DATA EVALUATION REPORT

CHLORPYRIFOS

Study Type: SPECIAL STUDY: CHOLINESTERASE AND METABOLITE DETERMINATION – RAT

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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DATA EVALUATION RECORD

STUDY TYPE: Special Study: Cholinesterase and Metabolite Study - Rat
No specific guideline reference

EPA ID NO.S: EPA MRID NO.: 44648102 [*should be 44648101*]
EPA DP BARCODE: D249600
EPA SUBMISSION CODE: S548834
EPA P.C. CODE: 059101

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

SYNONYMS: *O,O*-diethyl, *O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate

CITATION: Mattsson, J.L., J.P. Maurissen, P.J. Spencer, K.A. Brzak, and C.L. Zablotny (1998): EFFECTS OF CHLORPYRIFOS ADMINISTERED VIA GAVAGE TO CD RATS DURING GESTATION AND LACTATION ON PLASMA, ERYTHROCYTE, HEART AND BRAIN CHOLINESTERASE, AND ANALYTICAL DETERMINATION OF CHLORPYRIFOS AND METABOLITES, Health and Environmental Research Laboratories , The Dow Chemical Company for Dow AgroSciences, August 31, 1998 (Unpublished Study); MRID 44648102 .

SPONSOR: Dow AgroSciences, 9330 Zionsville Road, Indiandapolis, IN 46286

EXECUTIVE SUMMARY: The present study (MRID 44648102) complements a Developmental Neurotoxicity study and was designed to evaluate cholinesterase inhibition and determination of chlorpyrifos and its principal metabolites in dams and pups. Pregnant Sprague-Dawley CD® rats were administered chlorpyrifos (99.8% a.i.; Lot No. MM930503-17; TSN 100227) by gavage at doses of 0, 0.3, 1.0, or 5.0 mg/kg/day beginning on gestation day (GD) 6 and continuing through lactation day 10. Five dams, as well as 5 male and 5 female pups/dose, were sacrificed on GD 20 and lactation days 1, 5, and 11 for chlorpyrifos and metabolite determinations. Milk samples were taken from the dams for chlorpyrifos and chlorpyrifos-oxon analyses. Blood samples were taken from dams and pups for chlorpyrifos, chlorpyrifos-oxon, and 3,5,6-trichloro-2-pyridinol (TCP) analyses. Cholinesterase (ChE) was determined in an additional 5 dams/dose and 5 pups/sex/dose on GD 20 and lactation days 1, 5, 11, 22, and 65 (pups only). ChE activity was determined in plasma, RBC, brain, and heart. For all analyses, samples were taken from dams and fetuses 4 hours postdosing, and from pups 2 hours postdosing of the dams.

No treatment-related clinical signs of toxicity in dams or pups and no differences in maternal body weights were observed at any time during the study. No differences in litter sizes at parturition or in the number of pups born dead were observed between the treated and control groups. Pup survival during lactation was similar for the treated groups as compared to the control.

Chlorpyrifos was detected in the blood of high-dose dams at a mean concentration of 108.78 ng/g on GD 20. Levels of chlorpyrifos then declined to 87% on lactation day 1, remained unchanged on lactation day 5, and were below the limit of detection by lactation day 11. Chlorpyrifos was detected at a low level (2.55 ng/g) in blood of mid-dose dams only on GD 20 and was not detected at any time in blood of low-dose dams. In milk, chlorpyrifos concentrations in the 0.3, 1.0, and 5.0 mg/kg/day groups were 20.57, 139.49, and 3022.00 ng/g, respectively on lactation day 1 and were 13.54, 81.76, and 1533.98 ng/g, respectively on lactation day 5. By lactation day 11, chlorpyrifos was detected only in the high-dose group at a level of 19.79 ng/g. Chlorpyrifos-oxon was not detected in the blood or milk of any dams at any time point.

Blood concentrations of chlorpyrifos in male and female fetuses from high-dose dams were 52.81 and 39.40 ng/g, respectively on GD 20. Concentrations in the pups declined to less than half of the GD 20 levels by lactation day 1 and were below the limit of detection by lactation day 5. Levels of chlorpyrifos in the blood of male and female fetuses from the mid-dose dams were 0.99 and 1.19 ng/g, respectively on GD 20, but were undetectable thereafter. Chlorpyrifos-oxon was detected in the blood of male and female fetuses from high-dose dams only on GD 20 at concentrations of 0.97 and 0.94 ng/g, respectively.

