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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

July 5, 2006
TXR # 0053984

SUBJECT: DPX-X1179: Methomyl technical. Comparative Cholinesterase Activity Study in Rats

PC Code: 090301
DP Barcode: D324879

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I. CONCLUSIONS

The lowest-observed-adverse effect level (LOAEL) for young adults is 0.5 (M)/0.3 (F) mg/kg at 30 min post-dosing based on brain cholinesterase inhibition (12% in males and 14% in females) and increased erythrocyte cholinesterase inhibition (43% in males and 12% in females). A NOAEL was observed in male adults at 0.3mg/kg. A NOAEL was not observed for female adults.
The lowest-observed-adverse effect level (LOAEL) for 11 day old male/female pups is 0.1 mg/kg at 30 min post-dosing based on brain cholinesterase inhibition (12% in males and females) and erythrocyte cholinesterase inhibition (14% in male pups and 19% in female pups). A NOAEL was not observed in male/female pups.

II. ACTION REQUESTED

Conduct a benchmark dose analysis on comparative cholinesterase activity data.

III. BACKGROUND

IV. RESULTS/DISCUSSION

Subset 1 of the study provided peak ChE inhibition data on male/female pups dosed acutely with 0 or 0.3 mg/kg methomyl and measured at 30 min post-dosing. Male pups given a single 0.3 mg/kg oral gavage dose demonstrated the lowest RBC cholinesterase activity at 30 min post-dosing; females demonstrated the lowest RBC cholinesterase activity at 30 and 60 min post-dosing. RBC cholinesterase activities were similar to controls at 90 min post-dosing in males, and at 180 min in females. Male and female pups dosed with 0.3 mg/kg demonstrated the lowest brain cholinesterase activity at 30 min post-dosing. Brain cholinesterase activities were similar to controls at 90 min post-dosing in males, and at 60 min in females.

Subset 2 of the study provided dose response ChE activity data on male/female pups dosed acutely with 0, 0.1, 0.2, 0.3, or 0.4 mg/kg methomyl and measured at 30 or 240 min post-dosing. RBC and brain cholinesterase activities were decreased in a dose-dependent manner in pups 30 min after administration of a single oral gavage dose of methomyl. At 0.1 mg/kg and above in both sexes, RBC and brain cholinesterase were inhibited to similar levels.

Subset 3 of the study provided dose response at peak ChE inhibition and at recovery on male/female adults dosed acutely with 0, 0.3, 0.5, or 0.75 mg/kg methomyl and measured at 30 or 240 min post-dosing. At 30 min post-dosing, RBC cholinesterase in adult males was more sensitive to inhibition than brain cholinesterase, while in adult females, RBC and brain cholinesterase demonstrated similar levels of inhibition. However, it was stated that these apparent sex differences were likely the result of the marginal inhibition due to the low administered doses and the variability of the cholinesterase measurements. At 240 min post-dosing, RBC and brain cholinesterase activities were similar to controls in both sexes, again due to the inherent variability of cholinesterase measurements. Additionally, a previous study demonstrated complete recovery of cholinesterase activity within three hours of dosing with 3.0 mg/kg of the test compound.

Comparison of ChE activity between adults and pups shows baseline cholinesterase activities were age-dependent. RBC cholinesterase activities were higher in control pups than in control adults, while brain cholinesterase activities were lower in control pups than in control adults. The magnitude of inhibition was similar for RBC and brain cholinesterase activities in pups. In adults, RBC cholinesterase was inhibited to a greater extent than brain cholinesterase in males, while in females RBC and brain cholinesterase demonstrated similar levels of inhibition. Mean RBC cholinesterase inhibition was generally similar between pups dosed with 0.3-0.4 mg/kg and adults dosed with 0.3 and 0.5 mg/kg. Mean brain cholinesterase inhibition was slightly greater in pups dosed with 0.3-0.4 mg/kg than in adults dosed with 0.3 and 0.5 mg/kg, suggesting that pup brain cholinesterase is slightly more sensitive to inhibition than adult brain cholinesterase.

