UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

JANUARY 18, 2006

MEMORANDUM

Subject: PC Code 097301. Formetanate hydrochloride: Review of the comparative cholinesterase inhibition study in rats (2005, MRID No.: 46618901)

TXR No.: 0053305.
DP Barcode: D320464
PC Code: 097301

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Conclusions and Comments

ReRegistration Branch III (RRBIII) has reviewed the special comparative cholinesterase inhibition study (2005, MRID No.: 46618901) in which 11 day old pups and young adult were dosed with the N-methyl carbamate formetanate hydrochloride at predetermined the time of peak effect for cholinesterase inhibition. The study was classified as Acceptable/Non-Guideline and to satisfy the intended purpose. Both red blood cell (RBC) and brain acetylcholinesterase (AChE) was assessed for activity and inhibition. The data for RBC AChE was considered to have poor dose responses in both sexes of both pups and adults. However, brain AChE data were considered reasonable.

The study demonstrated that brain AChE was inhibited at the lowest test dose in both sexes in both pups and adults. RRBIII considered the approach to benchmark dose

(BMD) analysis as submitted by the registrant's contractor (refer to report by Thomas B. Starr, dated August 4, 2005 entitle Benchmark dose estimates for cholinesterase inhibition by formetanate HCL, MRID No.: 46618902). However, Health Effects Division determined that Dr. Starr's approach did not use the preferred model and did its own analysis (refer to the memo from Philip Villanueva and Anna Lowit entitled "Benchmark dose analysis of cholinesterase levels from the oral gavage acute relative sensitivity study of formetanate HCL in neonatal and adult rats" dated December 15, 2005, TXR # 0053699). Based on the BMDL (benchmark dose lower limit 10), derived from female pup brain AChE inhibition data, a reference dose of BMDL10 = 0.065 mg/kg was determined and recommended for risk assessments.

A copy of the DER for the comparative cholinesterase inhibition study is attached. The study is further identified in the following table together with the Executive Summary.

Submission	Executive Summary or Comments
Special Non-guideline Comparative Cholinesterase Inhibition Study- rats. Charles River Laboratories, Study No.: WJI000007, August 4, 2005, MRID No.: 46618901.	This series of non-guideline studies was undertaken to evaluate any differences between neonatal and adult rats with regard to acetylcholinesterase (AChE) inhibition. In this series of non-guideline cholinesterase inhibition studies (2005, MRID 46618901), formetanate HCL (97.8% a.i.; Lot #: 930904) in deionized water was administered once via gavage (5 mL/kg) to 5 adult and 5 PND 11 Sprague Dawley rats/sex/sacrifice time at doses of 0 or 5.0 mg/kg to determine the time of peak cholinesterase inhibition. Erythrocyte and brain AChE was determined at 0.25, 0.5, 1, 4, 8, and 24 hours. In the definitive dose response studies, formetanate HCL was administered once via gavage to 10 adult and 10 PND 11 rats/sex/dose at doses of 0, 0.6, 1.5, or 3.0 mg/kg. Erythrocyte and brain AChE was determined at 0.5 hours post-dosing (estimated time of peak-effect) in all groups. AChE was assessed at 26° C rather than at 37° C to minimize reversal of inhibition by the carbamate inhibitor. *Clinical signs.* There were no treatment-related effects on mortality or clinical signs. *RBC ACHE.* RBC AChE inhibition data did not exhibit informative time response curves in pups or adults in that the apparent inhibitory effects lacked dose responses. The apparent peak inhibition ($p<=0.05$) occurred at 15 minutes post-dosing in the male pups and at 30 minutes in the female pups. Recovery occurred between 8 and 24 hours post-dosing. In the adults, decreases in RBC AChE were similar to those in the pups. Comparative dose response studies at 30 minutes post dosing showed decreased ($p<=0.01$) RBC AChE in all dose groups in the pups of both sexes (decr. 21-30%) and in the 3 mg/kg adult males (decr. 29%). Overall, the RBC AChE data were of limited value and no reliable or definite indication that the pups were more sensitive than the adults was established. . *Brain AChE.* Brain AChE in pups was decreased in both sexes beginning at 15 minutes and continuing through 8 hours post-dosing, with recovery evident at 24 hours. In the adults, brain AChE was decreased in both sexes beginning at 15 minutes and continuing through 4 hours post-dosing. At 8 hours post-dose, recovery was marginal in the males and was evident in the females. Comparative dose response studies conducted at 30 minutes post dosing revealed dose-dependently decreased ($p<=0.05$) brain cholinesterase activity at all doses in both sexes of pups (decr. 34-74%) and adults (decr. 16-50%). **The LOAEL for brain AChE inhibition is < 0.6 mg/kg. The NOAEL was not established. Separate benchmark dose analysis demonstrated that the BMDL10 for female pup brain AChE inhibition was 0.065 mg/kg.** The BMD analysis of the brain AChE data indicatec that both male and female pups had lower BMDs than the adults indicating that the pups were more sensitive than the adults to the inhibitory effects of formetanate HCl.

