OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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

OFFICE OF PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

**TXR:** 0052426

**DATE:** **July 26, 2005**

**SUBJECT:** ***Trichlorfon*-Toxicology Study Reports**
**PC Code:** 057901 **Reregistration Case #:** 0104

**FROM:** Abdallah Khasawinah, Ph.D.
Reregistration Branch 4
Health Effects Division (7509C)

**TO:** Kylie Rothwell
Reregistration Branch
Special Review and Reregistration Division (7508C)

**THRU:** Susan V. Hummel, Branch Senior Scientist
Reregistration Branch 4
Health Effects Division (7509C)

**TASK ID:** DP Code: D272254

**Registrant**: Bayer CropScience LP, @ T.W. Alexander Dr., Research Triangle Park, NC

**Action Requested**: Review and Prepare DER for the following MRID: 46205301

**Agency's Response:** HED toxicologists have reviewed and prepared DER of the above MRID and the findings are presented below. The DER is attached to this memo.

In this developmental toxicity study, the **LOAEL** for maternal systemic toxicity is 150 ppm (13.4 mg/kg/day; LDT) based on the inhibition of red blood cell cholinesterase activity. A **NOAEL** for maternal systemic toxicity is not established. The **LOAEL** for the offspring toxicity is 150 ppm (13.4 mg/kg/day, LDT) based on decreased startle amplitude in males and females on PND 22. An offspring NOAEL is not established.

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes,

however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control data.

# DATA EVALUATION RECORD

DYLOX® (TRICHLORPHON)

Study Type: §83-6; Developmental Neurotoxicity Study in the Rat

Work Assignment No. 1-01-30 (MRID 46205301)

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

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

Primary Reviewer:
Ronnie J. Bever Jr, Ph.D.

Signature: Ronnie J. Bever Jr.
Date: 6/18/04

Secondary Reviewer:
David A. McEwen, B.S.

Signature: David A. McEwen
Date: 6/18/04

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/18/04

Quality Assurance:
Steven Brecher, Ph.D.

Signature: Steven Brecher
Date: 6/18/04

Disclaimer

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

**EPA Reviewer:** Abdallah Khasawinah, Ph.D. **Signature:** A. Khasawinah
**Reregistration Branch 4, Health Effects Division (7509C)** **Date** July 11, 2005
**Work Assignment Manager:** P.V. Shah, Ph.D. **Signature:** PV Shah
**Registration Action Branch 1, Health Effects Division (7509C)** **Date** 7/12/05



**TXR#:** 0052426

# DATA EVALUATION RECORD

**STUDY TYPE:** Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

**PC CODE:** 057901 **DP BARCODE:** D272254

**TEST MATERIAL (PURITY):** Dylox® Technical (Trichlorphon; 100.0-100.4% a.i.)

**SYNONYMS:** Dimethyl (2,2,2-trichloro-1-hydroxyethyl)-phosphonate

**CITATION:** Sheets, L.P. (2003) A developmental neurotoxicity screening study with technical grade trichlorfon (Dylox®) in wistar rats. Bayer CropScience LP, Toxicology, Stilwell, KS. Laboratory Study No.: 01-D72-CT, February 26, 2003. MRID 46205301. Unpublished.

**SPONSOR:** Bayer CropScience LP, 2 T.W. Alexander Drive, Research Triangle Park, NC

**EXECUTIVE SUMMARY** - In a developmental neurotoxicity study (MRID 46205301) Dylox® Technical (Trichlorphon; 100.0-100.4% a.i.; Batch #: 103-0228) was administered in the diet to pregnant Crl:WI (Glx/BRL/Han) IGS BR rats (30/dose) at doses of 0, 150, 500 or 1750 ppm (equivalent to 0, 13.4, 49.0, and 145.6 mg/kg/day during gestation and 0, 33.1, 103.4, and 264.6 mg/kg/day during lactation) from gestation day (GD) 0 through lactation day (LD) 21. Dams were allowed to deliver naturally. All surviving dams were sacrificed on LD 21 (weaning). The P females that did not deliver a litter were sacrificed on GD 24. Brain and blood cholinesterase activity were determined in the dams (6-9/dose) on LD 21, in the pups culled on post-natal day (PND) 4 (representing as many litters as possible), and in the Subset D pups (5-10 pups/sex/dose, representing 15-20 litters) on PND 21. The remaining pups and dams not selected for further evaluations were sacrificed and discarded without further examination. On PND 4, 15-23 litters/dose were standardized (6-8 pups/litter with approximately equal numbers of males and females) to reduce variation. Subsequently, 1-2 pups/litter/dose were allocated to Subsets A-D for examinations of detailed clinical signs and functional observational battery, motor activity, auditory startle habituation, passive avoidance and water maze learning and memory tests, brain weight and measurements, ophthalmology, neuropathology, and/or brain and blood cholinesterase determinations. Select offspring were sacrificed at PND 21 for brain weights and measurements and neuropathology, and the remaining offspring were sacrificed at PND 75 (±5 days). Satisfactory positive control data were provided , but the lab proficiency was not demonstrated for the various behavioral tests.

## Maternal Toxicity

There were no clinical signs of toxicity in any treated female. Two females in the 1750 ppm dose were found dead one each on Lactation Day (LD) 10 and 20 and another was sacrificed at LD 20 after the last of her pups was found dead, without clinical signs of toxicity or injury. There were no treatment-related FOB findings recorded during gestation or lactation. The fertility index was decreased (not statistically significant) at 500 (↓7%) and 1750 (↓10%) ppm. These values were within the range of controls in other studies. The mating index was 100% for all dose groups.

Compound related effects in the parental females were death at the high dose and reduction in cholinesterase activity ($p \leq 0.05$) in a dose-related manner as follows: (i) in plasma by 43-55% at ≥500 ppm; (ii) in erythrocytes by 26-71% in all treated groups; and (iii) in brain by 16-72% in all treated groups. Other treatment related effects were decreased body weight (↓7%; $p \leq 0.05$) and body weight gain (↓53% ) in the 1750 ppm females during lactation.

**The LOAEL for maternal systemic toxicity is 150 ppm (13.4 mg/kg/day; LDT) based on the inhibition of red blood cell cholinesterase activity. A NOAEL for maternal systemic toxicity is not established.**

## Offspring

Litter data were not summarized adequately in the study report and no conclusions can be made regarding the pup mortality and survival. No treatment-related clinical signs were reported at any dose.

Body weight and weight gain of pups were significantly decreased at the mid (8%) and high dose (37%) by PND 21, but not at the low dose. Cumulative body weight gains (PNDs 0-4 and 4-21) were decreased ($p \leq 0.01$) by 31-43% in the 1750 ppm group. Cumulative body weight gains (PNDs 4-21) were decreased ($p \leq 0.05$) by 8-9% in the 500 ppm group. Gradual recovery of body weights occurred during the post weaning period.

Sexual maturation (prepuptial separation and vaginal patency) was delayed by 4-6% ($p \leq 0.05$) in the 1750 ppm group, but this effect may have been related to decreased growth. Body weight was decreased ($p \leq 0.01$) by 9-10% at sexual maturation in these animals. Pupil constriction was evident in all control and treated pups at PND 21. Ophthalmology examination revealed no treatment-related effects.

No treatment-related effects were observed during the functional observational battery. Increased motor and locomotor activity was seen in the high dose group offspring males and females on PND 17. Habituation was unaffected by treatment. Decreased ($p \leq 0.05$) maximum amplitude of the auditory startle reflex was observed in all blocks at PND 22 in the 1750 ppm group (↓50-63%). Decreased ($p \leq 0.05$) amplitude was also observed in 1 or 2 blocks in the 150 and 500 ppm groups (↓27-34%) at PND 22. Total amplitude at PND 22 decreased dose-dependently in both sexes (↓19-61%). Incidental decreases ($p \leq 0.05$) were observed in all treated females in 1 or 2

blocks at PND 38, and these effects were unrelated to dose. Habituation was demonstrated and was unaffected by treatment. In the passive avoidance test, a statistically significant decrease ((↓17%; $p \leq 0.05$) in the latency period of Trial 1 in the Retention session was observed in males at the highest dose on PND 29. In the water maze test, there was a minor increase in errors in the 1750 ppm females at PNDs 58-62 during trial 2 of the learning phase (0.9 treated vs 0.2 controls).

Terminal body weights were decreased ($p \leq 0.05$) in the 1750 ppm males and females (↓29-42%) and in the 500 ppm females (↓13%, perfused only) at PND 21, and in the 1750 ppm males (↓9-11%) on PND 75. Absolute brain weights (perfused or non-perfused) were 7-18% significantly ($p<0.05$) less than in the controls in the 1750 ppm male and female pups sacrificed on PND 21. Absolute brain weight of the other groups were unaffected. At study termination, the absolute non-perfused brain and cerebullum weights were decreased ($p<0.05$) by 18% and 13% respectively in the 1750 ppm group male pups and cerebellum length was decreased 7% in the 1750 ppm males at study termination.

Macroscopic neuropathological examination revealed no treatment-related gross pathological findings in any treated group at either PND 21 or 75 (±5 days). Microscopic neuropathological examination revealed no treatment-related effects on histopathology findings or linear brain measurements in any treated group at either PND 21 or 75. Various lesions were observed, but the incidence was minor, such as (n=10) minimal perivascular cuffing in the lumbar spinal cord in one 1750 ppm perfused female at ~PND 70 (vs 0 controls), hydrocephalus and brain atrophy in one 150 ppm female at ~PND 70 (vs 0 controls).

At 1750 ppm, plasma and erythrocyte cholinesterase levels were decreased ($p \leq 0.05$) by 25% and 28%, respectively, in the pooled male and female samples at PND 4. On PND 21, plasma and brain cholinesterase levels were decreased ($p \leq 0.05$) in both sexes by 29-39% and 16-23%, respectively. Additionally on PND 21, decreases ($p \leq 0.05$) were also observed in brain cholinesterase levels in the 500 ppm males (↓7%) and the 150 and 500 ppm females (↓7-11%). However the decrease in the brain ChE activity level in the 150 ppm female pups was attributed to the unusually high control levels and thus not considered to be treatment related.

**The LOAEL for the offspring toxicity is 150 ppm (13.4 mg/kg/day) based on decreased startle amplitude in males and females on PND 22. An offspring NOAEL is not established.**

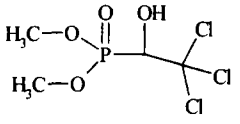
This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control data.

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

# I. MATERIALS AND METHODS

## A. MATERIALS

**1. Test Material:** Dylox® technical

| | |
|---|---|
| **Description:** | White solid |
| **Batch #:** | 103-0228 |
| **Purity:** | 100.0-100.4% a.i. |
| **Compound Stability:** | Stable for at least 7 days at room temperature and 42 days at freezer conditions |
| **CAS # of TGAI:** | 52-68-6 |
| **Structure:** |  |

**2. Vehicle** - Diet

**3. Test animals (P)**

| | | |
|---|---|---|
| **Species:** | Rat | |
| **Strain:** | Crl:WI (Glx/BRL/Han) IGS BR | |
| **Age at study initiation:** | Approximately 12 weeks | |
| **Group mean weight at study initiation:** | 200-202 g (females) | |
| **Source:** | Charles River Laboratories, Inc., Raleigh, NC | |
| **Housing:** | Dams were housed individually in stainless steel cages, except during breeding. Dams and their litters were kept together from LD 1-21, and the litter mates remained together until PND 28. All animals were housed individually thereafter. | |
| **Diet:** | Rodent Lab Chow meal 5002 (Purina Mills, Inc. Laboratories, St. Louis, MO), *ad libitum* except during neurobehavioral testing | |
| **Water:** | Tap water, *ad libitum* except during neurobehavioral testing | |
| **Environmental conditions:** | **Temperature:** | 19-25°C |
| | **Humidity:** | 30-70% |
| | **Air changes:** | ≥10/hr |
| | **Photoperiod:** | 12 hours light/12 hours dark |
| **Acclimation period:** | ≥6 days | |

## B. PROCEDURES AND STUDY DESIGN

**1. In life dates** - Start: 5/28/01 End: 8/31/01

**2. Study schedule** - The maternal animals were mated and assigned to study. The P females were administered the test substance via diet from gestation day (GD) 0 until lactation day (LD) 21. Dams were sacrificed on LD 21 (weaning). The P females that did not deliver a litter were sacrificed on GD 24. Brain and blood cholinesterase activity were determined in the dams (6-9/dose) on LD 21, in the pups culled on post-natal day (PND) 4 (representing as many litters as possible), and in the Subset D pups (5-10 pups/sex/dose, representing 15-20 litters) on PND 21. The remaining pups and dams not selected for further evaluations were sacrificed and discarded without further examination. On PND 4, 15-23 litters/dose were standardized (6-8 pups/litter with approximately equal numbers of males and females) to reduce variation. Select offspring were sacrificed at PND 21 for brain weights and measurements and neuropathology, and the remaining offspring were sacrificed at PND 75 (±5 days).

