Toxicity Studies

- Study of the effects on cholinesterase levels in juvenile and young adult Wistar rats (age sensitivity study) (MRID 46247601)
- Range finding developmental neurotoxicity study in Wistar rats (MRID 46240802)
- Study of the effects on cholinesterase levels in juvenile and adult Wistar rats after single administration ("time-to-peak" study) (MRID 46240801)

PC Code: 105001
DP Number: D305170

FROM: Paul Chin, Ph.D.
Reregistration Branch I
Health Effects Division (7509P)

Linda Taylor, Ph.D.
Reregistration Branch I
Health Effects Division (7509P)

TO: Tracy Perry/Susan Lewis
Reregistration Branch I
Special Review & Reregistration Division (7508P)

THROUGH: Michael Metzger, Branch Chief
Reregistration Branch I
Health Effects Division (7509P)

I. CONCLUSIONS

The registrant, BASF Corporation, submitted three studies with terbufos. One study (MRID 46247601) was reviewed by Health Effects Division (HED), OPP. Two studies (MRIDs 46240801 and 46240802) were reviewed by the contractor, Oak Ridge National Laboratory, and

Received in RIC 3/27/2008

went through the secondary review process in HED. All studies were classified as acceptable/non-guideline. The DERs for these studies are attached to this memorandum and the citation of each is presented below.

II. ACTION REQUESTED

The registrant, BASF Corporation, submitted three studies. SRRD requested RRB1, HED to review and prepare DERs for these studies.

The following lists each study with the MRID number:

Schneider, S.; Deckardt, K.; van Ravenzwaay, B. (2004). BAS 316 I (Terbufos) - Study of the Effects on Cholinesterase Levels in Juvenile and Young Adult Wistar Rats (Age Sensitivity), Oral Administration (Gavage): Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Final Report. Project Number: 06R0090/02059, 2004/1011013, April 2, 2004. MRID 46247601. Unpublished

Schneider, S.; Deckardt, K.; van Ravenzwaay, B. (2004). BAS316 I (terbufos) – Study of the effects on cholinesterase levels 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. Project Number 06R0090/02032, March 22, 2004. MRID 46240801. Unpublished.

Schneider, S.; Deckardt, K.; van Ravenzwaay, B. (2004). BAS316 I (terbufos) – 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. Project Number 29R0090/02006, March 19, 2004. MRID 46240802. Unpublished.

EPA Reviewer: Paul Chin, Ph.D. **Signature:** Paul Chin
Reregistration Branch 1, Health Effects Division (7509P) **Date:** 3/13/08
EPA Secondary Reviewer: Linda Taylor, Ph.D. **Signature:** Linda Taylor
Reregistration Branch 1, Health Effects Division (7509P) **Date:** 3/13/08

DATA EVALUATION RECORD

TXR#: 0052717

STUDY TYPE: Non-guideline Age Sensitivity Study (Study of the Effects on Cholinesterase Levels in Juvenile and Young Adult Rats)

DP BARCODE: D349105
P.C. CODE: 105001

TEST MATERIAL (PURITY): BAS 316 I (Terbufos), 88.8% a.i.

SYNONYMS: S-tert-butylthiomethyl O,O-diethyl phosphorodithioate

CITATION: Schneider, S.; Deckardt, K.; van Ravenzwaay, B. (2004). BAS 316 I (Terbufos) - Study of the Effects on Cholinesterase Levels in Juvenile and Young Adult Wistar Rats (Age Sensitivity), Oral Administration (Gavage): Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Final Report. Project Number: 06R0090/02059, 2004/1011013, April 2, 2004. MRID 46247601. Unpublished

SPONSOR: BASF Corporation, Agricultural Products Division, Research Triangle Park, NC.

EXECUTIVE SUMMARY:

In a special non-guideline study (MRID 46247601), terbufos (batch #AC12251-100, 88.8% a.i.) was administered to groups of Wistar (CrlGlxBrlHan:WI) rats (8/sex/age group) *via* gavage at dose levels of 0 (corn oil), 0.01, 0.08 and 0.15 mg/kg to assess whether there is an age-sensitivity in the effects of an acute oral dose of terbufos on cholinesterase (ChE) levels in rats. Treatment groups consisted of 11- and 21-day old juvenile and 60-day old young adult rats. In addition, groups of 60-day old young adult rats (8/sex) were given the same doses of terbufos by gavage from post-natal day (PND) 60 through PND 70 to compare the effects of terbufos on ChE levels in young adult rats following 11-day repeated oral administration. ChE activity was determined by a modified Ellman method in serum, red blood cell (RBC) and brain approximately 4 hours after test material administration. No further examinations were performed.

There were no clinical signs of toxicity in any of the juvenile animals in either age group or in the young adult animals. At the high-dose level, a significant decrease in serum (55% inhibition in both sexes) and RBC (30% inhibition in males; 20% inhibition in females not statistically significant) ChE was observed in the 11-day old rats administered 0.15 mg/kg of terbufos. The brain ChE inhibition (24% in males; 14% in females) in the 0.15 mg/kg group did not attain statistical significance; however, the magnitude of the response is considered treatment-related and adverse. In the 0.08 mg/kg group, a slight but significant decrease in serum (15% inhibition in females only) ChE activity was observed.

Four hours after a single administration of 0.15 mg/kg of terbufos to the 21-day old rats, a significant decrease in serum (38%/30% inhibition in males/females) ChE activity was observed. The brain ChE inhibition in 0.15 mg/kg (24% in males) and 0.08 mg/kg (22% in males) groups did not attain statistical significance; however, the magnitude of the response is considered treatment-related and adverse.

Four hours after a single administration of terbufos to the 60-day old young adult animals, no treatment-related changes were noted in serum, RBC or brain cholinesterase activity in either sex at any dose level examined. This is consistent with the NOAEL (0.15 mg/kg) identified in the acute neurotoxicity study in Sprague-Dawley rats.

After 11-day repeated administrations of 0.15 mg/kg of terbufos to the young adult rats, a significant decrease in serum (43%/85% inhibition in males/females), RBC (32/%75% inhibition in males/females), and brain (43% inhibition in females; 12% inhibition in males) ChE activity was observed. In both sexes of the 0.08 mg/kg group, a statistically significant decrease in serum (17%/59 % inhibition in males/females) and RBC (16%/18% inhibition in males/females) ChE activity was observed although the magnitude of RBC (males and females) and plasma (males) cholinesterase inhibition was not biologically significant. The brain ChE inhibition in 0.08 mg/kg group (21%/33% inhibition in males/females) did not attain statistical significance; however, the magnitude of the response is considered treatment-related and adverse although there was the lack of dose-response in males for brain ChEI (21% at 0.08 mg/kg group and 12% at 0.15 mg/kg group). In the 0.01 mg/kg group, a slight but significant decrease in serum (19 % inhibition in females) ChE activity was observed. The magnitude of the inhibition in each compartment was greater in the young adult female than in the young adult male.

The results of this study demonstrated an age-sensitivity in the effects of an acute dose of terbufos on ChE levels; *i.e.*, the younger rat demonstrated the greater response. Four hours after acute exposure to terbufos, the 11-day old rat showed a greater response than the 21-day old rat with respect to the magnitude of ChE inhibition in two compartments (serum and RBC) and the age-sensitivity was similar with respect to the magnitude of ChE inhibition in the brain compartment (males). Additionally, males displayed a slightly greater response (RBC and brain) than the females. In the 60-day old young adult rats, ChE activity was not inhibited in any of the compartments following acute exposure.

The results of this study demonstrated the effects of repeated exposure to terbufos on ChE levels in young adult rats. Marked ChE inhibition in all compartments was observed in the young adult rats following 11 days of repeat dosing, at the dose levels that had no effect on ChE levels in any compartment following acute exposure. Although repeat dosing of the PND 11 and PND 21 rats was not performed for comparison, it can be assumed that a greater response (ChE inhibition) would be seen in the PND 11 and PND 21 rats in all compartment following repeat exposure than was seen following acute exposure.

This study is classified as **acceptable/non-guideline**. The stated purpose of comparing the effects of terbufos on cholinesterase levels in juvenile and young adult rats after oral administration was accomplished.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Terbufos

Description:	Liquid/colorless to pale yellow
Batch #:	AC 12251-100
Purity:	88.8 % a.i.
Compound Stability:	"Proven by reanalysis" (certificate of analysis 131312_1)
CAS # of TGAI:	13071-79-9

2. Vehicle: corn oil

3. Test animals (P):

Species:	Rat	
Strain:	Wistar (CrlGlxBrlHan:WI)	
Age at study initiation:	Time-mated females: 10-12 wks	
Wt. at study initiation:	136-182 g (on post-coital day 0)	
Source:	Charles River Laboratories, Germany	
Housing:	Individually in stainless steel cages, except from gestation day 18 to lactation day 21 when dams, litters and subset II were in Makrolon type M III cages	
Diet:	ground or pelleted Kliba maintenance diet rat/mouse/hamster (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum	
Water:	Tap water, ad libitum	
Environmental conditions:	Temperature:	20-24°C
	Humidity:	30-70%
	Air changes:	Not provided
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	6 days	

B. PROCEDURES AND STUDY DESIGN:

IN LIFE DATES: Start: 7-7-03; End: 9-6-03

ANIMAL ASSIGNMENT

Animals were randomly assigned to the test groups noted in Table 1.

Table 1. Study design [a]

Test group	Dose (mg/kg)	Actual dose (mg/kg)	No. of animals/ sex
Pups (treated on PND 11 , 21, or 60)			
0	0	0	8
1	0.01	0.011	8
2	0.08	0.090	8
3	0.15	0.169	8
Young Adult animals (treated on PND 60 through 70)			
0	0	0	8
1	0.01	0.011	8
2	0.08	0.090	8
3	0.15	0.169	8

a Data obtained from the study report, Tables I-25 and I-26.

Time mating of females was carried out at Charles River Laboratories, Germany. The day that a vaginal plug or sperm in a vaginal smear was detected was designated gestation day (GD) 0. Females presumed to be pregnant were delivered to the testing laboratory on GD 0. The litters from these females (dams not treated with terbufos) were the test animals used in the study.

On postnatal day (PND) 4, individual litters were standardized in such a way that, where possible, each litter contained 4 male and 4 female pups. Pups were weaned from the dam on PND 21 and dams were sacrificed after the weaning.

Groups of PND 11- and 21-day old juvenile and 60-day old young adult rats (8/sex/age group) were given a single oral (gavage) dose of terbufos at a dose of 0 (corn oil), 0.01, 0.08, or 0.15 mg/kg. In addition, 60-day old young adults (8/sex) were given the same doses of terbufos by gavage for 11 days (from post-natal days 60 through 70). The volume administered was 5 mL/kg of body weight. Dosing was based on the most recent body weight determination.

PUPS (11- and 21-day old juvenile rats)

The live pups were examined daily for clinical symptoms during the clinical inspection of the dams. On PNDs 11 and 21, 8 pups/sex/group were sacrificed after isofluran-anesthetic by decapitation. Blood samples were collected (4 hours post dose) from the vena cava cranialis. Brain samples were collected from the same animals following blood collection.

YOUNG ADULT ANIMALS (60 day old rats):

The young adult animals were examined each day for clinical symptoms and, during the administration period, for overt signs of toxicity. Groups of 8 young adult rats of each gender were sacrificed by decapitation (following isoflurane anesthesia) at 4 hours after administration

of the test material. Blood samples from the young adult rats were collected from the retroorbital venous plexus. Brain samples were collected from the same animals following blood collection.

CHOLINESTERASE MEASUREMENTS

The blood and brain samples were analyzed using a spectrophotometric procedure, based on modified Ellman's method that was adapted to a Cobas Fara analyzer. Blood and brain samples were kept on ice during collection and processed as soon as possible. After all the brains were collected, the samples were deep frozen and stored at -80°C until analysis. Hematocrit or protein content of the brain was determined in order to calculate the cholinesterase activity of red blood cells per liter erythrocytes and specific cholinesterase activity of the brain, respectively.