Submission	Executive Summary or Comments
	These studies are classified as **acceptable/non-guideline**. These studies do not satisfy a guideline requirement for formetanate. Although the data with RBC AChE were of limited value, the data with brain AChE achieved the objectives of the study.
BMD report. Prepared by Dr. T.A. Starr, TBS Associates, Report No.: TBS/GOW/050804, August 4, 2005, MRID No.: 46618902.	This report consists of a benchmark dose analysis of the comparative ChE study (MRID No.: 46618901). However, HED determined that the analytical method used was not consistent with HED's current program and HED used an alternate method. Please refer to the memo from Philip Villanueva and Anna Lowit entitled "Benchmark dose analysis of cholinesterase levels from the oral gavage acute relative sensitivity study of formetanate HCL in neonatal and adult rats" dated December 15, 2005, TXR # 0053699).
Laboratory Report - Analysis of active ingredient in technical Formetanate Hydrochloride, PTRL, Study No.: 1356W, Feb. 24, 2005, MRID No.: 46618903.	This analytical report establishes that the purity of Lot. No.: 930904 formetanate hydrochloride used in the comparative cholinesterase inhibition study was 97.8%. This information was incorporated into the DER for MRID No.: 46618901.

DATA EVALUATION RECORD

FORMETANATE HCl

Study Type: Non-guideline; Time of Peak Cholinesterase Inhibition and Comparative Cholinesterase Studies in Preweaning and Adult Rats

Work Assignment No. 3-01-85 (MRID 46618901)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; Time of Peak Cholinesterase Inhibition and Comparative Cholinesterase Studies in Pup and Adult Rats.

PC CODE: 097301 **DP BARCODE:** D320464
TXR#: 053305

TEST MATERIAL (PURITY): Formetanate HCl (97.8% a.i.)

SYNONYMS: 3-Dimethylaminomethyleneiminphenyl-N-methylcarbamate, hydrochloride

CITATION: Barnett, J.F. (2005) Oral (gavage) acute relative sensitivity study of formetanate HCl in neonatal and adult rats. Charles River Laboratories, Preclinical Services, Horsham, PA. Laboratory Project ID.: WJI00007, August 4, 2005. MRID 46618901. Unpublished.

SPONSOR: Gowan Company, 370 S. Main Street, Yuma, AZ

EXECUTIVE SUMMARY - This series of non-guideline studies was undertaken to evaluate any differences between neonatal and adult rats with regard to acetylcholinesterase (AChE) inhibition. In this series of non-guideline cholinesterase inhibition studies (2005, MRID 46618901), formetanate HCL (97.8% a.i.; Lot #: 930904) in deionized water was administered once via gavage (5 mL/kg) to 5 adult and 5 PND 11 Sprague Dawley rats/sex/sacrifice time at doses of 0 or 5.0 mg/kg to determine the time of peak cholinesterase inhibition. Erythrocyte and brain AChE was determined at 0.25, 0.5, 1, 4, 8, and 24 hours. In the definitive dose response studies, formetanate HCL was administered once via gavage to 10 adult and 10 PND 11 rats/sex/dose at doses of 0, 0.6, 1.5, or 3.0 mg/kg. Erythrocyte and brain AChE was determined at 0.5 hours post-dosing (estimated time of peak-effect) in all groups. AChE was assessed at 26° C rather than at 37° C to minimize reversal of inhibition by the carbamate inhibitor.

Clinical signs. There were no treatment-related effects on mortality or clinical signs.
RBC ACHE. RBC AChE inhibition data did not exhibit informative time response curves in pups or adults in that the apparent inhibitory effects lacked dose responses. The apparent peak inhibition ($p<=0.05$) occurred at 15 minutes post-dosing in the male pups and at 30 minutes in the female pups. Recovery occurred between 8 and 24 hours post-dosing. In the adults, decreases in RBC AChE were similar to those in the pups. Comparative dose response studies at 30 minutes post dosing showed decreased ($p<=0.01$) RBC AChE in all dose groups in the pups of both sexes (decr. 21-30%) and in the 3 mg/kg adult males (decr. 29%). Overall, the RBC

AChE data were of limited value and no reliable or definite indication that the pups were more sensitive than the adults was established. .

Brain AChE. Brain AChE in pups was decreased in both sexes beginning at 15 minutes and continuing through 8 hours post-dosing, with recovery evident at 24 hours. In the adults, brain AChE was decreased in both sexes beginning at 15 minutes and continuing through 4 hours post-dosing. At 8 hours post-dose, recovery was marginal in the males and was evident in the females. Comparative dose response studies conducted at 30 minutes post dosing revealed dose-dependently decreased ($p<=0.05$) brain cholinesterase activity at all doses in both sexes of pups (decr. 34-74%) and adults (decr. 16-50%). **The LOAEL for brain AChE inhibition is < 0.6 mg/kg. The NOAEL was not established. Separate benchmark dose analysis[1] demonstrated that the BMDL10 for female pup brain AChE inhibition was 0.065 mg/kg.** The BMD analysis of the brain AChE data indicated that both male and female pups had lower BMDs than the adults indicating that the pups were more sensitive than the adults to the inhibitory effects of formetanate HCl.

