A-80M

8-21-98

DATA EVALUATION RECORD

CHLORPYRIFOS 059101

Study Type: §83-6; Developmental Neurotoxicity Study of Chlorpyrifos Administered Orally via Gavage to Crl:CD®BR VAF/Plus® Presumed Pregnant Rats

Work Assignment No. 3-56 (MRID 44556901)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer: Guy R. Beretich, Ph.D.	Signature: Guy Beretich Date: 8-19-98
Secondary Reviewer: Ashlee E. Walker, M.S.	Signature: Ashlee E Walker Date: 20 Aug 98
Project Manager: Mary L. Menetrez, Ph.D.	Signature: Mary L Menetrez Date: 8/21/98
Quality Assurance: Steven Brecher, Ph.D.	Signature: Steven Brecher Date: 8/21/98

Disclaimer

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

EPA Reviewer: Susan Makris, M.S.
Toxicology Branch 1 (7509C)

Susan L Makris 11-13-98

EPA Secondary Reviewer: Kathleen Raffaele, Ph.D.
Toxicology Branch 2 (7509C)

Kathleen C. Raffaele 11/13/98

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat
OPPTS Number: 870.3600

OPP Guideline Number: §83-6

DP BARCODE: D247891
P.C. CODE: 059101

SUBMISSION CODE: S546162
TOX. CHEM. NO.: 660

TEST MATERIAL (PURITY): Chlorpyrifos (99.8% a.i.)

SYNONYMS: Dursban

CITATION: Hoberman, A. M. (1998) Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD®BR VAF/Plus® presumed pregnant rats. Argus Research Laboratories, Inc., Horsham, Pennsylvania, Laboratory study No. 304-001, Sponsor study No. K-044793-109, May 1, 1998. MRID 44556901. Unpublished.

Hoberman, A.M. (1998) (pathology) Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD®BR VAF/Plus® presumed pregnant rats. Argus Research Laboratories, Inc., Horsham, Pennsylvania, Laboratory study No. 304-001, Sponsor study No. K-044793-109, May 1, 1998. MRID 44661001. Unpublished.

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268

EXECUTIVE SUMMARY:

In this developmental neurotoxicity study (MRID 44556901), 25 pregnant Sprague-Dawley rats/group were administered chlorpyrifos (99.8% a.i.) by gavage from gestation day 6 (GD 6) through lactation day 11 at 0, 0.3, 1, or 5 mg/kg/day. An additional 5 pregnant females/group were dosed at the same levels for the cholinesterase (ChE) phase of the study. Dams were examined for body weight, reproductive performance, number of viable pups, and postpartum behavior. During the dosing period, dams were observed daily for signs of autonomic function toxicity. Satellite dams were sacrificed four to five hours post-dosing on GD 20 for ChE analyses to be performed on brain, plasma, and erythrocytes. Offspring were examined for viability at birth, pup/litter survival, body weight, sex ratio, physical development landmarks (eye opening and pinna detachment), observed nursing behavior, and sexual maturation. F_1

generation litters were randomly standardized on lactation day 5 and assigned to 4 subsets for continued observation. On postnatal day (PND) 12, fixed brain weight measurements (10 pups/sex/dose) and neuropathological evaluations including morphometrics (6 pups/sex/dose) were performed on Subset 1 pups, with the remaining 10 pups/sex/dose necropsied for gross lesions. In Subset 2, 8 pups/sex/dose were selected for evaluation of learning and memory; evaluations were performed on PNDs 23-25 and 62-92. These Subset 2 animals were sacrificed on PNDs 97-101, following the last evaluation. The Subset 2 pups not selected for evaluation were necropsied for gross lesions on PND 22. The Subset 3 pups were tested for motor activity on PNDs 14, 18, 22, and 61 and auditory startle habituation on PNDs 23 and 62; all Subset 3 animals were sacrificed on PNDs 63 or 64 following the last evaluation. In Subset 4 pups, fixed brain weights were determined in 10 pups/sex/dose, neuropathological examinations were performed on 6 pups/sex/dose, and all remaining Subset 4 animals (10/sex/dose) were necropsied for gross lesions upon sacrifice on PND 66-77.

Maternal toxicity in the high-dose (5 mg/kg/day) animals was manifested as increased signs of autonomic function toxicity, apparent at the end of gestation as fasciculations (6/25 treated vs 0/25 controls), and during early lactation (days 1-5) as fasciculations (16/24 treated vs 0/25 controls), hyperpnea (8/24 treated vs 0/25 controls), and hyperreactivity (17/24 treated vs 2/25 controls). Dams with all pups dying were increased in the high-dose group (3/23 treated vs 0/25 controls). There were no significant effects on bodyweight, food consumption, or pregnancy parameters. There were no unscheduled deaths in the maternal animals.

Brain ChE activity was decreased in the high-dose (↓90%) and mid-dose (↓18%) dams as compared to control. Erythrocyte (↓41-99%) and plasma (↓43-92%) ChE activities were decreased in a dose-dependent manner in all treated groups.

The maternal toxicity NOAEL was not observed.
The maternal LOAEL was < 0.3 mg/kg/day, based on plasma and RBC cholinesterase inhibition.

For the F_1 generation pups, the high-dose group bodyweights were significantly reduced (↓8-15%) at PND 1 and 5 (pre- and post-culling). Bodyweights were also reduced from birth to PND 22 in Subset 4 high-dose animals (↓5-19%); bodyweight gains were reduced in these animals during the same period (↓5-30%). Additionally, compared to the controls, reduced terminal body weights were observed in the Subset 1 (PND 12) high-dose animals (↓17-19%) and the Subset 4 (PND 66) high-dose males (↓10%). For the F_1 generation adults, body weights of the high-dose males were decreased at PND 22 through 66 (↓11-17% vs controls). High-dose F_1 adult females also weighed less than controls at PND 22 (↓17% vs controls), but were of similar weight at PND 66. Bodyweight gains were also decreased in the high-dose males for the PND 22-40 interval (↓13% vs controls) and PND 40-66 interval (↓7%). Food consumption was decreased immediately after weaning (PND 23-30) in high-dose males and females (↓13% vs controls).

Development as assessed by pinna unfolding was delayed (4.0 days in treated vs 3.5 days in controls) in the high-dose group. Sexual maturation was delayed as assessed by time to preputial

separation (106% of controls) and vaginal patency (103% of controls).

Pup viability was reduced as assessed by the following parameters: surviving pups/ litter (↓27%) and live litter size at culling (↓16%), pup viability index (↓29%), and pups found dead or presumed cannibalized (day 1 - 7.2 % treated vs 0.0% controls; days 2 to 5 - 24.7% treated vs 1.3% controls).

There were no statistically significant differences between the groups in the average acquisition and delay training. Additionally, there were no differences among dose groups when comparing retention of information during PNDs 23-25 and 62-92. Motor activity was decreased in high dose male and female pups on PND 14 (↓56% in males and ↓37% in females), and increased in high dose females on PNDs 18 and 22 (↑51% on both days). There was a statistically significant increase (↑16-25%) in the latency to peak response during the auditory startle habituation assessments on PND 23 in the high-dose animals compared to concurrent controls. At PND 62, the latency to peak response in the high-dose animals was 10-12% higher than in the controls. Additionally, the peak response amplitudes in the high-dose animals were decreased by 9 to 29% on PNDs 23 and 62 (not statistically significant) compared to the controls.

There were no gross or microscopic lesions of the nervous system in Subset 1 or 4 offspring. Subset 1 high-dose males at PND 12 had reduced absolute brain weights (↓9% vs controls), increased relative brain weights (↑13% vs controls), reduced anterior to posterior measurement of the cerebellum (↓24% vs controls), reduced height of the cerebellum (↓14% vs controls), decreased thickness of the parietal cortex (↓6% vs controls), and decreased thickness of the hippocampal gyrus (↓9% vs controls). High-dose female pups had reduced absolute brain weights (↓9% vs controls), increased relative brain weights (↑14% vs controls), thickness of the parietal cortex (↓6% vs controls), width of the caudate-putamen (↓10% vs controls), and thickness of the hippocampal gyrus (↓12% vs controls). In Subset 4 F1 animals, killed on PND 66, morphometric analysis revealed decreased parietal cortex measurements (↓5%) and decreased thickness of the hippocampal gyrus (↓7%) in high-dose females. These measurements were also decreased in mid-dose females (parietal cortex,↓4%; hippocampal gyrus, ↓4%). The statistical significance of the differences in mid-dose females was not evaluated, and there was no evaluation of low dose females. Brain weight in high dose females was similar to control brain weight at day 66 (↓0.3%).

Due to inadequate presentation of the statistical data analysis, it was not possible to determine the definitive developmental neurotoxicity NOAEL and LOAEL for the offspring.
The tentative developmental neurotoxicity LOAEL is 5 mg/kg/day.
The tentative NOAEL is 1 mg/kg/day.