TCP was detected in the blood of dams from all treated groups on GD 20, lactation day 1, and lactation day 5. In the 0.3, 1.0, and 5.0 mg/kg/day groups, TCP levels in blood were 114.40, 322.01, and 1974.00 ng/g, respectively on GD 20, and were 142.93, 536.53, and 1449.92 ng/g, respectively on lactation day 5. On lactation day 11, TCP was detected only in the mid- and high-dose groups at levels of 9.87 and 71.40 ng/g, respectively.

TCP was detected in the blood of male and female fetuses from all dose groups in a dose dependent pattern on GD 20. In the 0.3, 1.0, and 5.0 mg/kg/day groups, TCP levels in blood on GD 20 were 93.93, 361.00, and 1680.00 ng/g, respectively for males, and were 99.49, 339.13, and 1884.00 ng/g, respectively for females. TCP was essentially not detectable in the blood of low- and mid-dose pups by lactation day 5. On lactation day 11, TCP was detected in the high-dose male and female pups at levels of 42.29 and 47.01 ng/g, respectively.

ChE activity in fore- and hindbrain from high-dose dams was 11.1-22.7% and 19.5-42.8%, respectively of the control level on GD 20 through lactation day 11 and 57.9% and 80.4%, respectively of controls on lactation day 22. ChE activity in the heart of high-dose dams was 16.9% of controls on GD 20, but recovered to 93.6% of controls on lactation day 22. Mid-dose dams had inhibition of brain ChE activity to 87.8-93.1% of controls from GD 20 through lactation day 11. In low-dose dams, brain and heart ChE activities were unaffected by treatment. In high-dose dams plasma and RBC ChE activities relative to the controls were 12.0% and 4.9%, respectively on GD 20, and 48.9% and 7.4%, respectively on lactation day 11. By lactation day 22, plasma ChE had recovered but RBC activity remained inhibited at 53.6% of the control level.

In mid-dose dams plasma and RBC ChE activities relative to the controls were 38.5% and 17.6%, respectively on GD 20, and 65.5% and 22.3%, respectively on lactation day 11. By lactation day 22, plasma ChE had recovered but RBC activity remained inhibited at 66.6% of the control level. In the low-dose group, plasma and RBC activities were 67.2% and 73.7%, respectively of controls on GD 20, but recovered to 83.8% and 75.5%, respectively on lactation day 11 and were similar to controls on lactation day 22.

No effects on ChE activity were seen in tissues from pups from the low- or mid-dose dams. In pups from the high-dose group, forebrain activity was 40.2% of controls on GD 20 and 63.3% on lactation day 1; hindbrain activity was 46.1%, 67.2%, and 88.4% of control levels on GD 20, lactation day 1, and lactation day 5, respectively. ChE activity in the heart of high-dose pups was 18.4%, 34.7%, and 83.9% of control levels on GD 20, lactation day 1, and lactation day 5, respectively. ChE activity in plasma from the high-dose pups was inhibited to 15.3% of controls on GD 20, 40.0% of controls on lactation day 1, and 81.5% of controls on lactation day 5. ChE activity in RBC from the high-dose pups was inhibited to 7.9% of controls on GD 20, 14.7% of controls on lactation day 1, and 86.4% of controls on lactation day 11. Complete recovery of ChE activity occurred in high-dose pups by lactation day 5 for forebrain, by lactation day 11 for hindbrain, heart, and plasma, and by lactation day 22 for RBC.

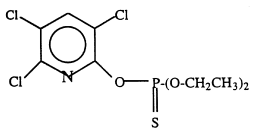
This study is classified as **ACCEPTABLE-NONGUIDELINE**. This is a special study intended to investigate specific parameters and does not fit into a guideline study classification. It is acceptable for the purposes for which it was intended.

COMPLIANCE: A signed and dated QUALITY ASSURANCE STATMENT, STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS, AND a statement of COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS was provided. A Flagging statement was not included since this was not a guideline study.