DATA EVALUATION RECORD

METHOMYL

Study Type: Non-guideline; Comparative Cholinesterase Activity Study in Rats

Work Assignment No 3-1-112 (MRID 46646401)

Prepared for
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Office of Pesticide Programs
U.S. Environmental Protection Agency
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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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DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; Comparative Cholinesterase Activity Study in Rats (gavage)

PC CODE: 090301 **DP BARCODE:** D324879
TXR#: 0053984

TEST MATERIAL (PURITY): Methomyl technical (98.08% a.i.; doses corrected for purity)

SYNONYMS: Methyl *N*-[[(methylamino)carbonyl]oxy]ethanimidothioate; DPX-X1179

CITATION: Malley, L.A. (2005) Methomyl (DPX-X1179 technical: comparison of cholinesterase activity in adult and pre-weanling rats. E.I. du Ponte de Nemours and Company, Haskell^{SM} Laboratory for Health and Environmental Sciences, Newark, DE. Laboratory Project ID: DuPont-15433, September 12, 2005. MRID 46646401. Unpublished

SPONSOR: E.I. du Ponte de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a non-guideline comparative cholinesterase activity study (MRID 46646401), a single dose of methomyl technical (Batch # DPX-X1179-512; 98.08% a.i.; doses corrected for purity) was administered in deionized water by oral gavage, in a dosing volume of 2 mL/kg, to three subsets of Sprague Dawley (Crl:CD®[SD]IGS BR) rats/sex/dose. The purpose of this study was to determine the relative sensitivity of pre-weanling pups and adult rats to inhibition and recovery of cholinesterase activity following the administration of a single oral dose of methomyl technical. In Subset 1, the time to peak cholinesterase activity inhibition and recovery in pre-weanling pups (5/sex/dose) was determined. Post-natal day (PND 11) pups were dosed with either 0 or 0.3 mg/kg of the test substance. Control pups were then killed at 60, 120, or 240 min post-dosing, while pups treated with 0.3 mg/kg were killed at 30, 60, 90, 120, 180, 240, or 360 min post-dosing. In Subset 2, the dose response of cholinesterase activity was measured in pre-weanling pups (10/sex/dose). PND 11 pups were dosed with 0, 0.1, 0.2, 0.3, or 0.4 mg/kg of the test substance and killed at the time of peak inhibition (30 min post-dosing). In Subset 3, the dose response at peak inhibition and at recovery was determined in young adult rats (10/sex/dose). The rats were dosed with 0, 0.3, 0.5, or 0.75 mg/kg of the test substance and killed at either 30 or 240 min post-dosing. At termination, blood and brain were collected from all animals for determination of red blood cell (RBC) and brain cholinesterase activities.

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In Subset 1, male pups given a single 0.3 mg/kg oral gavage dose demonstrated the lowest RBC cholinesterase activity (1968 ± 346 U/L) at 30 min post-dosing; females demonstrated the greatest decrease in RBC cholinesterase activity at both 30 (36% inhibition) and 60 (38% inhibition) min post-dosing. RBC cholinesterase activities were similar to controls at 90 min post-dosing in males, and at 180 min in females. Male and female pups dosed with 0.3 mg/kg demonstrated the lowest brain cholinesterase activity (M: 4.5 ± 0.6 U/g, F: 4.7 ± 0.7 U/g) at 30 min post-dosing. Brain cholinesterase activities were similar to controls at 90 min post-dosing in males, and at 60 min in females. In Subset 2, at 0.1 mg/kg and above, RBC (14-49% inhibition) and brain (12-42% inhibition) cholinesterase activities were decreased at 30 min post-dosing in a dose-dependent manner and by similar magnitudes in pups.