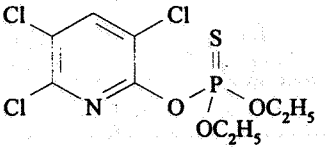
This study in the rat is classified **unacceptable (§83-6)** and does not satisfy the guideline requirements for a developmental neurotoxicity study. The study may be upgradable, following submission of more complete statistical analysis.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Chlorpyrifos
   Description: Technical, white solid
   Lot/Batch #: MM930503-17 (TSN 100227)
   Purity: 99.8 ± 0.1% a.i.
   Storage conditions: Room temperature
   CAS #: 2921-88-2
   Structure:



2. Vehicle: Mazola® corn oil
   Lot/Batch #: 108A7

3. Test animals: Species: Rat
   Strain: Crl:CD®BR VAF/Plus® (Sprague-Dawley)
   Age at mating: Approximately 3½ months
   Weight at mating: 227-293 g
   Source: Charles River Laboratories, Inc., Portage, Michigan
   Housing: Individually in stainless-steel wire-bottomed cages; all dams moved to individual nesting boxes with corn cob bedding (bed-o'cobs®) no later than GD 20
   Diet: Certified Rodent Diet® #5002, ad libitum except as noted during behavior evaluations
   Water: R.O.(reverse osmosis) water, ad libitum except as noted during behavior evaluations
   Environmental conditions:
   - Temperature (nominal): 64 to 79°F
   - Humidity (nominal): 30-70%
   - Air changes: ≥10/hr, HEPA filtered
   - Photoperiod: 12 hrs dark/12 hrs light

   Acclimation period (P): 6 weeks

B. PROCEDURES AND STUDY DESIGN (see appended Figure 1, a study schematic extracted from study report, page 24)

1. In life dates - start: 7/29/97 end: 1/9/98

2. Mating: One female was placed with one breeder male of the same strain and source in the male's cage for a cohabitation period of maximum 7 days. The day of positive identification of spermatozoa in the vaginal smear and/or a copulatory plug observed *in*

situ was termed gestation day (GD) 0; at this time, both animals were returned to their individual cages.

3. Animal Assignment: F_0 females were randomly assigned (weight-ordered) to dose groups as indicated in Table 1. F1 pups (5/sex/litter) were randomly selected from available litters on postnatal day 5. Of these, 4 pups/sex/litter were selected (Table 1) and assigned to testing subsets (Table 2) as available.

Table 1. Animal assignment.

Test Group	Dosage (mg/kg/day)	F_0 generation females	F_1 generation pups c	
			Males	Females
Control [a]	0	25 [b]	80	78
Low	0.3	25 [b]	80	79
Mid	1	25 [b]	80	80
High	5	25 [b]	64	69

a Control animals received vehicle only.
b An additional 5 rats/dose F_0 generation female rats were mated, assigned to a satellite portion of the study, and sacrificed on gestation day 20 for blood and brain sample collection and cholinesterase (ChE) analysis.
c Number of available litters for control through high-dose groups: 25, 24, 24, 23; nominal number of pups per sex to be assigned per group = 80.

4. Dose selection rationale: The low- and mid- doses were chosen based on a chronic study indicating that dosages of 1 mg/kg/day had no effect on the level of brain ChE. The high-dose was chosen based on substantial inhibition of brain ChE and histopathological changes in the adrenal zona fasciculata at 5 mg/kg/day.

5. Dosage preparation and analysis: Test substance formulations were prepared once at the beginning of the study. Analyses for substance stability and concentration verification were performed. Homogeneity and concentration analyses were performed on 0 (vehicle), 0.3, 1, and 5 mg/ml dose formulations. Stability analyses were performed on 0.06 and 1 mg/ml formulations that were stored for 42 days (5/13/97-6/24/97). Storage temperature was not reported.

Results - Homogeneity and concentration analysis: 96-100% of nominal.

Stability Analysis: The 0.06 mg/ml formulation was 103 ± 0.0006- 0.0028% of nominal; the 1 mg/ml dose was 103 ± 0.01% of nominal.

The analytical data indicated that the mixing procedure was adequate and the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered once daily to F_0 generation females by gavage on GD 6 through of lactation day 11 for dams that delivered a litter (day of delivery designated as lactation day or postnatal day 1) and through GD 24 for rats that did not deliver a litter. The dosing volume of the solutions was 1 mg/kg. Dosing was based on the daily body weights.

C. OBSERVATIONS

1. F_0 Generation Observations and Evaluations - Dams were inspected weekly during the predosing period, on GD 0, and twice daily during the dosing period for signs of toxicity and mortality. During the dosing period, the animals were observed daily at 3-4 hours postdose by someone unfamiliar with the rat's dose group for indications of autonomic dysfunction, abnormal posture, abnormal movement and behavior, and unusual appearance. Body weight data were evaluated and recorded weekly during the pretreatment period, on GD 0, and daily during the dosing period, lactation, and day of sacrifice. F_0 generation rats were also evaluated for the following:

   - pregnancy status
   - duration of gestation (GD 0 to the time the first pup was delivered)
   - delivered litters
   - gestation index
   - implantation sites
   - natural delivery
   - number of stillborn or dying pups
   - litter sizes (all pups delivered)
   - pup viability at birth
   - clinical observations during lactation

   Food consumption data were recorded on GD 0 during the predosing interval and then daily during the dosing period though lactation day 14. Maternal behavior was evaluated daily during the 22-day postpartum period and was recorded on lactation days 1 (birth), 5, 12, 18, and 22; any variations in behavior were recorded on the day observed. In addition, 20 mated F_0 generation females (5/dose group) were assigned to a satellite portion of the study for blood and brain sample collection and subsequent ChE analyses of the brain, plasma, and erythrocyte samples.

2. F_1 Generation Observations and Evaluations - The F_1 generation pups were examined for viability at birth. Pup viability was evaluated for each litter and evaluations of viability continued at least twice daily for the 22-day postpartum period. On lactation day 5, the litters were randomly standardized to 10 pups/litter (5 males/5 females when possible) and pups were randomly assigned to one of four subsets for continued observation. On lactation day 12, the litters were randomly standardized to eight pups/litter (4 males/4

females when possible). All pups were evaluated for the following:

- pups found dead or cannibalized (Day 1 and Days 2 to 5)
- surviving pups/litter (Day 1, Day 5 [preculling] and Day 5 [postculling])
- live litter size (Day 1, Day 5 [preculling] and Day 5 [postculling])
- time to pinna detachment
- time to eye opening

The pups in each litter were counted and evaluated for any abnormal physical signs once daily during the 22-day postpartum period. Body weight, sex, and observed nursing behavior were recorded on lactation days 1, 5, 12, 18, and 22, and any variations in behavior were recorded on the day observed.

3. F_1 Generation Subset Observations and Neurobehavioral Evaluations: On PND 5, 80 pups/sex/dose (nominally one male and one female/litter/dose) were randomly assigned to Subsets 1, 2, 3, or 4 (Table 2).

All pups in Subset 1 were sacrificed on PND 12. Fixed brain weights were determined in 10 Subset 1 pups/sex/dose and neuropathological examinations were performed on 6 of those pups/sex/dose. The remaining 10 pups/sex/dose were necropsied for gross lesions.

In Subset 2, 8 pups/sex/dose were selected for evaluation of learning and memory; evaluations were performed on PNDs 23-25 and 62-92; these Subset 2 animals were sacrificed on PNDs 97-101, following the last evaluation. The Subset 2 pups not selected for evaluation were necropsied for gross lesions on PND 22.

The Subset 3 pups (1 pup/sex/litter/dose) were tested for motor activity on PNDs 14, 18, 22, and 61 and auditory startle habituation on PNDs 23 and 62; all Subset 3 animals were sacrificed on PNDs 63 or 64 following the last evaluation.

In Subset 4 pups, the following were performed:

- Clinical observations were recorded on PNDs 40 and 66. Body weights were recorded on PNDs 1, 5, 12, 18, 22, 40, and 66. Food consumption was recorded on PNDs 23-30, 40-47, and 59-66;

- Pinna unfolding and eye opening were monitored daily beginning on PND 2 and PND 12, respectively, until each pup achieved the criterion; (pinna unfolding was also monitored in all other rats because monitoring started prior to litter standardization and subset assignment on PND 5);

- Males were evaluated for the age of preputial separation beginning on PND 39 and females were evaluated for the age of vaginal patency beginning on PND 28. Body weights were recorded on the day the criterion was attained; and

- Between PNDs 66-71, fixed brain weights were determined in 10 pups/sex/dose, neuropathological examinations were performed on 6 of these 10 pups/sex/dose and all remaining animals (10/sex/dose) were necropsied for gross lesions.

Table 2. F_1 generation subset observations and neurobehavioral evaluations.

Subset Number	Observations	Days of Sacrifice
1	A total of 80 pups/sex (20/sex/dose) were assigned to Subset 1. 10 pups/sex/dose were selected for fixed brain weights on PND 12; of these, 6 pups/sex/dose were subsequently selected for neuropathological examination on PND 12 (total of 24 male and 24 female pups evaluated).	All pups were sacrificed on PND 12.
2	A total of 80 pups/sex (20/sex/dose) were assigned to Subset 2. 8 pups/sex/dose were selected for evaluation of learning and memory between PNDs 23- 25 and between PNDs 62- 92 (total of 32 male and 32 female rats evaluated).	All rats were sacrificed on PNDs 97 to 101 following the last test.
3	1 pup/sex/litter/dose (a total of 80 male and 80 female pups) were tested for motor activity on PNDs 14, 18, 22, and 61 and auditory startle habituation on PNDs 23 and 62.	All rats were sacrificed on PNDs 63 and 64 following the last auditory startle test.
4	1 pup/sex/litter/dose (total of 80 male and 80 female pups) were assigned to Subset 4. 10 pups/sex/dose were selected for fixed brain weights between PNDs 66- 71; of these, 6 pups/sex/dose were subsequently selected for neuropathological examination between PNDs 66- 71 (total of 24 male and 24 female rats evaluated).	All rats were sacrificed between PNDs 66 and 71.