**3. Mating procedure** - After acclimation, females were paired (1:1) with males of the same strain and source. The cohabitation period lasted a maximum of four days and was discontinued when successful mating was verified by the presence of a vaginal plug or sperm in a vaginal smear. The day of successful mating was designated as GD 0, and the females were assigned to individual cages.

**4. Animal assignment** - Mated females were randomly assigned to test groups as shown in Table 1. When the litter did not have at least 3 pups/sex or 7 survivors at PND 4, both dam and pups were sacrificed without necropsy. Up to 23 litters/dose were randomly selected with preference given to those litters with ≥4 pups/sex. Litters were then standardized to a maximum of 8 pups/litter of which approximately 50% were male. Subsets A-D consisted of random allocation (assumed by reviewers) of 1 male and/or female/litter, representing from 15-23 litters/dose. In the learning and memory tests, the same animals were subjected to two different types of tests. The same individual animals assigned to FOB and motor activity testing were evaluated at all schedule time points.

**Table 1.** Study design. [a]

| Experimental Parameter | Dose (ppm) 0 | 150 | 500 | 1750 | Subset |
|---|---|---|---|---|---|
| **Maternal Animals** | | | | | |
| No. of maternal animals assigned | 30 | 30 | 30 | 30 | — |
| Detailed clinical/FOB<br>GDs 6 and 20<br>LDs 11 and 21 | <br>30<br>10 | <br>30<br>10 | <br>30<br>10 | <br>30<br>10 | — |
| Brain and blood cholinesterase determination LD 21 | 9 | 9 | 6 | 9 | — |
| **Offspring** | | | | | |
| Detailed clinical/FOB (PNDs 4, 11, 21, 35 [±1 day], 45 [±1 day], and 60 [±2 days]) | 16/sex | 16/sex | 16/sex | 9-16/sex | C |
| Motor activity (PNDs 13, 17, 21, and 60 [±2 days]) | 15-16/sex | 15-16/sex | 14-16/sex | 11-16/sex | A |
| Auditory startle habituation (PNDs 22, 38 [±2 days], and 60 [±2 days]) | 16/sex | 16/sex | 16/sex | 12-16/sex | B |
| Learning and memory<br>Passive avoidance (PNDs 22 and 29)<br>Water maze (PNDs 60 [±2 days] and 67 [±2 days]) | <br>15-16/sex<br>16/sex | <br>15-16/sex<br>15-16/sex | <br>16/sex<br>15-16/sex | <br>13-16/sex<br>12-14/sex | C |
| Brain weight and measurements<br>PND 21 (perfused)<br>PND 21 (non-perfused)<br>PND 75 (±5 days, perfused)<br>PND 75 (±5 days, non-perfused) | <br>10/sex<br>9-10/sex<br>10/sex<br>10/sex | <br>10/sex<br>9-10/sex<br>10/sex<br>10/sex | <br>9-10/sex<br>8-9/sex<br>10/sex<br>10/sex | <br>8-9/sex<br>7/sex<br>10/sex<br>10/sex | <br>D[b]<br>D[b]<br>A-C[b]<br>A-C[b] |
| Ophthalmology (PNDs 50-60) | 13/sex | 13/sex | 9-10/sex | 10-11/sex | A-C[c] |
| Neuropathology<br>PND 21<br>PND 75 [±5 days] | <br>10/sex<br>10/sex | <br>0<br>1 Female | <br>0<br>0 | <br>8-9/sex<br>10/sex | <br>D[b]<br>A-C[b] |
| Brain, erythrocyte, and plasma cholinesterase activity determination<br>PND 4[d]<br>PND 21 | <br>21<br>9-10/sex | <br>18<br>7-9/sex | <br>13<br>7-9/sex | <br>13<br>5-6/sex | <br>NA<br>D[b] |

*(table continues next page)*

a Data were obtained from MRID 46205301 (pages 25-26, 28, 34, 102-125, 188, 212, 222, 223, 225, 226, 829, 856-859, 891-911, and 913-923).

b Animals were randomly selected and represented 15-20 litters/dose. These animals were used for brain weight and measurements, neuropathology, and cholinesterase activity determination. Brain measurements were performed on perfused animals only.

c Animals were randomly selected and represented at least 20 litters/dose. These animals were also selected for perfusion and brain weights and measurements, and neuropathology was conducted at termination.

d Total animals; males and females from the same litter were pooled

NA Samples were collected from randomly selected pups that were to be culled. As many litters were represented as possible. Samples from male and female pups in the same litter were pooled.

**5. Dose-selection rationale** - Dose selection was based on the results of a two-generation reproduction study in Sprague-Dawley rats (MRID 42228301), where rats were treated with 0, 150, 500, or 1750 ppm Dylox® in the diet. The Sponsor stated that the NOAEL for the $F_1$ rats was 500 ppm. At 1750 ppm, decreased body weights (↓10-26%) and plasma, and brain cholinesterase inhibition (10-31%) were observed.

**6. Dosage administration** - The untreated control and treated diets were administered to the appropriate dose groups from GD 0 through LD 21. Mean compound intake (mg/kg bw/day) was calculated from the food consumption and body weight gain data.

**7. Dosage preparation and analysis** - The formulations were prepared weekly by mixing appropriate amounts of test substance with Purina Mills Rodent Lab Chow meal 5002, without the use of a solvent. Formulations were stored frozen until presented to the animals. Homogeneity was assessed in the 150 and 1750 ppm formulations prepared for Week 1, by analyzing 9 samples from each formulation. Stability was assessed in the 150 and 1750 ppm formulations prepared for Week 6 at freezer temperature for up to 42 days and at room temperature for up to 7 days. Concentration analyses were performed on Weeks 1, 2, 3, and 6 for all dose levels.

**Results:** **Homogeneity Analysis** (range as % coefficient of variation): 3.0-8.8%

**Stability Analysis** (range as % of Day 0): frozen, 106%; room temperature, 90.8-93.4%

**Concentration Analysis** (range of replicate means):

| Dose (ppm) | % of Nominal |
|---|---|
| 150 | 105-111 |
| 500 | 110-117 |
| 1750 | 97-103 |

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

## C. OBSERVATIONS

### 1. In-life observations

**a. Maternal animals** - Animals were observed at least once daily for clinical signs, mortality, and moribundity during acclimation (except for clinical signs) and throughout the study. Detailed physical observations were conducted once daily on gestation day (GD) 0 through lactation day (LD) 21. Signs of toxicity were recorded as they were observed, including the time of onset, degree, and duration. All presumed pregnant rats (30/dose) were subjected to a FOB on GDs 6 and 20. Ten dams/dose were also evaluated on LDs 11 and 21. At all time points the technicians were blind as to the dose groups of the animals. The following parameters were recorded:

| Observations During Handling | Open Field Observations |
|---|---|
| Ease of removal | Piloerection |
| Reaction to handling | Respiration abnormalities |
| Muscle tone | Posture |
| Palpebral closure | Involuntary motor - clonic |
| Lacrimation | Involuntary motor - tonic |
| Salivation | Stereotypy |
| Nasal discharge | Bizarre behavior |
| Stains | Gait abnormalities |
| Exophthalmia | Vocalization |
| Other | Arousal |
| | Rearing |
| | Defecation |
| | Urination |
| | Pupil size |
| | Pupil response |

Further details of the functional observational battery methodology were not provided.

Individual maternal body weights and food consumption were measured on GDs 0, 6, 13, and 20, and on LDs 0, 4 (weights only), 7, 14, and 21. Measurements of food consumption may have included consumption by the litter, as well as the dams.

Plasma, erythrocyte, and brain cholinesterase inhibition was measured in 9 dams/dose (except 6 of the 500 ppm group) at LD 21.

**b. Offspring**

**1) Litter observations** - The day of completion of parturition was designated as PND 0. As soon as possible following parturition, the pups were sexed and weighed. Each litter was observed for mortality and moribundity at least once daily. Additionally, detailed observations for clinical signs were made once daily before weaning. Any gross signs of toxicity in the

offspring were recorded as they were observed, including the time of onset, degree, and duration. Surviving pups were weighed on PNDs 0, 4, 11, 17, and 21. Additionally, pupil constriction was evaluated in all pups on PND 21.

Standardization occurred on PND 4. When the litter did not have at least 3 pups/sex or 7 survivors at PND 4, both dam and pups were sacrificed without necropsy. Up to 23 litters/dose were randomly selected to be assigned to the study with preference given to those litters with ≥4 pups/sex. Litters were then standardized to a maximum of 8 pups/litter of which approximately 50% were male. Subsets A-D consisted of random allocation (assumed by reviewers) of 1 male and/or female/litter, representing from 15-23 litters/dose.

**2) Developmental landmarks** - Beginning on PND 38, all male offspring were examined daily for preputial separation. Beginning on PND 29, all female offspring were examined daily for vaginal patency. The age of onset was recorded.

**3) Postweaning observations** - Each litter was observed for mortality and moribundity at least once daily. Additionally, detailed observations for clinical signs were made once weekly. Surviving pups were weighed weekly, as well as on the day of sexual maturation. Food consumption was measured weekly from the week of PND 28.

**4) Neurobehavioral evaluations** - Tests were conducted in a standard animal room during the light phase. The order of testing and assignment of animals to specific devices was semi-random, such that groups were balanced across test times and devices and no animal was tested more than once in the same device, except that animals were purposely tested in the same water maze on both test dates, as per standard procedures. Observations and the schedule for those observations are summarized as follows from the report.

**i) Functional observational battery (FOB)** - A functional observational battery was conducted on 9-16 offspring/sex/dose (1 male and/or female/litter, representing at least 20 litters/dose; Subset C) on PNDs 4, 11, 21, 35 (±1 day), 45 (±1 day), and 60 (±2 days) by individuals who were unaware of dose group assignment. The same parameters assessed in the maternal FOB were examined in the offspring, except that no open field observations were made on PND 4 and 11. Further details of the methodology were not provided.

**ii) Motor activity testing** - Motor activity measurements were performed on 11-16 offspring/sex/dose (1 male and/or female/litter, representing at least 20 litters/dose; Subset A) on PNDs 13, 17, 21, and 60 (±2 days) before dosing. Movement of individual animals was monitored for 60 minutes in 10 minute epochs in a figure-eight maze by infrared sensors, and data were collected automatically using a Columbus Instruments (Columbus, OH) Universal Maze Monitoring System and a personal computer. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Broad spectrum background noise (74±2 db[A]) was provided during testing. The uniformity of light intensity (100±70 Lux) over each maze was verified daily.

**iii) Auditory startle reflex habituation** - Auditory startle response and habituation testing was performed on 12-16 offspring/sex/dose (1 male and/or female/litter, representing at least 20 litters/dose; Subset B) on PNDs 22, 38 [±2 days], and 60 [±2 days]). Animals were evaluated in groups of 4 (maximum) in an integrated startle response test system (Coulbourn Instruments, Allentown, PA). Animals were placed in restraining cages over a load cell/force transducer assemblies to measure their reaction to a 50 msec burst of 118 dB(lin) white noise. Spectral characteristics of the stimulus was confirmed with a Bruel and Kjaer Real Time Frequency Analyzer fitted with a half-inch free-field microphone. The animals were allowed a 5 minute adaptation period at ambient noise levels followed by 50 trials (5 blocks of 10 trials) where stimuli were separated by 10 second intervals. Data collection began at stimulus and continued for 200 msec. Peak response amplitude (maximum value [g] of the average curve minus the individual's baseline [body weight]) and latency (the time [msec] following the onset of stimulus when the peak response amplitude occurs) were determined.

**iv) Learning and memory testing** - Learning and memory testing was performed on 12-16 offspring/sex/dose (1 male and/or female/litter, representing at least 20 litters/dose; Subset C). Passive avoidance testing was performed on PNDs 22 and 29. Water maze testing was performed on PNDs 60 (±2 days) and 67 (±2 days). Both short and long term recall were evaluated.

