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

012858

SEP 15 1998

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carbaryl - Review of Developmental Neurotoxicity Study (MRID # 44393701)

DP Barcode: D239920
PC Codes: 056801
Case: 818954
Submission: S531722

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer
Reregistration Branch I, Health Effects Division (7509C)
Virginia A Dobozy 9/14/98

TO: Linda Propst/Kathryn Boyle
Special Review and Reregistration Division (7508W)

THRU: Whang Phang, Ph.D., Branch Senior Scientist
Reregistration Branch I, Health Effects Division (7509C)
Whang Phang 9/15/98

Action Requested: Review developmental neurotoxicity study.

Recommendation: Reregistration Branch I has completed review of the study and found it acceptable. However, the registrant should address the deficiencies listed at the end of the DER. An Executive Summary of the DER is attached.

1

DATA REVIEW

CITATION: Robinson, K. and B. Broxup (1997) A Developmental Neurotoxicity Study of Orally Administered Carbaryl, Technical Grade, in the Rat. ClinTrials BioResearch, Ltd.. Quebec, Canada. Laboratory Project I.D. 97391, September 23, 1997. MRID 44393701. Unpublished

EXECUTIVE SUMMARY:

In this developmental neurotoxicity study (MRID # 44393701), 26 pregnant female Sprague-Dawley rats/group were administered carbaryl (99.1% a.i.) by gavage from Gestation Day (GD) 6 through Lactation Day (LD) 10 at doses of either 0, 0.1, 1.0 or 10 mg/kg/day. An additional 6 pregnant females/group were dosed at the same levels for the cholinesterase (ChE) phase of the study. ChE measurements were done pre-dosing (GD 6) and post-dosing at time of peak effect (1 hour post-dosing) on GD 6, 15 and 20 and LD 4 and 10. Functional Observational Battery (FOB) measurements were performed at approximately 0.5 and 2 hours post-dosing on the same days as body weight measurements during the dosing period (GD 0, 6, 9, 12, 15, 18 and 20 and LD 4, 7, 11, 13 and 21). Measures of reproductive performance were evaluated. Offspring were examined for body weight, physical development landmarks (tooth eruption and eye opening), FOB assessments (days 4, 7, 11, 13, 17 and 21) and motor activity (days 13, 17 and 21). On LD 11, 1 animal/sex/litter was sacrificed for brain weights; of these, six/sex were randomly selected for neuropathological evaluation. The eyes from all dose groups were examined. After LD 21, 3 animals/sex/litter were separated from the dams and constituted the F1 adult generation. These animals were evaluated for body weight, physical development (vaginal opening and preputial separation), motor activity (day 60), startle habituation response (days 22 and 60), passive avoidance (day 23) and water maze behavior (day 60). After completion of the behavior test period (at approximately 10 weeks of age), 12 animals/sex/group were anesthetized and perfused for post-mortem examination. Tissues from 6 animals/sex of the control and high dose group were processed for neuropathological evaluation and morphometric measurements; the eyes from the low and mid-dose group of all perfused animals were examined.

For the F0 generation animals, there were no carbaryl-associated deaths. No treatment-related clinical signs of toxicity were observed. There was a statistically significant decrease (92%) in body weight gain for females in the 10 mg/kg/day group for the period GD 6-9. Unfortunately, food consumption was not measured during the study. During the FOB measurements, the incidence of females in the 10 mg/kg/day group with decreased pupil size (pinpoint pupils) was increased on all occasions during the dosing period. An increased incidence of dams with slight tremors affecting the head, body and/or limbs was noted on the majority of assessment occasions in the dosing period. There were also occasional occurrences of ataxic gait/overall gait incapacity which was considered to be of toxicological significance due to other effects upon gait.

For the 10 mg/kg/day group, RBC and whole blood ChE levels were statistically significantly decreased (28% and 32-34%, respectively) on GD 20 and LD 10. Although the plasma ChE levels were not statistically significantly altered, the percentage decreases on GD 20, LD 4 and LD 10

2

were 32-39%. Brain ChE levels were statistically significantly decreased (42%). There were no treatment-related effects on gross necropsy findings for the F0 generation animals.

There were no effects observed on maternal performance parameters of pregnancy rate, gestation index, length of gestation, numbers of live pups, dead or malformed pups, implantation scars, sex ratio or post-implantation loss. There was a slight ($P>0.05$) increase in the number of dead pups in the 10 mg/kg/day group, however the value was within the historical control range for this strain.

For the F1 generation pups, there were no treatment-related effects on pup weight, pup survival indices, developmental landmarks (tooth eruption and eye opening), FOB measurements or motor activity assessments. At sacrifice on LD 11, there were no treatment-related effects on brain weight and gross or microscopic pathology. Significant differences noted in the morphometric measurements included an increase in Line B of the right forebrain and Line F of the right cerebellum in the 10 mg/kg/day males. In the 10 mg/kg/day females, Line F through both the right and left cerebellum was decreased (15% and 22%, respectively).

For the F1 generation adults, there were no treatment-related effects on clinical condition, body weight, physical development (vaginal opening and preputial separation), motor activity, auditory startle response, passive avoidance and water maze measurements. At sacrifice, there were no gross or microscopic neuropathological lesions observed for animals examined in this study that were attributable to treatment with the test article. There was an increased incidence of retinal fold/rosette in the 10 mg/kg/day group (1/12 for control vs. 4/12 for males; 0/12 for control vs. 2/12 for females). The finding was not considered of toxicological significance since the incidence was within the historical control range for males, occurred at a low rate and was not dose-dependent. For the morphometric measurements, there was a significant decrease in Line B through the left forebrain and a significant increase in Line F through the right cerebellum of the 10 mg/kg/day males. For the 10 mg/kg/day females, there were significant increases in Line G through the right and left cerebellum (18.8% and 29.8%, respectively).

The maternal toxicity LOEL was 10 mg/kg/day based on decreased body weight gain, alterations in Functional Observational Battery measurements and RBC, plasma, whole blood and brain cholinesterase inhibition. The maternal NOEL was 1.0 mg/kg/day.

The developmental neurotoxicity tentative LOEL was 10 mg/kg/day based on alterations in the morphometric measurements at Days 11 and 60; the tentative NOEL was 1 mg/kg/day because the morphometric analyses at the lower doses were not conducted.
The developmental effect persisted in F1 adults, thus confirming that this developmental effect is not transitional.

STUDY CLASSIFICATION: This developmental neurotoxicity study is classified acceptable and **does satisfy** the guideline requirement for a developmental neurotoxicity study (OPPTS 870.6300) in rats. However, the registrant should address the study deficiencies which are listed at the end of this review.

3

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia a Dobozy 5/27/98
Reregistration Branch I, Health Effects Division (7509C)

Secondary Reviewer: Susan Makris, M.S. Susan J Makris 5/28/98
Toxicology Branch I, Health Effects Division (7509C)

Secondary Reviewer: William Sette, Ph.D. William Sette 5/28/98
Science Analysis Branch, Health Effects Division (7509C)

Branch Senior Scientist: Whang Phang, Ph.D. Whang Phang 5/29/98
Reregistration Branch I, Health Effects Division (7509C)

012858

DATA EVALUATION REPORT

STUDY TYPE: Developmental Neurotoxicity Study - gavage (OPPTS 870.6300)

DP BARCODE: D240442; D240995[1]
P.C. CODE: 056801

SUBMISSION CODE: S531722
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Carbaryl (99.1% a.i.)

SYNONYMS: none

CITATION: Robinson, K. and B. Broxup (1997) A Developmental Neurotoxicity Study of Orally Administered Carbaryl, Technical Grade, in the Rat. ClinTrials BioResearch, Ltd.. Quebec, Canada. Laboratory Project I.D. 97391, September 23, 1997. MRID 44393701. Unpublished

SPONSOR: Rhone-Poulenc

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[1] An electronic copy of the study summary was supplied by the registrant. Some of the information in the Data Evaluation Report (DER) has been extracted from this summary. All of the data in the summary has been verified before inclusion in the DER. The Reviewer's Conclusions and Executive Summary were prepared by the EPA reviewer.

post-dosing on the same days as body weight measurements during the dosing period (GD 0, 6, 9, 12, 15, 18 and 20 and LD 4, 7, 11, 13 and 21). Measures of reproductive performance were evaluated. Offspring were examined for body weight, physical development landmarks (tooth eruption and eye opening), FOB assessments (days 4, 7, 11, 13, 17 and 21) and motor activity (days 13, 17 and 21). On LD 11, 1 animal/sex/litter was sacrificed for brain weights; of these, six/sex were randomly selected for neuropathological evaluation. The eyes from all dose groups were examined. After LD 21, 3 animals/sex/litter were separated from the dams and constituted the F1 adult generation. These animals were evaluated for body weight, physical development (vaginal opening and preputial separation), motor activity (day 60), startle habituation response (days 22 and 60), passive avoidance (day 23) and water maze behavior (day 60). After completion of the behavior test period (at approximately 10 weeks of age), 12 animals/sex/group were anesthetized and perfused for post-mortem examination. Tissues from 6 animals/sex of the control and high dose group were processed for neuropathological evaluation and morphometric measurements; the eyes from the low and mid-dose group of all perfused animals were examined.

