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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

June 13, 2006
TXR # 0053063

SUBJECT: Data Evaluation Record (DER) of comparative cholinesterase inhibition data in neonatal and adult rats (MRID nos. 46688912, 46688913, 46688914) following exposure to Carbofuran

PC Code: 090601
DP Barcode: D326245

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I. CONCLUSIONS

This memorandum transmits the evaluation of the definitive comparative cholinesterase study with carbofuran (MRID no. 46688914). In this study, adult and postnatal day (PND) 11 rats were given a single dose of carbofuran via gavage at the following doses: 0.3, 0.6, or 1.0 mg/kg. HED concluded that the adult and PND 11 LOAEL for cholinesterase inhibition in the brain is 0.3 mg/kg (the lowest dose tested). The adult and PND 11 LOAEL for clinical signs (tremors) was also 0.3 mg/kg. Thus, there was no NOAEL in this study. It was further determined that the red blood cell (RBC) cholinesterase results were not acceptable.

JUN 21 2006

EPA Reviewer: Kathleen Raffaele, Ph.D. Signature
Toxicology Branch, Health Effects Division (7509C) Date
EPA Secondary Reviewer: Louis Scarano, Ph.D. Signature
Toxicology Branch, Health Effects Division (7509C) Date

TXR#: 0053063

DATA EVALUATION RECORD

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

PC CODE: 090601

DP BARCODE: D326245
SUBMISSION NO.: none

TEST MATERIAL (PURITY): Carbofuran technical (99% a.i.)

SYNONYMS: 2,3-dihydro-2,2-dimethyl-benzofuran-7-yl methylcarbamate

CITATIONS: Tyl, R.W. (2005) Acute dose-response study of carbofuran technical administered by gavage to adult and postnatal day 11 male and female CD®(Sprague-Dawley) rats. RTI International, Center for Life Sciences and Toxicology, RTP, NC, RTI Identification No. 65C-09728.000.005, FMC Study No. A2005-5981. November 7, 2005. MRID 46688914. Unpublished.

Tyl, R.W. (2005) Acute time-course study of carbofuran technical administered by gavage to adult and postnatal day 11 male and female CD®(Sprague-Dawley) rats. RTI International, Center for Life Sciences and Toxicology, RTP, NC, RTI Identification No. 65C-09728.000.004, FMC Study No. A2005-5982. November 7, 2005. MRID 46688913. Unpublished.

Tyl, R.W. (2005) Acute range-finding study of carbofuran technical (CAS No. 1563-66-2) administered by gavage to postnatal day 11 male and female CD®(Sprague-Dawley) rat pups. RTI International, Center for Life Sciences and Toxicology, RTP, NC, RTI Identification No. 65C-09728.000.003, FMC Study No. A2005-5983. September 30, 2005. MRID 46688912. Unpublished.

SPONSOR: FMC Corporation

EXECUTIVE SUMMARY: In a series of special comparative cholinesterase inhibition (ChEI) studies, Carbofuran technical (99% a.i., LOT No. PL04-0056) was administered by gavage to groups of Crl:CD®(SD) rats. In the range-finding study (MRID 46688912), groups of 3 postnatal day (PND) 11 rats/sex were given single oral doses of 0.3, 0.6, or 1.0 mg/kg. Clinical signs were evaluated at 15, 30, 60, 90, 240, and 360 min post-dosing; animals were sacrificed at 360 min post-dosing and cholinesterase activity (brain and red blood cell [RBC]) was evaluated. In the time-course evaluation study (MRID 46688913), 5 rats/sex/time point, aged PND11 and PND 60, were administered a single oral dose of 0.6 mg/kg. Clinical signs were evaluated at 15, 30, 60, 90, 120, 240, and 360 min post-dosing. Five animals/sex/age of animals was sacrificed at each time point (controls were sacrificed at 0 and 360 minutes only) and cholinesterase activity (brain and RBC) was assessed. In the main study (MRID

46688914), 10 rats, aged PND11 or PND 60, per sex/time point were administered a single oral dose of 0, 0.3, 0.6, or 1.0 mg/kg. Clinical signs were evaluated at 0, 15, 30, 60, 120, 240, and 720 minutes post-dosing. Animals were sacrificed at 15 min or 720 min post-dosing, and cholinesterase activity (brain and RBC) was assessed.

Based on the analytical data for the dosing formulations, the mixing procedure was adequate and the difference between nominal and actual dosage to the study animals was acceptable. No information was provided regarding the mixing procedures used during dosing to ensure that homogeneity was maintained during dose administration.

