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

OFFICE OF PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

TXR: 0054212

DATE: **August 22, 2006**

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

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

TO: Kylie Rothwell
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THRU: Susan V. Hummel, Branch Senior Scientist
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TASK ID: DP Code: D322591 and D321550

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

Action Requested: Review and Prepare DER for the following Cholinesterase inhibition Studies MRIDs: 46647401, 46647402, 46635401, 46647404, 46647403, 46635601, and 46635501

Agency's Response: HED toxicologists have reviewed and prepared DERs of the above MRIDs and the findings are presented below. These studies supplement the developmental neurotoxicity study (MRID 46205301) and they are classified Acceptable/Nonguideline. The DERs are attached to this memo.

MRID 46635501. Klaus, A.M., Holzum (2005) Cholinesterase inhibition in maternal and fetal rats following gestational exposure via the diet with technical grade trichlorfon. Bayer HealthCare AG, Wuppertal Germany. Unpublished Report No. AT02232. 109 pages.

In a comparative cholinesterase (ChE) inhibition study, trichlorfon (100% a.i.) was administered to 13 inseminated female rats/group in the diet at concentrations of 150, 500, and 1750 ppm (10.4, 37.2, 134 mg/kg/day, respectively) from gestation day 0 to 20. On Day 20 of gestation, pups were retrieved by cesarean section and all animals were sacrificed for blood and tissue collection. No adverse clinical signs of toxicity were observed. Inhibition of ChE activity was apparent in all compartments of female rats in the mid- and high dose treatment groups. Significant inhibition of brain ChE activity was 20 and 58%, respectively, erythrocyte ChE activity was 31 and 54%, and plasma was 22 and 61%. In the corresponding fetuses (pooled by litter) significant inhibition of brain ChE activity (10 to 36%) was observed at all dose levels, while inhibition of erythrocyte ChE activity (49%) was observed at 1750 ppm only. Fetal plasma ChE inhibition was significant at the 500 ppm level (23%) and at 1750 ppm (38%). The overall maternal LOAEL for dietary exposure of rats to trichlorfon on gestation days 0 to 20 for cholinesterase activity inhibition is 500 ppm (37.2 mg/kg/day) based on enzyme inhibition in brain, plasma and, erythrocytes. The maternal NOAEL is 150 ppm (10.4 mg/kg). The overall fetal LOAEL for dietary exposure of rats to trichlorfon for cholinesterase activity inhibition in rats on gestation days 0 to 20 is 150 ppm (10.4 mg/kg/day) based on enzyme activity inhibition in brain. The fetal NOAEL was not identified.

MRID 46647402. Klaus A.M. (2005) Study to establish the time of peak cholinesterase inhibition in young-adult Wistar rats treated by gavage with an acute dose of technical grade trichlorfon. Bayer HealthCare AG, PH-R&D-PD Toxicology International, 42096 Wuppertal, Germany. Report No. AT02016, Study No. T0073929. March 31, 2005. 44 pages.

MRID 46647401. Langewische, F.W. (2005) Cholinesterase inhibition in young-adult Wistar rats treated by gavage with an acute dose of technical grade trichlorfon. Bayer HealthCare AG, PH-R&D-PD Toxicology International, 42096 Wuppertal, Germany. Report No. AT02063, Study No. T5073933. May 30, 2005. Unpublished. 79 pages.

MRID 46635401. Klaus A.M. (2005) Cholinesterase inhibition in young-adult Wistar rats treated daily by gavage for 11 days with technical grade trichlorfon. Bayer HealthCare AG, PH-R&D Toxicology, 42096 Wuppertal, Germany. Report No. AT02197, Study No. T0073352. July 20, 2005. Unpublished. 95 pages.

These studies were reviewed together in one DER. In a series of special non-guideline comparative cholinesterase inhibition (ChEI) studies, trichlorfon (Dylox technical; 99.6% a.i., lot #1030228) was administered by oral gavage to groups of young adult Wistar rats. For time-course evaluation (MRID 46647402) 6 animals/sex/group were given a single oral dose of 0 or 75 mg/kg and sacrificed 1, 2, 4, or 8 hours later. In an acute study (MRID 46647401)

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groups of 6 rats/sex were given a single oral dose of 0, 10, 25, or 50 mg/kg and sacrificed 1 hour post-dosing, at the time of peak effect. Finally, repeated administration was studied (MRID 46635401) by giving eleven daily doses of 0, 5, 10, 20, or 40 mg/kg/day to groups of 6 rats/sex; animals were sacrificed 1 hour after the last dose. Plasma, red blood cell (RBC), and brain cholinesterase (ChE) activities were measured in all animals in each study. All animals survived to scheduled sacrifice. No adverse clinical signs of toxicity were observed in any animal in any study. Body weight gain was not adversely affected by treatment in the repeated dose study.

During time-course investigations, the 1 hour time point consistently had the highest degree of ChE inhibition in all three compartments in both males and females, thus the time of peak effect was 1 hour. Females were more severely affected than males at all time points and in all compartments. For acute oral exposure to trichlorfon, the overall adult LOAEL for cholinesterase inhibition in rats is 25 mg/kg based on enzyme inhibition in brain from females and the adult NOAEL is 10 mg/kg. For repeated oral exposure to trichlorfon, the overall adult LOAEL for cholinesterase inhibition in rats is 20 mg/kg/day based on enzyme inhibition in brain in males and females and in red blood cells in females; the adult NOAEL is 10 mg/kg/day. The ChE activity measurements following acute and repeated oral dosing with trichlorfon demonstrate that adult females are more susceptible than adult males. Compared to plasma (52-55% inhibition) or brain (50-57% inhibition) enzyme activity in adult females, the RBC ChE activity appeared to be the least sensitive tissue to acute trichlorfon exposure (37% inhibition) but the most sensitive tissue following repeated exposure (66% inhibition).

MRID 46647404. Langewische, F.W. (2005) Study to determine the time of peak cholinesterase inhibition in preweaning Wistar rats treated by gavage with an acute dose of technical grade trichlorfon. Bayer HealthCare AG, PH-R&D-PD Toxicology International, 42096 Wuppertal, Germany. Report No. AT02017, Study No. T4073932. April 15, 2005. Unpublished. 48 pages.

MRID 46647403. Langewische, F.W. (2005) Study to determine cholinesterase inhibition in postnatal day 11 Wistar rats treated by gavage with an acute dose of technical grade trichlorfon. Bayer HealthCare AG, PH-R&D-PD Toxicology International, 42096 Wuppertal, Germany. Report No. AT02064, Study No. T8073936. May 30, 2005. Unpublished. 80 pages.

MRID 46635601. Klaus A.-M. (2005) Study to determine cholinesterase inhibition in postnatal day 11 Wistar rats treated by gavage for eleven days with technical grade trichlorfon. Bayer HealthCare AG, PH-R&D Toxicology, 42096 Wuppertal, Germany. Report No. AT02252, Study No. T5073357. August 5, 2005. Unpublished. 73 pages.

These studies were reviewed together in one DER. In a series of special non-guideline comparative cholinesterase activity inhibition studies, trichlorfon (Dylox technical; 100% a.i., lot #1030228) was administered by oral gavage to groups of preweaning Wistar rats. For time-course evaluation (MRID 46647404) 9-10 animals/sex/group were given a single oral dose of 0 or 50 mg/kg on post-natal day (PND) 11 and sacrificed 1, 2, 4, 8, or 24 hours later.

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In an acute study (MRID 46647403) groups of 10 rats/sex were given a single oral dose of 0, 5, 10, or 30 mg/kg on PND 11 and sacrificed 2 hours post-dosing, at the time of peak effect. Finally, repeated administration was studied (MRID 46635601) by giving eleven daily doses of 0, 5, 10, or 20 mg/kg/day to groups of 10 rats/sex on PNDs 11-21; animals were sacrificed 1 hour after the last dose. Plasma, red blood cell (RBC), and brain cholinesterase (ChE) activity was measured in all animals in each study. No adverse clinical signs of toxicity or treatment-related deaths were observed in any animal in any study.

During time-course investigations, maximum ChE inhibition occurred at 1-2 hours post-dosing. In male pups, the highest levels of inhibition of plasma and RBC cholinesterase activity were 82 and 75%, respectively, observed at 1 hour after dosing, and the greatest inhibition of brain cholinesterase activity was 58%, observed at 2 hours after dosing. In female pups, the highest level of cholinesterase activity inhibition was observed at 2 hours for all compartments with inhibition for plasma, RBC, and brain, at 81, 65, and 66%, respectively. After 24 hours, there was still significant inhibition of plasma (38%) and RBC (23%) enzyme activity in males, while brain enzyme activity recovered to control levels. In females after 24 hours, there was significant inhibition of plasma ChE (34%), while RBC and brain enzyme activity recovered to control levels. The time of peak effect of cholinesterase inhibition after acute trichlorfon administration was determined to be 2 hours post dosing. After an acute dose of trichlorfon, there was significant inhibition of plasma (68-75%), RBC (24-37%), and brain (39-45%) ChE activity in males and females at the high dose (30 mg/kg). After an acute dose of 10 mg/kg, there was significant inhibition in male and female plasma (26-29%) and brain (9-13%) ChE activity, but no significant effects on RBC enzyme activity. After an acute dose of 5 mg/kg, plasma ChE activity was inhibited by 12% in both males and females. For acute oral exposure to trichlorfon, the overall LOAEL for cholinesterase activity inhibition in PND 11 rats is 10 mg/kg based on enzyme inhibition in plasma and brain in males and females; the preweaning NOAEL is 5 mg/kg. Following repeated dosing with 20 mg/kg/day, plasma ChE activity was significantly inhibited in both males (24%) and females (39%). RBC enzyme activity was inhibited in high-dose females by 34% although statistical significance was not attained. Brain ChE activity was significantly inhibited in males at the high dose (17%), and in females at all doses (6, 11, and 26% at 5, 10, and 20 mg/kg/day, respectively). For repeated oral exposure to trichlorfon, the overall LOAEL for cholinesterase activity inhibition in PND 11 rats is 5 mg/kg/day based on enzyme inhibition in brain in females; the preweaning NOAEL is not identified. The ChE activity measurements following acute oral dosing with trichlorfon did not demonstrate a difference in susceptibility between preweaning male and female rat pups. By 24 hours post-dosing females showed a greater recovery in RBC activity, but no differences between the sexes were seen in the other compartments. Following repeated dosing, however, females appeared slightly more susceptible than males. The RBC ChE activity was the least sensitive compartment following acute and repeated trichlorfon exposure.