I. MATERIALS AND METHODS

A. MATERIALS (further details on methodology are available from the study report pages 16-32)

1. Test Material: Chlorpyrifos
   Description: not stated
   Lot No.: MM930503-17; TSN 100227
   Purity: 99.8% a.i. by weight
   Stability of compound: not stated
   CAS No.: not stated
   Structure:



2. Vehicle and/or positive control: Corn oil was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals

Species: female rats, time-mated at Charles River Breeding Laboratories
Strain: Sprague-Dawley CD®
Age and weight at study initiation: age not stated; 200-240 g
Source: Charles River Breeding Laboratories, Portage, MI
Housing: Animals were housed individually in suspended wire-mesh cages. No later than GD 19, dams were housed in plastic shoe box cages containing nesting material.
Diet: Purina® Certified Lab Diet #5002 and tap water were available *ad libitum*.
Environmental conditions:
   Temperature: 22 ± 3°C
   Humidity: 40-70%
   Air changes: 12-15/hour
   Photoperiod: 12 hour light/dark
Acclimation period: at least 4 days prior to initiation of dosing

B. PROCEDURES AND STUDY DESIGN

This study complements a Developmental Neurotoxicity study and was designed to evaluate cholinesterase inhibition and chlorpyrifos and its principal metabolites in dams and pups, after dosing of the dams from GD 6 through lactation day 10, inclusive.

1. In life dates

   Start: not given; end: not given

2. Mating

   Time-mated females were received from the supplier where the animals had been mated with a male of the same strain. Copulation was confirmed by the presence of a vaginal copulatory plug. The day evidence of mating was detected was designated gestation day (GD) 0.

3. Animal assignment and dose selection is presented in Table 1. Mated females were stratified by body weight and then randomly assigned to a vehicle control group and three treatment groups using a computer program. Allocation of animals to different subgroups of activities or measurements was done randomly using a computer program.

TABLE 1. Animal assignment

Group	Dose (mg/kg/day)	Number of dams	Number of pups[a]
Chlorpyrifos(-oxon) and TCP determination			
Control	0	20	40
Low Dose	0.3	20	40
Mid Dose	1.0	20	40
High Dose	5.0	20	40
Cholinesterase determination			
Control	0	30	60
Low Dose	0.3	30	60
Mid Dose	1.0	30	60
High Dose	5.0	30	60

Data taken from Table 1, p. 68.
[a]Pups were exposed to the test article in utero or through the milk; they were not dosed directly.

4. Dose selection rationale

   Doses used in the present study were the same as those used in a Developmental Neurotoxicity study. The high dose was expected to result in substantial maternal brain ChE inhibition and the mid dose was expected to cause plasma and RBC ChE inhibition and possibly minimal brain ChE inhibition in the dams. The low dose was chosen because it was close to a dose used in a Developmental Toxicity

study with chlorpyrifos conducted by NIEHS (reference: Robert Chapin, Personal Communication, 1997).

5. Dosing

Rats were given test substance or vehicle control once daily beginning on GD 6 and continuing through lactation day 10. Dams were not dosed on the day of parturition if they were delivering at the time of dosing. Doses were administered in a volume of 1 mL/kg body weight/day based on daily body weights.

6. Dose solution preparation and analysis

The method and frequency of dose solution preparation were not described in the study report. Concentration of the dose solutions was determined prior to the start of the study and again during the study. Homogeneity was determined for the low- and high-dose solutions prior to study initiation. Stability of the test article in the vehicle was not analyzed.

Absence of test article was confirmed in the vehicle. Mean concentrations of the 0.3, 1, and 5 mg/kg/day solutions were 98%, 103%, and 106%, respectively of nominal. Samples from the top, middle, and bottom of the low- and high-dose solutions ranged from 96-99% and 101-114%, respectively of nominal.

C. OBSERVATIONS

1. Maternal observations and evaluations

All animals were observed twice daily for morbidity, moribundity, and mortality and once daily for clinical signs of toxicity. Maternal body weights were recorded on GD 0 and GD 6 through lactation day 10. Body weights were used to calculate dose and were not analyzed statistically. Food consumption was not measured.

2. Litter observations

Dams were allowed to litter naturally. Visible physical abnormalities or behavioral changes in the neonates were recorded daily during the lactation period. On lactation day 4, the goal was to have 5 male and 5 female pups for all litters. The pups were sexed and the litters with too few males or females were supplemented with pups from other litters and then culled to 5 males and 5 females. These supplementary pups were clearly identified, were for litter size only, and were not used for any testing. On lactation day 11, litters were randomly culled to 4 male and 4 female pups. All litters were weaned on lactation day 21. Pups were not weighed.