With the exception of one mis-dosed PND11 female, all animals survived to scheduled sacrifice. Treatment-related clinical signs, most notably tremors, were observed in PND11 pups at all doses in all studies, starting as early as 2 minutes post-dosing and persisting for up to 120 min. Tremors were also noted in adults, at lower incidence rates, in a dose-related manner. In adults, the observation was noted as early as 7 min post-dosing, persisting up to 60 min. There were no treatment-related differences in body weight or brain weight during the observation period (up to 720 min post-dosing) for either age group.

No inhibition in RBC cholinesterase was observed in any group, at any time point. These data were quite variable; for example, there were substantial differences in control values at 15 and 360 minutes post-dosing in the time-course study (MRID 46688913). Since no inhibition was seen, time of peak effect and relative sensitivity could not be determined for RBC cholinesterase. Plasma cholinesterase activity was not evaluated.

There was a dose-related decrease in brain cholinesterase activity at all doses in both age groups. Peak inhibition occurred at the earliest time point measured (15 min in the time-course study). Substantial recovery had occurred by 720 min post-dosing in the main study (all groups were within 10% of control values). At 15 min post-dosing, inhibition in pups was considerably larger than that seen in adults at the same dose: at 0.3 mg/kg, brain cholinesterase activity in PND11 pups was 52% of control levels; for adults at the same dose activity was 74-76% of control levels.

For acute exposures:

the adult and PND 11 LOAEL for brain ChEI is 0.3 mg/kg (both sexes)
the adult and PND 11 NOAEL for brain ChEI is <0.3 mg/kg (both sexes);

the adult and PND 11 LOAEL for red blood cell ChEI is >1.0 mg/kg (both sexes)
the adult and PND 11 NOAEL for red blood cell ChEI is 1.0 mg/kg (both sexes);

For acute exposure, the overall adult and PND11 LOAEL for cholinesterase inhibition in rats is 0.3 mg/kg based on enzyme inhibition in brain; the NOAEL is <0.3 mg/kg.
For acute exposure, the overall adult and offspring LOAEL for clinical signs in rats is 0.3 mg/kg based on tremors; the NOAEL is <0.3 mg/kg.

Taken together these studies are classified **Acceptable/Nonguideline** for the determination of brain cholinesterase activities following treatment with a single dose of carbofuran technical in adult and PND11 rats, pending receipt of additional requested information (see deficiency section). They are **Unacceptable/Nonguideline** for the determination of RBC cholinesterase activity, as no effects were seen on that measure at any dose.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Carbofuran

Description:	Off-white solid with a mildly phenolic odor
Lot/Batch #:	LOT No. PL04-0056
Purity:	99 % a.i. (Certificate of analysis not provided)
Compound Stability:	Not provided
CAS # of TGAI:	1563-66-2

2. Vehicle and/or positive control: Corn oil (CAS No. 8001-30-7, supplier not specified) was used as the vehicle in all studies. No positive control was used.

3. Test animals (P):

Species:	Rat	
Strain:	Crl:CD®(Sprague Dawley)	
Age and wt. at study initiation:	**MRID 46688912**: lactating females with litters, received on PND3; pups were 'reconstituted litters' with 5 M/5F per litter. Body weight for females was not provided; Body weight for pups at dosing (on PND11) ranged from 24-33 g for females, 23-33 g for males ***MRID 4668913***: *lactating females with 'reconstituted' litters* (as above), received on PND4; pup body weight range at dosing (on PND11) 23-33g for females, 23-38g for males Young adult rats approximately 53 days of age, body weight range at dosing (60 days of age) was 204-233g for females, 278-318g for males **MRID 4668914**: lactating females with 'reconstituted' litters (as above), received on PND4; pup body weight range at dosing (on PND11) 22-33 g for males, 20-33 g for females Young adult rats approximately 60 days old at dosing; body weight range 275-324 for males; 194-237 for females	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Young adults (singly housed) and females with litters in solid-bottom polycarbonate cages (8x19x10.5 inches) with stainless wire lids and Sani-Chip7 cage litter	
Diet:	Certified Rodent Diet7 #5002, PMI Nutrition International, *ad libitum*	
Water:	Tap water (City of Durham, Department of Water Resources, Durham, NC) *ad libitum* by polycarbonate or polypropylene bottles with butyl rubber stoppers and stainless steel sipper tubes	
Environmental conditions:	**Temperature:**	64-79EF (nominal)
	Humidity:	30-70% (nominal)
	Air changes:	10/hour
	Photoperiod:	12 hrs light/dark
Acclimation period:	At least 7 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates: MRID 46688912: Start: August 10, 2005; End: August 18, 2005
MRID 46688913: Start: August 15, 2005; End: August 25, 2005