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DATA EVALUATION RECORD

TRICLORFON (DYLOX TECHNICAL)

Study Type: SPECIAL STUDIES, CHOLINESTERASE INHIBITION [NON-GUIDELINE]
MRID 46647402 (time-course); 46647401 (acute-young adult); 46635401 (repeated - young adult)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
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Task Order No. 124-2006A

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC., for the U.S. Dept. of Energy under contract DE-AC05-00OR22725.

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Reregistration Branch 4, Health Effects Division (7509P) **Date** Aug. 15, 2006
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date** 8/16/06
Template version 08/05

TXR#: 0054212

DATA EVALUATION RECORD

STUDY TYPE Non-guideline special study, Effects on Cholinesterase in Young Adult Rats: Companion Study to Developmental Neurotoxicity Study 870.6300

PC CODE: 057901 **DP BARCODE**: DP322591
SUBMISSION NO.: none

TEST MATERIAL (PURITY): Trichlorfon (99.6% a.i.)

SYNONYMS: Dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate; Dylox Technical

CITATION: Klaus A.M. (2005) Study to establish the time of peak cholinesterase inhibition in young-adult Wistar rats treated by gavage with an acute dose of technical grade trichlorfon. Bayer HealthCare AG, PH-R&D-PD Toxicology International, 42096 Wuppertal, Germany. Report No. AT02016, Study No. T0073929. March 31, 2005. MRID 46647402. Unpublished. 44 pages.

Langewische, F.W. (2005) Cholinesterase inhibition in young-adult Wistar rats treated by gavage with an acute dose of technical grade trichlorfon. Bayer HealthCare AG, PH-R&D-PD Toxicology International, 42096 Wuppertal, Germany. Report No. AT02063, Study No. T5073933. May 30, 2005. MRID 46647401. Unpublished. 79 pages.

Klaus A.M. (2005) Cholinesterase inhibition in young-adult Wistar rats treated daily by gavage for 11 days with technical grade trichlorfon. Bayer HealthCare AG, PH-R&D Toxicology, 42096 Wuppertal, Germany. Report No. AT02197, Study No. T0073352. July 20, 2005. MRID 46635401. Unpublished. 95 pages.

SPONSOR: Bayer CropScience AG, 40789 Monheim, Germany

EXECUTIVE SUMMARY: In a series of special non-guideline comparative cholinesterase inhibition (ChEi) studies, trichlorfon (Dylox technical; 99.6% a.i., lot #1030228) was administered by oral gavage to groups of young adult Wistar rats. For time-course evaluation (MRID 46647402) 6 animals/sex/group were given a single oral

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dose of 0 or 75 mg/kg and sacrificed 1, 2, 4, or 8 hours later. In an acute study (MRID 46647401) groups of 6 rats/sex were given a single oral dose of 0, 10, 25, or 50 mg/kg and sacrificed 1 hour post-dosing, at the time of peak effect. Finally, repeated administration was studied (MRID 46635401) by giving eleven daily doses of 0, 5, 10, 20, or 40 mg/kg/day to groups of 6 rats/sex; animals were sacrificed 1 hour after the last dose. Plasma, red blood cell (RBC), and brain cholinesterase (ChE) activities were measured in all animals in each study.

All animals survived to scheduled sacrifice. No adverse clinical signs of toxicity were observed in any animal in any study. Body weight gain was not adversely affected by treatment in the repeated dose study.

During time-course investigations, the 1 hour time point consistently had the highest degree of ChE inhibition in all three compartments in both males and females, thus the time of peak effect was 1 hour. Females were more severely affected than males at all time points and in all compartments. One hour after dosing enzyme inhibition in all three compartments was 63-66% in females compared to 36-46% inhibition in males. Brain ChE activity was statistically significantly inhibited through 8 hours post-dosing (17% inhibition, males; 11% inhibition, females), while RBC ChE activity was inhibited through 4 (39% inhibition, females) and 8 (12% inhibition, males) hours post-dosing. Plasma ChE activity was statistically significantly inhibited only at 1 hour post-dosing (37% inhibition males, 64% inhibition females).

A single 50 mg/kg dose of the test article significantly inhibited plasma (29% and 55% inhibition), RBC (36% and 37% inhibition), and brain (36% and 50% inhibition) ChE activity in male and female rats, respectively. At 25 mg/kg, brain ChE activity was significantly decreased by 14% in females while RBC ChE activity was significantly decreased by 16% in males. No significant differences in ChE activity occurred in the 10 mg/kg group of either gender compared to controls.

Following repeated dosing with 40 mg/kg/day, significant inhibition of ChE activity was apparent in all compartments in female rats and in RBC and brain of male rats. Plasma activity was inhibited by 52% in females; RBC activity was inhibited by 22% and 66%, and brain activity was inhibited by 25% and 57% in males and females, respectively. At 20 mg/kg/day, RBC and brain enzyme activities were inhibited in females by 27% and 25%, respectively, and brain enzyme activity in males was inhibited by 7%. No biologically significant enzyme inhibition was found in any compartment in males and females treated with 5 or 10 mg/kg/day.

For acute exposure:

the adult LOAEL for brain ChEI is 25 mg/kg (females)
the adult NOAEL for brain ChEI is 10 mg/kg;

the adult LOAEL for plasma ChEI is 50 mg/kg
the adult NOAEL for plasma ChEI is 25 mg/kg;

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the adult LOAEL for red blood cell ChEI is 50 mg/kg
the adult NOAEL for red blood cell ChEI is 25 mg/kg;

For acute oral exposure to trichlorfon, the overall adult LOAEL for cholinesterase inhibition in rats is 25 mg/kg based on enzyme inhibition in brain from females and the adult NOAEL is 10 mg/kg.

For repeated exposure:

the adult LOAEL for brain ChEI is 20 mg/kg/day
the adult NOAEL for brain ChEI is 10 mg/kg/day;

the adult LOAEL for plasma ChEI is 40 mg/kg/day (females)
the adult NOAEL for plasma ChEI is 20 mg/kg/day;

the adult LOAEL for red blood cell ChEI is 20 mg/kg/day (females)
the adult NOAEL for red blood cell ChEI is 10 mg/kg/day;

For repeated oral exposure to trichlorfon, the overall adult LOAEL for cholinesterase inhibition in rats is 20 mg/kg/day based on enzyme inhibition in brain in males and females and in red blood cells in females; the adult NOAEL is 10 mg/kg/day.

The ChE activity measurements following acute and repeated oral dosing with trichlorfon demonstrate that adult females are more susceptible than adult males. Compared to plasma (52-55% inhibition) or brain (50-57% inhibition) enzyme activity in adult females, the RBC ChE activity appeared to be the least sensitive tissue to acute trichlorfon exposure (37% inhibition) but the most sensitive tissue following repeated exposure (66% inhibition).

Benchmark dose levels of 10% and 20% inhibition of ChE activity were calculated by the study investigators using the National Center for Environmental Assessment Benchmark Dose Software (version 1.3.2). The BMD_{10} of plasma, RBC, and brain ChE activity were 28.5, 18.9, and 27.6 mg/kg, respectively for males and 22.3, 29.2, and 23.7 mg/kg, respectively for females following acute exposure. The BMD_{10} of plasma, RBC, and brain ChE activity were 33.6, 24.3, and 24.1 mg/kg/day, respectively for males and 16.6, 9.0, and 8.2 mg/kg/day, respectively for females.

Taken together these studies are classified **Acceptable/Nonguideline** for the determination of plasma, RBC, and brain ChE inhibition following treatment with trichlorfon in adult rats.

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

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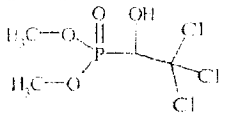
I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:		Trichlorfon
	Description:	Whitish powder; $C_4H_8Cl_3O_4P$
	Lot/Batch #:	1030228 (Bayer CropScience AG)
	Purity:	99.6 % a.i.
	Compound Stability:	Room temperature, 8 days
	CAS # of TGAI:	52-68-6
	Structure:	

2. **Vehicle and/or positive control:** Demineralized water was used as a vehicle. No positive control was used.

3. Test animals:			
	Species:	Rat	
	Strain:	Wistar (Crl:GI x Brl Han:WI)	
	Age and wt. at study initiation:	Adults: 9-10 weeks; males 245-311 g, females 160-195 g (nulliparous and non-pregnant)	
	Source:	Charles River Laboratories, Germany	
	Housing:	Individually in Type IIIh Makrolon® cages with low-dust wood shavings	
	Diet:	Kliba mouse and rat maintenance diet (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*	
	Water:	Tap water, *ad libitum*	
	Environmental conditions:	**Temperature:**	18-22 °C
		Humidity:	approximately 50%
		Air changes:	at least 10 times/hr
		Photoperiod:	12 hrs light/dark
	Acclimation period:	At least 7 days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** MRID 46647402: Start: October 19, 2004; End: October 20, 2004
MRID 46647401: Start: November 17, 2004;End: November 18, 2004
MRID 46635401: Start: May 9, 2005;End: May 19, 2005

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

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TABLE 1. Study design for cholinesterase inhibition studies			
MRID	Dose(s) (mg/kg/day)	Sex: No. of animals/ group	Treatment and termination
46647-02	0, 75	M&F; 6	Single oral dose at approx. 9 and 10 weeks of age to females and males, respectively; animals terminated 1, 2, 4, or 8 hours post-dosing; control animals terminated 2 hours post-dosing
46647401	0, 10, 25, 50	M&F; 6	Single oral dose at approx. 10 weeks of age; animals terminated 1 hour post-dosing
46635401	0, 5, 10, 20, 40	M&F; 6	Eleven daily oral doses beginning at approx. 10 weeks of age; terminated 1 hour after the last dose

1. **Mating procedure**: No mating was required. Animals were delivered as young adults.

1. **Animal assignment**: Animals were allocated to experimental groups (6/sex/group) according to a randomization plan generated on a personal computer (HP Vectra PC). Randomization was performed taking into consideration body weight on the day prior to dosing. In the time course study (MRID 46647402), 30 males and 30 females were allocated to five experimental groups. In the acute study (MRID 46647401), a total of 24 males and 24 females were allocated to four experimental groups. In the repeated dose study (MRID 46635401), 30 males and 30 females were allocated to five experimental groups.

1. **Dose selection rationale**: The dose levels used in all studies were selected as requested by the sponsor. Treatment should result in inhibition of ChE activity but not induce overt toxicity.