For brain weight measurements and neuropathological evaluations, the brains from 10 pups/sex/dose from Subset 1 (PND 12) and Subset 4 (PNDs 66-71) were weighed following fixation with neutral buffered 10% formalin. Of the 10 pups/sex/dose, 6 from each group were selected for neuropathology evaluation. Brains from Subset 1 animals were fixed by immersion in neutral buffered 10% formalin. Subset 4 animals (PND 66-71) were perfused in situ with neutral buffered 10% formalin after administration of heparin and an anesthetic (not specified). Subsequently, the spinal cord and peripheral nerves from the hindlimbs were exposed and immersed in neutral buffered 10% formalin prior to shipment to a consulting lab for processing.

Spatial delayed alternation was evaluated in 8 pups/sex/dose from Subset 2 on PNDs 23-25 and 62-92 in a Plexiglas® T-maze. The test was conducted on food and/or water deprived animals. F_1 generation pups were deprived of food and water and the adult rats were deprived of water only. The test included maze acclimation, acquisition training, and delay training. Maze acclimation included goal box training and forced runs. Acquisition training consisted of a forced run immediately followed by a choice run. Delay training was conducted similarly to acquisition training with a time delay added

between the forced and the choice runs.

Motor activity was evaluated for a 1 hour period on one Subset 3 pup/sex/litter on PNDs 14, 18, 22, and 61. The number of movements was tabulated at 5-minute intervals. The apparatus monitored up to 32 cages on one rack during each session. Each animal was tested in the same location on the rack during the 4 test sessions. On PNDs 22 and 61, body temperature measurements were performed on all animals immediately following completion of the motor activity testing.

On PND 23 and 62, an auditory startle habituation test was performed on the same animals tested for motor activity. The animals were given a 5 minute acclimation period and then the startle response was measured in 50 identical trials at a sound level of 120 dBA with a 10 second inter-trial interval. For each rat, peak response amplitude (corrected for baseline) and response latency were averaged over ten-trial blocks; the averages of these 10-trial blocks were then computed for each dose group (yielding 5 blocks per group), and these averages were compared among the dose groups.

4. Sacrifice and Pathology - F_0 Generation - All maternal rats were sacrificed by carbon dioxide asphyxiation. Rats that died or were killed for humane reasons were examined for the cause of death or moribund condition on the day the observation was made. These rats were evaluated for gross lesions, pregnancy status, and uterine contents. Any female that mated, but did not deliver a litter was euthanized on GD 25 and examined for gross lesions. To confirm the absence of implantation sites, the uteri were stained with 10% ammonium sulfide. Any dams whose entire litter was born dead or died prior to weaning of their litter were killed after the last remaining pup was found dead or missing, or presumed cannibalized; of these F_0 females, a gross necropsy was performed on the thoracic, abdominal, and pelvic viscera. Dams that littered were sacrificed on Lactation day 22, a gross necropsy with examination of the thoracic, abdominal, and pelvic viscera was performed, and the number and distribution of implantation sites was recorded. Satellite dams were sacrificed on GD 20, four to five hours post dosing. Blood was collected from the inferior vena cava and the brain was removed; ChE analyses were performed on brain, plasma, and erythrocyte samples.

5. Sacrifice and Pathology - F_1 Generation - All pups were euthanized by carbon dioxide asphyxiation, excluding the Subset 1 and 4 pups assigned to brain weight measurements and neuropathology examination.

Pups Found Dead on PND 1: To assess viability of any pups that appeared stillborn or that died before the initial viability examination of the litter, the lungs were removed and immersed in water. Pups with lungs that sank were considered stillborn, while pups with lungs that floated were considered liveborn and to have died shortly after birth. Pups with gross lesions were placed in Bouin's fluid for possible future examination.

Pups Found Dead or Moribund Sacrificed on PNDs 2 to 22: Pups found dead in the nesting boxes were necropsied and evaluated for cause of death. Pups with gross lesions were preserved in Bouin's fluid.

Pups Not Selected for Continued Observation: All pups chosen for culling were sacrificed and necropsied on PND 5 and offspring with gross lesions were placed in Bouin's solution. F_1 generation pups not assigned to one of the four subsets were sacrificed on PND 22, examined for gross lesions, and necropsied.

Scheduled Sacrifice of F_1 Generation Pups Selected for Continued Observation: On PND 12, all pups in Subset 1 were sacrificed and examined for gross lesions; pups selected for neuropathological evaluation were prepared as described in section 3. Upon completion of all postweaning behavior and developmental examinations, all Subset 2 and 3 rats were sacrificed and examined for gross lesions. On PND 66 to 71, Subset 4 pups were sacrificed and examined for gross lesions; any Subset 4 pups chosen for brain weight and neuropathological evaluation were sacrificed and processed as previously described in section 3.

Neuropathological evaluation: For Subset 1 pups selected for neuropathological evaluation, the following parameters were evaluated: fixed brain weight, external measurements (cerebrum and cerebellum), eight brain sections (paraffin embedded, stained with hematoxylin and eosin), seven additional linear morphometric measurements. All dose groups were evaluated, for a total of 48 rats (6/sex/group). For Subset 4 pups selected for neuropathological evaluation, the following parameters were evaluated: fixed brain weight; external measurements (as above); sections from brain, spinal cord, Gasserian ganglia, nerve roots, and dorsal root ganglia (paraffin embedded, stained with hematoxylin and eosin, luxol fast blue/cresyl violet, and Bielschowsky's technique); sections of sciatic, tibial, peroneal, and sural nerves (embedded in glycol methacrylate, stained with hematoxylin and eosin, toluidine blue, and Bielschowsky's technique); six additional linear morphometric measurements. Control and high dose group animals were evaluated for both sexes (6/sex/group), and mid-dose group females were evaluated, for a total of 30 rats.

D. DATA ANALYSIS

1. Statistical Analyses: Parametric data were analyzed using Bartlett's Tests (run at α = 0.001) or factorial repeated-measure analyses (Rep-ANOVA). In the event of statistical significance, one dose level was removed and the Rep-ANOVA was rerun to determine if a significant difference still existed between the remaining groups. Step-down analyses were conducted at $\alpha = 0.02$. Nonparametric data were evaluated using either the Kruskal-Wallis ($\alpha = 0.02$) or Chi-square test for proportions ($\alpha = 0.02$).

Note that although study authors used $p<0.02$ as their cut-off for statistical significance, most studies consider findings to be statistically significant if $p<0.05$. In addition, note that the above-described analyses do not include any pairwise comparisons (i.e., the statistical significance of the differences between, for instance, the high dose and low dose for specific measured values at any particular time point were not evaluated). Note also that only selected main effects and interactions from the statistical analysis were presented. For example, in the analysis for auditory startle habituation, p-values were presented for main effect of group, and for the interactions of [Group X Sex], [Group X Time], and [Group X Time X Block]; main effects for [Sex], [Time], and [Block], or interactions of (e.g.) [Group X Block], [Group X Sex X Time], etc. were not presented.

Parental Data: Group mean bodyweights and food consumption were analyzed using a Rep-ANOVA. Clinical signs were analyzed using the Chi-square test for proportions.

Maternal Data: The number of pregnant rats, gestation index (number of rats with live offspring/number of pregnant rats), live and stillborn pups, and dams with live/stillborn pups were analyzed using the Kruskal-Wallis and Wilcoxon tests. The duration of gestation, total pups delivered, dams with all pups dying days 1 to 5 postpartum, pups found dead or presumed cannibalized, and implantation sites were analyzed using the Chi-square test for proportions.

Litter Data: Litter size at weighing and sex ratio were analyzed using the Kruskal-Wallis and Wilcoxon tests. Pup viability indices (number of live pups on day 5 [preculling]/number of pups on day 1) were analyzed using Chi-square test for proportions. Bodyweights, food consumption, and body temperature were analyzed by Rep-ANOVA. Clinical signs were analyzed using the Chi-square test for proportions.

Behavioral Data: Auditory startle, motor activity, and spatial delayed alternation were analyzed using Rep-ANOVA.

Developmental Data: Pinna unfolding, vaginal patency, and preputial separation were tested by Kruskal-Wallis followed by a Wilcoxon test. Eye opening was analyzed using the Chi-square test for proportions.

Morphometric Data: All morphometric data were analyzed by ANOVA.

2. Historical Control Data: No data were submitted.

3. Positive Control Data: Positive control data for spatial delayed alternation methodology, motor activity, developmental neurotoxicity, and morphometric measurement were provided.

A brief methodology was submitted for spatial delayed alternation to assess learning and working memory of adult and pup rats. Testing consisted of three phases: maze

acclimation, acquisition training, and delay training. Results showed an increase in the learning curve during the acquisition training phase and an increase in the delay between the forced and the choice run indicating a decrease in the retention curve. Maze acclimation data were not submitted.