MRID 46688914: Start: August 30, 2005; End: September 15, 2005

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

TABLE 1. Study design for cholinesterase inhibition studies

MRID	Dose(s) (mg/kg/day)	Sex/ No. of animals/ sex/ dose	Treatment and termination
46688912	0.3, 0.6, 1.0	M&F/3	Single oral dose to pups; clinical observations at 15, 30, 60, 90, 120, 240, 360 min post-dosing; cholinesterase activity (brain and RBC) evaluated at 360 min post-dosing (acute range-finding PND11 rats)
46688913	0, 0.6	M&F/5	Single oral dose on PND 11 or to young adults; five rats/sex/group terminated at 0, 15, 30, 60, 90, 120, 240, 360 min post-dosing, except that controls were evaluated at 0 and 360 minutes only (time course evaluation – neonatal and young adult rats)
46688914	0, 0.3, 0.6, 1.0	M&F/10	Single oral dose on PND 11 or PND60; sacrifice 15 or 720 min post-dose; clinical observations at 0, 15, 30, 60, 120, 240, and 720 min post-dose (definitive acute toxicity study - pre-weaning and adult rats)

PND = postnatal day.
GD = gestation day.

3. Mating procedure: For all three studies, dams with 'reconstituted litters' were ordered from suppliers to arrive on PND3 (2 dams with litters; MRID 46688912) or PND4 (10 dams with litters, MRID 46688913; 18 dams with litters, MRID 46688914). Studies state that the 'reconstituted litters' consisted of 5M and 5F pups/dam, taken from 'the pool of available pups', but no information was provided regarding the possibility that unequal proportions of siblings might be included in various litters. If some test groups included a greater proportion of siblings than others (or if siblings were included in the same dose groups) variability and reliability of the data could be impacted. Registrant should provide additional information about this procedure.

4. Animal assignment: For MRID 46688912, pup assignment to doses was 'arbitrary': the first 3/sex from each litter were assigned one/dose group; the last two from the first litter were assigned to the low and mid dose groups, the 4th from the second litter were assigned to the high dose group, and the last from the second litter was not assigned. For MRID 46688913, the study report states that five PND11 pups/sex and five young adults/sex were randomly distributed into 9 groups (no other information was provided). For MRID 46688914, the study report states that "10 PND11 pups/sex were arbitrarily distributed and 10 young adults/sex were randomly distributed into 8 groups." (p. 12). No information was provided regarding whether these allocations controlled for litter of origin, and no definition of 'arbitrary' was provided. Failure to randomly distribute subjects among treatment groups could adversely affect the validity of the study. Additional information about this procedure should be provided.

5. Dose selection rationale: No information was provided regarding dose selection rationale for MRID 46688912 (the dose-range finding study). Dose levels for MRIDs 46688913 and 46688914 were selected on the basis of MRID 46688912. Sampling times for 46688914 were based on the results of the time-of-peak effect study in MRID 46688913.

6. Dosage administration: All doses were administered to the adult males and females and PND11 pups in the groups shown in Table 1, suspended in corn oil, by oral gavage at a volume of 5 mL/kg body weight. (NOTE: The dose-range finding study had no controls and the time-course study had a "control group" that apparently were not gavaged at all.)

7. Dosage preparation and analysis: Stock dosing solutions were prepared by suspending test material in corn oil at a concentration that would result in a dosing volume of 5 ml/kg. Homogeneity was achieved by stirring with a magnetic stir bar for at least 15 minutes and then subjecting the resulting suspension to ultrasonication, until a uniform suspension was achieved (15 minutes). No information was provided regarding the stability of carborfuran during ultrasonication. Dose formulations were stored at room temperature. No information was provided regarding the procedure used to maintain homogeneity during dose administration. Concentration was analyzed using HPLC with UV detection at 280 nm, with no internal standard.

   Homogeneity was evaluated by collecting 25 ml aliquots from the top, middle, and bottom of the beaker during preparation of the formulation. Formulations were then transferred to amber bottles, where they remained for the duration of the stability studies. Stability following room temperature storage was evaluated at 0, 3, 7, 14, and 21 days.

   Results of homogeneity studies indicated that concentration for the low dose formulation ranged from 0.0604-0.0615 mg/ml (101-103% of nominal). At the high dose, concentration ranged from 0. 196-0.199 mg/ml (98-99.5% of nominal). Results of stability studies indicated that carbofuran was stable in the formulation at room temperature for up to 21 days, with concentrations between 97.6-101% of day 0 for all evaluation times at the low dose (0.06 mg/ml), and between 98.6 and 100% of day 0 for all evaluation times at the high dose (0.20 mg/ml)

   Results - Absence of test material in the vehicle was confirmed in MRID 46688914 only.

   MRID 46688912: Concentrations of samples from all dosage formulations prepared on 8/16/05 (nominal concentration 0.06, 0.12, and 0.20 mg/ml) ranged from 100-105% of the nominal concentration.