These studies are classified as **acceptable/non-guideline**. These studies do not satisfy a guideline requirement for formetanate. Although the data with RBC AChE were of limited value, the data with brain AChE achieved the objectives of the study.

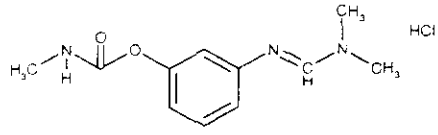
COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

[1]Refer to memo entitled "Benchmark dose analysis of cholinesterase levels from the oral gavage acute relative sensitivity toxicity study with formetanate HCl in neonatal and adult rats" from P. Villanueva and A. Lowit, dated December 15, 2005, TXR No.: 0053699).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Formetanate HCL

Description:	Yellow powder
Batch #:	930904
Purity (w/w):	97.8% a.i. (verified in accompanying report dated 2/ 24/05, MRID No.: 46618903).
Stability:	Not reported
CAS #:	23422-53-9
Structure:	

2. Vehicle - Reverse osmosis deionized water

3. Test animals

Species:	Rat
Strain:	Crl:CD(SD)
Adult age/weight at study initiation:	9 weeks old/ 274-381 g males and 212-252 g females (for both studies)
Pup Age/weight at dosing:	11 days old/ 12.8-25.4 g males and 15.2-25.3 g females (both studies)
Source:	Charles River Laboratories (Raleigh, NC) for time of peak effects study in pups and dose-response study in pups and adults (St. Constant, Quebec, Canada) for time of peak effects study in adults
Housing:	Each dam was housed with its litter in a nesting box during the post-natal period. Adults were individually housed in stainless steel, wire bottomed cages.
Diet:	Certified Rodent Diet #5002 (PMI Nutrition International, Inc., St. Louis, MO), *ad libitum*
Water:	Reverse osmosis water with chlorine added as a bacteriostat, *ad libitum*
Environmental conditions:	
Temperature:	18-26°C
Humidity:	30-70%
Air changes:	At least 10/hr
Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Adults, 6-7 days; pups 2-3 days

B. STUDY DESIGN

1. Study purpose - This non-guideline study was undertaken to evaluate any differences between neonatal and adult rats with regard to acetylcholinesterase (AChE) activity as a biomarker for more general neurological effects. General guidance for the study design was provided by the U.S. EPA (Agency) Office of Pesticide Programs in the July 20, 2004 draft memo entitled *Acute Comparative Cholinesterase Activity Study for Formetanate*. The guidance

provided by the Agency specified that the following work be conducted: (i) an initial abbreviated dose response study in post-natal day (PND) 11 neonatal pups; (ii) a study to determine the time to peak AChE inhibition and time to 90% recovery to control values in adults and neonates; and subsequently, (iii) a definitive acute comparative dose response study.

2. **In-life dates** - Start: 04/25/05 End: 05/10/05

3. **Animal assignment and treatment** - Pups were naturally bred by the supplier using male rats of the same source and strain. Dams were allowed to deliver their litters at the supplier and were shipped to arrive at the performing laboratory on PND 8-9. In the time of peak effect study (Table 1a), pups in each of 11 litters were assigned to one of 6 sampling time points in control and treated groups to achieve 5 pups/sex/dose/time point. In the dose-response study (Table 1b), pups from 10 standardized litters (4/sex/litter) were assigned by consecutive numeric order to one of 4 dose groups to achieve 10 pups in each dose group (1/sex/litter/group). For the studies using adult rats, the animals were randomly assigned (stratified by body weight) to the test groups noted in Tables 1a and 1b. Adults and pups (on PND 11) received a single gavage dose at a volume of 5 mL/kg body weight.

Table 1a. Study design for time of peak effect of cholinesterase inhibition.[a]

Group	# of Animals (M/F)	Dose (mg/kg)		Sample Time (Hrs post-dosing)
		Males	Females	
Adults				
Control	5/5	0	0	0.25
	5/5	0	0	0.5
	5/5	0	0	1
	5/5	0	0	4
	5/5	0	0	8
	5/5	0	0	24
Treated	5/5	5	5	0.25
	5/5	5	5	0.5
	5/5	5	5	1
	5/5	5	5	4
	5/5	5	5	8
	5/5	5	5	24
Pups				
Control	5/5	0	0	0.25
	5/5	0	0	0.5
	5/5	0	0	1
	5/5	0	0	4
	5/5	0	0	8
	5/5	0	0	24
Treated	5/5	5	5	0.25
	5/5	5	5	0.5
	5/5	5	5	1
	5/5	5	5	4
	5/5	5	5	8
	5/5	5	5	24

a The data were obtained from pages 22-24 of the study report.