Passive avoidance was evaluated using an integrated system of equipment and computer programs from Coulbourn Instruments (Allentown, PA) and a personal computer. The test device was a 7x7 inch shuttle cage with two compartments that were separated by a guillotine-type door. The floor was a stainless steel grid. The compartments were identical, except one was lined with a black film and the other was not, but could be illuminated by a high intensity lamp. After adaptation, an individual rat was placed in the lighted compartment, facing the light (turned off) with the trap door shut. After 20 seconds, the trial began with the light being turned on, and the trap door opening. Movement was monitored by a photocell system. When the animal crossed to the dark compartment, the trap door shut, a 0.5 second pulse of 0.5 mA distributed shock was delivered to the floor, and the light turned off. If the rat failed to cross within 180 seconds, the light was turned off and the animal was returned to a holding cage to await the next trial. The trials were repeated until the rat remained in the lighted compartment for two consecutive trials or 15 trials had elapsed. Reported parameters included the number of trials-to-criterion, latency to cross on Trial 1 and Trial 2 (learning phase only), and the number of rats/dose that failed to reach criterion within 15 trials (learning phase only). Animals that either failed to reach criterion performance within 15 trials or failed to cross during the first two trials during acquisition were excluded from the retention phase of the experiment.

The mazes used in the water maze test were constructed of opague Plexiglass with corridors 5" wide and walls that were 16" high. The mazes were filled with 22±1°C water to a 7.5" depth. On each test trial, the rat was placed into the starting position (base of the M-maze stem farthest from the two arms) and required to swim to one of the two goals of the M-maze, in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed from the water. The initial arm chosen on trial 1 was designated the incorrect goal during the remaining trials. Rats that failed to make a correct goal choice within

60 seconds in any given trial were guided to the correct goal and were then removed from the water. A 15±5-second inter-trial interval separated each trial. Each rat was required to reach a criterion of 5 consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was 15. Latency (measured in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial.

Animals that performed 5 consecutive errorless trials were retested seven days later to determine their retention. The correct goal and criterion were the same for both test sessions. Dose groups were compared for the following dependent measures: the number of trials to criterion on the first testing (this measure was also used to compare groups for overall learning performance), the average number of errors (incorrect turns in the maze) for each trial on the first day of testing (this measure was also used to compare groups for overall learning performance), the latency (in seconds) to reach the correct goal on trial 2 of the first day of testing (this measure was used to compare groups for short-term retention), the number of trials to criterion on the second testing (this measure was used to compare groups for long-term retention), the average number of errors on each trial on the second day of testing (this measure was also used to compare groups for long-term retention), and the latency (in seconds) to reach the correct goal on trial 1 of the second testing (this was another indicator of long-term retention).

**5) Cholinesterase determination** - Brain, erythrocyte, and plasma cholinesterase activities were determined from blood and brain samples collected from 13-21 offspring/dose (randomly selected from pups that were culled, representing as many litters as possible) on PND 4, and 5-10 offspring/sex/dose from Subset D (representing approximately 15-20 litters) on PND 21. On PND 4, samples from male and female pups within a litter were pooled. Blood was collected by decapitation on PND 4 or via the orbital plexus on PND 21. Brains were collected to assay immediately after collection of blood. Animals were not fasted. The analytical method is as described by Ellman, except 6,6'-dithiodinicotinic acid was used as the coupling reagent and absorbance was measured at 340 nm.

**6) Ophthalmology** - At PNDs 50-60, ophthalmological examinations were performed on 9-13/sex/dose offspring (representing at least 20 litters/dose) randomly selected from Subsets A-C. The pupillary reflex was tested using a penlight or transilluminator following application of a mydriatic agent to each eye. A slit lamp microscope and an indirect ophthalmoscope equipped with a condensing lens allowed evaluation of the eyelid, conjunctiva, cornea, aqueous humor, lens, vitreous humor, retina, choroid, and optic disc.

**2. Postmortem observations**

**a. Maternal animals** - Animals were sacrificed by $CO_2$ asphyxiation. Females that were found dead or sacrificed *in extremis* were necropsied; tissues were collected and examined, if necessary, to determine the cause of death. Necropsy included an examination of all organs, brain, body cavities, cut surfaces, external orifices, and surfaces. Gross lesions in neural tissues or skeletal muscle were typically collected for microscopic examination, while other gross lesions were not collected. Dams were sacrificed on LD 21, after litter weaning. Females that were found to be

sperm positive and/or with a vaginal plug, but did not deliver, were sacrificed on GD 24. Maternal animals that were sacrificed on schedule were typically not necropsied.

**b. Offspring** - At PND 4, offspring that were selected for culling and not required for cholinesterase activity study were sacrificed and discarded without necropsy. Offspring selected for cholinesterase activity study were decapitated.

Offspring selected for brain weight and measurements and neuropathology included animals that were perfused and animals that were not perfused. In each case, 7-10 offspring/sex/dose from Subset D were examined at PND 21 and 10 offspring/sex/dose from randomly selected animals from Subsets A-C were examined at PND 75 [±5 days]. The terminal body weight and absolute and relative brain weights were measured in all these pups (Subset D and 10/sex/dose from Subsets A-C). Perfusion was performed by anesthetizing the animal with an intraperitoneal dose of pentobarbital, then perfusing the animal via the left ventricle with sodium nitrite followed by 1% (w/v) glutaraldedhyde and 4% (w/v) EM-grade formaldehyde. In perfused pups, the brain with olfactory bulbs was removed and weighed. The length (anterior to posterior) of the cerebrum and cerebellum were measured. In the offspring that were not perfused, the brain was weighed, and then the cerebellum was weighed. The relative to brain cerebellum weight was calculated.

Gross examinations were performed on animals sacrificed *in extremis*, and tissues were collected at the study director's discretion. Gross examinations were also performed on animals found dead, but tissues were not collected from these animals. Gross examination was performed on the perfused animals at PND 21 (Subset D; 8-10/sex/dose) and the non-perfused and perfused animals at PND 75 (±5 days; randomly selected animals from Subsets A-C; 10/sex/dose). The necropsy included an examination of all organs, brain, body cavities, cut surfaces, external orifices, and surfaces. Gross lesions in neural tissues or skeletal muscle were collected for microscopic examination. Other lesions were typically not examined. Except for the animals specified above, animals from Subsets A-C were sacrificed without gross or microscopic examination at PND 75 (±5 days).

Histological samples of the brain were obtained from the perfused animals sacrificed at PND 21 (Subset D; 8-10/sex/dose) and the perfused animals sacrificed at PND 75 (±5 days; randomly selected animals from Subsets A-C; 10/sex/dose). Only the tissue samples from the 1750 ppm and control groups were routinely processed for histological examination. The protocol said that other dose groups would be examined only if a treatment-related effect was found or in the event of a gross lesion. The CHECKED (X) tissues were evaluated.

| | CENTRAL NERVOUS SYSTEM | | PERIPHERAL NERVOUS SYSTEM |
|---|---|---|---|
| | BRAIN | | OTHER [a] |
| X | Olfactory bulbs | X | Gasserian ganglion |
| X | Olfactory region | X | Optic nerve |
| X | Forebrain (optic nerve) | X | Eyes |
| X | Forebrain (optic chiasma) | X | Skeletal muscle |
| X | Midbrain | X | Sural Nerve |

| | | | |
|---|---|---|---|
| X | Mesencephalon | X | Tibial Nerve |
| X | Cerebellum/pons | X | Sciatic nerve |
| X | Cerebellum/medulla oblongata | X | Lumbar spinal nerve roots and dorsal root ganglion |
| | **SPINAL CORD**[a] | X | Cervical spinal nerve roots and dorsal root ganglion |
| X | Cervical | X | Cauda equina |
| X | Lumbar | | |
| X | Thoracic | | |

a Only regions of the brain were examined in the animals from PND 21.

Sections from the brain, spinal cord, cauda equina, eyes, optic nerves, and gastrocnemius muscle were embedded in paraffin and sectioned at 5 µm. Sections were stained with hematoxylin and eosin; the forebrain, midbrain, and cerebellum/pons sections were also stained with luxol fast blue/cresyl echt violet. Dorsal root ganglia, gasserian ganglia, and peripheral nerves were embedded in glycol methacrylate resin, sectioned at 2-3 µm, and stained with a modified Lee's stain.

The thickness of the frontal cortex, parietal cortex, corpus callosum, and hippocampal gyrus; horizontal width of the caudate putamen; and cerebellum/pons height were measured in the perfused animals sacrificed at PND 21 (Subset D, 8-10/sex/dose) and the perfused animals sacrificed at PND 75 (±5 days; randomly selected animals from Subsets A-C; 10/sex/dose).

## D. DATA ANALYSIS

**1. Statistical analyses** - Equality of variance of continuous data was generally assessed using Bartlett's test ($p \leq 0.001$). Significance was denoted at $p \leq 0.05$ and 0.01, when using the following statistical procedures:

| Parameter | Statistical Methods |
|---|---|
| Total motor and locomotor activity (total and interval), acoustic startle response peak amplitude, water maze latency | ANOVA and Dunnett's |
| Interval motor and locomotor activity, acoustic startle response amplitude data for each block | Repeated measures ANOVA, ANOVA, and Dunnett's test |
| Passive avoidance latency data | Wilcoxon test |
| Passive avoidance and water maze - number of trials-to-criterion | Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention |
| Micropathology | Chi-square and one-tailed Fischer's Exact Test |

**2. Indices**

**a. Reproductive indices** - Mating index (number of inseminated females/number of females co-housed with males x 100) and fertility index (number of pregnant females/number of inseminated females x 100) were reported for each dose group.

**b. Offspring viability indices** - For each dose group, the following parameters were reported: (i) proportion of pups that were stillborn; (ii) live birth index (number of live pups born per litter/total number of pups per litter x 100); (iii) viability index (number of live pups on PND 4 pre-culling per litter/number of live pups born per litter x 100; and (iv) lactation index (number of live pups on PND 21 per litter/number of live pups on PND 4 post-culling per litter x 100.

**3. Positive control data** - Summaries of seven studies (MRIDs 45540501 through 45540507) performed to generate positive control data and validate the procedures and observers of the performing lab to conduct the FOB and to assess motor activity, neurotoxicity and behavioral effects were previously provided. Exposure to **3,3-Iminodipropionitrile** (2000 mg/kg, single i.p. dose) induced the following in both sexes: (i) decreased body weight; (ii) FOB effects (eg. ataxia, females only); (iii) decreased fore- and hindlimb grip strength; (iv) corneal opacities; (v) blood lacunae in the iris; (vi) anisocoria; and (vii) hematobulbus. Additionally, the following histopathological effects were noted: (i) axonal atrophy in the distal segments of the tibial, sural, and sciatic nerves; (ii) intraoccular hemorrhage; (iii) retinal degeneration with atrophy; and (iv) degeneration and atrophy of the optic nerve. **Acrylamide** (40 mg/kg, 11 daily gavage doses in 2 weeks) induced the following in both sexes: (i) abnormal gait (ataxia, splay of toes of the hindlimbs and/or splay of the hind limbs); (ii) decreased fore- and hindlimb grip strength; and (iii) increased hindlimb foot splay. Additionally in the males, body weight and body weight gains were decreased, and decreased activity, reduced tail pinch response, and increased reaction time to hot-plate test were observed. In addition to decreased brain weight in both sexes, the following histopathological effects were noted: (i) selective Purkinje cell necrosis and vacuolation of the molecular layer of the cerebellar cortex; (ii) cytoplasmic remodeling in the lumbar spinal ganglia cells which resembles chromatolysis; (iii) Wallerian-like axonal degeneration of the sciatic, sural, tibial, and plantar nerves; (iv) neurofilament accumulation, decrease in or loss of synaptic vessicles, and swelling of synaptic terminals in the gastrocnemius muscle; and (v) neuronal necrosis in the mesencephalic trigeminal nucleus region of the midbrain in one male. In addition to the effects given above, acrylamide (30 mg/kg, daily gavage doses up to 4 weeks) induced mortality in both sexes. **Trimethyltin chloride** (6, 9, or 12 mg/kg, single i.p. dose) induced ataxia, tremors, convulsions, decreased grip strength, increased foot splay, and increased motor activity. Additionally, the following neuropathological effects were noted: (i) neuronal necrosis of the olfactory bulbs and midbrain; (ii) axonal degeneration of the cervical ganglia and peripheral nerves; (iii) hydrocephalus internus of the frontal and parietal lobes; (iv) Purkinje cell necrosis in the pons with cerebellar cortex, mid-cerebellum, and medulla oblongata; (v) chromatolysis of alpha motor neurons in the cervical and lumbar spinal cord; and (vi) vacuolar degeneration of the lumbar ganglia. Inter-observer reliability was demonstrated using **carbaryl** (10 or 30 mg/kg, single i.p. dose), **nomifensin** (10 mg/kg, gavage on 2 days), and **diazepam** (3 mg/kg, i.p. on 2 days). All observers detected the FOB effects from carbaryl (abnormal body posture, tremors, repetitive chewing, impaired gait, and reduced rearing), the increased motor activity from nomifensin, and the decreased motor activity from diazepam.