   MRID 46688913: Concentrations of samples taken from the single dosage formulation prepared on 8/18/05 (nominal concentration 0.12 mg/ml) was 102-103% of the nominal concentration.

   MRID 46688914: Concentrations of samples from all dosage groups (nominal concentrations of 0.06, 0.12, or 0.20 mg/mL), ranged from 97.5-101% of the nominal concentrations.

The results of these studies indicate that concentration stability and homogeneity in the formulations was adequately demonstrated, and concentrations of the dosage formulations were within the acceptable range. Additional information regarding the procedure used to maintain homogeneity during dose adminstration should be provided.

C. OBSERVATIONS:

1. **In-life observations:**

   a. **PND 11 and young adult animals:** In the range-finding and time of peak effect studies (MRIDs 46688912 and 46688913), clinical observations of pups and/or adults were recorded prior to

dosing and at approximately 15, 30, 60, 90, 120, 240, and 360 min post-dosing. In the main study (MRID 46688914) clinical observations were made at approximately 15, 30, 60, 90, 120, 240, and 720 min post-dosing. Observations included:

- Any response with respect to body position, activity, coordination or gait
- Any unusual behavior such as head flicking, compulsive biting or licking, circling, etc.
- The presence of convulsions or tremors, pintpoint pupils, increased salivation, increased lacrimation, increased or decreased urination or defecation (including diarrhea), piloerection, unusual respiration (fast, slow, gasping), unusual vocalization.

[From study reports, p. 12 or 13)

There was no indication in the study report that observed behaviors were ranked or scored in any way, nor was there any indication that observers were unaware of animals' treatment groups. There was also no indication in the study report as to whether animals were removed from their cages for observation. Statements in the discussion sections of MRIDs 46688913 and 46688912 that the technicians observed the pups 'unobtrusively' and 'with the least intrusion possible,' along with statements that salivation, lacrimation, urination, and defecation were not observed due to interactions between pups and dams, would indicate that pups were observed while in their home cages with their dams, although there was no specific statement to that effect. This information about the observation procedures should be clarified.

Body weight was recorded prior to dosing and at sacrifice, on the day of exposure.

2. Termination schedule and sample collection: Adults and pups were terminated according to the schedule shown in Table 1. All animals were killed by carbon dioxide asphyxiation. In all studies, whole blood samples were collected via cardiac puncture using a 1 ml glass or plastic syringe (22 gauge needle); syringes were pre-coated with EDTA. Blood (at least 200 µl per animal) was transferred into a 500 µl Microtainer (Becton Dickinson) collection tube (also pre-coated with EDTA and chilled in ice. Brain was removed, weighed, and stored on dry ice. Samples were transported to RTI Clinical Pathology Laboratory, where they were immediately processed (including separation of blood into RBC and plasma fractions) and then frozen at –70°C until cholinesterase assays were performed.

3. Cholinesterase determination: Cholinesterase assays were performed on all RBC and brain samples using a modified Ellman method. Assays were performed using a COBAS MIRA® Plus CC clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Absorbance was measured at 405 nm and 37°C. Detailed information on the sample processing and analysis is provided in the study reports (e.g., MRID 46688913, pp. 14-15). Plasma samples were stored but not analyzed.

4. Necropsy procedures: No information was provided regarding necropsy procedures.

D. DATA ANALYSIS: Statistical analyses were similar for all studies, using Levene's Test for homogeneity of variances, Wald Chi-Square Test and individual t-tests for data sets lacking homogeneity of variance, and SAS GLM procedures for Analysis of Variance (ANOVA) for data sets with homogeneous variance. Pairwise comparisons were performed using Dunnett's test. A detailed description of statistical procedures is provided on p. 14 for MRID 46688912, pp. 15-16 for MRID 46688913, and p. 15 for MRID 46688914.

II. RESULTS:

A. **MORTALITY AND CLINICAL OBSERVATIONS**: In all three studies, all adult and PND11 animals survived to scheduled sacrifice, with the exception of one PND11 female dosed with 0.6 mg/kg in the main study (MRID 46688914), removed due to a dosing error (misdirected dose), resulting in only nine PND11 females available for the 720 min sacrifice time. In the same study, one PND11 male noted to be moribund at 14 min post-dosing was sacrificed on schedule at 15 min post-dosing. Due to the lack of a fifth male in one litter at 0 mg/kg, only nine PND11 males were available for the 720 min time point.