STATISTICS

The following parameters were analyzed using the Dunnett's test (two-sided) for the hypothesis of equal means: food consumption (dams), body weight and body weight gain (dams and pups), duration of gestation and number of pups delivered per litter.

Cholinesterase data were analyzed using Kruskal-Wallis test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of the dose groups with the control was performed using Wilcoxon test (two-sided) for the hypothesis of equal medians.

II. RESULTS

Test substance analysis

Homogeneity analysis: Homogeneity analysis was not performed since the test material was considered a true solution and uniformly distributed in the corn oil.

Stability analysis: Analyses were also performed on the stability of terbufos in corn oil up to 7 days at room temperature. The analyses demonstrated that test article concentrations corresponded to the nominal concentrations within the range of tolerance (±10%).

Concentration analysis: Concentrations of samples ranged from 91 to 102 % of nominal. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

PUPS (11- and 21-day old juvenile rats)

There were no clinical signs of toxicity in any of the pups. It is noted that in mid-dose pups, increased mean body weights gains (11% increase, $p \leq 0.05$) were observed on PNDs 11-21. This observation is considered as spontaneous event due to the lack of dose-response relationship.

YOUNG ADULT ANIMALS (60 day old rats):

One control male animal (No. 113) was found dead on PND 69 without showing any remarkable clinical signs before the unscheduled death and mid dose male (No. 137) was sacrificed moribund on PND 67. The animal No. 137 showed a poor general state, respiratory sounds and piloerection presumably due to gavage error. No clinical signs of toxicity were observed in male or female young adult animals except for the animal No. 137 mentioned above.

No biologically significant treatment-related effects on body weights/body weight gains were noted in the young adult animals. It is noted that in mid-dose males, decreased mean body weights (10% decrease, $p \leq 0.05$) were observed on PNDs 21 and 67. These observations are considered as spontaneous event due to the lack of dose-response relationship.

CHOLINESTERASE EXAMINATIONS

Pups treated on PND 11 (4 hours after a single administration of terbufos)

Results of cholinesterase (ChE) activity assessment in pups on PND 11 or 21 4 after a single oral administration of terbufos are presented in Table 2. In the 0.15 mg/kg group, a significant decrease in serum (55% inhibition in both sexes; $p \leq 0.01$) and RBC (30% inhibition in males; $p \leq 0.05$) ChE activity was observed. In the 0.15 mg/kg group, the brain ChE inhibition (24% in males) did not attain statistical significance; however, the magnitude of the response is considered treatment-related and adverse. In the 0.08 mg/kg group, a significant slight decrease in serum (15% inhibition in females; $p \leq 0.01$) ChE activity was observed. No treatment-related effects on ChE activity in any compartment were noted in the 0.01 mg/kg test groups.

Pups treated on PND 21 (4 hours after a single administration of terbufos)

In the 0.15 mg/kg group, a significant decrease in serum (38%/30% inhibition in males/females; $p \leq 0.01$) ChE activity was observed. The brain ChE inhibition in the 0.15 mg/kg (24% in males) and 0.08 mg/kg (22% in males) groups did not attain statistical significance; however, the magnitude of the response is considered treatment-related and adverse. No treatment-related effects on ChE activity in any compartment were noted in the 0.01 mg/kg test groups.

Young Adult animals treated on PND 60 (4 hours after a single administration of terbufos)

No treatment-related changes were noted in serum, RBC or brain cholinesterase activity for male or female young adult (60 day old) animals after a single administration of terbufos (Table 3).

Young Adult animals treated on PNDs 60-70 (4 hours after 11-day repeated administration of terbufos)

Results of cholinesterase (ChE) activity assessment in young adult animals after 11-day repeated administration of terbufos are presented in Table 3. In the 0.15 mg/kg group, a significant decrease in serum (43%/85% inhibition in males/females; $p \leq 0.01$), RBC (32%/75% inhibition in males/females; $p \leq 0.01$), and brain (12% inhibition in males; not significant and 43% inhibition in females; $p \leq 0.05$) ChE activity was observed. In the 0.08 mg/kg group, a significant decrease

TABLE 2. Cholinesterase activity in 11- or 21-day old rats 4 hours after a single oral administration of terbufos

Dose (mg/kg)	Cholinesterase [mean ± SD (% inhibition relative to control)]					
	Males			Females		
	Serum	RBC	Brain	Serum	RBC	Brain
11-day old rats						
0	18.92 ± 2.26	21.32 ±2.85	1.71±0.48	18.79 ±1.76	21.23±3.92	1.58 ±0.41
0.01	18.57 ±1.08 (2)	20.62 ±3.65 (3)	1.58 ±0.41 (7)	18.59 ±1.48 (1)	21.32 ±2.93	1.52 ±0.29 (3)
0.08	17.02 ±1.73 (10)	18.83 ±3.05 (12)	1.78 ±0.45	**16.03 ±1.49** (15)**	20.76 ±3.67 (2)	1.45 ±0.31 (8)
0.15	**8.47 ±2.24** (55)**	**14.85 ±5.30* (30)**	1.30 ±0.44 (24)	**8.39 ±1.54** (55)**	16.93 ±3.59 (20)	1.35 ±0.31 (14)
21-day old rats						
0	14.50 ± 1.18	39.71 ±4.82	2.38±0.85	13.74 ± 1.28	34.52 ±5.47	2.05 ±0.60
0.01	13.77 ±1.73 (5)	38.02±7.82 (4)	2.17 ±0.54 (9)	13.60 ±1.77 (1)	36.15±6.07	2.30 ±0.51
0.08	13.72 ± 1.93 (5)	40.00 ±6.22	1.85±0.53 (22)	13.10 ± 1.22 (5)	35.12 ±7.53	2.09±0.64
0.15	**8.93 ±1.19** (38)**	32.53 ±5.69 (18)	1.80±0.61 (24)	**9.67 ±0.84** (30)**	33.89 ±5.08 (2)	1.98±0.39 (3)

Data obtained from the study report, pages 87-90, Table IB N=8 * Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$ Units: Serum=µkat/L serum; RBC= µkat/L RBC; Brain= µkat/g protein

TABLE 3. Cholinesterase activity in 60-day old rats (treated once with terbufos) and 70-day old rats (treated with terbufos from PND 60 through 70)

Dose (mg/kg)	Cholinesterase [mean ± SD (% inhibition relative to control)]					
	Males			Females		
	Serum	RBC	Brain	Serum	RBC	Brain
Adult animals treated once on PND 60						
0	10.20 ± 1.16	27.23 ±4.82	1.65±0.65	33.46 ±7.57	23.98±4.25	2.63 ±1.16
0.01	10.65 ±1.45	26.20 ±3.68	2.27 ±0.80	36.89 ±8.85	24.57 ±5.10	2.07 ±0.71
0.08	9.64 ±1.78	28.34 ±4.91	2.10 ±1.06	31.45 ±11.26	28.95 ±4.71	2.55 ±1.23
0.15	10.14 ±1.95	23.28 ±3.83	2.72 ±1.48	30.19 ±5.74	24.97 ±3.89	2.31 ±1.19
Adults animals on PND 70 (treated with terbufos from PND 60 through 70)						
0	10.57± 1.53	30.97 ±3.65	3.22±1.46	42.51 ± 6.95	29.40 ±3.22	2.50 ±1.10
0.01	11.71 ±1.34	28.04±3.15 (9)	3.21± 1.42 (0)	**34.27 ±9.89* (19)**	29.84±4.20	2.50 ±0.91
0.08	**8.76 ± 1.16* (17)**	**26.08 ±2.79* (16)**	2.56±1.06 (21)	**17.39 ± 4.14** (59**	**24.23 ±6.92* (18)**	1.66±0.37 (33)
0.15	**6.00 ±0.63** (43)**	**20.93 ±2.02 (32)**	2.83±0.90 (12)	**6.18 ±1.69** (85)**	**7.35 ±1.21** (75)**	**1.43±0.57* (43)**

Data obtained from the study report, pages 91-94, Table IB N=8
* Statistically different from control, $p<0.05$ ** Statistically different from control, $p<0.01$
Units: Serum=µkat/L serum; RBC= µkat/L RBC; Brain= µkat/g protein

in serum (17%/59 % inhibition in males/females; p≤0.05 or 0.01) and RBC (16%/18% inhibition in males/females; p≤0.05) ChE activity was observed. The brain ChE inhibition in 0.08 mg/kg group (21%/33% inhibition in males/females) did not attain statistical significance; however, the magnitude of the response is considered treatment-related and adverse although there was the lack of a dose-response in males for brain ChEI (21% at 0.08 mg/kg group and 12% at 0.15 mg/kg group). In the 0.01 mg/kg group, a slight but significant decrease in serum (19 % inhibition in females; p≤0.05) ChE activity was observed. However, this decrease in serum ChE activity is not considered to be biologically significant.

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The investigators concluded that administration of a single dose of 0.15 and 0.08 mg/kg of turbufos to male and female pups inhibited ChE activities in both sexes on PNDs 11 and 21 in a comparable manner. Marked ChE inhibition in all compartments was observed in the young adult rats following 11 days of dosing, while ChE levels in all compartments were not inhibited in the 60-day old young adult rats 4 hours after acute oral exposure to terbufos.

B. REVIEWER COMMENTS:

This was a well-designed and conducted study that adequately accomplished the purpose of comparing the effects of terbufos on cholinesterase levels in juvenile and young adult rats after acute and repeat oral administration.

There were no clinical signs of toxicity in any of the juvenile animals in either age group or in the young adult animals. At the high-dose level, a significant decrease in serum (55% inhibition in both sexes) and RBC (30% inhibition in males; 20% inhibition in females not statistically significant) ChE was observed in the 11-day old rats administered 0.15 mg/kg of terbufos. The brain ChE inhibition (24% in males; 14% in females) in the 0.15 mg/kg group did not attain statistical significance; however, the magnitude of the response is considered treatment-related and adverse. In the 0.08 mg/kg group, a slight but significant decrease in serum (15% inhibition in females only) ChE activity was observed.

Four hours after a single administration of 0.15 mg/kg of terbufos to the 21-day old rats, a significant decrease in serum (38%/30% inhibition in males/females) ChE activity was observed. The brain ChE inhibition in 0.15 mg/kg (24% in males) and 0.08 mg/kg (22% in males) groups did not attain statistical significance; however, the magnitude of the response is considered treatment-related and adverse.

Four hours after a single administration of terbufos to the 60-day old young adult animals, no treatment-related changes were noted in serum, RBC or brain cholinesterase activity in either sex at any dose level examined. This is consistent with the NOAEL (0.15 mg/kg) identified in the acute neurotoxicity study in Sprague-Dawley rats.

After 11-day repeated administrations of 0.15 mg/kg of terbufos to the young adult rats, a significant decrease in serum (43%/85% inhibition in males/females), RBC (32/%75% inhibition in males/females), and brain (43% inhibition in females; 12% inhibition in males) ChE activity was observed. In both sexes of the 0.08 mg/kg group, a statistically significant decrease in serum (17%/59 % inhibition in males/females) and RBC (16%/18% inhibition in males/females) ChE activity was observed although the magnitude of RBC (males and females) and plasma (males) cholinesterase inhibition was not biologically significant. The brain ChE inhibition in 0.08 mg/kg group (21%/33% inhibition in males/females) did not attain statistical significance; however, the magnitude of the response is considered treatment-related and adverse although there was the lack of dose-response in males for brain ChEI (21% at 0.08 mg/kg group and 12% at 0.15 mg/kg group). In the 0.01 mg/kg group, a slight but significant decrease in serum (19 % inhibition in females) ChE activity was observed. The magnitude of the inhibition in each compartment was greater in the young adult female than in the young adult male.