On page 29 of MRID 46205301, the Sponsor stated that positive control agents including triadimefon, chlorpromazine, methimazole, and (presumably) the startle equipment were used to verify the lab's ability to detect positive findings (MRIDs 42770301, 45464602, 45441302, and 45441303). Data were not provided in this study, but a separate summaries of these studies was

available. The material presented in this section of the DER were past positive control study data from this Sponsor. MRID 45441302 is a Method Validation Study for a Developmental Neurotoxicity Screen: Untreated (Nomative) and Perinatal **Methimazole** Treatment in Wistar Rats. A summary of this study is one dose of methimazole from GD16 to PND10 at 0.1 mg/ml in the drinking water was administered to Wistar rats. Results indicated 14% decrease in maternal body weight and decrease in pup weights postnatally with recovery in males not in females by PND~60. Most endpoints were affected. Exceptions included: no change in FOB, no change in PND24 passive avoidance, and no evidence of histopathology from standard subjective assessments. Motor activity was only affected on PND 13 and no other ages. No effect on startle on PND23, increases on PND 38 and 60 only in males. Only effect in males on learning phase in water maze test. No effects in females. There was an 84% decrease in T4 and a 16% decrease in T3. The study appears to be adequate data to support proficiency for developmental exposure to one agent. Problems include: only decrease seen in MA testing and only at one age, no increases; no effects on FOB measures, PND24 learning/menory testing or std histopath. Only effects in one sex in learning portion of water maze, no effects on retention testing. The Overall Conclusion: Proficiency was marginal.

MRID 45441303 was Historical Control and Method Validation Studies for a Developmental Screening Battery (Auditory Startle Habituation and Coginitive Function (Passive Avoidance and Water Maze Conditioning). A summary of this study: For startle used a fairly standard 50 trial habituation paridigm. Recorded peak amplitude. Testing was immediately post-dosing. Cognitive testing used an M-maze and one dose of **scopolamine**, 1.0 mg/kg administered between 30 and 60 days prior to testing. Training consisted of 15 trials. Retention testing was conducted 24 hours later. Variables were trials to criterion, number of errors and latency to goal. Passive avoidance used a standard paridigm: trials to criterion was maxed out at 15. Results: For startle there was no effect of the mCPP 8-OHDPAT caused an increase at the highest dose. Results for the water maze testing are not very good. There were no statistically significant effects on any measure in males, in females there was a decrease in latency (??), and an increase in the number that failed to meet criterion (controls = 2; scoploamine = 5). For passive avoidance the data are not very impressive. For the learning phase there was a small increase in the trials to criterion and a small decrease in the latency on trial 2, but not trial 1. For retention testing there was a small increase in trials to criterion, a decrease in latency on trial 1 and no effect on trial 2. Not very big effects compared to published literature on scopolamine and passive avoidance. These data alone are marginal to non-aacceptable as evidence of proficiency. There are only males for the PA testing and the effects of 1.0 mg/kg scopolamine are rather small. M-maze performance was not affected at all in males and affected only slightly in females. Startle shown to be increased, but not decreased by reference compounds used previously by the author. **Overall Conclusion: Proficiency was not demonstrated for startle, PA, or M-maze.**

MRID 45464601: A Motor Activity Historical Control and Method Validation Study using Triadimefon and Chlorpyrifos. **Triadimefon** (90 min prior to testing, po) and **chlorpromazine** (60 min prior, ip) were administered to 70 day old Wistar rats. Testing in figure-8 mazes lasted 90 min, summed in 10 min bins. Data analyzed by SAS. Results: Triadimefon resulted in increased activity of about 300% and a decrease in habituation.. Chlorpromazine resulted in

decreased activity, about 50% for total counts. Summary: Triadimefon and chlorpromazine data are inadequate due to males only and only adults at 70 days of age. Data demonstrate ability to detect decreases and increases, as well as decreased habituation. Sensitivity is unknown due to lack of dose response. Overall Conclusion: Marginal.

The overall conclusion from these studies is that the lab proficiency was not demonstrated for the various behavioral tests.

## II. RESULTS

### A. PARENTAL ANIMALS

1. **Mortality, clinical signs, and functional observations** - At 1750 ppm, females were found dead at LDs 10 (Animal # 3113) and 20 (Animal # 3105), and another was sacrificed at LD 20 after the last of her pups was found dead. These animals exhibited no clinical signs of toxicity beforehand and were uninjured. There were no clinical signs of toxicity in any treated female. There were no treatment-related FOB findings recorded during gestation or lactation. In the FOB, the following changes ($p \leq 0.05$) relative to the controls were observed, but were unrelated to dose: (i) decrease in minimal resistance with vocalization (ease of removal) at 1750 ppm (20% treated vs 47% control) observed during handling at GD 20; (ii) decrease in number of fecal boluses in all treated females (0.6-0.9 treated vs 1.7 controls; but not statistically significant [NS] at 150 ppm) observed in the open field at GD 20; and (iii) increase in rearing at 150 ppm (4.9 treated vs 2.6 control) observed in the open field at LD 21.

2. **Body weight and food consumption** - Selected group mean body weights and food consumption values for pregnant and nursing dams are presented in Tables 2a and 2b. Body weight was decreased ($p \leq 0.05$) in the 1750 ppm females at LDs 14 and 21 by 7% each day, and body weight gain during lactation (LD 0-21) was decreased by 53%. This large change in body weight gain is an artifact of the small changes in the body weight in the controls. Differences ($p \leq 0.05$) in food consumption were observed in the ≥500 ppm dams during GD 13-20 (↑9-12%) and LD 7-21 (↓9-35%). No other differences in body weight or food consumption were observed.

**Table 2a.** Selected mean (± SE) body weights (g) for P females administered Dylox® from GD 0 through LD 21. [a]

| Day(s) | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| **Gestation** (n=25-30) | | | | |
| 0 | 200.8±1.84 | 199.8±2.45 | 199.6±1.73 | 201.9±2.47 |
| 6 | 220.1±2.17 | 218.6±2.64 | 217.8±2.17 | 218.0±3.42 |
| 20 | 304.3±2.97 | 303.8±3.99 | 304.5±3.45 | 304.9±4.84 |
| Gain, GD 0-20 | 103.5±1.98 | 104.0±3.00 | 104.9±2.55 | 103.1±2.95 |
| **Lactation** (n=17-30) | | | | |
| 0 | 235.7±2.22 | 231.7±3.45 | 236.1±2.80 | 234.0±3.61 |
| 7 | 256.9±3.87 | 253.9±5.16 | 255.6±2.99 | 249.2±4.42 |
| 14 | 272.8±4.49 | 275.3±4.41 | 270.6±4.72 | 254.4±3.95* (↓7) |
| 21 | 269.9±3.40 | 271.9±3.78 | 272.8±3.24 | 250.0±4.23** (↓7) |
| Gain, LD 0-21 | 34.2 | 40.2 | 36.7 | 16 (↓53) |

a Data were obtained from pages 63 and 69 of MRID 46205301. Percent difference from control (presented parenthetically) and lactation body weight gain were calculated by reviewers.

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

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

**Table 2b.** Mean (± SE) cumulative food consumption (g/animal/day) in P females administered Dylox® from GD 0 through LD 21. [a]

| Interval (days) | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| **Gestation** (n=24-30) | | | | |
| 0-6 | 16.5±0.53 | 16.5±0.55 | 16.8±0.47 | 15.9±0.56 |
| 6-13 | 18.0±0.40 | 18.3±0.76 | 19.1±0.69 | 18.7±0.68 |
| 13-20 | 19.1±0.38 | 19.1±0.53 | 20.9±0.49* (↑9) | 21.4±0.70* (↑12) |
| **Lactation** (n=16-23) | | | | |
| 0-7 | 34.5±1.16 | 37.0±2.34 | 34.4±1.72 | 32.9±2.46 |
| 7-14 | 51.2±1.01 | 48.9±0.97 | 45.3±1.17** (↓12) | 38.3±2.23** (↓25) |
| 14-21 | 65.2±1.29 | 69.2±2.02 | 59.4±1.65* (↓9) | 42.2±1.90** (↓35) |

a Data were obtained from pages 65 and 71 of MRID 46205301. Percent difference from control (presented parenthetically) were calculated by reviewers.

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

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

**3. Test substance intake** - Based on maternal food consumption and body weight, the doses expressed as mean daily mg test substance/kg body weight during the gestation and lactation periods are presented in Table 3.

**Table 3.** Mean (± SE) maternal test substance intake (mg/kg body weight/day) [a]

| Interval (days) | Dose (ppm) | | |
|---|---|---|---|
| | 150 | 500 | 1750 |
| GD 0-20 [b] | 13.4 | 49.0 | 145.6 |
| LD 0-21 [b] | 33.1 | 103.4 | 264.6 |

a Data were obtained from pages 41, 73, and 74 of MRID 46205301.
b Calculated by the Sponsor: µg of active ingredient/g feed/1000 x feed consumed in g/kg bw/day

**4. Reproductive performance** - The fertility index was decreased (not statistically significant [NS]) at 500 (↓7%) and 1750 (↓10%) ppm (Table 4). The Sponsor reported that these values were within the range of controls in other studies (MRIDs 45475501, 45537501, 45666401, and 45711201); however, data were not provided. The mating index was 100% for all dose groups.

**Table 4.** Delivery observations in P females administered Dylox® from GD 0 through LD 21. [a]

| Observation | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| # of animals co-housed and mated | 30 | 30 | 30 | 30 |
| # of animals found dead during gestation | 0 | 0 | 0 | 0 |
| Mating index | 100 | 100 | 100 | 100 |
| Fertility index | 100 | 100 | 93.3 | 90.0 |

a Data were obtained from page 59 of MRID 46205301.

**5. Maternal postmortem results** - Cholinesterase activity was decreased ($p \leq 0.05$) dose-dependently in P females as follows: (i) in plasma by 43-55% at ≥500 ppm; (ii) in erythrocytes by 26-71% in all treated groups; and (iii) in brain by 16-72% in all treated groups (Table 5).

**Table 5.** Mean (± SD) cholinesterase activity in P females at LD 21 following administration of Dylox® from GD 0 through LD 21. [a]

| Compartment | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| Plasma (IU/mL) | 0.74±0.20 | 0.62±0.13 | 0.42±0.05* (↓43) | 0.33±0.07* (↓55) |
| Erythrocyte (IU/mL) | 1.18±0.25 | 0.87±0.22* (↓26) | 0.40±0.23* (↓66) | 0.34±0.17* (↓71) |
| Brain (IU/g) | 13.2±0.8 | 11.1±0.9* (↓16) | 6.8±1.4* (↓48) | 3.7±0.6* (↓72) |

a Data (n=6-9) were obtained from page 859 of MRID 46205301. Percent difference from control (presented parenthetically) was calculated by reviewers.

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

## B. OFFSPRING

1. **Viability and clinical signs** - The number of litters born are shown in Table 6a. Two females in the 500 ppm group and 3 females in the 1750 ppm group were non-pregnant. No treatment-related clinical signs of pups were observed at any dose. On PND 4, a number of litters were electively sacrificed as shown in Table 6a. According to the study report "if a dam delivered fewer than three pups per sex or if the litter size decreased to fewer than seven pups by PND 4, the dam and the litter were sacrificed without necropsy examination." A summary of the remaining litters is presented in Table 6b. Due to sacrifice of pups from litters not selected for further behavioral testing, the effects of the treatments on the litters and the survival of the pups cannot be discerned from the way the data was summarized and presented in the study report.

**Table 6a.** $F_1$ live litter size and viability before culling. [a]

| Observation | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| Number pregnant dams | 30 | 30 | 28 | 27 |
| Dams found dead | 0 | 0 | 0 | 3 (LD 10 & 20) |
| Total number of litters born | 30 | 30 | 28 | 27 |
| Total number of pups delivered | 306 | 337 | 295 | 293 |
| Total # of pups born dead | 0 | 0 | 1 | 5 |
| Total number of litters PND 4 (preculling) [b] | 28 | 27 | 19 | 20 |
| Number of litters electively sacrificed by PND 4. | 7 | 7 | 9 | 8 |

[a] Data were obtained from pages 228 to 235 of MRID 46205301.