At 30 min post-dosing in Subset 3, RBC cholinesterase in adult males was more sensitive to inhibition than brain cholinesterase [41% (RBC) vs. 19% (Brain)], while in adult females, RBC and brain cholinesterase demonstrated similar levels of inhibition [25% (RBC) vs. 29% (Brain)]. However, it was stated that these apparent sex differences were likely the result of the marginal inhibition due to the low administered doses and the variability of the cholinesterase measurements. At 240 min post-dosing, RBC and brain cholinesterase activities were considered to be similar to controls in both sexes, again due to the inherent variability of cholinesterase measurements. Additionally, a previous study demonstrated complete recovery of cholinesterase activity within three hours of dosing with 3.0 mg/kg of the test compound.

When pre-weanling pup and adult cholinesterase activities were compared, baseline activities were found to be age-dependent, with RBC cholinesterase activities higher in control pups than in control adults (pups:2764-3473 U/L, adults: 1890-2230 U/L), while brain cholinesterase activities were lower in control pups than in control adults (pups:5.3-6.3 U/g, adults: 9.5-10.2 U/g),. The magnitude of inhibition was similar for RBC and brain cholinesterase activities in pups, while in adults, RBC cholinesterase was inhibited to a greater extent than brain cholinesterase in males. In adult females, RBC and brain cholinesterase demonstrated similar levels of inhibition. Mean RBC cholinesterase inhibition was generally similar between pups dosed with 0.3-0.4 mg/kg and adults dosed with 0.3 and 0.5 mg/kg. Mean brain cholinesterase inhibition was slightly greater in pups dosed with 0.3-0.4 mg/kg than in adults dosed with 0.3 and 0.5 mg/kg, suggesting that pup brain cholinesterase is slightly more sensitive to inhibition than adult brain cholinesterase.

The lowest-observed-adverse effect level (LOAEL) for young adults is 0.5 (M)/0.3 (F) mg/kg at 30 min post-dosing based on brain cholinesterase inhibition (12% in males and 14% in females) and increased erythrocyte cholinesterase inhibition (43% in males and 12% in females). A NOAEL was observed in male adults at 0.3mg/kg. A NOAEL was not observed for female adults.

The lowest-observed-adverse effect level (LOAEL) for 11 day old male/female pups is 0.1 mg/kg at 30 min post-dosing based on brain cholinesterase inhibition (12% in males and females) and erythrocyte cholinesterase inhibition (14% in male pups and 19% in female pups). A NOAEL was not observed in male/female pups.

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

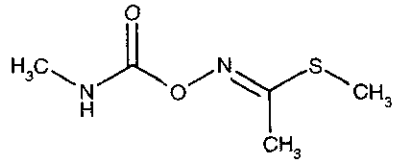
The study was included in the benchmark dose analysis of the N-methyl Carbamate cumulative risk assessment.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Methomyl technical

Description:	Off-white solid
Batch No.:	DPX-X1179-512
Purity:	98.08% a.i.
Stability of compound:	Stable in deionized water for up to 5 hours at room temperature
CAS #:	16752-77-5
Structure:	

2. Vehicle: Deionized (NanoPure®) water

3. Test animals

Species:	Rat
Strain:	Crl:CD®(SD)IGS BR
Age at initiation of treatment:	Post-natal day (PND) 11 – pups Approximately 42 days old – young adults
Source:	Charles River Laboratories, Inc., Raleigh, NC
Housing:	Lactating females and their litters were individually housed in shoe box cages. Young adults were housed individually in suspended stainless steel wire mesh cages.
Diet:	Pelleted Certified Rodent LabDiet® 5002 (PMI® Nutrition International, LLC, St. Louis, MO) , *ad libitum*
Water:	Filtered tap water, *ad libitum*
Environmental conditions	
Temperature:	18-26°C
Humidity:	30-70%
Air changes:	Not provided
Photoperiod:	12 h light/12 h dark
Acclimation period:	Approximately 4-6 days

B. STUDY DESIGN

1. In life dates: Start: 03/09/05 End: 03/17/05

2. **Purpose**: The purpose of this study was to determine the relative sensitivity of pre-weanling pups and adult rats to inhibition and recovery of cholinesterase activity following the administration of a single oral dose of methomyl technical.