The results of this study demonstrated an age-sensitivity in the effects of an acute dose of terbufos on ChE levels; *i.e.*, the younger rat demonstrated the greater response. Four hours after acute exposure to terbufos, the 11-day old rat showed a greater response than the 21-day old rat with respect to the magnitude of ChE inhibition in two compartments (serum and RBC) and the age-sensitivity was similar with respect to the magnitude of ChE inhibition in the brain compartment (males). Additionally, males displayed a slightly greater response (RBC and brain) than the females. In the 60-day old young adult rats, ChE activity was not inhibited in any of the compartments following acute exposure.

The results of this study demonstrated the effects of repeated exposure to terbufos on ChE levels in young adult rats. Marked ChE inhibition in all compartments was observed in the young adult rats following 11 days of repeat dosing, at the dose levels that had no effect on ChE levels in any compartment following acute exposure. Although repeat dosing of the PND 11 and PND 21 rats was not performed for comparison, it can be assumed that a greater response (ChE inhibition) would be seen in the PND 11 and PND 21 rats in all compartment following repeat exposure than was seen following acute exposure.

This study is classified as **acceptable/non-guideline**. The stated purpose of comparing the effects of terbufos on cholinesterase levels in juvenile and young adult rats after oral administration was accomplished.

C. STUDY DEFICIENCIES:

There were no deficiencies that affected the conduct or outcome of the reviewed study. Brain cholinesterase activity for the 60-day old adult male rats in the control and the test groups had large variation based on the lower variability usually seen in this tissue in comparison to the blood measures. For example, the brain ChE activity for the control group was 1.65±0.65 and it was 2.27±0.80 for the 0.01 mg/kg group or an unexplained increase of 38% in activity. The standard deviations were also **extremely large** for brain cholinesterase activity being 39% for the control group and they ranged 35-54% for the test groups. Although ChE inhibition in the young adult male brain (following repeat exposure) did not demonstrate a dose-response, based on the dose-response seen in the female for this compartment and the variations displayed in the

brain ChE data, the effect observed at 0.08 and 0.15 mg/kg/day are considered treatment related and biologically significant.

No justification was given for using the dose not higher than the NOAEL (0.15 mg/kg) identified in the acute neurotoxicity study in Sprague-Dawley rats. Also, ChE activity was not assessed at 6 hours post dose which was the peak-effect time chosen for the acute neurotoxicity study.

DATA EVALUATION RECORD

BAS 316 I (TERBUFOS)
NON-GUIDELINE

STUDY TYPE: TIME-TO-PEAK EFFECT – CHOLINESTERASE INHIBITION - RAT
MRID 46240801

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 175-2007

Primary Reviewer:
Sylvia S. Talmage, Ph.D., D.A.B.T.
Signature: Sylvia S. Talmage
Date: MAY 04 2007

Secondary Reviewers:
Carol S. Wood, Ph.D., D.A.B.T.
Signature: Carol S. Wood
Date: MAY 04 2007

Robert H. Ross, M.S., Group Leader
Signature: Robert H. Ross
Date: MAY 04 2007

Quality Assurance:
Lee Ann Wilson, M.A.
Signature: L. A. Wilson
Date: MAY 04 2007

Disclaimer

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

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Paul Chin, Ph.D. **Signature:** Paul Chin
Reregistration Branch 1, Health Effects Division (7509P) **Date:** 2/20/08
EPA Secondary Reviewer: Linda Taylor, Ph.D. **Signature:** Linda Taylor
Reregistration Branch 1, Health Effects Division (7509P) **Date:** 02-20-08
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** MOttley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 2/20/08

Template version 02/06

TXR#: 0052717

DATA EVALUATION RECORD

STUDY TYPE: Time-to-Peak Effect – Cholinesterase Inhibition - Rats (Non-Guideline)

PC CODE: 105001 **DP BARCODE:** D 305170

TEST MATERIAL (PURITY): BAS 316 I (Terbufos), 88.8% a.i.

SYNONYMS: S-tert-butylthiomethyl O,O-diethyl phosphorodithioate

CITATION: Schneider, S.; Deckardt, K.; van Ravenzwaay, B. (2004). BAS316 I (terbufos) – Study of the effects on cholinesterase levels 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. Project Number 06R0090/02032, March 22, 2004. MRID 46240801. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, Research Triangle Park, NC.

EXECUTIVE SUMMARY: In a special non-guideline study (MRID 46240801), groups of non-fasted 11-13 week-old adult and 22-day old juvenile Wistar (CrlGlxBrlHan:WI) rats (25/sex/age group) were given a single oral (gavage) dose of technical BAS 316 I (terbufos; batch #AC12251-100, 88.8% a.i.) in corn oil at a dose of 0.2 mg/kg bw in order to determine the time of peak effect on cholinesterase activity. Groups of 25 controls/sex in each age group were administered an equal volume of corn oil. Cholinesterase activity was determined by a modified Ellman method in 5 adult and 5 juvenile rats/sex/dose in serum, erythrocytes, and whole brain at 0.5, 1, 2, 4, and 8 hours after test material administration. No further examinations were performed.

All animals in both age groups survived until scheduled sacrifice, and there were no clinical signs of toxicity.

In the adult animal, maximum inhibition of serum and erythrocyte cholinesterase activity occurred at 8 hours after test substance administration (males 28% and 17%/females 57% and 23%, respectively). Brain cholinesterase inhibition was not observed in the adult male. The

cholinesterase inhibition observed in the adult female brain compartment (21% at 8 hours) did not attain statistical significance; however, the magnitude of the response is considered treatment-related and adverse. The magnitude of the inhibition in each compartment was greater in the adult female than in the adult male.

In the juvenile animal, maximum inhibition in all compartments was observed at 4 hours post dose (serum 60%; RBC 35%; brain 20%) in the male. In the juvenile female, maximum inhibition was observed at 4 hours post dose in serum (56%) and at 8 hours post dose in the RBC (38%) and brain (22%) compartments. The magnitude of the inhibition in each compartment was comparable between the sexes.

Based on the results of this one-dose study, assuming peak effect time is before or at 8 hours post dose, the juvenile male rat showed a greater response than the adult male rat with respect to the magnitude of cholinesterase inhibition in all compartments, and the time of maximum inhibition was shorter in the juvenile male (all compartments). The adult and juvenile female rats displayed a similar response with respect to the magnitude of cholinesterase inhibition in the serum and brain compartments, and the maximum inhibition was the same in the RBC and brain compartments for both age groups. The peak-effect time in serum may be shorter in the juvenile female (4 hours) than in the adult female (8 hours). The magnitude of cholinesterase inhibition in the brain compartment (20%-22%) was comparable between the adult females and the juvenile rats of both sexes.

Based on the lack of dose-response data and an assessment of cholinesterase activity at the 6-hour time point (peak-effect time in acute neurotoxicity study) and a time point greater than 8 hours, a definitive conclusion is not possible regarding the peak-effect time and magnitude of effect for either age group.

This study is classified as **Acceptable/Nonguideline**. There were no guideline requirements.

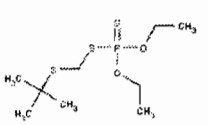
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** BAS 316 I, technical

Description:	Liquid, colorless to pale yellow
Batch #:	AC 12251-100
Purity:	88.8 % a.i.
CAS # of TGAI:	13071-79-9
Structure:	



2. **Vehicle and/or positive control:** Test material was prepared in corn oil.

3. **Test animals:**

Species:	Rat	
Strain:	Wistar, CrlGlxBrlHan:WI	
Age/weight at dosing:	Adults: about 11-13 weeks/ males: 296.4±11.27 g (control) and 298.8±12.01g (test group); females: 218.7±8.4 g (control) and 217.6±8.92g (test group) Juveniles: 22 days old/ males: 39.8±3.14 g (control) and 39.9±3.22g (test group); females: 39.9±3.59 g (control) and 40.8±3.29g (test group). (presumed pregnant females supplied by breeder for in-house delivery of litters approximately 22 days prior to test material administration)	
Source:	Charles River Laboratories, Germany	
Housing:	Adults: individually in type DK III stainless steel wire mesh cages Juveniles: Makrolon type I cages Pregnant females with litters: Makrolon type M III cages	
Diet:	Adults: ground Kliba maintenance diet, *ad libitum*; juveniles: pelleted Kliba maintenance diet, *ad libitum* (both from Provimi Kliba SA, Kaiseraugst, Switzerland),	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	20-24 °C
	Humidity:	30-70%
	Air changes:	Not given (air-conditioned rooms)
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	Adults: 6 days	

B. STUDY DESIGN:

1. **In life dates:** Start: 10-28-2002; End: 10-30-2002

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1. No information on randomization was provided; however, animals were weighed prior to dosing and a statistical analysis was performed comparing group mean body weight relative to control for equality of group means body weight. Groups of 25 adult and 25 juvenile rats of each gender were administered a single dose of 0.23 mg BAS 316 I /kg in corn oil (dose of 0.20 mg/kg bw based on a.i.); groups of 25 adult and 25 juvenile control rats of each gender received the corn oil vehicle. Dose volumes were 5 mL/kg bw. All animals were examined for clinical signs before and after administration. Groups of 5 adult and 5 juvenile rats of each gender were sacrificed by decapitation (following isoflurane anesthesia) at 0.5, 1, 2, 4, and 8 hours after administration of the test material. Blood and brain were collected for cholinesterase activity determination. Blood was collected from adults and juveniles from the retroorbital venous plexus and vena cava cranialis, respectively. Animals were discarded after sacrifice. Dose selection was not discussed.

TABLE 1. Study design				
Experimental parameter	Adults		Juveniles	
	Control	0.2 mg/kg	Control	0.2 mg/kg
Total number of animals/sex/group	25	25	25	25
Blood and brain cholinesterase determination				
0.5 hours	5/sex	5/sex	5/sex	5/sex
1 hour	5/sex	5/sex	5/sex	5/sex
2 hours	5/sex	5/sex	5/sex	5/sex
4 hours	5/sex	5/sex	5/sex	5/sex
8 hours	5/sex	5/sex	5/sex	5/sex

3. **Test Substance preparation and analysis:** An appropriate amount of the test substance was weighed, corn oil was added, and the materials were mixed by shaking. A dose of 0.23 mg/kg of the technical material was prepared in order to deliver a dose of 0.2 mg/kg based on a.i. Homogeneity was not verified. Concentration of frozen samples was verified analytically by high-performance liquid chromatography. Stability was tested following storage for 3 or 7 days at ambient temperature. No information on how the samples were collected was provided.

Results:

Homogeneity analysis: Homogeneity analysis was not performed since the test material was considered a true solution and uniformly distributed in the corn oil.

Stability analysis: Samples stored for 3 and 7 days at ambient temperature were 91.9 and 91.4%, respectively, of the nominal day 0 value.

Concentration analysis: The mean value of the two samples was 91.3% of nominal.

The analytical data indicated that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics:** Mean body weight prior to test material administration was analyzed by simultaneous comparison of all dose groups with the respective control group using the Dunnett two-sided test for the hypothesis of equal means. For cholinesterase activity, the dose group was compared with the control group using the Wilcoxon test (two sided) for the equality of means. Statistical analyses were not pertinent to additional study results.

C. METHODS / OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed for clinical signs before and following administration of the test material.

2. **Body weight:** Animals were weighed prior to dosing.

3. **Food consumption:** Animals were sacrificed within 8 hours of test material administration. Food consumption was not relevant to the study results.

4. **Cholinesterase determination:** Cholinesterase activity was determined in 5 animals/sex/group (both age groups) at 0.5, 1, 2, 4, and 8 hours after test material administration. Blood (approximately 1 mL) was collected from adults and juveniles from the retroorbital venous plexus and vena cava cranialis, respectively. Brains were collected from the same animals immediately following blood collection. Samples were kept on ice during sampling and processed as quickly as possible after collection. Brain samples were deep-frozen at -80 °C and stored until analysis. Analysis for cholinesterase activity used a spectrophotometric procedure based on a modified Ellman method (adapted to a Cobas Fara analyzer [Hoffmann LaRoche, Germany]). Hematocrit values were determined in order to calculate cholinesterase activity of RBC per liter erythrocytes, and brain protein was analyzed in order to calculate specific cholinesterase activity in brain.