Positive control data were submitted for the following chemicals (doses): acrylamide (45 mg/kg/day), d-amphetamine (0.75 mg/kg/day), MK-801 (10 mg/kg/day) and trimethyltin chloride (8 mg/kg/day) in a study evaluating motor activity, startle habituation, and neurohistological effects. No startle habituation data were provided. Several statistically significant differences were found in male rats during the motor activity testing when comparing mean ± S.D. between vehicle control and the test chemical. Statistically significant parameters for the male rats in motor activity testing follow: during session 2, differences were found in the number of movements when treated with acrylamide and d-amphetamine and time spent in movement when dosed with d-amphetamine; session 3 revealed differences in the number of movements when treated with acrylamide and d-amphetamine and time spent in movement when dosed with d-amphetamine; session 4 showed significant differences in the number of movements and time spent in movement when rats were treated with acrylamide and d-amphetamine. Increased mortality and increased frequencies and numbers of neurological clinical observations were found when male rats were treated with acrylamide. No significant changes were found in the males with regard to mean ± S.D. for terminal body weights, brain weights, or ratios (%) of brain weight to terminal body weight between treated groups and control.

Statistically significant differences were also found in female rats during the motor activity testing when comparing mean ± S.D. between vehicle control and the test chemical. Statistically significant parameters for the female rats in motor activity testing follow: sessions 2, 3, and 4 indicated significant differences in the number of movements and time spent in movement when females were treated with acrylamide and d-amphetamine. Increased frequencies and numbers of neurological clinical observations were found in female rats when treated with acrylamide, MK-801, and trimethyltin. No significant changes were found in the females with regard to mean ± S.D. for terminal body weights, brain weights, or ratios (%) of brain weight to terminal body weight between treated groups and control.

Another study submitted related to the developmental neurotoxicological effects of lead nitrate. Pregnant rats were given lead nitrate orally by gavage from GD 6 through lactation day 10 at doses of 0 (vehicle), 5, or 50 mg/kg/day. Observations made in the high-dose group included: significant increases in the number and frequency of neurotoxicological clinical observations in the dams and significantly reduced food consumption values throughout gestation and early lactation, significant decreases in maternal body weights during lactation, significant numbers of stillborn and dying pups on PND 1 to 4, average pup weights per litter were reduced on PND 1-7, and significant differences in body weights and food consumption values in the F_1 generation during the early postweaning period. Brain weights of high dose PND 11 F_1 generation females were significantly different from the vehicle control. In the low-dose, females of the F_1

generation showed significant differences from the vehicle control in whole brain weights, telencephalon, and cerebellum weights. No differences existed between the three dose groups in the functional evaluations of the F_1 generation rats which included: motor activity monitored for 1 hour periods on PND 13, 17, 21, and 60 (± 2), auditory startle habituation measured on PND 22 and 60 (± 2), performance in a passive avoidance task during the early postweaning period, and performance of adult rats in an M-maze watermaze task. Indices of sexual maturation were unaffected by treatment. Neurohistological examination of high dose group adult rats did not reveal any pathology.

The final positive control study submitted compared the gross and microscopic morphometric measurements of 10- and 12-day old pups. The ages of 10 and 12 days were selected to possibly predict either an *in utero* neurotoxic effect or delayed brain development. Results indicated the mean values for all of the neuroanatomic measurements were higher for the 12 day-old rat pups than for the 10 day-old pups, but considerable overlap existed in the data. Despite the data overlap between the two age groups and the presence of moderate intra-age group variability in the measurements for each location, statistically significant increases (day 12 vs. day 10 rat pups) were found for the following brain regions: anterior-posterior length of the cerebrum, anterior-posterior length of the cerebellum, thickness of the frontal cortex, width of the caudate-putamen, and height of the cerebellar cortex.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations - There were no unscheduled mortalities in the F_0 maternal animals. No treatment-related clinical signs occurred.

2. Autonomic Function - High-dose (5 mg/kg/day) animals had increased signs of autonomic function toxicity, manifested at the postdose examination at the end of gestation (day 21 or 22) as fasciculations (6/25 treated vs 0/25 controls, p=0.001), and during early lactation (days 1-5) as fasciculations (16/24 treated vs 0/25 controls, p=0.001), hyperpnea (8/24 treated vs 0/25 controls, p=0.001), and hyperreactivity response to handling (17/24 treated vs 2/25 controls, p=0.001). In the mid-dose group (1 mg/kg/day), hyperpnea was only observed in 1/24 dams and was not considered to be treatment-related due to the low incidence. Hyperreactivity was also observed in the low- (7/24 treated) and mid-dose (2/24 treated) groups, but was not considered treatment-related because the response was not dose-dependent. Data are shown in Table 3.

Table 3. Autonomic function toxicity observations during gestation and lactation in dams dosed with chlorpyrifos from gestation day 6 to lactation day 11 (total number of times finding was observed/no. of animals with finding observed at least once during the designated period).[a]

Observation	Dosage (mg/kg/day)			
	0	0.3	1	5
	Gestation			
Total # Animals	25	25	25	25
Fasciculations	0	0	0	6/6*
Ataxia	0	0	0	1/1
	Lactation			
Total # Animals	25	24	24	24
Hyperreactivity	3/2	7/7	3/2	27/17*
Fasciculations	0	0	0	30/16*
Hyperpnea	0	0	1/1	10/8*

a Data extracted from the study report Table B1, pages 120 and 121.
* Significant at p=0.001; p values represent overall significance of analysis when all groups are included, findings during lactation were no longer significant when the high dose was removed from the analysis.

3. Bodyweight - No significant differences occurred in maternal bodyweights and bodyweight gains during gestation or lactation. There was, however, a significant [Group X Time] interaction for body weight changes during gestation (i.e., there were differences among groups at some time points, but not at other time points, p=0.0059; p=0.0419 when the high dose group was removed from the analysis; no further analyses were done). Slightly lower gestation day 20 body weight values at the high dose were attributed by the study author to higher mean litter size and lower average pup weights in this group as compared to control. Bodyweight gains in the high-dose group were reduced during days 16-20 of gestation (↓11%, Table 4a). Although the statistical significance of bodyweight gains for this specific time period is unknown, the lower body weight gain was considered to be related to treatment by the study author. During lactation, maternal bodyweight gains in the high-dose group were reduced (↓16%, Table 4b) immediately after parturition. However, the bodyweight gains of all groups were small compared to absolute bodyweight, and the values were highly variable; therefore, the differences were not considered to be unequivocal evidence of treatment-related toxicity.

Table 4a. Mean bodyweight gains (g) during gestation in dams dosed with chlorpyrifos from gestation day (GD) 6 to lactation day 11.[a]

Interval	Dosage (mg/kg/day)			
	0	0.3	1	5 (*)
GD 0-6	26.2	22.4	26.1	23.8
GD 6-12	18.9	23.3	23.6	22.4
GD 12-16	22.2	25.0	22.1	22.2
GD 16-20	49.2	51.4	51.6	43.9
GD 6-20	90.2	99.7	97.3	88.5
Overall - GD 0-20	116.5	122.2	123.4	112.3

a Data extracted from the study report Table B3, page 123.
(*) Rep-ANOVA: significant effect of [Group X Time], p=0.0059; the analysis was no longer statistically significant when the high dose group was removed; data were not further analyzed.

Table 4b. Mean bodyweight gains (g) during lactation in dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Interval	Dosage (mg/kg/day)			
	0	0.3	1	5 (*)
Days 1-4	6.8	4.6	5.0	1.1
Days 12-14	3.0	1.0	1.6	7.0
Days 12-22	-0.6	1.8	9.2	5.0
Days 1-12	33.4	31.0	29.6	33.5
Overall - Days 1-22	32.9	32.7	38.8	38.6

a Data extracted from the study report Table B5, page 126.
(*) Rep-ANOVA: [Group X Time] interaction, p=0.0406; data not further analyzed.

4. Food Consumption - Food consumption values (g/kg/day and g/animal/day) in all treated groups were not significantly different from controls during gestation or lactation (Rep-ANOVA, main effect of [Group] for absolute food consumption during lactation, p=0.0352; not further analyzed). High-dose dams consumed slightly less food (g/day) during early lactation (↓4-15% vs control), and relative food consumption (g/kg/day) values were decreased (↓5-12%) during treatment. Although the differences were minor and not significantly different (defined as $p<0.02$), they were attributed to treatment by the study author.

5. Test Substance Intake - This parameter was not necessary since this was a gavage study and daily doses were based on daily bodyweight measurement.

6. Pregnancy Status and Litter Data - There were no observed effects on maternal performance parameters of pregnancy rate, gestation index, length of gestation, number of implantation sites, and number of live or dead pups. There were no abortions. Observations are presented in Table 5.