3. **Animal assignment**: Animals were assigned to the subsets shown in Table 1. Lactating females and their litters were received on post-natal day (PND) 5. The day after arrival, the pre-weanling pups were cross-fostered among the dams so that each dam was assigned five pups/sex. Unassigned pups were discarded. Litters were then randomly assigned to the test groups. Young adult rats were randomly assigned, stratified by body weight, to the test groups so that there were no significant differences among group body weight means within a sex.

Table 1. Study design [a]

Dose (mg/kg)	Number/sex	Sacrifice time (min post-dosing)
Subset 1: Time to peak cholinesterase activity inhibition and recovery in pre-weanling (PND 11) pups		
0	5	60
		120
		240
0.3	5	30
		60
		90
		120
		180
		240
		360
Subset 2: Dose response of cholinesterase activity in pre-weanling (PND 11) pups		
0	10	30
0.1		
0.2		
0.3		
0.4		
Subset 3: Dose response at peak inhibition and at recovery for young adult rats		
0	10	30
0.3		
0.5		240
0.75		

a Data were obtained from pages 12-13 of the study report.

4. **Study design**: All animals were administered a single oral gavage dose of the test substance. In Subset 1, pre-weanling pups were given either 0 or 0.3 mg/kg of the test substance. After dosing, the pups were returned to their litters and killed at the times indicated in Table 1. In Subset 2, pre-weanling pups were dosed with the test substance at 0, 0.1, 0.2, 0.3, or 0.4 mg/kg, returned to their litters, and killed 30 min post-dosing. In Subset 3, young adult rats were dosed with the test substance at 0, 0.3, 0.5, or 0.75 mg/kg, and killed at either 30 or 240 min post-dosing.

5. **Dose-selection rationale:** The Sponsor stated that doses were selected based on the results of a previously conducted range-finding study in pre-weanling pups, and on the results of an acute oral neurotoxicity study and a reversibility study with carbamate insecticides in adult rats (data not provided). No further information was provided.

6. **Dosage preparation and analysis:** On the day of dosing, an appropriate amount of the test substance (corrected for purity) was dissolved in deionized (NanoPure®) water to achieve the desired concentration. Duplicate samples of each dose formulation were collected and analyzed on the first day of dosing for homogeneity (top, middle, bottom) and concentration. Additionally, stability of the test substance in deionized water was evaluated for up to five hours at room temperature.

   Results:

   Homogeneity (% CV): 0.0-0.6%

   Stability (% of nominal): 94.4-100%

   Concentration (% of nominal): 94.9-100%

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

7. **Dosage administration:** All doses were administered once by oral gavage at a dose volume of 2 mL/kg. The doses administered were based on the body weight of each animal on the day of dosing. All pups were dosed on PND 11; adult rats were approximately 42 days old at dosing.

8. **Statistics:** No statistical analysis beyond calculation of group means, standard deviations, and coefficients of variation were performed.

C. METHODS

1. **Clinical observations and body weights:** It was stated that cage-side clinical observations were performed at least once daily after arrival, young adult rats were weighed prior to group assignment, and all animals (pups and adults) were weighed on the day of dosing. However, these data were not provided.

2. **Sacrifice and sample collection:** Pre-weanling pups were anesthetized with an intraperitoneal injection of Beuthansia® solution, and blood was collected by cardiac puncture. Adult rats were euthanized by CO_2 asphyxiation, and blood was collected from the vena cava. Blood samples were held on wet ice, and generally were centrifuged and analyzed within 15 min of collection. Brains from all animals were removed immediately after blood

collection, weighed, snap frozen in liquid nitrogen, and stored at approximately −80°C until analysis.

On PND 11, the dams were killed by CO_2 asphyxiation, and unassigned pups were killed by injection of Beuthansia® solution. Following sample collection, all dams, pups, and adult rats were discarded without necropsy.