3. Chlorpyrifos and metabolites determination

Five dams, as well as 5 male and 5 female pups/dose, were sacrificed on GD 20 and lactation days 1, 5, and 11. Milk samples were taken from the dams for chlorpyrifos and chlorpyrifos-oxon analyses. Blood samples were taken from dams and pups for chlorpyrifos, chlorpyrifos-oxon, and 3,5,6-trichloro-2-pyridinol (TCP) analyses. Animals were anesthetized prior to sampling. Two hours after dosing of the dams, the pups were removed and a blood sample taken immediately; two hours later (4 hours postdosing), the dams were sampled. Blood samples were taken from GD 20 fetuses when the dams were sampled 4 hours postdosing. If not enough blood was obtained from one pup/fetus, the blood of several individuals of the same litter was pooled to have a minimum of 0.2 mL blood. When sampling was complete, the anesthetized animals were killed by decapitation. Concentrations of chlorpyrifos and its metabolites were determined by gas chromatography/mass spectrometry using radiolabeled internal standards.

4. Cholinesterase determination

Plasma, RBC, heart, and brain ChE determinations were conducted on 5 dams/dose and 5 pups/sex/dose on GD 20 and on lactation days 1, 5, 11, 22, and 65 (pups only). After blood collection, the anesthetized animals were decapitated and the brain and heart were collected and weighed. If necessary, blood of several pups/fetuses was pooled to have a minimum of 0.25 mL; if several pups were used for blood collection, their brains and hearts were also pooled. Samples were taken from dams and fetuses 4 hours postdosing and from pups 2 hours post-dosing in order to be consistent with milk sampling. ChE activity was measured spectrophotometrically using a Hitachi 911 Automatic Analyzer (Boehringer Mannheim, Indianapolis, IN).

D. DATA ANALYSIS

Statistical analysis

Plasma, RBC, and brain ChE activities were analyzed by Multivariate Analysis of Variance (MANOVA). Heart ChE activity was analyzed by ANOVA. Bartlett's test was used to determine homogeneity of variance. The Pillai trace statistic was used to determine the statistical significance of the MANOVAs or ANOVAs. No inferential statistics were calculated for chlorpyrifos and metabolite concentrations in blood and milk. Since many of the metabolite samples were below the limit of detection, in groups containing at least 3 samples above the limit of detection, the one or two remaining samples were assigned a value of one-half the limit of detection value. This allowed calculation of means from as many dose groups and time points as possible. For chlorpyrifos and chlorpyrifos-oxon the limit of detection was 0.7 ng/g and for TCP the limit of detection was 10 ng/g.

II. RESULTS

A. ANIMAL OBSERVATIONS (Dams and Pups)

1. Mortality and clinical signs

No treatment-related clinical signs of toxicity were observed in dams at any time during the study. One pup from a high-dose litter had generalized muscle tremor associated with movement. However, this observation was a single incidence on lactation day 57 and is not considered due to treatment. No differences in litter sizes at parturition or in the number of pups born dead were observed between the treated and control groups. Pup survival during lactation was similar for the treated groups as compared to the control.

2. Maternal body weight

Selected maternal body weight data are given in Table 2. Although these data were not analyzed statistically by the study author, it is apparent that no differences occurred between the treated and control groups at any time during gestation or lactation. Pups were not weighed during this study.

TABLE 2: Selected maternal body weights and body weight gains[1] during gestation and lactation (g)

Day of study	0 mg/kg/day	0.3 mg/kg/day	1.0 mg/kg/day	5.0 mg/kg/day
GD 0	216.0	217.3	215.5	216.7
GD 10	279.2	276.0	276.7	278.1
GD 20	388.2	384.5	385.5	389.3
Lactation day 1	294.5	295.6	299.0	300.0
Lactation day 5	317.1	316.0	321.6	321.7
GD 0-10	63.2	58.7	61.2	61.4
GD 0-20	172.2	167.2	170.0	172.6
LD 1-5	22.6	20.4	22.6	21.7

[1] = calculated by the reviewers
Data taken from Tables 8 and 9, pp. 77 and 78, respectively.

3. Food consumption

Maternal food consumption was not measured.

4. Gross pathology

Gross necropsy findings were not reported for dams or pups.