Tremors were seen in both pups and adults, but occurred at lower doses and/or higher incidence rates in pups than adults. Due to limitations in reporting, no information on severity was available. In addition, since pups were apparently observed in their home cages, it is unclear how reliable the pup incidence data are, especially in that in some studies the number of incidences/pup appears to be lower in animals sacrificed later (e.g., in MRID 46688913, tremors were observed only 6 times in females observed for up to 120 minutes, but were observed 15 times in females observed for only 30 minutes; in both cases, all 5 females had at least one observation of tremors). It is notable that tremors in pups were often noted during or following handling (e.g. in the weighing pan), reinforcing concerns regarding the adequacy of these observations (if they were conducted cage-side). A summary of the reported incidence data is provided in Tables 2 and 3, for pups and adults, respectively. Since tremors were never observed in control groups, no control data are included in the table.

There was a discrepancy in the number of individuals observed for clinical signs in the main study (MRID 46688914): although 10 females were assigned to study, results for 11 were reported at the 15 min time point (we noted that one animal [number 51, p. 88-89] was included in the report as both male and female). At the 720 min time point, no clinical observations were reported for female No. 24; cholinesterase activity data for this animal were included in the study report. The registrant should be asked to explain these discrepancies.

Table 2. Incidence of tremors in pups, separately by study							
Dose (mg/kg)	Time of Sacrifice (minutes)	Number of animals with tremors		Number of observations		Time of observations (minutes after dosing)	
		M	F	M	F	M	F
MRID 46688912, n=3/sex/group							
0.3	360	3	3	5	3	2-42	8-21
0.6	360	2	2	3	3	8-30	7-21
1.0	360	3	3	11	7	2-54	8-60
MRID 46688913, n=5/sex/group							
0.6	15	5	5	10	10	7-16	8-15
0.6	30	5	5	15	15	6-30	6-30
0.6	60	5	5	16	12	9-60	8-60
0.6	90	5	5	16	16	4-90	6-90
0.6	120	5	5	12	6	7-118	7-119
0.6	240	5	5	11	13	7-60	6-90
0.6	360	5	5	16	10	5-60	13-60
MRID 46688914, n=10/sex/group (except 0.6 mg/kg females; n=11 for 15 min. sacrifice, 8 for 720 min sacrifice							
0.3	15	10	10	19	17	8-15	5-15
0.3	720	10	10	27	17	5-30	7-30
0.6	15	10	11	18	22	6-15	5-15
0.6	720	10	8	27	15	6-60	7-60
1.0	15	10	10	21	21	6-15	6-15
1.0	720	10	10	39	34	5-120	6-120

Table 3. Incidence of tremors in adults, separately by study							
Dose (mg/kg)	Time of Sacrifice (minutes)	Number of animals with tremors		Number of observations		Time of observations (minutes after dosing)	
		M	F	M	F	M	F
MRID 46688913, n=5/sex/group							
0.6	15	2	3	2	4	14	7-15
0.6	30	5	4	14	7	8-30	6-30
0.6	60	4	5	6	6	11-30	15-30
0.6	90	5	5	16	15	8-60	8-60
0.6	120	5	2	12	4	10-60	13-38
0.6	240	5	4	13	6	11-30	10-30
0.6	360	5	3	7	3	15-30	15-18
MRID 46688914, n=10/sex/group							
0.3	15	0	0	0	0	-	-
0.3	720	3	1	5	1	10-60	15
0.6	15	1	2	1	4	15	8-15
0.6	720	8	4	16	8	10-60	9-30
1.0	15	7	7	9	11	8-15	6-15
1.0	720	10	10	25	20	7-60	7-60

B. **BODY WEIGHT:** Body weight was measured at the time of dosing and at the time of sacrifice. For MRID 46688912, there were no differences in body weight among treatment groups or between measurement times. For MRID 46688913, there were statistically significant differences in pup body weight among treatment groups at the time of dosing, with mean weights ranging from 27.3 to 36.15 g for male pups, and from 25.6 to 30.9 g for female pups. These differences were mirrored in the body weights at sacrifice, with no apparent effect of dosing on body weight within a treatment group. For MRID 46688914, there were again statistically significant differences in pup body weight at dosing, for males only. Pre-dosing body weights ranged from 24.4 to 27.5 g for male pups, 24.7 to 28.0 g for female pups; as for MRID46688913, these differences were mirrored post-dosing, with no obvious treatment-related effect. For adult body weights, there were no differences among treatment groups for either study, either pre- or post-dosing.

It is unlikely that the differences in pup body weight had any impact on the cholinesterase measures in this study. Administered doses were calculated based on body weights, so it is possible, if response was not proportional to body weight, that those pups with higher body weights might show more inhibition than those with lower weights, increasing the variability of the data.

C. **BRAIN WEIGHT:** For MRID 46688912, there were no statistically significant or biologically significant differences in brain weights among treatment groups. For males, mean brain weight (±s.e.m.) ranged from 0.9526±0.0730 to 1.0516±0.0254 g; for females, the range was from 0.8726±0.0365 to 0.9629±0.0454 g.