5. **Neurobehavioral assessment:** Neurobehavioral assessments were not performed.

6. **Sacrifice and pathology:** Carcasses were discarded following blood and brain sampling.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** There were no test material-related clinical signs in either adult or juvenile rats.

2. **Mortality:** There were no mortalities in any test group.

B. FOOD CONSUMPTION:

Food consumption was not measured as part of the study protocol.

C. CHOLINESTERASE ACTIVITY:

Serum and RBC cholinesterase (ChE) activities are summarized in Table 2 and brain cholinesterase activities are summarized in Table 3.

Adult animals

Serum ChE: Adult males displayed statistically-significant serum cholinesterase inhibition (28% *) at the 8-hour time point and non-statistically significant inhibition (22%) at the 4-hour time po int. Similarly, adult females displayed statistically-significant serum cholinesterase inhibition (57 %**) at the 8-hour time point and non-statistically significant inhibition (35%) at the 4-hour time point. The response in females was greater than in males at both time points. In both cases, alth ough the maximum inhibition was observed at 8 hours, it is not possible to determine the peak-ef fect time for adult animals without a subsequent time point.

RBC ChE: Adult males did not display a statistically-significant inhibition in RBC cholinesterase activity at any time point; the highest level of inhibition occurred at 8 hours (17%). Similarly, the adult females displayed a non-significant inhibition (23%) at the 8-hour time point. In both cases, although the maximum inhibition was observed at 8 hours, it is not possible to determine the peak-effect time for adult animals without a subsequent time point.

Brain ChE: Adult males did not display any brain cholinesterase inhibition at any time point. Adult females displayed non-significant inhibition at the 2-hour (37%), 4-hour (13%), and 8-hour (21%) time points. Persual of the individual data shows a large variation over time in the controls (both sexes). For example, control females displayed a 61% increase in activity between the 0.5-hour and 2-hour time points. No historical control data on brain cholinesterase activity were discussed or presented. The inhibition observed at the 8-hour time point was considered the peak-effect time by the study authors.

Juvenile animals

Serum ChE: Juvenile animals displayed statistically-significant inhibition at the 2-, 4-, and 8-ho ur time points (males 45%**, 60%**, and 43%**, respectively; females 41%**, 56%**, and 44 %**, respectively). The % inhibition was similar between the sexes at their respective peak-effe ct times. In both cases, although the maximum inhibition was observed at 4 hours, without a 6 ho ur time point, it is not possible to determine the peak-effect time for juvenile animals.

RBC ChE: Juvenile males displayed statistically-significant inhibition at the 2-, 4-, and 8-hour time points (25%*, 35%**, and 27%?*, respectively). Juvenile females displayed a non-significant inhibition at the 4-hour time point (21%) and a statistically-significant inhibition at the 8-hour time point (38%**). The % inhibition was similar between the sexes at their respective peak-effect times, with the males displaying the earlier peak-effect time. In both cases, although the maximum inhibition was observed at 4 hours, it is not possible to determine the peak-effect time for juvenile animals without a 6 hour time point.

Brain ChE: Juvenile males displayed significant inhibition at the 2- and 4-hour time points (18%** and 20%**, respectively) and a non-significant inhibition (14%) at the 8-hour time point. Juvenile females displayed a significant inhibition only at the 8-hour time point (22%*). The magnitude of the inhibition was comparable between the sexes, but the maximum inhibition occurred earlier in the males. In both cases, although the maximum inhibition was observed at 4 and 8 hours in males and females, respectively, it is not possible to determine the peak-effect time for juvenile animals without a 6 hour time point.

Comparison of adult and juvenile response: **Males:** Juvenile males displayed brain cholinesterase inhibition (18%-20%) following exposure while adult males did not. The magnitude of the response (ChE inhibition) in the juvenile males was greater (approximately 2-fold) in both the serum (60%** *vs.* 28%*) and RBC (35%** *vs.* 17%) compartments compared

TABLE 2. Blood cholinesterase activity

Age/time	Males		Females	
	Control	0.2 mg/kg	Control	0.2 mg/kg
Serum ChE (µkat/L)				
Adults				
0.5 hours	11.78±3.17	13.81±2.68	66.77±11.32	63.38±7.11 (-5%)
1 hour	13.45±3.07	13.58±1.72	71.29±5.45	68.38±21.94 (-4%)
2 hours	12.21±1.02	11.94±2.51 (-2%)	60.24±9.35	58.19±22.69 (-3%)
4 hours	13.14±1.50	10.28±1.60 (-22%)	69.57±10.41	45.48±12.43 (-35%)
8 hours	14.12±1.95	10.18*±2.12 (-28%)	56.84±10.84	24.62**±11.85 (-57%)
Juveniles				
0.5 hours	14.80±1.63	15.27±1.14	13.54±1.76	14.96±2.55
1 hour	14.72±1.48	13.62±1.76 (-7%)	13.77±1.91	12.87±2.18 (-7%)
2 hours	14.45±1.33	7.96**±2.13 (-45%)	13.25±1.73	7.78**±1.59 (-41%)
4 hours	14.06±1.40	5.65**±0.63 (-60%)	13.23±1.45	5.84**±0.79 (-56%)
8 hours	13.28±1.19	7.56**±1.43 (-43%)	14.08±1.41	7.93**±1.25 (-44%)
RBC ChE (µkat/L RBC)				
Adults				
0.5 hours	32.88±4.54	32.74±2.11	32.50±3.85	35.63±5.29
1 hour	34.74±4.21	33.87±3.89 (-2%)	37.15±5.29	34.56±2.07 (-7%)
2 hours	33.75±2.80	31.00±3.90 (-8%)	37.98±4.35	34.98±2.71 (-8%)
4 hours	34.71±10.88	30.50±2.82 (-12%)	37.00±3.74	33.64±4.12 (-9%)
8 hours	37.00±5.01	30.67±3.16 (-17%)	35.73±5.72	27.65±5.90 (-23%)
Juveniles				
0.5 hours	46.55±5.45	45.91±6.28 (-1%)	42.22±10.16	43.42±3.70
1 hour	46.34±10.35	46.19±5.22	47.21±6.30	48.98±7.06
2 hours	48.64±8.41	36.63*±5.74 (-25%)	44.30±5.32	42.45±4.81 (-4%)
4 hours	44.83±6.26	29.32**±2.78 (-35%)	43.19±2.28	33.98±6.12 (-21%)
8 hours	43.39±5.09	31.83±2.4 (-27%)(a)	47.37±5.86	29.27**±6.27 (-38%)

Data were extracted from pp. 34, 35, and 49-68, MRID 46240801. Values represent means ± s.d.; values in parentheses represen t % inhibition. **=p<0.01, *=p<0.05, when compared to control mean. n=5.
(a): Value represents mean ± s.d without one outlier. 8 hour value for juvenile male RBC cholinesterase activity was 53.24±47.92 (no inhibition) due to an obvious outlier (138.89).

TABLE 3. Brain cholinesterase activity (µkat/g protein)

Age/time	Males		Females	
	Control	0.2 mg/kg	Control	0.2 mg/kg
Adults				
0.5 hours	1.94±0.51	2.13±0.89	1.93±0.64	2.25±1.09
1 hour	2.13±0.42	2.09±0.55 (-2%)	2.62±1.04	3.04±1.17
2 hours	2.77±1.22	2.67±1.05 (-4%)	3.15±1.19	1.98±0.53 (-37%)
4 hours	2.12±0.35	2.07±0.68 (-2%)	2.75±0.89	2.39±1.29 (-13%)
8 hours	2.35±1.47	2.73±0.65 (+16%)	2.65±1.13	2.09±1.26 (-21%)
Juveniles				
0.5 hours	1.92±0.18	2.09±0.17	1.88±0.22	1.86±0.16 (-1%)
1 hour	1.91±0.16	1.98±0.16	2.06±0.25	1.95±0.24 (-6%)
2 hours	2.00±0.26	1.64**±0.07 (-18%)	1.92±0.07	1.83±0.34 (-5%)
4 hours	1.93±0.15	1.54**±0.13 (-20%)	1.87±0.20	1.74±0.17 (-7%)
8 hours	1.88±0.13	1.61±0.15 (-14%)	1.98±0.17	1.54*±0.21 (-22%)

Data were extracted from pp. 34, 35, and 49-68, MRID 46240801.
Values represent means ± s.d.; values in parentheses represent % inhibition. **=p<0.01, *=p<0.05, when compared to control me an. n=5.

20

to the adult male. **Females:** Cholinesterase inhibition (magnitude of the response) in each of the compartments was comparable between the juvenile and adult females, although the time of occurrence in the serum was earlier in the juvenile females. At the 8-hour time point, both the adult and juvenile females displayed greater than 20% brain cholinesterase inhibition (adult 21%; juvenile 22%*).

D. NEUROBEHAVIORAL RESULTS:

Neurobehavioral assessments were not performed.

E. SACRIFICE AND PATHOLOGY:

Pathological examination was not part of the study protocol.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

In adult females, maximum inhibition of serum, erythrocyte, and brain cholinesterase activity occurred at 8 hours after test substance administration. At 8 hours, serum and erythrocyte cholinesterase activity were also maximally reduced in adult males. Maximum inhibition for all three cholinesterase activities occurred in male pups at the 4-hour interval. In female pups, serum cholinesterase was maximally inhibited at 4 hours after BAS 316 I administration. Thus, the maximal inhibition occurred in adult rats at the 8-hour interval and between 4 and 8 hours in pups. The degree of inhibition of cholinesterase activities was similar in adult females and juvenile animals (56-60% inhibition of serum cholinesterase).

B. REVIEWER COMMENTS:

The purpose of the study was to determine the time period when the maximum suppression of cholinesterase activity in blood and brain occurs after a single oral administration in both age groups. However, only one dose level was utilized (0.2 mg/kg), and no justification for its selection was provided. Although the dose appears to be an effect dose in the juvenile animals (inhibition observed in all compartments), the response in the adult animals, especially the males (no significant response in RBC and brain compartments), is minimal. NOTE: In the acute neurotoxicity study in **Sprague-Dawley** rats, ***peak-effect time was 6 hours post dose,*** and the doses tested were 0.15, 0.3, and 0.9 mg/kg. The NOAEL was 0.15 mg/kg; male plasma ChE inhibition was 51% at 0.3 mg/kg (LOAEL). The dose used in the current study in Wistar rats is less than the LOAEL identified in the Sprague-Dawley rats following an acute oral dose.

Although the results in the adult animals suggest that the 8-hour time point is the peak-effect time, a higher level of inhibition may have occurred at 6-hour and after 8-hour time points. Additionally, based on the minimal response in the adult male, a higher dose may have resulted in an earlier occurrence of cholinesterase inhibition. Based on the lack of dose-response data and the lack of an

assessment of cholinesterase activity at the 6-hour and after 8-hour time points, a definitive conclusion is not possible regarding the peak-effect time for either age group.

Based on the results of this one-dose study, the juvenile male rat showed a greater response than the adult male rat with respect to the magnitude of cholinesterase inhibition in all compartments, and the peak-effect time was shorter in the juvenile male. The adult and juvenile female rats displayed a similar response with respect to the magnitude of cholinesterase inhibition in the serum and brain compartments, and the time to peak effect was the same in the RBC and brain compartments for both age groups.

C. STUDY DEFICIENCIES:

No information was provided regarding dose selection rationale. Only one dose was used, and the results indicate that the dose was not adequate in the adult animals since minimal or no cholinesterase inhibition was observed in any of the compartments, especially in the males. For example, adult males showed less than 20% RBC cholinesterase inhibition throughout the 8-hour observation period and similarly, adult females displayed a non-significant 23% RBC inhibition at 8 hours. No brain cholinesterase inhibition was observed in the adult male. Dose-response information is lacking. Brain weight data and historical control cholinesterase activity (serum, RBC, and brain) were not provided.