B. CHLORPYRIFOS AND METABOLITE DETERMINATION

1. Dams

a. Blood

Mean concentrations of chlorpyrifos and TCP in the blood of dams are given in Table 3. Chlorpyrifos was detected in the blood of high-dose dams at a mean concentration of 108.78 ng/g on GD 20. Levels of chlorpyrifos then declined to 87% on lactation day 1 and remained unchanged on lactation day 5. By lactation day 11 (approximately 28 hours after the last dose), chlorpyrifos levels were below the limit of detection in high-dose dams. Chlorpyrifos was detected at a low level (2.55 ng/g) in blood of mid-dose dams only on GD 20 and was not detected at any time in blood of low-dose dams. Chlorpyrifos-oxon was not detected in the blood of any dams at any time point.

TCP was detected in the blood of dams from all treated groups on GD 20, lactation day 1, and lactation day 5. In the 0.3, 1.0, and 5.0 mg/kg/day groups, TCP levels in blood were 114.40, 322.01, and 1974.00 ng/g, respectively on GD 20, were 111.46, 394.54, and 2718.00 ng/g, respectively on lactation day 1, and were 142.93, 536.53, and 1449.92 ng/g, respectively on lactation day 5. On lactation day 11, TCP was detected only in the mid- and high-dose groups at levels of 9.87 and 71.40 ng/g, respectively.

TABLE 3: Chlorpyrifos and TCP concentrations in blood of dams

	0 mg/kg/day	**0.3 mg/kg/day**	**1.0 mg/kg/day**	**5.0 mg/kg/day**
Chlorpyrifos (ng/g±sd)				
GD 20	NQ[1]	NQ	2.55±0.88	108.78±58.74
Lactation day 1	NQ	NQ	NQ	14.53±6.20
Lactation day 5	NQ	NQ	NQ	14.79±10.64
Lactation day 11	NQ	NQ	NQ	NQ
TCP				
GD 20	NQ	114.40±23.87	322.04±13.10	1974.00±472.26
Lactation day 1	NQ	111.46±26.07	394.54±65.23	2718.00±1509.00
Lactation day 5	NQ	142.93±37.37	536.5358.16	1449.92±95.27
Lactation day 11	NQ	NQ	9.87±5.08	71.40±22.60

Data taken from Tables 10 and 11, pp. 79 and 80, respectively.
[1]NQ = not quantified; below limit of detection

b. Milk

Concentrations of chlorpyrifos in the milk are given in Table 4. Chlorpyrifos levels in the milk were much greater than those measured in blood and declined over time. In the 0.3, 1.0, and 5.0 mg/kg/day groups, chlorpyrifos concentrations in milk were 20.57, 139.49, and 3022.00 ng/g, respectively on lactation day 1 and were 13.54, 81.76, and 1533.98 ng/g, respectively on lactation day 5. By lactation day 11, chlorpyrifos was detected only in the high-dose group at a level of 19.79 ng/g. Chlorpyrifos-oxon was not detected in the milk at any dose level at any time point during the study.

TABLE 4: Chlorpyrifos concentrations in milk of dams (ng/g±sd)

	0 mg/kg/day	0.3 mg/kg/day	1.0 mg/kg/day	5.0 mg/kg/day
GD 20	N/A[1]	N/A	N/A	N/A
Lactation day 1	NQ[2]	20.57±8.47	139.49±35.54	3022.00±1153.63
Lactation day 5	NQ	13.54±3.88	81.76±6.62	1533.98±192.24
Lactation day 11	NQ	NQ	NQ	19.79±6.52

Data taken from Table 12, p. 81.
[1]N/A = not applicable; milk samples not obtained on GD 20.
[2]NQ = not quantified; below limit of detection.

2. Pups

Chlorpyrifos and TCP blood concentrations in male and female pups are given in Table 5. Levels of chlorpyrifos detected in the blood of fetuses/pups were approximately half that measured in dams from the same dose groups and time points. Blood concentrations of chlorpyrifos in male and female fetuses from high-dose dams were 52.81 and 39.40 ng/g, respectively on GD 20. Concentrations in the pups declined to less than half of the GD 20 levels by lactation day 1 and were below the limit of detection by lactation day 5. Levels of chlorpyrifos in the blood of male and female fetuses from the mid-dose dams were 0.99 and 1.19 ng/g, respectively on GD 20, but were undetectable thereafter. Chlorpyrifos-oxon was detected in the blood of male and female fetuses from high-dose dams only on GD 20 at concentrations of 0.97 and 0.94 ng/g, respectively.