[b] The other litters were electively sacrificed

**Table 6b.** $F_1$ live litter size and viability after culling. [a]

| Observation | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| Total number of litters PND 4 (postculling) | 23 | 23 | 19 (↓17) | 17 (↓26) |
| Total # of pups delivered | 245 | 265 | 218 (↓11) | 194 (↓21) |
| # of stillborn | 0 | 0 | 0 | 2 |
| # found dead | 0 | 2 | 0 | 20 |
| # missing | 0 | 1 | 1 | 11 |
| Mean live pups/litter (total pups) | | | | |
| PND 0 | 11 | 12 | 11 | 11 |
| PND 4 (Pre-culling) | 11 | 11 | 11 | 11 |
| PND 4 (Post-culling) | 8 | 8 | 8 | 8 |
| PND 21 | 8 | 8 | 8 | 7 |
| Live birth index (mean ± SE) | 100±0 | 100±0 | 100±0 | 99.2±0.84 |
| Viability index (mean ± SE) | 100±0 | 99.6±0.40 | 99.5±0.53 | 94.9±2.42 |
| Lactation index (mean ± SE) | 100±0 | 98.9±1.09 | 100±0 | 94.9±2.64 |

a Data were obtained from pages 76 and 77 of MRID 46205301. Percent difference from control (presented parenthetically) was calculated by reviewers. However, these differences are difficult to interpret because of the elective sacrifice (see text for details),

**2. Body weight** - In the 1750 ppm group, body weight was initially decreased ($p \leq 0.01$) by 7-8%, and decreased ($p \leq 0.01$) more with time during the preweaning period (↓17-18% at PND 4; ↓37% at PND 21; Table 7a). Body weight was also decreased in the 500 ppm group during the preweaning period, but only significantly ($p \leq 0.05$) in the 500 ppm females at PND 21 (↓8%). Cumulative body weight gains (PNDs 0-4 and 4-21) were decreased ($p \leq 0.01$) by 31-43% in the 1750 ppm group. Cumulative body weight gains (PNDs 4-21) were decreased ($p \leq 0.05$) by 8-9% in the 500 ppm group. After administration of treated food to the dams was discontinued (post-weaning period), the body weights of the 1750 ppm pups remained decreased ($p \leq 0.05$) by 22-25% at PND 29-30 compared to controls; however, the animals began to recover body weight over time (↓6-9% at PND 71-72; Table 7b). Cumulative body weight gain (PND 29-71) was decreased (NS) by only 4% in the 1750 ppm males. Body weight and body weight gains for other treated animals were similar to controls.

**Table 7a.** Selected mean (± SD) $F_1$ animal pre-weaning body weights and body weight gains (g).[a]

| Postnatal Day | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| Males (n=17-23) | | | | |
| 0 | 6.0±0.10 | 6.0±0.10 | 5.8±0.09 | 5.5±0.10** (↓8) |
| 4 (Pre-culling) | 10.0±0.25 | 9.7±0.23 | 9.5±0.20 | 8.2±0.31** (↓18) |
| 4 (Post-culling) | 9.9±0.25 | 9.7±0.24 | 9.5±0.21 | 8.2±0.31** (↓17) |
| 11 | 24.7±0.61 | 24.3±0.66 | 23.8±0.40 | 18.3±0.80** (↓26) |
| 17 | 37.1±0.89 | 37.6±0.87 | 34.8±0.63 | 25.7±0.84** (↓31) |
| 21 | 47.7±1.14 | 48.4±1.09 | 44.4±0.77 | 30.0±1.02** (↓37) |
| Gain, Days 0-4 | 3.9±0.17 | 3.7±0.15 | 3.7±0.14 | 2.7±0.24** (↓31) |
| Gain, Days 4-21 | 37.8±0.97 | 38.7±0.91 | 34.9±0.63* (↓8) | 21.8±0.84** (↓42) |
| Females (n=17-23) | | | | |
| 0 | 5.7±0.10 | 5.7±0.10 | 5.5±0.09 | 5.3±0.09** (↓7) |
| 4 (Pre-culling) | 9.6±0.25 | 9.4±0.22 | 9.1±0.19 | 8.0±0.31** (↓17) |
| 4 (Post-culling) | 9.6±0.25 | 9.4±0.21 | 9.1±0.19 | 8.0±0.32** (↓17) |
| 11 | 24.2±0.62 | 23.9±0.63 | 23.1±0.43 | 18.1±0.81** (↓25) |
| 17 | 36.2±0.91 | 36.8±0.76 | 33.8±0.63 | 24.9±0.83** (↓31) |
| 21 | 46.6±1.10 | 47.3±1.03 | 42.7±0.84* (↓8) | 29.2±1.05** (↓37) |
| Gain, Days 0-4 | 3.9±0.16 | 3.7±0.13 | 3.6±0.12 | 2.7±0.24** (↓31) |
| Gain, Days 4-21 | 37.1±0.93 | 37.9±0.87 | 33.7±0.71* (↓9) | 21.3±0.86** (↓43) |

a Data were obtained from pages 85-87 and 89-90 of MRID 46205301. Percent difference from controls (calculated by reviewers) is presented parenthetically.

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

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

**Table 7b.** Selected mean (± SD) $F_1$ animal post-weaning body weights and body weight gains (g). [a]

| Postnatal Day | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| Males | | | | |
| 29 | 77.0±6.4 | 76.9±7.6 | 75.6±4.8 | 58.1±9.0* (↓25) |
| 71 | 303.4±26.6 | 302.8±32.4 | 303.2±24.3 | 275.1±26.7* (↓9) |
| Gain, Days 29-71 | 226.4 | 225.9 | 227.6 | 217 (↓4) |
| Females | | | | |
| 30 | 78.1±7.7 | 78.3±6.1 | 76.1±5.2 | 61.0±8.6* (↓22) |
| 72 | 189.6±16.7 | 187.1±12.8 | 185.9±11.4 | 178.4±14.4* (↓6) |
| Gain, Days 30-72 | 111.5 | 108.8 | 109.8 | 117.4 |

a Data were obtained from pages 96-97 of MRID 46205301. Percent difference from controls (calculated by reviewers) is presented parenthetically. Body weight gains were calculated by the reviewers from data in this table.

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

## 3. Developmental landmarks

**a. Sexual maturation** - Sexual maturation was delayed ($p \leq 0.05$) in the 1750 ppm group by 4-6% (Table 8), but this effect may have been related to decreased growth. Body weight was decreased ($p \leq 0.01$) by 9-10% at sexual maturation in these animals.

**Table 8.** Sexual maturation (mean days ± SE) in $F_1$ generation rats. [a]

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| N (M/F) | 69/68 | 69/68 | 56/57 | 39/43 |
| Preputial separation (days to) | 43.7±0.28 | 43.5±0.34 | 43.5±0.34 | 45.4±0.53* (↑4) |
| Body weight at criterion | 176±1.8 | 177±1.9 | 175±2.2 | 160±2.4** (↓9) |
| Vaginal patency (days to) | 34.1±0.32 | 34.8=0.35 | 34.5±0.38 | 36.0±0.58* (↑6) |
| Body weight at criterion | 106±1.6 | 108±1.3 | 104±1.7 | 95±2.0** (↓10) |

a Data were obtained from page 94 of MRID 46205301. Percent difference from controls (calculated by reviewers) is presented parenthetically.

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

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

**b. Physical landmarks** - Pupil constriction was evident in all control and treated pups at PND 21.

## 4. Behavioral assessments

**a. Functional observational battery** - No treatment-related effects were observed during the functional observational battery.

**b. Motor activity** - Total motor activity (Table 9a), locomotor activity (Table 9b), subsession motor activity (Tables 9c and 9d), subsession locomotor activity (Tables 9e and 9f) data are presented below. The following increases ($p \leq 0.05$, unless otherwise stated) in motor activity were noted in the 1750 ppm group on PND 17: (i) total motor activity (↑54-63%, NS); (ii) subsession motor activity in the males (↑127-171%) at intervals 2, 5, and 6, and in the females (↑173-357%) at intervals 4-6; (iii) total locomotor activity (↑48-58%, NS); and (iv) subsession locomotor activity in the males (↑133-200%) at intervals 5 and 6, and in the females (↑600%) at interval 5. The Sponsor stated that these differences from control on PND 17 may represent a modest delay in the development of habituation, which is consistent with the marked effect of this dose on body weight and secondary delays in preputial separation and vaginal patency. Otherwise, habituation was unaffected by treatment. Other differences in locomotor subsession activity (observed in males only) were regarded as incidental. Total and locomotor activity increased in all groups throughout the study (i.e. values at PNDs 60 > 21 > 17 > 13).

**Table 9a.** Mean (±SD) total motor activity data (number of movements) in $F_1$ pups in Subset A.[a]

| Postnatal Day | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| Males | | | | |
| 13 | 80±76 | 67±60 | 65±60 | 62±86 |
| 17 | 129±80 | 138±60 | 137±82 | 199±82 (↑54) |
| 21 | 270±84 | 263±120 | 231±64 | 263±115 |
| 60 | 586±149 | 505±104 | 469±129 | 495±191 |
| Females | | | | |
| 13 | 73±86 | 65±70 | 73±55 | 52±98 |
| 17 | 112±67 | 157±67 | 149±79 | 182±101 (↑63) |
| 21 | 227±106 | 256±93 | 249±92 | 227±92 |
| 60 | 733±250 | 674±148 | 664±123 | 654±135 |

a Data (n=11-16) were obtained from pages 188-189 of MRID 46205301.

**Table 9b.** Mean (±SD) locomotor activity data (number of movements) in $F_1$ pups in Subset A.[a]

| Postnatal Day | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| Males | | | | |
| 13 | 8±13 | 8±16 | 3±5 | 5±8 |
| 17 | 26±23 | 27±17 | 25±17 | 41±25 (↑58) |
| 21 | 64±16 | 54±20 | 54±12 | 61±25 |
| 60 | 399±124 | 332±85 | 294±98 | 305±148 |
| Females | | | | |
| 13 | 6±13 | 5±4 | 4±8 | 2±2 |
| 17 | 25±19 | 35±18 | 28±18 | 37±22 (↑48) |
| 21 | 51±14 | 61±20 | 54±16 | 52±15 |
| 60 | 485±180 | 455±137 | 444±107 | 441±131 |

a Data (n=11-16) were obtained from pages 191-192 of MRID 46205301.

**Table 9c.** Motor activity sub-sessions in $F_1$ males (mean ± S.D. activity counts) in Subset A.[a]

| Sub-session | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| PND 13 | 1 | 23±22 | 23±25 | 23±30 | 12±12 |
| | 2 | 18±23 | 12±20 | 12±18 | 8±11 |
| | 3 | 12±13 | 7±12 | 16±16 | 10±22 |
| | 4 | 8±13 | 8±14 | 6±14 | 13±24 |
| | 5 | 9±15 | 10±16 | 4±7 | 9±18 |
| | 6 | 11±19 | 8±23 | 5±7 | 10±21 |
| PND 17 | 1 | 42±29 | 59±30 | 60±42 | 46±27 |
| | 2 | 14±19 | 22±17 | 24±21 | 38±20* (↑171) |
| | 3 | 21±28 | 19±21 | 23±24 | 32±15 |
| | 4 | 30±24 | 16±22 | 14±17 | 29±17 |
| | 5 | 12±18 | 11±21 | 8±15 | 30±16* (↑150) |
| | 6 | 11±16 | 10±12 | 8±9 | 25±18* (↑127) |
| PND 21 | 1 | 98±39 | 98±45 | 86±27 | 67±21 |
| | 2 | 66±34 | 67±30 | 54±26 | 52±32 |
| | 3 | 37±21 | 40±24 | 33±23 | 44±23 |
| | 4 | 30±26 | 32±26 | 26±23 | 35±23 |
| | 5 | 23±24 | 13±17 | 9±14 | 37±24 |
| | 6 | 17±23 | 13±19 | 23±27 | 29±21 |
| PND 60 | 1 | 116±35 | 110±22 | 99±22 | 96±21 |
| | 2 | 96±28 | 84±17 | 87±31 | 79±41 |
| | 3 | 111±40 | 89±36 | 72±29 | 87±50 |
| | 4 | 98±26 | 78±23 | 70±27 | 80±42 |
| | 5 | 87±22 | 78±22 | 72±28 | 82±35 |
| | 6 | 78±31 | 66±36 | 69±29 | 70±41 |

a Data (n=11-16) were obtained from pages 194-197 of MRID 46205301.