Brain cholinesterase activity for the male and female control groups had large variation over time. For males, for example the 0.5 hour sampling had 1.94±0.51 and at 2 hours it was 2.77±1.22 or an unexplained increase of 43% in activity. For females, the 0.5 hour sampling was 1.93 ±0.64 and at 2 hours it was 3.15±1.19 or 63% increase in activity. The brain cholinesterase activity for the control group should remain constant over the 8 hour period and it did not. The standard deviations were also very large for brain cholinesterase activity being 44% for the 2 hour male control group and 38% for the 2 hour female control group.

Based on the lack of dose-response data and an assessment of cholinesterase activity at the 6-hour time point (peak-effect time in acute neurotoxicity study) and a time point greater than 8 hours, a definitive conclusion is not possible regarding the peak-effect time or magnitude of effect for either age group.

DATA EVALUATION RECORD

BAS 316 I (TERBUFOS)
NON-GUIDELINE

STUDY TYPE: RANGE-FINDING – DEVELOPMENTAL NEUROTOXICITY
MRID 46240802

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 175-2007

Primary Reviewer:
Sylvia S. Talmage, Ph.D., D.A.B.T.
Signature: Sylvia S. Talmage
Date: MAY 04 2007

Secondary Reviewers:
Carol S. Wood, Ph.D., D.A.B.T.
Signature: Carol S Wood
Date: MAY 04 2007

Robert H. Ross, M.S., Group Leader
Signature: Robert H. Ross
Date: MAY 04 2007

Quality Assurance:
Lee Ann Wilson, M.A.
Signature: L.A. Wilson
Date: MAY 04 2007

Disclaimer

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

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Paul Chin, Ph.D. **Signature:** Paul Chin
Reregistration Branch 1, Health Effects Division (7509P) **Date:** 2/20/08
EPA Secondary Reviewer: Linda Taylor, Ph.D. **Signature:** Linda Taylor
Reregistration Branch 1, Health Effects Division (7509P) **Date:** 02-20-08
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** MOttley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 2/20/08

Template version 02/06

TXR#: 0052717

DATA EVALUATION RECORD

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

PC CODE: 105001 **DP BARCODE:** D 305170

TEST MATERIAL (PURITY): BAS 316 I (Terbufos), 88.8% a.i.

SYNONYMS: S-tert-butylthiomethyl O,O-diethyl phosphorodithioate

CITATION: Schneider, S.; Deckardt, K.; van Ravenzwaay, B. (2004). BAS316 I (terbufos) – 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. Project Number 29R0090/02006, March 19, 2004. MRID 46240802. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, Research Triangle Park, NC .

EXECUTIVE SUMMARY:

In a range-finding study (MRID 46240802) to determine dose level selection for a subsequent developmental neurotoxicity study, BAS 316 I (terbufos; Batch No. AC 12251-100; 88.8% a.i.) was administered to 20 presumed pregnant female Wistar (CrlGlxBrlHan:WI) rats/dose via gavage at dose levels of 0 (corn oil), 0.03, 0.1, or 0.3/0.2 mg a.i./kg/day. Due to marked toxicity in the high-dose group, the high dose was reduced to 0.2 mg/kg/day on GD 15. Eight of the dams in each group were dosed from gestation day (GD) 6 through GD 20, and these dams and their offspring were sacrificed on GD 20 (2-3 hours post dose) for blood (serum and red blood cell) and brain cholinesterase activity assessment. The remaining 12 dams in each group were dosed from GD 6 through postnatal day (PND) 10. These dams were allowed to litter and rear their pups until PND 4 (litters culled to 4/sex), PND 11, or PND 21. The test material was administered directly to the pups orally (via gavage) at their dam's dose levels [0 (corn oil), 0.03, 0.1, or 0.2 mg a.i./kg/day] from PND 11 through PND 21. Blood and brain cholinesterase activity was assessed in male and female offspring on PNDs 4 (culled pups), 11, and 21 and pups from each group on PNDs 11 and 21 (approximately 2 hours post dose) and in surviving dams on PND 21. However, times of cholinesterase activity assessment after dosing were not clearly identified for fetuses of each group on gestation day 20, culled pups from each group on PND 4, and surviving dams on PND 21.

Maternal toxicity was observed at the 0.3 mg/kg dose level, as evidenced by the deaths of seven dams during gestation days 14-15 and 18-21, and the death of another high-dose dam on GD 22 due to an inability to deliver her litter. The high-dose level was reduced to 0.2 mg/kg/day on gestation day 15. Clinical signs (unsteady gait, tremor, salivation, piloerection, accelerated/labored breathing, lacrimation, and diarrhea) consistent with cholinesterase inhibition were observed during the dosing period, mainly at the high-dose level. One mid-dose dam was found dead on gestation day 14, and clinical signs consistent with cholinesterase inhibition (salivation, lacrimation, piloerection, accelerated breathing) were observed in a few mid-dose dams. Decreased body-weight gain (13%) and food consumption were observed in the high-dose dams following the first week of dosing. There was a reduction in the number of liveborn pups and an increase in the number of stillborn and dead pups at the high dose only. Live birth, viability, and lactation indices were reduced at the high-dose level also.

Decreased cholinesterase activity was observed in all three compartments in the high-dose **dams on gestation day 20** (serum 91%**; RBC 89%**; brain 77%**) and in the RBC (39%*) and brain (32%) compartments of high-dose **dams on postnatal day 21** (11 days after cessation of dosing). Decreased cholinesterase activity was observed in all three compartments in the mid-dose dams on **gestation day 20** (serum 69%**; RBC 66%**; brain 35%*) and in the RBC (31%**) compartment of **dams on postnatal day 21** (11 days after cessation of dosing).

GD 20 fetuses displayed a dose-related decrease in cholinesterase activity in the serum (males 24%** and 52%**/females 29% and 53%), RBC (males 66%** and 89%**/females 54%** and 59%**), and brain (males 19%** and 39%**/females 6% and 32%**) compartments at the mid- and high-dose levels, respectively.

There were no clinical signs in the offspring during lactation, but there was a decrease in pup viability during early lactation (PND 0-4) at the high-dose level. Pup body weight/body-weight gains were reduced at the high-dose level during the initial days of lactation (PND 0-10) when the dams were being dosed. Pup body-weight gain was not affected during PNDs 11-21 when the pups were dosed directly.

PND 4 pups displayed a dose-related (slight) decrease in cholinesterase activity in the RBC compartment only (males 36%** and 48%/females 26% and 47% at the mid- and high-dose, respectively). Cholinesterase activity was inhibited in all three compartments in pups of both sexes on PND 11 at the high-dose level after one direct dose (serum: males 47%**/females 57%**; RBC: females 25%*; brain: males 23%/females 27%) and on PND 21 at the mid- (serum: males 56%**/females 58%**; RBC: males 42%**/females 40%**; brain: males 38%**/females 42%**) and high-dose (serum: males 74%**/females 84%**; RBC: males 72%*/females 83%*; brain: males 50%*/females 69%*) levels following direct dosing for 11 days.

Under the conditions of this range-finding study, **the LOAEL is 0.1 mg/kg/day, based on clinical signs (salivation, lacrimation, piloerection, accelerated breathing) in the maternal animals, and inhibition of serum, erythrocyte (RBC), and/or brain cholinesterase activity in GD 20 dams and PND 21 dams, GD 20 fetuses, and PND 4 (RBC only), 11, and 21 pups. The**

NOAEL is 0.03 mg/kg/day.

Based on these results, the study authors recommended dose levels of 0.01, 0.08, and 0.15 mg/kg/day for the definitive developmental neurotoxicity study in rats. Direct dosing of the pups *via* gavage was recommended also.

This range-finding study is classified **Acceptable/Nonguideline** and was not intended to satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft).

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

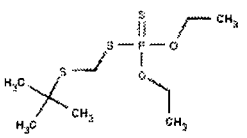
I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** BAS 316 I

Description:	Liquid, colorless to pale yellow
Batch #:	AC 12251-100
Purity:	88.8% a.i.
Compound stability:	Stable; stored at ambient temperature in the dark
CAS # of TGAI:	13071-79-9

Structure:



2. **Vehicle and/or positive control:** Test material was administered in corn oil.

3. **Test animals (P):**

Species:	Rat
Strain:	Wistar, CrlGlxBrlHan:WI
Age at study initiation:	11-13 weeks
Wt. at study initiation:	167.8-215.8 g
Source:	Charles River Laboratories, Germany
Housing:	Individually in Type DK III stainless steel wire mesh cages with the following exception: from the day 18 of gestation until day 21 after birth, the pregnant rats and their litters were housed in Makrolon type M III cages (Becker & Co., Germany). Cellulose wadding was provided as nesting material.
Diet:	Kliba maintenance diet meal, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	Temperature: 20-24°C Humidity: 30-70%

Air changes: Air-conditioned room
Photoperiod: 12 hrs dark/ 12 hrs light

Acclimation period: Presumed pregnant rats supplied by breeder on day 0 post coitum; acclimated approximately 7 days until test material administration

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: June 10, 2002; End: July 18, 2002

2. **Study schedule:** The maternal animals were mated by the breeder and supplied on the same day (presumed day 0 post coitum). The test substance was administered to groups of 8 maternal animals from GD 6 through 20. The remaining dams (groups of 12) were dosed from GD 6 through PND 10. Pups were treated from PND 11-21, at which time all surviving dams and offspring were sacrificed.

3. **Mating procedure:** Females were mated by the breeder. Pregnancy was determined by the presence of a vaginal plug or sperm (designated gestation day 0). No details on the mating procedure were provided. After gestation day 18, pregnant females were housed in individual cages and supplied with cellulose wadding nesting material.

4. **Animal assignment:** The maternal animals were "time mated" by the breeder, and the presumed pregnant rats were supplied to the testing facility on presumed day 0 *post coitum*. The mated females were assigned to one of two dosing intervals at the dose levels indicated in Table 1. No information was provided on how the animals were assigned to the groups (pilot study). **Interval I**. The test material was administered to groups of 8 dams/dose level from GD 6 through 20, and the dams and their fetuses were sacrificed on GD 20 for blood and brain cholinesterase activity determination. **Interval II.** The test material was administered to groups of 12 dams/dose level from GD 6 through PND 10. Pups were administered test material from PND 11 through PND 21. Offspring blood and brain cholinesterase activity was assessed on PND 4 (culled pups), PND 11, and PND 21. All surviving dams were sacrificed on PND 21, and blood and brain cholinesterase activity was determined.

TABLE 1. Study design

Experimental parameter	Dose (mg/kg/day)			
	0	0.03	0.1	0.3/0.2[a]
Maternal animals				
No. of maternal animals assigned	20	20	20	20
Dosed on GD 6 through 20; sacrificed on GD 20 for blood and brain cholinesterase activity determination	8	8	8	8
Dosed on GD 6 through day 10 post partum; sacrificed on PND 21 for blood and brain cholinesterase activity determination	12	12	12	12
Clinical observations	20	20	20	20
Offspring				
Clinical observations (PND 0-21)	10/sex	10/sex	10/sex	10/sex
Blood cholinesterase activity determination				
Fetuses (GD 20)	8-10/sex	11-12/sex	10/sex	10/sex
Brain cholinesterase activity determination				
Fetuses (GD 20)	8/sex	8/sex	8/sex	8/sex
Dosed from PND 11 through PND 21				
Blood cholinesterase activity determination				
PND 4 (culled pups)	6-7/sex	6-11/sex	5-6/sex	2/sex
PND 11	11/sex	10/sex	10/sex	3/sex
PND 21	11/sex	9-10/sex	9/sex	2/sex
Brain cholinesterase activity determination				
PND 4 (culled pups)	7-8/sex	7-12/sex	7/sex	2/sex
PND 11	11/sex	10/sex	10/sex	3/sex
PND 21	11/sex	9-10/sex	9/sex	2/sex

[a] High-dose reduced from 0.3 mg/kg bw/day to 0.2 mg/kg bw/day on GD 15; offspring treated with 0.2 mg/kg bw/day. GD = gestation day. PND = postnatal day. Data from pages 200-211 of study report

5. **Dose selection rationale:** The dose levels used in the rat developmental toxicity study were 0.05, 0.1, and 0.2 mg/kg/day (GD 6-15). NOAEL for maternal toxicity was 0.2 mg/kg/day, so the selected doses are appropriate.