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There were no effects observed on maternal performance parameters of pregnancy rate, gestation index, length of gestation, numbers of live pups, dead or malformed pups, implantation scars, sex ratio or post-implantation loss. There was a slight ($P > 0.05$) increase in the number of dead pups in the 10 mg/kg/day group, however the value was within the historical control range for this strain.

For the F1 generation pups, there were no treatment-related effects on pup weight, pup survival indices, developmental landmarks (tooth eruption and eye opening), FOB measurements or motor activity assessments. At sacrifice on LD 11, there were no treatment-

5

related effects on brain weight and gross or microscopic pathology. Significant differences noted in the morphometric measurements included an increase in Line B of the right forebrain and Line F of the right cerebellum in the 10 mg/kg/day males. In the 10 mg/kg/day females, Line F through both the right and left cerebellum was decreased (15% and 22%, respectively).

For the F1 generation adults, there were no treatment-related effects on clinical condition, body weight, physical development (vaginal opening and preputial separation), motor activity, auditory startle response, passive avoidance and water maze measurements. At sacrifice, there were no gross or microscopic neuropathological lesions observed for animals examined in this study that were attributable to treatment with the test article. There was an increased incidence of retinal fold/rosette in the 10 mg/kg/day group (1/12 for control vs. 4/12 for males; 0/12 for control vs. 2/12 for females). The finding was not considered of toxicological significance since the incidence was within the historical control range for males, occurred at a low rate and was not dose-dependent. For the morphometric measurements, there was a significant decrease in Line B through the left forebrain and a significant increase in Line F through the right cerebellum of the 10 mg/kg/day males. For the 10 mg/kg/day females, there were significant increases in Line G through the right and left cerebellum (18.8% and 29.8%, respectively).

The maternal toxicity LOEL was 10 mg/kg/day based on decreased body weight gain, alterations in Functional Observational Battery measurements and RBC, plasma, whole blood and brain cholinesterase inhibition. The maternal NOEL was 1.0 mg/kg/day.

The developmental neurotoxicity tentative LOEL was 10 mg/kg/day based on alterations in the morphometric measurements at Days 11 and 60; the NOEL was 1 mg/kg/day.

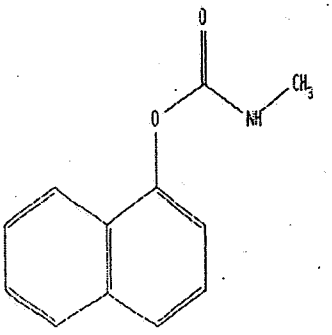
This developmental neurotoxicity study is classified **acceptable** and **does satisfy** the guideline requirement for a developmental neurotoxicity study (OPPTS 870.6300) in rats. However, the registrant should address the study deficiencies which are listed at the end of this review.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Carbaryl
   Description: coarse white powder
   Lot/Batch #: 201085006
   Purity: 99.1% ai.
   Storage conditions: refrigerated, protected from light
   CAS #: 63-25-2
   Chemical Structure:



2. Vehicle and/or positive control: Suspensions of carbaryl were prepared in aqueous 0.5% (w/v)carboxymethyl cellulose (high viscosity) and 0.1% (w/v) Tween 80.

3. Test animals: Species: Albino rat (Outbred)
   Strain: Sprague-Dawley [Crl:CD®(SDBR)]
   Age and weight at study initiation: Females- 76-81 days old weighing 211-278 gms.
   Source: Charles River Canada, Quebec, Canada
   Housing: Parental animals were individually housed in stainless steel wire mesh cages with an automatic watering valve except during mating. On Gestation Day 18, each mated female was housed in solid-bottomed cages on corn-cob bedding.
   Diet: certified rodent diet, No. 5002 ad libitum
   Water: automated watering system utilizing tap water that had been further treated by reverse osmosis and ultraviolet sterilization; available ad libitum
   Environmental conditions: Temperature: 22 ± 3°C
   Humidity: 50 ± 20%
   Air changes: 12-15 air changes/hour
   Photoperiod: 12 hour light/dark cycle
   Acclimation period: approximately 2 weeks

7

B. STUDY DESIGN:

1. In life dates -

Study Initiation: January 10, 1997
Start of Treatment: January 19, 1997
Necropsy: Final day of necropsy was April 18, 1997

2. Mating: One female was placed with 1 proven male of the same strain and source. The females were examined daily for evidence of mating by examination of the vaginal lavage for spermatozoa. The day of positive identification of spermatozoa in the vaginal lavage was termed Day 0 of gestation.

3. Animal Assignment: Mated females were assigned to dose groups as indicated in Table 1. Assignment was made using a computer-based randomization procedure.

TABLE 1 Parental Animal Assignment

Test Group	Dose (mg/kg/day)	No. of Females-Developmental Neuro. Phase	No. of Females-Cholinesterase Phase
Control	0	26	6
Low (LDT)	0.1	26	6
Mid (MDT)	1	26	6
High (HDT)	10	26	6

Females were dosed via gavage (10 ml/kg/day) from Gestation Day 6 through Lactation Day 10.

Pregnant females were allowed to give birth. On Day 4 of lactation, all litters with greater than eight pups were reduced to that number, with equal numbers/sex when possible.

4. Dose Selection: Dose selection was based on the results of three studies.[2] In the 13-week study, at doses of 10 and 30 mg/kg/day, animals showed tremors and decreased pupil size at the time of peak effect. Blood and brain cholinesterase (ChE) were also significantly affected at these doses. In the "Time of Peak Effects" study, the most marked clinical observations at doses of 50 and 125 mg/kg/day and the largest ChE inhibition occurred at 0.5 and 1 hour post-dosing, respectively. In the "Acute" study, clinical signs, including tremors and salivation, occurred at doses of 30 and 90 mg/kg with decreases in ChE levels at 10, 30 and 90 mg/kg at 1 hour post-dosing. Based on these findings, the dose level of 10 mg/kg/day in the study under review was expected to produce clear effects on nervous system function, in terms of behavior and neurochemistry. The Functional Observational Battery was performed at 0.5 and 2 hours post-dosing and ChE measurements at 1 hour post-dosing, also based on the results of the preliminary studies.

TABLE 2: F1 NEUROTOXICITY STUDY DESIGN

Test Group	Dose Level (mg/kg/day)	Number of F1 Animals (pups)							
		Motor Activity[b]		Auditory Startle		Passive Avoidance/ Water Maze		Neuropath[c]	
		M	F	M	F	M	F	M	F
Control[a]	0	26	26	26	26	26	26	16	16
Low	0.1	26	26	26	26	26	26	16	16
Mid	1	26	26	26	26	26	26	16	16
High	10	26	26	26	26	26	26	16	16

[a] Control animals received vehicle only

[b] Motor activity, auditory startle, passive avoidance/water maze testing and memory were performed on one pup/sex/litter.

[c] Neuropathology was performed on at least 16 pups/sex/group on Day 11 and 6 pups/sex/group on Day 60.

[2] The following studies were used for determining the doses in the present study: 1) A 13 Week Study of the Potential Effects of Orally Administered Carbaryl, Technical Grade, on Behavior, Neurochemistry and Neuromorphology in Rats. Bioresearch Project No. 97390; 2) A Time of Peak Effects Study of a Single Orally Administered Dose of Carbaryl, Technical Grade, in Rats. Bioresearch Project No. 97388; 3) An Acute Study the Time Course of Cholinesterase Inhibition by Orally Administered Carbaryl, Technical Grade, in the Rat. Bioresearch Project No. 97392.

9

5. Dosing preparation and analysis

Suspensions of carbaryl, technical grade, were prepared approximately weekly by direct dilution by adding aqueous 0.5% (w/v) carboxymethyl cellulose (high viscosity)/0.1% (w/v) Tween 80 to an appropriate amount of carbaryl, and mixing using a sonicator and then placed on a stir plate. Suspensions were stored refrigerated and aliquots prepared for daily use were resuspended on a stir plate prior to dosing. The homogeneity of the dose formulation for the 0.1 mg/kg/day dose level was analyzed. Previously, homogeneity and stability for the 1 and 10 mg/kg/day dosing suspensions had been established as part of other studies. The accuracy of formulation was measured on Weeks 1, 2, 3, and 5.

Results -

Concentration Analysis: Dose suspensions sampled to assess concentration during the study, were within specification on all occasions except for Week 1. The Analytical Chemistry Report (pages 39-45) indicates that the acceptance criteria was changed during Week 1. For the 0.1 mg/kg/day group, the first preparation at that time showed an average recovery of 79.0%, which deviated from the acceptance criteria of ± 15% of the nominal value. A second and third preparation had average recoveries of 55.0% and 73%, respectively. The acceptance criteria was then revised to ±25%. The study report states that the cause of the low recoveries was suspected to be absorption of the test article by the weighing paper used. This paper was replaced by glass weighing boats. The average recovery for this level was 80% at Week 2 and all other samples were within specification thereafter. For the 1 mg/kg/day group, the average recoveries for the Week 1 preparation were 84.6% and 74.1%. For the 10 mg/kg/day group, the average recoveries for the Week 1 preparation was 82.3% and 91.3%. All other samples for these groups were within specifications.