Table 1b. Study design for dose-response studies of cholinesterase inhibition at time of peak effect. [a]

Group	# of Animals (M/F)	Dose (mg/kg)		Sample Time (Hrs post-dosing)
		Males	Females	
Adults				
1 (control)	10/10	0	0	0.5
2	10/10	0.6	0.6	0.5
3	10/10	1.5	1.5	0.5
4	10/10	3.0	3.0	0.5
Pups				
1 (control)	10/10	0	0	0.5
2	10/10	0.6	0.6	0.5
3	10/10	1.5	1.5	0.5
4	10/10	3.0	3.0	0.5

a The data were obtained from pages 24-26 of the study report.

4. Dose selection and sampling time rationale

a) Dose selection - In a dose range-finding study (WJI00006), 5 pups/sex/dose were administered a single gavage dose of 0 (vehicle), 0.3, 1.0, or 3.0 mg/kg formetanate HCl. AChE activity in erythrocytes (RBC) was unaffected in the 0.3 mg/kg group and only marginally inhibited at 1.0 mg/kg, whereas brain AChE activity was dose-dependently inhibited. Thus, dose levels of 0, 0.6, 1.5, and 3.0 mg/kg were selected for the current dose-response study. Because inhibition of AChE at 3 mg/kg was minimal in RBC, a higher dose of 5 mg/kg was selected for the time to peak effect study to observe a response and recovery in this compartment.

b) Time point selection - In a range-finding study with adults, 5 rats/sex/dose were treated with 0 or 1 mg/kg formetanate HCL to compare to the results obtained previously at a different laboratory (Beyrouty, MRIDs 45255501 and 45314201). Results of blood and brain AChE activities were consistent with those previously observed. Specifically, in the brain, maximal inhibition was present at 15 minutes and continued through 1 hour with marginal effects in the blood at 15 and 30 minutes. Regarding the time to peak effect, there were no differences between pups and adults in either compartment. Thus, times of 15 minutes, 30 minutes, and 1 hour were selected to verify that 30 minutes was the time of peak effect; times of 4, 8, and 24 hours were included to determine recovery.

5. Test substance preparation and analysis - Using individual body weights, dose formulations were prepared on the day of dose administration by mixing the appropriate amount of the test substance with vehicle. Dose formulations were stored at room temperature and used within 6 hours of preparation. Quadruplicate samples were taken from the middle of each dose formulation on the day of preparation, and two samples from each quadruplicate set were stored frozen (-70°C) until analysis for actual concentration. It was stated that homogeneity and stability of the test substance in the vehicle were verified prior to the study; however, no data were reported.

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Results: **Concentration (% nominal):** 96.9-108.9%

Based on the data presented, the analytical data indicated that the variation between nominal and actual dosage to the study animals was acceptable.

6. Statistics - Significance was denoted at $p \leq 0.05$ and 0.01 in the study report tables.

a) Time to peak effect study - AChE values for RBC and brain were analyzed as separate dependent variables using two-way analysis of variance (ANOVA), with dose level and sampling time as independent variables. Control group values at each time point were compared at each combination of sex and age. Data were subjected to Bartlett's test for homogeneity of variance. If variances were homogeneous, ($p>0.001$), data were compared using one-way ANOVA. If ANOVA was significant ($p \leq 0.05$), the control group at 15 minutes was compared with those at other intervals using Dunnett's test. If variances were heterogeneous, ($p \leq 0.001$), data were analyzed using the following non-parametric procedures. If ≤75% of the scores in all groups are tied, data were compared using Kruskal-Wallis test. If the Kruskal-Wallis test was significant ($p \leq 0.05$), Dunn's test was used for pair-wise comparisons of the treated groups with controls. If >75% of the scores in all groups are tied, data were compared using Fisher's exact test.

b) Dose response study - The statistical analyses for the dose response study was similar to the scheme described for the time to peak effect study, except that there were no multiple time points in the dose response study. In other words, data were subjected to the same decision tree to determine if parametric or non-parametric procedures were appropriate, with the only difference being that AChE values for RBC and brain were analyzed as separate dependent variables in a one-way (and not a two-way) ANOVA at each combination of sex and age, with dose level as the independent variable.

C. METHODS

1. Observations

a. Adults - All adult rats, including dams of offspring, were observed at least twice daily for mortality. Clinical observations and general appearance were recorded weekly during the acclimation period and before dosing on the day of dose administration.

b. Pups - Litters were checked for live and dead pups, and viability recorded, at least twice daily.

2. Body weight - Body weights of all adults and pups were recorded on the day after arrival and on the day of dose administration to determine individual doses.