2. **Dosage administration**: The animals were treated with trichlorfon by oral gavage in a dose volume of 10 mL/kg body weight at dose levels described in Table 1. The control animals received only vehicle (demineralized water) at the same volume. The body weight of the animals was determined prior to dosing. In the acute studies (MRIDs 46647401 and 46647402) males were treated on the first day of the in-life part of the study and females were dosed on the second day. Gavage was selected since oral exposure is a possible route of exposure for humans.

3. **Dosage preparation and analysis**: The test material was dissolved in demineralized water and stored at room temperature for the duration of use. Stability analyses were performed in 0.1 mg/mL and 20 mg/mL samples prior to the start of these studies. Analysis of concentration of the active ingredient in samples was carried out the first day of dosing for each study. Homogeneity of the dosing solutions was not measured. The results for each study are given below.

   Results: For all studies, absence of test article was confirmed in the vehicle. Stability data confirmed stability over a period of 8 days at room temperature. After 4 days of storage at room temperature, the concentration of the 0.1 and 20 mg/mL solutions

was 95 and 97% of the initial measured concentration, respectively. After 8 days of storage at room temperature, the concentration of the 0.1 and 20 mg/mL solutions was 96 and 94% of the initial measured concentration, respectively.

MRID 46647402: The mean concentration was 105% of nominal for the 7.5 mg/mL solution.

MRID 46647401: The mean concentrations were 100, 112, and 106% of nominal for the 1.0, 2.5, and 5.0 mg/mL solutions, respectively.

MRID 46635401: The mean concentrations were 91%, 93%, 94 % and 96% of nominal for the 0.5, 1.0, 2.0, and 4.0 mg/mL dose preparations, respectively.

The analytical data indicated that the difference between nominal and actual dosage to the study animals was acceptable for all studies.

C. OBSERVATIONS:

1. **In-life observations**: All animals were inspected on the day of dosing for general tolerance of the test compound; appearance, behavior, and mortality were evaluated and recorded. Body weight was determined prior to dosing to establish the appropriate dose volume for each animal. In the repeated dose study, observations and body weight were recorded daily. Food and water consumption were not measured in any study.

2. **Termination schedule and sample collection**: Animals were terminated according to the schedule shown in Table 1. After the last blood sampling, animals were sacrificed by cervical dislocation while under deep carbon dioxide anesthesia. A gross pathological examination of animals was not performed. Brain weight was recorded after dissection of brains.

   Blood samples were collected from anesthetized (ether) animals from the retroorbital venus plexus before necropsy. The brain was collected immediately after sacrifice and stored at -18 °C or lower until analysis. In all studies, ChE activity was determined in RBC, plasma, and brain tissue.

3. **Cholinesterase activity determination**: Cholinesterase assays were performed on all blood and brain samples using a modified Ellman method with 6,6'-dithiodinicotinic acid as the coupling reagent and measuring the change in absorbance at 340 nm.

A. **DATA ANALYSIS**: Statistical analyses of ChE activity data was performed using SAS routines on the actual data, not on the percent inhibition values. An adjusted Welch test was used for statistical evaluation. In order to control the familywise type-one error rate within each sex × date constellation, Holm's sequentially rejective multiple test was applied. Benchmark dose levels of 10% and 20% inhibition of ChE activity were calculated using the National Center for Environmental Assessment

Benchmark Dose Software (version 1.3.2), the ChE data and the analytically confirmed doses. Statistical significance at $p \leq 0.05$ and $p \leq 0.01$ were designated by * and **, respectively.

In the repeated dose study, analysis of variance (ANOVA) and, in case of significance, Dunnett's test were used to evaluate body weight data.

II. RESULTS:

A. MORTALITY AND CLINICAL OBSERVATIONS: Neither mortality nor clinical signs were observed in any animal in the studies.

B. BODY WEIGHT: Body weight was not recorded in the acute studies. Repeated exposure up to and including 40 mg/kg bw/day did not have an effect on body weight or body weight gain in either sex. Selected body weight data following repeated dosing are presented in Table 2.

TABLE 2. Mean body weight (g ± S.D.) in adult rats following repeated exposure to trichlorfon

Day of study	Dose (mg/kg bw/day)				
	0	5	10	20	40
	Males				
Day 1	282.0 ± 12.00	281.8 ± 10.57	280.8 ± 17.81	285.5 ± 22.12	282.2 ± 15.46
Day 5	291.7 ± 11.59	289.8 ± 12.97	290.2 ± 22.24	294.5 ± 23.48	292.3 ± 18.17
Day 11	297.2 ± 6.05	296.5 ± 15.31	298.5 ± 22.36	302.0 ± 24.19	301.8 ± 22.15
	Females				
Day 1	185.7 ± 6.09	186.8 ± 6.56	179.7 ± 4.68	183.2 ± 7.76	185.8 ± 3.31
Day 5	189.3 ± 6.68	189.7 ± 6.95	182.8 ± 5.53	187.5 ± 8.14	190.2 ± 5.08
Day 11	198.2 ± 5.98	198.3 ± 7.15	192.3 ± 5.32	195.8 ± 7.11	201.2 ± 6.43

Data extracted from Annex, pp. 34-37, MRID 46635401
N = 6/sex group

C. BRAIN WEIGHT: Brain weight data were not obtained in any study

D. CHOLINESTERASE ACTIVITY: The plasma, RBC, and brain ChE activity data for treated adult male and female rats are shown in Tables 3, 4 and 5 for time-course, acute, and repeated dose studies, respectively.

1. Time-course of inhibition (MRID 46647402): Cholinesterase activity data for treated adult rats are shown in Table 3. In both males and females, the lowest levels of ChE activity, and thus the highest levels of inhibition, were seen in plasma, RBC, and brain one hour after administration. All were statistically significant. Females were more severely affected (63-66% inhibition) than were males (36-46% inhibition). In both sexes, recovery of plasma ChE activity was evident 2 hours after

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administration while statistically significantly reduced ChE activity of RBC and brain tissue was seen at all time points including 8 hours in males and through 4 (RBC) and 8 (brain) hours in females. The time of peak effect of ChE inhibition after acute trichlorfon administration was determined to be 1 hour post dosing.

TABLE 3. Plasma, RBC, and brain ChE activity in adult rats treated with 75 mg trichlorfon/kg bw: Acute exposure with termination 1, 2, 4, and 8 hours post-dosing

Time of sample collection after treatment (hr)	2	1[a]	2	4	8
Males					
Plasma (kU/L)	0.41±0.084	0.26*±0.078 (37)	0.32±0.054 (22)	0.40±0.079 (2)	0.38±0.045 (7)
RBC (kU/L)	1.69±0.102	0.92**±0.155 (46)	1.18**±0.207(30)	1.34*±0.216 (21)	1.49*±0.159 (12)
Brain (U/g)	11.36±0.519	7.28**±0.905 (36)	8.10**±0.605 (29)	10.22*±0.686 (10)	9.47*±1.207 (17)
Females					
Plasma (kU/L)	1.59±0.472	0.58*±0.083 (64)	1.16±0.222 (27)	1.00±0.174 (37)	1.52±0.454 (4)
RBC (kU/L)	1.91±0.220	0.65**±0.126 (66)	1.04**±0.160 (46)	1.17**±0.111 (39)	1.69±0.220 (12)
Brain (U/g)	11.08±0.716	4.12**±0.650 (63)	6.65**±0.619 (40)	7.91**±0.914 (29)	9.89*±0.619 (11)

Data extracted from Table 8-1 and Table 8-2 pp. 25 and 26, and Annex, pp. 32-33, MRID 46647402.
N = 5-6/sex/group
[a] One female was excluded from the plasma ChE statistical evaluation because the value was equivocal (0.00)
Numbers in parenthesis are percent inhibition relative to control from Table 8-2, p. 26.
Significantly different from control: *p≤ 0.05, **p ≤ 0.01.

2. **Acute exposure (MRID 46647401)**: Cholinesterase activity data for rats treated with a single dose of the test article are shown in Table 4. Plasma, RBC, and brain ChE activity was decreased in males and females treated with 50 mg/kg. At 25 mg/kg, brain enzyme activity was significantly decreased in females while RBC ChE activity was significantly decreased in males. No significant differences in ChE activity occurred in the 10 mg/kg group of either sex compared to controls.

Benchmark dose estimates for 10% inhibition (BMD_{10}) of plasma, RBC, and brain ChE activity were 28.5, 18.9, and 27.6 mg/kg, respectively for males and 22.3, 29.2, and 23.7 mg/kg, respectively for females. Benchmark dose estimates for 20% inhibition (BMD_{20}) of plasma, RBC, and brain ChE activity were 40.3, 33.8, and 39.0 mg/kg, respectively for males and 31.5, 41.3, and 33.6 mg/kg, respectively for females. The reviewer did not verify the benchmark calculations presented in the study.

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TABLE 4. Plasma, RBC, and brain ChE activity in adult rats treated with trichlorfon: Acute exposure with termination 1 hour post-dosing				
Dose (mg/kg bw)	**0**	**10**	**25**	**50[a]**
Males				
Plasma (kU/L)	0.41±0.058	0.48±0.105	0.41±0.084	0.29*±0.056 (29)
RBC (kU/L)	1.72±0.184	1.61±0.228 (6)	1.45*±0.115 (16)	1.10**±0.207 (36)
Brain (U/g)	11.67±0.288	11.83±0.534	10.77±1.051 (8)	7.45**±1.657 (36)
Females				
Plasma (kU/L)	1.43±0.206	1.57±0.254	1.35±0.383 (6)	0.65**±0.139 (55)
RBC (kU/L)	2.16±0.257	1.86±0.175 (14)	1.99±0.354 (8)	1.36**±0.232 (37)
Brain (U/g)	11.97±0.400	11.63±0.643 (3)	10.32**±0.891 (14)	6.00**±1.103 (50)

Data extracted from Table 6-1 and Table 6-2 pp. 24 and 25 and Annex, pp. 31-32, MRID 46647401.
N = 5-6/sex group
[a] One male was excluded from the plasma ChE statistical evaluation because the value was not valid (0.00)
Numbers in parenthesis are percent inhibition relative to control from Table 6-2, p. 25.
Significantly different from control: *p≤ 0.05, **p ≤ 0.01.

3. **Repeated Exposure (MRID 46635401):** Cholinesterase activity data for repeatedly treated adult rats are shown in Table 5. Significant inhibition of plasma ChE activity was observed in females at the 40 mg/kg/day dose level. Inhibition of RBC enzyme activity was observed in males at all dose levels tested, however, without a clear dose response and in females only at the 20 and 40 mg/kg/day dose levels. In brain, ChE activity was significantly reduced in males at the 5, 20, and to a more pronounced degree at the 40 mg/kg/day dose levels. In females, inhibition of brain ChE activity was seen at the 20 and 40 mg/kg/day dose levels.