The analytical data indicated that the mixing procedure was adequate for the 0.1 mg/kg/day group, although the recoveries were low, ranging from 73.4% to 84.1%.

C. METHODS:

1. Observations on Parental Females:

Parental animals were inspected twice daily for signs of toxicity and mortality. On days of body weight measurement (except day 0 *post partum*) during the treatment period, an observational battery was performed at approximately 0.5 to 2 hours post dosing.

The observational battery was performed with equipment built for this purpose. The arena was a 2' square of plexiglass placed on a raised platform. The tests were conducted in the room housing the animals, where temperature, humidity and photo-period were measured. Observations in the arena included: ataxic, hypotonic and impaired gait; overall gait incapacity; bizarre/stereotypic behavior; palpebral closure; tremors, twitches, convulsions; piloerection; defecation; and urination. Handling observations included: lacrimation; pupil size; salivation; urinary staining; and diarrhea. Testing was performed by the same trained technicians, wherever possible, who were blind to the animals' treatment.

Females were observed at least 3 times each day from Day 20 of gestation for signs of parturition. Where possible, parturition was observed, the times of onset and completion of parturition were recorded and any sign of dystocia noted. The females' behavior immediately *post partum* was observed. The day of completion of littering was termed Day 0 *post partum*.

Body weights of mated females were obtained on Days 0, 6, 9, 12, 15, 18, and 20 of gestation. During lactation, females with litters were weighed on Days 0, 4, 7, 11, 13, and 21. Food consumption was not recorded.

Animals assigned to the cholinesterase phase were bled, pre-dosing (Day 6 of gestation), and post-dosing at the time of peak effect (1 hour post dosing) on days 6, 15, and 20 of gestation and days 4 and 10 *post partum*. Non-terminal blood samples were taken from the lateral tail vein on gestation Day 6 for some animals and subsequently from the orbital sinus under isoflurane anesthesia. Terminal samples were taken from the abdominal aorta. Whole blood, plasma, RBC and brain (homogenized) cholinesterase determinations were made as appropriate.

2. Observations on F1 Generation Pups:

Litters were observed as soon as possible after delivery (Day 0 of lactation) for the number of live and dead pups and pup abnormalities. Any pups found to be malformed were killed and along with any dead pups were placed in Bouin's fluid for subsequent examination. Thereafter, litters were observed daily. On Day 4 of lactation, the litters were culled to 8 pups (4 males and 4 females), where possible, and the culled pups were discarded. The number and general condition of the pups in each litter were evaluated and any pups found dead between Days 0 and 7 of lactation were stored in Bouin's fluid for a subsequent examination. Pups found dead between 8 and 21 days of lactation were given a complete gross pathological examination. Individual live pup body weights were recorded on Days 0, 4, 7, 11, 13, 17, and 21 of lactation. On days of body weight measurement an observational battery was performed at approximately the same time as the dam's body weight evaluation.

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The observational battery was performed with equipment built for this purpose. The arena was a 2' square of plexiglass placed on a raised platform. The tests were conducted in the room housing the animals, where temperature, humidity and photo-period were measured. Observations in the arena included (day observed from): ataxic, hypotonic and impaired gait (13); overall gait incapacity (13); bizarre/stereotypic behavior (4); palpebral closure (21); tremors, twitches, convulsions (7); piloerection (13); defecation (13); and urination (13). Handling observations included: lacrimation (21); pupil size (21); salivation (13); urinary staining (13); and diarrhea (13). Testing was performed by the same trained technicians, wherever possible, who were blind to the animals' treatment.

The following physical development parameters were assessed: tooth eruption from Day 7 of lactation and eye opening from Day 12 of lactation until all pups in the litter showed development. One male and one female pup were randomly selected from each litter, to provide at least 16 males and 16 females per group, for neuropathological evaluation or brain weights on Day 11 of lactation. The activity of 1 male and 1 female per litter was measured individually in a Figure 8 activity maze for a 1 hour period on lactation days 13, 17, and 21.

After testing on Day 21 of lactation, the F1 generation pups were separated from their dams and 3 males and 3 females from each litter were randomly selected to provide the F1 adult generation. Animals retained post-weaning were observed to identify the day on which vaginal patency (from Day 26 - females) and preputial separation (from Day 34 - males) occurred.

3. Observations on F1 Generation Adults:

All animals were examined twice daily for mortality and clinical signs. Body weights were taken weekly and on the day of perfusion for those selected for neuro-pathology.

4. Neurobehavioral Studies on F1 Generation:

For motor activity, one male and one female pup in each litter was measured for a 1 hour period on Postnatal Day 60 in a Figure 8 activity maze. All sessions consisted of 6 ten minute intervals. The sound level was kept constant at approximately 70 dBA in the test room using exterior white noise generation. Room illumination was approximately 1000 Lux.

An auditory startle habituation test was performed on one male and one female pup/litter on Postnatal day 22 and 60 ($\pm$2). The animals were given a 4 minute

acclimation period and then the startle response was measured in 50 identical trials at a sound level of 120 dBA with an 8 second inter-trial interval. Data were collected on startle at start of trial (voltage), maximum startle (voltage) and time to maximum startle.

Passive avoidance was assessed on Day 23 *post partum* by placing the rat into the illuminated side of a two-compartment rodent shuttle cage and the time elapsed before crossing to the darkened side recorded. When the animal crossed to the darkened side it received a foot shock of 0.5 MA for 1 second (conditioning trial). After approximately 30 seconds this trial was repeated a second time, and if necessary a third time. Animals remaining on the lighted side for 2 minutes were considered to have met criteria for the conditioning test. One hour after the conditioning trial(s), those animals which achieved criteria were tested with the step-through latency being recorded for a 2 minute period. No shock was delivered to those entering the darkened side. On Day 24 *post partum*, at least 25 hours after the conditioning trial(s), those animals which achieved criteria were tested with the step-through latency being recorded for a 2 minute test period. No shock was delivered to those animals re-entering the darkened side.

On Days 60 and 65 post-partum, the animals were placed in the center arm of the 'E' Water Maze and the exit ramp placed at one end arm (randomly selected for each animal). The time to exit the maze and the number of errors (incorrect turns) were recorded for 5 tests, each of a maximum time of 1 minute on the first day of testing. Each test was performed at least 15 minutes apart. On the following day two tests were performed (at least 25 hours after the end of the first day's trials). These evaluations were conducted between Days 60 and 65 *post partum*. Any abnormality in swimming was also recorded.

5. Sacrifice and Pathology - Parental Animals

Complete macroscopic postmortem examinations were performed on all parental animals (euthanized by carbon dioxide). Any female that mated but did not litter was euthanized on Day 26 *post coitum* and particular attention was given to the examination of the reproductive tract for any abnormalities which may have prevented pregnancy. Any dams whose whole litters were born dead or died prior to weaning were killed, the number of implantation site scars recorded and their mammary tissue examined and a sample retained. The dams were killed on Days 21, 22, or 23 *post partum* and the number of implantation site scars recorded.

Animals designated for the cholinesterase phase were anesthetized with isoflurane and a blood sample taken from the abdominal aorta. Subsequently, the animals

were killed by exsanguination, the brain removed and weighed. These animals were not given a gross pathological evaluation.

On completion of the gross pathology examination, the following tissues and organs were retained in neutral buffered 10% formalin for fixation and preservation for possible future histopathological examination: mammary glands (thoracic and inguinal); ovaries; uterus; vagina; and any abnormalities.

6. Sacrifice and Pathology - F1 Generation

Dead Pups: Pups dying on or before Day 7 *post partum* were placed in Bouin's fluid for subsequent examination using a modified Barrow and Taylor technique. Pups dying between Days 8 and 21 *post partum* were immediately given a complete gross examination.

Day 11 Sacrifice: On Day 11 of sacrifice, one male and one female pup/liter were removed from each litter. Of these animals, at least 6 male and 6 female pups were randomly selected for eventual or possible neuropathological/morphological evaluation. The remaining selected pups, were killed by an intraperitoneal injection of sodium pentobarbital and the brains carefully removed, blotted, and weighed. Each brain was divided into four regions and these regions weighed - the telencephalon, the diencephalon/mid-brain, the medulla oblongata and pons, and the cerebellum.

The 6 male and 6 female pups per group selected for neuropathological evaluation were killed by an intraperitoneal injection of sodium pentobarbital and the brains removed and weighed. The brains were then immersion fixed in neutral buffered 10% formalin. The brains from the 6 high dose and 6 control group pups per sex were prepared for histological evaluation by embedding in paraffin wax and sectioning at 6 microns. Sections of brain (7 levels) were stained with hematoxylin and eosin and then examined by light microscopy. The brains from the low and mid dose groups were not examined. Planimetry (morphometry) was undertaken on these 6 high dose and 6 control group pups per sex, to assess the structural development of the brain. Estimates of the thickness of major layers at representative locations within the neocortex, hippocampus and cerebellum were determined. Histomorphometric evaluation was performed as follows:

Neocortex: 2 perpendicular lines (A and B) were drawn from the dorsal pial surface of the cerebral cortex to the cingulum and from the parietalcortex, i.e., from the somatosensory area to the pial surface of the cerebral cortex.