3. **Red blood cell (RBC) and brain cholinesterase assays:** RBC and plasma were separated by centrifugation, and the plasma was discarded. 50 µl RBC sample was then added to phosphate buffer containing 0.5% Triton X-100 to prepare a 1:20 RBC hemolysate. The sample was vortexed for 5-10 seconds. Blood samples were processed and analyzed within 15 minutes of collection. The RBC hemolysates were assayed using modified reagents (SOP CP124-P; Roche Reagent 124117) and analyzed for cholinesterase activity using a Roche Behringer-Mannheim AHitachi 717 automated chemistry analyzer. Sample blanks were run to correct for any interference.

   Thawed brain tissue was placed in 0.1M phosphate buffer and homogenized, and then diluted 3-fold with phosphate buffer containing 0.5% Triton X-100. The diluted brain homogenates were also analyzed for cholinesterase activity using the same automated chemistry analyzer and sample blanks.

4. **Calculations:** Sample blank values greater than 0 were subtracted from the measured cholinesterase results. Brain cholinesterase activities were calculated using the following formulae:

   (Tissue weight [g] x homogenate aliquot removed for dilution [mL] x 10^3) ÷ (volume buffer used for homogenizing [mL] x total volume of triton and homogenate aliquot [mL]) = g/L

   Analyzed amount of enzyme activity (U/L) ÷ amount of sample (g/L) = U/g

   Where appropriate, mean % inhibition values were calculated for RBC and brain cholinesterase using the following formula:

   (Mean control group cholinesterase activity – mean dose group cholinesterase activity) ÷ mean control group cholinesterase activity x 100

   Where appropriate, individual % inhibition values were calculated for RBC and brain cholinesterase using the following formula:

   (Mean control group cholinesterase activity – post-dose cholinesterase activity) ÷ mean control group cholinesterase activity x 100

   For Subset 1, the number of pups used was minimized by using fewer time points for control pups than for pups dosed with 0.3 mg/kg. Therefore, for the calculations described above, cholinesterase data from the treated pups were compared to the most appropriate values as listed below:

Sacrifice time for dosed pups (min)	Sacrifice time for control pups (min)
30	60
60	60
90	Mean of 60 and 120
120	120
180	Mean of 120 and 240
240	240
360	240

II. RESULTS

A. OBSERVATIONS: It was stated that clinical signs of toxicity and body weights were recorded but not included with this study. No other systemic observations were performed.

B. CHOLINESTERASE ASSAYS

1. Subset 1: Time to peak cholinesterase activity inhibition and recovery in pre-weanling (PND 11) pups: Male pups given a single 0.3 mg/kg oral gavage dose demonstrated the lowest RBC cholinesterase activity at 30 min post-dosing (36% inhibition); females demonstrated the lowest RBC cholinesterase activity at 30 and 60 min post-dosing (36% and 38% inhibition, respectively; Table 2a). RBC cholinesterase activities were similar to controls at 90 min post-dosing in males, and at 180 min in females.

Table 2a. Time course of mean (±SD) red blood cell cholinesterase activity (U/L) in pre-weanling (PND 11) pups given a single oral gavage dose of methomyl [a]

Time point (min)	Males (n = 5)		Females (n = 5)	
	0 mg/kg	0.3 mg/kg	0 mg/kg	0.3 mg/kg
30	ND	1968±346 (36)[b]	ND	1952±423 (36)[b]
60	3080±354	2292±164 (26)[b]	3068±586	1896±377 (38)[b]
90	ND	2648±382 (9)[c]	ND	2320±497 (22)[c]
120	2764±194	2680±279 (3)[d]	2864±366	2384±384 (17)[d]
180	ND	2576±389 (7)[e]	ND	3080±297[e]
240	2776±492	2404±412 (13)[f]	2912±468	2956±126[f]
360	ND	2676±408 (4)[f]	ND	3048±278[f]

a Data (n=5) were obtained from Tables 1 and 2 on pages 37-38 of the study report. Percent inhibition is included in parentheses.
b Compared to 60 min controls
c Compared to mean of 60 and 120 min controls
d Compared to 120 min controls
e Compared to mean of 120 and 240 min controls
f Compared to 240 min controls
ND Not determined

Male and female pups dosed with 0.3 mg/kg demonstrated the lowest brain cholinesterase activity at 30 min post-dosing (19% and 18% inhibition, respectively; Table 2b). Brain cholinesterase activities were similar to controls at 90 min post-dosing in males, and at 60 min in females.