Table 5. Pregnancy observations.[a]

Observation	Dose (mg/kg/day)			
	0	0.3	1	5
Animals Assigned (Mated)	25	25	25	25
Animals Pregnant Pregnancy Rate (%)	25 (100)	24 (96)	24 (96)	24 (96)
Number with Live Litters	25	24	24	23
Number with No Liveborn Pups	0	0	0	1
Gestation Index (%)	100	100	100	95.8
Duration of Gestation (days)	23.1±0.5	23.2±0.4	23.0±0.5	23.0±0.2
Total Implantations(FTG) Implantations/Dam	339 13.6±3.2	333 14.5±2.0	340 14.2±2.4	341 14.2±1.6
Total Live Pups(FTG)[b] Live Pups/Dam	308 12.3±3.1	319 13.3±1.9	311 13.0±2.4	292 12.7±2.4
Total Dead Pups(FTG)[b] Dead Pups/Dam	1 0.0±0.2	1 0.0±0.2	5 0.2±0.5	3 0.1±0.6
Mean % Male Pups	53.0	51.9	53.0	49.0

a Data extracted from the study report Tables B10 and B11, pages 131 through 133.
b Includes only animals with live litters.
FTG - Full term gestating females

7. Cholinesterase Activity - Brain ChE activity was decreased in the high-dose (↓90%) and the mid-dose (↓18%) dams (Table 6) as compared to control. Erythrocyte (↓41-99%) and plasma (↓43-92%) ChE activity were decreased in a dose-dependent manner in all treated groups. The study report does not indicate whether these data were analyzed statistically.

Table 6. Mean (± S.E.) plasma, erythrocyte, and brain cholinesterase activity as % of control group at gestation day 20 in dams (5/group) dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Sample	Dosage (mg/kg/day)		
	0.3	1	5
Plasma	56.70±2.69	31.13±4.07	8.46±1.19
Erythrocyte	58.74±16.10	15.59±6.80	0.13±0.15
Brain	99.72±1.48	82.11±2.80	10.18±0.92

a Data extracted from the study report Appendix K, pages 606 through 613. Cholinesterase measurements conducted by Dr. Stephanie Padilla (USEPA, NHEERL, RTP, NC).

B. F_1 GENERATION TOXICITY - PUPS

1. Pup Bodyweights: Body weights for all F_1 pups, recorded prior to selection to Subsets, are presented in Table 7a. In the high-dose group, pup (male and female) body weights were significantly reduced (↓8-15%, p=0.0001) at PND 1 and 5 (pre- and post-culling). Analysis of male and female bodyweights separately showed that these two groups reacted similarly to treatment.

   F_1 pup bodyweights (Table 7b) were reduced from birth to PND 22 in Subset 4 high-dose males (↓5-19%) and females (↓6-17%). Additionally, F_1 pup bodyweight gains (Table 7c) were reduced from birth to PND 22 in Subset 4 high-dose males (↓5-28%) and females (↓8-30%).

Table 7a. Mean pup body weights (g) (recorded prior to selection to Subset) from dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Interval (days postpartum)		Dosage (mg/kg/day)			
		0	0.3	1	5 (*)
Day 1	Males	6.6	6.7	6.4	6.1
	Females	6.3	6.2	6.1	5.6
Day 5 (Preculling)	Males	9.9	10.2	10.3	8.8
	Females	9.6	9.7	9.7	8.2
Day 5 (Postculling)	Males	9.8	10.2	10.1	8.8
	Females	9.4	9.6	9.5	8.2

a Data extracted from the study report Table B11, page 134.
(*) Rep-ANOVA, significant main effect of [Group] (p=0.0001), and significant [Group X Time] interaction (p=0.0001). The analysis was no longer statistically significant after removal of the high dose group; data were not further analyzed.

Table 7b. Selected, mean bodyweights of F_1 pups (Subset 4) from dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Day Postpartum	Dosage (mg/kg/day)			
	0	0.3	1	5 (*)
Males				
1	6.6	6.7	6.7	6.3
5	9.8	10.3	10.2	8.6
12	24.4	25.6	25.4	19.8
18	40.4	41.9	41.2	35.0
22	54.3	55.8	55.2	45.1
Females				
1	6.2	6.2	6.0	5.8
5	9.4	9.7	9.6	8.3
12	23.7	24.6	23.8	19.6
18	39.0	40.1	39.0	33.8
22	51.8	53.4	51.6	42.8

a Data extracted from the study report Tables F3 and F4, pages 419 and 420.

(*) Rep-ANOVA, analysis included PND 1, 5, 12, 18, 22, 40, and 66; main effect of [Group], p=0.0001, [Group X Time], p=0.0001; analysis was no longer statistically significant when the high dose group was removed; note that the analysis includes PND 40 and 66 (see Table 13a). No further statistical analysis was submitted.

Table 7c. Selected, mean bodyweight gains of F_1 pups (Subset 4) from dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

PND Interval	Dosage (mg/kg/day)			
	0	0.3	1	5 (*)
Males				
1-5	3.2	3.6	3.6	2.3
5-12	14.6	15.3	15.2	11.2
12-18	16.0	16.3	15.8	15.2
18-22	13.8	13.9	14.0	10.1
Females				
1-5	3.2	3.4	3.6	2.5
5-12	14.3	14.9	14.2	11.3
12-18	15.4	15.5	15.2	14.2
18-22	12.8	13.3	12.6	8.9

a Data extracted from the study report Tables F5 and F6, pages 421 and 422.

(*) Rep-ANOVA, analysis included days 1-5, 5-12, 12-18, 18-22, 22-40, and 40-66; significant main effect of [Group[(p=0.0001), significant [Group X Sex] interaction (p=0.0307), significant [Group X Time] interaction (p=0.0001). Analysis was no longer statistically significant when the high dose group was removed. Note that analysis included days 22-40 and 40-66 (see Table 13b).

2. Pup Survival Indices: There were overall significant group differences due to the high-dose group for the following parameters: surviving pups per litter (↓27%, p=0.002) and live litter size at PND 5 (↓16%, p=0.018), pup viability index (↓29%, p=0.001), and pups found dead or presumed cannibalized (day 1 - 7.2 % treated vs 0.0% controls, p=0.001; days 2 to 5 - 24.7% treated vs 1.3% controls, p=0.001). Dams with all pups dying were increased in the high-dose group (3/23 treated vs 0/25 controls, p=0.024). Data are shown in Table 8.

Table 8. Pup survival data from dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Observation (days postpartum)	Dose (mg/kg/day)			
	0	0.3	1	5 (*)
Viability Index (day 5)	98.7	98.7	98.1	69.9 c
Surviving Pups/Litter (day 5)	12.2	13.1	12.7	8.9 c
Live Litter Size (day 5)	12.2	13.1	12.7	10.2 b
Pups Found Dead or Missing (day 1, %)	0.0	0.3	0.6	7.2 c
Pups Found Dead or Missing (days 2-5, %)	1.3	0.9	1.3	24.7 c
Dams with all Pups Dying (days 1-5)	0	0	0	3 a

a Data extracted from the study report Tables B10 and B11, pages 131 through 134.
(*) Statistically significant effects, a: p=0.024; b: p<0.02; c: p<0.002; no longer significant after removal of high dose group.

3. Developmental Landmark Data: The mean day of pinna unfolding was delayed (4.0 days in treated vs 3.5 days in controls, p=0.0265) in the high-dose group (Table 9). Although statistical significance (at p<0.02) was not achieved, this finding was judged to be treatment-related and was considered to be consistent with findings of decreased pup body weight at the high-dose. Eye opening was not significantly impacted by treatment with chlorpyrifos (p=0.099).

Table 9. Development Landmark Data.[a]

Observation (Mean Day to Criteria for all Pups)		Dose (mg/kg/day)			
		0	0.3	1	5
Pinna Unfolding		3.5	3.5	3.6	4.0
Eye opening	Males	14.6	14.6	14.7	14.9
	Females	14.4	14.0	14.4	14.6

a Data extracted from the study report Table B13, page 136.

4. Clinical Observations: There were no clinical observations for the F_1 pups (Subset 4) that were considered to be treatment-related. There was no effect on body temperature in adult Subset 3 F_1 rats at PND 22 and 61.

5. Neurobehavioral Evaluations

Spatial Delayed Alternation - A total of 31-32 Subset 2 pups/sex were evaluated for the effect of exposure to chlorpyrifos on learning and memory on PNDs 22-25 and 62-92. The data were recorded as percent correct responses during acclimation over multiple

block trials and as response time and percent correct responses as a function of a variable time delay. A regression line was fitted to the individual rat data and the slope and intercept were analyzed. The slope represented the decay of information just learned, while the intercept at extrapolated time 0 represented non-memory factors such as motivation, sensory-motor processes, and attention. There were no statistically significant differences between the groups in the average acquisition and delayed response (statistical analysis compared the slope and intercept of regression lines fitted to delalyed response data for each group). Additionally, there were no differences among dose groups when comparing retention of information during PNDs 23-25 with PNDs 62-92. Maze acclimation data were not presented. (See also attached Figures 2A-2D, from study report pages 102-105).

Motor Activity - A total of 78-79 Subset 3 pups/sex were evaluated for motor activity. There was no statistically significant main effect of [Group] for the Rep-ANOVA evaluating mean motor activity for all treatment groups on PNDs 14, 18, 22, and 61 ($p=0.630$). However, there was a statistically significant ($p=0.0141$) [Group X Time] interaction (attributed to the high dose group). On day 14, total activity was decreased for both high dose males and females (37-56%). Although total activity remained decreased for males at day 18 (15%) and day 22 (4%), there was a large increase for females (51%) at both time points. On day 61, activity was increased for both males and females (16-17%). Insufficient statistical analyses were performed to determine the statistical significance of the differences at these various time points (for example, [Group X Sex X Time] and [Group X Sex X Time X Block] interactions were not presented). The study authors stated this effect was spurious because no consistent change in motor activity was noted across time points. Agency reviewers consider the effects on motor activity at the high dose to be treatment related. (See also attached Figures 3A-3H, from study report pages 106-113).