3. Cholinesterase activity determination - At each sacrifice time, blood was collected by cardiac puncture in pups or from the inferior vena cava of each adult rat under isoflurane anesthesia for erythrocyte AChE activity determination. Following blood collection, the animals were euthanized by exsanguination. The brain was excised, weighed, and homogenized in 0.1% Tween buffer. AChE activity determination was performed using a modification of the Ellman

method. These modifications were specifications by the Agency regarding three parameters to control in order to minimize reactivation of the inhibited AChE: (i) concentration of the tissue homogenate and final dilution of the samples (initial dilution <1:4; final dilution <1:30); (ii) assay reaction times; and (iii) assay temperature. The performing laboratory incorporated these modifications as follows. All samples were incubated for 2 minutes at 26°C, rather than the 10 minutes at 37°C outlined in the standard operating procedure (SOP). Brain sample analysis was conducted at 26°C rather than 37°C. RBC samples were analyzed for 12 minutes including a 2 minute lag period to minimize initial high and low spikes. Regarding dilution of the samples, the Sponsor stated that the usual laboratory procedures were sufficiently similar to the Agency's recommendations that additional modifications were unnecessary.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity

a) Pups - One 5.0 mg/kg male pup was cold to the touch, and one female in this group had a portion of its tail missing prior to AChE evaluation. No additional incidental clinical signs were noted prior to or after dose administration.

b) Adults - No clinical signs of toxicity were noted in the adults.

2. Mortality

a) Pups - One male pup (#4704) in the 3.0 mg/kg dose group was found dead shortly after dose administration. Necropsy of this animal revealed no adverse findings. This pup was subsequently replaced with another male (#9906). All other pups survived to scheduled termination.

b) Adults - All adult animals survived until scheduled termination.

B. BODY WEIGHTS

a) Pups - Body weights of the treated pups were comparable to controls on the day of dose administration.

b) Adults - Body weights of the treated adults were comparable to controls on the day of dose administration.

C. CHOLINESTERASE ACTIVITY - The cholinesterase data for all compartments and time points are summarized and presented below in Tables 2a and 2b (time of peak effect studies) and 3a and 3b (comparative dose-response cholinesterase inhibition studies).

1. Time to peak effect studies

a) Erythrocyte AChE - Pups. In the time to peak effect studies, erythrocyte AChE was decreased throughout the study in the male pups (↓6-28%); and throughout the first 8 hours post-dosing in the females (↓18-37%). The peak inhibition ($p \leq 0.05$) occurred at 15 minutes post-dosing in the males (↓28%) and at 30 minutes post-dosing in the females (↓37%; $p \leq 0.05$). However, because comparably decreased (↓37%; $p \leq 0.05$) AChE was observed at 8 hours post-dosing in the females, a second run was conducted at this time point for which the inhibition was not as great (↓18%; not significant [NS]). At 24 hours post-dosing in these animals, cholinesterase activity was comparable to controls, indicating apparent recovery between 8 and 24 hours post-dosing. Due to the marginal response and inter-animal variability, the time response curve was generally uninformative.

Adults. In the adult rats, decreases in erythrocyte AChE were similar to those in the pups in that they were marginal and variable, with a time response curve that was generally uninformative. In both sexes, the first run at 30 minutes post-dosing yielded inhibition of a low magnitude (↓1-7%; NS) compared to the time to peak effect studies previously conducted by Beyrouty (MRIDs 45255501 and 45314201). To resolve this apparent discrepancy, a second run was conducted at this time point, resulting in inhibition values of a greater magnitude than the first run in males (↓27%; $p \leq 0.01$) and females (↓13%; NS). Again, there did not appear to be a trend in inhibition of AChE with time, as inhibition at 15 minutes post-dose (↓23-24%; $p \leq 0.05$) was comparable to inhibition at 8 hours post-dose (↓26-30%; $p \leq 0.01$) in both sexes.

b) Brain AChE- pups- In the time to peak effect studies, brain AChE was decreased in the pups of both sexes beginning at 15 minutes and continuing through 8 hours post-dosing (↓20-78%; $p \leq 0.05$). Recovery was evident at 24 hours post-dose, with AChE levels comparable to or increased over controls.

In the adults, brain AChE was decreased (↓24-52%; $p \leq 0.05$) in both sexes beginning at 15 minutes and continuing through 4 hours post-dosing. At 8 hours post-dose, recovery was marginal in the males (↓19%; NS) and was evident in the females, with AChE levels comparable to or increased over controls thereafter.

Based on the time to peak effect studies, 30 minutes was selected as the appropriate sampling interval for all ages, sexes, and compartments for the dose response studies.