6. **Dose administration:** All doses were administered once daily to maternal animals by gavage either from gestation day 6 (GD 6) through GD 20 or from GD 6 through postnatal day 10 (PND 10), in a volume of 5 mL/kg body weight/day. Dosing was based on the most recent body weight measurement. Because of obvious maternal toxicity in the high-dose group, the dose was lowered from 0.3 mg/kg/day to 0.2 mg/kg/day on GD 15. An interval of one to three days occurred before dosing was resumed, depending on the severity of the clinical signs observed. Pups from the dams dosed through PND 10 were administered the same dose/volume as their dams by gavage on PNDs 11 through 21.

7. **Dosage preparation and analysis:** Formulations were prepared daily by mixing appropriate amounts of test substance with corn oil which yielded doses of 0.03, 0.1, and 0.3/0.2 mg/kg/day based on a.i. Prior to the start of the study, stability of the test substance in corn oil was evaluated in samples stored for a period of 3 or 7 days in the dark at room temperature. Homogeneity (top, middle, and bottom of containers) was evaluated once at the start of the study. During the study, samples of test mix were analyzed three times for concentration (June

18, and 26 and July 16 at one or more dose levels). Analysis was by HPLC.

Results:

Homogeneity analysis: The standard deviations of the nominal 0.0068 and 0.068 mg/mL samples were 3.1 and 3.0 (range of values 0.0043-0.0047 and 0.0599-0.0653, respectively). The authors noted that the first sample of the 0.0068 mg/mL concentration was only 65.7% of the nominal, but later samples were 94.1 and 89.7% of nominal. No homogeneity analyses were preformed on the latter samples.

Stability analysis: Samples of a nominal 0.0068 mg/mL sample stored in the dark at room temperature for 3 or 7 days were 91.9 and 91.4% of nominal, respectively (no range of values provided).

Concentration analysis: With the exception of the first sample of the nominal 0.0068 mg/mL concentration, concentrations of three additional samples ranged from 89.7-94.1% of nominal. The two 0.022 mg/mL sample values were 98.2 and 91.4% of nominal. The two 0.45 mg/mL samples were 97.8 and 93.1% of nominal.

The analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Twice daily checks for mortality or moribundity (once daily on weekends) and daily cage-side observations were conducted for maternal animals. Nesting, littering, and lactation behavior of the dams was checked in the morning during the daily clinical inspection.

Individual maternal body weight data were recorded on GD 0, 6, 13, and 20. Females with litters were weighed on the day of delivery, and on lactation days 1, 7, 14, and 21. Food consumption was determined on GD 0, 6, 13, and 20 and during lactation days 1, 7, 14, and 21.

b. **Offspring: Litter observations:** The day of completion of parturition was designated as lactation day (postnatal day) 0. Live pups were counted, sexed and weighed individually for each litter on postnatal days 1, 4 (before litter standardization), 11, 14, and 21. Twice daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity. Clinical symptoms were recorded daily.

On day 4 postpartum, litters were standardized (choosing the first pups/sex/litter) to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were used for blood and brain sampling for cholinesterase measurement. Litters with fewer than 8 pups were removed from the study.

The numbers of pups dying between days 1-4, 5-7, 8-14, and 15-21 of lactation were determined. The sex ratio was determined on days 1 and 21 after birth. Offspring were not subjected to neurotoxicity tests.

2. **Cholinesterase determination:** Blood and brain cholinesterase activity was determined in the dams (2-3 hours post dose) and fetuses of each group on gestation day 20; culled pups from each group on PND 4, pups from each group on PNDs 11 and 21 (approximately 2 hours post dose), and surviving dams on PND 21. Following isoflurane anesthesia, approximately 1 mL of blood was collected from adults and juveniles from the retroorbital venous plexus and vena cava cranialis, respectively. Brains were collected from the same animals immediately following blood collection. Samples were kept on ice during sampling, and brain samples were stored frozen. Analysis for cholinesterase activity used a spectrophotometric procedure based on a modified Ellman method (adapted to a Cobas Fara analyzer [Hoffmann LaRoche, Germany]). Hematocrit values were determined in order to calculate red blood cell cholinesterase activity, and brain protein was analyzed in order to calculate specific cholinesterase activity in brain.

3. **Postmortem observations:**

   a. **Maternal animals:** Maternal animals were sacrificed by cervical dislocation on GD 20 (8 dams/group; no anesthesia) or on PND 21 (remaining dams; isoflurane anesthesia) and subjected to a gross necropsy. Animals without a litter were subjected to gross necropsy. The uterus was removed and stained in 10% ammonium sulfide solution for evidence of early resorptions. Females used for cholinesterase determination on PND 21 were examined for number of implantation site scars. No tissues or organs were examined microscopically.

   b. **Offspring:** Pups culled on PND 4 and those sacrificed on days 11 or 21 after birth were killed by decapitation. Pups sacrificed on PND 21 were subjected to gross necropsy. All stillborn pups were subjected to gross necropsy. No tissues or organs were examined microscopically.

D. DATA ANALYSIS:

1. **Statistical analyses:** For food consumption (females), body weight and body weight gain (dams and pups [litter means]), duration of gestation, and number of pups delivered/litter, dose groups were compared with the control group using the Dunnett two-sided test for the hypothesis of equal means. The following parameters were analyzed using Fischer's Exact test for the hypothesis of equal proportions: female fertility index, gestation index, females with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, and viability and lactation indexes. Group necropsy findings per litter were compared pairwise using the Wilcoxon test for the hypothesis of equal medians. These parameters were evaluated at the 1 and 5% levels. Cholinesterase activity was compared non-parametrically one-way using

the Kruskal-Wallis test (two-sided). If the resulting p value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the two-sided Wilcoxon test for equal medians.

2. **Indices:**

The following indices were calculated from lactation records of litters in the study:

$$\text{Viability Index (\%)} = \frac{\text{No. of live pups on day 4 after birth}}{\text{No. of live pups on the day of birth}} \times 100$$

$$\text{Lactation Index (\%)} = \frac{\text{No. of live pups on day 21 after birth}}{\text{No. of live pups/litter on day 4 after birth (after - culling)}} \times 100$$

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical observations:** Mortality data and clinical observations are summarized in Table 2. Deaths were first recorded on day 14 of gestation: one female in the high-dose group and one female in the mid-dose group died. By the end of gestation (day 22/23), seven animals in the high-dose group and one animal in the low dose group had been found dead. An eighth dam in the high-dose group died at the end of gestation due to inability to deliver pups. One animal in the low-dose group was sacrificed after abortion (day 21). One dam in the control group died due to gavage error. During lactation, one animal was found dead in the low-dose group.

The following clinical signs were observed during gestation. Beginning on day 9, salivation was observed after treatment in 2, 7, and 15 dams in the low through high-dose groups respectively. Beginning on day 15 tremor was observed in 11 dams in the high-dose group. Piloerection was observed in 1-4 dams in all treated groups. In addition, one to two dams in the high-dose group showed additional signs of poor general health, crusted noses, and gait abnormalities.

During lactation, tremor was still observed in 3 females in the high-dose group (days 0-4). Piloerection was observed in one female in the mid-dose group (day 4-5). Cannabalization appeared to be limited to stillborn pups.

TABLE 2. Maternal mortality and clinical observations[a]

Observation	Dose (mg/kg/day)			
	Control	0.03	0.1	0.3/0.2
Gestation				
Mortality	0	1	1	8
Clinical signs				
Salivation after treatment	0	2	7	15
Tremor	0	0	0	11
Urine stains	0	1	0	6
Lacrimation	0	0	1	1
Piloerection	0	2	1	4
Increased respiration	0	1	1	1
Poor general health	0	0	0	2
Lactation				
Mortality	1[b]	1	0	0
Clinical signs				
Tremor	0	0	0	3
Piloerection	0	0	1	0

[a] Data obtained from pages 66-71, MRID 46240802.

[b] Died after gavage error.

N = 20 during gestation; n = 11, 11, 10, and 3 in the control through high-dose groups, respectively, during lactation.
Statistics were not provided.

2. **Body weight and food consumption:** Selected group mean body weight and food consumption values for pregnant or nursing dams are summarized in Table 3. At the end of gestation, final mean body weights of the treated groups were 3-6% lower than the mean control weight. These differences were not statistically significant. Body weight gain in the high-dose group during dosing period (GD 6-20) was lower by 24% ($p<0.01$) relative to the control group. Overall food consumption during gestation was not significantly reduced, although the value for days 13-20 in the high-dose group was reduced by 15% ($p<0.05$) relative to the control value. Food consumption in the high-dose group reflected the slight decrease in body weight in this group (6%).

   All groups lost weight on the first day of lactation as did the control group between days 14-21. During the PND 14-21 day interval, when treatment was discontinued, the high-dose group gained 10.3 g ($p<0.05$); whereas, gains in the control, low-, and mid-dose groups were -3.4, 0.0, and 2.0 g, respectively (data not shown). Body weight gain was reduced by 19% in the high-dose group over lactation days 1-21. Although mean food consumption over days 1-21 (mean of means) was not statistically significantly lower by 40% relative to the control value, values for the intervals 1-7, 7-14, and 14-21 were significantly lower by 39-42% ($p<0.01$). Final body weight, weight gain, and food consumption were generally unaffected in the mid- and low-dose groups.

TABLE 3. Mean (±SD) maternal body weight and food consumption [a]

Observations/study week	Dose (mg/kg/day)			
	Control	0.03	0.1	0.3/0.2
Gestation				
Mean body weight (g)				
Gestation day 0	162.2±9.47	162.1±9.24	163.5±11.05	164.2±8.67
Gestation day 6	187.9±8.12	188.1±11.78	189.6±11.81	190.0±9.91
Gestation day 13	214.3±11.30	211.9±15.57	210.3±14.63	212.9±12.80
Gestation day 20	268.4±10.81	260.6±30.30 (3%)	257.4±18.18 (4%)	252.8±24.69 (6%)
Mean weight gain (g)				
Gestation days 6-13	26.4±5.69	23.8±7.8	20.7±7.99	23 ±9.25 (15%)
Gestation days 13-20	54.1±8.09	48.7±18.52	45.9±8.79	**35.6**±19.72** (34%)
Gestation days 6-20	80.6±8.3	72.5±22.97	67.5±11.28	**61.1**±20.97** (24%)
Mean food consumption (g/animal/day)				
Gestation days 13-20	17.5±1.45	16.3±3.84	15.9±2.09	**14.8*±3.64** (15%)
Gestation days 6-20	16.6±1.14	15.9±0.58	15.4±0.64	15.2±0.51 (24%)
Lactation				
Mean body weight (g)				
Lactation day 21	239.5±17.73	246.7±12.82	241.4±14.35	236.3±12.80 (1%)
Mean weight gain (g)				
Lactation days 1-21	20.0±11.44	22.0±13.72	23.2±9.13	16.2±12.55 (19%)
Mean food consumption (g/animal/day)				
Lactation days 1-21	35.7±7.61	35.9±8.31	33.1±8.05	21.4±4.06 (40%)

[a] Data obtained from pages 72-77, MRID 46240802.
n = 19 for body weight throughout gestations, except for low dose (20) and GD 20 (mid 18/high 14)
3-11 for body weight, lactation;
2 for food consumption, gestation;
10-11 (control, low, and mid dose) and 3-4 (high dose) for food consumption, lactation.
Values in parenthesis represent decrease relative to control, calculated by Reviewer.
* Statistically significant different from control, $p<0.05$. * * Statistically significant different from control, $p<0.01$.