Corpus callosum: the thickness (line C) of the corpus callosum was taken at the midline.

Hippocampus: 2 perpendicular lines (D and E) were drawn from the highest point of Ammon's horn at the CA1 area to the lowest point of the dentate gyrus (greatest dorso-ventral extent of the hippocampus) and from the greatest medio-lateral extent of the hippocampus to the midline.

Cerebellum: 2 lines were drawn along the widest point of the long axis (line F) and perpendicularly to and at the midpoint of the long axis (line G).

Calculation of the group mean and standard deviation was done. Statistical analysis with the Dunnett's test was conducted since the homogeneity of the group variance was demonstrated with the Bartlett's test at the 0.001 level of significance.

Post Weaning Pups: Following weaning, weanlings of the F1 generation not selected for the adult F1 generation were euthanized by carbon dioxide asphyxiation followed by exsanguination from the abdominal aorta and given a complete gross pathological examination. Pups of dams in the Cholinesterase phase were euthanized, by carbon dioxide asphyxiation on Day 10 *post partum*, and discarded without examination.

F1 Adults: A complete necropsy of the carcass was conducted immediately on any animal that was killed during the study. The method of euthanasia for these animals was carbon dioxide asphyxiation followed by exsanguination from the abdominal aorta. If an animal was found dead, necropsy was completed as soon as possible after the time of death. No tissues from these animals were retained.

On completion of the behavioral test period (approximately 10 weeks of age), all surviving animals not selected for neuropathology were euthanized by carbon dioxide asphyxiation and discarded.

At least 12 adult animals per sex per group (derived from different litters) were randomly selected and deeply anesthetized by intraperitoneal injection of sodium pentobarbital. When anesthesia was deep, the thorax was opened. A 16 gauge needle was inserted into the left ventricle and the right atrium opened. Perfusion with lactated Ringer's solution containing heparin and sodium nitrite was initiated and continued until the auricular effluent was essentially free of blood. The perfusion fluid was then changed to a mixture of glutaraldehyde, paraformaldehyde, calcium chloride and picric acid in cacodylate buffer.

For 6 adult animals per sex per group following the perfusion, the thoracic and

abdominal organs were removed as two groups of tissues and placed in a separate tissue bag in neutral buffered 10% formalin, for possible future analysis. The remaining carcass containing the brain, spinal cord and limbs was placed in another tissue bag containing neutral buffered 10% formalin. Tissues from the high dose and control animals were processed for neuropathological evaluation as well as the eyes from the low and mid dose groups and the eyes from all animals perfused for morphology. The trimmed tissues from the low and mid dose groups were kept in 10% neutral buffered formalin for possible future analysis. The remaining carcasses from all animals were retained. The nervous system of animals in all groups was grossly examined at the time of sampling and any pathology observed was recorded and reported.

Tissues for paraffin embedding and sectioning included:

brain (7 levels) - olfactory bulbs, forebrain (through septum), center of the cerebrum (through the hypothalamus), midbrain, cerebellum and pons, midcerebellum and medulla oblongata, medulla oblongata
eyes - retina and optic nerve
spinal cord - cervical, thoracic, lumbar (longitudinal and cross-sections)
skeletal muscle - gastrocnemius (longitudinal and cross-section)
grossly abnormal central nervous system tissues

These tissues were prepared for examination by embedding in paraffin wax and sectioning at 6 microns. The skeletal muscle was stained with hematoxylin and eosin. Adjacent sections of brain and spinal cord were stained with hematoxylin and eosin, Kluver-Barrera, Holmes, and PTAH stains and then examined by light microscopy.

Tissues for epoxy embedding and sectioning included:

sciatic nerve (mid-thigh region) (longitudinal and cross-sections)
sciatic nerve (at sciatic notch) (longitudinal and cross-sections)
sural nerve (at knee) (longitudinal and cross-sections)
tibial nerve (at knee) (longitudinal and cross-sections)
Gasserian ganglion (cross-section)
lumbar dorsal root ganglion (L4) (cross-section)
lumbar dorsal root (L4) (cross-section)
lumbar ventral root (L4) (longitudinal and cross-sections)
cervical dorsal root ganglion (C5) (cross-section)
cervical dorsal root (C5) (cross-section)

cervical ventral root (C5) (cross-section)
grossly abnormal central or peripheral nervous system tissues

Planimetry (morphometry), as described for Day 11, was undertaken on a further 6 high dose and 6 control group adults per sex, to assess the structural development of the brain. Estimates of the thickness of major layers at representative locations within the neocortex, hippocampus and cerebellum were determined.

D. Data Analysis

1. Statistical Analyses (pages 29-31 of study report) -

Parental Data: Group mean body weights and cholinesterase data were analyzed using an analysis of variance (one-way classification); where the F value is of significance ($P<0.05$), differences between the control and treated groups were analyzed using Dunnett's test.

Maternal Data: The number of live pups was statistically evaluated using the Kruskal-Wallis and Mann-Whitney "U" tests, and the incidence of dead pups and malformed pups was analyzed using the chi-square or Fisher's exact probability test. The group mean number of implantation scars was analyzed using the Kruskal-Wallis and Mann-Whitney "U" tests. The post-implantation loss was calculated as follows:

$$\text{Post implantation Loss} = \frac{\text{No. of implantation site scars - No. of live pups at birth}}{\text{No. of implantation scars}} \times 100$$

The group mean length of gestation was analyzed using the Kruskal-Wallis and Mann-Whitney "U" tests. The pregnancy rate and gestation index were calculated as follows:

$$\text{Pregnancy Rate} = \frac{\text{No. of rats pregnant}}{\text{No. of rats mated}} \times 100$$

$$\text{Gestation Index} = \frac{\text{No. of rats with live litters}}{\text{No. of pregnant rats}} \times 100$$

Litter Data: The group mean viability, survival and lactation indices were calculated for each litter using the formulas below and analyzed using the Kruskal-Wallis and Mann Whitney "U" tests.

$$\text{Viability Index} = \frac{\text{No. of live pups on Day 4 } \textit{post-partum} \text{ (pre-cull)}}{\text{No. of live pups on Day 0 } \textit{post-partum}} \times 100$$

$$\text{Survival Index} = \frac{\text{No. of live pups on Day 7 or 10 } \textit{post-partum}}{\text{No. of live pups on Day 4 } \textit{post-partum} \text{ (post cull)}} \times 100$$

$$\text{Lactation Index} = \frac{\text{No. of live pups on Day 21 } \textit{post-partum}}{\text{No. of live pups on Day 11 } \textit{post-partum} \text{ (post-cull)}} \times 100$$

Intergroup variation in litter mean pup body weight and group mean litter weight were analyzed using a one-way analysis of variance; significant differences between control and treated groups were analyzed using the Dunnett's test.

Behavioral Data: The mean day of physical development per litter by sex and for the sexes combined were analyzed using the Kruskal-Wallis and Mann Whitney "U" tests. Motor activity counts were analyzed using repeated measures analysis and graphical presentation of the data was made. Startle habituation data were averaged for each 10 counts and then analyzed using repeated measures analysis. Group mean latency times for the passive avoidance test were analyzed using the Fisher exact probability test. Group escape mean times for the water maze were analyzed using a one-way analysis of variance; significant differences of control and treated groups were compared using the Dunnett's test. The errors for the water maze were analyzed using the Kruskal-Wallis and Mann Whitney "U" tests.

Morphometry Data: Group variances were compared using Bartlett's test. When differences between group variances were not significant ($P>0.001$), Dunnett's test was used to compare the control and high dose group. When differences between group variances were significant ($P<0.001$) by Bartlett's test, the control and high dose group were compared using Dunn's or Wilcoxon's test.

2. Historical Control Data - provided in Appendix 50, page 1785. Data on the following measurements were submitted: length of gestation, number of pups at birth (live and dead), number of implantation scars, post-implantation loss (%) and gestation index (%).

3. Positive Control Data - no data were submitted.

II. RESULTS

A. MATERNAL TOXICITY (F0 Generation)

1. Mortality and Clinical Observations: There were no deaths or sacrifices in poor condition in the carbaryl-treated groups which were attributed to treatment. Animal no. 1528 in the Control group died on Day 11 of gestation after showing salivation and irregular respiration. There were no findings at necropsy examination which

indicated the cause of death. In the 10 mg/kg/day group, one animal (no. 4530) was found dead on gestation Day 6, without prior clinical findings. Since this animal was in the cholinesterase phase and death occurred after the blood sample had been obtained, it was considered that the bleeding procedure may have contributed to the death. A Control group animal (no. 1512) was euthanized in poor condition on gestation day 12. Clinical findings for this animal included a skin lesion on the tail and vocalization. There were no treatment-related clinical signs seen in the remaining animals.