2. Comparative dose response studies

a) Erythrocyte AChE - In the comparative dose response studies, erythrocyte AChE was decreased ($p \leq 0.01$) in all dose groups in the pups of both sexes (↓21-30%) and in the 3 mg/kg adult males (↓29%). AChE in the low and mid dose males and in the adult females in all treated groups were either comparable to controls or were minimally and not significantly decreased.

b) Brain AChE- In the comparative dose response studies, brain AChE was dose-dependently decreased ($p \leq 0.05$) at all doses in the male (↓34-70%) and female (↓47-74%) pups and in the male (↓16-50%) and female (↓24-33%) adults.

Table 2a. Time course data - Mean (±SD) AChE data in PND 11 pups treated once via gavage with Formetanate HCL. [a] -

Time post-dose (hours)	Dose (mg/kg)	Red Blood Cells		Brain	
		AChE (U/mL)	% inhibition [b]	AChE (U/g)	% inhibition [b]
Males					
0.25	0	1.909 ± 0.478	---	7.207 ± 0.521	---
	5.0	1.374 ± 0.102*	28	1.808 ± 0.101**	75
0.5	0	1.792 ± 0.099	---	8.252 ± 0.505	---
	5.0	1.689 ± 0.235	6	1.835 ± 0.377**	78
1	0	2.051 ± 0.418	---	6.639 ± 0.378	---
	5.0	1.784 ± 0.214	13	1.629 ± 0.162**	76
4	0	1.697 ± 0.502	---	7.441 ± 0.723	---
	5.0	1.288 ± 0.176	24	1.657 ± 0.120**	78
8	0	1.941 ± 0.140	---	8.551 ± 1.053**	---
	5.0	1.691 ± 0.321	13	5.056 ± 2.207	41
24	0	2.113 ± 0.298	---	7.462 ± 0.515	---
	5.0	1.885 ± 0.470	11	6.358 ± 1.285	15
Females					
0.25	0	2.001 ± 0.610	---	7.207 ± 0.521	---
	5.0	1.510 ± 0.188	25	1.984 ± 0.457**	76
0.5	0	1.874 ± 0.538	---	7.562 ± 1.222	---
	5.0	1.184 ± 0.322**	37	2.027 ± 0.506**	73
1	0	1.949 ± 0.192	---	7.228 ± 0.755	---
	5.0	1.452 ± 0.469*	26	1.779 ± 0.270**	75
4	0	1.798 ± 0.571	---	6.910 ± 0.686	---
	5.0	1.254 ± 0.044	30	2.057 ± 1.076**	70
8 (combined)	0	2.030 ± 0.349	---	5.733 ± 1.179	---
	5.0	1.505 ± 0.354**	26	3.562 ± 1.021**	38
8 (1st run)	0	2.007 ± 0.251	---	5.999 ± 1.578	---
	5.0	1.262 ± 0.158**	37	2.778 ± 0.851**	54
8 (2nd run)	0	2.049 ± 0.441	---	5.401 ± 0.349	---
	5.0	1.688 ± 0.361	18	4.346 ± 0.260*	20
24	0	1.842 ± 0.128	---	7.350 ± 0.744	---
	5.0	1.886 ± 0.152	-2	9.562 ± 0.951**	-30

a Data were obtained from Text Tables 1 and 2 on pages 37 and 39 of the study report. n = 3-5, except for the combined 1st and 2nd runs for the 8 hour time point in the females where n = 7-9.

b Percent inhibition (decreased activity) was calculated by the reviewers as 100% - the % control presented in the study report.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

Table 2b. Time course data. Mean (±SD) AChE data in adult rats treated once via gavage with Formetanate HCL. [a]

Time post-dose (hours)	Dose (mg/kg)	Red Blood Cells		Brain	
		AChE (U/mL)	% inhibition [b]	AChE (U/g)	% inhibition [b]
Males					
0.25	0	1.525 ± 0.240	---	13.146 ± 0.822	---
	5.0	1.154 ± 0.106*	24	6.370 ± 1.846**	52
0.5 (combined)	0	1.573 ± 0.226	---	12.431 ± 1.698	---
	5.0	1.288 ± 0.163*	18	7.835 ± 2.504**	37
0.5 (1st run)	0	1.472 ± 0.239	---	13.547 ± 1.200	---
	5.0	1.369 ± 0.208	7	10.132 ± 0.802*	25
0.5 (2nd run)	0	1.674 ± 0.181	---	11.315 ± 1.391	---
	5.0	1.223 ± 0.094**	27	5.538 ± 0.520**	51
1	0	1.534 ± 0.271	---	13.192 ± 1.080	---
	5.0	1.194 ± 0.163*	22	8.071 ± 3.139**	39
4	0	1.506 ± 0.152	---	13.068 ± 2.238	---
	5.0	1.209 ± 0.158	20	8.782 ± 1.698**	33
8	0	1.617 ± 0.311	---	12.307 ± 1.947	---
	5.0	1.199 ± 0.203**	26	9.982 ± 0.783	19
24	0	1.225 ± 0.282	---	13.534 ± 4.540	---
	5.0	1.551 ± 0.492	-27	12.297 ± 1.862	9
Females					
0.25	0	1.552 ± 0.245	---	11.002 ± 0.499	---
	5.0	1.202 ± 0.076**	23	6.015 ± 1.657**	45
0.5 (combined)	0	1.335 ± 0.161	---	12.320 ± 1.954	---
	5.0	1.232 ± 0.208	18	7.866 ± 2.061**	36
0.5 (1st run)	0	1.282 ± 0.221	---	13.855 ± 0.633	---
	5.0	1.270 ± 0.084	1	9.778 ± 0.596**	29
0.5 (2nd run)	0	1.377 ± 0.099	---	10.786 ± 1.518	---
	5.0	1.201 ± 0.280	13	5.954 ± 0.245**	45
1	0	1.415 ± 0.158	---	11.532 ± 1.456	---
	5.0	1.107 ± 0.179*	22	7.336 ± 3.224**	36
4	0	1.318 ± 0.192	---	12.726 ± 1.171	---
	5.0	1.118 ± 0.155	15	9.615 ± 2.859*	24
8	0	1.676 ± 0.150	---	11.001 ± 1.379	---
	5.0	1.169 ± 0.153**	30	10.564 ± 1.151	4
24	0	1.553 ± 0.159	---	11.649 ± 0.421	---
	5.0	1.372 ± 0.246	12	12.076 ± 1.352	-4