3. Cholinesterase activity:

Results of blood and brain cholinesterase activity assessment in dams, fetuses, and offspring are presented in Tables 4 and 5, respectively.

GD 20 dams: On GD 20, dams displayed a dose-related inhibition in cholinesterase activity in the serum (mid- 69%** and high- 91%** dose), RBC (mid- 66%** and high- 89%** dose), and brain (mid- 35%* and high- 77%** dose) compartments.

GD 20 fetuses: On GD 20, male fetuses displayed a dose-related inhibition in serum cholinesterase activity at the mid- (24%**) and high- (52%**) dose levels, while a similar inhibition in serum cholinesterase activity was observed in the female fetuses at the mid- (29%) and high- (53%) dose levels, although statistical significance was not attained. A dose-related inhibition in RBC cholinesterase activity was observed in the male fetus on GD 20 at the mid- (51%**) and high- (69%**) dose levels, and a similar inhibition in RBC cholinesterase activity was observed in the female fetuses at the mid- (54%**) and high- (59%**) dose levels. Brain cholinesterase activity

was inhibited in male fetuses on GD 20 at the mid- (19%**) and high- (39%**) dose levels and in female fetuses at the high- (32%**) dose level. The response in the serum and RBC compartments was similar between the sexes, but the response in the brain compartment at the mid-dose level in males was 3 times greater (19%) than in the female (6%).

In all compartments, the GD 20 dams displayed a higher level of inhibition than the GD 20 fetuses.

PND 21 dams: On PND 21, serum cholinesterase activity was comparable among the groups, but the dams displayed a dose-related inhibition in RBC cholinesterase activity at the mid- (31%**) and high- (39%*) dose levels. Although not statistically significant, brain cholinesterase activity was inhibited at the high-dose level (32%), and the magnitude of the response is considered treatment-related and adverse.

Offspring:

On PND 4, serum cholinesterase activity was comparable among the pups in each group in both sexes. RBC cholinesterase activity was inhibited in both sexes (dose-related; males 15%, 36%** and 48%/females 23%*, 26%, and 47% with increasing dose), although statistical significance was attained only in the male mid-dose and female low-dose groups (Table 4). The magnitude of the effect at the low-dose in both sexes is not considered adverse by itself. Brain cholinesterase activity was not significantly inhibited in either sex at any dose level, but the high-dose males displayed a 15% reduction in activity, which is considered treatment-related and adverse (Table 5).

On PND 11, serum cholinesterase activity was inhibited in both sexes at the high-dose level (males 47%**/females 57%**). RBC cholinesterase activity was inhibited only in the high-dose females (25%*). Although inhibition of brain cholinesterase activity was not statistically significant (n=3), the magnitude of the reduction is considered treatment-related and adverse (males23%/females 27%).

On PND 21, serum cholinesterase activity was inhibited in both sexes (males 56%** and 74%**/females 58%** and 84%*) at the mid- and high-dose levels, respectively (dose-related). RBC cholinesterase activity was inhibited in both sexes (males 42%** and 72%*/females 40%** and 83%*) at the mid- and high-dose levels, respectively (dose-related). Brain cholinesterase activity was inhibited in both sexes (males 38%** and 50%*/females 42%** and 69%*) at the mid- and high-dose levels, respectively (dose-related).

TABLE 4. Blood cholinesterase activity

Observation	Dose level (mg/kg bw/day)			
	Control	0.03	0.1	0.3/0.2
Serum ChE (µkat/L)				
Dams				
GD 20 (n = 8)	53.20±11.61	52.36±14.01	16.45**±5.68 (69)	4.90**±2.61 (91%)
PND 4	—	—	—	—
PND 11	—	—	—	—
PND 21 (n = 2-11)	17.46±3.28	17.83±3.26	21.38±4.67	25.94±7.30
Male fetuses/offspring				
GD 20 (n = 5-8)	5.85±0.26	5.88±0.57	4.46**±0.45 (24%)	2.83**±0.65 (52%)
PND 4 (n = 2-6)	13.17±1.45	13.79±0.99	13.41±0.66	12.65±1.01
PND 11 (n = 3-11)	17.61±2.53	18.12±1.33	17.15±1.19	9.31**±3.91 (47%)
PND 21 (n = 2-11)	12.93±2.12	12.25±1.15	5.68**±1.22 (56%)	3.38**±0.70 (74%)
Female fetuses/offspring				
GD 20 (n = 2-5)	6.33±0.15	6.40±0.46	4.48±0.29 (29%)	2.98±0.78 (53%)
PND 4 (n = 2-11)	11.90±1.15	12.51±0.88	13.04±0.93	13.82±0.97
PND 11 (n = 3-11)	16.98±2.40	17.82±1.54	16.35±1.13	7.30**±1.21 (57%)
PND 21 (2-11)	13.13±2.11	12.23±1.93	5.46**±0.97 (58%)	2.17*±0.15 (84%)
RBC ChE (µkat/L)				
Dams				
GD 20 (n = 8)	42.30±5.00	40.68±4.00	14.42**±4.04 (66%)	4.46**±1.64 (89%)
PND 4	—	—	—	—
PND 11	—	—	—	—
PND 21 (n = 2-11)	34.54±2.96	32.19±3.16	23.76**±3.33 (31%)	21.17*±3.85 (39%)
Male fetuses/offspring				
GD 20 (n = 7-9)	5.16±1.48	4.63±1.86	2.51**±0.86 (51%)	1.62**±0.69 (69%)
PND 4 (n = 2-6)	13.53±3.51	11.51±2.16 (15%)	8.66**±1.43 (36%)	7.04±0.03 (48%)
PND 11 (n = 3-11)	20.87±3.96	19.89±4.44	19.64±3.18	18.26±5.85
PND 21 (n = 2-11)	42.66±8.91	38.60±8.81	24.82**±3.93 (42%)	12.11*±1.18 (72%)
Female fetuses/offspring				
GD 20 (n = 6-9)	4.32±0.85	4.52±0.99	1.99**±1.09 (54%)	1.76**±0.75 (59%)
PND 4 (n = 2-10)	13.23±2.57	10.21*±1.71 (23%)	9.77±0.79 (26%)	6.99±0.53 (47%)
PND 11 (n = 3-11)	23.83±4.74	19.98*±2.73 (16%)	20.73±4.15	17.89*±0.57 (25%)
PND 21 (n = 2-11)	41.63±8.69	37.52±10.66	25.04**±4.10 (40%)	6.90*±1.88 (83%)

Data were extracted from pp. 92-101, MRID 46240802. Values represent mean ± s.d. (% decrease relative to control mean).
GD = gestation day. PND = post-natal day.
**=$p<0.01$, *=$p<0.05$, when compared to control mean.

TABLE 5. Brain cholinesterase activity				
Observation	Dose level (mg/kg bw/day)			
	Control	0.03	0.1	0.3/0.2
Dams				
GD 20 (n = 8)	3.00±1.12	3.00±0.79	1.96*±0.68 (35%)	0.69**±0.19 (77%)
PND 4	—	—	—	—
PND 11	—	—	—	—
PND 21 (n = 2-11)	1.82±0.51	2.03±0.83	1.69±0.72	1.22±0.22 (32%)
Male fetuses/offspring				
GD 20 (n = 5-8)	0.59±0.11	0.53±0.05	0.48**±0.04 (19%)	0.36**±0.09 (39%)
PND 4 (n = 2-6)	1.19±0.12	1.22±0.08	1.15±0.09	1.01±0.01 (15%)
PND 11 (n = 3-11)	1.80±0.37	1.58±0.18	1.61±0.14	1.39±0.11 (23%)
PND 21 (n = 2-11)	1.56±0.29	1.54±0.32	0.97**±0.24 (38%)	0.79*±0.49 (50%)
Female fetuses/offspring				
GD 20 (n = 2-5)	0.53±0.04	0.57±0.04	0.50±0.05	0.36**±0.07 (32%)
PND 4 (n = 2-11)	1.13±0.05	1.14±0.06	1.17±0.10	1.10±0.05
PND 11 (n = 3-11)	1.72±0.27	1.61±0.31	1.71±0.23	1.26±0.18 (27%)
PND 21 (2-11)	1.71±0.58	1.75±0.51	0.99**±0.17 (42%)	0.53*±0.02 (69%)

Data were extracted from pp. 91-101, MRID 46240802.
Values represent mean ± s.d. (% decrease relative to control mean).
GD = gestation day.
PND = post-natal day.
**=p<0.01, *=p<0.05, when compared to control mean.

4. **Maternal postmortem results:** The following maternal postmortem findings were noted.

In the high-dose group

thoracic cavities with exudates (4 animals),
yellow-white deposition in the thoracic cavity (3 animals),
dark red heart (3 animals),
lungs with acute fibrinous purulent pneumonia (1 animal),
dark-red lungs (3 animals),
diaphragms with yellow-white deposition (2 animals),
intestines with yellow-white discoloration (8 animals),
a liver with foci (1 animal).

In the mid-dose group

thoracic cavities with exudates (3 animals),
yellow-white deposition in the thoracic cavity (3 animals),
dark red heart (1 animal),
dark-red lungs (1 animal),
diaphragms with yellow-white deposition (1 animal),
hydrometra (1 animal).

In the low-dose group

thoracic cavities with exudates (2 animals),
yellow-white deposition in the thoracic cavity (3 animals),
an abscessed thoracic cavity (1 animals),

intestines with yellow-white discoloration (1 animal).

In the control group

a thoracic cavity filled with bloody fluid (1 animal), lungs with acute fibrinous purulent pneumonia (1 animal), intestines with redden areas (1 animal), a light brown-gray kidney (1 animal).

B. OFFSPRING:

1. **Cesarean section observations:** Data collected at cesarean section are summarized in Table 6. The number of liveborn and stillborn pups and pup deaths were affected only in the high-dose group. The total number of pups born was lowest in the high-dose group, and the number of stillborn pups was highest in this group (note: only 5 females in the high-dose group survived to the end of gestation, and of these 5, one died delivering and one underwent elective sacrifice following delivery of 3 stillborn pups). Thus, the data in Table 6 for the high-dose treatment group pertain to litters from 3 females. Live-birth, viability, and lactation indices were all reduced in the high-dose group. The mean number of implantation sites did not differ among groups, but the total implantation sites were significantly less in the 0.2 mg/kg/day group than in the control group.

   There were no treatment-related clinical signs in the offspring during days 0-21 of lactation.

2. **Body weight:**

   Selected mean preweaning pup body weight data are presented in Table 7. Male pup body weight in the 0.2 mg/kg/day dose group was lower than the control weight at all time intervals, but the difference was statistically significant ($p<0.05$) only during days 7-11 (19-20% reduction). On PND 4, both before and after culling, male and female pup weights were lower than control values by 18-19% (no statistical analyses on the small number of pups). In the high-dose group, preweaning final pup body weight was reduced by 8% and 3% in males and females, respectively. The low- and mid-dose groups were unaffected.

   Body weight changes are shown in Table 8. Body weight changes for males and females in the high-dose group were lower during days 1-4 (32%, $p<0.05$), 4-7 (20-29%, $p<0.05$) and 7-11 (13%, not statistically significant), but day 11-21 body weight gains for male and female pups were similar among the control and treatment groups. The apparent lack of effect may be attributed to very few pups per litter at high dose.

3. **Developmental landmarks:** Sexual maturation data were not collected as part of the study protocol.

4. **Postmortem results:** Brains were not weighed or measured, and neuropathology data on pups were not collected. Necropsy findings were normal.