For MRID 46688913, there were several statistically significant differences in brain weight among the treatment times, but none were considered biologically significant. For male pups, mean brain weight (±s.e.m.) ranged from 0.9354±0.0106 to 1.1401±0.0139 g; for female pups, the range was from 0.9583±0.0389 to 1.0283±0.0208 g. For adult males, mean brain weight (±s.e.m.) ranged from 1.8458±0.0234 to 1.9681±0.0138 g; for adult females, the range was from 1.7340±0.0463 to 1.8149±0.0234 g.

For MRID 46688914, there were no statistically or biologically significant differences in brain weights for adults or pups at either time point. For male pups, mean brain weight (±s.e.m.) ranged from 0.9929±0.0233 to 1.0559±0.0221 g; for female pups, the range was from 0.9772±0.0181 to 1.0255±0.0225 g. For adult males, mean brain weight (±s.e.m.) ranged from 1.8389±0.0231 to 1.9612±0.0194 g; for adult females, the range was from 1.7996±0.0180 to 1.8403±0.0227 g.

D. CHOLINESTERASE ACTIVITY: The RBC and brain cholinesterase activity data for treated adult male and female rats and offspring are shown in Tables 4, 5, and 6 for the range-finding, time course, and main acute exposure studies of adults and pups, respectively. As noted previously, plasma cholinesterase inhibition was not evaluated. Note that variance in the tables (as provided in the study report) is reported as standard error of the mean (s.e.m.), not standard deviation (s.d.).

Range-finding study, PND11 pups (MRID 46688912): Since no controls were evaluated in this study, percent of control values could not be calculated (statistical significance was evaluated by comparison with the low dose group activity levels). No difference was seen in RBC cholinesterase activity among groups, at any dose level. In brain, there was a clear dose-related decrease in activity levels in males, but not in females. The lack of clear findings in this study may be related to the small sample sizes (3/sex/group), or to the late sampling time (360 min post-dosing). Data are included in Table 4 (below).

TABLE 4. RBC and brain ChE activity in male and female PND 11 pups acutely treated with carbofuran technical: termination at 360 min after dose			
	Dose (mg/kg)		
Age Group/ Tissue	0.3	0.6	1.0
Male -			
RBC (U/mL)	3307±202	3353±217	3400±171
Brain (U/g)	7.74±0.03	7.06±0.17**	5.47±0.09***
Female -			
RBC (U/mL)	2780±171	3393±267	3020±40
Brain (U/g)	7.01±0.79	7.45±0.28	6.37±0.14

Data from pp. 23-24, MRID 46688912.
N = 3 pups/sex/group. [a] Numbers in parenthesis are percent inhibition relative to low dose (no control group was used).
Significantly different from low dose: *p# 0.05, **p # 0.01, ***p<0.001.

Time to peak effect (MRID 46688913): No clear inhibition of RBC cholinesterase was seen in either sex, at either age, at any measurement time point. In contrast, clear inhibition was seen in brain cholinesterase starting at the earliest measured time point (15 minutes), and continuing through 360 minutes in pups, and 240 minutes in adults. Data are included in Table 5 (below).

In brain, inhibition was greatest at 15 minutes, with both ages showing some recovery toward baseline starting at the 30 minute time point. At all time points, brain cholinesterase inhibition was greater in PND11 pups (both sexes) than in adults of either sex. In addition, recovery appeared to be slower in pups than in adults, with substantial inhibition still present in pups at 360 minutes (the final assessment time point); adults had recovered to with 10% of control values by that time.

RBC cholinesterase data were quite variable for both pups and adults, increasing and decreasing around control levels with no apparent relationship to the time of sampling. For example, in treated adult males, RBC cholinesterase activity was increased over control levels at all time points evaluated. Given this level of variability, it is unlikely that a time of peak effect could have been reliably identified, even if inhibition had occurred.

For the statistical analysis, cholinesterase data from the 0 and 360 min control groups were combined. Evaluation of individual data for these groups indicates very little overlap in the values for the two time points, especially for RBC data (means were 3192+/-482 and 3000 +/- 466 for the 0 time point [F/M, respectively], and 4349 +/-1212, 3988 +/- 1061 at the 360 min time point), indicating a 36% and 33% increase, respectively [F/M] for the two time points. Whether this indicates a lack of stability in the assay over time, or is simply a reflection of the noise in the data, the results certainly raise concern regarding the reliability of these results. Brain data also seemed to be somewhat more variable than usual; examination of the coefficients of variation provided in Appendix III of the study report indicate a range of 9-27%. The upper end of this range is higher than that seen for brain data in many cholinesterase studies.