Body Temperature - Body temperature measurements taken on PNDs 23 and 61 immediately following completion of the motor activity testing were similar in all groups. (See also attached Figure 4, from study report page 114).

Auditory Startle Habituation - A total of 76-79 Subset 3 pups/sex were evaluated for auditory startle habituation on PNDs 23 and 62. There was an increase (↑16-25%) in the latency to peak response on PND 23 in the high-dose animals compared to concurrent controls (Table 10). At PND 62, the latency to peak response in the high-dose animals was 10-12% higher than in the controls (main effect of [Group], days 23 and 62, $p=0.0049$; [Group X Time] interaction, $p=0.1076$; when Group 4 is removed, the analysis is no longer significant). Additionally, the peak response amplitudes in the high-dose animals were decreased by 9 to 29% on PNDs 23 and 62 compared to the controls (main effect of [Group], $p=0.0737$; [Group X Time] interaction, $p=0.0442$). Note that not all potential interactions were presented in the analysis, most notably [Group X Sex X Time]

and [Group X Sex X Time X Block] (see also attached Figures 5A-5D, from study report pages 115-118).

Table 10. Auditory startle habituation [mean peak response amplitude (g) and mean latency (sec) to peak response] in F1 offspring (Subset 3)].[a]

	Dose Group (mg/kg/day)			
Observation/Study Days	0	0.3	1	5 (*)
Males				
Mean Peak Response/PND 23	56.6 ± 22.9	63.7 ± 30.1	56.9 ± 21.2	40.5 ± 10.0
Mean Peak Response/PND 62	219.7 ± 100.2	156.3 ± 69.5	171.3 ± 92.4	168.3 ± 80.5
Latency to Peak Response/PND 23	39.3 ± 7.1	38.5 ± 8.4	39.2 ± 9.4	49.1 ± 16.0
Latency to Peak Response/PND 62	36.5 ± 6.5	39.0 ± 9.2	37.5 ± 5.6	40.8 ± 11.6
Females				
Mean Peak Response/PND 23	57.9 ± 18.1	57.6 ± 16.0	55.7± 17.4	48.7 ± 20.5
Mean Peak Response/PND 62	146.6 ± 81.2	145.5 ± 89.2	97.0 ± 47.6	133.7 ± 82.3
Latency to Peak Response/PND 23	37.1 ± 8.8	36.8 ± 7.0	38.2± 7.0	43.0 ± 7.5
Latency to Peak Response/PND 62	39.3 ± 9.2	41.4 ± 9.4	45.6 ± 11.3	43.1 ± 8.8

a Data extracted from the study report Tables E3 and E4, pages 304-307; results for peak response amplitude were adjusted for baseline response (in the absence of stimulus).

(*) Rep-ANOVA: significant main effect of latency to peak response (main effect of [Group], days 23 and 62, p=0.0049; [Group X Time] interaction, p=0.1076; when high-dose was removed, the analysis was no longer significant). Additionally, the peak response amplitudes were decreased on PNDs 23 and 62 (main effect of [Group], p=0.0737; [Group X Time] interaction, p=0.0442).

6. Sacrifice and Pathology at PND 12

Macroscopic Pathology F_1 Generation Pups - There were no treatment-related gross pathology lesions.

Terminal Body Weights and Brain Weights of F_1 Generation Pups - Compared to the controls, reduced terminal body weights were observed in the Subset 1 (PND 12) high-dose animals (↓17- 19%) and the Subset 4 (PND 66) high-dose males (↓10%). Absolute brain weights of the Subset 1 high-dose animals were reduced (↓9%) and their brain/bodyweight ratios were increased (113-114%) compared to the controls (Table 11).

The study pathologist concluded that the differences in brain size in the Subset 1 animals may be due to undernutrition. Brain size in the Subset 4 animals was similar in all groups (PND 66).

Table 11. Mean terminal bodyweights, brain weights, and brain/bodyweight ratios from F_1 pups (Subset 1) at PND 12.[a]

Observation	Dosage (mg/kg/day)			
	0	0.3	1	5 (*)
Males (n=10)				
Body weight (g)	23.5	27.4	25.9	19.4
Brain weight (g)	1.284	1.409	1.356	1.173
Brain/body weight (%)	5.480	5.161	5.260	6.205
Females (n=10)				
Body weight (g)	23.1	23.2	23.1	18.8
Brain weight (g)	1.284	1.279	1.273	1.171
Brain/body weight (%)	5.585	5.535	5.540	6.355

a Data extracted from the study report Tables C2 and C4, pages 228 and 230.

(*) Statistical findings: Note that both day 12 and day 66 findings were included in the same statistical analysis; 1) Terminal body weight: main effect of [Group], p=0.004; [Group X Sex] interaction, p=0.0149; when high dose was excluded from analysis, [Group X Sex] interaction p=0.0273; 2) Brain weight: main effect of [Group], p=0.0001, [Group X Time] interaction, p=0.0276; when high dose was excluded from analysis, main effect of [Group], p=0.0275; 3) Brain/body weight (%): main effect of [Group], p=0.0001, [Group X Time] interaction, p=0.001; when high dose was excluded from analysis, there were no significant findings.

Microscopic Pathology F_1 Generation Pups - There were no treatment-related microscopic neuropathology lesions.

Brain Morphometry - Morphometry measurements performed on PND 12 pups (Subset 1) detected a number of alterations, including: reduced anterior to posterior measurement of the cerebellum (↓24%), reduced height of the cerebellum (↓14%), a decrease in parietal cortex thickness (↓6%), and a decrease in the thickness of the hippocampal gyrus (↓9%) in the high-dose males (Table 12). The same treatment-related findings were noted in high-dose female pups: Subset 1 high-dose female pups had decreased cerebellar height (↓9%) and anterior to posterior length (↓4%), reduced thickness of the parietal cortex (↓6%), reduced width of the caudate-putamen (↓10%), and reduced thickness of the

hippocampal gyrus (↓12%). The study pathologist concluded that no other neuropathologic alterations were detected in any of the animals and that the differences that were detected should be attributed to the observed reduction in brain size only. However, we consider these effects to be treatment-related at both time points (see below for day 60 findings). Note that findings from both day 12 and day 60 were included in the same statistical analysis; day 12 findings included all treatment groups, day 60 findings included control males and females, high-dose males and females, and mid-dose females (see table footnote). No analysis excluding the high dose group were performed for morphometric findings, therefore the statistical significance cannot be attributed to high dose animals only. Since both time points were included in the analysis, treatment cannot be attributed to a single treatment day. Several possible interactions were not evaluated, including [Group X Time X Sex].

Table 12. Selected, mean morphometric data at PND 12 from F1 pups (Subset 1) from dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Observation	Dosage (mg/kg/day)			
	0	0.3	1	5 (*)
Males (n=6)				
Cerebellum (Ant. to Post.; mm)	3.267±0.308	3.450±0.345	3.333±0.186	2.467±0.550
Cerebellum (Height; µm)	3504±128.8	3456±17.17	3416±200.0	3008±504.0
Parietal Cortex Thickness (µm)	1336±56.1	1448±58.1	1448±32.8	1256±138.0
Caudate-Putamen Width (µm)	2240±84.1	2240±108.0	2312±93.16	2224±147.7
Hippocampal Gyrus Thickness (µm)	904±93.2	1004±114.0	972±54.2	824±65.6
Females (n=6)				
Cerebellum (Ant. To Post.; mm)	3.183±0.223	3.033±0.320	3.300±0.167	3.000±0.310
Cerebellum (Height; µm)	3512±200.0	3176±130.3	3120±328.4	3208±226.0
Parietal Cortex Thickness (µm)	1380±54.2	1376±19.6	1368±80.3	1304±72.3
Caudate-Putamen Width (µm)	2384±131.2	2224±116.3	2288±108.0	2152±133.8
Hippocampal Gyrus Thickness (µm)	936±81.7	912±50.3	932±96.5	828±78.5

a Data extracted from the pathology report Tables 1 and 2, pages 666 through 669.

(*) Statistical findings are as follows (analysis included both PND 12 and 60): 1) Cerebellum (ant. to post.): main effect of [Group], p=0.0029; [Group X Time] interaction, p=0.0249; 2) Cerebellum (height): main

effect of [Group], p=0.0063, [Group X Time] interaction, p=0.0291; 3) Parietal cortex thickness: main effect of [Group], p=0.0199; [Group X Time] interaction, p=0.2596; 4) Caudate-putamen width: main effect of [Group], p=0.2792, [Group X Time] interaction, p=0.0528; 5) Hippocampal gyrus thickness: main effect of [Group], p=0.0011, [Group X Time] interaction, p=0.6434. Further statistical analysis was not conducted.

C. F_1 GENERATION TOXICITY - ADULTS

1. Body Weights and Bodyweight Gains: Body weights (Table 13a) of the high-dose F_1 adult males (Subset 4) were decreased at PND 22 through 66 (↓11-17% vs controls; p=0.0001). High-dose F_1 adult females (Subset 4) also weighed less than controls at PND 22 (↓17% vs controls) and 40 (↓9%), but were of similar weight at PND 66. Bodyweight gains (Table 13b) were also decreased in the high-dose males for the PND 22-40 interval (↓13% vs controls) and PND 40-66 interval (↓7%). Mid- and low-dose males and females did not show significant differences in bodyweight gains.