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

**Table 9d.** Motor activity sub-sessions in $F_1$ females (mean ±S.D. activity counts) in Subset A[a]

| Sub-session | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| PND 13 | 1 | 18±20 | 20±25 | 15±20 | 13±15 |
| | 2 | 21±26 | 7±9 | 16±30 | 12±25 |
| | 3 | 8±12 | 12±17 | 15±22 | 7±13 |
| | 4 | 8±17 | 10±18 | 13±17 | 8±16 |
| | 5 | 9±21 | 9±15 | 9±11 | 8±27 |
| | 6 | 9±19 | 8±15 | 5±9 | 5±17 |
| PND 17 | 1 | 49±26 | 55±18 | 62±39 | 32±31 |
| | 2 | 22±20 | 34±24 | 27±25 | 33±17 |
| | 3 | 15±20 | 21±20 | 23±19 | 24±22 |
| | 4 | 7±12 | 13±23 | 16±22 | 32±35* (↑357) |
| | 5 | 8±12 | 17±25 | 13±20 | 32±25* (↑300) |
| | 6 | 11±18 | 17±24 | 9±14 | 30±26* (↑173) |
| PND 21 | 1 | 83±42 | 91±25 | 89±44 | 74±22 |
| | 2 | 55±35 | 57±29 | 46±27 | 37±21 |
| | 3 | 36±21 | 46±26 | 48±24 | 33±25 |
| | 4 | 21±25 | 29±24 | 25±19 | 29±23 |
| | 5 | 21±21 | 16±17 | 22±19 | 35±27 |
| | 6 | 11±17 | 17±19 | 20±21 | 19±19 |
| PND 60 | 1 | 131±45 | 131±38 | 128±23 | 128±16 |
| | 2 | 111±36 | 104±32 | 113±26 | 100±31 |
| | 3 | 127±46 | 110±34 | 112±24 | 113±40 |
| | 4 | 127±54 | 116±39 | 123±39 | 119±31 |
| | 5 | 126±65 | 109±19 | 104±44 | 105±28 |
| | 6 | 110±56 | 104±41 | 84±45 | 89±39 |

a Data (n=11-16) were obtained from pages 198-201 of MRID 46205301.

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

**Table 9e.** Locomotor activity sub-sessions in $F_1$ males (mean ±S.D. activity counts) in Subset A.[a]

| Sub-session | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| PND 13 | 1 | 2±2 | 3±5 | 1±1 | 1±1 |
| | 2 | 2±5 | 2±4 | 0±0 | 0±1 |
| | 3 | 1±2 | 1±3 | 1±2 | 1±2 |
| | 4 | 1±3 | 1±3 | 0±1 | 1±4 |
| | 5 | 1±2 | 1±3 | 1±2 | 1±2 |
| | 6 | 1±3 | 1±3 | 0±1 | 0±1 |
| PND 17 | 1 | 9±8 | 11±7 | 11±8 | 7±7 |
| | 2 | 3±5 | 5±5 | 5±4 | 7±4 |
| | 3 | 4±6 | 4±4 | 4±4 | 7±5 |
| | 4 | 6±7 | 4±6 | 3±4 | 7±6 |
| | 5 | 3±5 | 2±3 | 1±3 | 7±6* (↑133) |
| | 6 | 2±3 | 2±2 | 2±3 | 6±5* (↑200) |
| PND 21 | 1 | 27±7 | 23±9 | 25±6 | 18±6* (↓33) |
| | 2 | 14±6 | 13±5 | 11±5 | 12±7 |
| | 3 | 8±4 | 8±4 | 7±5 | 9±5 |
| | 4 | 7±5 | 5±5 | 5±5 | 8±6 |
| | 5 | 5±5 | 2±3 | 2±3 | 8±5 |
| | 6 | 3±5 | 3±4 | 4±5 | 6±4 |
| PND 60 | 1 | 83±28 | 76±18 | 72±17 | 66±16 |
| | 2 | 62±26 | 52±15 | 55±28 | 47±32 |
| | 3 | 80±35 | 62±27 | 45±23* (↓44) | 53±38 |
| | 4 | 69±21 | 51±20 | 43±22* (↓38) | 41±33* (↓41) |
| | 5 | 55±17 | 49±18 | 41±18 | 50±27 |
| | 6 | 49±26 | 41±27 | 39±17 | 48±32 |

a Data (n=11-16) were obtained from pages 203-206 of MRID 46205301.

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

**Table 9f.** Locomotor activity sub-sessions in $F_1$ females (mean ±S.D. activity counts) in Subset A.[a]

| Sub-session | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| PND 13 | 1 | 2±2 | 1±1 | 2±2 | 1±2 |
| | 2 | 2±4 | 1±1 | 2±5 | 0±1 |
| | 3 | 0±2 | 1±1 | 0±1 | 0±0 |
| | 4 | 1±2 | 1±2 | 1±1 | 0±1 |
| | 5 | 0±1 | 1±1 | 0±1 | 0±1 |
| | 6 | 1±4 | 0±1 | 0±1 | 0±0 |
| PND 17 | 1 | 11±9 | 15±5 | 12±8 | 6±6 |
| | 2 | 6±5 | 6±4 | 5±4 | 6±5 |
| | 3 | 3±5 | 4±5 | 4±3 | 5±7 |
| | 4 | 1±3 | 4±7 | 3±5 | 6±5 |
| | 5 | 1±3 | 3±6 | 2±4 | 7±7* (↑600) |
| | 6 | 2±5 | 3±5 | 2±4 | 7±8 |
| PND 21 | 1 | 24±9 | 26±9 | 23±8 | 20±5 |
| | 2 | 9±4 | 13±5 | 10±5 | 9±3 |
| | 3 | 8±5 | 10±6 | 8±5 | 6±4 |
| | 4 | 5±7 | 5±5 | 5±4 | 7±5 |
| | 5 | 4±3 | 3±3 | 5±4 | 5±4 |
| | 6 | 2±4 | 3±4 | 3±4 | 5±5 |
| PND 60 | 1 | 87±28 | 89±34 | 88±23 | 86±18 |
| | 2 | 73±31 | 67±24 | 71±19 | 63±26 |
| | 3 | 87±42 | 75±31 | 78±23 | 77±40 |
| | 4 | 88±43 | 79±39 | 87±34 | 82±30 |
| | 5 | 82±45 | 75±18 | 67±33 | 74±29 |
| | 6 | 69±38 | 71±38 | 53±34 | 59±34 |

a Data (n=11-16) were obtained from pages 207-210 of MRID 46205301.
* Significantly different from controls at $p \leq 0.05$

c. **Auditory startle reflex habituation** - Decreased ($p \leq 0.05$) maximum amplitude of the auditory startle reflex was observed in all blocks at PND 22 in the 1750 ppm group (↓50-63%; Table 10). Decreased ($p \leq 0.05$) amplitude was also observed in 1 or 2 blocks in the 150 and 500 ppm groups (↓27-34%) at PND 22. Total amplitude at PND 22 decreased at all doses in a dose-dependent manner in both sexes (↓19-61%). Incidental decreases ($p \leq 0.05$) were observed in all treated females in 1 or 2 blocks at PND 38, and these effects were unrelated to dose. Habituation was demonstrated and was unaffected by treatment. Latency was unaffected by treatment.

**Table 10.** Mean (±SD) auditory startle reflex maximum amplitude (g) data from $F_1$ rats in Subset B. [a]

| Observation [b] | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| Males | | | | | |
| PND 22 | Block 1 | 53±18 | 44±17 | 35±14* (↓34) | 20±11* (↓62) |
| | Block 2 | 52±17 | 41±18 | 38±16* (↓27) | 19±10* (↓63) |
| | Block 3 | 52±22 | 38±16* (↓27) | 38±14 | 20±12* (↓62) |
| | Block 4 | 46±20 | 37±18 | 36±14 | 18±11* (↓61) |
| | Block 5 | 41±15 | 37±15 | 31±12 | 18±10* (↓56) |
| | Total | 244 | 197 (↓19) | 178 (↓27) | 95 (↓61) |
| PND 38 | Block 1 | 107±61 | 70±45 | 88±42 | 75±39 |
| | Block 2 | 91±59 | 69±40 | 68±41 | 64±38 |
| | Block 3 | 83±59 | 65±32 | 63±47 | 59±40 |
| | Block 4 | 70±48 | 51±27 | 62±38 | 53±35 |
| | Block 5 | 69±51 | 43±19 | 57±38 | 52±23 |
| | Total | 420 | 298 | 338 | 303 |
| PND 60 | Block 1 | 249±195 | 252±203 | 178±107 | 195±121 |
| | Block 2 | 220±173 | 216±176 | 141±113 | 168±115 |
| | Block 3 | 180±124 | 189±120 | 106±66 | 125±89 |
| | Block 4 | 162±115 | 126±81 | 93±59 | 103±40 |
| | Block 5 | 120±74 | 101±66 | 104±81 | 85±36 |
| | Total | 931 | 884 | 622 | 676 |
| Females | | | | | |
| PND 22 | Block 1 | 50±17 | 42±18 | 39±14 | 25±12* (↓50) |
| | Block 2 | 55±15 | 46±30 | 38±18 | 24±11* (↓56) |
| | Block 3 | 52±19 | 37±22 | 35±18* (↓33) | 23±11* (↓56) |
| | Block 4 | 44±16 | 31±15* (↓30) | 35±15 | 19±8* (↓57) |
| | Block 5 | 44±17 | 30±19* (↓32) | 34±14 | 19±7* (↓57) |
| | Total | 245 | 186 (↓24) | 181 (↓26) | 110 (↓55) |
| PND 38 | Block 1 | 68±51 | 53±37 | 51±24 | 66±39 |
| | Block 2 | 88±74 | 41±26* (↓53) | 46±30* (↓48) | 46±18* (↓48) |
| | Block 3 | 64±47 | 37±32 | 38±23 | 40±25 |
| | Block 4 | 49±27 | 33±24 | 37±19 | 39±25 |
| | Block 5 | 43±19 | 25±13* (↓42) | 33±21 | 32±16 |
| | Total | 312 | 189 | 205 | 223 |

*(table continues next page)*

| Observation [b] | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| Females | | | | | |
| PND 60 | Block 1 | 123±107 | 87±43 | 106±42 | 133±98 |
| | Block 2 | 105±92 | 91±57 | 78±44 | 163±181 |
| | Block 3 | 74±36 | 62±35 | 69±37 | 119±106 |
| | Block 4 | 77±46 | 57±31 | 57±33 | 90±88 |
| | Block 5 | 67±50 | 52±20 | 67±26 | 82±69 |
| | Total | 446 | 349 | 377 | 587 |

a Data (n=12-16) were obtained from pages 215-220 of MRID 46205301.
b Block=10 consecutive trials
* Significantly different from controls at $p \leq 0.05$

**d. Learning and memory testing** - No treatment-related differences in learning or memory were noted in the low or mid dose groupsany treated group relative to concurrent controls in the passive avoidance and water maze tests (Tables 11a and 11b). In the passive avoidance test, a treatment-related effect manifested as a slight decrease ($p \leq 0.05$) in the latency period of trial 1 in the Retention session in high dose males at PND 29 (↓17%). In the water maze test, a minor increase in errors was observed in the 1750 ppm females at PNDs 58-62 during trial 2 of the learning phase (0.9 treated vs 0.2 controls).

**Table 11a.** Passive avoidance performance (mean±SD) in $F_1$ rats in Subset C. [a]

| Session/Parameter | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| Males | | | | | |
| Session 1 Learning PND 22 | Trials to criterion | 3.3±0.4 | 3.4±0.8 | 3.3±0.6 | 3.1±0.5 |
| | Latency trial 1 (sec) | 34.8±36.5 | 41.6±44.4 | 33.0±36.1 | 52.0±52.2 |
| | Latency trial 2 (sec) | 164.7±43.5 | 159.6±39.8 | 172.4±23.3 | 166.9±33.5 |
| | Failed to learn | 0 | 1 | 0 | 1 |
| Session 2 Retention PND 29 | Trials to criterion | 2.3±0.6 | 2.3±0.7 | 2.2±0.4 | 2.5±0.5 |
| | Latency trial 1 (sec) | 176.7±9.1 | 180.0±0.0 | 171.0±26.4 | 147.1±49.2* (↓17) |
| | Latency trial 2 (sec) | 173.3±26.6 | 171.6±27.7 | 180.0±0.0 | 180.0±0.0 |
| Females | | | | | |
| Session 1 Learning PND 22 | Trials to criterion | 2.9±0.3 | 3.2±0.5 | 3.1±0.3 | 2.9±0.3 |
| | Latency trial 1 (sec) | 36.5±49.2 | 33.2±25.0 | 26.7±24.5 | 41.0±55.4 |
| | Latency trial 2 (sec) | 180.0±0.0 | 178.6±5.5 | 170.1±31.1 | 180.0±0.0 |
| | Failed to learn | 1 | 0 | 0 | 2 |
| Session 2 Retention PND 29 | Trials to criterion | 2.3±0.6 | 2.1±0.3 | 2.6±0.7 | 2.1±0.4 |
| | Latency trial 1 (sec) | 174.1±15.6 | 174.0±24.0 | 158.5±54.6 | 178.4±4.9 |
| | Latency trial 2 (sec) | 176.7±12.8 | 180.0±0.0 | 170.7±25.4 | 180.0±0.0 |

a Data (n=13-16) were obtained from pages 222-223 of MRID 46205301.