Table 2b. Time course of mean (±SD) brain cholinesterase activity (U/g) in pre-weanling (PND 11) pups given a single oral gavage dose of methomyl [a]				
Time point (min)	Males (n = 5)		Females (n = 5)	
	0 mg/kg	0.3 mg/kg	0 mg/kg	0.3 mg/kg
30	ND	4.5±0.6 (19)[b]	ND	4.7±0.7 (18)[b]
60	5.6±0.5	4.8±0.5 (13)[b]	5.8±0.3	5.4±1.0 (7)[b]
90	ND	5.5±0.7 (8)[c]	ND	5.7±0.6 (5)[c]
120	6.3±0.4	5.9±0.3 (7)[d]	6.1±0.2	5.7±0.6 (6)[d]
180	ND	5.5±1.0 (8)[e]	ND	5.8±0.5[e]
240	5.7±0.6	5.8±0.1[f]	5.3±0.6	5.7±0.5[f]
360	ND	6.0±0.2[f]	ND	5.8±0.6[f]

a Data (n=5) were obtained from Tables 1 and 2 on pages 37-38 of the study report. Percent inhibition is included in parentheses.
b Compared to 60 min controls
c Compared to mean of 60 and 120 min controls
d Compared to 120 min controls
e Compared to mean of 120 and 240 min controls
f Compared to 240 min controls
ND Not determined

2. **Subset 2: Dose response of cholinesterase activity in pre-weanling (PND 11) pups:** RBC and brain cholinesterase activities were decreased in a dose-dependent manner in pups 30 min after administration of a single oral gavage dose of methomyl (Table 3). At 0.1 mg/kg and above in both sexes, RBC cholinesterase was inhibited 14-49%, while brain cholinesterase was inhibited 12-42%.

Table 3. Dose response of mean (±SD) cholinesterase activity in pre-weanling (PND 11) pups 30 min after administration of a single oral gavage dose of methomyl [a]					
Parameter	Dose (mg/kg)				
	0	0.1	0.2	0.3	0.4
Males (n = 10)					
Red blood cells	3060±709	2636±463 (14)	2478±661 (19)	1848±521 (40)	1574±389 (49)
Brain	5.7±0.4	5.0±0.6 (12)	4.2±0.7 (26)	3.6±0.5 (36)	3.3±0.5 (41)
Females (n = 10; RBC control n = 9)					
Red blood cells	3473±333	2802±550 (19)	2234±531 (36)	2000±824 (42)	1768±445 (49)
Brain	5.5±0.3	4.9±0.7 (12)	4.4±0.5 (20)	3.7±0.9 (33)	3.2±0.6 (42)

a Data (n=9-10) were obtained from Tables 3 and 4 on pages 39-40 of the study report. Percent inhibition is included in parentheses.

3. **Subset 3: Dose response at peak inhibition and at recovery for young adult rats:** At 30 min post-dosing (Table 4a), RBC cholinesterase (19-43% inhibition) in adult males was more sensitive to inhibition than brain cholinesterase (2-19% inhibition), while in adult females, RBC and brain cholinesterase demonstrated similar levels of inhibition (12-29% inhibition). However, it was stated that these apparent sex differences were likely the result of the marginal inhibition due to the low administered doses and the variability of the cholinesterase measurements. Additionally, a previous study demonstrated complete recovery of cholinesterase activity within three hours of dosing with 3.0 mg/kg of the test compound. At

240 min post-dosing (Table 4b), RBC and brain cholinesterase activities were similar to controls in both sexes.