Table 13a. Selected, mean bodyweights of F_1 adults (Subset 4) from dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Day Postpartum	Dosage (mg/kg/day)			
	0	0.3	1	5 (*)
Males				
22	54.3	55.8	55.2	45.1
40	186.7	191.2	190.5	160.3
66	388.9	385.4	389.8	348.0
Females				
22	51.8	53.4	51.6	42.8
40	146.5	148.8	145.4	133.3
66	228.7	238.1	228.8	220.3

a Data extracted from the study report Tables F3 and F4, pages 419 and 420.

(*) Rep-ANOVA, analysis included PND 1, 5, 12, 18, 22, 40, and 66; main effect of [Group], p=0.0001, [Group X Time], p=0.0001; analysis was no longer statistically significant when the high dose group was removed (see alsoTable 7b). No further statistical analysis was submitted.

Table 13b. Selected, mean bodyweight gains of F_1 adults (Subset 4) from dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Day Postpartum Interval	Dosage (mg/kg/day)			
	0	0.3	1	5 (*)
Males				
22-40	132.4	135.4	135.2	115.2
40-66	202.2	194.2	199.3	187.8
Females				
22-40	94.7	95.4	93.8	90.5
40-66	82.2	89.3	83.0	87.0

a Data extracted from the study report Tables F5 and F6, pages 421 and 422.
(*) Rep-ANOVA, analysis included days 1-5, 5-12, 12-18, 18-22, 22-40, and 40-66; significant main effect of [Group[(p=0.0001), significant [Group X Sex] interaction (p=0.0307), significant [Group X Time] interaction (p=0.0001). Analysis was no longer statistically significant when the high dose group was removed (see also Table 13b).

2. Food Consumption: Food consumption was decreased immediately after weaning (PND 23-30) in high-dose male and females (↓13% vs controls). In the later intervals (PND 40-47 and PND 59-66), food consumption was decreased only 3-10% versus controls. Data are shown in Table 14.

Table 14. Mean food consumption of F_1 adults (Subset 4) from dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Day Postpartum Interval	Dosage (mg/kg/day)			
	0	0.3	1	5 (*)
Males				
23-30	13.6	14.0	13.4	11.9
40-47	26.2	26.6	26.5	23.7
59-66	30.4	29.9	30.0	28.2
Females				
23-30	12.0	12.9	12.1	10.4
40-47	19.4	19.6	18.8	18.3
59-66	20.5	21.3	20.0	19.9

a Data extracted from the study report Tables F7 and F8, pages 423 and 424.
(*) Statistical analysis indicated a significant main effect of [Group] (p=0.0001); analysis was no longer significant when high dose group was removed.

3. Mortality and Clinical Signs: No treatment-related clinical signs were observed in adult F_1 rats. F_1 adult mortality was limited to 1 death in the mid-dose group (Subset 2), and 3 deaths in the high-dose group (Subsets 2 and 3).

4. Sexual Maturation: Sexual maturation was delayed as assessed by time to preputial separation (106% of controls; not significant [p=0.0535]) or vaginal patency (103% of controls; p=0.0130). Data are shown in Table 15. Such delays are not inconsistent with decreased body weights.

Table 15. Mean (±S.D.) day of sexual maturation observation in F_1 adult rats (Subset 4) from dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Observation	Dosage (mg/kg/day)			
	0	0.3	1	5
Males				
Preputial Separation	44.2±1.9	43.4±1.9	45.2±3.2	47.0±5.9
Females				
Vaginal Patency	32.4±1.0	31.5±1.5	32.1±2.3	33.4±2.2*

a Data extracted from the study report Table F9, page 425.
* Significantly different from control group, p<0.02.

5. Sacrifice and Pathology at PND 66

Macroscopic Pathology - There were no treatment-related gross pathology lesions.

Terminal Body Weights and Brain Weights of F_1 Generation Adults: In the high-dose males, a decrease in terminal bodyweight (↓10% vs controls), decrease in brain weight (↓1%), and increase in the brain to body weight ratio (111% of controls) were observed. In the high-dose females, a slight decrease in brain weight (↓0.4%) and increase in brain to body weight ratio (↑2%) were also observed. The findings were considered to be marginal. Mid- and low-dose males and females did not show any treatment-related effect on body weight, brain weight, or brain to body weight ratio. Data are shown in Table 16.

Table 16. Terminal bodyweight, brain weight, and brain/bodyweight ratio of F_1 adult rats (Subset 4) at day postnatal day 66 from dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Observation	Dosage (mg/kg/day)			
	0	0.3	1	5
Males				
Terminal Body weight	410.7	384.8	408.2	368.7
Brain Weight	2.270	2.257	2.288	2.238
Brain/Body weight (%)	0.549	0.588	0.565	0.610
Females				
Terminal Body weight	234.5	254.2	233.1	230.4
Brain Weight	2.093	2.178	2.122	2.085
Brain/Body weight (%)	0.893	0.864	0.919	0.908

a Data extracted from the study report Tables F12 and F13, pages 428 and 429.
(*) Statistical findings (see also Table 11): Note that both day 12 and day 66 findings were included in the same statistical analysis; 1) Terminal body weight: main effect of [Group], p=0.004; [Group X Sex] interaction, p=0.0149; when high dose was excluded from analysis, [Group X Sex] interaction p=0.0273; 2) Brain weight: main effect of [Group], p=0.0001, [Group X Time] interaction, p=0.0276; when high dose was excluded from analysis, main effect of [Group], p=0.0275; 3) Brain/body weight (%): main effect of [Group], p=0.0001, [Group X Time] interaction, p=0.001; when high dose was excluded from analysis, there were no significant findings.

Microscopic Pathology - There were no microscopic neuropathological lesions.

Brain Morphometry - Morphometric measurements performed on PND 66 (Subset 4) revealed some slight intergroup differences (Table 17). There were no apparent treatment-related differences between control and high dose male brain measurements, but in high dose females, the parietal cortex measurement was decreased (5%) and the thickness of the hippocampal gyrus was decreased (7%). Results of the statistical analysis are discussed above and in the footnote for Table 12. Without an analysis excluding the high-dose group, statistical significance cannot be attributed at the high-dose only. Evaluation of mid-dose females indicated a possible dose-related trend in the findings, but the study pathologist dismissed them as representative of random variation. Although the pathologist concluded that the results demonstrated a recovery from any delays in brain development observed at PND 12, there remains some concern that a marginal deficit in brain morphometry (in particular for hippocampus and parietal cortex) still exists for the high-dose females at PND 66, even after body and brain weight are no longer significantly decreased as compared to control. Due to incompleteness of the statistical analysis, it is not clear whether there are significant effects at the mid-dose. If signifcant effects were foundat that dose, brains from the low dose females would need to be evaluated at this time

point.

Table 17. Selected, mean (±S.D.) morphometric data at PND 66 from F1 offspring (Subset 4) of dams dosed with chlorpyrifos from gestation day 6 to lactation day 11.[a]

Observation	Dosage (mg/kg/day)			
	0	0.3	1	5 (*)
Males (n=6)				
N	6	0	0	6
Cerebellum (Ant. to Post.; mm)	5.683±0.232	--	--	5.667±0.216
Cerebellum (Height; µm)	5152±218.2	--	--	5104±351.0
Parietal Cortex Thickness (µm)	1756±79.5	--	--	1792±58.1
Caudate-Putamen Width (µm)	2800±176	--	--	2744±98.0
Hippocampal Gyrus Thickness (µm)	1640±91.9	--	--	1612±95.3
Females (n=6)				
N	6	0	6	6
Cerebellum (Ant. To Post.; mm)	5.517±0.232	--	5.500±0.261	5.383±0.098
Cerebellum (Height; µm)	5016±120.5	--	4888±150.0	4968±207.6
Parietal Cortex Thickness (µm)	1792±36.1	--	1716±36.4	1700±55.6
Caudate-Putamen Width (µm)	2576±131.2	--	2552±178.1	2704±112.2
Hippocampal Gyrus Thickness (µm)	1708±57.6	--	1644±129.5	1592±86.8

a Data extracted from the pathology report Tables 1 and 2, pages 32 through 33.

(*) Statistical findings are as follows (analysis included both PND 12 and 60; see also Table 12): 1) Cerebellum (ant. to post.): main effect of [Group], p=0.0029; [Group X Time] interaction, p=0.0249; 2) Cerebellum (height): main effect of [Group], p=0.0063, [Group X Time] interaction, p=0.0291; 3) Parietal cortex thickness: main effect of [Group], p=0.0199; [Group X Time] interaction, p=0.2596; 4) Caudate-putamen width: main effect of [Group], p=0.2792, [Group X Time] interaction, p=0.0528; 5) Hippocampal gyrus thickness: main effect of [Group], p=0.0011, [Group X Time] interaction, p=0.6434. Further statistical analysis was not conducted.