   Benchmark dose estimates for 10% inhibition (BMD_{10}) of plasma, RBC, and brain ChE activity were 33.6, 24.3, and 24.1 mg/kg/day, respectively for males and 16.6, 9.0, and 8.2 mg/kg/day, respectively for females. BMD_{20} for 20% inhibition of plasma, RBC, and brain ChE activity were 48.9, 48.6, and 34.8 mg/kg/day, respectively for males and 23.5, 16.1, and 15.6 mg/kg/day, respectively for females. The reviewer did not verify the benchmark calculations presented in the study.

TABLE 5. Plasma, RBC, and brain ChE activity in adult rats treated with trichlorfon: Repeat exposure with termination 1 hour post-dosing					
Dose (mg/kg/day)	**0**	**5**	**10**	**20**	**40**
Males					
Plasma (kU/L)	0.34±0.055	0.34±0.042	0.39±0.096	0.31±0.062 (9)	0.31±0.051 (9)
RBC (kU/L)	1.69±0.157	1.39*±0.324 (18)	1.41*±0.194 (17)	1.48*±0.104 (12)	1.31*±0.180 (22)
Brain (U/g)	11.60±0.340	11.04*±0.353 (5)	11.29±0.238 (3)	10.77*±0.454 (7)	8.65**±1.079 (25)
Females					
Plasma (kU/L)	1.41±0.217	1.19±0.217 (16)	1.73±0.341	1.18±0.390 (16)	0.67**±0.305 (52)
RBC (kU/L)	1.76±0.190	1.44±0.243 (18)	1.57±0.211 (11)	1.29*±0.240 (27)	0.60**±0.127 (66)
Brain (U/g)	11.43±0.516	11.02±0.37 (4)	10.42±1.934 (9)	8.53**±0.343 (25)	4.95**±0.654 (57)

Data extracted from Table 6-1 and Annex, pp. 27 and 28-34, MRID 46635401
N = 6/sex group
Numbers in parenthesis are percent inhibition relative to control from Table 6-2, p. 28.
Significantly different from control: *p≤ 0.05, **p ≤ 0.01.

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III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS: Conclusions were made separately for each of the studies submitted. In both sexes and all tissues (plasma, RBC, brain) evaluated, the time of peak effect for ChE inhibition was 1 hour after treatment.

Acute administration of 25 or 50 mg/kg trichlorfon caused dose dependent inhibition of plasma, RBC, and brain ChE activities in females. In males, 50 mg/kg caused ChE inhibition in all three compartments while 25 mg/kg caused inhibition of RBC enzyme activity only. No significant differences in ChE activity occurred in the 10 mg/kg group compared to controls.

Repeated administration of 20 or 40 mg/kg/day trichlorfon caused dose dependent inhibition of ChE activity in RBC and brain of females. Plasma ChE activity was only inhibited at the 40 mg/kg dose level in females. In males, plasma ChE activity was not affected by treatment, while RBC and brain enzyme inhibition was significant at all doses tested (with the exception of brain ChE activity at the 10 mg/kg dose). There was no clear dose relationship and inhibition was less than 20% in males treated with trichlorfon up to and including 20 mg/kg/day. Thus, biological significance of these findings is considered questionable. Due to the more pronounced inhibition of ChE activity at the 40 mg/kg dose level (inhibition in RBC by 22% and in brain by 25%), toxicological relevance might be assumed for findings at this dose level.

B. DISCUSSION AND REVIEWER COMMENTS: A series of studies was conducted to determine ChE inhibition resulting from acute or repeated oral exposure of rats to trichlorfon.

No clinical signs were observed in animals in any study. Neither survival nor body weight was affected by treatment with the test article.

Following a single dose of the test article, the greatest inhibition of ChE activity was observed 1 hour after dosing, thus the time of peak effect was 1 hour. There was some variability within the time course of ChE inhibition at individual time points, but the 1 hour time point consistently had the highest degree of inhibition in all three compartments in both males and females. Females were affected to a higher degree (63-66% inhibition) compared to males (36-46% inhibition). Brain ChE activity was statistically significantly inhibited until 8 hours post-dosing, while RBC ChE activity was inhibited through 4 (females) and 8 (males) hours post-dosing. Although not statistically significant, RBC ChE activity was inhibited 12% in females 8 hours post-dosing, the same degree of inhibition of males at this time point which did achieve statistical significance; thus, biological significance is questionable. Plasma ChE activity was statistically significantly inhibited only at 1 hour post-dosing; however, biological significance might be assumed through 2 (22% inhibition, males) or 4 hours (37% inhibition, females) post-dosing. Regardless of biological or statistical

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significance beyond 1 hour post-dosing, a clear time of peak effect was established at 1 hour for all three tissue compartments in both males and females.

Following acute exposure, a clear dose-related inhibition of enzyme activity was not observed in any compartment in either sex. While biologically significant enzyme inhibition was seen in all compartments in males and females at 50 mg/kg, at 25 mg/kg/day only brain enzyme activity in females was biologically significantly inhibited.

After eleven daily doses of 40 mg/kg/day of trichlorfon, biologically significant inhibition of ChE activity was apparent in all three compartments in female rats and in RBC and brain in male rats. At 20 mg/kg/day, biologically significant inhibition of brain enzyme activity was observed in males and females and of RBC enzyme activity was observed in females. In males, RBC ChE activity was statistically inhibited at all doses and brain ChE activity was statistically inhibited at 5 mg/kg/day, but not at 10 mg/kg/day. These findings in males are not considered biologically significant due to lack of a dose response, the small magnitude of the effects, and no statistically significant inhibition in RBC and brain enzyme activity in females treated with 5 or 10 mg/kg/day.

The ChE activity measurements following acute and repeated oral dosing with trichlorfon demonstrate that adult females are more susceptible than adult males. The sex differences in sensitivity were more pronounced following repeated dosing than after a single dose. Compared to plasma (52-55% inhibition) or brain (50-57% inhibition) enzyme activity in adult females, the RBC ChE activity was the least sensitive tissue after acute trichlorfon exposure (37% inhibition) but the most sensitive tissue following repeated exposure (66% inhibition). In general, benchmark dose estimates reflect this hierarchy of susceptibility.

For acute exposure:

the adult LOAEL for brain ChEI is 25 mg/kg (females)
the adult NOAEL for brain ChEI is 10 mg/kg;

the adult LOAEL for plasma ChEI is 50 mg/kg
the adult NOAEL for plasma ChEI is 25 mg/kg;

the adult LOAEL for red blood cell ChEI is 50 mg/kg
the adult NOAEL for red blood cell ChEI is 25 mg/kg;

For acute oral exposure to trichlorfon, the overall adult LOAEL for cholinesterase inhibition in rats is 25 mg/kg based on enzyme inhibition in brain in females; the adult NOAEL is 10 mg/kg.

For repeated exposure:

the adult LOAEL for brain ChEI 20 mg/kg/day
the adult NOAEL for brain ChEI is 10 mg/kg/day;

the adult LOAEL for plasma ChEI is 40 mg/kg/day (females)
the adult NOAEL for plasma ChEI is 20 mg/kg/day;

the adult LOAEL for red blood cell ChEI is 20 mg/kg/day (females)
the adult NOAEL for red blood cell ChEI is 10 mg/kg/day;

For repeated oral exposure to trichlorfon, the overall adult LOAEL for cholinesterase inhibition in rats is 20 mg/kg/day based on enzyme inhibition in brain in males and females and in red blood cells in females; the adult NOAEL is 10 mg/kg/day.

STUDY DEFICIENCIES: No major deficiencies were identified in the conduct of any study.

DATA EVALUATION RECORD

TRICLORFON (DYLOX TECHNICAL)

Study Type: SUPPLEMENTAL STUDY, CHOLINESTERASE INHIBITION [NON-GUIDELINE]
MRID 46635501

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
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Task Order No. 124-2006

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

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Work Assignment Manager: P. V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date** 8/16/06

Template version 08/05

TXR#: 0054212

DATA EVALUATION RECORD

STUDY TYPE: Supplement to Developmental Neurotoxicity Study - Rat; Nonguideline

PC CODE: 057901 DP BARCODE: DP322591

TEST MATERIAL (PURITY): Trichlorfon (100% a.i.)

SYNONYMS: Dylox Techinal

CITATION: Klaus, A.M., Holzum (2005) Cholinesterase inhibition in maternal and fetal rats following gestational exposure via the diet with technical grade trichlorfon. Bayer HealthCare AG, Wuppertal Germany. Unpublished Report No. AT02232. MRID 46635501

SPONSOR: Bayer CropScience AG, Germany

EXECUTIVE SUMMARY:

In a comparative cholinesterase (ChE) inhibition study (MRID 46635501) that was supplemental to a developmental neurotoxicity study (MRID 46205301), trichlorfon (100% a.i.) was administered to 13 inseminated female rats/group in the diet at concentrations of 150, 500, and 1750 ppm (10.4, 37.2, 134 mg/kg/day, respectively) from gestation day 0 to 20. On Day 20 of gestation pups were retrieved by cesarean section and all animals were sacrificed for blood and tissue collection. No adverse clinical signs of toxicity were observed. Inhibition of ChE activity was apparent in all compartments of female rats in the mid- and high dose treatment groups. Significant inhibition of brain ChE activity was 20 and 58%, respectively, erythrocyte ChE activity was 31 and 54%, and plasma was 22 and 61%. In the corresponding fetuses (pooled by litter) significant inhibition of brain ChE activity (10 to 36%) was observed at all dose levels, while inhibition of erythrocyte ChE activity (49%) was observed at 1750 ppm only. Fetal plasma ChE inhibition was significant at the 500 ppm level (23%) and at 1750 ppm (38%).

For maternal exposure:

The maternal LOAEL for brain ChE activity inhibition is 500 ppm (37.2 mg/kg/day)
The maternal NOAEL for brain ChE activity inhibition is 150 ppm (10.4 mg/kg/day)
The maternal LOAEL for plasma ChE activity inhibition is 500 ppm (37.2 mg/kg/day)
The maternal NOAEL for plasma ChE activity inhibition is 150 ppm (10.4 mg/kg/day)

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The maternal LOAEL for erythrocyte ChE activity inhibition is 500 ppm (37.2 mg/kg/day)
The maternal NOAEL for erythrocyte ChE activity inhibition is 150 ppm (10.4 mg/kg/day)

The overall maternal LOAEL for dietary exposure of rats to trichlorfon on gestation days 0 to 20 for cholinesterase activity inhibition is 500 ppm (37.2 mg/kg/day) based on enzyme inhibition in brain, plasma and, erythrocytes. The maternal NOAEL is 150 ppm (10.4 mg/kg).