2. Body Weight: At the 10 mg/kg/day level, there was reduced body weight gain ($p < 0.01$) between days 6 and 9 of gestation. There were no other differences in body weights or body weight gains during gestation and lactation in the treated groups. Selected body weight and body weight gain data are summarized in Tables 3 and 4 below.

TABLE 3: MATERNAL BODY WEIGHT (gm)[a]

Interval	Dose in mg/kg/day			
	Control	0.1	1.0	10.0
Pretreatment:				
GD[b] 0	236.9	236.4	238.7	239.9
GD 6	270.6	271.1	273.3	274.1
Treatment:				
GD 9	277.3	278.8	280.5	274.6
GD 12	289.1	290.9	294.2	287.5
GD 15	306.9	306.7	309.7	303.0
GD 20	370.0	363.5	374.5	364.5
LD 0	278.0	282.0	287.6	272.9
LD 4	294.9	297.9	303.5	289.8
LD 11	320.3	320.4	329.1	321.0
LD 21	308.9	316.2	320.8	315.0

[a] Data extracted from Tables 2 and 3 (pages 70-71) of the study report
[b] GD = Gestation Day; LD = Lactation Day

19

TABLE 4: MATERNAL BODY WEIGHT GAIN(gm)[a]

Interval	Dose in mg/kg/day			
	Control	0.1	1.0	10.0
Pretreatment: GD[b] 0-6	33.8	34.7	34.6	34.3
Treatment:				
GD 6-9	6.6	7.7	7.2	0.5**
GD 9-12	11.8	12.2	13.7	13.0
GD 12-15	16.2	15.8	15.5	15.4
LD 0-4	16.8	15.9	15.9	16.9
LD 7-11	13.9	14.4	17.2	18.4
LD 13-21	-14.3	-5.8	-8.4	-9.3

[a] Data extracted from Tables 4 and 5 (pages 72-73) of the study report
[b] GD = Gestation Day; LD = Lactation Day
**Statistically different from controls $p < 0.01$

3. Food Consumption: Food consumption was not measured in this study.

4. Test Substance Intake: This parameter was not necessary since this was a gavage study.

5. Pregnancy Status and Litter Data: There were no effects observed on maternal performance parameters of pregnancy rate, gestation index, length of gestation, numbers of live pups, dead or malformed pups, implantation scars, sex ratio or post-implantation loss. The number of dead pups in the 10 mg/kg/day group was slightly higher than the control value. The study report states that the mean number per litter (0.3) was within the historical control range (0.0 - 0.9), therefore this difference was not considered toxicologically important. The historical control data are based on 12 studies conducted with Sprague-Dawley rats from 1988-1994.The location of the studies is not specifically identified; the historical control data only lists Charles River - Kingston, which is most likely the supplier of the animals. The mean number of dead pups in the 12 studies was 0.29. The ranges for the group mean reproduction parameters follow:

20

	High	Low
Length of Gestation (days)	22.0	21.4
No. of live pups at birth	16.7	13.2
No. of dead pups at birth	0.88	0
No. of implantation scars	17.9	14.9
Post-implantation loss (%)	12.2	3.9
Gestation index (%)	100.0	95.0

There was a statistically significant difference in the sex ratio for the 1 and 10 mg/kg/day groups. The study report states that the difference was not of biological significance. The sex ratio for studies where dosing starts after implantation is used to assess whether any post-implantation loss is affecting primarily one sex. Therefore, since no effect upon the post implantation loss or number of live fetuses was seen, these differences must be related to biological variation. In addition, the percentage of males for the control group was unusually low, which is why the biologically normal values at the mid- and high-dose were statistically significant. These data are summarized in Table 5 below:

21

TABLE 5 Litter Data[a]

Observation	Dose (mg/kg/day)			
	0	0.1	1.0	10
Number Mated	26	26	26	26
Number Pregnant	22	24	24	24
Pregnancy Rate (%)	84.6	92.3	92.3	92.3
Gestation Index (%)	95.5	95.8	100.0	100.0
Gestation Length (days)	21.6	21.7	21.5	21.8
Number with Live Litters	21	23	24	24
Total Number of Pups Born	315	322	358	368
Live Pups Born/litter	14.9	13.9[b]	14.8	15.0
Pups Dead at Birth	2	2	3	8
Mean No. of Dead Pups at Birth	0.1	0.1	0.1	0.3
Sex Ratio (% males)	41.6	43.3	53.3**	52.0**
No. Of Implantation Scars/litter	15.9	15.3[b]	15.7	15.9
Post Implantation Loss (%)	6.1	8.8	6.5	5.8

[a] Data taken from Table 10 of the report
[b] Includes only animals with live litters
**Statistically different from controls $p<0.01$

6. Functional Observational Battery: A number of parameters were observed to be affected in the assessment of females in the 10 mg/kg/day group during the dosing period (gestation day 6 to *post partum* day 10). The incidence of females with decreased pupil size, as shown by pinpoint pupils, was increased on all occasions during the dosing period. An increased incidence of dams with slight tremors affecting the head, body and/or limbs was noted on the majority of assessment occasions in the dosing period. There were also occasional occurrences of ataxic gait/overall gait incapacity in the 10 mg/kg/day group. There was variability in the incidence of hypotonic gait, a common finding, particularly in late gestation. The study report states that, by itself, this finding is not considered of neurotoxicological significance; however, in the 10 mg/kg/day level it was seen in combination with other effects upon gait (ataxic gait/overall gait incapacity) (Table 6). For the 0.1 and 1 mg/kg/day groups there were no effects clearly attributable to treatment.

12

TABLE 6 FOB Data[a]

Group	Day of Observation										
	GD6[b]	GD9	GD12	GD15	GD18	GD20	LD4	LD7	LD11	LD13	LD21
Incidence (%) of Slight Ataxic Gait											
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	8.7	3.8	7.7	0	0	3.8	12.5	0	0	0	0
Incidence (%) of Slight Hypotonic Gait											
1	26.1	26.9	19.2	28.0	20.0	36.0	14.3	23.8	38.1	19.0	4.8
2	26.9	7.7	19.2	42.3	38.5	19.2	13.0	26.1	21.7	21.7	4.3
3	26.9	38.5	50.0	53.8	42.3	42.3	29.2	37.5	25.0	12.5	12.5
4	47.8	42.3	42.3	38.5	57.7	50.0	16.7	33.3	29.2	25.0	25.0
Incidence (%) of Slight Overall Gait Incapacity											
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	8.7	3.8	7.7	0	0	0	12.5	0	0	0	0

Table 6 continued

Group	Day of Observation										
	GD6[b]	GD9	GD12	GD15	GD18	GD20	LD4	LD7	LD11	LD13	LD21
Incidence (%) of Slight Tremors											
1	4.3	0	0	0	0	0	0	0	0	0	0
2	7.7	0	3.8	0	0	0	0	0	0	0	0
3	7.7	7.7	0	0	3.8	0	4.2	0	0	0	0
4	21.7	15.4	11.5	0	15.4	30.8	4.2	0	0	0	0
Incidence (%) of Pinpoint Pupil Size											
1	0	0	3.8	8.0	4.0	4.0	0	0	0[b]	0	0
2	3.8	0	0	11.5	3.8	0	4.3	0	4.3	0	0
3	0	0	0	0	7.7	3.8	0	12.5	0[c]	0	0
4	39.1	15.4	26.9	46.2	50.0	61.5	25.0	50.0	0	0	0

[a] Data extracted from Table 6 (pages 74-106) of the study report
[b] GD = gestation day; LD = lactation day
[c] Based on 20 animals
[d] Based on 23 animals

7. Blood and Brain Cholinesterase: RBC and whole blood ChE levels in the 10 mg/kg/day group were slightly or significantly decreased ($P<0.05$ or $p < 0.01$) for post-dosing assessment occasions, generally from gestation Day 20 through *post partum* day 10. Although there were no statistically significant differences from control for the plasma ChE values, the percentage decrease from control was >30% for GD 20, LD 4 and LD 10. The brain showed a significant decrease ($p < 0.01$) in cholinesterase levels at the 10 mg/kg/day dose level. No effects were noted in any of these parameters at 0.1 or 1.0 mg/kg/day (Tables 7, 8, 9, 10). It is noted that the standard deviations for the whole blood, RBC and plasma ChE measurements were very large as shown in Table 7.