TABLE 5. Time-course of RBC and brain cholinesterase activity in PND11 and adult rats				
Time point (minutes)/ Compartment	0.6 mg/kg		0.6 mg/kg	
	PND11 Male	PND11 Female	Adult Male	Adult Female
RBC (U/L)				
0	3494±295	3770±336	4208±261	4760±296
15	2988±199	3100±203	4332±299	4088±400
30	3056±96	3232±227	4456±390	4432±400
60	4092±251	4444±202	5124±233	5272±358
90	3052±139	3048±193	4288±240	4908±366
120	3896±87	3928±133	4732±297	4540±329
240	3340±118	3208±132	4796±611	3856±403
360	4320±199	5064±459	5164±298	4536±173
Brain (U/g)s				
0	7.33±0.33	8.38±0.38	12.98±0.22	13.06±0.12
15	3.50±0.29*** (48)	3.43±0.32*** (41)	8.04±0.55*** (62)	8.67±0.38*** (66)
30	4.02±0.33*** (55)	4.45±0.41*** (53)	8.47±0.39*** (65)	9.18±0.33*** (70)
60	4.29±0.25*** (59)	4.13±0.25*** (49)	11.28±0.17*** (87)	11.38±0.27*** (87)
90	5.31±0.39*** (72)	4.71±0.57*** (56)	10.74±0.12*** (83)	11.18±0.28*** (86)
120	4.62±0.18*** (63)	4.99±0.37*** (60)	11.68±0.26** (90)	12.12±0.20*** (93)
240	5.54±0.52*** (76)	6.87±0.52* (82)	11.78±0.39* (91)	13.04±0.56 (100)
360	6.18±0.05* (84)	6.36±0.20** (76)	12.00±0.32 (92)	12.24±0.30** (94)

Data taken from pages 30, 37, MRID 46688913; *p<0.05, **p<0.01, ***p<0.001

Data are mean±s.e.m., number in parenthesis represents percent of control values.

n = 5 rats/sex/dose group, except for controls (10/sex/group, 5 sacrificed at time 0, 5 at 360 min, combined); control data are reported as time 0.

Main study – single gavage dose to male and female adults and PND 11 pups (MRID 46688914): As in the time-to-peak-effect study, no significant inhibition of RBC cholinesterase was seen at either time point in the main study. Brain cholinesterase was significantly inhibited at all doses at the 15 min evaluation, with pups showing considerably greater inhibition than adults (see Table 6). By 6 h post-dosing, brain cholinesterase activity levels were greater than 90% of control levels; the differences remained statistically significant in some, but not all, groups.

TABLE 6. RBC and brain ChE activity in male and female adults and PND11 pups acutely treated with carbofuran technical: termination at 15 minutes or 6 hours after dose (mean ± s.e.m.)

Age Group/ Tissue	Dose (mg/kg)			
	0	0.3	0.6	1.0
15 minutes				
Adult Males				
RBC (U/mL)	4774±515	5110±353	4880±395	5276±390
Brain (U/g)	12.46±0.20	9.22±021*** (74)	8.44±0.28*** (68)	7.01±0.19*** (56)
Adult Females				
RBC (U/mL)	5390±441	5754±507	4718±267	5200±356
Brain (U/g)	12.88±0.18	9.84±0.35*** (76)	8.54±0.31*** (66)	7.16±0.35*** (56)
Male Pups -PND 11				
RBC (U/mL)	5436±437	5746±307	4944±449	5260±357
Brain (U/g)	6.62±0.14	3.47±0.09*** (52)	3.13±0.17*** (47)	2.53±0.11*** (38)
Female Pups - PND 11				
RBC (U/mL)	4782±472	6672±270	4646±319	5064±367
Brain (U/g)	6.90±0.11	3.56±0.19*** (52)	3.27±0.15*** (47)	2.42±0.09*** (35)
6 hours				
Adult Males				
RBC (U/mL)	5470±199	5570±264	5246±267	6884±237
Brain (U/g)	13.00±0.25	12.29±0.27* (95)	12.41±0.18 (95)	12.18±0.11* (94)
Adult Females				
RBC (U/mL)	5058±291	5146±96	5492±195	5910±279
Brain (U/g)	13.29±0.20	12.74±0.15 (96)	12.62±0.25* (95)	12.55±0.17* (94)
Male Pups - PND 11				
RBC (U/mL)	4791±430	4034±287	6093±499	4062±191
Brain (U/g)	7.03±0.12	6.49±0.15 (92)	6.55±0.24 (93)	6.53±0.16 (93)
Female Pups - PND 11				
RBC (U/L)	4558±215	4166±309	5427±471	4092±289
Brain (U/g)	7.17±0.12	6.87±0.17 (96)	7.10±0.16 (99)	7.20±0.16 (100)

Data from pp. 34 and 42), MRID 46688914.
N = 10 /sex/group. except n=9 for RBC, 0.6 mg/kg male pups at 15min and 6 h ., 0.6 mg/kg female pups at 6 h (both measures), .
[1] Numbers in parenthesis are percent of control values.
Significantly different from control: *p# 0.05, **p # 0.01, ***p<0.001 .