For fetal exposure:

The fetal LOAEL for brain ChE activity inhibition is 150 ppm (10.4 mg/kg/day)
The fetal NOAEL for brain ChE activity inhibition was not identified

The fetal LOAEL for plasma ChE activity inhibition is 500 ppm (37.2 mg/kg/day)
The fetal NOAEL for plasma ChE activity inhibition is 150 ppm (10.4 mg/kg/day)

The fetal LOAEL for erythrocyte ChE activity inhibition is 1750 ppm (134 mg/kg/day)
The fetal NOAEL for erythrocyte ChE activity inhibition is 500 ppm (37.2 mg/kg/day)

The overall fetal LOAEL for dietary exposure of rats to trichlorfon for cholinesterase activity inhibition in rats on gestation days 0 to 20 is 150 ppm (10.4 mg/kg/day) based on enzyme activity inhibition in brain. The fetal NOAEL was not identified.

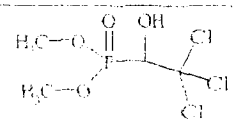
Benchmark dose (BMD) response estimates for ChE activity were calculated for each compartment in both dams and litters using the non-positive quadratic polynomial model of the BMDS software provided by the USEPA (Version 1.3.2). The OP cumulative model designed for ChE inhibition is the preferred model for this type of analysis. The BMD_{10} and BMD_{20} values for ChE activity inhibition were comparable for the fetuses and the dams in all three compartments, with the fetuses showing slightly lower sensitivity.

This study is classified Acceptable/Nonguideline and supplements the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft).

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:	Trichlorfon (technical)	
Description:	Whitish powder	
Lot/Batch #:	682721-Dylox technical	
Purity:	100 % a.i.	
Compound Stability:	Stable at room temperature	
CAS # of TGAI:	52-68-6	
Structure:		

2. **Vehicle and/or positive control**: None

3. Test animals (P):		
Species:	Rat	
Strain:	SPF-bred Wistar Crl:GLxBrl Han:WI	
Age at study initiation:	13-19 wks	
Wt. at study initiation:	Females: 200-250 g	
Source:	Charles River Wiga GmbH, Germany	
Housing:	Individually in plastic cages with low dust wood shavings	
Diet:	Provimi Kliba Rat and Mouse Maintenance No. 3883 9.25 *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	Temperature:	20 ± 2 °C
	Humidity:	50%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	At least 7 days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates** : Start: April 19, 2005; End: May 18, 2005

2 **Study schedule**: The maternal animals were mated and assigned sequentially to study groups as insemination was verified. The test substance was administered to the maternal animals in the diet from gestation Day (GD) 0 through 20 (Table 1). On Day 20 of gestation pups were retrieved by cesarean section and all animals were sacrificed for blood and tissue collection.

TABLE 1: Study design			
Test group	Nominal/actual conc. in diet (ppm)	Actual dose (mg/kg bw/day)[a]	No. Females
Control	0/0	0	13
Low	150/128	10.4	13
Mid	500/451	37.2	13
High	1750/1643	134.0	13

Data from p. 19 and 21, MRID 46635501
[a] Based on actual diet concentrations

3. **Mating procedure:** One or two females were placed into a cage with one male of the same strain. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated GD 0.

4. **Animal assignment:** Mated females were assigned sequentially to dose groups as insemination was verified as indicated in Table 1. On day 20 of gestation pups were retrieved by cesarean section and all animals were sacrificed for blood and tissue collections.

5. **Dose selection rationale:** Dose levels were chosen to be identical with a previous developmental neurotoxicity study (MRID 46205301).

6. **Dosage administration:** The test material was administered in the diet from GD0 through GD20.

7. **Dosage preparation and analysis:** Diet was prepared every 3-5 days by mixing appropriate amounts of test substance with the rodent feed and was stored at room temperature. Concentrations were measured twice for each treatment level (one day prior to the beginning of the study and 5 days prior to the end of the study). Fresh feed was supplied to the animals daily. Separate investigations on stability and content of the active ingredient in feed at nominal concentrations of 0, 150, 500, and 1750 ppm were conducted prior to the study.

Results

Concentration analysis: The variance of actual to nominal was 76% and 95% for the low concentration, 85% and 95% for the middle concentration, and 94% and 94% for the high concentration.

Homogeneity analysis: The analyses was 99∓6.21% of nominal (RSD; number of samples from each of the four diet preparations not specified) for the low concentration, 103 ∓ 3.45% for the middle concentration, and 99 ∓ 8.07% for the high concentration.

Stability analysis: Stability of the diet at room temperature was satisfactory over the 4-day time

frame for each preparation. The sample was 99% of nominal at the start, 87% after 4 days, and 75% after 8 days for the low concentration; 103% of nominal at the start, 90% after 4 days, and 82% after 8 days for the middle concentration; and 99% of nominal at the start, 92% after 4 days, and 83% after 8 days for the low concentration.

The analytical data indicated that the variance between nominal and actual dosage to the animals was acceptable except for the low dose preparation represented by the second concentration analysis. This preparation was administered to three animals for one day and to one animal for 5 days. This is not believed to be significant to the overall outcome of the study.

C. **OBSERVATIONS**:

1. **In-life observations:** Daily cage-side observations were conducted for maternal animals. Signs of toxicity were recorded as they were observed. Body weight was determined daily from GD 0 to 20.
2. **Postmortem observations:** Blood was collected from the retoorbital sinus of each ether-anesthetized dam on GD 20 for determination of erythrocyte and plasma ChE activity activity. Maternal animals were sacrificed by carbon dioxide asphyxia, the fetuses retrieved by cesarean section, and sacrificed by decapitation. Fetal blood was collected (method not specified) and pooled litterwise for determination of erythrocyte and plasma ChE activity. Maternal and fetal whole brain were taken out, weighed (individually for dams and pooled by litter for fetuses), and frozen until brain ChE activity was measured. No gross examination of dams or fetuses was performed.

3. **Food Consumption:** Food consumption was measured on GD 0-4, 4-7, 7-11, 11-14, 14-17 and 17-20.

4. **Cholinesterase determination:** Cholinesterase assays were performed on all blood and brain samples using a modified Ellman method (6,6'-dithiodinicotinic acid as the coupling reagent) and measuring the change in absorbance at 340 nm.

D. **DATA ANALYSIS**:

1. **Statistical analyses:** Body weight and food consumption data were evaluated by analysis of variance to establish the significance of variability among the groups. Significance was tested at the 5% and 1% levels ($p < 0.05$ and $p < 0.01$). If significant, Dunnett's test of significance was performed for confirmation. Statistical evaluations of ChE activity were performed using SAS routines with an adjusted Welch test. Holm's sequentially rejective multiple test procedures were used to control the familywise Type I error rate.

2. **Benchmark dose calculations:** Benchmark dose (BMD) response estimates for ChE activity were calculated for each compartment in both dams and litters using the non-positive quadratic polynomial model of the BMDS software provided by the USEPA (Version 1.3.2). The OP cumulative model designed for ChE inhibition is the preferred model for this type of analysis.

II. **RESULTS**:

A. MORTALITY AND CLINICAL OBSERVATIONS:

No mortality nor any clinical signs of toxicity were observed in this study.

B. BODY WEIGHT AND FOOD CONSUMPTION:

Body weight and food consumption data are summarized in Table 2. No substance-related effects on body weight were seen in any of the treatment groups. Neither were there any effects on food consumption observed in the study.

TABLE 2. Mean (∓SD) maternal body weight and food consumption [a]

Observations/study week	Diet concentration (ppm)			
	Control (N=11)	150 (N=12)	500 (N=12)	1750 (N=10)
Gestation				
Mean body weight (g) Gestation day 0	224 ∓ 12.9	229 ∓ 13.2	223 ∓ 16.4	219 ∓ 13.9
Mean body weight (g) Gestation day 20	327 ∓ 18.1	328 ∓ 23.3	321 ∓ 30.2	319 ∓ 18.9
Mean weight gain (g) Gestation days 0-20	103.3	99.6	98.5	100
Mean food consumption (g/animal/day) Gestation days 0-20 [b]	21.0	20.9	20.8	20.2

[a] Data obtained from pages 38-42, MRID 46635501
[b] Calculated by Reviewer; N = Number of animals in each group

C. TEST SUBSTANCE INTAKE:

The time-weighted average daily Trichlorfon intake is summarized in Table 1.

D. CHOLINESTERASE ACTIVITY:

Inhibition of ChE activity was apparent in all compartments of female rats in the mid- and high level treatment groups (Table 3). Inhibition ranged from 20 to 31% at 500 ppm and from 54 to 61% at 1750 ppm ($p<0.01$). In the corresponding fetuses (pooled by litter), statistically significant ($p<0.05$) inhibition of plasma and brain ChE activity (12 and 10%, respectively) was evident at 150 ppm and in brain at 500 ppm (14%), but the plasma inhibition at 150 ppm was not biologically significant. Significant fetal plasma ChE inhibition of 23% was observed at the 500 ppm exposure level ($p<0.01$), while inhibition in all three compartments ranging from 36 to 49% was observed at 1750 ppm.

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TABLE 3. Blood and brain ChE activity in dams and fetuses: maternal treatment on GDs 0-20				
Cholinesterase	Diet concentration (ppm)			
	0	150	500	1750
GD 20 Dams				
Plasma (kU/L)	2.06∓0.30	2.03∓0.26 **(1)**	1.60∓0.15**(22)	0.81∓0.16**(61)
Erythrocyte (ku/L)	2.11∓0.40	1.89∓0.48 **(10)**	1.46∓0.22**(31)	0.97∓0.44**(54)
Brain (U/g)	10.95∓0.65	10.5∓0.60 **(4)**	8.81∓0.92**(20)	4.55∓0.35**(58)
Litters				
Plasma (kU/L)	0.26∓0.04	0.23∓0.02*(12)	0.20∓0.02**(23)	0.16∓0.03**(38)
Erythrocyte (ku/L)	0.98∓0.08	0.94∓0.15 **(4)**	0.92∓0.12	0.50∓0.15**(49)
Brain (U/g)	1.84∓0.23	1.66∓0.17*(10)	1.59∓0.15*(14)	1.18∓0.16**(36)

Data from p. 30 & 31, MRID 46635501
Numbers in parenthesis are percent inhibition relative to control
Significantly different from control: *p≤ 0.05, **p ≤ 0.01

E. BMD CALCULATIONS:

Results of the BMD calculations are shown in Table 4. Benchmark dose 20% inhibition (BMD20) calculations for the dams were 43.3, 49.7, and 45.5 mg/kg bw/day for plasma, erythrocytes, and brain, respectively and the corresponding values for the fetuses were 73.8, 81.5, and 79.2. The BMD10 estimates for the litters in each compartment are also higher than the corresponding estimates for the dams in all three compartments. Therefore, the sensitivity of fetuses and dams to ChE activity inhibition was comparable, although the fetuses showed slightly lower sensitivity.