TCP was detected in the blood of male and female fetuses from all dose groups in a dose dependent pattern on GD 20. In the 0.3, 1.0, and 5.0 mg/kg/day groups, TCP levels in blood on GD 20 were 93.93, 361.00, and 1680.00 ng/g, respectively for males, and were 99.49, 339.13, and 1884.00 ng/g, respectively for females. By lactation day 1, TCP levels had declined to not quantified (NQ), 137.31, and 842.67 ng/g, respectively for males and 50.03, 133.90, and 433.29 ng/g, respectively for females. TCP was essentially not detectable in the blood of low- and mid-dose pups by lactation day 5. On lactation day 11, TCP was detected in the high-dose males and females at levels of 42.29 and 47.01 ng/g, respectively.

TABLE 5: Chlorpyrifos and TCP concentrations in blood of pups

	0 mg/kg /day	0.3 mg/kg/day	1.0 mg/kg/day	5.0 mg/kg/day	0 mg/kg/day	0.3 mg/kg/day	1.0 mg/kg/day	5.0 mg/kg/day
	Males				**Females**			
Chlorpyrifos (ng/g±sd)								
GD 20	NQ[1]	NQ	0.99±0.41	52.81±25.23	NQ	NQ	1.19±0.32	39.40±12.99
Lactation day 1	NQ	NQ	NQ	18.17±24.64	NQ	NQ	NQ	6.61±8.04
Lactation day 5	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Lactation day 11	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
TCP								
GD 20	NQ	93.93±14.47	361.00±64.11	1680.00±241.35	NQ	99.49±13.67	339.13±93.75	1884.00±234.05
Lactation day 1	NQ	NQ	137.31±85.65	842.67±293.51	NQ	50.03±27.51	133.90±27.74	433.29±262.82
Lactation day 5	NQ	NQ	NQ	47.37±18.81	NQ	NQ	NQ	50.23±50.23
Lactation day 11	NQ	NQ	NQ	42.29±8.94	NQ	NQ	9.50±2.69	47.01±47.01

Data taken from Tables 13 and 15, pp. 82 and 84, respectively.
[1]NQ = not quantified; below limit of detection.

C. CHOLINESTERASE DETERMINATIONS

1. Dams

Cholinesterase activities in dams for brain and heart are given in Table 6 and for RBC and plasma are given in Table 7. In the high-dose dams, fore- and hindbrain ChE activity was significantly ($p \leq 0.01$) inhibited throughout the study and heart activity was significantly inhibited through lactation day 11 as compared to controls. Activity in fore- and hindbrain from high-dose dams was 11.1-22.7% and 19.5-42.8%, respectively of the control level on GD 20 through lactation day 11 and 57.9% and 80.4%, respectively of controls on lactation day 22. ChE activity in the heart of high-dose dams was 16.9% of controls on GD 20, but recovered to 93.6% of controls on lactation day 22. Mid-dose dams had statistically significant ($p \leq 0.05$ or 0.01), although not biologically significant, inhibition of brain ChE activity of 87.8-93.1% of controls from GD 20 through lactation day 11. In the low-dose group, brain and heart ChE activities were unaffected by treatment.

Plasma and RBC ChE activities for dams are given in Table 7. Mid- and high-dose dams had significant ($p \leq 0.01$) inhibition of plasma activity through lactation day 11 and of RBC activity through lactation day 22. In the high-dose group,

plasma and RBC ChE activities relative to the controls were 12.0% and 4.9%, respectively on GD 20, 6.1% and 1.2%, respectively on lactation day 1, and 48.9% and 7.4%, respectively on lactation day 11. By lactation day 22, plasma ChE had recovered but RBC activity remained inhibited at 53.6% of the control level. In the mid-dose group plasma and RBC ChE activities relative to the controls were 38.5% and 17.6%, respectively on GD 20, 22.9% and 12.7%, respectively on lactation day 1, and 65.5% and 22.3%, respectively on lactation day 11. By lactation day 22, plasma ChE had recovered but RBC activity remained inhibited at 66.6% of the control level. In the low-dose group, plasma and RBC activities were 67.2% and 73.7%, respectively of controls on GD 20, but recovered to 83.8% and 75.5%, respectively on lactation day 11 and to 116.8% and 92.8%, respectively on lactation day 22.