TABLE 7: RBC ChE Data - F0 Generation (U/L)[a]

	Dose in mg/kg/day			
Interval	Control	0.1	1.0	10.0
Pretreatment:				
GD[b] 6	980.2	1039.8	942.8	1093.2
SD	333.87	130.98	151.35	175.94
Treatment:				
GD 6	988.3	990.0	913.5	800.0 (19)
SD	204.71	129.77	272.84	194.49
GD 15	1127.0	1245.8	1203.5	1064.3 (6)
SD	172.19	195.29	249.12	325.46
GD 20	1173.7	1171.7	1251.2	845.4** (28)
SD	86.84	91.94	179.24	93.90
LD 4	844.3	869.3	943.0	752.2 (11)
SD	170.02	181.60	113.56	127.43
LD 10	894.3	938.0	933.0	643.2** (28)
SD	14.84	91.64	127.11	41.25

(Percentage decrease from control value)

[a] Data extracted from Table 7 (pages 107-110) of the study report

[b] GD = Gestation Day; LD = Lactation Day; SD = Standard Deviation

**Statistically different from controls $p<0.01$

25

TABLE 8: Plasma ChE Data-F0 Generation (U/L)[a]

Interval	Dose in mg/kg/day			
	Control	0.1	1.0	10.0
Pretreatment: GD[b] 6	936.3	950.0	873.6	1089.2
Treatment:				
GD 6	844.8	964.4	902.0	697.8 (17)
GD 15	981.7	1097.5	1149.5	975.0 (1)
GD 20	1049.0	1134.2	1124.2	644.0 (39)
LD 4	729.0	696.7	702.0	498.2 (32)
LD 10	560.0	491.2	539.2	359.0 (36)

(Percentage decrease from control value)

[a] Data extracted from Table 7 (page 109) of the study report

[b] GD = Gestation Day; LD = Lactation Day

TABLE 9: Whole Blood ChE Data-F0 Generation (U/L)[a]

Interval	Dose in mg/kg/day			
	Control	0.1	1.0	10.0
Pretreatment: GD 6[b]	954.5	987.7	982.0	1088.8
Treatment:				
GD 6	899.5	973.6	905.7	735.7 (18)
GD 15	1013.3	1151.7	1164.0	1009.0 (0)
GD 20	1084.7	1147.3	1164.0	710.4* (34)
LD 4	771.3	761.3	770.0	585.6 (24)
LD 10	695.3	666.3	693.0	469.2* (32)

(Percentage decrease from control value)

[a] Data extracted from Table 7 (page 108) of the study report

[b] GD = Gestation Day; LD = Lactation Day

* Significantly different from control value ($P < 0.05$)

26

TABLE 10: Brain ChE Data - F0 Generation (U/G)[a]

Interval	Dose in mg/kg/day			
	Control	0.1	1.0	10.0
Treatment: LD 10	5.9	6.2	5.8	3.4** (42)

(Percentage decrease from control value)

[a] Data extracted from Table 8 (page 110) of the study report

[b] GD = Gestation Day; LD = Lactation Day

**Statistically different from controls $p<0.01$

8. Necropsy Findings

A summary table and individual animal data were provided for gross necropsy findings on the F0 generation animals sacrificed at Day 21 *post-partum*. There was no evidence of a treatment-related effect on these findings.

B. F1 GENERATION TOXICITY - PUPS

1. Pup Body Weights: Pup body weights (male, female and total) were unaffected by treatment. The pup body weight data are presented in Table 11.

TABLE 11: PUP BODY WEIGHT DATA[a]

Mean Pup Weight (grams)	Dose (mg/kg/day)			
	0	0.1	1.0	10.0
Lactation Day 0:				
Males-	6.2	6.2	6.1	6.0
Females-	5.8	5.9	5.7	5.7
Lactation Day 4 (post-Cull)[b]:				
Males-	9.3	9.5	9.2	8.9
Females-	8.9	9.2	8.7	8.6
Lactation Day 11:				
Males-	24.3	24.4	24.6	23.6
Females-	23.5	23.5	23.4	22.7
Lactation Day 17:				
Males-	40.9	40.8	40.9	40.2
Females-	39.3	39.3	39.0	38.7
Lactation Day 21:				
Males-	53.2	53.0	52.8	53.0
Females-	51.1	51.3	50.1	50.3

[a] Data taken from Table 14 (pages 121-122) of the study report
[b] Pre-cull data were not supplied

2. Pup Survival Indices: Pup survival was unaffected by treatment during gestation and lactation. The data are presented in Table 12 below:

TABLE 12: PUP SURVIVAL INDICES[a]

Observation	Dose (mg/kg/day)			
	0	0.1	1.0	10.0
Day 4 Viability Index-	99.1	99.1	99.5	98.3
Day 7 Survival Index-	99.4	100.0	100.0	100.0
Day 21 Lactation Index-	100.0	99.4	100.0	100.0

[a] Data taken from Table 13 (page 116) of the study report

3. Developmental Landmark Data: The mean day of development of tooth eruption and eye opening was not significantly impacted by treatment with carbaryl.

TABLE 13: DEVELOPMENTAL LANDMARK DATA[a]

Observation (Mean Day to Criteria for all Pups)	Dose (mg/kg/day)			
	0	0.1	1.0	10.0
Development of Tooth Eruption	10.0	10.0	9.9	9.7
Development of Eye Opening	14.2	14.4	14.5	14.2

[a] Data taken from Tables 15 and 16 (pages 124-129) of the study report

4. Clinical Condition and Observational Battery: There were no clinical observations for the F1 generation pups that were considered to be related to treatment. The FOB evaluations used to assess behavioral status also showed no treatment-related effects. There was an increased incidence of moderate hypotonic gait, slight impaired gait and moderate overall gait incapacity in the 1.0 mg/kg/day group at post-partum Day 13. However, the effect was not seen at the 10 mg/kg/day dose.

5. Motor Activity: The study report states that activity counts for the male and female pups on Days 13, 17, and 21 *post partum* were similar across all groups on all occasions. Although there were no statistically significant changes and no dose-response relationship, the mean for the total activity counts of the 10 mg/kg/day groups did differ from the control. The largest difference was for the Day 13 measurements when the mean total counts for the females was 66% increased as compared to the controls. However, there was wide variability in these data with some standard deviations exceeding the mean value. No historical or positive control data were submitted; therefore no definitive conclusions could be drawn at this time regarding treatment-related effects on pup motor activity. The means of the six intervals for each group are presented in Table 14.

29

Table 14: Mean Activity Counts[a]

	Dose Level (mg/kg/day)			
	0	0.1	1.0	10.0
Males				
Day 13 - Mean S.D.	59.9 53.2	62.4 47.9	60.2 53.0	68.3 76.2
Day 17 - Mean S.D.	143.4 112.5	137.2 101.0	140.6 102.5	100.9 93.4
Day 21 - Mean S.D.	98.0 66.2	83.7 31.7	85.6 41.1	92.3 52.0
Females				
Day 13 - Mean S.D.	67.1 75.6	75.1 63.3	52.1 55.8	111.0 114.8
Day 17 - Mean S.D.	129.0 89.0	113.7 108.9	160.9 128.7	108.4 64.7
Day 21 - Mean S.D.	98.3 56.4	88.2 27.0	114.8 56.0	105.8 64.6

a Extracted from Table 18 (pages 139-144) of the study report.

6. Sacrifice and Pathology:

a. Macroscopic and Microscopic Pathology F1 Generation Pups - There were few gross or microscopic neuropathology lesions observed for animals examined in this study and none were attributable to treatment with the test article.

b. Brain Weights F1 Generation Pups - Whole and regional brain weights showed no significant differences for male or female pups in the carbaryl-treated groups.

c. Brain Morphometry - Significant differences were noted in the measurements of the right parietal cortex (line B) in the 10 mg/kg/day males and of the widest point of the long axis of the left cerebellum (line F) in the males and females and of the right cerebellum (line F) in females. The decreases in the right and left cerebellum of the 10 mg/kg/day females were 15% and 22%, respectively. The data are presented in Table 15 below. The pathologist's report states that the differences are

30

not treatment-related because of the following observations: no significant difference was usually seen in the contralateral measurement; the variability of the group mean calculated for a similar line in controls (line F, right and left cerebellum, male) may have influenced the degree of significance between controls and high dose animals (line F, left cerebellum, male); a different trend was observed between males and females for the same structure (line F, left and right cerebellum, increased in males, decreased in females); normal tissue architecture with moderate individual variability in maturation was noted during microscopic evaluation; no obvious difference in the whole and regional brain weights in high dose pups compared to controls. In addition, some of the differences in the cerebellum could have been related to technical preparation of the slides.

Table 15: Brain Morphometric Measurements in Pups[a]

		Line B Rt. Forebrain Mean SD (μm)	Line B Left Forebrain Mean SD (μm)	Line F Rt. Cerebellum Mean SD (μm)	Line F Left Cerebellum Mean SD (μm)
Group 1 Control	Male	1497.71 80.875	1549.47 44.996	4380.93 500.246	3929.16 322.175
Group 4 10 mg/kg/day		1613.64* 40.089	1555.19 83.458	4734.26 515.940	4707.18* 563.820
Group 1 Control	Female	1554.70 126.754	1598.07 98.205	4440.99 535.429	4601.68 440.127
Group 4 10 mg/kg/day		1553.15 50.665	1635.92 50.112	3753.68* 387.062	3582.04** 417.496

a Extracted from Table 25 (pages 163-164) of the study report.
* Significantly different from control ($P < 0.05$, Dunnett's)
** Significantly different from control ($P < 0.01$, Dunnett's)

C. F1 GENERATION TOXICITY - ADULTS

1. Body Weights: Body weights of the F1 adults were unaffected by treatment. The adult body weight data are presented in Table 16.