a Data were obtained from Text Tables 3 and 4 on pages 42 and 44 of the study report.
n = 4-5, except for the combined 1st and 2nd runs for the 0.5 hour time point in both sexes where n = 9-10.

b Percent inhibition (decreased activity) was calculated by the reviewers as 100% - the % control presented in the study report.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

Table 3a. Dose response data. Mean (±SD) AChE data at the estimated time of peak effect (30 minutes) in PND 11 pups treated once via gavage with Formetanate HCL. [a]

Compartment	Dose (mg/kg)				Comment
	0	0.6	1.5	3.0	
Males					
Erythrocyte (U/mL)	2.131 ± 0.321	1.661 ± 0.251** (21)	1.506 ± 0.336** (29)	1.565 ± 0.300 ** (27)	No dose response.
Brain (U/g)	6.695 ± 0.837	4.440 ± 2.054* (34)	2.168 ± 0.264** (68)	2.017 ± 0.482** (70)	Good dose response.
Females					
Erythrocyte (U/mL)	2.180 ± 0.363	1.526 ± 0.254** (30)	1.524 ± 0.291** (30)	1.552 ± 0.531** (29)	No dose response
Brain (U/g)	6.876 ± 0.765	3.632 ± 1.365** (47)	2.186 ± 0.558** (68)	1.799 ± 0.573** (74)	Good dose response.

a Data were obtained from Text Tables 5 and 6 on pages 47 and 48 of the study report; n = 6-10 for RBC and n = 10 for brain.
b Percent inhibition (decreased activity) was calculated by the reviewers as 100% - the % control presented in the study report.
* Significantly different from controls at p≤0.05
** Significantly different from controls at p≤0.01

Table 3b. Dose response data. Mean (±SD) cholinesterase activity at the estimated time of peak effect (30 minutes) in adult rats treated once via gavage with Formetanate HCL. [a]

Compartment	Dose (mg/kg)				Comment
	0	0.6	1.5	3.0	
Males					
Erythrocyte (U/mL)	1.807 ± 0.253	1.685 ± 0.296 (7)	1.576 ± 0.342 (13)	1.292 ± 0.330** (29)	Sig. At high dose only.
Brain (U/g)	11.779 ± 1.427	9.923 ± 0.968* (16)	7.203 ± 1.583** (39)	5.878 ± 1.717** (50)	Good dose response.
Females					
Erythrocyte (U/mL)	1.566 ± 0.407	1.574 ± 0.316 (-1)	1.461 ± 0.189 (7)	1.379 ± 0.374 (12)	No sig. inhibition
Brain (U/g)	10.163 ± 1.904	7.719 ± 1.430** (24)	6.950 ± 1.428** (32)	6.808 ± 1.393** (33)	Good dose response.

a Data were obtained from Text Tables 7 and 8 on pages 49 and 50 of the study report; n = 6-10 for RBC and n = 10 for brain.
b Percent inhibition (decreased activity) was calculated by the reviewers as 100% - the % control presented in the study report.
* Significantly different from controls at p≤0.05
** Significantly different from controls at p≤0.01

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - Based on the time to peak effect studies in pups and adults, the investigators concluded that 30 minutes post-dosing was the appropriate time to sample blood and brain tissue for AChE in the comparative dose-response study. In the time to peak effect studies, erythrocyte AChE recovery was generally uninformative. In the brain,

substantial recovery was observed between 8 and 24 hours in pups and adults. In the dose-response study, the test substance did not substantially reduce erythrocyte AChE in either sex in either age group of rats. In the brain, AChE inhibition appeared more pronounced and marginally dose responsive in pups and adults, although inter-animal variation was apparent. Modifications in the methods requested by the Agency precluded comparison of the control values in the current study with historical controls from the performing laboratory.