TABLE 6: Brain and heart cholinesterase activity in dams (% of control)

	0.3 mg/kg/day	1.0 mg/kg/day	5.0 mg/kg/day
Forebrain			
GD 20	98.0±2.7	92.2**±3.7	13.3**±3.4
Lactation day 1	102.2±3.9	93.1*±3.2	11.1**±3.7
Lactation day 5	102.1±8.2	90.0*±3.5	16.1**±4.1
Lactation day 11	98.9±3.8	91.2*±4.5	22.7**±1.1
Lactation day 22	99.18.2	94.9±5.5	57.9**±3.6
Hindbrain			
GD 20	101.1±7.2	92.0*±2.2	24.0**±4.8
Lactation day 1	93.8±20.1	93.2±5.0	19.5**±4.5
Lactation day 5	101.3±4.8	87.8**±3.2	28.1**±7.4
Lactation day 11	104.5±4.2	97.1±7.3	42.8**±3.5
Lactation day 22	97.8±4.5	93.2±8.2	80.4**±5.0
Heart			
GD 20	100.3±20.1	51.1**±7.4	16.9**±4.3
Lactation day 1	90.1*±7.1	58.3**±8.9	11.4**±3.1
Lactation day 5	95.8±4.9	68.8**±10.3	16.9**±6.0
Lactation day 11	87.6±11.0	90.9±14.0	35.3**±6.2
Lactation day 22	110.3±12.7	93.3±21.3	93.6±12.5

Data taken from Tables 22-24, pp. 99-101.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

TABLE 7: Blood cholinesterase activity in dams (% of control)			
	0.3 mg/kg/day	**1.0 mg/kg/day**	**5.0 mg/kg/day**
Plasma			
GD 20	67.2**±10.1	38.5**±6.3	12.0**±2.9
Lactation day 1	47.8**±10.3	22.9**±4.1	6.1**±2.3
Lactation day 5	68.3**±6.6	30.7**±4.5	7.8**±1.4
Lactation day 11	83.8±11.5	65.5**±10.3	48.9**±15.6
Lactation day 22	116.8±21.8	131.7**±25.4	120.3±10.1
RBC			
GD 20	73.7**±14.5	17.6**±6.7	4.9**±2.8
Lactation day 1	60.8**±9.2	12.7**±3.3	1.2**±1.6
Lactation day 5	88.9±16.5	16.0**±2.7	2.7**±2.3
Lactation day 11	75.5**±8.0	23.3**±4.3	7.4**±4.1
Lactation day 22	92.8±6.8	66.6**±9.6	53.6**±5.6

Data taken from Tables 22-24, pp. 99-101.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

2. Pups

Brain and heart ChE activities for pups are given in Table 8 and plasma and RBC ChE activities for the pups are given in Table 9. No statistical differences in ChE activity were observed between male and female pups in any tissue at any time point, so the data are presented as combined male and female data. No effects on ChE activity were seen in tissues from pups from the low- or mid-dose dams. Pups from the high-dose group had significant ($p \leq 0.01$) inhibition of ChE activity in all tissues. Forebrain activity was 40.2% of controls on GD 20 and 63.3% on lactation day 1; hindbrain activity was 46.1%, 67.2%, and 88.4% of control levels on GD 20, lactation day 1, and lactation day 5, respectively. ChE activity in the heart of high-dose pups was 18.4%, 34.7%, and 83.9% of control levels on GD 20, lactation day 1, and lactation day 5, respectively.

ChE activity in plasma from the high-dose pups was significantly ($p \leq 0.01$) inhibited to 15.3% of controls on GD 20, 40.0% of controls on lactation day 1, and 81.5% of controls on lactation day 5. ChE activity in RBC from the high-dose pups was significantly ($p \leq 0.01$) inhibited to 7.9% of controls on GD 20, 14.7% of controls on lactation day 1, 57.2% of controls on lactation day 5, and 86.4% of controls on lactation day 11. Complete recovery of ChE activity occurred in high-dose pups by lactation day 5 for forebrain, by lactation day 11 for hindbrain, heart, and plasma, and by lactation day 22 for RBC.