TABLE 4. Benchmark dose (BMD) response estimates for ChE activity		
Cholinesterase	Benchmark dose (mg/kg bw/day)	
	BMD10	BMD20
GD 20 Dams		
Plasma	21.7	43.3
RBC	24.8	49.7
Brain	22.8	45.5
Litters		
Plasma	36.9	73.8
RBC	54.0	81.5
Brain	39.6	79.2

Data from p. 34, MRID 46635501.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

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The benchmark dose 20% inhibition (BMD20) calculations for the dams were 43.3, 49.7, and 45.5 mg/kg bw/day for plasma, erythrocytes, and brain, respectively and the corresponding values for the fetuses were 73.8, 81.5, and 79.2. Therefore, the sensitivity of fetuses and dams toward ChE inhibition was comparable, or fetal sensitivity may even be slightly lower.

B. **REVIEWER COMMENTS:**

The study results support the investigators' conclusions. No adverse clinical signs of toxicity were observed in any rat. Inhibition of ChE activity was apparent in all compartments of female rats in the mid- and high-level treatment groups. In the corresponding fetuses (pooled by litter) significant inhibition of brain ChE activity was observed at all dose levels, while inhibition of erythrocyte ChE activity was observed at 1750 ppm only. Fetal plasma ChE inhibition was significant at the 500 and 1750 ppm levels.

For maternal exposure:

The maternal **LOAEL** for plasma, erythrocytes and brain ChE activity inhibition is 500 ppm (37.2 mg/kg/day)

The maternal **NOAEL** for plasma, erythrocytes and brain ChE activity inhibition is 150 ppm (10.4 mg/kg/day)

The overall maternal **LOAEL** for dietary exposure of rats to trichlorfon on gestation days 0 to 20 for cholinesterase activity inhibition is 500 ppm (37.2 mg/kg/day) based on enzyme inhibition in brain, plasma and, erythrocytes. The maternal **NOAEL** is 150 ppm (10.4 mg/kg/day).

For fetal exposure:

The fetal LOAEL for brain ChE activity inhibition is 150 ppm (10.4 mg/kg/day)
The fetal NOAEL for brain ChE activity inhibition was not identified

The fetal LOAEL for plasma ChE activity inhibition is 500 ppm (37.2 mg/kg/day)
The fetal NOAEL for plasma ChE activity inhibition is 150 ppm (10.4 mg/kg/day)

The fetal LOAEL for erythrocyte ChE activity inhibition is 1750 ppm (134 mg/kg/day)
The fetal NOAEL for erythrocyte ChE activity inhibition is 500 ppm (37.2 mg/kg/day)

The overall fetal LOAEL for dietary exposure of rats to trichlorfon for cholinesterase activity inhibition in rats on gestation days 0 to 20 is 150 ppm (10.4 mg/kg/day) based on enzyme activity inhibition in brain. The fetal NOAEL was not identified.

This study is classified Acceptable/Nonguideline and supplements the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 draft.

C. **STUDY DEFICIENCIES:** None

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DATA EVALUATION RECORD

TRICLORFON (DYLOX TECHNICAL)

Study Type: SPECIAL STUDIES, CHOLINESTERASE INHIBITION [NON-GUIDELINE]
MRID 46647404 (peak effect - preweaning); 46647403 (acute - preweaning); 46635601 (repeated - preweaning)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
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Oak Ridge, TN 37831
Task Order No. 124-2006C

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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Registration Action Branch 1, Health Effects Division (7509P) **Date** 8/16/06

Template version 08/05

TXR#: 0054212

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline special study. Effects on Cholinesterase in Preweaning Rats (Companion Study to Developmental Neurotoxicity Study 870.6300)

PC CODE: 057901

DP BARCODE: DP322591
SUBMISSION NO.: none

TEST MATERIAL (PURITY): Trichlorfon (100% a.i.)

SYNONYMS: Dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate; Dylox Technical

CITATION: Langewische, F.W. (2005) Study to determine the time of peak cholinesterase inhibition in preweaning Wistar rats treated by gavage with an acute dose of technical grade trichlorfon. Bayer HealthCare AG, PH-R&D-PD Toxicology International, 42096 Wuppertal, Germany. Report No. AT02017, Study No. T4073932. April 15, 2005. MRID 46647404. Unpublished. 48 pages.

Langewische, F.W. (2005) Study to determine cholinesterase inhibition in postnatal day 11 Wistar rats treated by gavage with an acute dose of technical grade trichlorfon. Bayer HealthCare AG, PH-R&D-PD Toxicology International, 42096 Wuppertal, Germany. Report No. AT02064, Study No. T8073936. May 30, 2005. MRID 46647403. Unpublished. 80 pages.

Klaus A.-M. (2005) Study to determine cholinesterase inhibition in postnatal day 11 Wistar rats treated by gavage for eleven days with technical grade trichlorfon. Bayer HealthCare AG, PH-R&D Toxicology, 42096 Wuppertal, Germany. Report No. AT02252, Study No. T5073357. August 5, 2005. MRID 46635601. Unpublished. 73 pages.

SPONSOR: Bayer CropScience AG, 40789 Monheim, Germany

EXECUTIVE SUMMARY: In a series of special non-guideline comparative cholinesterase activity inhibition studies, trichlorfon (Dylox technical; 100% a.i., lot #1030228) was administered by oral gavage to groups of preweaning Wistar rats. For time-course evaluation (MRID 46647404) 9-10 animals/sex/group were given a single oral dose of 0 or 50 mg/kg on post-natal day (PND) 11 and sacrificed 1, 2, 4, 8, or 24 hours later. In an acute study (MRID 46647403) groups of 10 rats/sex were given a single oral dose of 0, 5, 10, or 30 mg/kg on PND 11 and sacrificed 2 hours post-dosing, at the time of peak effect. Finally, repeated administration was studied (MRID 46635601) by giving eleven daily doses of 0, 5, 10, or 20 mg/kg/day to

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groups of 10 rats/sex on PNDs 11-21; animals were sacrificed 1 hour after the last dose. Plasma, red blood cell (RBC), and brain cholinesterase (ChE) activity was measured in all animals in each study.

No adverse clinical signs of toxicity or treatment-related deaths were observed in any animal in any study.

During time-course investigations following a single oral dose of 50 mg/kg on PND 11, maximum ChE inhibition occurred at 1-2 hours post-dosing. In male pups, the highest levels of inhibition of plasma and RBC cholinesterase activity were 82 and 75%, respectively, observed at 1 hour after dosing, and the greatest inhibition of brain cholinesterase activity was 58%, observed at 2 hours after dosing. In female pups, the highest level of cholinesterase activity inhibition was observed at 2 hours for all compartments with inhibition for plasma, RBC, and brain, at 81, 65, and 66%, respectively. Overall, the greatest level of inhibition was observed in plasma cholinesterase activity in both males and females. After 24 hours, there was still significant inhibition of plasma (38%) and RBC (23%) enzyme activity in males, while brain enzyme activity recovered to control levels. In females after 24 hours, there was significant inhibition of plasma ChE (34%), while RBC and brain enzyme activity recovered to control levels. The time of peak effect of cholinesterase inhibition after acute trichlorfon administration was determined to be 2 hours post dosing.

After an acute dose of trichlorfon, there was significant inhibition of plasma (68-75%), RBC (24-37%), and brain (39-45%) ChE activity in males and females at the high dose (30 mg/kg). After an acute dose of 10 mg/kg, there was significant inhibition in male and female plasma (26-29%) and brain (9-13%) ChE activity, but no significant effects on RBC enzyme activity. After an acute dose of 5 mg/kg, plasma ChE activity was inhibited by 12% in both males and females.

Following repeated dosing with 20 mg/kg/day, plasma ChE activity was significantly inhibited in both males (24%) and females (39%). RBC enzyme activity was inhibited in high-dose females by 34% although statistical significance was not attained. Brain ChE activity was significantly inhibited in males at the high dose (17%), and in females at all doses (6, 11, and 26% at 5, 10, and 20 mg/kg/day, respectively).

For acute exposure of preweaning rats to trichlorfon:

the preweaning LOAEL for brain ChEI is 10 mg/kg
the preweaning NOAEL for brain ChEI is 5 mg/kg;

the preweaning LOAEL for plasma ChEI is 10 mg/kg
the preweaning NOAEL for plasma ChEI is 5 mg/kg;

the preweaning LOAEL for red blood cell ChEI is 30 mg/kg
the preweaning NOAEL for red blood cell ChEI is 10 mg/kg;

For acute oral exposure to trichlorfon, the overall LOAEL for cholinesterase activity inhibition in PND 11 rats is 10 mg/kg based on enzyme inhibition in plasma and brain in males and females; the preweaning NOAEL is 5 mg/kg.

For repeated exposure of preweaning rats to trichlorfon:

the preweaning LOAEL for brain ChEI 5 mg/kg/day (females)
the preweaning NOAEL for brain ChEI is not identified:

the preweaning LOAEL for plasma ChEI is 20 mg/kg/day
the preweaning NOAEL for plasma ChEI is 10 mg/kg/day:

the preweaning LOAEL for red blood cell ChEI is 20 mg/kg/day (females)
the preweaning NOAEL for red blood cell ChEI is 10 mg/kg/day:

For repeated oral exposure to trichlorfon, the overall LOAEL for cholinesterase activity inhibition in PND 11 rats is 5 mg/kg/day based on enzyme inhibition in brain in females; the preweaning NOAEL is not identified.