Table 4a. Dose response of mean (±SD) cholinesterase activity in young adult rats 30 min after administration of a single oral gavage dose of methomyl [a]

Parameter	Dose (mg/kg)			
	0	0.3	0.5	0.75
Males (n = 10)				
Red blood cells	2230±506	1806±422 (19)	1272±453 (43)	1326±546 (41)
Brain	9.5±0.9	9.3±0.8 (2)	8.4±0.8 (12)	7.7±0.8 (19)
Females (n = 10)				
Red blood cells	1942±476	1712±460 (12)	1526±799 (21)	1456±452 (25)
Brain	10.2±0.5	8.7±0.9 (14)	7.9±0.6 (23)	7.2±1.1 (29)

a Data (n=10) were obtained from Tables 5 and 6 on pages 41-42 of the study report. Percent inhibition is included in parentheses.

Table 4b. Dose response of mean (±SD) cholinesterase activity in young adult rats 240 min after administration of a single oral gavage dose of methomyl [a]

Parameter	Dose (mg/kg)			
	0	0.3	0.5	0.75
Males (n = 10)				
Red blood cells	2156±488	2068±726 (4)	1480±349 (31)	1700±489 (21)
Brain	10.0±0.6	10.0±0.4	9.5±0.9 (6)	10.1±0.7
Females (n = 10)				
Red blood cells	1890±551	2426±396	1710±1123 (10)	1486±373 (21)
Brain	9.6±0.9	9.7±0.6	10.0±0.8	9.9±0.8

a Data (n=10) were obtained from Tables 5 and 6 on pages 41-42 of the study report. Percent inhibition is included in parentheses.

4. **Comparison of cholinesterase activities between pups and adults:** It was stated that baseline cholinesterase activities were age-dependent. RBC cholinesterase activities were higher in control pups (M: 3060 ± 709, F: 3473 ± 333) than in control adults (M: 2230 ± 506, F: 1942 ± 488), while brain cholinesterase activities were lower in control pups (M: 5.7 ± 0.4, F: 5.5 ± 0.3) than in control adults (M: 9.5 ± 0.9, F: 10.2 ± 0.5). The magnitude of inhibition was similar for RBC and brain cholinesterase activities in pups. In adults, RBC cholinesterase was inhibited to a greater extent than brain cholinesterase in males, while in females RBC and brain cholinesterase demonstrated similar levels of inhibition. Mean RBC cholinesterase inhibition was generally similar between pups dosed with 0.3-0.4 mg/kg and adults dosed with 0.3 and 0.5 mg/kg. Mean brain cholinesterase inhibition was slightly greater in pups dosed with 0.3-0.4 mg/kg than in adults dosed with 0.3 and 0.5 mg/kg, suggesting that pup brain cholinesterase is slightly more sensitive to inhibition than adult brain cholinesterase (data not submitted).

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATOR'S CONCLUSIONS:** In control pups, RBC cholinesterase activities were higher and brain cholinesterase activities were lower compared to control adults. The greatest degree of cholinesterase inhibition in pre-weanling pups dosed with 0.3 mg/kg generally occurred at 30 min post-dosing; therefore, 30 min was chosen as the sampling time for peak effects in the dose-response subsets. RBC and brain cholinesterase activities were decreased in pre-weanling pups at 30 post-dosing, and returned to baseline by 240 min post-dosing. The magnitude of inhibition was similar for RBC and brain cholinesterase in pups. RBC and brain cholinesterase activities were only marginally affected in adults at 30 min post-dosing, and returned to baseline by 240 min. RBC cholinesterase was inhibited to a greater extent than brain cholinesterase in adult males, but to a lesser extent in adult females. However, these apparent sex differences in adults were likely due to the marginal inhibition present at the low doses administered, and to the variability in cholinesterase activity measurements. The difference in RBC cholinesterase inhibition between pups and adults was considered minimal due to the inherent variability in these measurements. Brain cholinesterase was slightly more sensitive to inhibition in pups than adults at high doses.