III. DISCUSSION

A. Investigator's Conclusions: No effects, other than cholinesterase inhibition in the dam, were seen in either the dam or pups at dosage levels of 0.3 or 1.0 mg/kg/day. The study report highlights brain cholinesterase inhibition at 5 mg/kg/day as the only adverse effect, while plasma and RBC cholinesterase inhibition at 1 and 0.3 mg/kg/day are not considered to be adverse. Administration of chlorpyrifos at 5 mg/kg/day produced clinical evidence of cholinergic toxicity in the dams, supported by non-statistically significant reductions in body weight change and food consumption. In the high-dose group (5 mg/kg/day), increased pup mortality in early lactation, decreased offspring body weight, related decreases in brain size and brain layer thickness on PND 12, transient changes in the auditory startle response (decreased response and increased latency to response, attributed by the study author to "motor slowing"), and delayed pinna detachment, vaginal opening and preputial separation were observed. Learning, memory, and habituation were not impaired in the pups at any time at any dosage. The study author concluded that chlorpyrifos is not a selective developmental neurotoxicant in the rat, and that the no-observed-adverse-effect-level (NOAEL) for maternal and pup toxicity in this study was 1 mg/kg/day.

B. Reviewer's Conclusions

In this developmental neurotoxicity study (MRID 44556901), 25 pregnant Sprague-Dawley rats/group were administered chlorpyrifos (99.8% a.i.) by gavage from gestation day 6 (DG 6) through lactation day 11 at 0, 0.3, 1, or 5 mg/kg/day. An additional 5 pregnant females/group were dosed at the same levels for the cholinesterase (ChE) phase of the study. Dams were examined for body weight, reproductive performance, number of viable pups, and postpartum behavior. During the dosing period, dams were observed daily for signs of autonomic function toxicity. Satellite dams were sacrificed four to five hours post-dosing on DG 20 for ChE analyses to be performed on brain, plasma, and erythrocytes. Offspring were examined for viability at birth, pup/litter survival, body weight, sex ratio, physical development landmarks (eye opening and pinna detachment), observed nursing behavior, and sexual maturation. F_1 generation litters were randomly standardized on lactation day 5 and assigned to 4 subsets for continued observation. On postnatal day (PND) 12, fixed brain weight measurements (10 pups/sex/dose) and neuropathological evaluations including morphometrics (6 pups/sex/dose) were performed on Subset 1 pups, with the remaining 10 pups/sex/dose necropsied for gross lesions. In Subset 2, 8 pups/sex/dose were selected for evaluation of learning and memory; evaluations were performed on PNDs 23-25 and 62-92. These Subset 2 animals were sacrificed on PNDs 97-101, following the last evaluation. The Subset 2 pups not selected for evaluation were necropsied for gross lesions on PND 22. The Subset 3 pups were tested for motor activity on PNDs 14, 18, 22, and 61 and auditory startle habituation on PNDs 23 and 62; all Subset 3 animals were sacrificed on PNDs 63 or 64 following the last evaluation. In Subset 4 pups, fixed brain weights were determined in 10 pups/sex/dose, neuropathological examinations were performed on 6 pups/sex/dose, and all remaining Subset 4 animals (10/sex/dose) were necropsied for gross lesions upon sacrifice on PND 66-77.

Maternal toxicity in the high-dose (5 mg/kg/day) animals was manifested as increased signs of autonomic function toxicity, apparent at the end of gestation as fasciculations (6/25 treated vs 0/25 controls), and during early lactation (days 1-5) as fasciculations (16/24 treated vs 0/25 controls), hyperpnea (8/24 treated vs 0/25 controls), and hyperreactivity (17/24 treated vs 2/25 controls). Dams with all pups dying were increased in the high-dose group (3/23 treated vs 0/25 controls). There were no significant effects on bodyweight, food consumption, or pregnancy parameters. There were no unscheduled deaths in the maternal animals.

Brain ChE activity was decreased in the high-dose (↓90%) and mid-dose (↓18%) dams as compared to control. Erythrocyte (↓41-99%) and plasma (↓43-92%) ChE activities were decreased in a dose-dependent manner in all treated groups.

The maternal toxicity NOAEL was not observed.
The maternal LOAEL was < 0.3 mg/kg/day, based on plasma and RBC cholinesterase inhibition.

For the F_1 generation pups, the high-dose group bodyweights were significantly reduced (↓8-15%) at PND 1 and 5 (pre- and post-culling). Bodyweights were also reduced from birth to PND 22 in Subset 4 high-dose animals (↓5-19%); bodyweight gains were reduced in these animals during the same period (↓5-30%). Additionally, compared to the controls, reduced terminal body weights were observed in the Subset 1 (PND 12) high-dose animals (↓17-19%) and the Subset 4 (PND 66) high-dose males (↓10%). For the F_1 generation adults, body weights of the high-dose males were decreased at PND 22 through 66 (↓11-17% vs controls). High-dose F_1 adult females also weighed less than controls at PND 22 (↓17% vs controls), but were of similar weight at PND 66. Bodyweight gains were also decreased in the high-dose males for the PND 22-40 interval (↓13% vs controls) and PND 40-66 interval (↓7%). Food consumption was decreased immediately after weaning (PND 23-30) in high-dose males and females (↓13% vs controls).

Development as assessed by pinna unfolding was delayed (4.0 days in treated vs 3.5 days in controls) in the high-dose group. Sexual maturation was delayed as assessed by time to preputial separation (106% of controls) and vaginal patency (103% of controls).

Pup viability was reduced as assessed by the following parameters: surviving pups/ litter (↓27%) and live litter size at culling (↓16%), pup viability index (↓29%), and pups found dead or presumed cannibalized (day 1 - 7.2 % treated vs 0.0% controls; days 2 to 5 - 24.7% treated vs 1.3% controls).

There were no statistically significant differences between the groups in the average acquisition and delay training. Additionally, there were no differences among dose groups when comparing retention of information during PNDs 23-25 and 62-92. Motor activity was decreased in high dose male and female pups on PND 14 (↓56% in males and ↓37% in females), and increased in high dose females on PNDs 18 and 22 (↑51% on both days). There was a statistically significant increase (↑16-25%) in the latency to peak response during the auditory startle habituation assessments on PND 23 in the high-dose animals

compared to concurrent controls. At PND 62, the latency to peak response in the high-dose animals was 10-12% higher than in the controls. Additionally, the peak response amplitudes in the high-dose animals were decreased by 9 to 29% on PNDs 23 and 62 compared to the controls.

There were no gross or microscopic lesions of the nervous system in Subset 1 or 4 offspring. Subset 1 high-dose males at PND 12 had reduced absolute brain weights (↓9% vs controls), increased relative brain weights (↑13% vs controls), reduced anterior to posterior measurement of the cerebellum (↓24% vs controls), reduced height of the cerebellum (↓14% vs controls), decreased thickness of the parietal cortex (↓6% vs controls), and decreased thickness of the hippocampal gyrus (↓9% vs controls). High-dose female pups had reduced absolute brain weights (↓9% vs controls), increased relative brain weights (↑14% vs controls), thickness of the parietal cortex (↓6% vs controls), width of the caudate-putamen (↓10% vs controls), and thickness of the hippocampal gyrus (↓12% vs controls). In Subset 4 F1 animals, killed on PND 66, morphometric analysis revealed decreased parietal cortex measurements (↓5%) and decreased thickness of the hippocampal gyrus (↓7%) in high-dose females. These measurements were also decreased in mid-dose females (parietal cortex, ↓4%; hippocampal gyrus, ↓4%). The statistical significance of the differences in mid-dose females was not evaluated, and there was no evaluation of low dose females. Brain weight in high dose females was similar to control brain weight at day 66 (↓0.3%).

The tentative developmental neurotoxicity LOAEL is 5 mg/kg/day.
The tentative NOAEL is 1 mg/kg/day.

In setting this developmental NOAEL based upon all available data from this study, the following issues are acknowledged:

1) No cholinesterase inhibition data were generated for offspring (fetuses and pups) on this study. Therefore, this study in and of itself may not provide a definative assessment of maternal vs fetal susceptibility following chlorpyrifos exposure. It is recognized that a separate study has been submitted by the Registrant to address pharmacokinetic issues and comparative cholinesterase inhibition in adult vs neonatal rats (MRID 44648102). The results of the developmental neurotoxicity study should be considered in context of the supplementary special study.
2) As discussed by the investigator, other confounding factors in the interpretation of these data include:
   - Appropriateness of the route of administration to the dams (gavage vs dietary) and pups (in utero and/or via the milk.
   - Time of exposure to the offspring (pre- vs postnatal) and its relevance to vulnerable periods of neurodevelopment in humans
   - Unknown pharmacokinetic and pharmacodynamic information in dams vs offspring and how such considerations may have affected study outcome or interpretation
   - The contribution of maternal toxicity to observation in the offspring (confounding interpretation of effects).

Study Deficiencies: The submitted stability data did not provide storage temperature.

The statistical analyses as presented in the text tables of the study report were in general difficult to interpret, due to lack of data on variations over time or due to differential responses by sex, e.g., body weight and motor activity data. Statistical analysis of some parameters was apparently not performed, e.g., cholinesterase inhibition data and PND 66 morphometric data. As a result, a definitive developmental neurotoxicity NOAEL and LOAEL could not be determined. Submission of more complete statistical analysis is required.

This study in the rat is classified **unacceptable (§83-6)** and does not satisfy the guideline requirements for a developmental neurotoxicity study. The study may be upgradable, following submission of more complete statistical analysis.