The ChE activity measurements following acute oral dosing with trichlorfon did not demonstrate a difference in susceptibility between preweaning male and female rat pups. By 24 hours post-dosing females showed a greater recovery in RBC activity, but no differences between the sexes were seen in the other compartments. Following repeated dosing, however, females appeared slightly more susceptible than males. Compared to plasma or brain enzyme activity in males and females, the RBC ChE activity was the least sensitive compartment after acute and repeated trichlorfon exposure.

Benchmark dose levels of 10% and 20% inhibition of ChE activity were calculated by the study investigators using the National Center for Environmental Assessment Benchmark Dose Software (version 1.3.2). The BMD_{10} of plasma, RBC, and brain ChE activity were 4.00, 8.82, and 10.72 mg/kg, respectively for males and 4.42, 16.25, and 7.75 mg/kg, respectively for females following acute exposure to trichlorfon. The BMD_{10} of plasma, RBC, and brain ChE activity were 12.3, 20.6, and 12.4 mg/kg/day, respectively for males and 9.2, 8.4, and 9.1 mg/kg/day, respectively for females following repeated exposure to trichlorfon. The reviewer did not verify these benchmark calculations.

Taken together these studies are classified **Acceptable/Nonguideline** for the determination of plasma, RBC, and brain ChE activity inhibition following treatment with trichlorfon in PND 11 rats.

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

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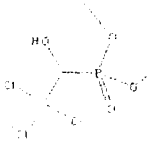
I. MATERIALS AND METHODS:

A. MATERIALS:

1.	**Test material:**	Trichlorfon
	Description:	whitish powder; $C_4H_8Cl_3O_4P$
	Lot/Batch #:	1030228 (Bayer CropScience AG)
	Purity:	100% a.i.
	Compound Stability:	room temperature, 8 days
	CAS # of TGAI:	52-68-6
	Structure:	

2. **Vehicle and/or positive control:** Demineralized water was used as a vehicle. No positive control was used.

3.	**Test animals:**		
	Species:	Rat	
	Strain:	SPF-bred Wistar (Crl:GL x Brl Han:WI)	
	Age and wt. at study initiation:	Postnatal day 11; body weight not reported	
	Source:	Charles River Wiga (Deutschland) GmbH, 97633 Su zfeld, Germany	
	Housing:	Dams with litter in Type IIIh Makrolon® cages	
	Diet:	Kliba mouse and rat maintenance diet (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*	
	Water:	Tap water, *ad libitum*	
	Environmental conditions:	**Temperature:**	20±2 °C
		Humidity:	approximately 50%
		Air changes:	at least 10 times/hr
		Photoperiod:	12 hrs light/12 hrs dark
	Acclimation period:	Adult females at least 7 days prior to mating	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** MRID 46647404: Start: December 14, 2004; End: December 17, 2004
MRID 46647403: Start: January 12, 2004; End: January 12, 2004
MRID 46635601: Start: June 19, 2005; End: June 29, 2005

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

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TABLE 1. Study design for cholinesterase activity inhibition studies			
MRID	**Dose(s) (mg/kg/day)**	**Sex; No. of animals/ group**	**Treatment and termination**
46647404	0, 50	M: 10 F: 9-10	Single oral dose on PND 11; animals terminated 1, 2, 4, 8, or 24 hours post-dosing; control animals terminated 2 hours post-dosing
46647403	0, 5, 10, 30	M&F: 10	Single oral dose on PND 11; animals terminated 2 hours post-dosing
46635601	0, 5, 10, 20	M&F: 10	Eleven daily oral doses beginning on PND 11; terminated 1 hour after the last dose on PND 21

1. **Mating procedure:** Animals were mated by placing two females in a cage with one male overnight. On the morning after mating, if vaginal smears indicated a vaginal plug or sperm, the day was considered day 0 of gestation.

1. **Animal assignment:** Using a within-litter treatment design, pups were consecutively allocated to the dose groups described in Table 1. In the peak effect study (MRID 46647404), 60 males and 59 females were allocated to six experimental groups. In the acute study (MRID 46647403), a total of 40 males and 40 females were allocated to four experimental groups. In the repeated dose study (MRID 46635601), 40 males and 40 females were allocated to four experimental groups.

1. **Dose selection rationale:** The dose levels used in all studies were selected as requested by the sponsor. Treatment should result in inhibition of ChE activity but not induce overt toxicity.

2. **Dosage administration:** The animals were treated with trichlorfon by oral gavage in a dose volume of 10 mL/kg body weight at dose levels described in Table 1. The control animals received only vehicle (demineralized water) at the same volume. The body weight of the animals was determined prior to dosing. Gavage was selected since oral exposure is a possible route of exposure for humans.

3. **Dosage preparation and analysis:** The test material was dissolved in demineralized water and stored at room temperature for the duration of use. Stability analyses were performed in 0.1 mg/mL and 20 mg/mL samples prior to the start of these studies. Analysis of concentration of the active ingredient in samples was carried out a few days before first day of dosing for each study. For the repeated dose study, the frequency of preparation was not stated; however, it was noted that the dosing solutions were stored for a maximum of 8 days. Homogeneity of the dosing solutions was not measured. The results for each study are given below.

   Results: For all studies, absence of test article was confirmed in the vehicle. Stability data confirmed stability over a period of 8 days at room temperature. After 4 days of storage at room temperature 0.1 and 20 mg/mL solutions were 95 and 97% of the initial measured

concentration, respectively. After 8 days of storage at room temperature, 0.1 and 20 mg/mL solutions were 96 and 94% of the initial measured concentration, respectively.

MRID 46647404: The mean concentration was 104% of nominal for the 5 mg/mL solution and 97% of nominal for the 20 mg/L solution.

MRID 46647403: The mean concentrations were 99, 99, and 101% of nominal for the 0.5, 1.0, and 3.0 mg/mL solutions, respectively.

MRID 46635601: The mean concentrations were 97, 101, and 99% of nominal for the 0.5, 1.0, and 2.0 mg/mL dose preparations, respectively.

The analytical data indicated that the difference between nominal and actual dosage to the study animals was acceptable for all studies.

C. OBSERVATIONS:

1. **In-life observations:** Dams were observed once daily for clinical signs, mortality and moribundity. Body weight gain, and feed and water consumption were not determined. The numbers of live and stillborn pups were recorded for each litter. Pups were observed once daily for clinical signs from birth until sacrifice. If a clinical sign was observed, the pup was removed from the cage for more detailed observation. On PND 4 litters were culled using a computer-generated randomization plan, to yield 4 males and 4 females. Litters with less than 8 pups were excluded. If there were less than 4 males or females, litters were adjusted to yield 3 of one sex and 5 of the other. Pup body weight was recorded after birth, on PND 4, and on each dosing day for determination of dose volume.

2. **Termination schedule and sample collection**: Pup blood samples were taken for cholinesterase determination at the timepoints specified in Table 1. Following decapitation, blood was collected in K-EDTA tubes for analysis of erythrocyte and plasma cholinesterase levels. Immediately following blood collection, the whole brain was removed, weighed, and stored at ≤18 °C until analysis. After the last pup was sacrificed, dams were sacrificed by cervical dislocation while under deep carbon dioxide anesthesia. Gross pathological examination of dams and pups was not performed.

3. **Cholinesterase activity determination**: Cholinesterase assays were performed on all blood and brain samples using a modified Ellman method with 6,6'-dithiodinicotinic acid as the coupling reagent and measuring the change in absorbance at 340 nm.

A. **DATA ANALYSIS**: Statistical analyses of ChE activity data was performed using SAS routines on the actual data, not on the percent inhibition values. An adjusted Welch test was used for statistical evaluation. In order to control the familywise type-one error rate within each sex × date constellation, Holm's sequentially rejective multiple test was applied. Benchmark dose levels of 10% and 20% inhibition of ChE activity were calculated using the National Center for Environmental Assessment Benchmark Dose Software (version 1.3.2), the ChE data and the analytically confirmed doses. Statistical significance at $p \leq 0.05$ and $p \leq 0.01$ were designated by * and **, respectively.

II. RESULTS:

A. Mortality and clinical observations: In the acute dose studies, no clinical signs or mortality were observed in the dams or the pups. In the repeated dose study, two female pups in the control group were found dead. One was found cannibalized on postnatal day 14 and another was found dead on postnatal day 16.

B. Body weight: Body weight data were not reported.

C. Brain weight: Brain weight data were not reported.

D. Cholinesterase activity: The plasma, RBC, and brain ChE activity data for treated preweaning male and female rats are shown in Tables 3, 4 and 5 for time-course, acute, and repeated dose studies, respectively.

1. **Time-course of inhibition (MRID 46647404):** ChE activity data for rat pups treated on PND 11 are shown in Table 2. In male pups, the highest levels of inhibition of plasma and RBC enzyme activity were 82 and 75%, respectively, observed at 1 hour after dosing, and the greatest inhibition of brain ChE activity was 58%, observed at 2 hours after dosing. In female pups, the highest level of cholinesterase activity inhibition was observed at 2 hours for all compartments with inhibition for plasma, RBC and brain enzyme activity, at 81, 65, and 66%, respectively. Overall, the greatest level of inhibition was observed in plasma cholinesterase activity in both males and females. At 24 hours, there was still significant inhibition of plasma and RBC enzyme activity in males, while brain ChE activity recovered to control levels. In females after 24 hours, there was significant inhibition of plasma ChE activity, while erythrocyte and brain enzyme activity recovered to control levels. The time of peak effect of cholinesterase inhibition after acute trichlorfon administration was determined to be 2 hours post dosing.

TABLE 2. Plasma, RBC, and brain ChE activity in PND 11 rats treated with trichlorfon:
Acute exposure with termination 1, 2, 4, 8, and 24 hours post-dosing

Dose	0 mg/kg	50 mg/kg	50 mg/kg	50 mg/kg	50 mg/kg	50 mg/kg
Time after treatment (hr)	2	1	2	4	8	24
Males						
Plasma (kU/L)	0.71±0.074	0.13**±0.024 (82)	0.17**±0.034 (76)	0.26**±0.080 (63)	0.33**±0.087 (54)	0.44**±0.040 (38)
RBC (kU/L)	2.13±0.362	0.54**±0.155 (75)	0.60**±0.185 (72)	0.86**±0.438 (60)	0.96**±0.318 (55)	1.63**±0.397 (23)
Brain (U/g)	5.63±0.234	2.34**±0.656 (54)	2.52**±0.278 (58)	3.38**±0.591 (46)	4.15**±0.557 (30)	5.74±0.239
Females						
Plasma (kU/L)	0.70±0.082	0.15**±0.044 (79)	0.13**±0.031 (81)	0.24**±0.087 (66)	0.42**±0.039 (40)	0.46**±0.082 (34)
RBC (kU/L)	1.86±0.333	0.64**±0.213 (65)	0.55**±0.222 (65)	0.85**±0.290 (46)	0.99**±0.149 (37)	1.63±0.276
Brain (U/g)	6.21±0.551	3.04**±0.614 (51)	2.14**±0.360 (66)	3.01**±0.557 (52)	4.41**±0.052 (29)	5.95±0.211

Data extracted from Annex pp. 33-36, MRID 46647403.
N = 10/sex/group
Numbers in parenthesis are percent inhibition relative to control From Table 6-2, p. 28.
Significantly different from control: **p < 0.01.