Table 6. Cesarean section observations

	mg/kg/day			
	0	0.03	0.1	0.2
Females on test	11	12	11	7 ♪
# mated	11	12	11	7
# pregnant	11	12	10	7
maternal wastage				
# died	1	0	1	8
#died pregnant	1	0	1	8♪♪
# aborted	0	1	0	0
# delivered early	0	0	0	
total implantations	97	96	86	29
mean implantations/dam	8.8	9.6	8.6	9.7
total # pups born	94	99	82	29
# females w/ liveborn (%)	11 (100)	11 (92)	10 (100)	3* (43)
total # live pups	92	99	78	20**
live pups/dam	8.4	9.0	7.8	5.0
total # stillborn pups	2	0	4	9**
# dams w/ stillborn	1	0	4	2
# dams w/ all stillborn	0	0	0	1
Mean litter size:				
Day 0	8.4 ±1.29	9.0±1.34	7.8±1.69	5.0±4.24
Day 4 [b]	8.3±1.10	8.3±2.97	7.8±1.69	3.5±3.42
Day 4 [c]	6.9±0.54	6.5±2.30	6.4±0.84	2.5±2.08
Day 14	4.7±0.65	4.6±1.69	4.4±0.84	1.0±1.41
Day 21	4.4±1.03	4.6±1.69	4.3±0.95	1.0±1.41
Sex Ratio Day 0 (%)	54	44	49	45
Live birth index (%)	98	100	95	69**
Viability index (%)	99	92	100	70
Lactation index (%)	63	71	67	40

[a] Data obtained from pages 80-83 and 156-159, MRID 46240802.
Values in parenthesis represent decrease relative to control, calculated by Reviewer.
[b] Before standardization (culling).
[c] After standardization (culling).
* Statistically different from control, $p<0.05$. ** Statistically different from control, $p<0.01$.
Viability and lactation index calculated by Reviewer.
♪ It's not clear why the report (page 80, study report) shows that only 7 were on study/mated in Group 3 and 11 in both the control and Group 2. Twelve presumed-pregnant females were dosed from GD 6-PND 10.
♪♪ one died at end of gestation due to inability to deliver pups; * $p<0.05$; ** $p<0.01$

TABLE 7. Mean (±SD) pre-weaning pup body weight (g) [a]								
Postnatal day	Dose (mg/kg/day)							
	Control	0.03	0.1	0.2	Control	0.03	0.1	0.2
	Males				Females			
1	6.8±0.71	6.6±0.78	6.3±0.85	5.8±0.20	6.5±0.68	6.2±0.73	6.1±0.86	5.7±0.63
4 [b]	10.5±1.32	10.1±1.21	9.6±1.47	8.4±1.22	10.3±1.29	9.7±1.11	9.4±1.48	8.3±1.01
7	16.7±2.09	15.9±1.84	15.3±2.19	13.0* ±1.88	16.4±2.02	15.5±1.52	15.0±2.34	13.2*±1.15
11	25.0±2.44	24.1±3.21	23.4±2.90	20.2*±2.58	24.8±2.26	23.5±2.90	23.1±3.11	20.5±1.64
14	34.3±3.37	33.1±4.20	32.2±4.15	28.2±5.52	33.4±3.01	32.2±3.59	32.2±4.03	31.1±0.00
21	55.0±4.17	53.2±7.81	53.6±5.96	50.5±7.14	54.1±3.37	50.9±6.23	51.9±6.36	52.5±0.00

[a] Data obtained from pages 84-87, MRID 46240802. n=1-2 for 0.2 mg/kg/day.

[b] Before standardization (culling).

[c] After standardization (culling).

* Statistically different from control, $p<0.05$.

Table 8. Pup Body-Weight Changes (grams)				
	mg/kg/day			
	0	0.03	0.1	0.2♪
Males				
days 1-4	3.8±0.68	3.5±0.50	3.3±0.77	2.6±1.14* (32)
days 4-7	6.2±0.83	5.9±0.88	5.7±0.88	4.4±0.85* (29)
days 7-11	8.3±1.04	8.1±1.52	8.1±0.85	7.2±0.78 (13)
days 11-21√	30.0	29.1	30.2	30.3
Females				
days 1-4	3.8±0.66	3.5±0.47	3.3±0.73	2.6±0.78* (32)
days 4-7	6.1±0.77	5.7±0.72	5.6±1.02	4.9±0.18 (20)
days 7-11	8.4±0.98	8.0±1.54	8.1±0.93	7.3±0.87 (13)
days 11-21√	29.3	27.4	28.8	32.0

√ calculated by EPA reviewer using mean data from pages 86-87 (no statistics); * $p<0.05$

n= 9-11 except ♪ n=2 (males)/1 (female)

Values in parenthesis represent decrease relative to control, calculated by reviewer.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

BAS 316 I administered to pregnant rats and pups at dose levels of 0.1 and 0.3/0.2 mg/kg bw/day caused a significant inhibition of the serum, erythrocyte, and brain cholinesterase activity in the dams and fetuses. In pups, a similar inhibition of this enzyme was only observed when they were directly exposed to the test material by gavage. Thus, transfer into the milk was limited or did not occur. No treatment-related effects on cholinesterase activity were seen in dams and fetuses in the 0.03 mg/kg bw/day dose group. Cholinesterase inhibition was associated with marked systemic toxicity and mortality only at the high-dose level of 0.3/0.2 mg/kg bw/day. Dose levels of 0.01, 0.08, and 0.15 mg/kg bw/day were recommended for the developmental neurotoxicity study. Pups should be exposed by gavage.

B. REVIEWER COMMENTS:

High-dose dams: Maternal toxicity was observed at the 0.3 mg/kg dose level, as evidenced by the deaths of seven dams during gestation days 14-15 and 18-21, and the death of another high-dose dam on GD 22 due to an inability to deliver her litter. The high-dose level was reduced on gestation day 15 to 0.2 mg/kg/day. Additionally, clinical signs (unsteady gait, tremor, salivation, piloerection, accelerated/labored breathing, lacrimation, and diarrhea) consistent with cholinesterase inhibition were observed during the dosing period. Decreased body-weight gain and food consumption were observed in the high-dose dams following the first week of dosing. There was a reduction in the number of liveborn pups and an increase in the number of stillborn and dead pups at the high dose only. Decreased cholinesterase activity was observed in all three compartments in dams on **gestation day 20** (serum 91%**; RBC 89%**; brain 77%**) and in the RBC (39%*) and brain (32%) compartments of **dams on postnatal day 21** (11 days after cessation of dosing). **Mid-dose dams**: One mid-dose dam was found dead on gestation day 14, and clinical signs consistent with cholinesterase inhibition (salivation, lacrimation, piloerection, accelerated breathing) were observed in a few mid-dose dams. Decreased cholinesterase activity was observed in all three compartments in dams on **gestation day 20** (serum 69%**; RBC 66%**; brain 35%*) and in the RBC (31%**) compartment of **dams on postnatal day 21** (11 days after cessation of dosing). **Low-dose dams**: Although salivation, piloerection, accelerated respiration, and diarrhea were observed in 1 to 2 low-dose dams, these clinical signs were not associated with cholinesterase inhibition and are not considered treatment-related.

GD 20 fetuses:
High-dose: Decreased cholinesterase activity was observed in all three compartments in both sexes on gestation day 20 (serum: males 52%**/females 53%; RBC: males 69%**/females 59%**; brain: males 39%**/females 32%**). **Mid-dose**: Decreased cholinesterase activity was observed in both sexes on gestation day 20 in the serum (males 24%**/females 29%) and RBC (males 51%**/females 54%**) compartments but in the brain compartment in males only (19%**). **Low-dose**: There was no inhibition of cholinesterase activity at this dose level in either sex.

Pups: There were no clinical signs in the offspring during lactation, but there was a decrease in pup viability during early lactation (PND 0-4) at the high-dose level. Decreased pup body weight was observed in both sexes (high-dose) during PNDs 1 through 10 during which time the dams were being dosed with the test material. From PND 11 to 21, when pups were dosed directly with the test material, pup body weight appeared to recover, although this is based on only 2 male pups and 1 female pup at the high dose. Pup body-weight gain was significantly lower than the control during the first week to PND 11 of weaning at the high-dose level when the dams were being dosed, but thereafter (PND 11 to 21), body-weight gains were comparable to or greater than the controls (both sexes).

On PND 21, following direct treatment of offspring for 11 days, there was a dose-related, statistically-significant, reduction in serum (males 56% and 74%/females 58% and 84%), erythrocyte (males 42% and 72%/females 40% and 83%), and brain (males 38% and 50%/females 42% and 69%) cholinesterase activities in both sexes at the mid- and high-dose levels,

respectively. At the mid-dose level, the magnitude of the inhibition in each compartment was comparable between the sexes; *i.e.,* serum (56% *vs.* 58%), RBC (42% *vs.* 40%), and brain (38% *vs.* 42%). At the high-dose level, the magnitude of the cholinesterase inhibition in each compartment was slightly higher in the female offspring compared to the male offspring; *i.e.*, serum (male 74% *vs.* female 84%), RBC (male 72% *vs.* female 83%), and brain (male 50% *vs.* female 69%).

A comparison of the results between the GD 20 **dams** (dosed during GD 6-20) and the PND 21 **dams** (dosed during GD6 through PND 11; cholinesterase activity assessed on PND 21): Following cessation of dosing for 10 days, serum cholinesterase activity recovered to control levels in the PND 21 dams, but RBC (mid- 33% and high- 39%) and brain (high- 32%) cholinesterase activity remained inhibited; however, the magnitude of the response was reduced compared to the GD 20 dams (RBC mid- 66% and high- 89%; brain mid- 35% and high- 77%).

Following 15 doses (GD 6-20), high-dose dams displayed slightly greater inhibition in all compartments compared to the 21-day-old high-dose pups who received 11 doses. [serum: pups (male 74%/female 84%) *vs.* dams (91%); erythrocyte: pups (male 72%/female 83%) *vs.* dams (89%); brain: pups (male 50%/female 69%) *vs.* dams (77%)]

A similar finding (slightly greater inhibition in dams *vs.* pups) also occurred at the mid-dose level, except the brain compartment, where pups displayed a slightly higher inhibition (males 38%/females 42% *vs.* dams 35%).

The GD 20 fetuses displayed a significant level of inhibition in all compartments (dosed *in utero*), whereas the PND 4 pups (dosed *in utero* and during PND 0-4 *via* the dam) displayed inhibition only in the RBC compartment in both sexes and in brain (males). The PND 11 pups (dosed *in utero* plus during PND 0-10 *via* the dam and one direct dose) displayed inhibition only at the high-dose level, although the magnitude of the response was somewhat comparable to the GD fetuses.

Under the conditions of this range-finding study, **the LOAEL is 0.1 mg/kg/day, based on clinical signs (salivation, lacrimation, piloerection, accelerated breathing) in the maternal animal, and inhibition of serum, erythrocyte (RBC), and/or brain cholinesterase activity in GD 20 dams and PND 21 dams, GD 20 fetuses, and PND 4 (RBC only), 11, and 21 pups. The NOAEL is 0.03 mg/kg/day.**

Based on these results, the study authors recommended dose levels of 0.01, 0.08, and 0.15 mg/kg/day for the definitive developmental neurotoxicity study in rats. Direct dosing of the pups *via* gavage was recommended also.

C. STUDY DEFICIENCIES:

The following deficiencies were noted, but do not alter the conclusions of this DER:

It was not clear if exudate in the thoracic cavity as the cause of death was due to gavage error or was a normal response to administration of an irritating substance.

The tables/contents in pages 92-101 are not clearly identified.

On page 21 (Test group 2 for fetuses), decreased brain ChE in the males (-35%) on gestation day 20 is in error. The decreased brain ChE in the males should be -19%.

Blood and brain cholinesterase activity was determined in the dams (2-3 hours post dose) and pups from each group on PNDs 11 and 21 (approximately 2 hours post dose). However, times of cholinesterase activity assessment after dosing were not clearly identified for fetuses of each group on gestation day 20, culled pups from each group on PND 4, and surviving dams on PND 21.

13544

R158250

Chemical: Terbufos

PC Code: 105001
HED File Code: 13000 Tox Reviews
Memo Date: 3/19/2008
File ID: TX0052717
Accession #: 000-00-0125

HED Records Reference Center
5/8/2008