TABLE 16: ADULT BODY WEIGHT DATA[a]

Mean Adult Weight (grams)	Dose (mg/kg/day)			
	0	0.1	1.0	10.0
Week 0:				
Males-	79.9	79.9	81.1	79.9
Females-	79.3	79.7	80.5	79.2
Week 1:				
Males-	132.4	133.3	135.5	132.2
Females-	120.0	122.4	123.3	120.7
Week 2:				
Males-	195.2	195.8	198.5	193.1
Females-	155.9	157.4	157.9	154.3
Week 4:				
Males-	318.1	320.8	327.0	316.3
Females-	200.9	202.7	206.2	199.3
Week 6:				
Males-	407.9	410.3	417.9	404.7
Females-	237.3	237.3	239.4	233.2

[a] Data taken from Table 27 (pages 166-167) of the study report

2.Clinical Condition: There were no clinical observations for the F1 generation pups that were considered to be related to treatment. Two female weanlings in the 1 mg/kg/day group (Nos. 3508 and 3509) and one male weanling (No. 2026) in the 0.1 mg/kg/day group died on Days 26, 25, and 24 post partum respectively, without prior clinical signs. Gross pathological findings for these animals showed dark areas on the gastric mucosa for No. 2026 and dark discoloration of the ingesta/digesta for Nos. 3508 and 3509.

3.Physical Development: The mean days for development of vaginal opening and preputial separation were not significantly affected by treatment with carbaryl (Table 17).

92

TABLE 17: DEVELOPMENTAL LANDMARK DATA[a]

Observation (Mean Day to Criteria for all Pups)	Dose (mg/kg/day)			
	0	0.1	1.0	10.0
Vaginal Opening	30.9	30.8	30.8	30.9
Preputial Separation	43.5	43.1	43.1	43.2

[a] Data taken from Tables 29 and 30 (pages 170-171) of the study report

4. Motor Activity: The mean activity counts on Day 60 for males and females were similar across all groups.

5. Auditory Startle Response: There were no toxicologically significant differences between groups for the mean startle data. For males on Day 22 *post partum* the linear constructed variable for maximum startle voltage was statistically significantly different ($P < 0.05$) at the 0.1 mg/kg/day. The study report states that this was not attributed to treatment since there was no dose response and there was no effect on Day 60 *post partum*. However, there was much variability in the data, especially for maximum startle voltage.

6. Passive Avoidance: For males and females the incidences and times for the learning trials (1 and 2) on Days 23/24 were similar. For the 1 and 25 hour memory trials there was some intergroup variation, but these differences did not attain statistical significance.

7. Water Maze: There were no significant differences between groups for the times to complete the maze or for the number of errors incurred on Days 60/65.

8. Sacrifice and Pathology:

a. Macroscopic and Microscopic Pathology F1 Generation Adults - There were no gross or microscopic neuropathological lesions observed for animals examined in this study that were attributable to treatment with the test article. There was an increased incidence of retinal fold/rosette in the 10 mg/kg/day group (1/12 for control vs. 4/12 for males; 0/12 for control vs. 2/12 for females.) The study report states that these findings were not attributed to treatment due to the lack of a dose dependent response, the minimal effects, and comparability with the historical control range. Historical control data in males from 5 studies (1995-1997) of 28-35 days duration in 52 animals showed a 11.5% mean incidence of retinal fold/rosette; the range was 0-40%. Historical control data in females

33

from 4 studies (1995-1997) in 42 animals showed a mean of 7.1% and range of 0-10%.

b. Brain Weights F1 Generation Pups - Whole and regional brain weights showed no significant differences for male or female adult animals in the carbaryl-treated groups.

c. Brain Morphometry - Slides prepared from the left cerebellum of 4/6 animals in the high dose group were not suitable for measurements (line F). Therefore, additional histomorphometric evaluation was done on the left and right cerebellum. Significant differences were noted in the measurement of the left neocortex - cingulum (line A) in males, of the widest point of the long axis of the right cerebellum (line F) in males (first evaluation) and of the line perpendicular to and at the midpoint of the long axis (line G) in females (second evaluation). The right and left cerebellar increases in Line G were 18.8% and 29.8% The data are presented in Tables 18 and 18a. The pathologist's report states that the differences were not treatment related for the reasons described for the pups. In addition, the brain length and width taken in adult animals were comparable between control and high dose animals. It was also noted that in the cerebellum, the statistically significant differences involved different structures (line F vs line G) and sexes (male vs female) when the first and second evaluations were compared.

Table 18: Brain Morphometric Measurements in Adults[a]

		Line B Rt. Forebrain Mean SD (μm)	Line B Left Forebrain Mean SD (μm)
Group 1 Control	Male	1743.74 200.029	1819.05 74.852
Group 4 10 mg/kg/day		1608.81 135.278	1641.06* 116.697
Group 1 Control	Female	1818.30 102.888	1764.47 120.376
Group 4 10 mg/kg/day		1723.89 128.136	1636.13 129.172

a Extracted from Table 39 (pages 194-196) of the study report.
* Significantly different from control ($P<0.05$, Dunnett's)

Table 18a: Brain Morphometric Measurements for Adults - First and Second Examinations[a]

		Line F Rt. Cerebellum Mean SD (μm)	Line F Left Cerebellum Mean SD (μm)	Line G Rt. Cerebellum Mean SD (μm)	Line G Left Cerebellum Mean SD (μm)
First Examination					
Group 1 Control	Male	6201.13 265.726	6139.81 298.621	4566.79 556.330	4602.84 491.233
Group 4 10 mg/kg/day	Male	6718.66* 310.685	6186.32 422.015	4870.79 283.794	4885.12 465.128
Group 1 Control	Female	6217.21 222.922	6219.86 450.270	4724.84 149.327	4557.05 372.813
Group 4 10 mg/kg/day	Female	6280.37 447.451	6287.54 440.619	4437.91 428.392	4473.14 334.580
Second Examination					
Group 1 Control	Male	6480.99 572.174	6643.26 363.806	5283.18 648.375	4971.00 314.405
Group 4 10 mg/kg/day	Male	6778.12 642.408	6749.61 387.596	4961.01 421.550	4767.13 392.040
Group 1 Control	Female	5209.11 463.305	6004.50 350.148	4422.60 374.102	4222.40 201.219
Group 4 10 mg/kg/day	Female	5260.90 434.138	5674.52 781.087	5256.32** 338.986	5480.01** 464.472

a Extracted from Table 39 (pages 194-196) of the study report.
* Significantly different from control ($P<0.05$, Dunnett's)
** Significantly different from control ($P<0.01$, Dunnett's)

III. DISCUSSION

A. Investigator's Conclusions - Transitory behavioral effects included autonomic effects and tremors for the high dose group (10 mg/kg/day) dams dosed from day 6 of gestation to day 10 *post partum*. Also at this dose level, RBC, plasma, and brain cholinesterase levels were decreased. Development of the offspring, including survival, growth, and physical development was unaffected. There were no behavioral changes among these offspring, nor was there any neuropathological finding which indicated

35

that carbaryl, technical grade, produced a developmental neurotoxic effect.

B. Reviewer's Conclusions

In this developmental neurotoxicity study, 26 pregnant female Sprague-Dawley rats/group were administered carbaryl (99.1% a.i.) by gavage from Gestation Day (GD) 6 through Lactation Day (LD) 10 at doses of either 0, 0.1, 1.0 or 10 mg/kg/day. An additional 6 pregnant females/group were dosed at the same levels for the cholinesterase (ChE) phase of the study. ChE measurements were done pre-dosing (GD 6) and post-dosing at time of peak effect (1 hour post-dosing) on GD 6, 15 and 20 and LD 4 and 10. Functional Observational Battery (FOB) measurements were performed at approximately 0.5 and 2 hours post-dosing on the same days as body weight measurements during the dosing period (GD 0, 6, 9, 12, 15, 18 and 20 and LD 4, 7, 11, 13 and 21). Measures of reproductive performance were evaluated. Offspring were examined for body weight, physical development landmarks (tooth eruption and eye opening), FOB assessments and motor activity. On LD 11, 1 animal/sex/litter was sacrificed for brain weights and neuropathological evaluation. After LD 21, 3 animals/sex/litter were separated from the dams and constituted the F1 adult generation. These animals were evaluated for body weight, physical development (vaginal opening and preputial separation), motor activity, startle habituation response, passive avoidance and water maze behavior. After completion of the behavior test period (at approximately 10 weeks of age), 12 animals/sex/group were anesthetized and perfused for post-mortem examination. Of 6 animals/sex/group, tissues from the control and high dose group were processed for neuropathological evaluation and morphometric measurements, as well as the eyes from the low and mid-dose group of all perfused animals.

