DATA EVALUATION RECORD

BAS 316 I (TERBUFOS)
NON-GUIDELINE

STUDY TYPE: RANGE-FINDING – DEVELOPMENTAL NEUROTOXICITY
MRID 46240802

Prepared for

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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 Unpublished.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**

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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.

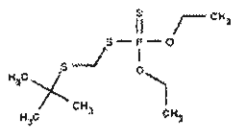
I. MATERIALS AND METHODS:

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) PND 11 PND 21	 6-7/sex 11/sex 11/sex	 6-11/sex 10/sex 9-10/sex	 5-6/sex 10/sex 9/sex	 2/sex 3/sex 2/sex
Brain cholinesterase activity determination PND 4 (culled pups) PND 11 PND 21	 7-8/sex 11/sex 11/sex	 7-12/sex 10/sex 9-10/sex	 7/sex 10/sex 9/sex	 2/sex 3/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

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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

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