B. **EPA COMMENTS:** Subset 1 of the study provided peak ChE inhibition data on male/female pups dosed acutely with 0 or 0.3 mg/kg methomyl and measured at 30 min post-dosing. Male pups given a single 0.3 mg/kg oral gavage dose demonstrated the lowest RBC cholinesterase activity at 30 min post-dosing; females demonstrated the lowest RBC cholinesterase activity at 30 and 60 min post-dosing. RBC cholinesterase activities were similar to controls at 90 min post-dosing in males, and at 180 min in females. Male and female pups dosed with 0.3 mg/kg demonstrated the lowest brain cholinesterase activity at 30 min post-dosing. Brain cholinesterase activities were similar to controls at 90 min post-dosing in males, and at 60 min in females.

Subset 2 of the study provided dose response ChE activity data on male/female pups dosed acutely with 0, 0.1, 0.2, 0.3, or 0.4 mg/kg methomyl and measured at 30 or 240 min post-dosing. RBC and brain cholinesterase activities were decreased in a dose-dependent manner in pups 30 min after administration of a single oral gavage dose of methomyl. At 0.1 mg/kg and above in both sexes, RBC and brain cholinesterase were inhibited to similar levels.

Subset 3 of the study provided dose response at peak ChE inhibition and at recovery on male/female adults dosed acutely with 0, 0.3, 0.5, or 0.75 mg/kg methomyl and measured at 30 or 240 min post-dosing. At 30 min post-dosing, RBC cholinesterase in adult males was more sensitive to inhibition than brain cholinesterase, while in adult females, RBC and brain cholinesterase demonstrated similar levels of inhibition. At 240 min post-dosing, RBC and brain cholinesterase activities were similar to controls in both sexes, again due to the inherent variability of cholinesterase measurements. Additionally, a previous study demonstrated complete recovery of cholinesterase activity within three hours of dosing with 3.0 mg/kg of the test compound.

Comparison of ChE activity between adults and pups shows baseline cholinesterase activities were age-dependent. RBC cholinesterase activities were higher in control pups than in control adults, while brain cholinesterase activities were lower in control pups than in control adults. The magnitude of inhibition was similar for RBC and brain cholinesterase activities in pups. In adults, RBC cholinesterase was inhibited to a greater extent than brain cholinesterase in males, while in females RBC and brain cholinesterase demonstrated similar levels of inhibition. Mean RBC cholinesterase inhibition was generally similar between pups dosed with 0.3-0.4 mg/kg and adults dosed with 0.3 and 0.5 mg/kg. Mean brain cholinesterase inhibition was slightly greater in pups dosed with 0.3-0.4 mg/kg than in adults dosed with 0.3 and 0.5 mg/kg, suggesting that pup brain cholinesterase is slightly more sensitive to inhibition than adult brain cholinesterase.

The lowest-observed-adverse effect level (LOAEL) for young adults is 0.5 (M)/0.3 (F) mg/kg at 30 min post-dosing based on brain cholinesterase inhibition (12% in males and 14% in females) and increased erythrocyte cholinesterase inhibition (43% in males and 12% in females). A NOAEL was observed in male adults at 0.3mg/kg. A NOAEL was not observed for female adults.
The lowest-observed-adverse effect level (LOAEL) for 11 day old male/female pups is 0.1 mg/kg at 30 min post-dosing based on brain cholinesterase inhibition (12% in males and females) and erythrocyte cholinesterase inhibition (14% in male pups and 19% in female pups). A NOAEL was not observed in male/female pups.

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

The study was included in the benchmark dose analysis of the N-methyl Carbamate cumulative risk assessment.

C. **STUDY DEFICIENCIES:** The following minor deficiencies were noted but do not alter the conclusions of this review:

- Pups and adults should have been administered identical doses to facilitate comparisons of inhibition and recovery of cholinesterase activity.
- No statistical analysis performed beyond calculation of group means, standard deviations, and coefficients of variation.
- Exact method for measurement of comparative cholinesterase inhibition should be provided.

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Chemical: 3-(2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethyl)glutarimide

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