For the F0 generation animals, there were no carbaryl-associated deaths. One animal in the 10 mg/kg/day group of the cholinesterase phase was found dead on GD6 without prior clinical signs of toxicity. Since death occurred after a blood sample was taken, the bleeding procedure may have contributed to the death. No treatment-related clinical signs of toxicity were observed. There was a statistically significant decrease (92%) in body weight gain for females in the 10 mg/kg/day group for the period GD 6-9. No food consumption data were available. During the FOB measurements, the incidence of females in the 10 mg/kg/day group with decreased pupil size (pinpoint pupils) was increased on all occasions during the dosing period. An increased incidence of dams with slight tremors affecting the head, body and/or limbs was noted on the majority of assessment occasions in the dosing period. There were also occasional occurrences of ataxic gait/overall gait incapacity in the 10 mg/kg/day group. There was variability in the incidence of hypotonic gait, a common finding, particularly in late gestation. The study report states that, by itself, this finding is not considered of neurotoxicological significance; however, in the 10 mg/kg/day level it was seen in combination with other

3C

effects upon gait (ataxic gait/overall gait incapacity).

There were no effects observed on maternal performance parameters of pregnancy rate, gestation index, length of gestation, numbers of live pups, dead or malformed pups, implantation scars, sex ratio or post-implantation loss. The number of dead pups in the 10 mg/kg/day group was slightly higher ($P > 0.05$) than the control value. The study report states that the mean number per litter (0.3) was within the historical control range (0.0 - 0.9), therefore this difference was not considered toxicologically important. The historical control data are based on 12 studies conducted with Sprague-Dawley rats at Charles River, Kingston, from 1988-1994. The mean number of dead pups in the 12 studies was 0.29.

For the 10 mg/kg/day group, RBC ChE levels were statistically significantly decreased (28%) on GD 20 and LD 10. Whole blood ChE levels were significantly decreased (32-34%) on the same days. Although the plasma ChE levels were not statistically significantly altered, the percentage decrease on GD 20, LD 4 and LD 10 were 32-39% in relation to the control value. Brain ChE levels were statistically significantly decreased (42%). There were no treatment-related effects on gross necropsy findings for the F0 generation animals.

For the F1 generation pups, there were no treatment-related effects on pup weight, pup survival indices, developmental landmarks (tooth eruption and eye opening) or FOB measurements. Although there were no statistically significant changes and no dose-response relationship for motor activity, the mean for the total activity counts of the 10 mg/kg/day groups did differ from the control. The largest difference was for the Day 13 measurements when the mean total counts for the males was 66% increased as compared to the controls. However, there was wide variability in these data with some standard deviations exceeding the mean value. The registrant should submit historical control data for motor activity in the age of rats.

At sacrifice on LD 11, there were no treatment-related effects on brain weights or gross and microscopic pathology. In the morphometric measurements, significant differences were noted in the measurements of the right parietal cortex (line B) in the 10 mg/kg/day males and of the widest point of the long axis of the left cerebellum (line F) in the males and females and of the right cerebellum (line F) in females. The decreases in the right and left cerebellum of the 10 mg/kg/day females was 15% and 22%, respectively. The pathologist's report states that the differences are not treatment-related because of the following observations: no significant difference was usually seen in the contralateral measurement; the variability of the group mean calculated for a similar line in controls (line F, right and left cerebellum, male) may have influenced the degree of significance between controls and high dose animals (line F, left cerebellum, male); a different trend was observed between males and females for the same structure (line F, left and right

37

cerebellum, increased in males, decreased in females); normal tissue architecture with moderate individual variability in maturation was noted during microscopic evaluation; no obvious difference in the whole and regional brain weights in high dose pups compared to controls. In addition, some of the differences in the cerebellum could have been related to technical preparation of the slides. In the opinion of the EPA reviewers, these cerebellar effects in the 10 mg/kg/day females should not be disregarded for the following reasons: 1) the decreases were seen bilaterally; 2) female rats also had decreases ($P > 0.05$) in cerebellar weights. The percentage decreases for the 0.1, 1.0 and 10.0 mg/kg/day groups compared to control were 6%, 11% and 5%, respectively.

For the F1 generation adults, there were no treatment-related effects on clinical condition, body weight, physical development (vaginal opening and preputial separation), motor activity, auditory startle response, passive avoidance and water maze measurements. At sacrifice, there were no gross or microscopic neuropathological lesions observed for animals examined in this study that were attributable to treatment with the test article. There was an increased incidence of retinal fold/rosette in the 10 mg/kg/day group (1/12 for control vs. 4/12 for males; 0/12 for control vs. 2/12 for females.) The study report states that these findings were not attributed to treatment due to the lack of a dose dependent response, the minimal effects, and comparability with the historical control range. Historical control data in males from 5 studies (1995-1997) of 28-35 days duration in 52 animals showed a 11.5% mean incidence of retinal fold/rosette; the range was 0-40%. Historical control data in females from 4 studies (1995-1997) in 42 animals showed a mean of 7.1% and range of 0-10%.

For the morphometric measurements, significant differences were noted in the measurement of the left neocortex - cingulum (line A) in males, of the widest point of the long axis of the right cerebellum (line F) in males (first evaluation) and of the line perpendicular to and at the midpoint of the long axis (line G) in females (second evaluation).The right and left cerebellar increases in Line G were 18.8% and 29.8% The pathologist's report states that the differences were not treatment related for the reasons described for the pups. In addition, the brain length and width taken in adult animals were comparable between control and high dose animals. It was also noted that in the cerebellum, the statistically significant differences involved different measurements (line F vs line G) and sexes (male vs female) when the first and second evaluations were compared. In the opinion of the EPA reviewers, these effects should not be disregarded since they are consistent with the findings in the Day 11 measurements, i.e., they occurred at the same area of the brain in the same sex.

The high dose selected for the study was based on three previous studies measuring the 13-week toxicity of the chemical, the time to peak effect and the time course of cholinesterase inhibition. The study report states that the dose level of 10 mg/kg/day was expected to produce clear effects on nervous system function, in terms of behavior

and neurochemistry. Based on the effects observed, the animals could have endured a higher dose which would have better tested the developmental effects of the chemical. The guidance on dose levels in the OPPTS 870.6300 guidelines states that the maximum dose should not induce *in utero* or neonatal deaths or malformations sufficient to preclude a meaningful evaluation of neurotoxicity, if the chemical has been shown to be developmentally toxic in other studies. The high dose should induce some overt maternal toxicity, but should not result in a reduction in weight gain exceeding 20% during gestation and lactation, if standard developmental studies have not been conducted. In the present study with carbaryl, the effects on body weight gain at 10 mg/kg/day appeared to be substantial (95% decrease) but were limited to a short time period (GD 6-9) and constituted only a 6 gram difference between the control and high dose mean values, after which the animals responded in GD 9-12 by gaining more weight than the controls. The only other effects on the maternal animals were observed in the FOB measurements and ChE inhibition. All of the increases in FOB measurements were in the slight category. The decreases in ChE were not observed until GD 20. The plasma, RBC and whole blood ChE levels never exceeded more than a 39 % depression; the most significant effect was the 42 % decrease in brain ChE.

The maternal toxicity LOEL was 10 mg/kg/day based on decreased body weight gain, alterations in Functional Observational Battery measurements and RBC, plasma, whole blood and brain cholinesterase inhibition. The maternal NOEL was 1.0 mg/kg/day.

The tentative developmental neurotoxicity LOEL was 10 mg/kg/day based on alterations in the morphometric measurements at Days 11 and 60; the NOEL was 1.0 mg/kg/day.

IV. STUDY DEFICIENCIES

1. The findings in the morphometric measurements are judged to be possibly treatment related. At Day 11, the 10 mg/kg/day females had a significant bilateral decrease in the length of the cerebellum accompanied by a slight decrease ($P > 0.05$) in the weight of the cerebellum. At Day 60, these animals had a significant bilateral increase in the width of the cerebellum. Forebrain measurements were also affected. **Therefore, the registrant/study author should conduct additonal morphometric measurements on the mid- and low-dose groups to define a NOEL and to describe more fully the cell layers in these areas as well as the thickness of cell layers, as described in the OPPTS.6300 guidelines.**

2. The level of carbaryl administered to the 0.1 mg/kg/day group was significantly less than the nominal amount. At Week 1, the range of the percentage of nominal value for three preparations was 55% - 79%. The acceptance criteria was then changed from ± 15% to ± 25 %. At Week 2, the average recovery was 80% of nominal and all other

samples were within the revised specification. This reduced dose to the low-dose group could affect the results of the study depending on the morphometric evaluations of the low- and mid-dose groups, as requested in item 1 above. For the 1.0 mg/kg/day group, the average Week 1 recoveries were 84.6 and 74.1%; the remaining weeks were within the original ±15% acceptance criteria. For the 10 mg/kg/day group, the average Week 1 recoveries were 82.3 and 91.3%; the remaining weeks were within the original ±15% acceptance criteria.

3. Positive control data should be submitted for motor activity in young pups to validate the data in this study, which were extremely variable.

4. The registrant/study author should explain the variability in the cholinesterase data.

cc: Susan Makris (HED, Toxicology Branch I), William Sette (HED, Science Analysis Branch)

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