2. **Acute exposure (MRID 46647403)**: Cholinesterase activity data for rats treated with a single dose of the test article are shown in Table 3. After an acute dose of trichlorfon, there was significant inhibition of plasma, RBC and brain ChE activity in males and females at the high dose (30 mg/kg). After an acute dose of 10 mg/kg, there was significant inhibition in male and female plasma and brain cholinesterase activity, but no significant effects on RBC cholinesterase activity. After an acute dose of 5 mg/kg, there was significant inhibition (12%) in plasma cholinesterase activity in both males and females.

Benchmark dose estimates for 10% inhibition (BMD_{10}) of plasma, RBC, and brain ChE activity were 4.00, 8.82, and 10.72 mg/kg, respectively for males and 4.42, 16.25, and 7.75 mg/kg, respectively for females. Benchmark dose estimates for 20% inhibition (BMD_{20}) of plasma, RBC, and brain ChE activity were 8.01, 17.64, and 17.68 mg/kg, respectively for males and 8.85, 26.53, and 15.49 mg/kg, respectively for females. The reviewer did not verify the benchmark calculations presented in the study.

TABLE 3. Plasma, RBC and brain ChE activity in PND 11 rats treated with trichlorfon: Acute exposure with termination 2 hours post-dosing

Dose (mg/kg bw)	0	5	10	30
Males				
Plasma (kU·L⁻¹)	0.68±0.062	0.60*±0.061 (12)	0.48**±0.079 (29)	0.17**±0.075 (75)
RBC (kU·L⁻¹)	1.93±0.341	1.60±0.250	1.58±0.347	1.22**±0.287 (37)
Brain (U·g)	5.89±0.279	5.78±0.248	5.34**±0.347 (9)	3.25**±0.336 (45)
Females				
Plasma (kU·L⁻¹)	0.69±0.079	0.61*±0.080 (12)	0.51**±0.114 (26)	0.22**±0.096 (68)
RBC (kU·L⁻¹)	1.66±0.352	1.64±0.351	1.57±0.423	1.26*±0.235 (24)
Brain (U·g)	5.66±0.223	5.30±0.409	4.90**±0.462 (13)	3.46**±0.763 (39)

Data extracted from Annex pp. 33-34, MRID 46647403.
N = 10/sex/group
Numbers in parenthesis are percent inhibition relative to control from Table 6-2, p. 27.
Significantly different from control: *p≤ 0.05, **p ≤ 0.01.

3. **Repeated Exposure (MRID 46035601)**: Cholinesterase activity data for repeatedly treated preweaning rats are shown in Table 4. Plasma cholinesterase activity was significantly inhibited at 20 mg/kg/day only, in both males and females. RBC enzyme activity was inhibited in high-dose females by 34% although statistical significance was not attained. Brain cholinesterase activity was significantly inhibited in males at the high dose (20 mg/kg/day), and in females at all doses.

Benchmark dose estimates for 10% inhibition (BMD_{10}) of plasma, RBC, and brain ChE activity were 12.3, 20.6, and 12.4 mg/kg/day, respectively for males and 9.2, 8.4, and 9.1 mg/kg/day, respectively for females. Benchmark dose estimates for 20% inhibition (BMD_{20}) of plasma, RBC, and brain ChE activity were 18.0, 31.7, and 21.6 mg/kg/day, respectively for males and 13.7, 14.0, and 16.2 mg/kg/day, respectively for females. The reviewer did not verify the benchmark calculations presented in the study.

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TABLE 4. Plasma, RBC, and brain ChE activity in preweaning rats treated with trichlorfon: Repeat exposure on PNDs 11-21 with termination 1 hour post-dosing on PND 21				
Dose (mg/kg/day)	**0**	**5**	**10**	**20**
Males				
Plasma (kU/L)	0.55±0.133	0.54±0.078	0.51±0.085	0.42*±0.079 (24)
RBC (kU/L)	1.11±0.234	1.25±0.367	1.03±0.168	1.06±0.376
Brain (U/g)	10.13±0.953	9.91±0.408	9.30±0.416	8.36**±0.772 (17)
Females				
Plasma (kU/L)	0.57±0.104	0.56±0.053	0.50±0.121	0.35*±0.174 (39)
RBC (kU/L)	1.75±0.599	1.60±0.345	1.54±0.567	1.15±0.664
Brain (U/g)	10.60±0.362	9.93**±0.259 (6)	9.43**±0.357 (11)	7.82**±0.962 (26)

Data extracted from Annex pp. 37-38, MRID 46635601
N = 10/sex/group
Numbers in parenthesis are percent inhibition relative to control from Table 6-2, p. 30.
Significantly different from control: *$p \leq 0.05$, **$p \leq 0.01$

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS: The study author concluded that after an acute dose of trichlorfon (50 mg/kg), the greatest cholinesterase activity inhibition was observed in male pups after 1 hour (plasma and erythrocytes) and after 2 hours (brain), and in female pups, the greatest cholinesterase inhibition was observed after 2 hours in all three compartments. Therefore, 2 hours after administration was recommended as the optimal time for sample collection in subsequent investigations.

Two hours after an acute dose of trichlorfon, cholinesterase activity was decreased in both males and females in a dose dependent manner, at doses up to 30 mg/kg, inclusive, in all three compartments (plasma, erythrocytes, and brain). Treatment related effects did not result in clinical signs at any dose level.

After treatment of pups for 11 consecutive days, with 0, 5, 10, or 20 mg/kg/day trichlorfon, at approximately 1 hour following the last dose, female brain ChE activity was significantly inhibited at all dose levels and male brain ChE activity was inhibited at 20 mg/kg only. Significant inhibition of plasma cholinesterase activity was observed only at the high dose level (20 mg/kg) in both males and females. At all dose of ≤10 mg/kg in both sexes, inhibition of cholinesterase activity was ≤12% in all compartments. Clinical signs were not observed at any dose level. In male pups at 20 mg/kg, ChE activity inhibition was ≤24% in all compartments, thus toxicological relevance is questionable. In female pups at 20 mg/kg, cholinesterase activity inhibition was ≥26% and toxicological relevance is assumed.

The US EPA has stated that comparisons based on NOAELs/LOAELs may under or overestimate relative sensitivity. Therefore, Bench Mark Dose (BMD) levels were

calculated for each compartment. These BMD estimates are also useful for comparison of ChE activity inhibition between adult and preweaning rats.

B. DISCUSSION AND REVIEWER COMMENTS: A series of studies was conducted to determine ChE activity inhibition resulting from acute or repeated oral exposure of preweaning rats to trichlorfon.

No clinical signs were observed in any study and survival was not affected by treatment with the test article.

Following a single dose of the test article, the greatest inhibition of ChE activity was observed between 1 and 2 hours after dosing. In males, the level of enzyme inhibition in all three compartments peaked at one hour post-dosing with a similar level of inhibition maintained up to two hours post-dosing. However, in females slightly greater inhibition occurred in all three compartments after two hours compared with inhibition measured after one hour. Thus the time of peak effect was chosen as one hour post-dosing. Little difference in enzyme inhibition in any compartment was seen between males and females up to 8 hours post-dosing. At 24 hours post-dosing females had recovered RBC and brain enzyme activity, while males had only recovered brain enzyme activity. Plasma ChE activity remained significantly inhibited through 24 hours.

Following acute exposure, a clear dose-related inhibition of enzyme activity was observed in all compartments in both sexes. Biologically significant enzyme inhibition was seen in plasma and brain in males and females at ≥10 mg/kg, while RBC enzyme activity was significantly inhibited only at 30 mg/kg/day.

After eleven daily doses of 20 mg/kg/day of trichlorfon, biologically significant inhibition of ChE activity was apparent in all three compartments in female rats and in plasma and brain in male rats. No other effects were noted in males from repeated dosing. In females, inhibition of brain ChE activity was seen in all treated groups in a dose-related manner.

The ChE activity measurements following acute oral dosing with trichlorfon did not demonstrate a difference in susceptibility between preweaning male and female rat pups. By 24 hours post-dosing females showed a greater recovery in RBC activity, but no differences between the sexes were seen in the other compartments. Following repeated dosing, however, females appeared slightly more susceptible than males. Compared to plasma or brain enzyme activity in males and females, the RBC ChE activity was the least sensitive compartment after acute and repeated trichlorfon exposure. In general, benchmark dose estimates reflect this hierarchy of susceptibility.

For acute exposure of preweaning rats to trichlorfon:

the preweaning LOAEL for brain ChEI is 10 mg/kg

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the preweaning NOAEL for brain ChEI is 5 mg/kg;

the preweaning LOAEL for plasma ChEI is 10 mg/kg
the preweaning NOAEL for plasma ChEI is 5 mg/kg;

the preweaning LOAEL for red blood cell ChEI is 30 mg/kg
the preweaning NOAEL for red blood cell ChEI is 10 mg/kg;

For acute oral exposure to trichlorfon, the overall LOAEL for cholinesterase activity inhibition in PND 11 rats is 10 mg/kg based on enzyme inhibition in plasma and brain in males and females; the preweaning NOAEL is 5 mg/kg.

For repeated exposure of preweaning rats to trichlorfon:

the preweaning LOAEL for brain ChEI 5 mg/kg/day (females)
the preweaning NOAEL for brain ChEI is not identified;

the preweaning LOAEL for plasma ChEI is 20 mg/kg/day
the preweaning NOAEL for plasma ChEI is 10 mg/kg/day;

the preweaning LOAEL for red blood cell ChEI is 20 mg/kg/day (females)
the preweaning NOAEL for red blood cell ChEI is 10 mg/kg/day;

For repeated oral exposure to trichlorfon, the overall LOAEL for cholinesterase activity inhibition in PND 11 rats is 5 mg/kg/day based on enzyme inhibition in brain in females; the preweaning NOAEL is not identified.

STUDY DEFICIENCIES: No major deficiencies were identified in the conduct of these studies.

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