III. DISCUSSION AND CONCLUSIONS:

INVESTIGATORS' CONCLUSIONS: Investigators concluded that there were no effects on RBC cholinesterase activity at any age or dose. Brain cholinesterase was inhibited at all doses in both sexes and age groups; peak inhibition occurred in both ages at 15 min post-dosing, with recovery to control levels by 720 minutes post-dosing. The absolute amount of cholinesterase inhibition in adults was greater than that in pups, except in females at 0.3 mg/kg, 15 minutes post-dosing. Thus, there was no differential sensitivity seen in pups when compared to adults.

REVIEWER=S COMMENTS: No treatment-related inhibition was seen in RBC cholinesterase in any group at any time point. In addition, as discussed above, the RBC cholinesterase activity levels were variable even in control animals, raising questions about the adequacy of this assessment. In the absence of inhibition, the relative sensitivity of this compartment cannot be adequately assessed.

There was treatment-related inhibition of brain cholinesterase in all dose groups for both ages evaluated. There were no apparent differences in susceptibility between males and females, for either age group. The relative magnitude of the inhibition was much greater in pups than in adults, with similar levels of inhibition seen at the 0.3 mg/kg dose in pups (activity 52% of control levels) and at 1.0 mg/kg in adults (activity 56% of control levels). Since the baseline activity levels were much lower in pups than in adults (approximately half, with 12.5-13 U activity/g tissue in adults, versus 6.5-7 U/g in pups), it is inappropriate to compare absolute differences in activity, as registrants have done in drawing their conclusion that no differential sensitivity was seen.

The increased sensitivity of pups to carbofuran as demonstrated by the inhibition of brain cholinesterase is supported by the increased incidence of tremors seen during the clinical evaluations. Even given the limitations of the assessments, as discussed above, there was a clear increase in sensitivity, with pups showing 100% incidence of tremors at all doses for both sexes. In contrast, while adults showed a dose-related increase in incidence, tremors were seen at the low dose of 0.3 mg/kg in only 3 males and 1 female (seen only in the main study, at the 720 min time point). The only adult group showing 100% incidence of tremors was the high dose, 720 min observation group, in the main study. Tremors also appeared to persist longer in pups, with some incidence occurring as late as 120 minutes following treatment; the latest incidence in adults occurred at 60 min post-dosing.

No treatment-related effects were seen on brain or body weight in any group. Given the short duration of the study, effects on these parameters would not be expected.

For single acute exposures:

the adult and PND 11 LOAEL for brain ChEI is 0.3 mg/kg (both sexes)
the adult and PND 11 NOAEL for brain ChEI is <0.3 mg/kg (both sexes);

the adult and PND 11 LOAEL for red blood cell ChEI is >1.0 mg/kg (both sexes)
the adult and PND 11 NOAEL for red blood cell ChEI is 1.0 mg/kg (both sexes);

For acute exposure, the overall adult and PND11 LOAEL for cholinesterase inhibition in rats is 0.3 mg/kg based on enzyme inhibition in brain; the NOAEL is <0.3 mg/kg.

For acute exposure, the overall adult and offspring LOAEL for clinical signs in rats is 0.3 mg/kg based on tremors; the NOAEL is <0.3 mg/kg.

Taken together these studies are classified **Acceptable/Nonguideline** for the determination of brain cholinesterase activities following treatment with a single dose of carbofuran technical in adult and PND11 rats, pending receipt of additional requested information (see deficiency section). They are **Unacceptable/Nonguideline** for the determination of RBC cholinesterase activity, as no effects were seen on that measure at any dose.

STUDY DEFICIENCIES:

1. Insufficient information was provided about randomization of subjects and the selection of pups for the reconstituted litters, as described above. This information should be provided

2. Insufficient information was provided about the procedures for the clinical observations, including whether or not animals were observed outside the home cage; clinical observations were not performed 'blind', which limits the value of this information. More complete descriptions of these procedures should be provided.

3. No information was provided regarding the mixing procedure for the formulations during dosing. Since the formulation was a suspension, some mixing procedure would be needed to ensure the concentration would remain homogeneous during dose administration. Information regarding this procedure should be provided.

4. As no inhibition was seen in RBC cholinesterase, this compartment may not have been adequately assessed.

5. An explanation for the discrepancies in reporting of the individual animal data for clinical signs, as discussed above for female #24 in the main study, should be provided.

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