TABLE 8: Brain and heart cholinesterase activity in pups (% of control)			
	0.3 mg/kg/day	**1.0 mg/kg/day**	**5.0 mg/kg/day**
Forebrain			
GD 20	103.9±22.4	91.1±5.2	40.2**±8.9
Lactation day 1	97.8±8.8	93.8±5.1	63.3**±8.6
Lactation day 5	105.5±26.9	124.3±45.3	103.4±37.3
Lactation day 11	101.9±3.9	102.3±4.1	97.3±8.4
Lactation day 22	102.5±2.6	99.4±2.8	99.6±8.2
Hindbrain			
GD 20	107.0±5.0	99.7±5.6	46.1**±9.3
Lactation day 1	103.7±11.3	100.5±9.1	67.2**±10.5
Lactation day 5	99.5±8.2	96.4±3.5	88.4**±4.6
Lactation day 11	92.6±6.2	92.9±5.4	95.5±10.1
Lactation day 22	99.6±10.6	101.8±5.6	104.1±5.9
Heart			
GD 20	112.4*±16.0	94.3±15.4	18.4**±3.6
Lactation day 1	101.3±20.4	97.0±20.3	34.7**±12.7
Lactation day 5	106.2±10.0	108.4±12.6	83.9**±12.6
Lactation day 11	95.5±8.6	99.7±9.8	94.3±9.0
Lactation day 22	102.4±10.7	102.0±10.7	109.0±21.1

Data taken from Tables 25-27, pp. 102-104.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

TABLE 9: Blood cholinesterase activity in pups (% of control)			
	0.3 mg/kg/day	**1.0 mg/kg/day**	**5.0 mg/kg/day**
Plasma			
GD 20	103.8±6.1	95.6±7.0	15.3**±4.3
Lactation day 1	97.6±6.4	94.1±8.3	40.0**±10.8
Lactation day 5	104.5±11.5	108.1±9.5	81.5**±11.4
Lactation day 11	92.6±8.6	95.6±8.8	91.2±8.7
Lactation day 22	95.7±4.7	96.7±6.2	96.7±14.9
RBC			
GD 20	102.2±20.3	106.4±16.7	7.9**±4.3
Lactation day 1	111.1±16.4	101.0±24.4	14.7**±5.4
Lactation day 5	104.4±15.2	104.6±18.2	57.2**±14.8
Lactation day 11	101.4±10.8	96.7±10.7	86.4**±6.7
Lactation day 22	101.6±25.5	109.6±28.7	103.6±15.7

Data taken from Tables 25-27, pp. 102-104.
Significantly different from control: **$p \leq 0.01$.

III. DISCUSSION

A. CHLORPYRIFOS AND METABOLITE DETERMINATIONS

Dose-dependent levels of chlorpyrifos and TCP were measured in the blood of dams and fetuses/pups. Levels of chlorpyrifos detected in the blood of fetuses/pups were approximately half that measured in dams from the same dose groups and time points. Chlorpyrifos concentrations in pups decreased rapidly after birth despite continued exposure to substantial concentrations in the milk. While blood TCP levels were similar in dams and fetuses on GD 20, levels in pups declined rapidly during lactation but remained constant in dams during the dosing interval. It is interesting that chlorpyrifos-oxon was detected in the blood of fetuses from high-dose dams on GD 20, but not in dams or pups at any time point. Therefore, it appears that maternal circulation, not milk, is of greater concern for exposure of fetuses/pups to chlorpyrifos and/or its metabolites.

B. CHOLINESTERASE DETERMINATIONS

ChE inhibition was much greater for dams than for pups at all time points. While dams had inhibition in the brain and heart at the mid- and high-doses and in the blood at all doses, the fetuses/pups were affected only at the highest maternal dose. In the dams, recovery of enzyme activity was rapid in most tissues following cessation of dosing. In contrast, ChE activity in the pups began to recover after birth and was near control levels in all tissues (except RBC) by lactation day 11. Recovery in pups occurred during lactation despite continued exposure to chlorpyrifos in the milk. Therefore, it appears that pups were not uniquely sensitive to ChE inhibition by chlorpyrifos following maternal dosing and that the pups were able to regenerate their ChE.

C. STUDY DEFICIENCIES

There were no major deficiencies in the conduct of this study.

D. CLASSIFICATION

This study is classified as **ACCEPTABLE-NONGUIDELINE**. This is a special study intended to investigate specific parameters and does not fit into a guideline study classification. It is acceptable for the purposes for which it was intended.

SignOff Date:	12/7/1998
DP Barcode:	D237217
HED DOC Number:	013012
Toxicology Branch:	RRB3