**Table 11b.** Water maze performance (mean±SD) in $F_1$ rats in Subset C. [a]

| Session/Parameter | | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 150 | 500 | 1750 |
| Males | | | | | |
| Session 1 Learning PND 60 (±2 days) | Trials to criterion | 7.0±1.9 | 9.5±2.9 | 7.9±3.1 | 7.9±3.2 |
| | Trial 1 errors | 1.0±1.2 | 1.1±1.2 | 0.8±1.0 | 0.5±0.8 |
| | Trial 1 duration (sec) | 19.2±15.5 | 24.0±16.7 | 20.8±15.9 | 15.1±15.6 |
| | Trial 2 errors | 1.3±1.9 | 0.6±0.9 | 0.9±1.0 | 0.9±1.3 |
| | Trial 2 duration (sec) | 21.3±20.4 | 16.0±13.5 | 19.9±17.7 | 20.1±18.1 |
| | Failed to learn | 0 | 1 | 1 | 1 |
| Session 2 Retention PND 67 (±2 days) | Trials to criterion | 6.1±2.5 | 5.2±0.4 | 5.5±1.3 | 5.8±2.6 |
| | Trial 1 errors | 0.4±0.8 | 0.2±0.4 | 0.3±0.6 | 0.0±0.0 |
| | Trial 1 duration (sec) | 7.5±5.3 | 6.1±4.0 | 7.7±5.7 | 4.2±1.5 |
| | Trial 2 errors | 0.2±0.5 | 0.0±0.0 | 0.0±0.0 | 0.1±0.3 |
| | Trial 2 duration (sec) | 4.8±2.9 | 3.3±0.5 | 3.8±1.4 | 4.9±2.8 |
| Females | | | | | |
| Session 1 Learning PNDs 58-62 | Trials to criterion | 7.4±3.2 | 7.6±2.3 | 7.1±2.3 | 8.1±3.2 |
| | Trial 1 errors | 0.7±0.8 | 0.8±1.0 | 0.9±1.0 | 1.1±1.2 |
| | Trial 1 duration (sec) | 11.9±4.7 | 18.3±14.9 | 17.1±11.0 | 22.7±17.6 |
| | Trial 2 errors | 0.2±0.4 | 0.3±0.8 | 0.1±0.3 | 0.9±1.0* |
| | Trial 2 duration (sec) | 10.6±5.7 | 12.4±13.0 | 8.4±5.5 | 17.6±11.9 |
| | Failed to learn | 0 | 0 | 0 | 1 |
| Session 2 Retention PNDs 65-69 | Trials to criterion | 6.4±2.8 | 6.2±2.7 | 8.4±4.0 | 8.0±3.1 |
| | Trial 1 errors | 0.5±0.9 | 0.1±0.3 | 0.4±0.8 | 0.4±0.8 |
| | Trial 1 duration (sec) | 7.8±5.8 | 5.5±3.8 | 6.6±5.5 | 6.5±5.9 |
| | Trial 2 errors | 0.2±0.8 | 0.1±0.3 | 0.1±0.5 | 0.4±0.8 |
| | Trial 2 duration (sec) | 4.7±4.4 | 3.8±1.6 | 4.9±3.8 | 6.2±6.5 |

a Data (n=12-16) were obtained from pages 225-226 of MRID 46205301.
* Significantly different from controls at $p \leq 0.05$

## 5. Postmortem results

**a. Brain weights** - Terminal body weights were decreased ($p \leq 0.05$) in the 1750 ppm group (↓29-42%) and in the 500 ppm females (↓13%, perfused only) at PND 21, and in the 1750 ppm males (↓9-11%) on PND 75 (Tables 12a and 12 b). Absolute brain weights (perfused or non-perfused) were 7-18% significantly ($p<0.05$) less than in the controls in the 1750 ppm male and female pups sacrificed on PND 21. Absolute brain weight of the other groups were unaffected. At study termination, the absolute non-perfused brain and cerebullum weights were decreased ($p<0.05$) by 18% and 13% respectively in the 1750 ppm group male pups and cerebellum length was decreased 7% in the 1750 ppm males at study termination.

**Table 12a.** Mean (±SD) absolute and relative (to body) brain weights and measurements in $F_1$ rats from Subset D at PND 21.[a]

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| **Males** | | | | |
| Perfused Tissue | | | | |
| Terminal Body (g) | 43.1±6.1 | 47.9±6.2 | 44.9±4.3 | 30.4±4.8* (↓29) |
| Absolute Brain (g) | 1.354±0.067 | 1.399±0.050 | 1.393±0.049 | 1.256±0.053* (↓7) |
| Cerebrum (mm) | 13.43±0.33 | 13.52±0.28 | 13.58±0.22 | 13.20±0.36 |
| Cerebellum (mm) | 7.44±0.43 | 7.37±0.24 | 7.29±0.25 | 7.36±0.18 |
| Non-perfused Tissue | | | | |
| Terminal Body (g) | 44.3±5.5 | 45.9±3.4 | 40.8±3.9 | 28.0±3.0* (↓37) |
| Absolute Brain (g) | 1.464±0.060 | 1.495±0.056 | 1.424±0.080 | 1.225±0.091* (↓16) |
| Absolute Cerebellum (g) | 0.323±0.043 | 0.342±0.032 | 0.333±0.058 | 0.293±0.027 |
| **Females** | | | | |
| Perfused Tissue | | | | |
| Terminal Body (g) | 47.3±3.5 | 46.4±3.9 | 41.1±3.7* (↓13) | 27.2±3.5* (↓42) |
| Absolute Brain (g) | 1.370±0.078 | 1.367±0.039 | 1.352±0.055 | 1.201±0.043* (↓12) |
| Cerebrum (mm) | 13.48±0.24 | 13.46±0.21 | 13.44±0.27 | 12.90±0.26* (↓4) |
| Cerebellum (mm) | 7.63±0.59 | 7.20±0.21 | 7.27±0.32 | 7.22±0.41 |
| Non-perfused Tissue | | | | |
| Terminal Body (g) | 45.2±6.4 | 43.1±4.2 | 42.3±3.5 | 27.7±3.8* (↓39) |
| Absolute Brain (g) | 1.441±0.095 | 1.411±0.081 | 1.411±0.055 | 1.184±0.122* (↓18) |
| Absolute Cerebellum (g) | 0.328±0.032 | 0.321±0.046 | 0.313±0.025 | 0.311±0.074 |

a Data (n=7-10) were obtained from pages 891-895 of MRID 46205301.

**Table 12b.** Mean (±SD) absolute and relative (to body) brain weights and measurements in $F_1$ rats from Subsets A-C at PND 75 (±5 days).[a]

| Parameter | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| **Males** | | | | |
| Perfused Tissue | | | | |
| Terminal Body (g) | 311.3±26.1 | 284.9±43.6 | 302.6±19.2 | 276.6±17.0* (↓11) |
| Absolute Brain (g) | 1.830±0.093 | 1.835±0.086 | 1.796±0.082 | 1.745±0.065 |
| Cerebrum (mm) | 14.70±0.57 | 14.83±0.43 | 14.65±0.21 | 14.62±0.31 |
| Cerebellum (mm) | 7.86±0.40 | 7.80±0.41 | 7.54±0.15 | 7.33±0.33* (↓7) |
| Non-perfused Tissue | | | | |
| Terminal Body (g) | 308.1±26.6 | 310.2±29.9 | 305.7±20.3 | 280.8±20.6* (↓9) |
| Absolute Brain (g) | 1.932±0.082 | 1.962±0.073 | 1.909±0.049 | 1.811±0.077* (↓18) |
| Absolute Cerebellum (g) | 0.472±0.032 | 0.489±0.032 | 0.442±0.111 | 0.412±0.035* (↓13) |
| **Females** | | | | |
| Perfused Tissue | | | | |
| Terminal Body (g) | 188.4±18.1 | 198.4±6.3 | 186.3±11.6 | 176.1±12.7 |
| Absolute Brain (g) | 1.703±0.076 | 1.749±0.111 | 1.709±0.037 | 1.637±0.080 |
| Cerebrum (mm) | 14.30±0.40 | 14.37±0.53 | 14.52±0.29 | 14.21±0.33 |
| Cerebellum (mm) | 7.69±0.37 | 7.90±0.50 | 7.46±0.40 | 7.41±0.52 |
| Non-perfused Tissue | | | | |
| Terminal Body (g) | 186.3±16.6 | 188.7±15.0 | 191.7±11.9 | 182.6±11.8 |
| Absolute Brain (g) | 1.789±0.087 | 1.747±0.062 | 1.781±0.083 | 1.726±0.056 |
| Absolute Cerebellum (g) | 0.417±0.033 | 0.404±0.034 | 0.412±0.037 | 0.411±0.039 |

a Data (n=10) were obtained from pages 897-901 of MRID 46205301.

## b) Neuropathology

1) **Macroscopic examination** - No treatment-related gross pathological findings were noted in any treated group at either PND 21 or 75 (±5 days).

2) **Microscopic examination** - No treatment-related effects on histopathology findings or linear brain measurements were noted in any treated group at either PND 21 or 75 (±5 days; Table 13). Various lesions were observed, but the incidence was minor, such as (n=10) minimal perivascular cuffing in the lumbar spinal cord in one 1750 ppm perfused female at ~PND 70 (vs 0 controls), hydrocephalus and brain atrophy in one 150 ppm female at ~PND 70 (vs 0 controls).

**Table 13.** Microscopic linear brain measurements [a]

| Length (mm) | Dose (ppm) | |
|---|---|---|
| | 0 | 1750 |
| **Males** | | |
| **Day 21** | | |
| Frontal cortex | 1.8761±0.0084 | 1.8216±0.0092 |
| Parietal cortex | 1.9579±0.0090 | 1.9164±0.0158 |
| Caudate putamen | 3.1412±0.0255 | 3.1797±0.0093 |
| Corpus callosum | 0.4402±0.0089 | 0.3784±0.0052 |
| Hippocampal gyrus | 1.6290±0.0140 | 1.5725±0.0097 |
| Cerebellum | 4.5484±0.0284 | 4.2443±0.1362 |
| **Day 75 (±5 days)** | | |
| Frontal cortex | 1.7785±0.0124 | 1.7880±0.0177 |
| Parietal cortex | 1.8389±0.0177 | 1.8570±0.0178 |
| Caudate putamen | 3.2965±0.0419 | 3.4143±0.0663 |
| Corpus callosum | 0.5956±0.0134 | 0.5768±0.0267 |
| Hippocampal gyrus | 1.7313±0.0223 | 1.7034±0.0156 |
| Cerebellum | 4.6589±0.0781 | 4.4823±0.0633 |
| **Females** | | |
| **Day 21** | | |
| Frontal cortex | 1.9612±0.0175 | 1.8829±0.0048 |
| Parietal cortex | 2.0243±0.0070 | 1.9036±0.0148 |
| Caudate putamen | 3.2176±0.0113 | 3.0724±0.0106 |
| Corpus callosum | 0.4443±0.0099 | 0.4656±0.0130 |
| Hippocampal gyrus | 1.5912±0.0205 | 1.5263±0.0026 |
| Cerebellum | 4.2687±0.1799 | 4.3990±0.0807 |
| **Day 75 (±5 days)** | | |
| Frontal cortex | 1.7189±0.0235 | 1.8287±0.0136 |
| Parietal cortex | 1.7759±0.0329 | 1.8842±0.0030 |
| Caudate putamen | 3.4038±0.0112 | 3.3358±0.0208 |
| Corpus callosum | 0.5483±0.0081 | 0.5625±0.0078 |
| Hippocampal gyrus | 1.6423±0.0128 | 1.5945±0.0135 |
| Cerebellum | 4.6294±0.1132 | 4.4734±0.1122 |

a Data (n=8-10) were obtained from pages 903-911 of MRID 46205301.