B. REVIEWER COMMENTS - There were no treatment-related effects on mortality or clinical signs of toxicity.

Erythrocyte AChE did not exhibit informative time response curves in pups or adults. Peak inhibition ($p \leq 0.05$) occurred at 15 minutes post-dosing in the male pups and at 30 minutes post-dosing in the female pups. Recovery occurred between 8 and 24 hours post-dosing. In the adult rats, decreases in erythrocyte AChE were similar to those in the pups. In both sexes, the first run at 30 minutes post-dosing yielded inhibition of a magnitude lower than expected compared to the time to peak effect studies previously conducted by Beyrouty (MRIDs 45255501 and 45314201). Thus, a second run was conducted at this time point, resulting in inhibition values of a greater magnitude than in the first run in both sexes. Comparative dose response studies at 30 minutes post dosing showed decreased ($p \leq 0.01$) erythrocyte AChE in all dose groups in the pups of both sexes (↓21-30%) and in the 3 mg/kg adult males (↓29%). Overall, the RBC AChE data were of limited value.

Brain AChE in the pups was decreased in both sexes beginning at 15 minutes and continuing through 8 hours post-dosing, with recovery evident at 24 hours post-dose. In the adults, brain cholinesterase activity was decreased in both sexes beginning at 15 minutes and continuing through 4 hours post-dosing. At 8 hours post-dose, recovery was marginal in the males and was evident in the females. Comparative dose response studies conducted at 30 minutes post dosing revealed dose-dependently decreased ($p \leq 0.05$) brain cholinesterase activity at all doses in both sexes of pups (↓34-74%) and adults (↓16-50%).

This study is classified as **acceptable/non-guideline.**

C. STUDY DEFICIENCIES - No data for homogeneity or stability of the test substance in the vehicle were reported. However, it was stated that homogeneity and stability were verified prior to the study.

Appendix 1. Special note on AChE assessments added by the HED reviewer.

The study report (pages 11 and 51) stated regarding the changes in the standard protocol as requested by EPA (see methods and materials of this DER) that "these changes were associated with notably higher control levels of brain AChE in both pups and adults as compared to the Test Facility's historical backgrounds obtained using the unmodified conditions. Acetylcholinesterase responses at the lowest dose tested in the definitive dose response study were in the range of historical control values in both pups and adults". The historical control data were provided by the registrant and are presented in Special Table #1 together with the control data from both the time course and the definitive study.

Special Table #1. Variability in the control groups for AChE in RBC and brain and in pups and adults and historical control data for pup and adult RBC and Brain AChE.

Source	Pups		Adults	
	Males	Females	Males	Females
RBC AChE				
(a)	1.909 ± 0.478 (25%)	2.001 ± 0.610(30%)	1.525 ± 0.240 (16%)	1.552 ± 0.245(16%)
(b)	2.131 ± 0.321 (15%)	2.180 ± 0.363 (17%)	1.807 ± 0.253 (14%)	1.566 ± 0.407(26%)
Historical	1.641	1.705	1.758	1.811
Brain AChE				
(a)	7.207 ± 0.521 (7.2%)	7.207 ± 0.521 (7.2%)	13.146 ± 0.822 (6.2%)	11.002 ± 0.499 (4.5%)
(b)	6.695 ± 0.837 (13%)	6.876 ± 0.765 (11%)	11.779 ± 1.427 (12%)	10.163 ± 1.904 (19%)
Historical	4.977	4.596	9.184	9.871

(a) Time course study.
(b) definitive dose response study.
(c) Historical control data from pages 350-352 of the study report. No standard deviation data were presented for the historical control data.

Special Table #1 demonstrates that the control values from this study which was conducted under different experimental conditions than the data in the historical control data base are as the study author indicated higher for all pup groups and for brain (but not RBC and female adult brain is only slightly higher) adult groups. This might imply that the inhibition seen in the low in the dose response study is an artifact of the higher concurrent control group. However, the time course study (see Tables 2 a and 2 b indicate that following inhibition, the activity level of brain AChE in the group dosed with 5 mg/kg of formetanate returns to about the same level as the control groups. Thus, there is no basis to discount the observation that there is inhibition of brain AChE at the lower dose in the definitive study.

Special Table #1 also shows that RBC AChE had variability for the control (or 15 minute group for the time course study) ranging from 14% to 30%. The pup variability ranged from 15 to 30% and the adult 14% to 26%. Brain AChE had variability ranging from 4.5% to 19%. The pup variability ranged from 7.2 to 13% and adult variability ranged from 4.5 to 19%. Thus there was not much difference between adults and pups to say that the pup data were less reliable than the adult data.

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