**c) Cholinesterase determinations** - At 1750 ppm, plasma and erythrocyte cholinesterase levels were decreased ($p \leq 0.05$) by 25% and 28%, respectively, in the pooled male and female samples at PND 4 (Table 14a). On PND 21, plasma and brain cholinesterase levels were decreased

($p \leq 0.05$) in both sexes by 29-39% and 16-23%, respectively. Additionally on PND 21, minor decreases ($p \leq 0.05$) were also observed in brain cholinesterase levels in the 500 ppm males (↓7%) and the 150 and 500 ppm females (↓7-11%). The brain ChE activity in the female controls appears to be high, particularly three of the individual values seemed to be outliers (Table 14b). If these are excluded, the mean control value will be 11.8 ±0.3 and the decrease in the female brain ChE activity relative to the control at 150, 500, and 1750 ppm will be 4%, 8% and 14%, respectively. Furthermore, the brain ChE activity in the female controls in the current trichlorfon study are relatively higher than the comparable control values in other studies from the same Bayer laboratory conducted around the same time period (Table 14c). This gives more support that the decreased brain ChE activity in the current trichlorfon study at 150 ppm is marginal and insignificant.

**Table 14a.** Mean (± SD) cholinesterase activity in $F_1$ rats[a]

| Cholinesterase activity | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 150 | 500 | 1750 |
| PND 4 (pooled males and females; n=13-21) | | | | |
| Plasma (IU/mL) | 0.63±0.06 | 0.64±0.08 | 0.58±0.06 | 0.47±0.13* (↓25) |
| Erythrocyte (IU/mL) | 1.25±0.32 | 1.32±0.37 | 1.08±0.40 | 0.90±0.35* (↓28) |
| Brain (IU/g) | 4.5±0.4 | 4.5±0.4 | 4.5±0.5 | 4.2±0.5 |
| PND 21 males (n=5-10) | | | | |
| Plasma (IU/mL) | 0.62±0.08 | 0.61±0.05 | 0.56±0.11 | 0.38±0.13* (↓39) |
| Erythrocyte (IU/mL) | 1.25±0.18 | 1.33±0.14 | 1.47±0.38 | 1.31±0.37 |
| Brain (IU/g) | 11.6±0.4 | 11.7±0.5 | 10.8±0.8* (↓7) | 8.9±1.1* (↓23) |
| PND 21 females (n=6-9) | | | | |
| Plasma (IU/mL) | 0.59±0.09 | 0.64±0.07 | 0.52±0.08 | 0.42±0.05* (↓29) |
| Erythrocyte (IU/mL) | 1.20±0.19 | 1.21±0.28 | 1.24±0.22 | 1.37±0.27 |
| Brain (IU/g) | 12.2±0.7 | 11.3±0.4* (↓7) | 10.9±0.8* (↓11) | 10.2±0.3* (↓16) |

a Data obtained from pages 856-858 of MRID 46205301.
* Significantly different from controls at $p \leq 0.05$

**Table 14b.** Individual Brain ChE activity in $F_1$ Pups (IU/g)[a]. Mean ± SD are bolded

| Control | 150 ppm | 500 ppm | 1750 ppm |
|---|---|---|---|
| PND 21 male pups | | | |
| 11.2, 11.5, 11.5, 11.7, 11.1, 12.1, 11.9, 11.4, 12.2, 11.8<br>**11.6±0.4** | 11.6, 12.0, 10.5, 12.0, 11.9, 11.6, 12.1<br>**11.7±0.5** | 11.1, 10.9, 10.8, 10.1, 11.9, 9.6, 10.1, 11.5, 11.5<br>**10.8±0.8** * (↓7%) | 9.9, 7.7, 9.8, 7.9, 9.2<br>**8.9±1.1** *(↓23%) |
| PND 21 female pups | | | |
| 13.6,12.9, 12.4, 12.3,12.0, 11.9, 11.7, 11.6, 11.3<br>**12.2±0.7** | 11.9, 11.6, 11.5, 11.5, 11.3, 11.3, 11.2, 10.9, 10.7,<br>**11.3±0.4** * (↓7%) | 11.3, 12.3, 10.6, 10.2, 11.4, 10.9, 10.0<br>**10.9±0.8** * (↓11%) | 10.4, 10.6, 9.8, 9.9, 10.6, 10.2<br>**10.2±0.3** * (↓16%) |

[a] Data obtained from pages 949-956 of MRID 46205301
* Significantly different from controls at $p \leq 0.5$

**Table 14c.** Female brain ChE activity in control group females for several other studies from the Bayer lab (mean value ± SD)

| Chemical | Year | MRID | Female Brain AChE (IU/gm) |
|---|---|---|---|
| Trichlorfon | 2003 | 46205301 | 12.2 ± 0.7 |
| Coumaphos | 2003 | 45912101 | 11.6 ± 0.5 |
| Disyston | 2002 | 45827601 | 11.5 ± 0.4 |
| Tribufos | 2001 | 45499501 | 11.2 ± 0.5 |
| Azinphos methyl | 2002 | 45711201 | 11.4 ± 0.5 |
| Fenamiphos | 2004 | 46203401 | 11.5 ± 0.5 |
| Methamidphos | 2002 | 45666401 | 11.6 ± 0.3 |

Mean for six (not including trichlorfon) = 11.47 ±0.2

**d) Ophthalmology** - No treatment-related effects were observed during ophthalmology.

## III. DISCUSSION and CONCLUSIONS

**A. INVESTIGATORS' CONCLUSIONS** - The investigators concluded that the maternal LOAEL was 500 ppm, based on inhibition of plasma, erythrocyte, and brain cholinesterase activity measured on LD 21. The offspring LOAEL was also set at 500 ppm, based on slight inhibition of brain cholinesterase activity at PND 21, decreased startle amplitude at PND 22, and decreased body weight gain at weaning in both sexes.

## B. REVIEWER'S COMMENTS

There were no clinical signs of toxicity in any treated female. Two females in the 1750 ppm dose were found dead one each on lactation day (LD) 10 and 20 and another was sacrificed at LD 20 after the last of her pups was found dead, without clinical signs of toxicity or injury. No treatment-related effects were seen on clinical signs, body weight, body weight gain, food consumption, FOB, or reproductive parameters. There was a significant ($p \leq 0.05$), dose-related decreases in: (i) plasma (43-55%) at ≥500 ppm; (ii) erythrocytes ( 26-71%) at all doses; and (iii) brain (16-72%) at all doses.

**The LOAEL for maternal systemic toxicity is 150 ppm (13.4 mg/kg/day; LDT) based on the inhibition of red blood cell cholinesterase activity. A NOAEL for maternal systemic toxicity is not established.**

Viability and lactation indicies were each decreased by 5.1% at the high dose. No treatment-related clinical signs were reported at any dose. Body weight and weight gain of pups were significantly decreased at the mid (8%) and high dose (37%) by PND 21, but not at the low dose. Cumulative body weight gains (PNDs 0-4 and 4-21) were decreased ($p \leq 0.01$) by 31-43% in the 1750 ppm group. Cumulative body weight gains (PNDs 4-21) were decreased ($p \leq 0.05$) by 8-9% in the 500 ppm group. Gradual recovery of body weights occurred during the post weaning period.

Sexual maturation (prepuptial separation and vaginal patency) was delayed by 4-6% ($p \leq 0.05$) in the 1750 ppm group, but this effect may have been related to decreased growth. Body weight was decreased ($p \leq 0.01$) by 9-10% at sexual maturation in these animals. Pupil constriction was evident in all control and treated pups at PND 21. Ophthalmology examination revealed no treatment-related effects.

No treatment-related effects were observed on FOB at any dose level or learning and memory at the low and mid dose groups. Increased motor and locomotor activity was seen in the high dose group offspring males and females on PND 17. Habituation was unaffected by treatment. There was a clear dose-depended deceases in auditory startle response in both sexes at all dose levels at different time periods (ages). Decreased ($p \leq 0.05$) maximum amplitude of the auditory startle reflex was observed in all blocks at PND 22 in the 1750 ppm group (↓50-63%). Decreased ($p \leq 0.05$) amplitude was also observed in 1 or 2 blocks in the 150 and 500 ppm groups (↓27-34%) at PND 22. Total amplitude at PND 22 decreased dose-dependently in both sexes (↓19-61%). Habituation was demonstrated and was unaffected by treatment. Latency was unaffected by treatment. In the passive avoidance test, a slight decrease ((↓17%; $p \leq 0.05$) in the latency period of Trial 1 in the Retention session was observed in males at the highest dose on PND 29. A minor increase in errors was observed in the 1750 ppm females at PNDs 58-62 during trial 2 of the learning phase (0.9 treated vs 0.2 controls) in the water maze test.

Terminal body weights were decreased ($p \leq 0.05$) in the 1750 ppm males and females (↓29-42%) and in the 500 ppm females (↓13%, perfused only) at PND 21, and in the 1750 ppm males (↓9-11%) on PND 75. Absolute brain weights (perfused or non-perfused) were 7-18% significantly

($p<0.05$) less than in the controls in the 1750 ppm male and female pups sacrificed on PND 21. Absolute brain weight of the other groups were unaffected. At study termination, the absolute non-perfused brain and cerebullum weights were decreased ($p<0.05$) by 18% and 13% respectively in the 1750 ppm group male pups and cerebellum length was decreased 7% in the 1750 ppm males at study termination.

Macroscopic neuropathological examination revealed no treatment-related gross pathological findings in any treated group at either PND 21 or 75 (±5 days). Microscopic neuropathological examination revealed no treatment-related effects on histopathology findings or linear brain measurements in any treated group at either PND 21 or 75. Various lesions were observed, but the incidence was minor, such as (n=10) minimal perivascular cuffing in the lumbar spinal cord in one 1750 ppm perfused female at ~PND 70 (vs 0 controls), hydrocephalus and brain atrophy in one 150 ppm female at ~PND 70 (vs 0 controls).

At 1750 ppm, plasma and erythrocyte cholinesterase levels were decreased ($p\leq0.05$) by 25% and 28%, respectively, in the pooled male and female samples at PND 4. On PND 21, plasma and brain cholinesterase levels were decreased ($p\leq0.05$) in both sexes by 29-39% and 16-23%, respectively. Additionally on PND 21, decreases ($p\leq0.05$) were also observed in brain cholinesterase levels in the 500 ppm males (↓7%) and the 150 and 500 ppm females (↓7-11%). However the decrease in the brain ChE activity level in the 150 ppm female pups was attributed to an unusually high control level and therefor was not considered to be treatment related.

**The LOAEL for the offspring toxicity is 150 ppm (13.4 mg/kg/day) based on decreased startle amplitude in males and females on PND 22. An Offspring NOAEL is not established.**

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) at this time pending a comprehensive review of all available positive control data.

**C. <u>STUDY DEFICIENCIES</u>** The following suggestions and requirements noted in the Agency memo dated September 20, 2000 were not performed by the Registrant in this study:

- *On page 3, it is stated that the EPA recommends that cholinesterase activity be measured in: (i) pups and dams on PND/LD 21; (ii) fetuses and dams on GD 20; and (iii) pups during early and mid lactation.* CheA was determined on LD/PND 21 in dams and pups and in pups on PND 4 (early lactation); however, no activity determinations were performed on GD 20 or mid lactation.
- *On page 3, it stated that the EPA highly recommends that measurements of cholinesterase activity in a variety of peripheral nervous system tissues be performed. Additional tissues (atria of the heart, skeletal muscle, lung, diaphragm, and salivary glands) can also be evaluated.* The registrant did not perform these measurements.
- *On page 5, the EPA stated that the Registrant should weigh the cerebellum and perform histological examination and morphometric assessment of the external granule layer, the molecular layer, and Purkinje cells of the cerebellum.* The Registrant didn't weigh the cerebellum or measure the external granule layer, the molecular layer, or Purkinje cells of the cerebellum.

13544

# R112181

| | |
|---|---|
| **Chemical:** | **Trichlorfon** |
| **PC Code:** | **057901** |
| **HED File Code** | **13000 Tox Reviews** |
| **Memo Date:** | **07/26/2005** |
| **File ID:** | **TX0052426** |
| **Accession Number:** | **412-05-0099** |

**HED Records Reference Center**
**08/15/2005**